Anatomic Pathology Abstracts, 9/15

Editors: Michael Cibull, MD, professor emeritus, University of Kentucky College of Medicine, Lexington; Rouzan Karabakhtsian, MD, attending pathologist, Department of Pathology, Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; Thomas Cibull, MD, dermatopathologist, Evanston Hospital, NorthShore University HealthSystem, Evanston, Ill.; and Rachel Stewart, DO, resident physician, Department of Pathology and Laboratory Medicine, University of Kentucky.

Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy in select breast cancers

Modulation of immunologic interactions in cancer tissue is a promising therapeutic strategy. To investigate the immunogenicity of HER2-positive and triple-negative breast cancers, the authors evaluated tumor-infiltrating lymphocytes and immunologically relevant genes in the neoadjuvant GeparSixto trial. GeparSixto investigated the effect on pathologic complete response of adding carboplatin to an anthracycline-plus-taxane combination. A total of 580 tumors were evaluated before random assignment for stromal tumor-infiltrating lymphocytes and lymphocyte-predominant breast cancer (LPBC). The mRNA expression of immune-activating (CXCL9, CCL5, CD8A, CD80, CXCL13, IGKC, CD21) and immunosuppressive (IDO1, PD-1, PD-L1, CTLA4, FOXP3) factors was measured in 481 tumors. Increased levels of stromal tumor-infiltrating lymphocytes predicted pathologic complete response in univariable ($P<0.001$) and multivariable ($P<0.001$) analyses. The pathologic complete response rate was 59.9 percent in LPBC and 33.8 percent in non-LPBC ($P<0.001$). Pathologic complete response rates of 75 percent or more were observed in patients with LPBC tumors treated with an anthracycline-plus-taxane combination with carboplatin, with a significant test for interaction with therapy in the complete ($P=0.002$) and HER2-positive ($P=0.006$), but not the triple-negative breast cancer, cohorts. Hierarchic clustering of mRNA markers revealed three immune subtypes with different pathologic complete response rates ($P<0.001$). All 12 immune mRNA markers were predictive for increased pathologic complete response. The highest odds ratios were observed for PD-L1 (odds ratio, 1.57; 95 percent confidence interval [CI], 1.34–1.86; $P<0.001$) and CCL5 (odds ratio, 1.41; 95 percent CI, 1.23–1.62; $P<0.001$). The authors concluded that immunologic factors were highly significant predictors of therapy response in the GeparSixto trial, particularly in patients treated with carboplatin. After further standardization, these factors could be included in the histopathologic assessment of breast cancer.

Denkert C, von Minckwitz G, Brase JC, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-

negative primary breast cancers. *J Clin Oncol.* 2015;33:983–991./p>

Correspondence: Dr. Carsten Denkert at carsten.denkert@charite.de

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Genomic and transcriptomic heterogeneity in metaplastic breast carcinomas

Metaplastic breast carcinoma is a rare and aggressive histologic type of breast cancer, preferentially displaying a triple-negative phenotype. The authors sought to define the transcriptomic heterogeneity of metaplastic breast cancers on the basis of gene-expression, microarray-based classifiers and determine whether these tumors display gene copy-number profiles consistent with those of BRCA1-associated breast cancers. Twenty-eight consecutive triple-negative metaplastic breast carcinomas were reviewed, and the metaplastic component present in each frozen specimen was defined—that is, spindle-cell, squamous, or chondroid metaplasia. RNA and DNA extracted from frozen sections with tumor cell content greater than 60 percent were subjected to gene expression (Illumina HumanHT-12 v4 BeadChip) and copy-number profiling (Affymetrix SNP Array 6.0), respectively. Using the best practice PAM50/claudin-low microarray-based classifier, all metaplastic breast carcinomas with spindle-cell metaplasia were of claudin-low subtype, whereas those with squamous or chondroid metaplasia were preferentially of basal-like subtype. Triple-negative breast cancer subtyping using a dedicated website (http://cbc.mc.vanderbilt.edu/tnbc/) revealed that all metaplastic breast carcinomas with chondroid metaplasia were of mesenchymal-like subtype, spindle-cell carcinomas preferentially of unstable or mesenchymal stem-like subtype, and those with squamous metaplasia were of multiple subtypes. None of the cases was classified as immunomodulatory or luminal androgen receptor subtype. Integrative clustering, combining gene expression and gene copy-number data, revealed that metaplastic breast carcinomas with spindle-cell and chondroid metaplasia were preferentially classified into integrative clusters four and nine, respectively, whereas those with squamous metaplasia were classified into six different clusters. Eight of the 26 metaplastic breast cancers subjected to SNP6 analysis were classified as BRCA1-like. The diversity of histologic features of metaplastic breast carcinomas is reflected at the transcriptomic level, and an association between molecular subtypes and histology was observed. BRCA1-like genomic profiles were found only in a subset (31 percent) of metaplastic breast cancers and were not associated with a specific molecular or histologic subtype.

Weigelt B, Ng CK, Shen R, et al. Metaplastic breast carcinomas display genomic and transcriptomic heterogeneity. *Mod Pathol.* 2015;28:340–351.

Correspondence: Dr. A. Vincent-Salomon at anne.salomon@curie.fr or Dr. J. S. Reis-Filho at reisfilj@mskcc.org

[hr]

Quantification of histochemical stains using whole slide imaging

Histochemical staining of tissue is a fundamental technique in tissue diagnosis and research, but it suffers from significant variability. Laboratory quality controls and quality assurance schemes have been used to address this issue, but these rely on subjective interpretation of stain quality and are laborious and have low reproducibility. The authors reported on their efforts to develop a method for histochemical stain quantification using whole slide imaging and image analysis and to demonstrate its usefulness in measuring staining variation. A method to quantify the individual stain components of histochemical stains on virtual slides was developed. It was evaluated for repeatability and reproducibility and then applied to control sections of an appendix to quantify H&E staining (H/E intensities and H:E ratio) between automated staining machines and to measure differences between six regional diagnostic laboratories. The method was validated with less than 0.5 percent variation in H:E ratio measurement when using the same scanner for a batch of slides (that is, it was repeatable), but it was not highly reproducible between scanners or over time, with variation of seven percent. Application of the method showed that H:E ratios between three staining machines varied from 0.69 to 0.93. H:E ratio variation over time was

observed. Interlaboratory comparison demonstrated differences in H:E ratio of 0.57 to 0.89 between regional laboratories. The authors concluded that a simple method using whole slide imaging can be used to quantify and compare histochemical staining. This method could be deployed in routine quality assurance and quality control. Work is needed to improve reproducibility on whole slide imaging devices.

Gray A, Wright A, Jackson P, et al. Quantification of histochemical stains using whole slide imaging: development of a method and demonstration of its usefulness in laboratory quality control. *J Clin Pathol.* 2015;68:192–199.

Correspondence: Dr. Darren Treanor at darren treanor@nhs.net

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Assessment of percutaneous renal needle core biopsy

While biopsies are increasingly being used to diagnose renal cortical neoplasms, the influence of the pathological diagnoses on clinical management is rarely documented. The authors reported their experience with consecutively performed renal biopsies and the potential impact of the diagnoses on subsequent clinical management. Material from needle biopsies performed consecutively at the authors' institution between 2006 and 2011 was reviewed. The influence of the reported pathology results on clinical management was determined from patient follow-up medical record review. In total, 218 percutaneous biopsies for renal masses were performed during this period. Among them, 181 (83 percent) yielded neoplastic tissue, including 81 clear cell renal cell carcinomas, 29 low-grade oncocytic neoplasms, seven papillary renal cell carcinomas, five clear cell papillary renal cell carcinomas, five angiomyolipomas, and 14 urothelial carcinomas. Fourteen (six percent) additional cases contained lesional material from clinically known nonneoplastic processes, for a total diagnostic yield of 89 percent. Twenty-three (11 percent) were nonrepresentative of lesional tissue. In 10 of these, repeat biopsies or resections established the diagnosis of renal tumors. Biopsy diagnosis was confirmed in 29 of 30 (97 percent) cases on subsequent nephrectomy. Following biopsy diagnosis, there were significant differences in clinical management: overall, 79 percent of patients with clear cell renal cell carcinoma received therapeutic interventions, and 17 percent were put on active surveillance. In contrast, 77 percent of patients with benign or low-grade lesions were put on active surveillance. The authors concluded that accurate and specific diagnosis can be rendered on renal core biopsy in most renal tumors, and biopsy diagnosis can play a definitive role in clinical management of renal tumors.

Gellert LL, Mehra R, Chen YB, et al. The diagnostic accuracy of percutaneous renal needle core biopsy and its potential impact on the clinical management of renal cortical neoplasms. *Arch Pathol Lab Med.* 2014;138:1673–1679.

Correspondence: Dr. Satish K. Tickoo at tickoos@mskcc.org

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Neuroendocrine prostate cancer progressing from conventional prostatic adenocarcinoma

An often underrecognized late manifestation of prostate adenocarcinoma is the development of treatment-related neuroendocrine prostate cancer (NEPC). The authors conducted a study to identify the risk factors related to survival after NEPC diagnosis (NEPCS) and time from initial diagnosis of prostate adenocarcinoma to development of NEPC (TTNEPC). A literature search on NEPC was performed using such databases as MedLine and Embase. Studies were eligible if outcomes data (NEPCS or TTNEPC, or both) were reported in patients with a prior history of prostate adenocarcinoma and histopathologically confirmed NEPC. NEPCS and TTNEPC were evaluated using the Cox regression model with the robust sandwich estimates of the covariance matrix. Fifty-four eligible publications contributed 123 patients. The median TTNEPC was 20 months. In multivariate analyses, Gleason score was significantly associated with shorter TTNEPC (hazard ratio [HR], 1.66; P=0.032). The median NEPCS was seven months. Furthermore, in multivariable analyses, the number of organs with metastatic disease at NEPC was

significantly associated with shorter NEPCS (HR, 3.31; P=0.001). Type of treatment after NEPC was significantly associated with longer NEPCS, with hazard ratios of 0.66 (radiotherapy versus palliative therapy; P=0.034), 0.38 (chemotherapy versus palliative therapy; P=0.018), and 0.29 (chemoradiotherapy versus palliative therapy; P=0.012), respectively. The authors concluded that treatment-related NEPC is an often under-recognized late manifestation of prostate adenocarcinoma with poor prognosis. Gleason score was the only independent factor contributing to TTNEPC in this study. Once NEPC was diagnosed, type of treatment and number of organs with metastatic disease were the most important factors related to survival.

Wang HT, Yao YH, Li BG, et al. Neuroendocrine prostate cancer (NEPC) progressing from conventional prostatic adenocarcinoma: factors associated with time to development of NEPC and survival from NEPC diagnosis—a systematic review and pooled analysis. *J Clin Oncol.* 2014;32:3383–3390.

Correspondence: Dr. Hai Tao Wang at peterrock2000@126.com

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Analysis of wntless expression in gastric, ovarian, and breast cancers

The oncogenic role of WNT is well-characterized. Wntless, also known as GPR177 or Evi, which is a key modulator of WNT protein secretion, was recently found to be highly overexpressed in malignant astrocytomas. The authors hypothesized that this molecule may be atypically expressed in other cancers known to possess aberrant WNT signaling, such as ovarian, gastric, and breast cancers. Immunohistochemical analysis using a tissue microarray platform revealed wntless (WLS) overexpression in a subset of ovarian, gastric, and breast tumors. This overexpression was associated with poorer clinical outcomes in gastric cancer (P=0.025). Furthermore, a strong correlation was observed between WLS expression and human epidermal growth factor receptor 2 (HER2) overexpression. Indeed, 100 percent of HER2-positive intestinal gastric carcinomas, 100 percent of HER2-positive serous ovarian carcinomas, and 64 percent of HER2-positive breast carcinomas coexpressed WLS protein. Although HER2 protein expression or gene amplification is an established predictive biomarker for trastuzumab response in breast and gastric cancers, a significant proportion of HER2-positive tumors display resistance to trastuzumab, which may, in part, be explained by a possible mechanistic link between WLS and HER2.

Stewart J, James J, McCluggage GW, et al. Analysis of wntless (WLS) expression in gastric, ovarian, and breast cancers reveals a strong association with HER2 overexpression. *Mod Pathol.* 2015;28:428–436.

Correspondence: Dr. M. Salto-Tellez at m.salto-tellez@qub.ac.uk

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Evaluation of the stage IB designation of the AJCC staging system for breast cancer

The seventh edition of the American Joint Committee on Cancer staging system for breast cancer differentiates patients with T1 tumors and lymph node micrometastases (stage IB) from patients with T1 tumors and negative nodes (stage IA). The authors undertook a study to determine the utility of the stage IB designation. Two cohorts of patients with breast cancer were identified: 3,474 patients treated at The University of Texas MD Anderson Cancer Center from 1993 to 2007 and 4,590 patients from the American College of Surgeons Oncology Group (ACOSOG) Z0010 trial. Clinicopathologic and outcomes data were recorded, and disease was staged according to the seventh edition of the AJCC staging system. Recurrence-free survival, disease-specific survival, and overall survival were determined using the Kaplan-Meier method and compared using the log-rank test. Median follow-up times were 6.1 years and 9.0 years for the MD Anderson and ACOSOG cohorts, respectively. In both cohorts, no significant differences were found between patients with stage IA and stage IB disease in five- or 10-year recurrence-free, disease-specific, or overall survival. Estrogen receptor status and grade significantly stratified patients with stage I disease with respect to the aforementioned categories of survival. The authors concluded that among patients with

T1 breast cancer, individuals with micrometastases and those with negative nodes have similar survival outcomes. Estrogen receptor status and grade are better discriminants of survival than the presence of small-volume nodal metastases. In preparing the next edition of the AJCC staging system, consideration should be given to eliminating the stage IB designation and incorporating biologic factors.

Mittendorf EA, Ballman KV, McCall LM, et al. Evaluation of the stage IB designation of the American Joint Committee on Cancer staging system in breast cancer. *J Clin Oncol.* 2015;33:1119–1127.

Correspondence: Dr. Elizabeth A. Mittendorf at eamitten@mdanderson.org

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Exploring the rising incidence of neuroendocrine tumors

An increased incidence of neuroendocrine tumors has been reported worldwide, but the reasons underlying this rise have not been identified. By assessing patterns of metastatic presentation, the authors of this study sought to examine the epidemiologic characteristics of neuroendocrine tumors (NETs) and the contribution of early-stage detection to rising incidence. They conducted a population-based retrospective cohort study with prospectively maintained databases linked at the Institute for Clinical Evaluative Sciences. The study assessed adult patients given a NET diagnosis between 1994 and 2009, in Ontario, Canada. The main outcomes included overall and site-specific incidence, proportion of metastatic disease, overall survival, and recurrence-free survival. The study identified 5,619 NET cases. The incidence of NETs increased from 2.48 to 5.86 per 100,000 per year. Metastases were found in 20.8 percent at presentation and another 38 percent after the initial diagnosis. The proportion of metastases at presentation decreased from 1994 to 2009 (from 29 to 13 percent). Therefore, although the incidence of NETs increased, the overall incidence of metastases did not change (0.63–0.69 per 100,00 per year). The 10-year overall survival rate was 46.5 percent, and the recurrence-free survival rate was 64.6 percent. In addition to primary tumor site, independent predictors of worse overall survival included advanced age ($P<0.0001$), male gender ($P<0.0001$), low socioeconomic status ($P<0.0001$), and rural residency ($P<0.049$). The authors concluded that the incidence of NETs has markedly increased during the course of 15 years. This is the first study to provide evidence suggesting that the increase in the incidence of NETs may be due to improved detection. In addition to tumor characteristics, low income and rural residency portend worse survival rates for patients with NETs.

Hallet J, Law CH, Cukier M, et al. Exploring the rising incidence of neuroendocrine tumors: a population-based analysis of epidemiology, metastatic presentation, and outcomes. *Cancer.* 2015;121:589–597.

Correspondence: Dr. Julie Hallet at julie.hallet@sunnybrook.ca

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Prognostic significance of lymphatic invasion in lymph node-positive breast carcinoma

The poor prognostic significance of lymphatic invasion in breast carcinoma, as a whole, and in lymph node-negative patients, in particular, has been recognized in several studies. However, its prognostic role in lymph node-positive patients is still questionable. The authors conducted a study to assess the prognostic role of lymphatic invasion in lymph node-positive breast carcinoma specimens. Sections from 557 nonselected lymph node-positive breast carcinoma specimens were stained with antibody to podoplanin/D2-40. Lymphatic invasion was identified and correlated with clinicopathological features and patient outcome. Twenty-year overall survival, disease-free interval, and development of distant metastasis or recurrence were known for all patients. Lymphatic invasion was detected in 262 of the 557 (47 percent) specimens ranging from one to 350 lesions per tumor section. Its presence was associated with higher grade tumors ($P<0.0001$), negative hormonal receptors ($P<0.0001$), high human epidermal growth factor receptor 2 (HER2) expression ($P=0.006$), and increased number of positive lymph nodes

(P=0.019). In the whole lymph node-positive breast carcinoma, presence of lymphatic invasion was a poor prognostic factor for overall survival, disease-free interval, and development of distant metastasis in univariate and multivariate analysis. In further stratification of patients, lymphatic invasion was associated with poorer prognosis in patients with a single positive lymph node but not in patients with more than one positive lymph node. In T1N1 stage, lymphatic invasion was highly associated with poor overall survival (P=0.002), disease-free interval (P<0.0001), and distant metastasis (P<0.0001). In T2N1 patients, lymphatic invasion was associated with poorer disease-free interval (P=0.037) but not with death or distant metastasis. In the former two patient groups, lymphatic invasion lost significance in multivariate analysis. The authors concluded that lymphatic invasion is a poor prognostic factor in lymph node-positive breast carcinoma, particularly for patients having a single positive lymph node. Lymphatic invasion, therefore, would add further prognostic significance when considered in the treatment of those patients. The authors recommend incorporating lymphatic invasion in breast carcinoma staging and prognostic indices.

Mohammed RA, Menon S, Martin SG, et al. Prognostic significance of lymphatic invasion in lymph node-positive breast carcinoma: findings from a large case series with long-term follow-up using immunohistochemical endothelial marker. *Mod Pathol.* 2014;27:1568–1577.

Correspondence: Dr. R. A. Mohammed at rabab_aamh@yahoo.com