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; Sounak Gupta, MBBS, PhD, senior associate consultant, Mayo Clinic, Rochester, Minn.; Tauangtham Anekpuritanang, MD, molecular pathology fellow, Department of Pathology, OHSU; Hassan Ghani, MD, molecular genetic pathology fellow, Department of Pathology, OHSU; and Fei Yang, MD, assistant professor, Department of Pathology, OHSU.

Genomic characterization of origins and epidemiology of novel SARS-CoV-2 coronavirus

April 2020—Prior to the recent novel coronavirus outbreak, the vast majority of coronaviruses that were known to be pathogenic in humans caused mild symptoms, with the exception of two strains. These included SARS (severe acute respiratory syndrome coronavirus [SARS-CoV]) and MERS (Middle East respiratory syndrome coronavirus [MERS-CoV]). SARS was documented as having emerged in the Guangdong province in southern China in 2002 and having caused at least 774 reported fatalities worldwide, while MERS, which was first detected in Saudi Arabia in 2012, was responsible for at least 858 fatalities worldwide. In this initial report of the novel SARS-CoV-2 coronavirus, the authors discovered the etiologic cause of an epidemiologic cluster of viral pneumonias in three hospitals in Wuhan, China, each linked to this same novel strain of coronavirus. Initial epidemiologic studies suggested that at least eight of the nine patients had visited the Huanan seafood and live animal market prior to the onset of symptoms in late December. Bronchoalveolar lavage fluids were obtained for further analysis from the majority of these patients after they became ill. These samples were cultured in vitro to promote viral outgrowth, and the supernatants, presumed to contain the viral pathogen, were subjected to next-generation sequencing (NGS) to assess the viral genome. After removing human nucleic acids using a probe-capture technique, RNA from these specimens was reverse transcribed into cDNA. Sequences that mapped to the human reference genome were filtered out and the remaining sequences were aligned to a nucleotide database of published coronavirus sequences. Based on the assembled genome of the resulting novel coronavirus, a real-time polymerase chain reaction (PCR) assay was developed for more rapid diagnostic and epidemiologic applications. Phylogenetic analysis of the ~30 kb viral RNA genome showed high sequence similarity (more than 99.9 percent) in the viral isolates from the nine patients. This was suggestive of the recent introduction of this new virus into the human population within a short period of time, as RNA viruses have a high rate of mutations, with mutations arising in every replication cycle. Surprisingly, this new coronavirus strain was closely related to bat-derived coronaviruses, suggesting a bat reservoir. However, multiple lines of evidence suggest that another unknown organism may serve as an intermediate host. Among the findings was that most bat species in the Wuhan area were in hibernation at the time of the outbreak in December; no bats were sold or found at the Huanan seafood market; and the sequence similarity with known bat strains was less than 90 percent, which is suggestive of a long evolutionary branch between these species. Perhaps the most interesting observation was that although the 2019 novel coronavirus was closely related to bat coronavirus species at the genome level, it showed less similarity (68 percent sequence identity) at the C terminus of the S1 domain of the envelope spike (S) protein. This S1 domain is responsible for virus receptor binding and, therefore, host tropism and transmission capacity. Instead, the 2019 SARS-CoV-2 coronavirus showed significant similarity in its receptor-binding domain to the SARS coronavirus that circulated in humans in 2002 and 2003. This suggests an evolutionary basis for human tropism of the 2019-nCoV. In summary, this study provides insight into the origin of the 2019-nCoV and highlights the potential of hidden virus reservoirs in wild animals to spill into the human population.

Lu R, Zhao X, Li J, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*. 2020;395:565–574.

Correspondence: Dr. Wenjie Tan at tanwj@ivdc.chinacdc.cn and Dr. Weifeng Shi at wfshi@sdfmu.edu.cn

Pan-cancer efficacy of vemurafenib in *BRAF* V600-mutant nonmelanoma cancers

The authors conducted a study in which they embraced the concept of basket clinical trials. Most clinical trials focus on organ site-specific tumor types, often with a specific molecular alteration—for example, non-small cell lung cancer that harbors targetable gene alterations, such as alteration of the *ALK* gene. However, an emerging paradigm involves targeting specific molecular alterations, regardless of the underlying tumor's site of origin. Such an approach has led to FDA approval of tumor-agnostic immunotherapy agents, such as pembrolizumab, for microsatellite instability-high (MSI-high) tumors or, more recently, drugs that target tumors that exhibit alterations of the *NTRK* gene. For this study, the authors targeted activating *BRAF* alterations (*BRAF* V600E, in 99 percent of cases) in a tumor-agnostic manner in a basket clinical trial format. The authors excluded from their study melanoma, papillary thyroid carcinoma, and hairy cell leukemia because the bulk of the literature regarding the efficacy of *BRAF* V600-mutant selective RAF inhibitors, such as vemurafenib, is in the context of these tumor types. Therefore, the study provided information regarding the efficacy of *BRAF* V600-mutant selective RAF inhibitor monotherapy in 172 patients with 26 other unique tumor types. It is important to note that due to the rarity of these molecular alterations in many of the tumors, it would have been challenging to accrue a sufficient number of patients to represent every tumor type in a conventional clinical trial. This assertion is supported by the observation that 11 of the 26 tumor type categories had fewer than 10 patients enrolled. Tumor type categories with more than 10 patients included non-small cell lung cancer, histiocytosis, glioma, and anaplastic thyroid cancer. The clinical benefit rate, defined as a partial response of any duration or stable diseases lasting six months or more, was 42 percent, and objective responses were identified across 13 unique cancer types. This study also provided important safety information regarding vemurafenib. Notably, grade 3 or higher adverse events were identified in 73 percent of patients, with cutaneous squamous cell carcinoma being the most common, at 15 percent, while discontinuation of treatment secondary to drug-related adverse events occurred in 7.6 percent of patients. Although this study has limitations, the most notable being that it was launched before the advent of combination BRAF/MEK inhibition, it has several successes. For example, the study demonstrated objective responses in tumor types that are associated with a poor prognosis and treatment refractoriness, including gliomas, sarcomas, and cholangiocarcinomas. Data from this study also were used to support the approval of vemurafenib for the rare histiocytic neoplasm Erdheim-Chester disease. Furthermore, the study led to regulatory approval of combination RAF/MEK therapy in non-small cell lung cancer and anaplastic thyroid cancer and to inclusion of vemurafenib in National Comprehensive Cancer Network guidelines for managing non-small cell lung cancer. In summary, this study highlights the utility of basket clinical trials for tumor types with a low incidence of targetable molecular alterations.

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Correspondence: Dr. Vivek Subbiah at vsubbiah@mdanderson.org and Dr. David M. Hyman at dhyman@loxooncology.com