Incorporating Clinical Biomarkers into Clinical Trials

Paul W. Rhyne

Abstract Clinical trials are required for testing the safety and efficacy of new drugs produced by the pharmaceutical industry. These trials also serve to provide information on clinical and survival benefits, prediction of treatment responses, identification of patient subpopulations that will benefit from the drug, and many other important aspects of treatment. Clinical biomarkers are essential tools in these trials that enable these different aspects to be evaluated and defined. However, the incorporation of biomarkers into clinical trials requires a knowledge base that includes understanding the different types of clinical biomarkers, selection of the best biomarkers for the trial, best sample handling and processing practices for the biomarker, validation planning, selection of the best technology platform, and selection of the best laboratory to perform the analysis. This chapter provides an overview of how clinical biomarkers are used in clinical trials and discusses the different aspects of incorporating them into clinical trials.

Keywords

Biomarker	A characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention
Clinical trial	Research-based studies involving human volunteers that are assigned to receive one or more interventions so that researchers can evaluate the effects of the interventions on biomedical or health-related outcomes
Validation	Confirmation through laboratory testing that the performance characteristics of an assay are suitable and reliable for its intended analytical use
Critical reagent	Reagents such as antibodies, oligonucleotides, enzymes, or fluorescent molecules that are integral parts of an assay that influence assay performance or quality

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Why Incorporate Biomarkers into Clinical Trials?

A question that is often asked by clinical teams is why should biomarkers be incorporated into clinical trials? The answer to this question starts by defining exactly what a clinical trial is. The US National Institutes of Health defines clinical trials as research-based studies involving human volunteers that are "assigned to receive one or more interventions so that researchers can evaluate the effects of the interventions on biomedical or health-related outcomes". In these studies, participants receive specific interventions according to the research plan or protocol created by the investigators [18]. Clinical trials may include testing new medical products, new drugs, new treatment procedures, or comparing new medical approaches to existing ones. A major aspect of clinical trials is the evaluation of safety and efficacy of these interventions in the participants. However, there are other aspects of clinical trials equally important such as prediction of treatment benefit, evaluation of survival benefit, selection of drug dosing, demonstrating clinical benefit, verification of the therapeutic biological target, identification of patient subpopulations, etc. Incorporating biomarkers into clinical trials provides the necessary tools to evaluate many of these other important aspects in addition to drug safety and efficacy.

Biomarkers

Given the increased use of biomarkers in clinical trials, it is important to understand how biomarkers are defined and the differences between the different categories. The term biomarker has been defined as a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [1]. The National Cancer Institute Investigational Drug screening task force created a biomarker task force that was to provide recommendations for the use of biomarkers in clinical trials [2]. The task force also provided some definitions on different subcategories of biomarkers. Prognostic biomarkers provide evidence about the patients overall disease outcome independent of any specific intervention. Predictive biomarkers provide evidence about the probability of benefit or toxicity from a specific intervention. Surrogate biomarkers are intended to serve as a substitute for a clinically meaningful endpoint. Pharmacodynamic biomarkers are used to provide evidence of pharmacological effects of drugs [2]. Diagnostic biomarkers provide information that aids to establish or confirm a diagnosis. Exploratory biomarkers are used mainly for hypothesis generation. They are typically based on scientific literature and knowledge of biological pathways, and have not previously been shown to have clinical significance. There are so many different subcategories of biomarkers that have been described, it is important to remember that they are not always referring to the same thing.

Predictive Biomarkers

The incorporation of biomarkers into clinical trials can provide tools to predict how individual patients will respond or benefit from treatment. Clinical trials that incorporate the use of predictive biomarkers often involve pretesting potential trial subjects prior to enrollment. In some cases, only those subjects that have a "positive" or "negative" biomarker profile will be enrolled into the trial. This may be used to eliminate subpopulations that will not benefit from treatment. Alternatively, there may be a concern for putting a potentially harmful therapeutic drug into a non-disease state population. This is often the case for oncology and infectious diseases therapeutics, where the treatments create a safety concern in healthy individuals. Predictive biomarkers are typically genetic-based biomarkers such as KRAS [3] or BRAF [4]. However, other types of predictive biomarkers are increasingly being used such as cellular phenotyping [5], expression levels of proteins [6, 7], and others. Predictive biomarkers are also used during the trial to assess progress and subsequently to change aspects of the trial midstream. Examples of these type trial designs include BATTLE (biomarker integrated approaches of targeted therapy for non-small cell lung Carcinoma) [8] and I-SPY [9]. Trials can also be designed with the purpose of codeveloping a predictive biomarker with the therapeutic drug being developed. These require a robust hypothesis and high-quality clinical data, so that benefit and magnitude of the benefit of treatment can be determined.

Selection of Clinical Biomarkers

The development of new drug products involves a significant amount of investment in drug discovery and preclinical work, before clinical trials can be initiated. Biomarkers are incredibly valuable in these early stages and are often used to help evaluate early drug candidates before clinical trials begin. In the early stages of clinical trial planning, clinical teams need to identify biomarkers that will be used in these trials. Selection of potential clinical biomarkers include those identified cell lines, preclinical animal studies, those used in other clinical studies, and the published literature. Consideration should be given to ensure the selected biomarker is amenable for clinical use. Laboratory scientists performing these studies have control over the cellular growth conditions including viability, drug exposure, and cell numbers in each of the experiments. In contrast, this level of control is not available for samples collected from clinical trial participants. The expression level of the biomarker in clinical samples needs to be evaluated to ensure the biomarker is present in sufficient quantities to be useful.

Preclinical studies in cell lines often involve stimulation or treatment of cells to induce changes in the biomarker [10]. Performing these treatments or providing stimulation to cells from cell lines are relatively easy in comparison to using isolated

cells taken from clinical trial participants. The clinical sites where trial participants are being treated often do not have the needed expertise or the right equipment to implement complex pre-analytical sample collection and handling procedures. Isolating cells from blood and stimulating them at the clinical sites are often met with significant technical challenges. Another option is to send the samples to an external lab with the required expertise in the methodology. This will require additional logistics for the shipment of the samples to a different location which may expose the samples to conditions that change the cellular response generated from treatment [11, 12]. Finally, cellular responses to the stimulation conditions used on cell lines are likely to be different then in freshly isolated cells [10]. Thus, it is recommended that some level of investigation be done on cell-based biomarkers from freshly isolated cells before incorporating them into the clinical trial.

Clinical Biomarker Pre-analytic Sample Collection and Handling

One of the key attributes of incorporating clinical biomarkers into clinical trials is the collection and processing of the clinical samples. Biomarker assays are typically developed, tested, and validated using common laboratory buffers which do not reflect the clinical samples. The assay should be tested in the same matrix as the clinical samples before being used for sample analysis. The sample collection procedures, processing, and handling of the clinical samples has an impact on the biomarker measurement [12]. A prime example would be VEGF. While VEGF has measureable expression levels in serum and plasma, care must be taken in the collection and processing of the clinical samples to avoid inadvertent release of VEGF from platelets [11]. Improper sample collection will result in levels of VEGF that do not reflect the status of the test subject, but rather improper handling at the clinical site. Another example would be the addition of compounds or reagents directly to the clinical sample [13]. These additives are often added to a specified volume of the clinical sample, in which the premeasured volume may not be accurate. Thus, careful evaluation of the instrumentation and staff expertise at the clinical site should be done to ensure successful handing and processing of samples. This evaluation should be repeated at regular intervals, every 6–12 months and/or when key site personnel change.

Biomarker Assay Performance and Prevalidation Planning

Biomarker assays should have good clinical utility and have performance characteristics that are suitable for clinical trials. The first step in selecting the best assay is to define what question the biomarker data will be used to answer. This will be used

to define the required performance characteristics of the assay including analytical sensitivity, accuracy and precision, range of detection, and dilutional linearity [2, 14]. Once the performance requirements are defined, the search for an appropriate assay can begin. The assay format, technology base, availability of assay reagents, cost, level of technical expertise, lot-to-lot variability of kits and reagents, and data format should be considered in the selection of the best assay. The clinical team should strive for simple assay formats in lieu of more complex platforms, which are more difficult to perform and troubleshoot. The technology used in the assay will determine instrumentation needed, which could limit the number of available labs capable of running the assay. Technologies that are well established tend to be more widely available in laboratories than newer technologies. Newer technologies may offer or claim advantages over existing technologies, but often come with increased operating costs and the need for additional technical expertise technical expertise. Newer technologies also carry an increased risk of unknown or unresolved technical issues that may be encountered during the clinical trial. Thus, it is essential to have access to expertise to minimize this impact. Utilizing a technology that is provided by a single company carries an economic risk and a risk of not being able to complete sample analysis. There are examples of single source vendors that have gone out of business or are no longer able to support their technology. Cost of the instrumentation and needed reagents/supplies should be assessed before final selection of the technology. This is especially critical for highly labile samples where storing the sample for an extended period of time, while an alternative method is identified, is not feasible due to lack of long-term sample stability.

Importance of Critical Reagents

Biomarker assays, regardless of the assay format or the technology, rely on critical analytical reagents such as antibodies, oligonucleotides, enzymes, or fluorescent molecules. It is essential that these critical reagents are available throughout the clinical trial period in which the biomarker assay is used. Evaluation of supply and expiration dates should be done well before biomarker analysis is started [15, 16]. Protein-based reagents are often given an expiration date of one year, which presents problems for clinical trials that extend beyond a year. As a result, this would require multiple lots of reagents to be purchased and used. It is incumbent upon the bioanalytical scientist to evaluate multiple lots of reagents and determine if all lots perform as the original lot of reagent. In addition to reagents, other critical supplies such as assay plates, chips, and disposables fall into this category. Careful planning will ensure that delays from back ordered items are minimized as much as possible.

Lot changes of critical reagents and supplies often impact the performance of the assay, which can result in significant differences in reported biomarker results.

Examples of this include differences in precoated plates such as streptavidin plates, changes in lot of antibody, differences in the labeling of antibodies used to detect the biomarker, or differences in purity of critical reagents such as peptides or oligonucleotides. Instances where lot changes occur should be evaluated in bridging experiments to assess the impact on biomarker assay performance and reported results. This can be disruptive during a clinical trial that is dependent on biomarker data for enrollment. Careful planning is needed to minimize the impact of availability and lot changes during the course of the trail.

Biomarker Data Handling

Analyzing biomarkers in clinical samples will generate data that is reported back to the clinical team. Some level of evaluation must take place before sample analysis begins. The handling and generation of clinical data must be compliant with good clinical laboratory practices (GCP) [19], which are typically handled through specialized software that protects patient identification, reported results, and other important information in a secure manner. The transfer of biomarker data into clinical databases is not trivial and can be the rate-limited step to getting data to the clinical team. Data may need to be transformed into various formats such as text or comma separated values (csv) before the data can be imported into clinical databases. The final data should be checked for transcription errors that may have been occurred during the transfer of data into the clinical database.

Commercial Biomarker Assays

The selection of a suitable biomarker assay for clinical sample analysis is sometimes easier when the assay is available as a commercial kit. The ability to purchase a premade kit eliminates the need for assay development and simplifies the process of reagent procurement. Commercially available kits provide prewritten assay protocols, prepackaged reagents and supplies, and technical support from the kit manufacturer all with a fixed cost. Commercial kits that have the required assay performance needs provide an excellent and viable option for the clinical team. However, there are several risks that come with selecting a commercial kit for biomarker analysis [17]. Manufacturers of commercial kits often develop the kits using laboratory buffers and not clinical matrices such as serum or plasma. These kits require testing in the appropriate biological matrix before they can be reliably used to support clinic sample analysis. There are kit manufacturers that recognize this and have started to provide kit performance data in biological matrices. Clinical

teams selecting commercial sources of biomarker assays should make sure the assay can measure the biomarker in samples that are as close as possible to the clinical samples taken from trial participants.

Biomarker Assay Validation

Once a suitable biomarker assay has been developed or acquired, the clinical team should have the assay validated prior to use. The required performance characteristics should be used to generate a validation plan. The amount of effort, time, and performance characteristics tested for each biomarker assay is often referred to as a "fit-for-purpose" validation plan [14]. In some cases where the biomarker data is for informational or exploratory in nature, a minimal amount of validation may be appropriate. In other cases, where the biomarker will be used as clinical surrogate endpoints, used for enrollment or dosing decisions, or go/no-go decisions the level of analytical validation should be advanced and more comprehensive. The data generated during validation should be evaluated to ensure the assay meets the performance requirements and provide useful data to the clinical team before the assay is used in the clinical trial. The assay should also have run acceptance criteria that are used throughout sample analysis to ensure the assay performance is consistent. The performance of the assay throughout the sample analysis period should be reviewed and used to define the clinical performance characteristics of the assay for subsequent and future clinical trial use.

Selection of the Biomarker Laboratory

Selecting the right biomarkers and the best assays for a clinical trial should be done with careful planning and evaluation. However, selecting the best laboratory to perform the work is equally important. Several aspects of laboratory selection that need to be part of the decision process include instrumentation, technical expertise, SOPs, certifications, and cost. The majority of biomarker laboratories are equipped with instrumentation for well-established technologies, but may not always have newer technologies. Clinical teams should perform some level of laboratory qualification to ensure the laboratory has the right instrumentation, maintains the instrumentation in good working order, and keeps good maintenance records for the instrumentation. The qualification process should also include a review of laboratory staff qualifications (training, education, and experience) to ensure the level of expertise is present to run the assay correctly. Laboratories with experienced staff are likely to solve technical issues more efficiently and identify potential problems with the assay before they occur. Biomarker laboratories should have documented SOPs and work practices in place to ensure consistency in running assays regardless of the scientist performing the work.

Laboratory Certification and Operational Practices

There are multiple types of certifications and practices that laboratories can acquire or implement. Incorporating biomarkers into clinical trials may or may not require laboratory certification. Thus, clinical teams should have a working knowledge of these certifications and practices in addition to the requirements for biomarker analysis. A few examples of these would be Good Clinical Practice (GCP) [19], Good Laboratory Practice (GLP) [20], and certifications under the Clinical Laboratory Improvement Amendments (CLIA) [21].

GLP is for laboratories conducting nonclinical laboratory studies, which do not involve human subjects [20]. GLP ensures that all laboratory testing is performed by qualified personnel in adequate facilities and supervision. The equipment has to be well maintained, 21 CSF Part 11 compliant and calibrated prior to use. Written SOPs must be in place and all work is fully documented to ensure traceability and reproducibility. GLP also requires monitoring of the study by a separate, quality assurance unit. Good Clinical Practice, or GCP, provides a standard for the design, conduct, performance, monitoring, auditing, recording, analysis, and reporting of clinical trials. GCP provides assurance that the data and reported results are credible and accurate. Also, GCP provides assurance that the rights, integrity, and confidentiality of the trial subjects are protected. In 1988, congress passed the clinical laboratory improvement amendments (CLIA) [21]. CLIA establishes quality standards for all non-research laboratory testing performed on specimens derived from humans for the purpose of providing information for the diagnosis, prevention, treatment of disease, or impairment of, or assessment of health. Thus, if a biomarker assay is to be used on human samples for the diagnosis, prevention, or treatment of disease, the laboratory performing the assay would need to be CLIA certified and hold a CLIA certificate that corresponds to the test being performed. Laboratories that have a CLIA certification should have well-maintained instrumentation, documented training of staff, background information on staff, and have good documentation on reagents. Depending on the intended use of the biomarker, the lab may be required to implement GLP or GCP practices, or obtain CLIA certification.

Clinical trials are essential tools in the drug development and approval process. A significant amount of effort is needed to ensure a successful clinical trial outcome. Biomarkers can be used to predict treatment outcome, adjust drug dosing during the trial, verify targeted biological pathways, provide information of drug safety and set subject enrollment criteria, all of which increase the success rate of the clinical trial. Thus, the incorporation of biomarkers in clinical trials plays a crucial part in the success rate of clinical trials and subsequently the drug development process.

Chapter Summary

1. There are many types and definitions of clinical biomarkers.
2. Sample collection and handling should be carefully planned to minimize the impact on the measurement of the biomarker.
3. Assays used to measured clinical biomarkers should be evaluated with a fit-for-purpose validation plan to ensure the assay is suitable for the clinical trial.
4. Biomarker laboratories should have the proper instrumentation, experience, and work practices that are needed to support the clinical trial.

References

1. Atkinson AJ, Colburn WA, DeGruttola VG, DeMets DL, Downing GJ, Hoth DF, Oates JA, Peck CC, Schooley RT, Spilker BA, Woodcock J, Zeger SL (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69(3):89–95
2. Dancey JE, Dobbin KK, Groshen S, Jessup JM, Hruszkewycz AH, Koehler M, Parchment R, Ratain MJ, Shankar LK, Stadler WM, True LD, Gravell A, Grever MR (2010) Guidelines for the development and incorporation of biomarker studies in early clinical trials of novel agents. Clin Cancer Res 16:1745–1755
3. Lièvre A, Bachet JB, Le Corre D, Boige V, Landi B, Emile JF, Côté JF, Tomasic G, Penna C, Ducreux M, Rougier P, Penault-Llorca F, Laurent-Puig P (2006) KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 66:3992–3995
4. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA (2002) Mutations in the BRAF gene in human cancer. Nature 417:949–954
5. Younes A, Gopal AK, Smith SE, Ansell SM, Rosenblatt JD, Savage KJ, Ramchandren R, Bartlett NL, Cheson BD, de Vos S, Forero-Torres A, Moskowitz CH, Connors JM, Engert A, Larsen EK, Kennedy DA, Sievers EL, Chen R (2012) Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. J Clin Oncol 30(18):2183–2190
6. Lanari A, Parnetti L (2009) Cerebrospinal fluid biomarkers and prediction of conversion in patients with mile cognitive impairment: 4-year follow-up in a routine clinical setting. Sci World J 9:961–966
7. Mattsson N, Zetterberg H, Hansson O, Andreasen N, Parnetti L, Jonsson M, Herukka SK, van der Flier WM, Blankenstein MA, Ewers M, Rich K, Kaiser E, Verbeek M, Tsolaki M, Mulugeta E, Rosén E, Aarsland D, Visser PJ, Schröder J, Marcusson J, de Leon M, Hampel H, Scheltens P, Pirttilä T, Wallin A, Jönhagen ME, Minthon L, Winblad B, Blennow K (2009) CSF biomarkers and incipient Alzheimer disease in patients with mild cognitive impairment. J Am Med Assoc 302:385–393

8. Kim ES, Herbst RS, Wistuba II, Lee JJ, Blumenschein GR Jr, Tsao A, Stewart DJ, Hicks ME, Erasmus J Jr, Gupta S, Alden CM, Liu S, Tang X, Khuri FR, Tran HT, Johnson BE, Heymach JV, Mao L, Fossella F, Kies MS, Papadimitrakopoulou V, Davis SE, Lippman SM, Hong WK (2011) The BATTLE trial: personalizing therapy for lung cancer. Cancer Discovery 1:44–53 (2011)
9. Esserman LJ, Berry DA, Cheang MC, Yau C, Perou CM, Carey L, DeMichele A, Gray JW, Conway-Dorsey K, Lenburg ME, Buxton MB, Davis SE, van't Veer LJ, Hudis C, Chin K, Wolf D, Krontiras H, Montgomery L, Tripathy D, Lehman C, Liu MC, Olopade OI, Rugo HS, Carpenter JT, Livasy C, Dressler L, Chhieng D, Singh B, Mies C, Rabban J, Chen YY, Giri D, Au A, N Hylton (2012) Chemotherapy response and recurrence-free survival in neoadjuvant breast cancer depends on biomarker profiles: results from the I-SPY 1 TRIAL (CALGB 150007/150012; ACRIN 6657). Breast Cancer Res Treat 132:1049–4062
10. Schildberger A, Rossmanith E, Eichhorn T, Strassl K, Weber V (2013) Monocytes, peripheral blood mononuclear cells, and THP-1 cells exhibit different cytokine expression patterns following stimulation with lipopolysaccharide. Mediators Inflamm 2013:1–10
11. Schlingemann RO, Van Noorden JF, Diekman MJM, Tiller A, Meijers JCM, Kookwijk P, Wiersinga WM (2013) VEGF levels in plasma in relation to platelet activation, glycemic control, and microvascular complications in type 1 diabetes. Diabetes Care 36(6):1629–1634 (June)
12. Mallone R, Mannering SI, Brooks-Worrell BM, Durinovic-Bello I, Cilio CM, Wong FS, Schloot NC (2010) Isolation and preservation of peripheral blood mononuclear cells for analysis of islet antigen-reactive T cell responses: position statement of the T-Cell workshop committee of the Immunology of diabetes society. J Transl Immunol 163:33–49
13. Mueller SC, März R, Schmolz M, Drewelow B (2012) Intraindividual long term stability and response corridors of cytokines in healthy volunteers detected by a standardized whole-blood culture system for bed-side application. BMC Med Res Methodol 12:112–121
14. Lee JW, Devanarayan V, Barrett YC, Weiner RS, Allinson J, Fountain S, Keller S, Weinryb I, Green M, Duan L, Rogers JA, Millham R, O'Brian PJ, Sailstad J, Khan M, Ray C, Wagner JA (2006) Fit-for-purpose method development and validation for successful biomarker measurement. Pharm Res 23(2):312–328
15. O'Hara DM, Theobald V, Egan AC, Usansky J, Krishna M, TerWee J, Maia M, Spriggs FP, Kenney J, Safavi A, Keefe J (2012) Ligand binding assays in the 21st century laboratory: recommendations for characterization and supply of critical reagents. AAPS J 14(2):316–328
16. Geist BJ, Egan AC, Yang T, Dong Y, Shankar G (2013) Characterization of critical reagents in ligand-binding assays: enabling robust bioanalytical methods and lifecycle management. Bioanalysis 5(2):227–244
17. Khan M, Bowsher R, Cameron M, Devanarayan V, Keller S, King L, Lee J, Morimoto A, Rhyne P, Stephen L, Wu Y, Wyant T, Lachno R (2015) Recommendations for adaptation and validation of commercial kits for biomarker quantification in drug development. Bioanalysis 7(2):229–242

Website References

18. www.clinicaltrials.gov/ct2/about-studies/glossary#interventional-study
19. www.fda.gov/scienceresearch/specialtopics/runningclinicaltrials
20. www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=58
21. www.cdc.gov/clia

Author Biography

Paul Rhyne obtained his Ph.D. in Cellular Immunology from the University of Tennessee at Memphis where he studied the cellular interactions between B cells and T cells. Dr. Rhyne gained post-doctoral experience in Virology at St. Jude Children's Research Hospital focusing on the tumorgenicity of Epstein-Barr Virus. Following this, Dr. Rhyne began a career in industry working in a start-up biotechnology company focused on early cancer detection technologies. He subsequently worked in the commercial antibody industry where he developed a Luminex-based product line to measure phosphorylated proteins. He joined Bristol-Myers Squibb pharmaceutical company where he built and managed a clinical biomarker group. This biomarker group was responsible for developing and validating clinical biomarker assays for BMS clinical trials. Dr. Rhyne continued to expand his career in the contract research organization industry where he was responsible for the large molecule operations at Tandem Labs. This large molecule group provided services to pharmaceutical companies that included the development and validation of clinical biomarkers, pharmacokinetic assays, and Immunogenicity assays. Recently, Dr. Rhyne joined Quintiles bioanalytical services in Marietta Georgia where he is responsible for method development of large molecule pharmacokinetic and Immunogenicity assays. In summary, Dr. Rhyne has developed hundreds of both commercial and clinical biomarker assays for the pharmaceutical industry and has successfully used many of these assays in clinical trials.