FRIENDS of CANCER RESEARCH

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# CHARTING THE COURSE FOR PRECISION MEDICINE

## ADOPTING CONSENSUS ANALYTICAL STANDARDS AND STREAMLINING APPROVAL PATHWAYS FOR POST-MARKET MODIFICATIONS FOR NGS TESTS IN ONCOLOGY

## GOAL

This whitepaper aims to provide recommendations to establish minimum analytical performance characteristics for somatic mutation testing in oncology, particularly for Next Generation Sequencing (NGS)-based panels, using a standardized, transparent, and optimized approach. In addition, this whitepaper will propose a regulatory process that could reduce the need for premarket review to support modifications of US Food and Drug Administration (FDA)-approved NGS diagnostics to ensure tests reflect the most up-to-date information for clinical decision-making.

## INTRODUCTION

Transformative medicines are quickly changing the landscape of oncology treatment and care. Genomic information from NGS panels has led to a deeper understanding of tumor biology. As a result, treatment modalities are shifting from using primarily systemic cytotoxic chemotherapies to employing molecularly targeted therapies or a combination of both. The success of targeted therapies is dependent on diagnostic tools that can accurately identify patients with the appropriate molecular target(s) to confer a higher chance of benefit from these therapies. Currently, there are over 30 in vitro diagnostics (IVDs) approved as companion diagnostics by the FDA's Center for Devices and Radiological Health (CDRH). Many of these IVD tests are for a single biomarker and are linked to a single corresponding therapeutic product. In disease settings where there are multiple targeted therapeutic options, patients may require multiple tests that in turn neces-


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sitates the need for obtaining sufficient biopsy material to find all actionable mutations and thus an appropriate therapy. By maximizing the information obtained from diagnostics tests, patients can be assessed for all potential genomic variants of clinical relevance using the least number of tests necessary to achieve reliable answers.

Progress towards the goal of developing high content assays that can detect multiple biomarkers of clinical significance is rapidly increasing, and one key enabler is NGS technology. By sequencing multiple sections of a person's genome concurrently, NGS-based tests have the capability to detect hundreds of mutations simultaneously that could potentially be matched to a variety of approved targeted agents. Consequently, as the number of biomarkers and corresponding targeted agents continue to increase, test developers are focusing on NGS technology to query multiple markers in a single test. Three NGS-based oncology tests have been approved by the FDA and many laboratory developed tests (LDTs) have been reviewed under the College of American Pathologists (CAP) accreditation program and/or by New York State's Clinical Laboratory Evaluation Program. Despite these strong signs that NGS platforms are increasingly available and used by physicians, NGS tests have some issues that need to be addressed so that each patient receives results that appropriately inform the use of the many available therapeutic options.

One of the key issues to be addressed is the accuracy of results amongst diagnostic platforms. Due in part to the fragmented regulatory landscape for diagnostic tests in the United States, physicians and patients relying on these tests often do not know whether the test went through the FDA approval process or is being offered as an LDT. This bifurcated regulatory system may result in divergent analytical performance characteristics of similar tests used by physicians and patients. Many physicians and patients may expect that all tests offered in a clinical setting are equally accurate and interchangeable. In reality, tests may demonstrate variability in both accuracy and precision. This can be a barrier to selecting the most appropriate test and consequently the therapy for a given patient. Ideally, principles should be established that allow for identification of an agreed upon and modifiable set of clinically actionable genomic alterations, analytical performance characteristics for test comparisons, and the ability to rapidly add new information to test claims as science and medicine generate new associations between markers and therapies regardless of the regulatory path to the clinic. Addressing these issues in a concerted effort will help reduce the number of uncertainties that affect development, clinical use, and regulatory oversight of NGS-based tests. This will help ensure the regulatory pathway is sufficiently flexible to support future precision medicines while still ensuring that diagnostic tests remain safe and effective for patients.

This paper will discuss two major issues in the validation and approval of NGS-based oncology tests, as well as propose incentives for assuring test comparability:

1. The lack of consensus on what analytical performance characteristics are important to assess
2. The need for a more streamlined regulatory approval pathway for changes to NGS-based tests

## ESTABLISHING ANALYTICAL PERFORMANCE CHARACTERISTICS

There is no shortage of measurement parameters available to help establish a test as a valid tool for physicians to make treatment related decisions. For physicians and patients to benefit from this rapidly evolving technology, it is important that minimum baseline analytical performance characteristics are established to ensure consistency of test results. Reducing variability and establishing baseline analytical performance characteristics for diagnostic tests are critical to ensure high-quality patient care and aid in clinical decision-making processes. High analytical concordance can provide reassurance that the clinical outcomes of the drug/diagnostic pairing are likely to be similar in the absence of a clinical trial. Guidelines developed by several entities, including the New York State Department of Health, Association for Molecular Pathology (AMP) and CAP, and the FDA outline basic principles for establishing the analytical validity of NGS-based tests and/or mechanisms for testing proficiencies of laboratories that offer them (see appendix A for comparison of guidelines).

The relative importance of specific analytical performance criteria is an area of continual discussion but identifying and agreeing on the minimal measures critical for analytical standardization can help establish concordance between tests. These include accuracy, analytical sensitivity, limit of detection/quantitation, analytical specificity, precision, reproducibility, and coverage. To move the field forward, consensus should be established on the minimal analytical performance characteristics that every NGS diagnostic used in clinical care should meet, and these performance characteristics should be utilized uniformly. The evidence necessary to meet each core standard may vary depending on the type of diagnostic and its intended use.

Evaluation of analytical performance requires access to appropriate clinical samples and/or reference materials that can be used to demonstrate test performance and assess comparability between tests and laboratories. As samples with clinical outcomes from therapeutic trials (the "gold standard" of samples) are necessarily limited and not widely available, other sources and types of adequate samples or material standards need to be identified and developed as acceptable for analytical performance characterization. Solutions to address access to samples that will appropriately assess analytical performance of a test to infer clinical performance of follow-on tests need to be explored. An established set of criteria for samples that contain a range of analytes and analyte types (e.g., single nucleotide and copy number variants, indels, fusions, etc.) and a roadmap for how these materials should be utilized would likely incentivize their use and increase their availability by encouraging increased development and curation.

It is suggested that a multi-stakeholder group be convened to establish harmonized analytical performance characteristics for NGS-based oncology tests. Likewise, further multi-stakeholder efforts are needed to oversee the development of reference materials that can be used to evaluate assay performance across different test platforms and laboratories. Subsequently, there is a need to ensure that laboratories meet these established analytical performance standards and demonstrate appropriate accuracy when challenged by reference materials. There are

several approaches that could be performed alone or in some combination. First, laboratories could provide test performance characteristics in a standardized format available in a public database, on company websites, or on third party sites (e.g., NIH, ASCO, AMP, CAP, etc.). This transparency would allow physicians and patients the opportunity to assess potential limitations of individual tests because understanding test performance and how it was assessed is relevant to understanding how to use and interpret the test results. A second approach would be to provide a publicly available list of individual tests that meet the harmonized analytical performance characteristics and demonstrate appropriate performance using the reference materials. This would provide patients and their physicians with assurance that the test being used to guide their care is accurate and reliable, without placing the potential burden of test evaluation on the patient or treating physician. A third approach would be for laboratory accrediting agencies to mandate that labs performing NGS tests meet certain analytical performance characteristics. Ultimately, the incentive for performing these studies is to ensure maximum benefit for patients.

**Questions on Analytical Standards:**

- **What are the core performance characteristics and how can we get the necessary groups to reach consensus on the necessary performance characteristics to be assessed and how good performance should be?**

- **Should a Standards Development Organization, such as CLSI, be charged with developing an internationally recognized format for collecting data and a rigorous but reasonable method for establishing minimal analytical performance characteristics and assuring cut-offs (decision points) have been adequately set?**

- **Where should these standards be published to encourage adoption and should there be an enforcement strategy?**

- **How should the claims and limitations of a test be reported to patients and physicians?**

## ENCOURAGING RAPID INNOVATION OF NGS-BASED TESTS

Under the current FDA regulatory framework, proposed modifications for an approved IVD test must be submitted to the FDA via the supplemental Premarket Approval (PMA) process, which can take up to 180 days. However, this timeframe for review of modifications to an existing IVD may delay the incorporation of emerging, validated data and prevent physician and patient access to information critical to the clinical decision-making process. To deliver the best patient care, tests should evolve with technology and clinical science in a near simultaneous manner, which may require regulatory review timeframes faster than the currently available 180-day supplemental PMA pathway for such proposed device changes. Because high-throughput technologies, such as NGS-based tests, can rapidly generate large amounts of clinically relevant data leading to identification of new genomic alterations that can impact patient care, reevaluating the regulatory pathway to modify tests and update labels without compromising patient safety is necessary. FDA recognizes the need for an improved regulatory framework and has published two draft guidances,[1,2] proposing methods to streamline oversight of NGS-based tests incorporating adaptability and flexibility into the regulatory framework. The recommendations presented in this paper are intended to describe additional options that may be considered by FDA to help encourage innovation without compromising patient safety.

### The Establishment of a Process for a Pre-Specification Plan for Anticipated Expanded Claims or Test Modifications

We propose a pre-specified modification plan developed by sponsors in consultation with FDA prior to or at the time of PMA submission to streamline the incorporation of new analytical and clinical claims to FDA-approved NGS-based oncology tests. While the framing of the proposal is around the FDA approval process, a parallel process could be considered by other review bodies (e.g., New York State Department of Health, CLIA/CAP, etc.) as well. The pre-specification process could be used for modifications to variants, analytes, or clinical claims on tests. For instance, if clinical trial data is being collected for a variant of interest, an agreed upon pre-specification plan could streamline the incorporation of this information onto the label without the need to submit a supplemental PMA. Updates to NGS-based oncology tests can often be predicted in advance of specific analytes having established analytical and/or clinical validity, and will require routine validation to assure the performance meets preset goals. Ideally, with multiple tests making similar clinical claims available for clinical use, all (or most) tests should incorporate the same changes at nearly the same time, in order to provide optimal information for physician/patient clinical decision-making. The necessary data to support a modification change would be context dependent and would require the sponsor and FDA to agree on the necessary steps for a sponsor to follow. As part of the discussion, the sponsor and FDA could outline a pre-specification plan that may include the following steps:

1. **Develop a protocol and acceptance criteria for each analytical and clinical performance metric;**

2. **Outline a documentation plan to demonstrate that the modification meets the pre-determined performance parameters;**

3. **The sponsor and FDA should reach agreement on how and when modification validation will be communicated to the FDA; and**

4. **If the modification(s) will lead to a label change, the sponsor and FDA should reach agreement on the labeling update as part of the pre-specified plan.**

Once the plan has been agreed upon, subsequent modifications that follow the pre-specified plan would not need to be submitted to the FDA using a supplemental PMA, and the requirement for FDA approval, if acceptance criteria are met and labels are as anticipated, would be replaced by a "post-market" addition to the original PMA file. As such, the 180 day review time associated with the submission of a supplemental PMA would be avoided and modifications to tests would be more streamlined. Permitted modifications in this proposed system would be gated by approval of a new drug or label with altered Indications and Usage, Dosage and Administration, Contraindications, Warnings and Precautions, Use in Specific Populations, and approval of an IVD test that supports such changes. Data supporting the modification would be required to meet the agreed upon performance metrics in the pre-specification plan. The development of a portal to report modifications and whether the modifications are self-reported or independently verified may also be considered. The label would be updated as agreed upon in the pre-specification plan, and FDA would have the ability to audit the data within a pre-determined amount of time. This process could be implemented similarly to the FDA administrative and scientific process currently used to address replacement reagents[3] or FDA's new Software Pre-Certification pilot program, which is developer-focused rather than product-focused allowing for reduced or streamlined submissions. While such a system must be scientifically robust, it would generate up-front agreement on analytical validation of system modifications, which would result in consistency of biomarker data collected and thus lower variability in clinical study outcomes (e.g., ensuring homogeneity with respect to biomarker status in intent-to-treat (ITT) population), a reduced number of iterative submissions, and an expedited pathway to marketing new claims.

### Additional Considerations for Implementing a Pre-Specification Plan

To monitor the robustness of modifications, an evaluation of the data generated through the use of the pre-specification plan may be needed. Modifications should follow the defined criteria in the pre-specification plan and a summary of the results should be provided as part of the PMA annual report or other report as specified. A template prescribing how modification validation results will be reported should be part of the modification plan and may include the following: list of the new variants detected/reported, agreement between the previous and current sensitivity, description of changes, and labeling changes. An important process of the PMA and PMA supplement pathway is reviewing the information to be included on labels; and therefore, label changes should be specified and agreed upon in the modification work plan and followed closely.

**Questions on Streamlining Modifications to NGS-based Tests:**

- **What should the labeling process look like and what are the potential implications for drug labels?**
- **Is FDA review of the modification data needed? Should another entity review the data (e.g., CMS, CAP inspectors, peer medical reviewers)?**

## POLICY CHALLENGES AND OPPORTUNITIES FOR PRECISION MEDICINE

To fully consider and implement the processes and strategies outlined in this whitepaper, regulatory and legislative changes may be required. In addition, key stakeholders may need to be called upon to fully implement necessary steps to ensure these can be appropriately carried out. Several areas identified as requiring significant stakeholder input are listed below.

- **A survey should be performed of existing guidelines for establishing agreed upon analytical performance characteristics to avoid redundant standards and to build upon existing consensus standards.**
- **FDA should describe which materials are acceptable for validation of modifications given that clinical samples from clinical trials will not be widely available.**
- **Adopting analytical performance standards requires standardized reference material. Standard setting bodies such as National Institute of Standards and Technology (NIST) and others should be encouraged to develop reference materials such that they are made available to sponsors and labs for use to assure standardization of test results across test platforms.**

- **Multi-stakeholder groups should identify high quality reference materials that are available for establishing analytical performance characteristics, identify gaps in needed reference materials, and work toward development of these materials.**

- **Incentives should be identified and fostered for demonstrating analytical validation across laboratories.**

- **Where possible, real-world evidence should be gathered about test performance and patient outcomes through expanded use of registries and databases (clinical claims). This is keeping with FDA's draft guidance on the "Use of Public Human Genetic Variant Databases to Support Clinical Validity for Next Generation Sequencing (NGS)-Based In Vitro Diagnostics" use of databases.**

- **Organizations administering proficiency testing should make overall performance results widely available so that there is a better understanding of the comparability of analytical performance across platforms and laboratories.**

- **FDA expertise should be leveraged to develop innovative regulatory strategies for regulatory review and approval of modifications to NGS-based tests. FDA is familiar with reducing review burden in using a variety of methods, including use of special 510(k)s, use of migration studies for introducing new versions of old tests, and use of the replacement reagent protocol to reduce redundant review. While these strategies do not directly fit the regulatory paradigm currently being proposed, they may serve as the basis for creating a reliable but efficient mechanism for addressing the data opportunities and burdens of NGS technologies.**

- **Standardizing the information reported to patients and physicians, and ensuring the interpretability of lab report information.**

- **In addition to diagnostic modifications, stakeholders should be encouraged to propose novel approaches to the process of modifications to use of approved drugs. For example, if additional variants are shown to be clinically relevant to the use of an approved drug, patients and physicians would benefit from an expansion of not only the diagnostic label but also the drug label to reflect the expanded ITT population.**

- **Reimbursement and coverage challenges. The extensive efforts of sponsors that have demonstrated analytic and clinical validity of their IVDs via FDA review should be recognized in some way such that it provides an incentive for sponsors undergoing FDA review (e.g., differential reimbursement).**

## Appendix A. Comparison of Analytical Validation Guidelines from New York State; Association for Molecular Pathology (AMP) and College of American Pathologists (CAP); and U.S. FDA*

| | New York State[i] | AMP and CAP Joint Guidelines[ii] | FDA[iii] |
|---|---|---|---|
| **Identification of samples and performance characteristics** | • "Performance characteristics must be established and validated separately for each type of variant the assay is intended to detect."<br>• "Performance characteristics for each sample type must be established and validated, along with the demonstration of quality sequences for all target areas without sample type bias." | • "Massively parallel sequencing of multiple genes cannot be validated as if it were a single-analyte test. There is far too much variation in the types of samples, types of variants, allele burden, and targeted exons or regions."<br>• "Performance is certainly expected to vary considerably for different sample types, variant types, and allele burden, and therefore it is essential to establish performance characteristics by these factors. . . laboratories should strive to include samples with hotspot mutations relevant to the test's intended use."<br>• "The validation protocol should start with an explicit statement of the intended use, which will determine the types of samples and the performance characteristics that need to be addressed." | • "FDA believes that one approach for supporting the analytical validation of NGS-based tests may be through conformity with one or more FDA recognized standards (if available) or special controls."<br>• "FDA believes that for a standard to be recognized by FDA it should include, among other things, a description of the design activities that should be carried out and the performance characteristics that should be validated, as well as specific methodology, materials, and performance thresholds, where appropriate and justifiable."<br>• "Establish and document minimum acceptable thresholds for coverage, base quality, and other test run quality metrics relevant to the specific design and test processes." |
| **Accuracy** | • "Sequence a minimum of 3-well characterized reference materials to determine a robust laboratory specific error rate across all target areas. This error rate is expected to be <2%." | • "Accuracy should be stated in terms of PPA and PPV."<br>• "Because the performance will likely vary by mutation type, the PPA should be determined for each." | • "FDA recommends that PPA, NPA, and TPPV be set at no less than a point estimate of 99.9% with a lower bound of the 95% confidence interval of 99.0% for all variant types reported by the test."<br>• "The minimum acceptable overall and |

* This table contains the exact text found in the New York State guidelines, joint guidelines from the Association for Molecular Pathology and College of American Pathologist, and FDA guidance

## Appendix A. Comparison of Analytical Validation Guidelines from New York State; Association for Molecular Pathology (AMP) and College of American Pathologists (CAP); and U.S. FDA* (con't)

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| | | | target accuracy of an NGS-based test may vary depending on the type of variations and on whether variants are confirmed using an orthogonal assay."<br>• "Evaluate and document accuracy by comparison to a method identified as appropriate by FDA, such as bidirectional sequencing or another well-validated method."<br>• "Calculate PPA, NPA, and TPPV separately for each type of variant claimed." |
| **Initial Validation** | • "Must include a minimum of 50 patient samples comprising specimens of all intended sample and tumor types." | • "We recommend that the validation samples include. . . a minimum of 59 samples to assess quality metrics and performance characteristics... We recommend that PPA and PPV should be documented for each variant type."<br>• "By testing a minimum of 59 samples during validation, conclusions can be drawn as to the tolerance intervals of essentially any performance characteristic whether parametric or nonparametric in nature." | • "After design and development of the test, validation studies will indicate if the predefined performance is met. If the test does not meet any one of the predefined performance specifications, the test should be modified and revalidated. The cycle of design, development, and validation should continue until the test meets the predefined performance specifications." |
| **Full Validation** | • "10 positive samples for each type of intended variant in each target area must be sequenced and confirmed."<br>• "SNVs: Confirmation can be ceased once a minimum of 20 target areas have been fully | • "The quantitative analytical performance of a laboratory test does not necessarily predict performance at a clinical level."<br>• "We recommend that clinical validity and clinical utility of the NGS assays needs to be | • "The complete NGS-based test should be analytically validated in its entirety. . . prior to initiating clinical use of the test." |

| | | | |
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| | validated/confirmed with accuracy greater than or equal to your established specificity."<br>• "Indels: Confirmation can be ceased once a minimum of 29 target areas have been fully validated/confirmed with accuracy greater than or equal to your established specificity."<br>• "CNVs must always be fully validated." | defined during design of the test and need to be evaluated during the validation process."<br>• "Full scale of clinical validation is required for multianalyte NGS tests with prediction algorithms and should be performed using the guidelines and calculations as defined for an analytical validation."<br>• "It is expected that laboratories would be able to acquire quality metric data for 59 samples that contain SNCs. Ideally these 59 samples would also have other variants such as indels [but] it is acknowledged that ascertainment of samples containing indels is more challenging." | |
| **Precision (within run)** | • "For each type of variant a minimum of 3 positive patient samples containing variants near the stated sensitivity of the assay must be analyzed in triplicate in the same run using different barcodes." | • "Replicate (within run) and repeat (between run) testing should be performed."<br>• "Acceptance criteria need to be set before the acquisition of validation data." | • "FDA recommends thresholds for reproducibility and repeatability that meet or exceed 95.0% for the lower bound of the 95% CI, calculated by conditions tested and genomic context, separately for each variant type."<br>• "When presenting the results of reproducibility and repeatability studies, indicate the failed quality control rate, and list all "no calls" or "invalid calls." Data from runs that do not meet coverage depth, coverage uniformity, and other technical |

| | | | |
|---|---|---|---|
| | | | metrics are typically considered quality control failures." |
| **Reproducibility (between run)** | • "For each type of variant, a minimum of three positive patient samples containing variants near the stated sensitivity of the assay must be analyzed in three separate runs using different barcodes on different days by 2 different technologists." | • "Replicate (within run) and repeat (between run) testing should be performed."<br>• "Acceptance criteria need to be set before the acquisition of validation data."<br>• "It is recommended to asses a minimum of three samples across all steps and over an extended period to include all instruments, testing personnel, and multiple lots of reagent." | • "For reproducibility studies, document results for each variant or variant type. Indicate the number of replicates tested for each variant and the conditions that were tested (e.g., number 934 of runs, days, instruments, reagent lots, operators)." |

[i] NYSDOH "Next Generation" Sequencing (NGS) guidelines for somatic genetic variant detection (https://www.wadsworth.org/sites/default/files/WebDoc/Updated%20NextGen%20Seq%20ONCO_Guidelines_032016.pdf)
[ii] Jennings et al. Guidelines for Validation of Next-Generation Sequencing–Based Oncology Panels: A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists. 2017. J Mol Diagn. 19(3); 341-365.
[iii] Use of Standards in FDA Regulatory Oversight of Next Generation Sequencing (NGS)-Based In Vitro Diagnostics (IVDs) Used for Diagnosing Germline Diseases (https://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM509838.pdf)

# Appendix B. Considerations for Streamlining Diagnostic Development Requirements and Proposed Implementation of a Pre-specified Plan for OncomineDx

The Oncomine™ Dx Target Test is intended for use on the Ion PGM™ Dx Instrument System and is intended for in vitro diagnostic (IVD) use by trained personnel in a professional laboratory environment.

The device is indicated as a companion diagnostic to identify:

- **ROS1 fusion positive NSCLC patients for treatment with XALKORI® (crizotinib)**
- **BRAF V600E positive NSCLC patients for treatment with Tafinlar+Mekinist® (dabrafenib in combination with trametinib)**
- **EGFR L858R and Exon 19 deletions positive NSCLC patients for treatment with IRESSA® (gefitinib)**

The product's intended use:

The Oncomine™ Dx Target Test is a qualitative in vitro diagnostic test that uses targeted high throughput, parallel-sequencing technology to detect sequence variations in 23 genes in DNA and RNA isolated from formalin-fixed, paraffin-embedded tumor (FFPE) tissue samples from patients with non-small cell lung cancer (NSCLC) using the Ion PGM™ Dx System.

The test is indicated to aid in selecting NSCLC patients for treatment with the targeted therapies listed in Table 1 in accordance with the approved therapeutic product labeling.

Results other than those listed in Table 1 are indicated for use only in patients who have already been considered for all appropriate therapies (including those listed in Table 1). Safe and effective use has not been established for selecting therapies using this device for the variants in Table 1 in tissue types other than NSCLC.

Analytical performance using NSCLC specimens has been established for the variants listed in Table 2.

The test is not indicated to be used for standalone diagnostic purposes, screening, monitoring, risk assessment, or prognosis.

## Table 1 - List of variants for therapeutic use

| Gene | Variant | Targeted therapy |
|---|---|---|
| BRAF | BRAF V600E | TAFINLAR®(dabrafenib) in combination with MEKINIST® (trametinib) |
| ROS1 | ROS1 fusion | XALKORI® (crizotinib) |
| EGFR | L858R, Exon 19 deletions | IRESSA® (gefitinib) |

## Table 2 - List of variants with established analytical performance only

| Gene | Variant ID | Nucleotide change |
|---|---|---|
| KRAS | COSM512 | c.34_35delGGinsTT |
| KRAS | COSM516 | c.34G>T |
| MET | COSM707 | c.3029C>T |
| PIK3CA | COSM754 | c.1035T>A |

In the original Oncomine Dx Target Test assay pre-market approval (PMA), pre-clinical laboratory studies were assessed by comparing the effectiveness and concordance of the diagnostic test to that of externally validated comparator methods. No pre-clinical animal studies were conducted as part of the PMA. The clinical studies performed were used to determine the clinical utility of the product including selection of the correct patients for the designated therapy. The studies performed are listed in Table 3.

Sequence variations in DNA for the following 23 genes are reported: AKT1, ALK, BRAF, CDK4, DDR2, EGFR, ERBB2, ERBB3, FGFR2, FGFR3, HRAS, KIT, KRAS, MAP2K1, MAP2K2, MET, MTOR, NRAS, PDGFRA, PIK3CA, RAF1, RET, and ROS1. Sequence variation in RNA for ROS1 gene is also reported.

## Table 3 - Original PMA Submission Studies for the Oncomine Dx Target Test assay

| Pre-clinical laboratory studies | | Clinical Studies | |
|---|---|---|---|
| Parameters | | Parameters | |
| Analytical Accuracy | | Study Design | |
| Analytical Sensitivity | | | Inclusion and exclusion criteria |
| | Limit of detection (LoD) | | Follow-up schedule |
| | Nucleic acid input | | Clinical endpoints |
| | Tissue input | Accountability of PMA cohort | |
| | Tumor content | Study population demographics and baseline parameters | |
| Analytical Specificity | | Safety and effectiveness results | |
| | Inclusivity/cross-reactivity | | Concordance study |
| Interference | | | Bridging study |
| | Endogenous interference | | Sensitivity analysis |
| | Exogenous interference | | |
| | Antimicrobial testing | | |
| Precision and Reproducibility | | | |
| | Assay reproducibility across testing sites | | |
| | Sample processing reproducibility | | |
| | Assay precision | | |
| Tissue Heterogeneity | | | |
| Extraction Method Equivalency Studies for DNA/RNA | | | |
| Contrived Sample Functional Characterization Study | | | |
| Guard Band Studies | | | |
| | Workflow tolerances | | |
| | Tissue fixation | | |
| | Contamination studies | | |
| Stability Studies | | | |
| | Shelf-life stability | | |
| | In-use stability | | |
| | Designated hold times | | |
| | Kit lot interchangeability | | |
| | Extracted DNA and RNA sample stability | | |
| Pre-clinical laboratory studies | | Clinical Studies | |
| Parameters | | Parameters | |
| | Stored slide stability | | |
| | Stored block stability | | |
| | Transport stability | | |

Having a regulatory process such as the PMA application that establishes the minimum analytical performance characteristics for somatic mutation testing in oncology, particularly for Next Generation Sequencing (NGS)-based panels, using a standardized, transparent, and optimized approach is necessary. However, in order to reduce burden and decrease the time required for modifications to approved products, it is recommended to reduce the need for premarket review to support modifications of US Food and Drug Administration (FDA)-approved NGS diagnostics to ensure tests reflect the most up-to-date information for clinical decision-making.

In order to deliver the best patient care, tests should evolve with technology and clinical science in a near simultaneous manner, which may require regulatory review timeframes faster than the currently available 180-day supplemental PMA (sPMA) pathway for such proposed device changes. This case study identifies suggestions to reduce the regulatory burden and decrease the regulatory review time. These suggestions need to be vetted between NGS assay developers and the FDA to understand how these proposals can be put into action and utilized in the PMA and sPMA approval process.

In developing a streamlined modification process, the minimum analytical performance testing for initial development that is standardized and transparent needs to be defined. This will set the stage for a pre-specified modification plan process which is developed by sponsors in consultation with FDA prior to or at the time of PMA submission to streamline the incorporation of new analytical and clinical claims to FDA-approved NGS-based oncology tests. The pre-specification process could be used for modifications to variants, analytes, or clinical claims on tests. For instance, if clinical trial data is being collected for a variant of interest, an agreed upon pre-specification plan could streamline the incorporation of this information onto the label without the need to submit a supplemental PMA.

The following areas describe the potential changes to testing and development requirements for the PMA and sPMA process to enable FDA-approved NGS diagnostics to incorporate emerging, validated data and enable physician and patient access to information critical to the clinical decision-making process in real-time. The areas indicated in this case study require thoughtful review and consideration by the FDA and industry as they dramatically reduce time and cost. The areas for review include software, product controls, DNA origin from tissue type and representative validation, clinical sample availability, and validation.

## SOFTWARE

Software development is a prime area where the burden could be lessened for product modifications. The software validation submitted in the original PMA would contain all required validation needed to ensure safety and effectiveness following appropriate guidelines and standards.

Allowing the software to include multiple tissue types in the sample program menu regardless of the tissue type defined in the original approved indication would greatly benefit both industry and patients without compromising safety. This change would provide the user the ability to select the tissue type tested and would decrease the software development and validation burden on future programs as the information would already exist in the program menu.

Selecting from a multiple tissue menu would benefit users of clinical studies and allow the companies to progress on existing software development without requiring a new software version. In addition, this would allow clinical cases for which there are no other approved tests to use validated software and assay combinations.

## PRODUCT CONTROLS

Product controls increase the reliability of the results often through comparison of the control to other measures. Requiring a clinical biomarker to be present in each control, however, is burdensome and can cause delays in development.

Instead, a control would be considered a 'representative control' and each clinical marker would not need to be present as assay performance would be determined using the biomarkers for each class (SNV, SNP, insertions, deletions, etc.). A biomarker class-based approach would eliminate the need to update the control for each new clinical/therapeutic biomarker added and the requirement to manufacture a new control for each modification.

The classes that would be included in the "represented control" would represent:

- **SNV/ SNP**
- **Insertions**
- **Deletions**
- **CNV**
- **Fusion**

## DNA ORIGIN FROM TISSUE TYPE

The laboratory community and numerous researchers utilize the hypothesis that DNA extracted from each tissue type perform similarly when tested with a validated assay regardless of the tissue type and, therefore, DNA is DNA. In order to provide evidence for the FDA to accept this concept, which is well accepted within the industry, it is suggested that a well-controlled study of significant size and scope be performed across multiple tissue types showing that the variants across numerous tissue types perform similarly. This study could be leveraged for future NGS assay development.

The agreement that DNA performs the same regardless of tissue type would lessen the requirement to validate performance for each tissue type (i.e. sample stability [slide, block, nucleic acid] and sample reproducibility). With the acceptance of this hypothesis, testing would still be needed for tissue specific interfering substances specifically when there is a specific tissue with a specific interfering substance (i.e., melanoma); as well as marker specific testing, limit of detection, panel reproducibility, and accuracy.

In addition, regardless of tissue type, a representative analytical validation approach could be used where all biomarkers within the panel would be reported. As a result of the representative analytical validation, the need for additional updating of the software would be eliminated as all biomarkers would be unmasked. Software updates would only be needed to add clinical biomarker information/ therapeutic information. In this scenario, submissions would be for clinical information and require limited software information due to the addition of new clinical biomarkers. This approach would be less burdensome for the manufacturer and review timeframes would be faster than the currently available 180-day supplemental PMA pathway for such proposed device changes.

## REPRESENTATIVE VALIDATION

Representative/class-based analytical validation would lessen the burden with established minimum analytical performance characteristics for somatic mutation testing for Next Generation Sequencing (NGS)-based panels. Using a standardized, transparent, and optimized approach would potentially eliminate additional analytical validation requirements.

## CLINICAL SAMPLE AVAILABILITY

As described in the white paper, demonstrating analytical performance characteristics is required and it is necessary to have access to appropriate clinical samples and/or reference materials that can be used to demonstrate test performance and enable comparability between tests and laboratories. As samples with clinical outcomes from therapeutic trials (the "gold standard" of samples) are necessarily limited and not widely available, other sources and types of adequate samples or material standards need to be identified and developed as acceptable

for analytical performance characterization. Solutions to address access to samples that will appropriately assess analytical performance of a test to infer clinical performance of follow-on tests need to be explored. An established set of criteria for samples that contain a range of analytes and analyte types (e.g., SNVs, indels, CNAs, fusions, etc.) and a roadmap for how these materials should be utilized would likely incentivize their use and increase their availability by encouraging increased development and curation.

It is burdensome to the assay developer performing specific tissue/biomarker testing when a specific tissue type cannot be located due to rare variants or limited availability of tissue; in these instances, the use of a cell line or plasmids are needed, and in some instances, it may even be necessary to eliminate the test requirement. Requiring the manufacturer to develop a cell line or to pay to have a cell line developed is cost prohibitive and very lengthy. In most cases, the manufacturer will abandon the development process due to little or no return on investment.

## PRE-SPECIFIED MODIFICATION PLAN TO INCORPORATE ADDITIONAL BIOMARKERS INTO ONCOMINE DX TARGET TEST ASSAY

In order for the pre-specified modification plan to be successful there would need to be clear direction from the agency on requirements via a guidance document including information about needed studies.

In developing the pre-specified modification plan to incorporate additional biomarkers into the Oncomine Dx Target Test assay, tests to measure the following would be proposed:

- **Interfering substances**
- **Accuracy**
- **Clinical validation using samples from the intended use patient populations' tissue type to be added**
- **Small reproducibility study with enough samples, including those that can challenge the assay (e.g., samples near LoD, samples with low tumor content, etc.)**
- **Software validation**
- **Sample stability**

As part of the modification process, the following considerations need to be reviewed and resolved:

- **Same tissue type; is it the same intent to treat population as what is on the market already (NSCLC)? Is the biomarker already on panel (example ERBB2)?**
- **Is the biomarker already on panel with existing analytical data? Is it a new tissue type (example KRAS)?**

Table 4 - The proposed pre-specification plan would include the required tests to be performed with an appropriate justification

| Study Type | Description |
|---|---|
| Development | Integration Development Study and Test Pass/Fail Criteria Setting |
| Development | Detection of Variants Using In Vitro Transcripts |
| Analytical | Panel Reproducibility |
| Analytical | Analytical Accuracy |
| Analytical | Tumor Content |
| Analytical | Kit Lot Interchangeability |
| Clinical | ALK Clinical Study |
| Clinical | ROS1 Clinical Study |

## REFERENCES

[1] Use of Standards in FDA Regulatory Oversight of Next Generation Sequencing (NGS)-Based In Vitro Diagnostics (IVDs) Used for Diagnosing Germline Diseases (https://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM5098 38.pdf)

[2] Use of Public Human Genetic Variant Databases to Support Clinical Validity for Next Generation Sequencing (NGS)-Based In Vitro Diagnostics (https://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM5098 37.pdf)

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[5] Jennings et al. Guidelines for Validation of Next-Generation Sequencing–Based Oncology Panels: A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists. 2017. J Mol Diagn. 19(3); 341-365.

[6] Use of Standards in FDA Regulatory Oversight of Next Generation Sequencing (NGS)-Based In Vitro Diagnostics (IVDs) Used for Diagnosing Germline Diseases (https://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM509838.pdf)