Molecular Pathology Abstracts

Editors: Donna E. Hansel, MD, PhD, chief, Division of Anatomic Pathology, and professor, Department of Pathology, University of California, San Diego; James Solomon, MD, PhD, resident, Department of Pathology, UCSD; Charmi Patel, MD, molecular genetic pathology fellow, Department of Pathology, UCSD; and Sounak Gupta, MBBS, PhD, molecular pathology fellow, Memorial Sloan Kettering Cancer Center, New York.

Prevalence of clonal hematopoiesis mutations in tumor-only clinical genomic profiling of solid tumors

August 2018—Challenges to implementing next-generation sequencing-based comprehensive molecular profiling of solid tumors include reliably separating germline variants from somatic variants. This is an important consideration, particularly when a "tumor-only" profiling approach is used. Bioinformatic strategies in this setting involve filtering out germline variants based on annotated population frequencies in public datasets, such as 1000 Genomes, ExAC, and gnomAD, while reporting mutations occurring at cancer-associated hotspots. This study involves next-generation sequencing (NGS)-based profiling of solid tumors from more than 17,000 patients using a paired normal (blood) specimen to filter out germline variants. The results provide valuable insight into the potential pitfalls of molecular profiling using a tumor-only approach. This is highlighted by the documentation of clonal hematopoiesis events in the authors' institutional clinical sequencing cohort. Clonal hematopoiesis refers to clonal expansion of hematopoietic progenitor cells that have shared molecular alterations; these events are not uncommon in patients with solid tumors. Therefore, the presence of these alterations within tumor-infiltrating lymphocytes might lead to misattributing clonal hematopoiesis alterations as somatic variants in the solid tumor. These misattributions can have significant clinical consequences, particularly for actionable alterations that can be seen in solid tumors and hematologic malignancies. Specific examples documented by the authors include *IDH2* p.R140Q alterations, as well as a *KRAS* p.G12R alteration in a patient with colorectal carcinoma, both of which would alter targeted therapy strategies. Conversely, the authors highlighted scenarios in which the use of a matched normal (blood) sample could lead to misattributing a variant as a germline alteration. For instance, a *BRCA2* truncating alteration was detected in a solid tumor and a blood specimen of a patient with melanoma, suggesting a germline alteration. In this case, the sequencing of multiple normal specimens (saliva, buccal swab, non-neoplastic colon tissue) helped correctly attribute this alteration to clonal hematopoiesis. In summary, the authors noted that in the absence of matched normal (blood) sequencing, more than five percent of patients within their clinical sequencing cohort would have had at least one clonal hematopoiesis-related alteration erroneously attributed to the solid tumor. Furthermore, more than 95 percent of the clonal hematopoiesis variants likely would not be filtered out bioinformatically using a tumor-only profiling strategy as they were not present in common public databases.

Ptashkin RN, Mandelker DL, Coombs CC, et al. Prevalence of clonal hematopoiesis mutations in tumor-only clinical genomic profiling of solid tumors [published online June 5, 2018]. *JAMA Oncol.* doi:10.1001/jamaoncol.2018.2297.

Correspondence: Dr. Ahmet Zehir at zehira@mskcc.org

Use of urinary cell-free DNA for monitoring infections of the urinary tract

While there is an abundance of literature about the use of plasma circulating cell-free DNA in diagnostic assays, data on the use of urinary cell-free DNA (cfDNA) are limited. The authors conducted a study in which they tested the utility of urinary cfDNA to monitor host and pathogen interactions in bacterial and viral urinary tract infections. Urinary cfDNA is primarily composed of DNA released from host cells and microbes in the urinary tract and has a lesser contribution from plasma cfDNA that is filtered into urine. The authors extracted cfDNA from as little as 1 mL of urine and processed it using a single-stranded library preparation protocol followed by next-generation sequencing. Urine samples for cfDNA analysis collected from 82 kidney transplant recipients were assayed for

varying metrics that included the use of host and microbe cfDNA. This was a particularly relevant cohort as more than 15,000 patients receive renal transplants in the United States annually, and bacterial and viral infections are a significant cause of morbidity and mortality in this cohort. The authors profiled the urinary microbiome in patients without urinary tract infection (UTI) and showed a gender-specific signature that corresponded to transplant recipient gender. For instance, female recipients had significantly higher cfDNA from *Gardnerella, Ureaplasma*, and *Lactobacillus* compared with male recipients. Using relative genome equivalents (RGE), by normalizing microbial genome copies to human genome copies, the authors identified causative organisms for UTI using conventional culture and identification methods, with a high degree of sensitivity and specificity. This assay was also used to establish the nature of polymicrobial bacterial infections as well as to identify causative organisms in culture-negative cases. This technique was equally effective for viral identification, such as detecting BK polyomavirus (BKV), as well as detecting parvovirus B19 in the preclinical phase. Furthermore, estimation of bacterial replication index, based on sequencing coverage signatures and antimicrobial resistance profiles, could be further exploited to guide clinical decision-making. Assessment of host cfDNA revealed elevated donor-derived cfDNA, particularly in the setting of BKV nephropathy, while this metric was not informative in the setting of bacterial UTI, suggesting that donor-specific urinary cfDNA correlates with graft tissue injury in the setting of BKV nephropathy. In summary, this study shows that urinary cfDNA is a highly versatile analyte and may have immediate application in monitoring bacterial and viral infections and in allograft health in patients with renal transplants.

Burnham P, Dadhania D, Heyang M, et al. Urinary cell-free DNA is a versatile analyte for monitoring infections of the urinary tract. *Nat Commun.* 2018;9:2412. doi:10.1038/541467-018-04745-0.

Correspondence: Dr. John Richard Lee at jrl2002@med.cornell.edu