


A Clinical Approach to Detecting Germline Pathogenic Variants From Tumor-Only Sequencing

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In this issue of *JNCI Cancer Spectrum*, Klek and colleagues (1) describe a single institution's approach to identifying patients who carry a genetic cancer predisposition from tumor-only sequencing. They demonstrate that careful review of tumor-sequencing data substantially increased the percentage of cancer patients in their cohort diagnosed with a hereditary cancer susceptibility.

Tumor-only profiling by next-generation sequencing has been widely implemented in oncology practices to help guide therapeutic decisions, clarify diagnosis, and aid in prognostication. This approach has been adopted by both academic centers and commercial companies alike (2–5). Although tumor-only sequencing provides a cost-effective approach to identifying somatic variants present in the tumor, it will also detect any germline variants present in all cells of the body. Importantly, it can be difficult to distinguish somatic and germline alterations from tumor-sequencing data without a normal matched control (eg, blood sample) (6–8).

Klek et al. demonstrate that involvement of clinical genetics in a tumor-only sequencing data review process can improve the identification of patients and families with germline pathogenic variants in hereditary cancer predisposition genes. After implementing a formal tumor-only sequencing data review and genetic counseling referral process, the percentage of patients undergoing tumor-only sequencing with a detected pathogenic germline variant increased from 1.4% to 7.5%. Their findings are in agreement with recent studies demonstrating that the inclusion of germline genetics expertise in tumor-sequencing analysis improves the identification of germline cancer risk in various cancer-patient populations (6,9). Notably, some of these individuals would not have met standard criteria for germline testing, and the only indication for genetic testing was a variant identified on tumor-only sequencing.

Estimating the possibility of a variant having germline vs somatic origin can be complex and requires an expert review of multiple factors, including variant attributes in sequencing data and the prior probability for the individual to have a germline pathogenic variant in a given gene. A few automated approaches to tumor-sequence analysis have been developed to

predict which variants are likely to be germline in origin, and guidelines have been proposed for implementing such variant filters in laboratory practices (10). However, although these methods can detect a substantial proportion of germline pathogenic variants from tumor-sequencing data, any automated method will have some limitations because of the nature of the tumor genome. For example, variants with allele fractions close to 50% are assumed likely to be germline heterozygous variants, but this can be complicated by tumor purity and changes in tumor allele fractions because of somatic deletion and amplification events. Moreover, from a clinical perspective, assessing the prior probability of a patient having a hereditary cancer predisposition syndrome can also be difficult. Although genes such as *NF1* or *TSC1* are believed to have high penetrance, many patients may have milder presentations and may not be diagnosed until examined specifically for associated features. Several studies have shown that this is even more complex for diseases caused by lower penetrance genes, such as hereditary breast and ovarian cancer and Lynch syndrome. Guideline-directed genetic testing misses a substantial proportion of patients with pathogenic germline variants for these disorders (11–14). Having genetic counseling involvement in the review of tumor-sequencing results helps in multiple steps of the process to accurately identify and classify germline pathogenic variants, as well as clarify their implications.

Klek et al. show that the review of tumor-sequencing data by a molecular tumor board increases the yield for detecting pathogenic germline variants and that this methodology can contribute to detecting hereditary cancer susceptibilities in individuals who otherwise may not have had genetic testing. However, as the authors suggest, most tumor-only sequencing panels do not provide complete coverage of all target genes and are limited in their ability to detect certain variant types such as exon-level copy number variants and variants in high homology regions. Therefore, it should be recognized that tumor sequencing is not a substitute for clinical genetic testing where the gene panels are designed and validated specifically for germline variant detection, and all variants are scrutinized and interpreted according to American College of Medical Genetics and

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Genomics criteria (15). Moreover, although the criteria developed by Klek et al. to help identify variants of potential germline origin are well-thought-out heuristic methods, the only unambiguous method to immediately distinguish between germline and somatic origin is matched tumor-normal sequencing (12,16,17). However, the cost and challenges of coordinating a paired analysis may be limitations to its implementation in many institutions.

In this study, Klek et al. (1) clearly demonstrate that tumor sequencing can provide an opportunity to detect germline pathogenic variants if a proper system of manual review or automated flagging of variants on tumor-sequencing reports is implemented. Additionally, any institution that implements a workflow for identifying variants from tumor-sequencing reports with a high index of suspicion for germline origin must also have genetics professionals available to interpret these results for patients and provide expert genetic counseling for patients and their families. Such a comprehensive review process of tumor-sequencing data can help identify cancer patients who harbor a previously undiagnosed hereditary cancer predisposition.

Notes

The authors declare no conflicts of interest.

References

- Klek S, Heald B, Milinovich A, et al. Genetic Counseling and Germline Testing in the Era of Tumor Sequencing: A Cohort Study. *JNCS J Natl Cancer Inst.* 2020;112(2). doi: 10.1093/jncics/pkaa018
- Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. *J Mol Diagn.* 2015;17(3):251–264.
- Pritchard CC, Salipante SJ, Koehler K, et al. Validation and implementation of targeted capture and sequencing for the detection of actionable mutation, copy number variation, and gene rearrangement in clinical cancer specimens. *J Mol Diagn.* 2014;16(1):56–67.
- Wagle N, Berger MF, Davis MJ, et al. High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. *Cancer Discov.* 2012;2(1):82–93.
- Frampton GM, Fichtenholtz A, Otto GA, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. *Nat Biotechnol.* 2013;31(11):1023–1031.
- Garofalo A, Sholl L, Reardon B, et al. The impact of tumor profiling approaches and genomic data strategies for cancer precision medicine. *Genome Med.* 2016;8(1):79.
- Hiltemann S, Jenster G, Trapman J, van der Spek P, Stubbs A. Discriminating somatic and germline mutations in tumor DNA samples without matching normals. *Genome Res.* 2015;25(9):1382–1390.
- Sukhai MA, Misyura M, Thomas M, et al. Somatic tumor variant filtration strategies to optimize tumor-only molecular profiling using targeted next-generation sequencing panels. *J Mol Diagn.* 2019;21(2):261–273.
- Clark DF, Maxwell KN, Powers J, et al. Identification and confirmation of potentially actionable germline mutations in tumor-only genomic sequencing. *J Clin Oncol Precis Oncol.* 2019;3:1–11.
- Mandelker D, Donoghue M, Talukdar S, et al. Germline-focussed analysis of tumour-only sequencing: recommendations from the ESMO Precision Medicine Working Group. *Ann Oncol.* 2019;30(8):1221–1231.
- Meric-Bernstam F, Brusco L, Daniels M, et al. Incidental germline variants in 1000 advanced cancers on a prospective somatic genomic profiling protocol. *Ann Oncol.* 2016;27(5):795–800.
- Mandelker D, Zhang L, Kemal Y, et al. Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs guideline-based germline testing. *JAMA.* 2017;318(9):825–835.
- Whitworth J, Smith PS, Martin JE, et al. Comprehensive cancer-predisposition gene testing in an adult multiple primary tumor series shows a broad range of deleterious variants and atypical tumor phenotypes. *Am J Hum Genet.* 2018;103(1):3–18.
- Beitsch PD, Whitworth PW, Hughes K, et al. Underdiagnosis of hereditary breast cancer: are genetic testing guidelines a tool or an obstacle? *J Clin Oncol.* 2019;37(6):453–460.
- Richards S, Aziz N, Bale S, et al. on behalf of the ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405–424.
- Seifert BA, O'Daniel JM, Amin K, et al. Germline analysis from tumor-germline sequencing dyads to identify clinically actionable secondary findings. *Clin Cancer Res.* 2016;22(16):4087–4094.
- Schrader KA, Cheng DT, Joseph V, et al. Germline variants in targeted tumor sequencing using matched normal DNA. *JAMA Oncol.* 2016;2(1):104–111.