Molecular pathology selected abstracts

Correlation between tumor mutation burden and efficacy of combination immunotherapy in nonsmall cell lung cancer

July 2018—Checkpoint inhibitor therapy has dramatically improved outcomes in many cancer types, with treatments including antibodies against cytotoxic T-lymphocyte–associated protein 4 (CTLA-4), programmed cell death receptor-1 (PD-1), and its ligand (PD-L1). Combination immunotherapy using PD-1 plus CTLA-4 blockade has shown promising outcomes in nonsmall cell lung cancer (NSCLC).

However, the response is not universal in all patients, and it is difficult to predict those who will respond. Pathologist interpretation of the proportion of tumor cells showing membranous immunohistochemical staining with anti-PD-L1 antibody has been the mainstay for determining whether patients are eligible for treatment or clinical trials, or both. However, such interpretation shows interobserver variability, and more importantly, it is only modestly sensitive and specific for predicting response. Therefore, it is necessary to identify other predictive markers. There is increasing recognition of an association between tumor mutation burden (TMB) and response rates in multiple tumor types treated with checkpoint inhibitor monotherapy. In this study, the authors sought to determine whether this relationship between TMB and response rate also occurs in NSCLC treated with combination immunotherapy. They performed whole exome sequencing on tumor tissue and paired blood from 75 patients with advanced NSCLC treated with combination immunotherapy. A comparison of patients above and below the TMB median found that patients with a high TMB had significantly improved progression-free survival. To determine whether there were confounding variables, the authors compared the clinical characteristics between the two groups. Except for smoking status (patients with a high TMB were significantly more likely to be smokers), the clinical characteristics were similar. However, in a multivariate analysis that included smoking status, PD-L1 expression, histology, performance status, and amount of tumor, the authors confirmed that TMB was independently associated with overall response rate and progression-free survival. Finally, the authors examined whether other molecular features were associated with response to combination immunotherapy. They found that mutations in *STK11* and *PTEN* were associated with resistance. It has been hypothesized in the medical literature, and by the authors, that immunotherapy is more effective in tumors with a high TMB because these tumors have increased numbers of neoantigens, mutated proteins specific to the tumor, that can be recognized by the immune system. Although determining mutation burden using whole exome sequencing would be difficult, especially given the short time frame in which it would need to be performed in patients with advanced NSCLC, some studies have shown that TMB correlates with the number of mutations seen in the targeted next-generation sequencing panels that are routinely used clinically. Overall, TMB appears to be a strong predictor of the efficacy of combination immunotherapy in NSCLC.

Hellmann MD, Nathanson T, Rizvi H, et al. Genomic features of response to combination immunotherapy in patients with advanced non-small-cell lung cancer. *Cancer Cell.* 2018;33:843–852.

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Open-source algorithms for identifying structural variants in single-molecule sequencing

There is increasing evidence that structural variations, including insertions, deletions, duplications, inversions, and translocations, play a vital role in genetic diversity and disease. Large structural variations, such as aneuploidies and large translocations, are traditionally detected with optical microscopes. Submicroscopic structural variants are much more difficult to detect, even with modern techniques. The widely used short-read next-generation sequencing techniques lack sensitivity and are susceptible to high false-positive rates. They are also likely to misinterpret complex or nested structural variations. Long-read single-molecule sequencing, with average read

lengths of 10 kbp or higher, can be more accurately aligned to reference sequences and are more likely to span the breakpoints in the DNA that cause structural variants. However, the drawback of long-read methodologies is that the sequencing error rate is high with some platforms, showing up to a 20 percent error rate. The authors conducted a study in which they introduced two open-source algorithms: the NGMLR algorithm for long-read alignment, which can align reads even in the presence of small indels that occur because of sequencing errors, and the Sniffles algorithm, which can call true structural variants in the noisy background. The authors tested these algorithms using parent progeny trios, for which Mendelian discordance rates ranged from 3.4 to 5.6 percent, as compared with a 21.1 percent discordance rate using short-read analysis. The long-read algorithms were then tested in healthy and breast cancer genomes, for which Sniffles was able to detect far more structural variants overall but called far fewer translocations than the short-read methods. However, the authors demonstrated that more than 80 percent of the translocations that were called by the short-read methods were false, caused by a deletion or insertion that resulted in the short reads being mismapped to different areas of the genome. The long-read algorithms, on the other hand, accurately called these structural variants. Finally, the authors demonstrated that Sniffles was able to accurately call complex, nested structural variants, such as inverted duplications and inverted deletions. They concluded that identifying structural variants is challenging, especially with the short-read technology being used clinically. Long-read sequencing using novel alignment and variant-calling algorithms, such as NGMLR and Sniffles, could be used to accurately identify structural variants. Widespread use of long-read sequencing technologies, for clinical and research purposes, may shed light on the many structural variants that cause genetic diversity and that play a role in various diseases.

Sedlazeck FJ, Rescheneder P, Smolka M, et al. Accurate detection of complex structural variations using single molecule sequencing. *Nat Methods.* 2018. doi.org/10.1038/s41592-018-0001-7.

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