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Analytical Validation and Application of a Targeted Next-Generation Sequencing Mutation-Detection Assay for Use in Treatment Assignment in the NCI-MPACT Trial

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Robust and analytically validated assays are essential for clinical studies. We outline an analytical validation study of a targeted next-generation sequencing mutation-detection assay used for patient selection in the National Cancer Institute Molecular Profiling–Based Assignment of Cancer Therapy (NCI-MPACT) trial (NCT01827384). Using DNA samples from normal or tumor cell lines and xenografts with known variants, we assessed the sensitivity, specificity, and reproducibility of the NCI-MPACT assay in five variant types: single-nucleotide variants (SNVs), SNVs at homopolymeric (HP) regions (≥3 identical bases), small insertions/deletions (indels), large indels (gap ≥4 bp), and indels at HP regions. The assay achieved sensitivities of 100% for 64 SNVs, nine SNVs at HP regions, and 11 large indels, 83.33% for six indels, and 93.33% for 15 indels at HP regions. Zero false positives (100% specificity) were found in 380 actionable mutation loci in 96 runs of haplotype map cells. Reproducibility analysis showed 96.3% to 100% intraoperator and 98.1% to 100% interoperator mean concordance in detected variants and 100% reproducibility in treatment selection. To date, 38 tumors have been screened, 34 passed preanalytical quality control, and 18 had actionable mutations for treatment assignment. The NCI-MPACT assay is well suited for its intended investigational use and can serve as a template for developing next-generation sequencing assays for other cancer clinical trial applications. *(J Mol Diagn 2016, 18: 51–67; http://dx.doi.org/10.1016/j.jmoldx.2015.07.006)*

Cancer is a genetic disease in which accumulating somatic mutations eventually lead to the deregulation of cell proliferation and survival-signaling pathways. In some tumors, there is crucial dependence of cancer cell growth on somatic driver mutations. These deleterious mutations provide an opportunity for developing cancer-specific targeted therapies.[1,2] The development of U.S. Food and Drug Administration (FDA)–approved targeted cancer therapeutics has demonstrated that drug efficacy is dependent on the accurate detection of the presence of the target in tumor tissue. Such therapies require analytically validated diagnostic assays for the selection of patients for treatments.[3–6] Existing companion diagnostic assays are mainly single-analyte assays that require a dedicated biopsy specimen. Because pharmaceutical agents may target driver mutations that occur only in a small subset of cancers, and because each tumor may have different driver mutations, it

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is clear that, as more targeted treatments become available, the use of multianalyte assays that test for multiple mutations will be required for screening patients for the best possible treatment. This approach will add value in making the most efficient use of patients' biopsy samples.

The emergence of next-generation sequencing (NGS) technologies has dramatically transformed cancer research by aiding in the rapid discovery of genetic aberrations in a large number of tumors.[7–10] NGS technologies continue to evolve and are becoming comprehensive platforms for the development of diagnostic assays and for use in screening tumor specimens for somatic mutations in the clinical setting. Recent advances in NGS permit larger amounts of the genome to be sequenced in a single run, with lower-input DNA, faster turnaround time, and reduced costs, resulting in rapid adoption by laboratories.[7–12]

The Division of Cancer Treatment and Diagnosis, National Cancer Institute (NCI), is conducting a randomized pilot trial, the Molecular Profiling-Based Assignment of Cancer Therapy (NCI-MPACT), to assess the utility of applying sequencing data to the selection of treatment in cancer patients whose disease has progressed despite the administration of standard treatment (*https://clinicaltrials.gov/ct2/home*; trial number NCT01827384).[13] Apart from a few notable examples, it has not yet been established whether making therapeutic choices based on molecular profiling provides superior clinical benefit compared with treatment selection based on best clinical judgment. In NCI-MPACT, patients with actionable mutations will be randomly assigned, in a 2:1 ratio, to receive either a predefined targeted treatment agent based on their corresponding mutation status, or therapy from the complementary set of drugs (from the same predefined set of agents) not prospectively identified to target one of their mutations. The objectives of this trial are to assess whether the response rate (primary objective) or 4-month progression-free survival rate (secondary objective) will be improved in the treatment arm in which treatment was chosen based on sequencing results compared with the arm in which treatment was not selected based on sequencing results (Supplemental Figure S1).

In this trial, core needle biopsy samples obtained from patients' tumors on trial entry will be assayed on the Ion Torrent PGM (Thermo Fisher Scientific, Waltham, MA) using a customized AmpliSeq panel that interrogates genetic variants in targeted genomic regions. The FDA has the authority to review assays used for assigning treatment or stratifying patients (termed *integral assays*) in clinical trials for the necessity of an Investigational Device Exemption (Treatment Use of an Investigational Device, *http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=812.36*, last accessed July 2, 2015).[14,15] To do so, information on the validation of the NCI-MPACT assay, as well as the risk posed to the patient, were discussed with the FDA in presubmission meetings. We believe that this article will be of value to others and could serve as a template for preparing an NGS assay for clinical use.

Recent public discussions led by the FDA indicated a desire for all clinical laboratories using laboratory-developed NGS tests to follow some form of design control to ensure that tests are sufficiently robust for their intended clinical use. The intended use of the NCI-MPACT assay is to screen patients' tumor biopsy samples for actionable mutations of interest (aMOI), which would then be used for assigning a study treatment in the NCI-MPACT clinical study. To assign treatment arms in a timely manner, the NCI-MPACT assay workflow was streamlined to ensure a 7- to 10-day turnaround in reporting results (Figure 1). Patients eligible for this trial have histologically confirmed solid tumors that have progressed after at least one line of standard therapy, or for which there is no standard therapy that prolongs survival. This intended use of the assay that directed the desired criteria for the assay performance was agreed on by the study physicians (B.A.C. and A.P.C.) and the sequencing laboratory. In addition, it was agreed that the assessment of the NCI-MPACT assay result would require a high specificity threshold to prevent a patient from entering the study through a false-positive (FP) result.

Before the validation study, an assay-feasibility study was conducted to assess assay performance, to identify potential FP loci, to empirically determine assay quality-control (QC) metrics, and to establish and lock standard operating procedures. An initial feasibility study was performed by sequencing well-characterized specimens so that a preliminary assessment of assay sensitivity and specificity could be determined in combination with setting and examining different thresholds of assay quality metrics. An analytical validation plan was then developed that included the experimental design and expected performance criteria. A total of 191 NCI-MPACT assay runs were performed in this analytical validation study. The results of sensitivity, specificity, accuracy, and reproducibility assessments suggested that the NCI-MPACT assay was well-suited for the intended investigational use of the NCI-MPACT trial. This is the first report that details the analytical validation process of an NGS-based assay for use as an integral assay in a clinical study.

Materials and Methods

Cell Lines and Tumor Specimens

Cell lines (Supplemental Table S1) obtained from Frederick National Laboratory for Cancer Research (Frederick, MD), American Type Culture Collection (Manassas, VA), and Coriell Institute for Medical Research (Camden, NJ) were cultured using vendor-recommended conditions. A minimum of two core needle biopsy specimens (18 gauge) were obtained from each patient with cancer metastasis at the NIH Clinical Center after the completion of an informed-consent form using an Institution Review Board–approved protocol. Biopsy specimens were shipped in neutral buffered formalin and embedded in paraffin within