FRIENDS of CANCER RESEARCH

Innovative Validation and Regulatory Processes for Companion Diagnostic Tests for Rare Biomarkers or Indications

Friends of Cancer Research White Paper | 2025

Executive Summary

Precision therapy has become a leading approach for oncology treatment, showing continued success in improving outcomes for patients with cancer over the past 20 years. The U.S. Food and Drug Administration (FDA) reviews and approves the diagnostic tests critical for identifying patients who may benefit from precision therapy as companion diagnostics (CDx). The review process includes analytical and clinical test validation, which often requires an abundance of clinical samples. However, in situations where the biomarker or the cancer is rare, there are often limited clinical samples from the clinical trial, making it challenging to perform all necessary test validation studies. To overcome this challenge, drug sponsors and diagnostic test developers may consider using alternative sample sources for validation, such as procured human samples or contrived samples. While the use of alternative sample sources to support regulatory approval of a CDx for a rare biomarker has been in practice for some time, sponsors may lack an understanding of when this flexibility is warranted and how various alternative sample types should be considered for each validation analysis. Friends of Cancer Research convened a working group of experts to align on an approach to determine when regulatory flexibility might be considered, identify possible alternative samples, and suggest opportunities for using the samples in validation studies, including potential ways to support more streamlined discussions on validation plans and strategies between sponsors and FDA.

Authors

Hillary Andrews, Friends of Cancer Research

Lucia D'Apote, Amgen

Biswajit (Bishu) Das, Frederick National Laboratory (NIH)

Jennifer Dickey, PGDx/LabCorp

Megan Doyle, Eli Lilly and Company

Jonathan Freaney, Tempus AI

Elaine Katrivanos, Tempus AI

Laura Koontz, Foundation Medicine, Inc.

Joe Lennerz, BostonGene

Dun Liang, Eli Lilly and Company

Elizabeth Mansfield, Foundation Medicine, Inc.

Carly McWilliams, Roche Diagnostics

Gary Pestano, Biodesix

Brianna Phillips, Biodesix

Alain Silk, Tempus AI

Nino Sireci, Eli Lilly and Company

The authors thank the FDA for their participation as observers on the working group.

Table of Contents

Introduction

Rapid technological innovations and a deeper understanding of cancer biology have driven advancements in precision oncology. As treatments become increasingly tailored to the unique characteristics of each cancer, the need for diagnostic tests to identify rarer biomarkers for diagnosis, prognosis, and therapeutic decision-making has grown significantly. Especially for rare biomarkers and indications affecting a small subset of the population diagnosed with cancer (see definition of rare biomarker on page 6), there can be inherent challenges for validating assays as companion diagnostics (CDx), such as difficulty obtaining sufficient quantities of well-characterized samples and limited established reference materials. As such, it is critical to assess the current regulatory frameworks and propose strategies to facilitate continued advancements, particularly for the evaluation of diagnostic tests for rare biomarkers or indications. It is especially important to consider flexible validation approaches to ensure patients have access to validated CDx for rare biomarkers or indications in a timely manner. While the regulatory landscape continues to evolve, challenges with validating these diagnostic tests will likely remain.

One of the most frequent challenges for the validation* of a rare biomarker test is obtaining sufficient quantities of well-characterized clinical samples in a timely manner to perform analyses due to small patient numbers. In situations where the method employed to determine positivity by the test is novel and thus not regularly used in routine clinical practice, the ability to screen and identify positive samples is particularly challenging. To supplement these data, companies may need to invest considerable resources and time to acquire and screen a large number of samples to identify biomarker-positive samples. Identifying and employing alternative data or sample sources to support test validation is critical and needs to be conducted thoughtfully and collaboratively with drug sponsors, diagnostics developers, and the U.S. Food and Drug Administration (FDA).

Friends of Cancer Research (*Friends*) organized a collaborative working group of experts to propose potential approaches to facilitate oncology diagnostic test validation for rare biomarkers and indications. The considerations discussed here focused on scenarios where a diagnostic test is being validated for a rare biomarker or indication in preparation for a CDx premarket submission to FDA. These considerations may also be applicable in other regulatory contexts for biomarker testing.

The group identified three objectives for discussion:

- Identify situations where regulatory flexibilities would be appropriate and facilitate validation of diagnostics for rare biomarkers and indications in oncology.
- Develop approaches for leveraging alternative sample sources or data to support validation strategies.

* The term "validation" is used to refer to the establishment of specific performance characteristics, including (but not limited to) accuracy, precision, sensitivity, specificity, range, reference intervals, or other required performance characteristics.

- Outline a framework for capturing key information to support the proposed validation strategy, particularly when using alternative samples, to ensure clarity in premarket submissions.

Considerations for Regulatory Flexibility

Various biomarker, assay, and disease attributes inform the benefit-risk and safety and effectiveness assessment by regulators, which may influence the level of regulatory flexibility appropriate for a specific diagnostic test and validation strategies, such as the use of alternative samples or other data sources. Herein, we provide some broad categories of attributes to consider, ranked by their potential impact on decisions about flexibility. Categories for consideration should not be taken in isolation, but the sum of considerations can be used to support proposals for flexibility.

Biomarker and Indication Prevalence

A key aspect of when regulatory flexibility should be considered is the size of the population with the specific biomarker or indication, as a smaller population can make it more challenging to identify samples with the biomarker for assay validation. We propose that these flexibilities be considered for biomarker-defined subsets of cancer types with an estimated prevalence of 1% or lower (in the population of patients with that specific cancer type in the U.S.) or for rare cancer types with an estimated total prevalence of 1% or lower (in the overall population of patients with cancer in the U.S.) Determining whether a biomarker or indication qualifies as "rare" should rely on reasonable estimated prevalence with appropriate data and justification but should not be considered an exclusive criterion for applying flexibility. Biomarker or indication prevalence or incidence can be difficult to accurately identify, as it can change over time (e.g., if left undiagnosed or untreated), or can be unknown, especially among racial and ethnic minorities.[1–4] Additionally, the novelty of the biomarker may impact the degree to which testing is employed in routine clinical practice and the degree to which samples with the biomarker of interest are available at commercial biobanks.

Sample Availability

In addition to biomarker prevalence, other factors can influence the availability of clinical samples for assay validation. In certain populations, accessing adequate tissue to perform the necessary clinical and analytical validation analyses may be challenging. The location of the tumor and the risk associated with procedures required to obtain the sample may result in limited tissue availability (e.g., lung cancer). For liquid biopsies, sample volume is typically restricted, with blood providing the highest volume (albeit still limited) and other fluids, like cerebrospinal fluid and aqueous humor of the eye, yielding even less. Additionally, to represent the expected testing scenario, the sample would need to be collected in the appropriate compatible sample collection tube, which is not always the case. Some sample types, like whole blood, extracted mRNA, or frozen tissue, may degrade beyond usability faster than other sample types (e.g., FFPE), impacting the ability to do testing at later timepoints. This can be a practical issue when the time of sample collection and the time of validation extend beyond stability expectations for the analyte. Additionally, patients in biomarker driven trials are often initially screened at local sites for enrollment and, as a result, tissue for bridging studies or to support analytical validation may be more

difficult to obtain. This is especially challenging for rare biomarkers, where the test may not be part of standard practice so there are overall fewer patients who are screened at local sites. Additionally, samples may be exhausted by the time the trial initiates due to their use in supporting clinical care and management and other stakeholders (e.g., patients, providers, Institutional Review Boards) may be resistant to subjecting patients to an additional biopsy for the purposes of test validation. Ethical considerations related to biopsy for the sole or primary purpose of supporting analytical testing may preclude additional sample collection.

Unmet Needs and Expedited Review Pathways

In situations where the CDx is co-developed with a drug for a serious or unmet medical need that has Breakthrough Therapy Designation (BTD) or is ultimately approved through the Accelerated Approval pathway, development timelines may be condensed. Contemporaneous approval of the drug with a CDx ensures patients are appropriately identified and have access as soon as the product is approved.[5] However, aligning the timing of the development and approval of both the drug and the device can be challenging, particularly within the expedited timelines of FDA's drug development programs.

Approaches for Leveraging Data Sources for Validation

For rare biomarkers where accessing adequate tissue to perform all necessary assessments for assay validation may be difficult, other data sources could be considered to support the premarket submission for the CDx. Assay validation includes clinical validation, which for a CDx refers to the accuracy with which the test identifies the patients for whom the therapy is safe and effective, and analytical validation, which focuses on ensuring tests are accurate, precise, specific, and reliable.[5,6] The following sections outline various data sources, their proposed use in clinical or analytical validation, and opportunities and challenges for using each (also outlined in **Table 1**). The text and the table suggest prioritization for using samples in different types of validations. Examples are also provided for situations where the various data sources could be considered with appropriate justification.

Clinical Trial Samples

In general, clinical trial samples from the corresponding pivotal study should be prioritized for clinical validation as these samples represent the intended use population. Ideally, the candidate CDx will be used to identify all patients for inclusion in the therapeutic product's pivotal study; however, in some cases, the pivotal study enrolls patients using one or more Clinical Trial Assays (CTA) and may also include the candidate CDx test and local testing. Bridging studies assess agreement between assays (e.g., the enrollment tests vs. the candidate CDx) to bridge the intended use population clinical data from the enrollment tests to the candidate CDx to evaluate safety and effectiveness and support approval. Thus, remaining patient samples from the enrollment tests or local testing should be prioritized for conducting bridging studies. In some cases, these samples are saved as pre-processed samples such as extracted DNA or RNA from clinical trial studies and can be considered for use in CDx clinical validation. If samples are extracted using a different method/process than the one specified for the

candidate CDx, information is needed to demonstrate equivalent performance across the different method(s)/process(es).

Therapeutic clinical trial sponsors should prospectively plan for storing archival tissues or nucleic acids from the pivotal clinical trial and ensure they obtain and retain patient consent to use these tissues for test development. These archived samples may be useful for follow-on CDx development (e.g. to support the development of a liquid biopsy CDx if only a tissue-based CDx exists), or to support the need to develop multiple different CDx in various geographies. However, some archived patient specimens may be of lower-quality, and for the reasons noted above regarding ethical and practical challenges with obtaining additional biopsies, sponsors may not have sufficient samples for all activities and must therefore determine how to prioritize the use of available samples.

Provided the clinical trial samples from related clinical studies (e.g., earlier phase study in the same development program) were not used to develop the candidate CDx, these samples may be prioritized for clinical validation when the intended use population is the same as the pivotal trial and can supplement the available samples for clinical concordance studies. Biomarker-negative samples are necessary for bridging and clinical concordance studies; however, these may not be included in the target trial design due to the lack of anticipated effect in patients without the biomarker, raising ethical questions about the enrollment of biomarker-negative patients. In this scenario, it will be challenging or impossible to have sufficient clinical trial-enrolled biomarker-negative samples due to the selection criteria and limited inclusion of these patients with biomarker-negative tumors in the trial. However, this should not preclude development of a plan that includes storing biomarker negative specimens from patients that were not enrolled in the trial. In addition, well-characterized negative samples from related studies or normal healthy donors for blood-based biomarkers could be considered. Specifically, early phase trials may include biomarker negative samples that may be valuable for negative percent agreement (NPA) analyses. The value of these samples is that drug sponsors have control of the trial, the samples, and their availability, and thus similar performance could be expected. However, for analytical validation studies, the stage of the disease may not be significant in certain scenarios, such as when analyzing driver mutations in tissue samples. Stage can be highly relevant in other situations, like when dealing with resistance mutations or using ctDNA approaches, where the extent of tumor shedding can vary significantly. Therefore, whenever proposing to use samples from related trials or a different cancer stage, proper justification should be included.

Representative Clinical Approaches

Trial samples from related clinical studies, or samples from routine clinical testing of different cancer types (e.g., lung vs. colon) or specimen types (e.g., biopsy type or fixation) could be considered for analytical validation, provided such samples are applicable and relevant to the intended use of the candidate CDx. It is important to consider whether there are any differences in analytical validation due to the specimen or cancer type and to describe the rationale for using these samples. There is potential to use a more prevalent cancer type (e.g., lung cancer) for analytical validation of a CDx for a tissue agnostic indication being used in a rare tumor type (e.g., pediatric brain cancer). These samples could

also be considered when a drug is tested for a different indication where the sample type and biomarker tested are the same as the related study.

An alternative approach is to use clinical specimens that are not necessarily reflective of the intended use population to leverage representative validation approaches. Generalized conclusions about analytical validity can be based on a broad sampling of variants in the same class (i.e., substitutions, insertions, deletions, etc.) in various contexts across the queried genome. This approach may be particularly useful for assessing rare genetic variants in similar genomic contexts (e.g., GC regions, same chromosome) to other more prevalent variants. Whether there are opportunities to use a similar approach for other assay modalities beyond nucleic acid sequencing (e.g., IHC) should be explored.

Real-World Evidence (RWE)

There are opportunities to track patients in real-world settings who have been tested with a diagnostic that could be developed as a CDx and who have also received a therapy of interest. Such real-world data (RWD), when appropriately gathered and analyzed, may be proposed to support clinical validation. Leveraging RWD provides value not only for assessing clinical outcomes at single time points but also for tracking outcomes over time. However, RWD from electronic health records will likely differ from the data collected in a clinical trial, which may lead to inconsistencies in data interpretation. For example, measurements of progression in the real world often do not apply RECIST criteria and may occur with a different periodicity. These factors should be considered and addressed in proposals to use RWD in CDx regulatory filings.

RWE developed from incidence rates in developer databases can be supportive in post-market settings to demonstrate non-specific comparability with other assays measuring the same biomarker but would not be useful for demonstrating safety and effectiveness of a CDx. For example, knowing that the prevalence of ALK alterations is 3-7% in the general population, a developer might demonstrate the same rate of ALK alterations in their real-world NSCLC dataset. In any approach using RWD, use of different versions of an assay (e.g., design iteration) could confound analysis and clear explanations about the potential impact of assay versions should be described.

Procured Human Specimens

Procured human specimens that are similar to the intended use population can be purchased from a vendor, identified from data repositories or representative archival tissue, and are often useful for analytical validation, including determining the limit of detection (LOD), accuracy, precision, and other key analytical studies related to the specimen (e.g., stability). Additionally, since clinical trials often enroll only biomarker positive patients, sample procurement provides an alternative approach to identifying biomarker negative samples that could be used for analytical validation studies.

In some cases, the specimen may be from the appropriate intended use population, but the sample acquisition method may differ. Differences could include either the approach for sample collection (e.g., biopsy vs. a fine needle aspirate vs. a cytology smear) or the sample preservation approach (e.g., FFPE block vs. a frozen tissue that was secondarily fixed, or plasma collected in a K2EDTA tube and frozen vs. a Streck cfDNA BCT shipped at an ambient temperature). In each case, there may be implications for the

analytical analysis, which should be clearly described. These factors should be considered and addressed in proposals to use procured samples for validation in CDx regulatory filings.

Cell Lines

Cell lines, including immortalized cell lines with the biomarker of interest, those with CRISPR or other genetic modification to have the biomarker of interest, and primary cultures or organoids, can be considered for analytical studies. When appropriately validated, these cell lines may be beneficial for accuracy, precision, interference, reagent stability, guard banding, and other studies. Cell line identity and validity for use may vary depending on the supplier. The benefit of validated cell lines is that they can be processed to simulate tissue processing (i.e., freezing or FFPE embedding, as appropriate). However, the samples may not reflect the tumor tissue complexity of clinical samples and so may not be feasible for analyses that require tissue architecture (e.g., analytical validation of IHC) or where sample-based interfering substances are problematic. Further, prolonged culture of cell lines can lead to genetic drift, making them less representative of the original tumor. These factors should be considered and addressed in proposals to use cell line data for regulatory use in CDx regulatory filings. Such proposals should also include information to support that the performance in cell lines is not different from the performance in clinical intended use specimens.

Contrived Samples

Contrived samples such as analyte spike-in, synthesized DNA, and double-stranded DNA fragments may be useful to supplement human samples in analytical validation studies such as linearity, stability, precision, interfering substances, and dilution studies to assess limits of detection. These samples could be especially helpful in studies where large numbers of replicates are required. When appropriately validated, there is confidence that the biomarker is present. The variant type (e.g., substitutions, insertions, deletions) and level (e.g. allele frequency) can be specified and customized. Contrived samples may be especially beneficial when validating highly sensitive assays, such as liquid biopsies assessing ctDNA. In this case, distinguishing a few particles of cancerous DNA from billions of non-target molecules can be challenging. Purpose-built, patient-like contrived reference materials built using, for example, a 'plasma in plasma' approach could be used to address this challenge.[7] Ensuring "spike-in" samples are prepared using an appropriate background/matrix to mimic the intended use specimens to the extent possible is important. Strengths and weaknesses of contrived samples should be considered and addressed in CDx regulatory filings. Such proposals should include information to demonstrate the performance in contrived samples does not differ from clinical intended use specimens.

In Silico Datasets

In silico datasets can be considered for analytical validation, specifically focused on validating the bioinformatics pipeline and other informatics components. Appropriately constructed and relevant in silico datasets are stable and may be useful to re-validate an assay after a software or hardware change. It is important that the dataset used to train the algorithm is not used for validation. An in silico validation approach requires close alignment between the in silico dataset and the specific wet lab procedures,

making it challenging to establish a standardized, off-the-shelf solution using in silico reference datasets. Specific approaches to dataset construction and the ability to query important bioinformatics functions should be considered and addressed in proposals to use them for validation in CDx regulatory filings.

Table 1. Overview of Data Sources and Sample Types in Assay Validation

Data or Sample Type Category (Examples)	Use in Validation	Potential Advantages	Challenges
Clinical Trial Samples (e.g., tissue or nucleic acids from either the pivotal trial or other clinical trials with the same intended use population)	Prioritize for clinical validation (e.g., bridging studies)	• If from the therapeutic pivotal study, validation performance represents best evidence for clinical validity • Use of clinical specimens from related trials that were not used for diagnostic test development and are adequately representative of the intended use population may have well-characterized negative samples	• Pivotal trial samples are often limited and may not be available or appropriate for analysis with the CDx • Matched efficacy data may be unavailable for related trials
Representative Clinical Approaches (e.g., trial samples from related clinical study with different cancer (lung vs. colon) or specimen type (biopsy type or fixation), or clinical specimens that are not reflective of the intended use population)	Analytical validation	• Validation performance expected to be similar to validation performance in the intended use clinical specimens • For procured samples, broad sampling of variants in the same class may support generalized conclusions about analytical validity	• There may be nuanced differences between cancer types that could affect the interpretation of either analytical or clinical validation data. Therefore, any anticipated differences should be clearly described along with a biological and/or technical justification for the use of samples from different cancer types • The biomarker prevalence may be low in other cancer types as well
Real-World Evidence (e.g., tracking patients in real-world settings who have been tested with the candidate CDx and received therapy of interest)	Clinical validation	• Can include clinical outcomes and track outcomes over time • Data reflects real-world use of assays and therapies	• RWD (e.g., data from clinical practice in an EHR) will differ from data collected in a clinical trial, potentially leading to inconsistencies in data interpretation ○ Measurements of progression in the real world often do not apply RECIST and may occur with a different periodicity • Use of different versions of an assay (e.g., design iteration) could confound analysis

Data or Sample Type Category (Examples)	Use in Validation	Potential Advantages	Challenges
Procured Human Specimens (e.g., purchased from a vendor, identified from data repositories, or representative archival tissue)	Analytical validation (e.g., LOD, accuracy, precision, and other key analytical studies related to the specimen (e.g., stability))	• Commercially available • Includes the complexity/difficulty of the human specimen • Technological and/or biological justification can support representative variant detection across the genome, or within a specified genomic context • Existing data and metrics can support performance and justification	• Sometimes expensive • Commercial availability may still be a problem for some rare cancers or rare biomarkers
Cell Lines (e.g., CRISPR, immortalized cells, primary cultures/ organoids)	Analytical validation (e.g., interference, reagent stability, input of intermediate steps, guard banding, etc.)	• Materials processing can simulate tissue processing (e.g., freezing or FFPE embedding, as appropriate) • When appropriately validated, confidence that biomarker is present • Defined quality and abundant quantity • Useful when testing accuracy and reproducibility at lowest analyte levels	• May not reflect the tumor tissue complexity of clinical samples • Prolonged culture of cell lines can lead to genetic drift, making them less representative of the original tumor • Analytical validity may vary depending on the supplier • Not feasible for analyses that require tissue architecture (e.g., IHC) • Does not reflect the intended use population

Data or Sample Type Category (Examples)	Use in Validation	Potential Advantages	Challenges
Contrived Samples (e.g., analyte spike-in, synthesized DNA, double-stranded DNA fragments)	Analytical validation (e.g., linearity, stability, reproducibility, interfering substances, etc.)	• When appropriately validated, have confidence biomarker is present • Can use patient-derived materials (e.g., plasma in plasma approach) • Can perform robustness or process studies where large numbers of replicates are required • The variant type (e.g., SNPs with certain INDEL) and level (e.g. allele frequency) can be specified and customized • Can be sensitive enough to assess the most sensitive assay (e.g., ctDNA assay or highly sensitive flow cytometry)	• May not reflect the complexity or variability of actual patient samples • Does not reflect the intended use population
In Silico Datasets (e.g., sequence coverage at biomarker positions, can include synthetic approaches)	Validating bioinformatics pipeline and other informatics components	• Could be used to re-validate the assay after software or hardware changes • Does not expire	• Cannot be used to validate the wet lab portion of an assay • Requires sequencing to align with wet lab approach (i.e., challenging to establish off the shelf approach) • May not be useful across different platforms as the data processing can be mismatched to the specific analytical platform used by a test • The reference human genomes used may be different in different pipelines and can impact variant calls

Opportunities for Consistent Data Reporting and Regulatory Discussions

For discussions with FDA regarding premarket submissions of CDx for rare biomarkers, consistent descriptions of the validation strategy, including suggested samples and justifications, is important. In the **Appendix**, we provide an example snapshot to aid in sharing the validation strategy during the pre-submission and marketing application, which may be updated based on feedback throughout the development process. Using this or a similar approach would provide FDA with a clear understanding of the evidence used for validation and accompanying justification. Additionally, this snapshot could help drug sponsors and diagnostics companies align on the approach for the drug and CDx review and approval. We recommend discussing the co-development program with the FDA as early in development as possible.

Each development program will have different needs and considerations for the justification for flexibility and sample selection. However, some consistent recommendations apply. In general, clinical samples from the intended use population, particularly those with clinical outcomes data, should be prioritized for clinical validation. This is especially important for complex biomarkers, such as those incorporating sophisticated algorithmic analyses, to ensure accuracy in the clinical state or cutoff determination. For novel biomarkers, readily available reference standards and clinical samples may be limited, as testing for these biomarkers is not yet routine in clinical practice. In each case, adequate justification for the selected data source should be included.

Conclusions and Next Steps

Regulatory flexibilities can aid in demonstrating a favorable benefit-risk profile for rare biomarkers and indications, especially where there are limited clinical trial samples for validation studies. Various alternative evidence sources (e.g., samples, data, etc.) can support clinical and analytical validation for CDx biomarker tests when specimen availability is limited. Sponsors should provide an explanation for why samples would be limited and discuss plans for using alternative data or samples for validation, including a well-justified rationale for their use in early conversations with the FDA. Sponsors could consider using the proposed snapshot document in the Appendix to more effectively facilitate these discussions.

As drug development for cancers with rare biomarkers expands, consistent approaches to clinical sample storage and alternative sample selection for validation are increasingly important. To maximize the availability of trial samples, drug and device sponsors, working together, should establish proactive plans for preserving samples from all phases of clinical trials. Additionally, the field should consider aligned approaches for establishing validated reference materials and methods, for example, datasets with well-annotated samples that could support both already approved products and the rapid development of reference information for novel, rare biomarkers, which may allow for more standardized characterization of assay performance.

References

1 Aldrighetti CM, Niemierko A, Van Allen E et al. Racial and ethnic disparities among participants in precision oncology clinical studies. JAMA Netw Open 2021;4(11):e2133205. DOI: 10.1001/jamanetworkopen.2021.33205

2 Cheung ATM, Palapattu EL, Pompa IR et al. Racial and ethnic disparities in a real-world precision oncology data registry. NPJ Precis Oncol 2023;7(1):7. DOI: 10.1038/s41698-023-00351-6

3 Overman MJ, Morris V, Kee B et al. Utility of a molecular prescreening program in advanced colorectal cancer for enrollment on biomarker-selected clinical trials. Ann Oncol 2016;27(6):1068–74. DOI: 10.1093/annonc/mdw073

4 Dienstmann R, Garralda E, Aguilar S et al. Evolving landscape of molecular prescreening strategies for oncology early clinical trials. JCO Precis Oncol 2020;4:505–13. DOI: 10.1200/PO.19.00398

5 U.S. Food and Drug Administration. Principles for Codevelopment of an In Vitro Companion Diagnostic Device with a Therapeutic Product: Draft Guidance. Available from: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/principles-codevelopment-in-vitro-companion-diagnostic-device-therapeutic-productThe Clinical and

6 Laboratory Standards Institute (CLSI). https://clsi.org/

7 Stetson D, Labrousse P, Russell H et al. Next-generation molecular residual disease assays: do we have the tools to evaluate them properly? J Clin Oncol 2024;42(23):2736–40. DOI: 10.1200/JCO.23.02301

Appendix

Proposed Snapshot for Alternate Data Use for CDx in Rare Biomarker Validation

1. *Include a paragraph that provides justification for the biomarker of interest being considered as a rare biomarker with citations:*
    - What is the biomarker? What are the incidence and prevalence (either overall or in the specific cancer type of interest)?
    - How many clinical samples do you anticipate having access to? Explain why you believe the use of alternative evidence is necessary.
2. *Complete the table below for each proposed validation study (An example follows with proposed language in red. There should be one table for each validation study.)*

Category	Description
Validation Study Describe which study you will be using the proposed samples for	
Proposed Samples Describe the samples and include the anticipated sample size	
Sample Source Describe how the samples are procured	
Sample Justification Describe the justification behind using these samples	

Category	Description
Validation Study Describe which study you will be using the proposed samples for	Analytical validation - limit of detection
Proposed Samples Describe the samples and include the anticipated sample size	Human specimens containing the biomarker of interest from five different patients with alternative cancer types (i.e., samples representing different cancer types than the specific cancer type of interest).
Sample Source Describe how the samples are procured	All samples will be residual clinical specimens processed for routine laboratory testing and/or sourced from a biorepository. Dilutions to establish limit of detection will be prepared and analyzed by diagnostic test sponsor.
Sample Justification Describe the justification behind using these samples	Limit of detection confirmation studies require more samples than are available for [biomarker/specimen type], due to rarity of biomarker. Limit of detection is an analytical validation analysis that does not rely on clinical outcomes. As such, we are proposing to use procured samples that have [biomarker of interest] to support limit of detection confirmation analyses. The assay analyzes extracted nucleic acid. There are no unique biological characteristics of the biomarker, or biological differences between cancer types, that would make evaluation of limit of detection dependent on the cancer type from which nucleic acid is extracted. The specific variants tested for limit of detection using alternative cancer types will be representative of the specific variants relevant to the intended use population. Therefore, we believe that the limit of detection for [the biomarker of interest] can be appropriately confirmed using alternative cancer types.