The Journal of Molecular Diagnostics, Vol. 19, No. 1, January 2017

ELSEVIER

the Journal of Molecular Diagnostics

jmd.amjpathol.org

SPECIAL ARTICLE

Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer

A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists

Marilyn M. Li,[*†] Michael Datto,[*‡] Eric J. Duncavage,[*§] Shashikant Kulkarni,[*¶] Neal I. Lindeman,[*‖] Somak Roy,[*,**] Apostolia M. Tsimberidou,[*††] Cindy L. Vnencak-Jones,[*‡‡] Daynna J. Wolff,[*§§] Anas Younes,[*¶¶] and Marina N. Nikiforova[*,**]

From the Interpretation of Sequence Variants in Somatic Conditions Working Group of the Clinical Practice Committee, Association for Molecular Pathology, Bethesda, Maryland; the Department of Pathology and Laboratory Medicine,† Division of Genomic Diagnostics, the Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania; the Duke University School of Medicine,‡ Durham, North Carolina; the Department of Pathology and Immunology,§ Washington University School of Medicine, St. Louis, Missouri; Baylor Genetics,¶ Houston, Texas; the Brigham and Women's Hospital,‖ Harvard Medical School, Boston, Massachusetts; the University of Pittsburgh Medical Center,** Pittsburgh, Pennsylvania; the Department of Investigational Cancer Therapeutics,†† University of Texas MD Anderson Cancer Center, Houston, Texas; the Department of Pathology, Microbiology and Immunology,‡‡ Vanderbilt University Medical Center, Nashville, Tennessee; the Department of Pathology and Laboratory Medicine,§§ Medical University of South Carolina, Charleston, South Carolina; and the Memorial Sloan Kettering Cancer Center,¶¶ New York, New York*

Accepted for publication October 13, 2016.

Address correspondence to Marilyn M. Li, M.D., Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, University of Pennsylvania Perelman School of Medicine, 3615 Civic Center Blvd, ARC 716i, Philadelphia, PA 19104. E-mail: lim5@email.chop.edu.

Widespread clinical laboratory implementation of next-generation sequencing–based cancer testing has highlighted the importance and potential benefits of standardizing the interpretation and reporting of molecular results among laboratories. A multidisciplinary working group tasked to assess the current status of next-generation sequencing–based cancer testing and establish standardized consensus classification, annotation, interpretation, and reporting conventions for somatic sequence variants was convened by the Association for Molecular Pathology with liaison representation from the American College of Medical Genetics and Genomics, American Society of Clinical Oncology, and College of American Pathologists. On the basis of the results of professional surveys, literature review, and the Working Group's subject matter expert consensus, a four-tiered system to categorize somatic sequence variations based on their clinical significances is proposed: tier I, variants with strong clinical significance; tier II, variants with potential clinical significance; tier III, variants of unknown clinical significance; and tier IV, variants deemed benign or likely benign. Cancer genomics is a rapidly evolving field; therefore, the clinical significance of any variant in therapy, diagnosis, or prognosis should be

Supported by the Association for Molecular Pathology.

Disclosures: E.J.D. is the Medical Director for Cofactor Genomics and claims ownership in P&V Licensing, LLC; A.Y. is a consultant for Foundation Medicine; A.M.T. received research funding from Foundation Medicine, EMD Serono, Baxalta, Bayer, and Onyx.

The Interpretation of Sequence Variants in Somatic Conditions Working Group is a working group of the Association for Molecular Pathology Clinical Practice Committee with liaison representation from the American College of Medical Genetics and Genomics (S.K. and D.J.W.), American Society of Clinical Oncology (A.M.T. and A.Y), and College of American Pathologists (M.D. and N.I.L.). The 2015 and 2016 Clinical Practice Committee consisted of Marina Nikiforova (Chair 2015 to 2016), Monica Basehore, Christopher Coldren, Linda Cook, Jennifer Crow, Birgit Funke, Meera Hameed, Larry Jennings, Arivarasan Karunamurthy, Annette Kim, Bryan Krock, Mary Lowery-Nordberg, Melissa Miller, Benjamin Pinsky, Somak Roy, Mark Routbort, Ryan Schmidt, and David Viswanatha.

Standard of practice is not defined by this article and there may be alternatives. See *Disclaimer* for further details.

http://dx.doi.org/10.1016/j.jmoldx.2016.10.002

reevaluated on an ongoing basis. Reporting of genomic variants should follow standard nomenclature, with testing method and limitations clearly described. Clinical recommendations should be concise and correlate with histological and clinical findings. *(J Mol Diagn 2017, 19: 4–23; http://dx.doi.org/10.1016/j.jmoldx.2016.10.002)*

The sequencing of human DNA for the human genome project has led to the emergence of technologies that identify genomic, transcriptional, proteomic, and epigenetic alterations in patients' tumors. Precision medicine uses concepts of the genetic and environmental basis of disease to individualize prevention, diagnosis, and treatment and integrates tumor molecular data into decision making in medical practice.[1–4] Genomic information–based disease prognosis and the selective use of targeted therapy to target specific genotypic and biological biomarkers, combined with other therapeutic strategies based on the tumor biology of the individual patient, hold the promise of improving clinical outcomes and patient care.

In recent years, assays for single-target detection have been replaced by next-generation sequencing (NGS) or massively parallel sequencing. This technology allows for the simultaneous evaluation of many genes and the generation of millions of short nucleic acid sequences in parallel.[5,6] The NGS high-throughput platform is more efficient and less expensive and provides information that is not provided by single gene-by-gene Sanger DNA sequencing analysis or by gene-specific targeted hot spot mutation assays.[7] The vast number of variants identified by NGS in tumor tissue is attributed to the complexity of carcinogenesis, including the multistep process of genetic mutations and tumor heterogeneity (ie, multiple clones of cells with related but distinct molecular signatures within tumors).[8,9] Herein, tumor refers to tissue deriving from either a benign or malignant neoplasm. NGS results obtained using DNA or RNA extracted from tumor tissue frequently demonstrate a complex molecular signature that is different from that of normal tissue for any given patient. The significance of the change relative to tumorigenesis depends on the type of genetic aberration, the location of the variant, and the normal function of the protein. Genetic variants can be germline or somatic. A germline variant is defined as a genetic alteration that occurs within the germ cells (egg or sperm), such that the alteration can be passed to subsequent generations. A somatic variant is defined as a genetic alteration that occurs in any of the cells of the body, except the germ cells, and therefore is not passed on to subsequent generations. Genetic variations may be activating, resulting in a gain of function of the protein, such as a missense mutation in the functional or kinase domain of the protein, allowing for autophosphorylation of the protein, the loss of regulation for downstream signaling, and uncontrolled cell growth and proliferation. Conversely, the genetic alteration may be inactivating, such as nonsense, splice-site, and frameshift insertion/deletion mutations, thereby causing a loss of function of a tumor-suppressor gene. The types of variants observed include single-nucleotide variants (SNVs) that cause a missense, silent, or nonsense amino acid substitution, or a splice site alteration affecting normal splicing of the mRNA transcript. Alternatively, one or more nucleotides may be involved in duplications, deletions, insertions, or even a more complex pattern with a nucleotide(s) deletion coupled with a nucleotide(s) insertion (indels) at a particular location. Also common in the pathogenesis of cancer is a change in copy number of cancer-related genes. Generically identified as copy number variants (CNVs), these include copy number alterations of various types. Examples of CNVs include the common loss (deletion) of the tumor-suppressor *RB1* gene in retinoblastoma or the gain (amplification) of the oncogene *ERBB2* in invasive breast carcinoma. In addition, structural rearrangements, including chromosome translocations, deletions, duplication, or inversions, are frequently identified in tumor DNA and result in gene fusions and associated fusion proteins with unique cancer-promoting properties, such as the *EML4-ALK* recurrent inversion mutation that is seen in non-small cell lung cancer.

Molecular profiles obtained on tumor DNA and RNA can guide the clinical management of cancer patients. This information can provide diagnostic or prognostic information, identify a potential treatment regimen or targeted therapy, and determine eligibility for the following: i) a Food and Drug Administration (FDA)–approved medication for that tumor type, ii) a medication available as off-label treatment for the specific molecular alteration in a nonapproved tumor type, or iii) a targeted therapy available in clinical trials with investigational agents based on an identified molecular alteration. In the United States, the Clinical Laboratory Improvement Amendments of 1988 provide regulatory oversight to laboratories performing tumor genomics characterization (US Government Publishing Office, Electronic Code of Federal Regulations, Title 42, §Part 493.1, *http://www.ecfr.gov/cgi-bin/text-idx?SID=1248e3189da5e5f936e55315402bc38b&node=pt42.5.493&rgn=div5#se42.5.493_11*, last accessed July 6, 2016). Clinical Laboratory Improvement Amendment certification of laboratories, ongoing quality assurance/improvement, and appropriate proficiency testing are required to ensure accurate and reproducible molecular profiling.

Implementaion of NGS identifies large numbers of genetic variations in tumor DNA, which are crucial for optimal patient care, and treatment guidelines are developed based on specific molecular findings; therefore, it is imperative to unify the interpretation and reporting of molecular results among laboratiores performing these tests.[10–13] In the spring of 2015, a clinical laboratory–focused working group was formed to establish recommendations for the interpretation and reporting of sequence variants identified