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Clinical Genomic Profiling of a Diverse Array of Oncology Specimens at a Large Academic Cancer Center

Identification of Targetable Variants and Experience with Reimbursement

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Large cancer panels are being increasingly used in the practice of precision medicine to generate genomic profiles of tumors with the goal of identifying targetable variants and guiding eligibility for clinical trials. To facilitate identification of mutations in a broad range of solid and hematological malignancies, a 467-gene oncology panel (Columbia Combined Cancer Panel) was developed in collaboration with pathologists and oncologists and is currently available and in use for clinical diagnostics. Herein, we share our experience with this testing in an academic medical center. Of 255 submitted specimens, which encompassed a diverse range of tumor types, we were able to successfully sequence 92%. The Columbia Combined Cancer Panel assay led to the detection of a targetable variant in 48.7% of cases. However, although we show good clinical performance and diagnostic yield, third-party reimbursement has been poor. Reimbursement from government and third-party payers using the 81455 Current Procedural Terminology code was at 19.4% of billed costs, and 55% of cases were rejected on first submission. Likely contributing factors to this low level of reimbursement are the delays in valuation of the 81455 Current Procedural Terminology code and in establishing national or local coverage determinations. In the absence of additional demonstrations of clinical utility and improved patient outcomes, we expect the reimbursement environment will continue to limit the availability of this testing more broadly. *(J Mol Diagn 2016, ■: 1–11; http://dx.doi.org/10.1016/j.jmoldx.2016.10.008)*

Q4 The availability and accessibility of next-generation sequencing technologies, combined with the identification of increasing numbers of driver mutations from large-scale cancer sequencing projects, has led to evolving needs in the practice of oncology and precision medicine. Laboratory diagnostic tests that can identify actionable or targetable variants are routinely being incorporated into clinical practice and are moving beyond small panels that can identify well-established targets toward larger cancer panels that can guide eligibility for current and future clinical trials.[1–4]

Implementing a large cancer panel in a Clinical Laboratory Improvement Amendment–certified and College of American Pathologists–accredited clinical laboratory is challenging in many aspects. Test design and clinical validation, development of clinical expertise, and acquisition of the genomics and bioinformatics resources and infrastructure required are just some of the challenges and hurdles that clinical laboratories face.[5–8] Equally important, but less well described, is the challenge of providing a highly demanded test that provides clinically useful information in

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the current uncertain coding, reimbursement, and revenue cycle duration environment.[9,10]

Multigene panels performed on tumor tissue (solid or hematolymphoid) covering >50 genes inclusive of copy number and translocations are coded using a single Current Procedural Terminology (CPT) code (81455). This CPT code was adopted starting January 1, 2015, by the American Medical Association under Genomic Sequencing Procedures.[11] Before this, laboratories performing this testing, including our own, relied on listing codes for specific genes. Adoption of the 81455 code introduced uncertainty with regard to reimbursement because, unlike the prior gene-specific codes, the 81455 code had not yet been valued on the Clinical Laboratory Fee Schedule (CLFS) nor had any coverage determinations been rendered. Therefore, it was it was unclear what, if anything, Medicare and, as a result, commercial payers, would reimburse for this code. Past experience with introduction of new molecular CPT codes (ie, the introduction of tier 1 and 2 codes in 2012) suggested that Medicare administrative contractors and other payers could be inconsistent with payment decisions, leading to inadequate reimbursement and extended time to reimbursement.[12]

Given these challenges and uncertainties, many clinical laboratories have been cautious to develop and offer large cancer genomics tests. We report herein our experience with the Columbia Combined Cancer Panel (CCCP), in the hopes that our experience can provide some guidance to other clinical laboratories. The CCCP test is a 467-gene cancer panel, developed in collaboration with institutional oncologists and pathologists to interrogate cancer genes implicated in a broad range of solid and hematological tumors. This test was designed, developed, and validated in our Clinical Laboratory Improvement Amendment–certified and College of American Pathologists–accredited laboratory and is approved by the New York State Department of Health. We report both on clinical performance and diagnostic yield of this assay, as well as our experience with reimbursement with the 81455 CPT code. We find, despite the ability to detect targetable variants in a large proportion of clinical cases, reimbursements are lower than those for more traditional molecular assays, threatening access to cancer genomic profiling for some patients.

Materials and Methods

Tumor Specimens and DNA Extraction

This study was approved by our institutional review board. All consecutive cases from July 2014 through December 2015 were included in this study. Tumor samples included formalin-fixed, paraffin-embedded tissue, cell blocks from fine-needle aspirates, peripheral blood, and bone marrow aspirates. Hematoxylin and eosin–stained sections or flow cytometry reports were examined by a pathologist and assessed for tumor cell content. For formalin-fixed, paraffin-embedded tissue, manual macrodissection and microdissection was performed on unstained sections to enrich for tumor cells (minimum 30% to 40% tumor cells). Genomic DNA was extracted from paraffin tissue using a QIAcube (Qiagen, Hilden, Germany), and from peripheral blood or bone marrow using a QIASymphony (Qiagen) instrument. DNA quantification was performed using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA).

Sequencing, Variant Calling, and Interpretation

Genomic DNA (50 to 200 ng) was sheared using a Covaris S2 Sonication system (Covaris, Woburn, MA), and targeted sequences from 467 genes were captured using custom Agilent (Santa Clara, CA) SureSelect capture reagents. Sequencing was performed on the Illumina (San Diego, CA) HiSeq2500 as 2 × 100-bp paired-end reads. Analysis of resulting sequences was performed using NextGENe software (Softgenetics, State College, PA). The FASTQ files were demultiplexed and filtered on the basis of their quality metrics and converted into FASTA files. Samples with at least 6 Gb of data were used for mapping and variant calling. A minimum average coverage of at least 500-fold, as well as at least 50-fold coverage of >98.0% of coding sequences in the region of interest, was obtained on all samples. The reads were aligned to human genome reference sequence GRCh37, and variants were identified. For all variants, variants were called if the mutant allele was present at a minimum of 10% variant allelic fraction, and seen in a minimum of three variant reads. Single-nucleotide variants, and small insertions and deletions, were annotated by an in-house developed pipeline and were evaluated by a molecular pathologist. Variants were filtered by several criteria, including whether the variant was a known disease-associated mutation listed in the Catalogue of Somatic Mutations in Cancer database,[13] by the effect on protein (synonymous, nonsynonymous, nonsense, canonical splice site, or frameshift variants), and by presence in the 1000 Genomes,[14] Exome Variant Server (National Heart, Lung, and Blood Institute Gene Ontology Exome Sequencing Project, Seattle, WA, *http://evs.gs.washington.edu/EVS*, last accessed), or internal databases. Prediction of functional effects of missense substitutions was performed using the *in silico* algorithm Provean.[15] Variants (minimum variant allelic fraction of 10%) were reported using the following tiered system (developed in consultation with institutional oncologists and pathologists): tier 1, known actionable mutations in the patient's tumor type; tier 2, known actionable variants in other tumor types and mutations in well-established cancer genes; tier 3, other pathogenic mutations (ie, mutations that are predicted to be loss of function, such as nonsense mutations, canonical splice site mutations, or frameshift mutations, in genes on the panel in which the role in cancer is not well established); and tier 4, variants of uncertain significance. Variants that were considered to be benign or likely benign were not reported. Actionable mutations were

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