Molecular pathology selected abstracts

Editors: Donna E. Hansel, MD, PhD, chair of pathology, Oregon Health and Science University, Portland; Richard D. Press, MD, PhD, professor and director of molecular pathology, OHSU; James Solomon, MD, PhD, assistant professor, Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, New York; Erica Reinig, MD, assistant professor and medical director of molecular diagnostics, University of Wisconsin-Madison; Marcela Riveros Angel, MD, molecular genetic pathology fellow, Department of Pathology, OHSU; and Andrés G. Madrigal, MD, PhD, assistant professor, clinical, Ohio State University Wexner Medical Center, Columbus.

Creation of a methylation model index to predict ovarian cancer risk

May 2022—The most common cause of death related to gynecological malignancies is epithelial ovarian cancer. One of the biggest challenges to treating this disease is the lack of reliable biomarkers for identifying its underlying precancerous and early stages. The study of epigenetic changes in epithelial cells shows some promise for detecting early ovarian cancer. In previous studies, DNA methylation performed on blood samples demonstrated important epigenetic changes associated with ovarian cancer but did not yield realistic screening parameters due to the heterogeneity of blood samples. To identify ovarian cancer risk earlier, the authors conducted a molecular epigenetic analysis of cervical epithelial cells derived from the Mullerian duct and collected using the ThinPrep system to establish a methylation model index called the Women's Risk Identification for Ovarian Cancer [WID-OC] index. To design this index, they used a discovery set of samples from 242 women with ovarian cancer (before histologic diagnosis) and 869 without cancer and validation sets tested with external sets of samples from women with endometrial cancer and healthy women with cancer-predisposing *BRCA1* mutations. To obtain the discovery set and design the WID-OC index, the authors performed an epigenomic-wide DNA methylation analysis to determine the differential methylation of CpG loci between cancer cases and controls. Using biostatistical analysis and an internal validation set, they defined the WID-OC index as a function of the number of methylated CpG sites and achieved an optimal index performance with an area under the curve (AUC) of 0.78. The WID-OC index identified 71.4 percent of ovarian cancer patients younger than age 50 and 54.5 percent of ovarian cancer patients over age 50 with a specificity of 75 percent. The authors also calculated the index in the cells from the fimbria of the fallopian tubes, which are the cells of origin of most epithelial ovarian tumors. These results support the hypothesis that epigenetic changes in cervical cells from ovarian cancer patients are similar to those in the ovarian cancer cells of origin. The WID-OC index was not associated with family history, age at menarche, oral contraceptive use, or ethnicity for control subjects. The ability of the WID-OC index to discriminate between healthy controls and women with ovarian, endometrial, or breast cancer was highly consistent across study centers. The WID-OC index may be useful as a noninvasive screening method for the early identification of women who may have ovarian cancer or are at risk of developing the disease and who may benefit from early diagnostic interventions.

Barrett JE, Jones A, Evans I, et al. The DNA methylome of cervical cells can predict the presence of ovarian cancer. *Nat Commun*. 2022;13:448. https://doi.org/10.1038/s41467-021-26615-y

Correspondence: Dr. Martin Widschwendter at martin.widschwendter@uibk.ac.at

Use of cell-free DNA analysis to detect complications after HCT

Numerous neoplastic and immunologic conditions are treated with hematopoietic cell transplantation. Common complications of this life-saving procedure include graft-versus-host disease (GVHD), infections due to immunosuppression, graft failure, and primary disease relapse. A combination of laboratory methods, including routine pathology or microbiology, flow cytometry, and PCR, are required to diagnose these complications, but there is no comprehensive assay to detect them with a high degree of sensitivity. The authors conducted a proof-of-principle study in which they developed a molecular assay using cell-free DNA (cfDNA) from blood to monitor and predict the common complications of hematopoietic cell transplantation (HCT). They collected 170 blood samples from 27 HCT patients at different time points—before disease therapy or transplant, after transplant

engraftment, and during post-transplant monitoring. The authors analyzed cfDNA from these blood samples using whole genome bisulfite sequencing and bioinformatics analysis to generate epigenetic and genetic profiles. They used methylation profiles to trace the tissue of origin of the cfDNA and thereby infer tissue-specific injury consequent to GVHD. Prior to the onset of symptoms, the concentration of organ-specific cfDNA was significantly elevated in patients who developed GVHD. Therefore, cfDNA could not only predict presymptomatic GVHD (area under the curve, 0.88) but also the tissue site of future injury. To predict the development of future infections, the authors used metagenomic sequencing of patients' cfDNA and compared the results with various microbial reference genomes in databases. They found a remarkable increase in different pathogens after transplant. Compared to routine PCR analysis for the BK virus (the most common infection related to transplant), this cfDNA method was highly sensitive and specific. To analyze disease relapse, the authors assessed tumor-specific genomic aberrations for identifying tumor cell clones (some with novel mutations) prior to morphologic relapse. In post-transplant donor-host chimerism, the fraction of cfDNA that was donor derived was shown to be consistently high in patients with stable engraftment and decrease in patients with relapse or graft failure, or both. Because hematopoietic cell transplants remain the gold standard for treating aggressive hematologic malignancies and diseases, the need to find reliable assays for predicting the most common complications has risen. While the results of this study are promising, additional clinical studies with larger cohorts are needed to validate this sensitive and reliable single-method assay for simultaneously detecting many of the most common complications of stem cell transplant.

Cheng AP, Cheng MP, Loy CJ, et al. Cell-free DNA profiling informs all major complications of hematopoietic cell transplantation. *Proc Natl Acad Sci USA*. 2022;119(4). https://doi.org/10.1073/pnas.2113476118

Correspondence: Dr. Iwijn De Vlaminck at vlaminck@cornell.edu