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Analytical Validation of the Next-Generation Sequencing Assay for a Nationwide Signal-Finding Clinical Trial

Molecular Analysis for Therapy Choice Clinical Trial

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The National Cancer Institute—Molecular Analysis for Therapy Choice (NCI-MATCH) trial is a national signal-finding precision medicine study that relies on genomic assays to screen and enroll patients with relapsed or refractory cancer after standard treatments. We report the analytical validation processes for the next-generation sequencing (NGS) assay that was tailored for regulatory compliant use in the trial. The Oncomine Cancer Panel assay and the Personal Genome Machine were used in four networked laboratories accredited for the Clinical Laboratory Improvement Amendments. Using formalin-fixed paraffin-embedded clinical specimens and cell lines, we found that the assay achieved overall sensitivity of 96.98% for 265 known mutations and 99.99% specificity. High reproducibility in detecting all reportable variants was observed, with a 99.99% mean interoperator pairwise concordance across the four laboratories. The limit of detection for each variant type was 2.8% for single-nucleotide variants, 10.5% for insertion/deletions, 6.8% for large insertion/deletions (gap $\geq$4 bp), and four copies for gene amplification. The assay system from biopsy collection through reporting was tested and found to be fully fit for purpose. Our results indicate that the NCI-MATCH NGS assay met the criteria for the intended clinical use and that high reproducibility of a complex NGS assay is achievable across multiple clinical laboratories. Our validation approaches can serve as a template for development and validation of other NGS assays for precision medicine. *(J Mol Diagn 2017, 19: 313—327; http://dx.doi.org/10.1016/j.jmoldx.2016.10.007)*

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Precision medicine attempts to direct treatment for a patient based on molecular alterations known to exist in the patient's disease. The treatment of patients with cancer has been at the center of the evolution for precision medicine studies. Many recently developed treatments, including those approved by the US Food and Drug Administration (FDA), target specific genetic defects known to drive or significantly contribute to the cancer phenotype. Well-defined, reproducible, and robust molecular assays are therefore required that can efficiently assess tumor tissue to identify defects for which a treatment exists. Such assays play a pivotal role in the success of precision medicine.

The National Cancer Institute (NCI) initiated a large national precision medicine trial called the Molecular Analysis for Therapy Choice (referred to as NCI-MATCH) that is conducted through the National Clinical Trial Network and National Clinical Oncology Research Program and led by the Eastern Cooperative Oncology Group−American College of Radiology Imaging Network (ECOG-ACRIN) Cancer Research Group. The goal of this trial is to screen thousands of patients recruited from up to 2400 National Clinical Trial Network clinical sites who have relapsed or refractory solid tumors and lymphomas after standard systemic treatment for their cancer and then to assign the patients to a treatment appropriately matched to their cancer genotype. Details of the trial and protocol can be found at the NCI website (*http://cancer.gov/nci-match*, last accessed October 13, 2016).[1−3]

Targeted next-generation sequencing (NGS) panels can identify mutations in key genes with predictive value for approved or investigational cancer treatments.[4−7] Although NGS technology is a powerful tool, it is also new and not yet standardized among clinical laboratories. Different sample collection and processing methods, sequencing chemistries, instruments, protocols, and data analysis methods are known to affect NGS assay results.[8,9] In addition, regulatory compliance, such as the Code of Federal Regulations title 21 part 812 for investigational device exemption (Code of Federal Regulations, *http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRsearch.cfm?CFRPart=812*, last accessed October 13, 2016) for the FDA must be achieved for use of assays in clinical trials.

To provide a robust, standardized, and reproducible assay to support the NCI-MATCH trial and accommodate potentially large numbers of specimens, four clinical molecular diagnostics laboratories accredited through the Clinical Laboratory Improvement Amendments (CLIA) program formed a network to synergistically develop and validate the targeted NGS assay system with input from regulatory agencies. These four laboratories are located at Frederick National Laboratory for Cancer Research (FNLCR), Massachusetts General Hospital (MGH), the University of Texas MD Anderson Cancer Center (MDACC), and the Yale School of Medicine (YSM). We report on the methods used and the results obtained during the analytical performance testing and validation for analytical sensitivity, specificity, reproducibility, and limit of detection in each of the four laboratories and the overall combined data set and performance of the four laboratories. We believe our validation approach can serve as a template for development and validation of other clinical applications of NGS in support of precision medicine trials.

Materials and Methods

Tumor Specimens and Cell Lines

For evaluating analytical performance of this assay, archived formalin-fixed, paraffin-embedded (FFPE) clinical tumor specimens with various histopathologic diagnoses from the four network laboratories were chosen as samples of convenience to include a wide variety of known somatic variants encompassing all five variant types: single-nucleotide variants (SNVs), small insertions/deletions (indels), large indels (gap $\geq$4 bp), copy number variants (CNVs), and gene fusions. These variants were originally identified by orthogonal analytically validated assays (eg, digital PCR, Sanger sequencing, and fluorescent *in situ* hybridization [FISH]) in the CLIA-accredited laboratories. Tumor content for the specimens was assessed by board-certified pathologists.

Although every effort was made to include informative FFPE clinical specimens, a few FFPE cell line pellets were also included in this assay validation study because of the scarcity of specific variant types in available clinical specimens. Cell lines (Supplemental Table S1) were obtained from the Frederick National Laboratory for Cancer Research (Frederick, MD), American Type Culture Collection (Manassas, VA), and Coriell Institute for Medical Research (Camden, NJ) and were cultured using vendor-recommended conditions. Cultured cells were harvested and pelleted by centrifugation, fixed overnight in 10% neutral buffered formalin, and embedded in paraffin blocks.

Sections were cut from tumor and cell line FFPE blocks, and the relevant regions were collected for nucleic acid extraction. Numbers of specimens sequenced in each assay performance assessment in the validation study are summarized in Table 1, and the complete list of the specimens is provided in Supplemental Table S1.

Assay System and Content

The overall laboratory workflow and components in the NCI-MATCH assay system are depicted in Figure 1. Clinical biopsy samples were sent overnight to the central pathology laboratory of the MDACC for preanalytical histologic assessment followed by extraction of nucleic acids (DNA and RNA). Nucleic acid specimens from each patient's biopsy sample were shipped to one of the four clinical laboratories where the NCI-MATCH NGS assay was performed using locked standard operating procedures (SOPs) and validated personal genome machine (PGM) instruments. The work used a locked data analysis pipeline, Torrent Suite version 4.4.2 (Thermo Fisher Scientific,