PRECISION for medicine

Successful Strategies for Biomarker-Driven Research and Clinical Development

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Table of Contents

Published in FIERCE Biotech

Biospecimens

Clinical Trials

Specialty Labs

Central Lab Services

Data Science

Manufacturing

Introduction

Biomarkers—and the assays used to detect, measure, and characterize them—are the cornerstone of precision medicine, which aims to match the right patient with the right drug at the right dose and time to optimize treatment impact and patient outcome. This eBook is a compilation of Precision for Medicine's latest thinking on integrating biomarkers into development to optimize the therapeutic index of a drug and increase the likelihood of approval.

Over the past decade, the pace of progress in precision medicine has quickened due to advances in omics technologies that enable more rapid identification of mutations and more precise characterization of molecular targets. Greater understanding of the genetic bases for cancers and other diseases has created opportunities for clinical trials to leverage genetic variability for recruitment and stratification but may introduce complexity during development and study planning.

Today, more than half of all oncology trials involve the use of biomarkers and nearly two-thirds of clinical decisions are influenced by biomarker-based diagnostics.[1] Research has shown that biomarker-driven strategies increase the likelihood of drug approval by approximately 40 percent across all therapeutic indications.[2] In addition, precision medicines tend to require fewer and smaller trials and less time for approval than other medicines.[3] Further, a recent large-scale analysis of over 10,000 trials and 745 drugs found that inclusion of biomarker status dramatically increases clinical trial success rates in breast cancer, melanoma, and non-small cell lung cancer. Interestingly, this analysis revealed that even exploratory biomarkers improved success rates in oncology studies, though the benefit was less pronounced.[4]

As the use of biomarkers continues to expand across the research and care continuum, therapeutic developers are tasked with integrating clinical design and execution with a

clear biomarker-informed development plan. Lack of an appropriate biomarker plan can have downstream effects on clinical trial operations, including informed consent, electronic case report form design, site budgets, and even enrollment. As such, it is never too early to identify a defined pathway for taking an exploratory biomarker all the way through to diagnostic approval if data supports its use.

To be incorporated into clinical trials, biomarkers that have been discovered and characterized in preclinical models must be translated into fit-for-purpose biomarkers that can be seamlessly integrated into study designs. Biomarker assays will need to be developed and analytically validated, and if they are to be used as companion diagnostics, will also need to undergo more rigorous clinical validation. Developing a robust, effective biomarker strategy can be challenging as it requires in-depth understanding and integration of three separate disciplines: biomarker assay development and implementation, clinical trial operations, and data science. Only by working together can these disciplines ensure that the biomarker and assay selected are validated, clinically relevant, and feasible for use in the ultimate clinical setting.

The journey from biomarker discovery to biomarker-driven clinical trials requires thoughtful, proactive planning and collaboration among cross-functional teams at every stage of development. In this eBook, we explore the opportunities and challenges of biomarker-driven development, providing insight on topics ranging from biospecimen selection and clinical translation to companion diagnostics and study execution.

About Precision for Medicine

As a global precision medicine clinical research organization, Precision for Medicine has brought together new technologies, expertise, and operational scale to help the life sciences industry improve the speed, cost and success rate of bringing life-changing therapies to patients. Precision leverages the combined power of clinical trial solutions, biospecimens, specialty lab services and data sciences to accelerate discovery and drive faster, more efficient clinical development by maximizing insights into patient biology.

Purpose-built to advance biomarker-driven development, Precision understands how to realize the full potential of biomarker use in clinical use. Our experience in biomarker-driven development and diagnostics is unmatched, with more than 500 trials conducted and more than 250 marketing clearances and approvals for in vitro diagnostic submissions. Precision has also spearheaded an ambitious sequencing initiative to uncover new insights on oncology disease drivers, drug targets and novel biomarkers by screening both its extensive biorepository and prospectively collected liquid biopsy samples.

Choose Wisely. Biospecimens Can Make or Break Your Biomarker-Driven Cancer Research

Anuj Kalsy, Scientific Director, Precision for Medicine

Immunotherapy is transforming the treatment of cancer, with efficacy observed across a variety of tumors. Recent advances in biomarker analysis have been critical not only for identifying those patients who are likely to achieve benefit from immuno-oncology therapeutics, but also for providing insight into the mutational variations of tumors throughout treatment and disease progression. These advancements in biomarker discovery, validation, and clinical application have hinged on one critical resource: Quality biospecimens.

Access to a consistent supply of high-quality, deeply phenotyped biospecimens is required to support ongoing biomarker discovery and validation for any novel immuno-oncology treatment, from immune checkpoint and targeted therapeutics to chimeric antigen receptor T (CAR-T) cell therapies and other precision medicines. To extract the necessary data from these biospecimens, researchers must also consider which specimen type is most appropriate for the scientific question to be answered.

Below, we discuss key considerations for developing the right biospecimen strategy to advance immuno-oncology research and development (R&D), with a focus on applications of biospecimens to support biomarker discovery.

Optimizing Program Success with a Robust Biospecimen Strategy

Sourcing and selecting the right biospecimens is make or break in the identification of *de novo* biomarkers or therapeutic drug targets. The use of large cohorts of quality-controlled biospecimens that contain rich disease characterization has enabled the identification of tumor associated antigens and has led to the successful development of myriad targeted therapies, including monoclonal antibodies, antibody drug conjugates (ADCs), anti-cancer vaccines, and CAR-T cell therapies.

Researchers who are unable to procure the right biospecimens to validate and guide therapeutic or diagnostic development are more likely to see their programs fail in clinic. For example, many programs falter because the drug target is expressed only by a subset of patients or is also present in healthy patients, both of which could have been discovered early on by comparing biomarker prevalence in severe versus mild disease or versus controls. If the biospecimen strategy does not include disease severity characterization or a powered control group, this type of validation is impossible.

Quality biospecimens are also critical for the identification of novel cancer resistance mechanisms and oncogenic pathways. Research has shown that biomarker expression may change throughout cancer treatment and tumor progression. Repeated biospecimen profiling over time can provide insight into mechanisms of treatment-specific resistance or immunological escape and may also be used to inform disease management. Thus, procuring specimens with longitudinal collections can help guide researchers' treatment and study stratification plans.

Successful sourcing of these specimens requires a robust biospecimen strategy and a reliable biospecimen solutions partner. The Precision for Medicine Biospecimen Solutions team has a dedicated operation for specimen collection, processing, and logistics that supports 1,500 R&D clients globally. Their prospective collection capabilities include blood, biofluids, tissues, and cell products from almost any disease, including related scientific services.

The Critical Role of Biomarkers in Immuno-Oncology

The discovery that blocking the programmed death-1 (PD-1)/programmed death-ligand 1 (PD-L1) pathway could enhance anti-tumor T cell reactivity and promote immune control over cancer cells was a turning point in immuno-oncology. The seven PD-1 or PD-L1 immune checkpoint inhibitors approved to date have been a therapeutic breakthrough for patients with many types of cancer. However, only 20-40% of patients derive benefit from these therapies and, of those who do respond, a substantial proportion acquire resistance. Moreover, some patients whose tumor biopsies do not appear to show dysregulated expression of these biomarkers still respond to treatment. All these factors highlight the complex biological underpinnings of therapeutic response, heterogeneity and underscore the need to optimize immuno-oncology biomarkers for patient selection and treatment monitoring.[1]

Researchers who are unable to procure the right biospecimens... are more likely to see their programs fail in clinic.

Already, many immune checkpoint inhibitors use either an immunohistochemistry (IHC)-based companion or complementary diagnostics to evaluate the potential safety and effectiveness of the treatment in a particular patient, though none of these diagnostics are definitive. Other biomarkers, such as tumor mutational burden (TMB), microsatellite instability (MSI), and gene expression profile, may also be valuable for subclassifying tumor types and

assessing likelihood of response. In some tumor types, immune function genes, inflammatory markers, and human leukocyte antigen (HLA) expression may provide predictive information. More recently, molecular, and spatial profiling with multiplex immunofluorescence has been used to assess the tumor microenvironment, providing insight into the extent and distribution of immune cell infiltration, which affects the activity and efficacy of immune checkpoint inhibitors. The Precision for Medicine Translational Solutions team offers specialty lab capabilities, leveraging established technologies and proprietary approaches to generate robust biomarker data from the full range of biospecimens.

Given the diversity of biospecimens, it is essential to choose the specimen type that is most appropriate for generating the required data.

Selecting Fit-for-Purpose Biospecimens

Given the diversity of biospecimens, it is essential to choose the specimen type that is most appropriate for generating the required data. Formalin-fixed, paraffin-embedded (FFPE) tissues are versatile biospecimens with a range of applications. FFPE specimens are frequently used to determine tissue distribution of an antigen or biomarker of interest or to perform genomic profiling. With the development of newer transcriptomic technologies, it is also possible to perform gene expression analysis studies on FFPE tissues. In addition, FFPE samples of solid tumors can be used for molecular and spatial profiling or epigenetic profiling, an emerging approach to understanding the molecular basis of carcinogenesis.

Liquid biospecimens are valuable tools for evaluating biomarkers and identifying potential targets for further validation to shape immuno-oncology therapies. Plasma is one of the most widely used biospecimens for cancer biomarker discovery research and diagnostic or therapeutic development.[2] The plasma proteome can provide insight into normal physiological states and cancer-induced alterations in the body; however, studying it can be challenging. The plasma proteome comprises high-abundance proteins and a wide dynamic range of protein concentrations. Recent advances in mass spectrometry workflows have made it possible to perform unbiased, hypothesis-free characterizations of the plasma proteome to screen for and validate biomarkers.[3] These workflows eliminate the needs for separate immunoassay-based validation, accelerating biomarker discovery.

Due to their role in the innate and adaptive immune system, peripheral blood mononuclear cells (PBMCs) have long been used to study immunological mechanisms and responses. Immuno-oncology R&D generally requires PBMCs that have been characterized by cell type, quantity, and activity, and are accompanied by phenotypic data such as patient medical history. Such in-depth characterization is vital for any study seeking to elucidate the molecular differences or drivers of various disease severities or subtype or to compare against controls.

PBMCs can be used for both in vitro and in vivo studies. Common applications for in vitro PBMC studies include cell function investigations and disease modeling. *In vivo* analyses may involve reconstituting immunocompromised animal models with human PBMCs to study immune response to malignant tumors. PBMCs can also be used in adoptive cell therapy for expanding patient-derived T cells.

Precision for Medicine Biospecimen Solutions has developed an infrastructure for delivering fresh PBMCs with the highest viability, purity, and cell counts. In addition, their extensive biorepository enables access to deeply characterized health and disease state PBMCs from a diversity of

donors, often with matched plasma and tissue, facilitating evaluation of population differences in immunological responses.

Incorporating Advanced Liquid Biopsy Techniques into a Biospecimen Strategy

Blood- or biofluid-based biomarkers offer flexibility, as samples can be collected less invasively and more frequently than tissue biopsies. Liquid biopsies enable analysis of tumor cells or tumor cell products and, in principle, have the potential to reflect all subclones present at a single point in time for assessing tumor burden, tracking tumor evolution, or monitoring treatment response. In addition to enabling longitudinal assessments, liquid biopsies can also be used to complement tumor tissue, providing deeper molecular insights.

Currently, it is possible to analyze the genetic material of circulating tumor cells at the single cell level to evaluate both spatial and temporal dynamics. Liquid biopsy techniques have also expanded to include circulating tumor DNA (ctDNA), cell-free DNA or RNA (cfDNA or cfRNA), soluble proteins, and exosomes. The approvals of the Guardant360 CDx assay and the FoundationOne Liquid CDx test, both of which combine liquid biopsy with next generation sequencing (NGS) reflect the growing importance of genomic profiling in immuno-oncology and precision medicine. NGS has been widely adopted for genomic profiling due its massively parallel sequencing capability and its compatibility with low-quantity input DNA. It is also the preferred sequencing method when target mutations are not known, as in biomarker discovery.

Blood- or biofluid-based biomarkers offer flexibility, as samples can be collected less invasively and more frequently

Supporting New Biomarker Discovery and Development

Clinically meaningful, actionable mutations occur at extremely low frequencies and finding biospecimens with those specific mutations is a challenge for researchers and diagnostic or therapeutic developers. To help create a library of deeply characterized biospecimens that can be leveraged for immuno-oncology R&D, Precision for Medicine Biospecimen Solutions launched the Precision Oncology Sequencing Initiative (Project P.O.S.I.) Project P.O.S.I. is a large-scale, two phase NGS initiative to screen biospecimens for key biomarkers across cancer indications, designed to generate data from real clinical samples rather than contrived specimens. The screening panels used include the Oncomine Precision Assay, which detects 2,768 unique cancer variants across 50 genes, and the Illumina TSO500 assay, which includes pan-cancer biomarker content for a variety of solid tumor types and can also measure TMB and MSI.

The first phase of this undertaking, which is ongoing, focuses on NGS screening of thousands of FFPE tumor tissues from our extensive biorepository. The second phase of Project P.O.S.I. involves mass screening of liquid biopsies. From our global clinical network comprising over 55 countries, Precision for Medicine is prospectively collecting cfDNA from liquid biopsies obtained from patients with cancer. NGS data from these cfDNA samples is combined with clinical information and liquid biopsy metadata and then interrogated using QuartzBio®, our proprietary multi-omics data processing engine.

Precision for Medicine is dedicated to accelerating the discovery of clinically meaningful biomarkers to support the development of immuno-oncology therapeutics and diagnostics. With our extensive biospecimen repository, large specialty lab network, logistics capabilities, and full-service clinical research organization solutions, we are helping researchers and developers deliver on the promise of precision oncology.

Anuj Kalsy,
Scientific Director,
Precision for Medicine

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Bridging the Preclinical to Clinical Divide: Practical Considerations for Biomarker Success

Amanda Woodrooffe, PhD, Senior Vice President, General Manager UK Labs

Precision medicine is driven by advances in omics technologies that facilitate identification of mutations and the presence or absence of certain molecular targets, which, in turn, enables streamlined development of therapeutics that are precisely tailored to an individual's specific disease characteristics. Biomarkers are integral to the development of precision medicines; in fact, a recent report revealed that biomarker-driven strategies increase the likelihood of drug approval by approximately 40 percent. To successfully integrate biomarkers into clinical design and execution, developers need a clear plan for bridging the gap from bench to bedside to ensure biomarker feasibility and utility.

Understanding Categories of Biomarkers

Biomarkers can take many forms, and their role in successful development of targeted therapies and personalized medicines is increasing. Biomarkers fall broadly into 3 categories:

1. **Safety.** Preclinical biomarkers are often focused on safety, where correlations with toxicity—typically assessed through biochemical changes and histopathologic evidence of tissue injury—are evaluated and used to help define a drug's therapeutic index and clinical dose. To be suitable for transition

from bench to bedside, these biomarkers must be able to be monitored in a clinical-development setting.

2. **Predictive/prognostic.** These are biomarkers that allow for monitoring of drug efficacy and pharmacodynamics. For hypothesis-free biomarker discovery, multiplexed measurements of analytes are used to define panels of potentially valid biomarkers for future application. A key consideration for this type of biomarker discovery is how to manage the resulting complex datasets to enable extraction of relevant molecular signatures for the biological process or drug mechanism of action (MOA) in question.
3. **Diagnostic.** These are markers of specific disease, which are typically identified and validated by comparing well-characterized cohorts of ex vivo diseased human tissues with relevant healthy control tissues. An understanding of variations in biomarker expression among diseased vs nondisease populations can be effectively used to guide patient selection for clinical trials. Where expression of a particular biomarker or biomarker signature indicates that a patient's disease characteristics are a match for the therapeutic MOA, this provides a higher likelihood of clinical response.

Figure 1. **An example of a clinical biomarker workflow**

Assessing Biomarker Clinical Utility

To maximize its clinical utility, a biomarker must be monitorable, transferable, and reliable. Whether biomarkers are identified and characterized in animal models or using human biospecimens, it is important to ensure that these biomarkers will be suitable for use in the ultimate clinical setting. In particular, biomarkers must correlate with a clinically relevant endpoint that, ideally, is minimally invasive to the patient. Moreover, it is imperative that the right sample type be collected and processed in the right way to allow for the downstream clinical biomarker assay to generate high quality, actionable data.

Selection of the assay type is also important, as utility will be circumscribed by accessibility. If an assay requires a highly specialized platform or fresh samples, it will be more challenging to deploy not only in a global clinical trial, but also, if being developed as a diagnostic, in routine clinical practice. Assay turnaround times must also be factored into biomarker feasibility and utility, especially if biomarker data are being used to make real-time clinical decisions such as patient inclusion or exclusion or changes to the administered dose. Considering the assay format, with respect to the number of analytes measured, is also key for the management, integration, and interrogation of multiomic data sets. In particular, it's important to plan for how the interpretation of incidental findings from a multiplexed, nontargeted assay dataset is presented to regulators.

Planning the Preclinical-to-Clinical Transition

Planning for the transition of a biomarker from the preclinical to clinical setting should begin as early as possible during the development process and well before clinical collection plans are finalized and documented. The importance of early dialogue on what samples are required and why they need to be processed at a specific time in a particular way for downstream biomarker analyses cannot be overemphasized.

This is an area where Precision for Medicine creates significant value through early discussions around the feasibility of various assay options and through **custom assay development and validation**.

The analytical assays required will be defined by the nature and timing of the actionable biomarker data needed. Subsequently, the assay type will dictate the sample type and how, when, and where it needs to be processed. Early consideration of the feasibility of stabilizing samples, where required, to create an extended assay window will add a level of sample protection and maximize the ability to generate valuable biomarker data. Moreover, planning for broad informed consent for sample analysis is helpful, in case biomarker plans change and different assay types are required.

Another critical element of early planning is considering how the resulting data will be used, as this will define the level of assay validation required by regulators. As assay development and validation can take several months, it is advisable to work backwards from the first patient in (FPI) date to ensure that the assay is ready in time to support the trial. Precision for Medicine has extensive experience in evaluating assays and assay panels and determining whether they are fit-for-purpose or require modifications—either in design or in the level of validation—to meet clinical study needs.

Figure 2. **Practicalities of bridging the preclinical to clinical divide**

FACTOR	PRECLINICAL	CLINICAL
	Inherently well controlled animal population Less variable biological background	Diseased patients Inherent inter-subject variability
	Fresh blood collected on site Optimal sample quality / integrity	Fresh blood collected at multiple clinical sites
	On site	No local testing
	Immediate processing on site	Shipment to processing lab (min 8h, typically 24h) Consider requirement to stabilise sample

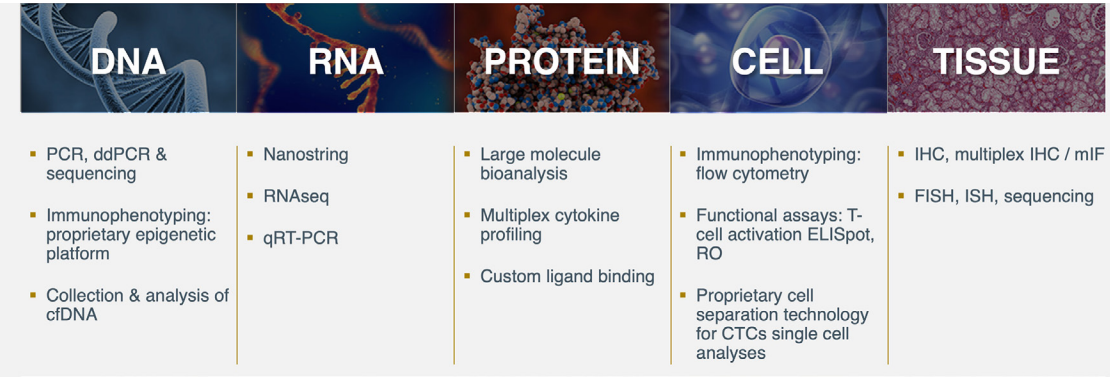
Ensuring Assay Practicalities Fit Within the Constraints of Clinical Trials

As mentioned above, many preclinical assays are developed in animal models, so it is critical to understand if and how a preclinical assay will translate to the reality of the clinical development environment.

Early communication between preclinical assay development teams and their clinical counterparts is essential for understanding the practicalities governing assays within the constraints of a global clinical trial setting. Key factors to consider include:

- **Variability.** The preclinical species comprises an inherently well-controlled population with less variable biological background than the clinical trial population, which will demonstrate significant inter-subject variability and, often, concomitant disease. Further, due to fundamental differences in biology between species, preclinical assays may need to be redesigned to suit the clinical biology
- **Sample type and logistics.** With preclinical animal model assays, fresh blood is typically collected and immediately processed on site, resulting in optimal sample quality and integrity and, thus, the best possible samples for downstream analyses. In clinical trials, however, patient samples will be collected from multiple, often global, sites and will then require shipment to a sample processing lab within a suitable timeframe for the downstream application. Careful consideration

Figure 3. **Analytes and platforms for biomarker analysis**



must be given to the location of clinical trial sites and the logistics of transporting precious samples to a processing lab in a safe and timely manner. Options for mitigating logistical challenges include identifying a CRO partner with global lab coverage, selecting a sample type, where appropriate, that is suitable for local processing and storage prior to batched analysis, or stabilizing the sample. Precision for Medicine has created an integrated solution for sample management, from collection through analysis, by designing and providing sample collection kits, supporting a worldwide network of specialty biomarker and sample processing labs, and developing a global infrastructure to support shipping, logistics, and storage

Optimizing Actionable Data from Biomarker Analysis

There is a broad array of platforms and technologies available for biomarker analysis, enabling interrogation of samples including nucleic acids, proteins, tissues, and biofluids, as well as measurement of cellular function. Platform selection will be driven by the assay type needed to generate the biomarker data necessary for on-trial decision making. The level of assay validation required is based upon the types of decisions those data will influence. For instance, assays used for patient selection or for an altered-dose regimen will require more comprehensive analytical and clinical validation.

While dose escalation through clinical cohorts is primarily driven by safety data and clinical observations or measurements, building in the right early or exploratory biomarker data can help drive decisions around adaptive trial design and confirm the utility and value of biomarker changes that can be applied as clinical development progresses. Here, the challenge lies in managing and making sense of all the resulting data. In addition to biomarker assay data, clinical trials produce a plethora of datasets in diverse formats from multiple sources across patients, sample types, and timepoints. Precision for Medicine has developed QuartzBio®, a powerful data platform that can collate, integrate, interrogate, and interpret these data, together with third-party, publicly available data sources, to generate actionable insights. This platform also includes a virtual sample inventory management tool that provides ongoing visibility into the location and status of clinical samples.

Conclusion

Preclinical data are a valuable and essential part of biomarker discovery, validation, and proof of concept. To successfully advance preclinical biomarker assays from the bench to the bedside, it is crucial to maintain engagement between discovery and clinical biomarker and operations teams and to develop a clear plan for delivering assays that can be utilized within the constraints of the clinical trial setting.

Precision for Medicine is focused on delivering personalized medicine approaches to match the right patient with the right drug at the right dose and time to optimize treatment impact and outcome. We support the entire development process, from design and execution of biomarker-driven clinical trials and kitting and logistics to specialty laboratory services, biomarker data management, and regulatory and commercialization support. Our goal is to provide comprehensive translational science-led research services to deliver biomarker science at scale.

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General Manager
UK Labs

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Recommended Strategies for Your Next Biomarker-Driven Clinical Trial

Tiziana Magnani, Senior Project Manager and
Stephanie Jarjabka, Director, Project Management

Over the past decade, there has been a paradigm shift in drug development. The rise of next generation precision medicines have fueled a dramatic increase in biomarker-driven clinical trials, which seek to optimize the likelihood of study success by enrolling only those patients who are most likely to demonstrate benefit. However, incorporating biomarkers into clinical development can be challenging, and innovators must carefully consider strategies for ensuring the selection and seamless integration of fit-for-purpose biomarkers into their study designs.

Precision for Medicine, a global CRO, is purpose-built around developing and executing biomarker-driven clinical trials. Based on the experience and feedback from our global project teams, who actively support cell and gene therapy studies and other biomarker-influenced trials, Precision offers the following strategies for overcoming 4 common challenges in biomarker-driven trials.

1. Effective Patient Identification in Biomarker-Driven Clinical Trials Requires a Clear Study Concept at the Outset

Common Challenge: Often, during early phase research, sponsors may still be tweaking planned biomarkers. This lack of clarity can impact patient identification, next-generation

sequencing (NGS) testing, patient stratification, site selection, study timelines, and costs.

Precision's Recommended Strategies:

- **Set up a pre-screening trial.** Suppose you are still deciding among biomarkers and have a large immunotherapy pipeline. In that case, you may want to consider setting up a pre-screening trial to create a funnel of patients who can then be enrolled into randomized trials across your portfolio.
- **Lock in your biomarkers at the beginning of your clinical trial.** The market is competitive, and biopharmaceutical sponsors must move at lightning speed, but not locking in your biomarkers at the time of study start-up (with a CRO engaged) will lead to scope, budget, and site creep. If you are still deciding among biomarkers, this can inflate the patient totals in your trial, with knock-on effects on overall study cost due to increases in the number of sites needed, the equipment required, and other resources. Having a solid protocol and clear biomarker foundation is critical for avoiding unnecessary expenditures and study delays caused by inevitable protocol amendments. This sounds simple, but taking a few more weeks or months to lock in your biomarkers at the front end will save your organization precious time and possibly millions on the back end.

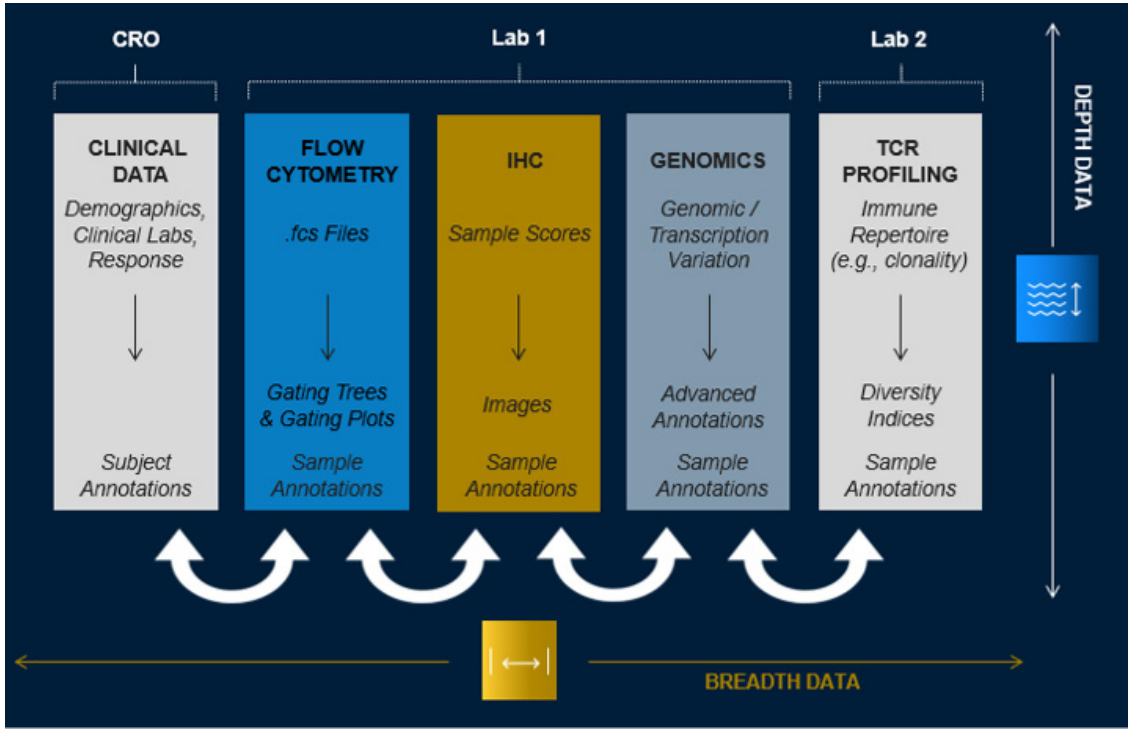
2. Awareness of NGS Testing Standards at Planned Sites within the Target Countries

Common Challenge: Harmonized NGS testing standards simply do not exist. The NGS testing standard done at an academic institution, such as MD Anderson, is going to differ dramatically from testing completed in a community-based hospital system – where it may or may not even be done on a routine basis. Similarly, testing will vary if performed at a large hospital in Paris versus one in rural France.

Precision's Recommended Strategies:

- **When evaluating site feasibility, ask about the standard NGS testing done by the site for patients in your targeted indication.** In our experience, many sponsors prefer to be deeply involved in site identification and may have already initiated site outreach and selection activities prior to engaging with their CROs. It is not uncommon for sponsors to overlook the NGS testing standards question during their initial conversations with study sites. This oversight can adversely impact rapid start-up if further feasibility is required.
- Including specific, detailed questions about NGS testing in your site feasibility evaluations will give you a more accurate and comprehensive assessment of that site's enrollment potential. If your selected biomarker is included in the site's standard NGS testing, the site may already have a database of known patients with that biomarker, which may lead to more rapid enrollment. If your biomarker is not included, enrollment at that site will likely be slower as the screening process will require additional testing. Understanding enrollment potential is critical for curating an effective and efficient group of trial sites from which to find the right patients, thus enabling you to hit your overall study timeline.
- **Plan for more screening failures than you would with a non-biomarker-driven trial.** In biomarker-driven trials, NGS testing is a necessary part of the screening process. Screening can be expensive, though, and the cost of screening failures must be accounted for in the study budget. The likelihood of screening failures—and the resultant budget impact—will depend on several factors, including your indication, the statistical incidence of your expressed biomarker, and the type of testing required, among others.

Figure 1. **Biomarker data management links the breadth and depth data generated in biomarker-driven trials, as described in Precision's use case, *From IHC to Immune Profiling: Managing Data Breadth and Depth to Inform On-Study Decisions.***



- **Carefully vet and select your NGS testing laboratory partner.** With the lack of NGS testing standards and differences in its "routine" application between hospitals globally and locally, this dramatically increases the importance and capabilities required of your selected NGS testing vendor. Sponsors and their CROs have to carefully consider the selected specialty labs capabilities and ensure that the logistics of sample collection, shipment, analysis, etc. must all be fully thought through and documented in an agreed plan for your study.

- **Ensure the capabilities of your CRO align with your biomarker requirements.** The growth of targeted treatments has increased the therapeutic importance of companion diagnostics. **If your study requires a new assay or companion diagnostic,** ensure that your selected CRO partner has the capability to support its development. Precision for Medicine is adept at supporting biopharmaceutical sponsors through the intricacies of companion diagnostic development, providing end-to-end expertise from specialty lab services and regulatory strategy to market access and commercialization. This is where a fully capable CRO, like Precision, can make a difference in your development to approval timeline.

3. Efficient integration of biomarker data to support study and patient decision making.

Common Challenge: Biomarker-driven clinical trials often involve more external data sources and thus require additional technologies to support data mapping and data integration. Turning data chaos into actionable insights is critical for supporting rapid decision-making at the screening stage and for informing treatment and maintenance, especially if your therapy is cell or gene based.

Precision's Recommended Strategy:

Implement a robust biomarker data management solution. Biomarker-driven trials generate large datasets across a wide range of assay modalities (see Figure 1), but the data often remains siloed. It is critical to break down and break through common data silos, so the full data picture can be leveraged to make crucial and time-sensitive decisions about a patient's participation or status in an immunotherapy-focused clinical trial. Precision's multiomic QuartzBio® data platform is designed to synthesize diverse biological data to reveal hidden insights—within and across studies.

4. Sample management is critical to patient identification and selection in biomarker-driven trials.

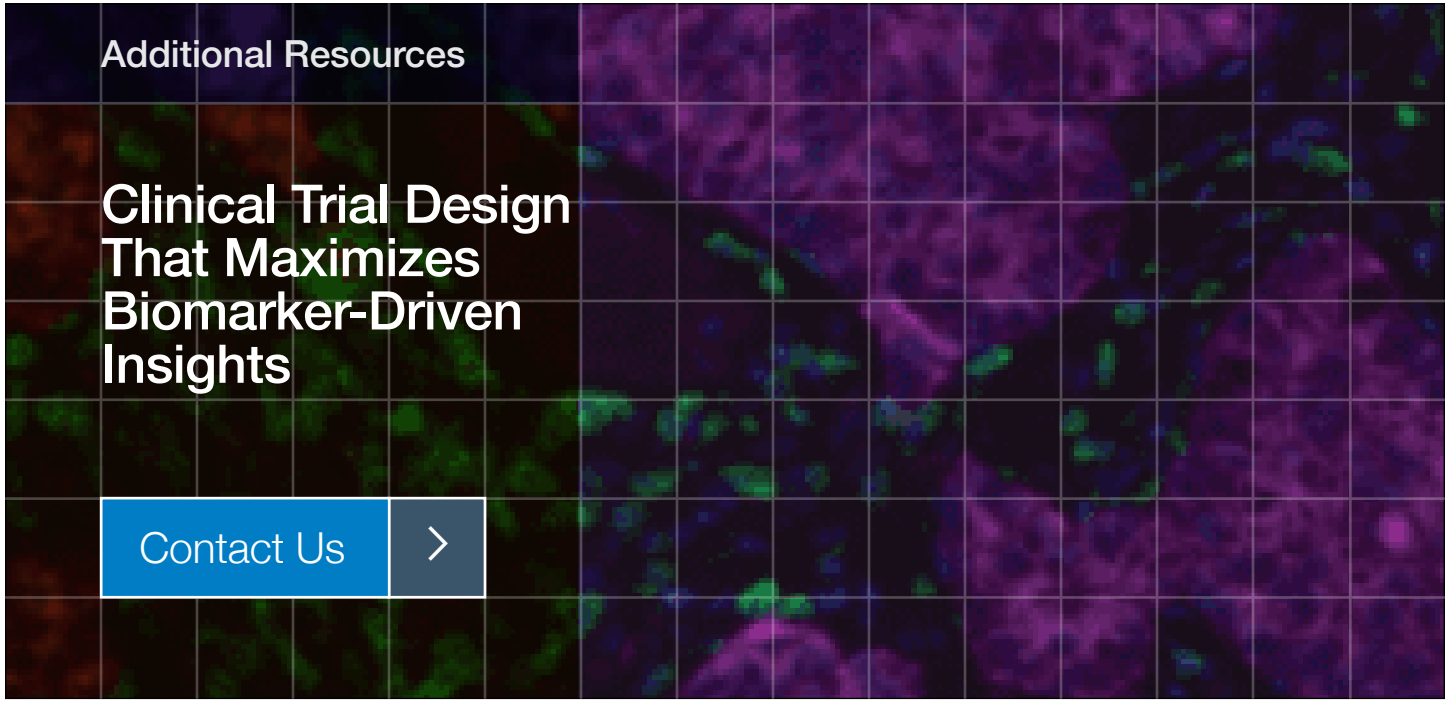
Common Challenge: Every sample collected in a biomarker-driven trial is vital to study success, especially if your therapeutic is cell-therapy based. Studies that do not have a well-thought-out logistics plan will have issues managing critical samples.

Precision's Recommended Strategy:

Assign a dedicated logistics coordinator to support the additional demands of your biomarker-driven clinical trial. The role of a logistics coordinator is to serve as the primary point of contact among the sites, the clinical research associates monitoring those sites, the specialty labs involved, and the manufacturing company when a cell therapy is involved. Having a dedicated logistics coordinator can be a difference-maker at the patient identification stage and during treatment.

Pair your logistics coordinator with a virtual sample inventory management solution (vSIM). In studies like these, if your internal and external team members are left tracking samples via massive spreadsheets, then errors are likely. In addition, too much time is spent manually wrangling data across participating sites, labs, vendors and biorepositories. By pairing your logistics coordinator with a vSIM solution, like Precision QuartzBio®, you can gain centralized visibility into sample collection, processing, and storage status across siloed source systems for key stakeholders in your trial.

The use of biomarkers to individualize treatment is a cornerstone of precision medicine. Successful incorporation of biomarkers for effective patient identification and selection requires careful planning. Working with a CRO who understands the nuances of biomarker-driven clinical trials ensures you have the support you need to realize the full potential of your study. At Precision for Medicine, we integrate clinical trial execution with deep scientific knowledge, specialized laboratory expertise, and advanced data sciences, helping innovators conquer their most complex work.


Additional Resources
Clinical Trial Design That Maximizes Biomarker-Driven Insights
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CDx Biomarkers for Oncology Research and Development

Dr. Margaret Curnutte, Senior Director, In Vitro Diagnostics & Quality (Precision for Medicine)

Precision medicine is built on the premise of identifying patients who are most likely to respond to a therapy while avoiding serious adverse effects, thus increasing the therapeutic index of a drug or biologic. Biomarkers—whether they are complex genomic signatures or single-gene mutations—can be critical for patient selection in therapeutic development.

In this article, we discuss the development of companion diagnostics (CDx) for oncology therapeutics, providing considerations for evaluating both complex genomic signatures and single-gene biomarkers across all phases of oncology clinical trials.

Precision for Medicine is a global leader in companion diagnostic development, biospecimen sourcing, and CRO services to the life sciences industries. Precision's regulatory experts offer comprehensive CDx regulatory and evidence-development strategies, anticipating areas of regulatory risk and monitoring developments in a shifting policy landscape.

Background on oncology companion diagnostics

A biomarker is a characteristic that can be measured as an "indicator of normal or pathogenic biological processes or of responses to an exposure or intervention".[1] The Biomarkers, EndpointS and other Tools

(BEST) glossary defines 7 biomarker categories: susceptibility/risk, diagnostic, monitoring, prognostic, predictive, pharmacodynamic/response, and safety.[2] In the context of oncology therapeutics, predictive biomarkers are used to identify individuals who are more likely to experience a favorable or unfavorable effect from exposure to a drug or biologic.[2]

Oncologic CDx are biomarker assays used to inform the management of patients by identifying treatment options that may be appropriate based on the unique drivers of their individual tumors. Approximately two-thirds of the breakthrough therapy designations granted by the US Food and Drug Administration (FDA) are accompanied by a CDx.[3] In oncology, CDx may be:

- Complex genomic signatures with a clinical cutoff, such as tumor mutational burden (TMB), microsatellite instability (MSI), or loss of heterozygosity (LOH)
- Single-gene biomarker assays based on mutations in known oncogenes or tumor suppressor genes, such as BRCA1/2, ALK, or EGFR

Over the past few years, FDA has approved a number of CDx across cancer types, including immunohistochemistry-based assays such as PD-L1 IHC 22C3 pharmDx for identifying individuals with non-small cell lung cancer (NSCLC) who may be candidates for pembrolizumab (Keytruda®) and VENTANA ALK for identifying individuals with ALK-positive NSCLC who may be candidates for lorlatinib (Lorbrena®).[4] FoundationOne® CDx, a tissue-based genomic test has been approved as a CDx for more than 20 therapies, with the following recent indications:

- NSCLC - Identifying individuals with EGFR exon 19 deletions and exon 21 (L858R) alterations who may be candidates for EGFR inhibitors approved by FDA[5]
- Solid tumors - Identifying individuals with MSI-high solid tumors who may be candidates for pembrolizumab[6]
- Melanoma - Identifying individuals with BRAF V600E and V600K who may be candidates for BRAF or MEK inhibitors, such as trametinib (Mekinist®), or BRAF/MEK inhibitor combinations approved by FDA[4]

FoundationOne® Liquid CDx is a blood-based test with approved CDx indications in NSCLC and prostate, ovarian, and breast cancers.[4] In March 2022, Myriad Genetics received approval for BRACAnalysis CDx for identifying patients with germline BRCA-mutated, HER2-negative breast cancer who are eligible for olaparib (Lynparza®).[7]

Evaluating biomarkers for therapeutics that may require a CDx

If a biomarker is to be used for patient selection, it must be qualified. Qualification is a formal regulatory process that ensures the biomarker—and not the biomarker measurement method—can be relied on to have a specific application and interpretation within the stated context of use.[1] The CDx will also need to undergo analytical validation, which can involve establishing a cutoff, and demonstrating that the assay accurately and reliably measures the biomarker.[8]

Transitioning a diagnostic from bench to bedside requires a disciplined strategy that balances product design considerations, regulatory requirements, and feasibility. Precision for Medicine integrates clinical development, biomarker assays, regulatory strategy, and commercialization capabilities within a single organization, giving us unique insight into what it takes to co-develop a targeted therapeutic and its CDx.

Potential biomarkers are often evaluated in prospective-retrospective studies in which biomarkers are retrospectively measured on archived specimens following the completion of prospective clinical trials.[9] Given that some biomarker assays can be costly, researchers may opt for random sampling designs where biomarker testing is only performed on a sub-sample of subjects selected on the basis of observed outcome or other variables. Group testing, which involves physically pooling specimens across subjects and performing biomarker testing on those pooled samples, is

another approach for estimating the prognostic and predictive values of biomarkers.[6]

Biomarkers may also be measured prospectively and used for enrollment or patient stratification. At Precision for Medicine, we have supported more than 250 marketing clearances and approvals for in vitro diagnostic submissions and assisted with market access on more than 100 diagnostics and CDx. Below, we offer considerations for developing both complex genomic signature and single-gene biomarkers through clinical trial phases.

In Phase 1

Successful drug/biologic-diagnostic co-development depends on the strength of the biomarker hypothesis, which is based on a thorough molecular understanding of disease pathology and therapeutic mechanism of action.[7] Researchers may have multiple biomarker hypotheses which are tested through prototype assays in the early stages of clinical development to evaluate their predictive potential.

In phase 1, researchers can perform retrospective analyses of clinical data from all study subjects to look for signals that a biomarker may be predictive. However, given that the number of subjects is small and the primary endpoint may be safety, any signal will only be an early indicator. Depending on the biomarker, prevalence may be very low, so it is not uncommon to see few, if any, patients who are biomarker-positive. Thus, biomarker development in phase 1 will rely heavily on preclinical data.

In Phase 2

For complex genomic signatures with a clinical cutoff, a key challenge in phase 2 studies is how to determine a clinical cutoff when additional clinical outcome data are needed to support that cutoff. In the absence of sufficient outcome data, published literature, preclinical data, and risk to the patient can be used to establish a cutoff range. Applying machine learning and modeling to these data may be useful for both refining the genomic signature and defining an appropriate starting point for the clinical cutoff.[10]

Another consideration is the level of validation required for the biomarker and the associated clinical trial assay (CTA) at this stage, particularly if the true cutoff is still unknown. Therapeutics developers may consider splitting the study into cutoff establishment and cutoff validation cohorts, a method that has been accepted by FDA. However, the feasibility of this approach will depend on disease prevalence. A robust limit of detection (LOD), limit of blank (LOB), or limit of quantification (LOQ) study may be useful for identifying a cutoff for enrollment. If multiple cutoffs will be examined in the study, developers may benefit from considering all-comers study design, as this allows for an unbiased analysis of potential cutoffs.

When designing phase 2 studies, developers should also understand the implications of prospective versus retrospective biomarker analysis. In prospective studies where patients are only enrolled if they are biomarker positive based on a pre-established clinical cutoff, analysis of other cutoffs may be limited based on patient selection. In retrospective studies, on the other hand, biomarker data can be bucketed and analyzed in many ways.

For single-gene biomarkers, the primary considerations in phase 2 are whether there is a clear, locked biomarker definition and whether it has been confirmed that the enrollment assay(s) can detect all aspects of that biomarker definition. Often phase 2 studies may utilize multiple laboratory developed tests (LDTs) to enroll patients, as some centralized biomarker CTAs may be costly and are not regularly performed as part of routine care. It may also be more burdensome for sites to have to use tests that required a centralized lab rather than a laboratory developed test (LDT). A central laboratory assay, like those offered by Precision for Medicine, can reduce the risks associated with the need for a bridging study to the final CDx. If these enrollment assays differ from the

final CDx, it is critical for sponsors to perform a gap analysis to thoroughly understand any differences between what is detected by the enrollment assay(s) and final CDx. If the enrollment assay(s) detects a variant that the final CDx does not, that variant cannot be included in the final biomarker definition.

Moreover, as a general rule, the biomarker definition can only include variants that are present in the clinical trial population, which can be challenging if the variant of interest is rare. FDA has made exceptions to this rule, though, for tumor suppressor genes. The agency may allow tumor suppressor gene variants not seen in the clinical trial population to be included in a final biomarker definition as variants in such genes are known to alter the tumor suppression function.

In Phase 3

In a drug/biologic-diagnostic co-development model, phase 3 studies are used not only to demonstrate safety and efficacy of the therapeutic, but also to clinically validate the CDx assay. The CDx must be shown to discriminate between likely responders and non-responders and, as such, clinical sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are important metrics to consider.

For complex genomic signatures with a clinical cutoff, phase 2 data should inform the locked clinical cutoff. If sponsors choose to continue examining multiple cutoffs, those cutoffs must be validated and specified in the statistical analysis plan (SAP) prior to trial initiation.

Sponsors should avoid using multiple CTAs to enroll patients in phase 3 registrational studies due to varying quality and levels of validation. Ideally, the final CDx assay is used for enrollment, with only one testing laboratory to reduce possible site to site variation. While using multiple CTAs may help accelerate enrollment, this speed may come at a cost. If a different assay(s) is used to enroll patients, a bridging study to the final CDx will be required.[11]

Of note, for genomic signatures with a clinical cutoff, biomarker negative data are not needed as enrollment should be based on the cutoff.

For single-gene biomarkers, sponsors should use the final CDx assay for enrollment whenever possible and ensure a locked biomarker definition. Just as with genomic signatures with a clinical cutoff, any discordances between local assays and the final CDx would need to be explained if multiple CTAs are used.

With single-gene biomarkers, the need for biomarker negative data will depend upon the assay used to enroll patients. If the final CDx is used to select the biomarker positive population, biomarker negative data are not needed. If bridging from local assays, however, biomarker-negative data would be needed to establish concordance between the enrollment assay and the final CDx, specifically for negative percent agreement (NPA). High NPA is necessary for confirming that there would only have been screen failures if central testing had been conducted.

Conclusion

Increasingly, cancer treatment is dependent on biomarkers for insight into prognosis and treatment selection. Thus, biomarker discovery and development are critical for advancing oncology therapeutics. In some cases, CDx assays are developed in parallel with therapeutics based on known targets or mechanisms of action. In others, CDx are developed based on studies that failed to reach their primary endpoints, where retrospective analysis reveals correlations between biomarkers and therapies, as was the case with TMB and pembrolizumab. Precision for Medicine supports drug/biologic-diagnostic co-development programs through our Diagnostic Solutions team, offering **scientific and regulatory strategy consulting**, biospecimens, specialty lab services, and a full-service CRO for designing and executing all phases of oncology clinical trials.

With comprehensive genomic profiling platforms and multi-gene panels, valuable study specimens can provide reliable information on complex

genomic signatures and single-gene mutations, supporting and accelerating CDx for oncology therapeutics. Continuous expansion of indications for marketed CDx fuels both personalized medicine and basic and clinical research related to drug/biologic response and mechanism of action.[12] Moreover, data from these investigations can be applied to other therapeutic areas, bringing us closer to realizing the full promise of precision medicine.

Dr. Margaret Curnutte,
Senior Director, In Vitro Diagnostics & Quality, Precision for Medicine

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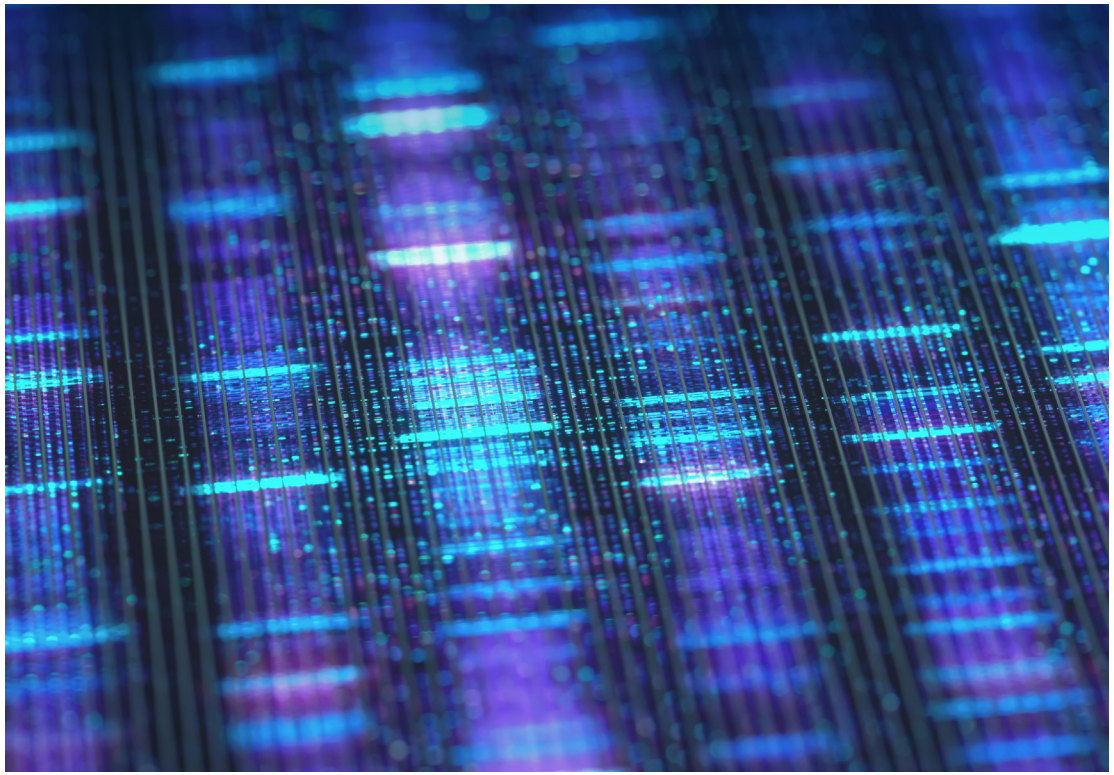


Building a Diverse, Variant-Rich Biobank to Fast-Track CDx Development—AMP 2021

Rob Fannon, MPH, MBA, General Manager, Biospecimen Solutions, Precision for Medicine

With the emergence of precision medicine, biomarkers have advanced from being exploratory endpoints to operating as clinical trial assays under fit-for-purpose validations to serve as inclusion and exclusion criteria. Genomic insights play a crucial role in the development of companion diagnostics (CDx) to support precision therapeutics. However, clinically meaningful, actionable mutations occur at very low frequency and securing biospecimens with those mutations is challenging.

Rob Fannon, General Manager for Biospecimen Solutions at Precision for Medicine, delivered a presentation entitled **Advancing CDx Development with a Novel Next Generation Sequencing Initiative at the Association for Molecular Pathology Annual Meeting – AMP 2021.** Precision for Medicine, a global leader in supplying biospecimens, lab services, and CRO services to the life sciences industries, was pleased to have Fannon share his strategic insights with the AMP audience.

A Novel Next Generation Sequencing Initiative

Generating data from real clinical samples, rather than contrived specimens, helps optimize biomarker and companion diagnostic development. In partnership with researchers and industry, Precision for Medicine has undertaken an ambitious next-generation sequencing (NGS) initiative called the Precision

Figure 1. **OncomIne Precision Assay Content**

Oncology Sequencing Initiative (Project P.O.S.I.). The objective of Project P.O.S.I. is to provide annotated, profiled specimens to support biomarker discovery. The overarching goal is to create a precompetitive environment that is agnostic to sequencing panel, chemistry, or technique, thus enabling head-to-head analysis of different panels and facilitating the development of products across a range of technologies.

The first phase of this two-phase initiative focuses on NGS screening of Precision's extensive library of formalin-fixed, paraffin-embedded (FFPE) specimens. These specimens are screened on the Thermo Fisher Ion Torrent Genexus System using the Oncomine Precision Assay, a clinically-curated cancer panel which features 2,768 of the most prevalent and potentially relevant cancer driver variants across 50 genes. Use of the Oncomine Precision Assay enables a total nucleic acid approach, as the panel can be run as a DNA- or RNA-only assay, or both. To date, approximately 7,000 FFPE specimens have been screened.

The second phase of Project P.O.S.I. focuses on replicating the work of phase 1 in the liquid biopsy fraction. In addition to serving as a complement to tumor tissue, liquid biopsy offers the advantage of flexibility, as sample collection is less invasive and can be performed more frequently than tissue biopsy.

As part of phase 2, Precision for Medicine has collaborated with Pillar Biosciences, a company with a novel amplicon-based sequencing chemistry. Pillar recently received FDA approval for its oncoReveal™ lung and colon cancer assay, an NGS tissue-based CDx, and is now focused on developing cell-free DNA (cfDNA) diagnostics. This collaboration leverages Precision's clinical network of oncology sites and laboratory services for collection and processing of whole blood samples to obtain cfDNA specimens. These specimens are sent to Pillar Biosciences for sequencing. This effort yields an extremely rich data set as it is a prospective collection done under informed consent, where more phenotypic information can be gathered.

Data generated from both phases of Project P.O.S.I. are combined with key clinical information and biopsy metadata for interrogation using QuartzBio®, Precision's proprietary multiomic data processing engine. QuartzBio utilizes AI and computational biology to find connections or relationships among diverse biological data to inform disease modeling, biomarker identification, pathway selection, and patient stratification.

A Purpose-Built Life Science Provider

Access to high-quality, data-rich specimens is just as—if not more—important as a regulatory pathway and commercialization strategy for optimizing the market for a companion diagnostic program. At Precision for Medicine, we understand that high-quality, ethically procured specimens that can be evaluated on a range of lab-based platforms and techniques

must be combined with a well-thought-out regulatory and commercialization plan to get innovations to market as quickly as possible.

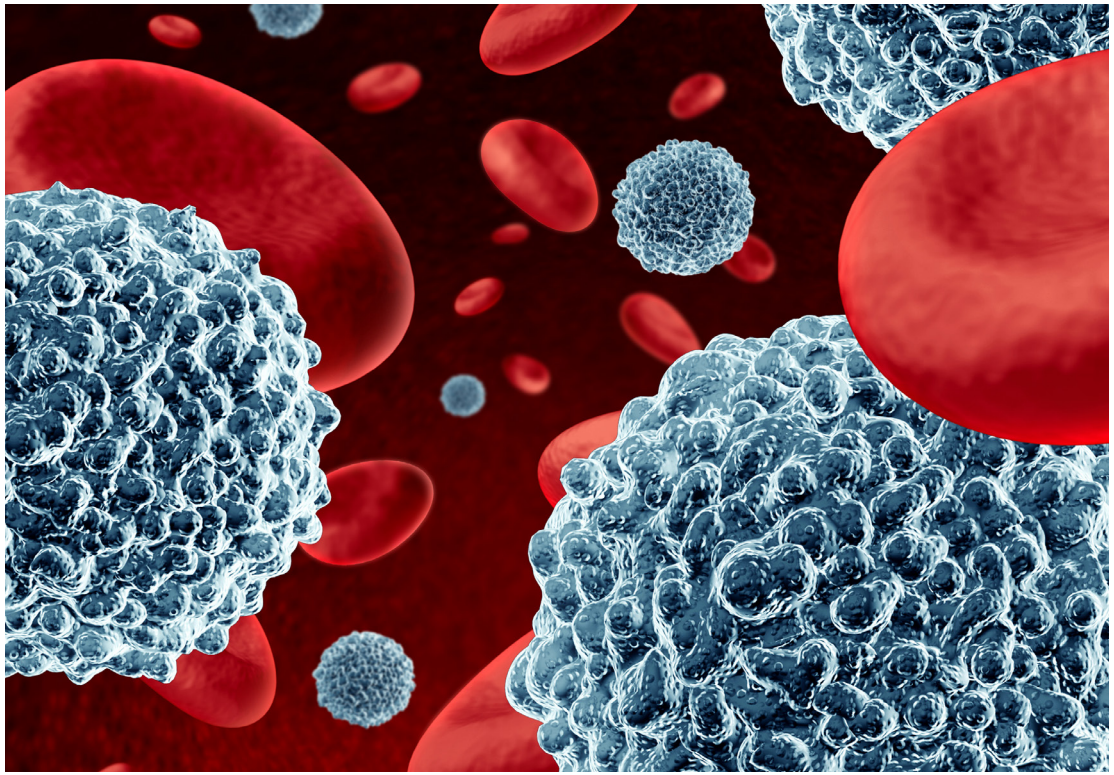
Precision Medicine Group, the parent company of Precision for Medicine, is a purpose-built life science provider focused on not only accelerating time to market for innovative tests and products, but also optimizing commercialization. We offer a full-service solution, from **biospecimens**, biorepositories, and sample management expertise to assay development, **specialty laboratory services**, and **clinical trial capabilities** for biomarker-anchored studies. We also offer expertise in regulatory strategy, data sciences, and commercialization.

Our world-class laboratory network includes seven wholly owned laboratories across the world, five in North America and two in Europe, all operating under the same quality system. Designed to be biomarker and analyte agnostic, our laboratory network offers a spectrum of advanced tissue and liquid biopsy profiling techniques, including immunohistochemistry, pathology, quantitative multiplex immunofluorescence, CTC isolation and analysis, and next generation sequencing.

Precision for Medicine, our dedicated in-house specimen unit, was designed to align with this laboratory network and enable our laboratory service offerings. Our specimen product offerings comprise three primary categories—biofluids, tissues, and viable cells—which are available either through our pre-existing inventory or through prospective collections under IRB-approved protocols within our clinical network.

To learn more about Precision for Medicine's services, watch full presentation **here**.

Rob Fannon, MPH, MBA
General Manager, Biospecimen Solutions, Precision for Medicine



Taking a Holistic Approach to Sample Management and Sample Processing for Your Clinical Trial

Precision for Medicine Research Team

Patient samples have always been critical assets for clinical trial success. In this era of precision medicine, in which researchers seek to tailor treatments on the basis of a specific patient's biomarker profile or disease characteristics, sample management has become even more important and valuable. It is critical to ensure that clinical samples collected during a trial constitute a robust sample set that can be used for elucidating mechanism of action, stratifying patients, and monitoring response to treatment. Consequently, having a plan to ensure sample quality, minimize processing time post-blood draw, and manage logistics is crucial for optimizing the accuracy and integrity of clinical trial data.

Clinical trial samples span a spectrum of urine, blood, cells, DNA, RNA, and protein and tissue specimens, requiring a broad range of technologies and approaches for analysis. To ensure sample quality and data accuracy, every step of sample management—including collection, labeling, shipment, storage, processing, and testing—must be carefully choreographed.

Key Tenets of Sample Management

While the nuances of sample management will vary on the basis of the specimen type, there are certain strategic principles that apply across the board:

- Document proper patient preparation prior to specimen collection
- Specify the container and transport medium required for each sample type
- Outline the labeling requirements, such as patient identifiers, site, and associated clinical sample collection information
- Establish sample processing and testing protocols to ensure consistency across laboratories
- Develop a plan for streamlined logistics and secure storage

Selecting the Right Samples

The samples needed to support a study will be determined by the clinical trial objectives and endpoints, along with feasibility of specimen collection. For instance, immune monitoring has become an essential strategy for understanding, predicting, and monitoring the immune system response to therapeutic interventions. Researchers who are seeking to perform immune cell profiling with flow cytometry will need to determine what analytes will be measured and which assay will be performed in order to choose the right sample. If looking for absolute cell counts per milliliter of blood, whole blood is appropriate. If looking for subtle changes in cell populations over time and the markers of interest are stable in cryopreserved cells, banked cryopreserved samples are likely to yield the most robust, accurate data.

Coordinating Sample Processing and Logistics

Biological samples often require specific conditions for collection, handling, and sample processing. For example, factors such as time, nutritional state, anticoagulant type, shipping conditions, and even method of isolation may influence the immunological function of peripheral blood mononuclear cells (PBMCs) isolated from whole blood. Studies have shown that when comparing antigen-specific T-cell responses in different individuals, it is critical to standardize the procedures for blood collection, processing, and preservation.[1] Minimizing the time between venipuncture and PBMC isolation helps ensure cell viability and freshness.

A sample management plan must include procedures for the proper collection, preparation, and shipment of samples at the required time points and in the required conditions. For example, if seeking to perform receptor occupancy assays using real-time flow cytometry, whole or stabilized blood will need to be processed by the lab on the day of receipt. Researchers who want to streamline immune monitoring may want to consider epigenetic immune cell quantification using Precision's patented Epiontis ID technology. A significant advantage of Epiontis ID is that it can be applied to fresh, frozen, or paper-spotted dried blood and other bodily fluids or tissues, eliminating the need for special care during transport and storage and potentially reducing overall cost.

Often, researchers may want to perform batch analyses of stored samples, not just for logistical convenience but also to reduce variability—particularly when samples are collected longitudinally from the same subjects. Understanding proper storage and the impact of the freeze/thaw process on cell viability and function will help shed light on the optimal processing approach.

Leveraging the Expertise of an End-to-End Specialty Lab Service Provider

Trials involving multiple sites and independent testing labs are subject to variability in the quality of processing and, hence, variability in the resulting data. At Precision for Medicine, we have focused on building a network of specialty labs with consistent protocols, with the goal of obtaining the highest-quality samples for the best possible downstream data. We offer a broad range of platform technologies and approaches, including flow cytometry, epigenetic immune cell phenotyping, cytokine analysis, genomic profiling, sequencing, liquid biopsy, and immunohistochemistry and multiplex immunofluorescence.

Our end-to-end solution offers real-time processing in more than 55 countries, custom assay and kit development capabilities, and state-of-the-art biobanking facilities for secure storage, management, and distribution of samples for future use. Precision also offers custom specialty lab solutions tailored to specific clinical trial requirements, helping researchers ensure high-quality samples and optimize the value of their study data.

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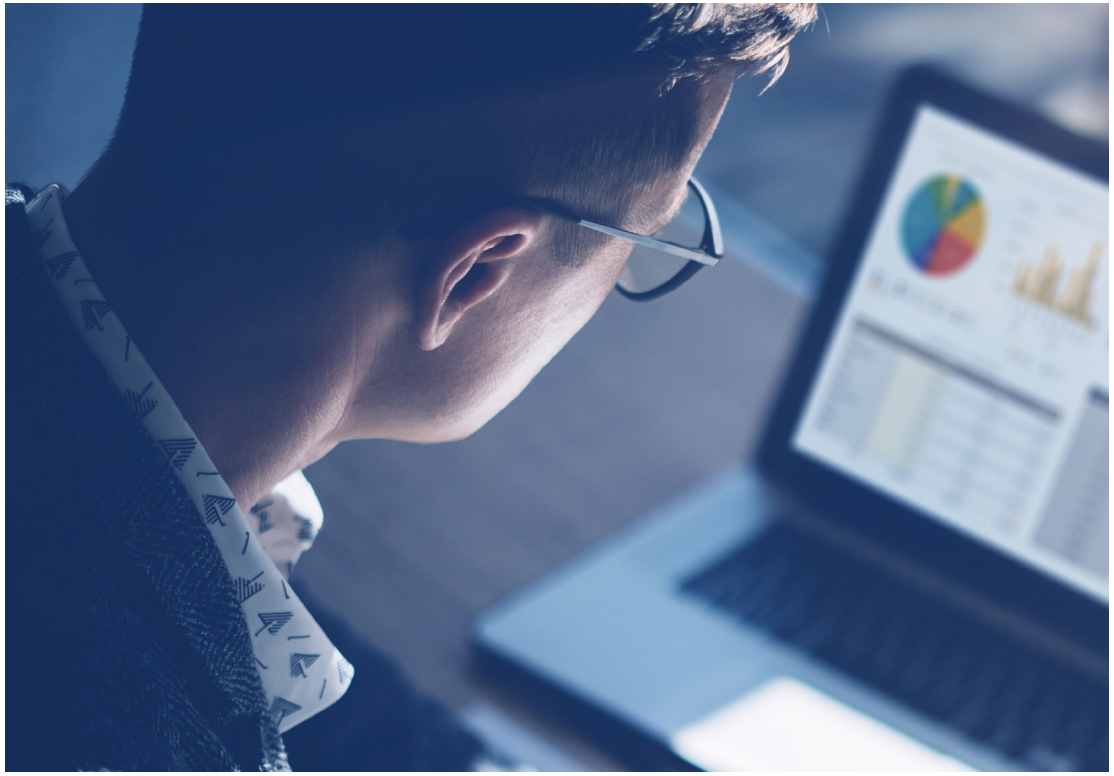


6 Critical Discrepancy Reports for Effective Clinical and Biomarker Operations

Precision for Medicine Research Team

PRECISION quartzbio®

Clinical operations leaders use the QuartzBio® virtual Sample Inventory Management (vSIM) solution to maintain robust visibility across sample collection and processing status, consent status, and sample and derivative quality information.

Traditionally, generating these reports is frustratingly time-consuming – often involving spreadsheets that attempt to manually link key information from the EDC, central labs, testing labs, and biorepositories to illuminate critical information like discrepancies or missed collections.

The QuartzBio team has identified six critical on-study reports that our clients prioritize as absolutely required for effective sample and biomarker operations.

Coupled with generating the reports continuously throughout the life of a study, our clients reiterate the need for teams to have collaborative visibility as new data are available.

In this quick guide, we highlight these six key reports – and introduce QuartzBio's proprietary processing and harmonization pipelines that make them a reality (including for ongoing studies).

#1 Missing Samples

Clinical operations teams must extract information from multiple sources, including protocols, protocol revisions, and optional consent forms, to gain visibility into what samples and derivatives are expected to be collected.

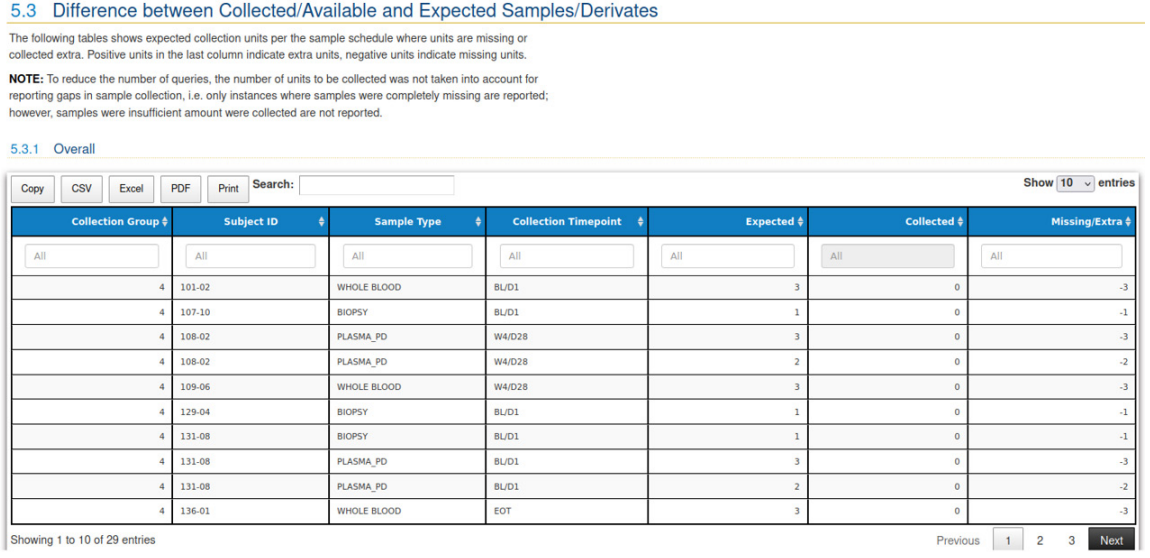
Teams then compare these expectations to actual collection data. The resulting report can be tedious to generate.

With QuartzBio's dynamic dashboards, the missing samples report is easy to access, filter, and share, saving clinical operations teams time and providing opportunities to intervene during the trial to minimize loss of critical specimens and data.

#2 Samples with Potential Consent Violations

When managing complex sample inventories across labs and repositories, clinical teams often spend time manually cross-referencing inventory data with informed consent forms and protocols.

QuartzBio accepts, reviews and houses the protocol under which each subject was enrolled, as well as consent forms. In addition to discrepancies in expected sample collection, QuartzBio can also detect and report instances where a sample was collected, but optional consent was not given.



5.3 Difference between Collected/Available and Expected Samples/Derivates

The following tables shows expected collection units per the sample schedule where units are missing or collected extra. Positive units in the last column indicate extra units, negative units indicate missing units.

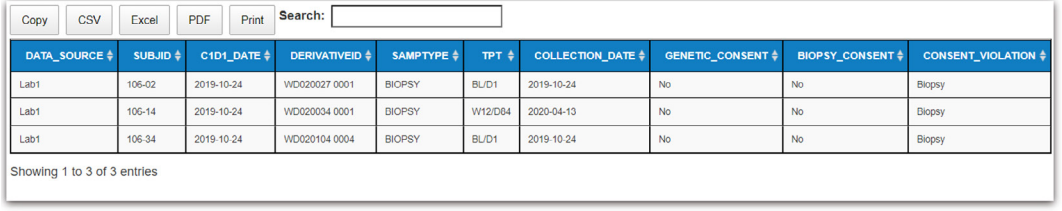
NOTE: To reduce the number of queries, the number of units to be collected was not taken into account for reporting gaps in sample collection, i.e. only instances where samples were completely missing are reported; however, samples were insufficient amount were collected are not reported.

5.3.1 Overall

Copy CSV Excel PDF Print Search: Show 10 entries

Collection Group	Subject ID	Sample Type	Collection Timepoint	Expected	Collected	Missing/Extra
All	All	All	All	All	All	All
4	101-02	WHOLE BLOOD	BL/D1	3	0	-3
4	107-10	BIOPSY	BL/D1	1	0	-1
4	108-02	PLASMA_PD	W4/D28	3	0	-3
4	108-02	PLASMA_PD	W4/D28	2	0	-2
4	109-06	WHOLE BLOOD	W4/D28	3	0	-3
4	129-04	BIOPSY	BL/D1	1	0	-1
4	131-08	BIOPSY	BL/D1	1	0	-1
4	131-08	PLASMA_PD	BL/D1	3	0	-3
4	131-08	PLASMA_PD	BL/D1	2	0	-2
4	136-01	WHOLE BLOOD	EOT	3	0	-3

Showing 1 to 10 of 29 entries Previous 1 2 3 Next



4.6 Samples with Invalid Collection Based on Consent

The following table lists **1** samples/derivatives where DNA or biopsy samples were collected when the subject had not consented to those collections per the clinical eCRF information.

Copy CSV Excel PDF Print Search:

DATA_SOURCE	SUBJID	C1D1_DATE	DERIVATIVEID	SAMPTYPE	TPT	COLLECTION_DATE	GENETIC_CONSENT	BIOPSY_CONSENT	CONSENT_VIOLATION
Lab1	106-02	2019-10-24	WD020027.0001	BIOPSY	BL/D1	2019-10-24	No	No	Biopsy
Lab1	106-14	2019-10-24	WD020034.0001	BIOPSY	W12/D84	2020-04-13	No	No	Biopsy
Lab1	106-34	2019-10-24	WD020104.0004	BIOPSY	BL/D1	2019-10-24	No	No	Biopsy

Showing 1 to 3 of 3 entries

In this example, the study has two optional consents, for genetic testing and biopsy sampling. In this report, three subjects did not consent to biopsy, but a biopsy was taken, violating consent.

#3 Subject IDs in Unrecognized Format

Pulling together sample inventories and metadata across sites, central, and testing labs is already a tedious task. Subject IDs that do not match the expected format prevent accurate reporting. Clinical teams frequently use the “Problematic Subject IDs” report in the QuartzBio® platform to quickly address these discrepancies.

#4 Missing EDC Collections or EDC Data Entry Errors

Samples without a recorded collection date in the EDC could either indicate a data entry error or, potentially, that a sample without a recorded collection in the EDC was received by the central or specialty lab. The latter could indicate a more serious issue at the site. With multiple labs and biorepositories, there are multiple points at which these errors can occur.

This QuartzBio® report lists samples whose collection date is more than 14 days prior to the last EDC export.

4.1 Problematic Subject IDs

The following subject IDs are erroneous and need to be corrected as they do not conform with expected format of a 3 digit site identifier and a 2 digit site-specific subject identifier:

Copy CSV Excel PDF Print Search:

DATA_SOURCE	SUBJID	SAMPID	TPT	SAMPTYPE	COLLECTION_DATE
Lab1	12-102	WD020299	W12/D84	PLASMA_PD	2020-03-16
Lab2	32-104	WD024740	W16/D104	PLASMA_PD	2020-04-20
Lab1	18-102	WD019406	W16/D104	PLASMA_PD	2020-04-20
Lab3	21102	WD019081	W20/D136	PLASMA_PD	2020-04-16

Showing 1 to 4 of 4 entries

4.3 Samples with Missing EDC Collection Dates

The following table lists **2** samples/derivatives with missing EDC entries. A sample was identified as missing if the sample collection date was more than *14* days prior to the last EDC export. The last EDC export was obtained on 2020-02-14 and thus any samples with collection dates before 2020-01-31 were considered to be lacking expected EDC entries.

Copy CSV Excel PDF Print Search:

DATA_SOURCE	SUBJID	SITEID	SAMPID	DERIVATIVEID	TPT	SAMPTYPE	COLLECTION_DATE	EDC_COLLECTION_DATE
Lab1	106-02	106	WD020027	WD020027 0001	BL/D1	BIOPSY	2019-10-24	
Lab1	122-02	122	WD020110	WD020110 0001	BL/D1	BIOPSY	2020-01-13	

Showing 1 to 2 of 2 entries

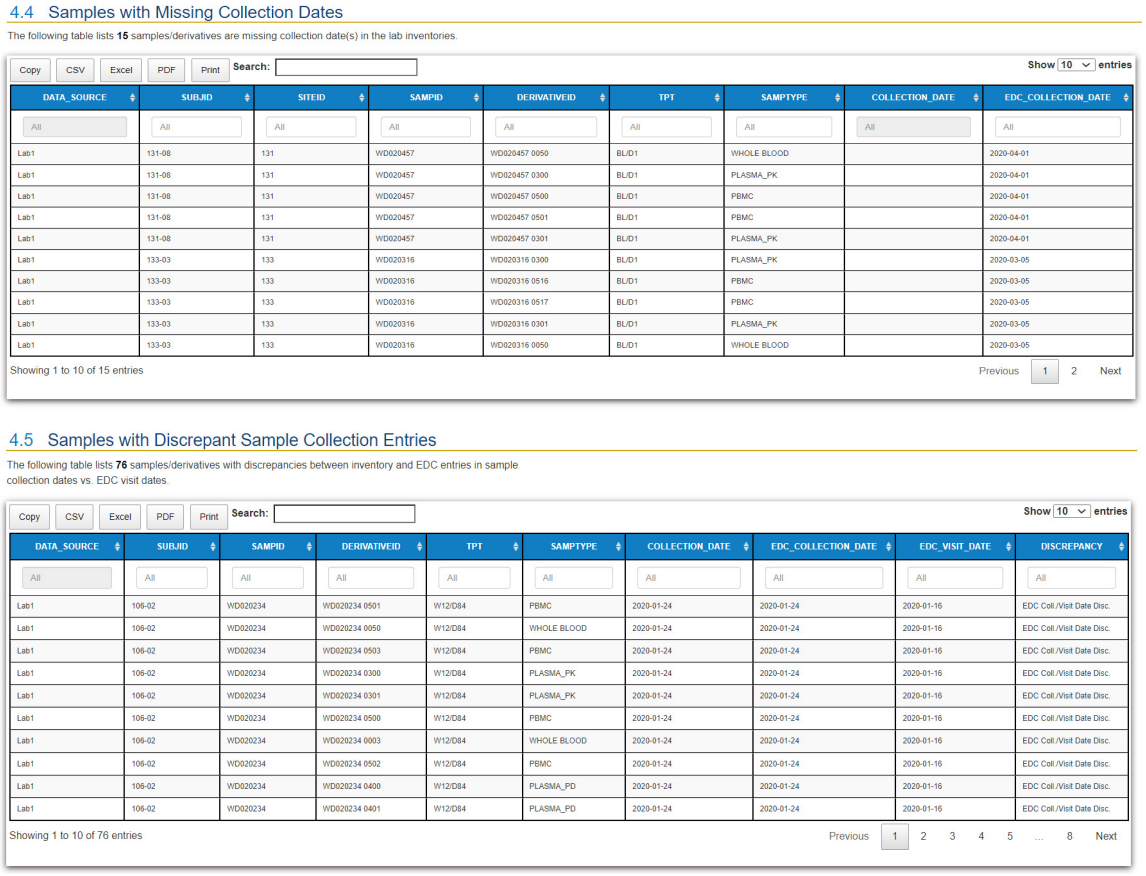
#5 Collection Dates Missing from Sample Inventory

The ability to maintain continuous visibility enables teams to address discrepancies before more serious issues occur.

Sometimes, the EDC is complete, but the inventory provided by a lab or repository is missing the corresponding collection data. This could occur where samples were either not accessioned properly, or where a given sample was never received. The ability to maintain continuous visibility enables teams to address discrepancies before more serious issues occur.

#6 Samples with Discrepant Sample Collection Entries (EDC vs. Inventory)

This report shows direct reconciliation issues between the EDC and inventories at central labs, specialty labs, and biorepositories. The vSIM solution is designed to streamline and automate the reconciliation, a time-consuming process that is typically manual and requires accessing multiple systems.



4.4 Samples with Missing Collection Dates

The following table lists **15** samples/derivatives are missing collection date(s) in the lab inventories.

Copy CSV Excel PDF Print Search: Show 10 entries

DATA_SOURCE	SUBJID	SITEID	SAMPID	DERIVATIVEID	TPT	SAMPTYPE	COLLECTION_DATE	EDC_COLLECTION_DATE
All	All	All	All	All	All	All	All	All
Lab1	131-08	131	WD020457	WD020457 0050	BL/D1	WHOLE BLOOD		2020-04-01
Lab1	131-08	131	WD020457	WD020457 0300	BL/D1	PLASMA_PK		2020-04-01
Lab1	131-08	131	WD020457	WD020457 0500	BL/D1	PBMC		2020-04-01
Lab1	131-08	131	WD020457	WD020457 0501	BL/D1	PBMC		2020-04-01
Lab1	131-08	131	WD020457	WD020457 0301	BL/D1	PLASMA_PK		2020-04-01
Lab1	133-03	133	WD020316	WD020316 0300	BL/D1	PLASMA_PK		2020-03-05
Lab1	133-03	133	WD020316	WD020316 0516	BL/D1	PBMC		2020-03-05
Lab1	133-03	133	WD020316	WD020316 0517	BL/D1	PBMC		2020-03-05
Lab1	133-03	133	WD020316	WD020316 0301	BL/D1	PLASMA_PK		2020-03-05
Lab1	133-03	133	WD020316	WD020316 0050	BL/D1	WHOLE BLOOD		2020-03-05

Showing 1 to 10 of 15 entries Previous 1 2 Next

4.5 Samples with Discrepant Sample Collection Entries

The following table lists **76** samples/derivatives with discrepancies between inventory and EDC entries in sample collection dates vs. EDC visit dates.

Copy CSV Excel PDF Print Search: Show 10 entries

DATA_SOURCE	SUBJID	SAMPID	DERIVATIVEID	TPT	SAMPTYPE	COLLECTION_DATE	EDC_COLLECTION_DATE	EDC_VISIT_DATE	DISCREPANCY
All	All	All	All	All	All	All	All	All	All
Lab1	106-02	WD020234	WD020234 0501	W12/D84	PBMC	2020-01-24	2020-01-24	2020-01-16	EDC Coll./Visit Date Disc.
Lab1	106-02	WD020234	WD020234 0050	W12/D84	WHOLE BLOOD	2020-01-24	2020-01-24	2020-01-16	EDC Coll./Visit Date Disc.
Lab1	106-02	WD020234	WD020234 0503	W12/D84	PBMC	2020-01-24	2020-01-24	2020-01-16	EDC Coll./Visit Date Disc.
Lab1	106-02	WD020234	WD020234 0300	W12/D84	PLASMA_PK	2020-01-24	2020-01-24	2020-01-16	EDC Coll./Visit Date Disc.
Lab1	106-02	WD020234	WD020234 0301	W12/D84	PLASMA_PK	2020-01-24	2020-01-24	2020-01-16	EDC Coll./Visit Date Disc.
Lab1	106-02	WD020234	WD020234 0500	W12/D84	PBMC	2020-01-24	2020-01-24	2020-01-16	EDC Coll./Visit Date Disc.
Lab1	106-02	WD020234	WD020234 0003	W12/D84	WHOLE BLOOD	2020-01-24	2020-01-24	2020-01-16	EDC Coll./Visit Date Disc.
Lab1	106-02	WD020234	WD020234 0502	W12/D84	PBMC	2020-01-24	2020-01-24	2020-01-16	EDC Coll./Visit Date Disc.
Lab1	106-02	WD020234	WD020234 0400	W12/D84	PLASMA_PD	2020-01-24	2020-01-24	2020-01-16	EDC Coll./Visit Date Disc.
Lab1	106-02	WD020234	WD020234 0401	W12/D84	PLASMA_PD	2020-01-24	2020-01-24	2020-01-16	EDC Coll./Visit Date Disc.

Showing 1 to 10 of 76 entries Previous 1 2 3 4 5 ... 8 Next

Summary

The six reports outlined in this quick guide represent the minimum requirements of most clinical operations teams as they monitor biospecimen operations in biomarker-informed trials generating complex data from multiple samples and derivatives. This foundation of visibility further enables clinical teams to project forward-looking sample collections and assay results data availability, confirm appropriate sample status prior to data generation, and monitor sample or consent expiry.

Continuously updated data and collaborative platform enable teams to work from a unified data set

Clinical teams frequently ask, “Is everyone working off the same data?”

With the QuartzBio® Data Platform, teams can rest assured that all team members across the organization, across functions, are accessing a centralized reliable source of sample information. QuartzBio’s data ingestion and harmonization pipeline continuously integrates sample information from multiple sources as it is generated over the course of the study, automatically performs edit checks, and highlights data inconsistencies.

When deployed on an ongoing study or even across an entire portfolio, QuartzBio’s vSIM solution can provide sponsors with up-to-date information on potential data issues as they arise throughout the course of their studies.

Sign up for our webinar, “Clinical Sample Journeys,” to see these reports and query tracker in action, as well as the latest reporting capabilities available to users of QuartzBio’s vSIM solution.

7 Applications for Formalin-Fixed Paraffin-Embedded (FFPE) Tissue Specimens

Cullen Taylor, MD, Medical Director at Precision for Medicine, Biospecimen Solutions

As technologies and methodologies advance, so do the potential applications for biospecimens. In this article, we explore seven uses of FFPE, ranging from the common to the cutting-edge.

1. Immunohistochemistry.

Formalin-Fixed Paraffin Embedded (FFPE) tissues are frequently used in immunohistochemistry (IHC) for determining the distribution of an antigen or biomarker of interest in tissues. IHC is used widely in oncology for making diagnoses, evaluating prognosis, and predicting response to therapy. Additionally, it plays a critical role in biological research and drug development for immunology, hematology, infectious diseases, and neurodegenerative disorders.

IHC is also utilized in the development of therapeutic antibodies or antibody-like molecules, particularly for preclinical tissue cross-reactivity (TCR) studies, where a series of IHC screening assays are used to not only identify off-target binding, but also to detect previously unknown sites of on-target binding for novel biotherapeutics. The FDA generally recommends **fresh frozen tissues** for TCR studies; however, certain cytoplasmic or nuclear antigens may be better preserved in **FFPE specimens**.[1] **Tissue microarrays** containing

FFPE tissue core samples may also be useful for preliminary evaluations of cross-reactivity focused on lead identification or optimization.

Advances in digital pathology platforms and artificial intelligence (AI) tools are improving the efficiency and accuracy of IHC. For example, there are approved digital pathology platforms that enable pathologists to score or diagnose from a computer, allowing for quicker turnaround times and remote collaboration. Precision for Medicine supported the development and approval of several of these platforms, including the first digital pathology solution for primary diagnostic use in the US, the **Philips PIPS (Philips IntelliSite Pathology Solution)**.

Additionally, given its ability to retain the spatial coordinates of every cell and the pathologist annotations, digital pathology can be used to quantify distances between cells and other objects. These cell-to-cell or cell-to-structure distances give us an idea of how different subpopulations of cells are interacting with each other, or how different markers are expressed on similar cell types, depending on their localization. For example, immune cells far away from the tumor might have different levels of expression of some markers compared to immune cells that are very close to the tumor, or even infiltrating the tumor. These distances also allow Precision for Medicine to use the tissue architecture to better assess the tumor microenvironment, much more so than the traditional percentage of positive cells or area.

AI algorithms are also making it easier for pathologists to classify tissues or regions, perform more accurate annotations more efficiently, quantify stained areas, and evaluate single cell-resolution data. Beyond their research applications, some of these algorithms are being developed and evaluated with the intention of validating them as companion diagnostics (CDx) at some point.

At Precision for Medicine we have millions of **FFPE specimens** readily available to researchers, and a full-service IHC lab staffed with expert, board-certified pathologists to support IHC analyses. We also provide multiple digital pathology services for IHC, including supervised annotations, AI-assisted tissue and object classifiers, and supervised automated scoring. Having all these capabilities integrated under one roof streamlines workflows, from sample procurement all the way through to professional interpretation.

2. Transcriptomic and gene expression analysis.

Historically, fresh frozen tissue has been the sample of choice for RNA sequencing as it contains relatively large amounts of RNA. However, collection and storage of fresh frozen tissue can be costly and involve complex logistics. FFPE samples can be challenging for molecular analysis due to high variability, low yield, and higher degradation of RNA compared to other tissue sources.

Newer transcriptomic technologies have made it possible to reliably perform gene expression analysis studies on FFPE specimens.

The ability to maintain continuous visibility enables teams to address discrepancies before more serious issues occur.

The NanoString nCounter® Analysis System, for example, does not require any amplification steps and can detect and quantify hundreds of unique transcripts in a single reaction.[2] Studies have shown that data generated by nCounter from FFPE correlates with data generated from matched frozen tissue and is superior to qPCR for gene expression analysis.[3]

RNA-seq with next-generation sequencing (NGS) is becoming the method of choice for studying the transcriptome. Compared to gene expression arrays, RNA-seq offers a broader dynamic range and is not limited by prior knowledge so it captures both known and novel

features—including transcript isoforms, gene fusions, and single nucleotide variants—in a single assay.[4]

Precision for Medicine offers both NanoString nCounter and RNA-seq, in addition to qPCR and ddPCR, for gene expression analysis.

3. Multiplex immunofluorescence for spatial signatures.

Multiplex immunofluorescence (mIF) is a technique that preserves the architectural features of a sample and reveals the spatial relationships between cells.[5] Multiplexing allows simultaneous analysis of multiple markers for exploration of the spatial relationships and physical interactions among them. FFPE samples of solid tumors can be used for mIF, providing insights into the tumor microenvironment and the extent and spatial distribution of immune cell infiltration, such as PD-L1 expression and the average distance to CD8 cells.

leveraging spatial context has the potential to significantly increase the ability to predict response to treatment.

For example, in one study, multiplex IHC/immunofluorescence showed diagnostic accuracy comparable to multimodality cross-platform composite approaches, including tumor mutational burden (TMB), gene expression profiling, and PD-L1 IHC, in predicting response to anti–PD-1/PD-L1.[6] While the optimal signatures are yet to be discovered, leveraging spatial context has the potential to significantly increase the ability to predict response to treatment.

In addition, multiplexing is developing rapidly and highly multiplexed technologies are emerging. Some of these are IF-based, while others use barcodes or mass cytometry. These technologies further increase the number of markers that can be analyzed on the same slide. While this may increase complexity of data, researchers are developing computational solutions that leverage AI to discover new signatures or relevant cell populations involved in patient response.

Precision for Medicine offers multiplexing of up to 8 markers on the same slide and image analysis that leverages AI tools. We also offer multiple approaches for spatial analysis.

4. Epigenetic profiling.

The study of epigenetic markers is an emerging approach to not only understand the molecular basis of carcinogenesis, but also to gain insight into cancer diagnosis and potential targeted therapies. As with gene expression analysis, the first methods for epigenetic profiling required high-quality DNA from fresh-frozen tissues. Now, technologies such as Precision for Medicine's proprietary **EpiontisID** can detect differential methylation in DNA extracted from FFPE samples. With over 69,000 samples analyzed across more than 100 clinical trials, EpiontisID is a qPCR-based immune cell phenotyping platform based on epigenetic cell counting. Studies have shown that EpiontisID results correlate strongly with flow cytometry, making it an effective solution for immune monitoring in both early- and late-stage clinical trials.[7]

EpiontisID can be performed on FFPE samples to monitor more than 30 immune cell types, for which fully validated, automated epigenetic assays have been developed. This includes B and T cells or subsets of T cells, such as Treg cells, all expressed as percentage of total cells. For instance, FFPE samples of atopic dermatitis or psoriasis can be characterized for a wide range of infiltrating immune cells including Th17 cells. Bisulfite-specific NGS, which characterizes the methylation status of a genomic region, can complement investigations with additional markers beyond the cell type-specific epigenetic biomarkers that are already validated and available.

Precision for Medicine offers epigenetic profiling using EpiontisID, our proprietary immune cell phenotyping technology.

5. Spatial transcriptomics.

This term describes the ability to locate and localize transcripts down to the subcellular level, providing a three-dimensional map of RNA targets throughout tissue sections.[8] NanoString's GeoMX® Digital Spatial Profiling platform provides spatial resolution of both RNA and protein detection on all sample types, including FFPE. NanoString has also developed protein assays for over 300 validated antibodies that enable multi-analyte analysis for both nCounter and next generation sequencing (NGS) readout.

10x Genomics has developed Visium Spatial Gene Expression for FFPE, which combines histological spatial information with whole transcriptome analysis. Visium can provide spatial profiling of RNA expression for over 18,000 genes in human and mouse FFPE samples. It can be combined with IF for simultaneous visualization of protein and gene expression or with H&E staining for morphological context.

The ability to understand transcriptomic profiles in a highly multiplexed manner, while still retaining tissue architecture, can provide a more comprehensive understanding of a tumor and its surrounding environment. Researchers are also developing clustering algorithms to better generate meaningful insights from these highly multiplexed data, and the potential to correlate spatial transcriptomics with spatial protein information would increase the data obtained from each sample.

Precision for Medicine is currently developing spatial transcriptomics solutions to provide our clients with comprehensive spatial-omics services.

6. Tumor profiling.

Cancer profiling using FFPE is commonly used for determining eligibility for treatment and for discovering new biomarkers. There are a number of commercially available assays, including the Oncomine Precision Assay and the TruSight Oncology 500 (TSO500) assay, which analyze variants across known cancer-related genes using NGS. The Oncomine Precision Assay analyzes known variants across 50 key genes. The TSO500 includes pan-cancer biomarker content that supports identification of all relevant DNA and RNA variants implicated in a variety of solid tumor types. The assay also measures microsatellite instability (MSI) and TMB, and is available in a high throughput version.

Precision for Medicine has created the Precision Oncology Sequencing Initiative (Project P.O.S.I), a large-scale NGS initiative to screen FFPE and liquid biopsy samples in its extensive biorepository for key biomarkers across cancer indications.

7. RNAscope®

This is a novel in situ hybridization (ISH) assay for detection of target RNA within intact cells. With its proprietary probe design that amplifies target-specific signals, but not background noise, the assay represents a major advance in RNA ISH approaches. RNAscope can be multiplexed and even combined with protein assays.

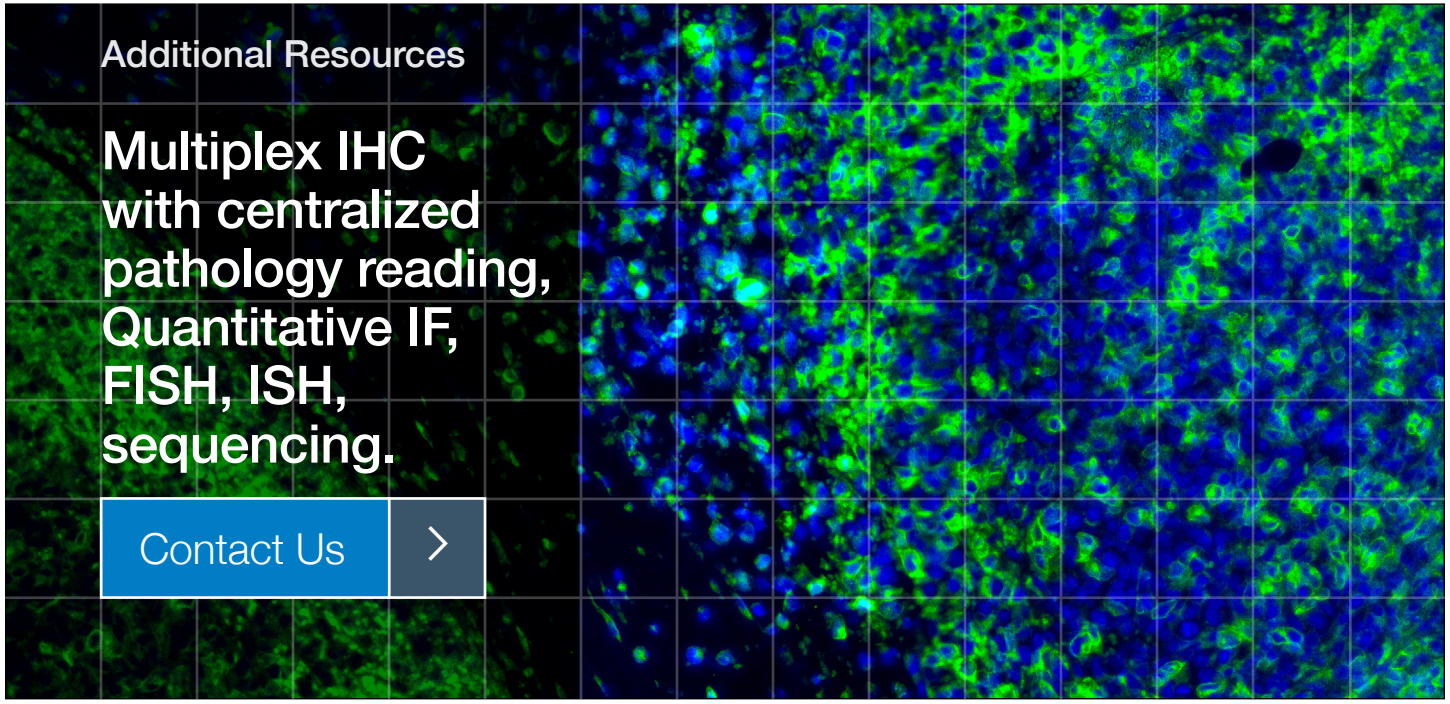
Precision for Medicine develops **FISH and ISH assays** and can develop and validate RNAscope biomarker assays for both research and clinical applications.

Key Considerations.

With technological and methodological advances, FFPE tissues are becoming an increasingly valuable source of DNA, RNA, and proteins for research and therapeutic applications. The quality of the sample is critical, and researchers should seek tissues that have been prepared using the most stringent standards and quality control measures. Precision for Medicine offers an extensive inventory of pathologist-reviewed FFPE tissue blocks, slides, curls, cores, and tissue microarrays from both normal and diseased

subjects, all collected under IRB-approved protocols. Each sample comes with pathology reports and, optionally, medical history, disease characterization, and outcomes data. To learn more about our FFPE tissue library, click here.

Precision for Medicine also has 7 full-service, accredited labs providing custom assay development, testing, and clinical trial support. You can learn more about these services at: www.PrecisionforMedicine.com



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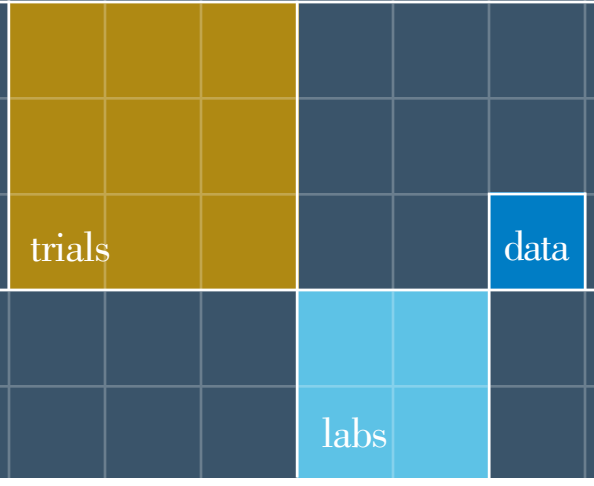
Biomarker-driven development is complex. Surmount it with Precision

We've been believers in biomarkers from the beginning. Precision was founded to help innovators conquer the complexity of biomarker-driven development through interconnected capabilities and scientists.

Get in touch with our experts here.

PRECISION for medicine



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