

# A Rethink for IO Biomarkers

*Initiatives aim to validate, harmonize assays that capture complex tumor-immune system interactions*

Tests for PD-L1 expression, tumor mutation burden (TMB), tumor-infiltrating lymphocytes, and other biological metrics can help determine which patients are most likely to respond to checkpoint blockade, but no assays have yet delivered consistent, robust, and actionable insights into the efficacy of immunotherapy that might guide decision-making for individual patients.

The underwhelming performance of putative biomarkers to date has prompted a growing realization that the immunoncology (IO) community needs to take a multipronged approach to biomarker development. Because of the multifactorial nature of cancer-immune system interactions, researchers are exploring new aspects of tumor-intrinsic, immune microenvironmental, and host-related factors associated with drug response. They are also working more openly and collaboratively to deliver consistent, comparable assays.

“Nothing by itself is perfect yet, and I continue to worry about the technical issue that, if you use one assay and I use another, we’re not going to get quite the same answer,” says Lisa Butterfield, PhD, of the University of Pittsburgh in Pennsylvania, president of the Society for Immunotherapy of Cancer.

Several initiatives are under way to promote the discovery, calibration, and harmonization of biomarkers for immunotherapeutics, with most focusing on checkpoint inhibitors. In October 2017, for example, the Foundation for the National Institutes of Health (FNIH) announced the Partnership for Accelerating Cancer Therapies (PACT), a 5-year, \$220 million public-private effort to validate and standardize biomarker assays. Twelve drug companies are each donating \$1 million annually to the alliance, and the NCI is spending nearly \$11 million per year for biomarker trials.

According to FNIH’s director of cancer research partnerships, Stacey Adam, PhD, PACT was inspired in part by the Blueprint Project, an initiative to compare PD-L1 assays on a common set of lung cancer specimens. The project showed that three commercially available assays, each of which uses a different anti-PD-L1 antibody, produced comparable results, but two others stained varied numbers of tumor cells (*J Thorac Oncol* 2018;13:1302-11).

Going forward, says Adam, the goal is to avoid having intertest variability—or at least correct any inherent inconsistencies in the assays. “What’s really needed is a baseline set of data so you can cross-compare trials,” she says.

Other cooperative efforts surrounding biomarker validation have been more targeted. For instance, two initiatives—one from Friends of Cancer Research (FOCR) in the United States and another from Quality Assurance Initiative Pathology in Germany—are working on standardizing quantification of TMB, through either whole-exome sequencing or targeted gene panels. FOCR recently completed phase I of a project comparing the performance

of 11 TMB assays on publicly available genomic data. According to Mark Stewart, PhD, vice president of science policy at FOCR, the assays were largely concordant, but “there were enough differences to warrant further discussions about how best to align the tests.”

Agreement among TMB tests, however, doesn’t necessarily mean they’re clinically useful, and simply tallying mutations per coding area of a tumor genome may be an inaccurate proxy for neoantigen load. Eliezer Van Allen, MD, of Dana-Farber Cancer Institute in Boston, MA, discovered this when he and his colleagues looked beyond TMB in tumor samples from 249 patients with a variety of cancer types who had received checkpoint inhibitors. As Van Allen’s team reported in August, alterations in particular driver genes often had an outsized effect on the usefulness of immunotherapy (*Nat Genet* 2018;50:1271-81). What’s more, integrating these genetic driver events with mutational signatures, measures of intratumoral heterogeneity, and TMB yielded a more accurate predictor of response to checkpoint blockade than TMB alone.

Beyond tumor-intrinsic biomarkers, many researchers are developing assays that assess aspects of host immunity, including the tumor microenvironment’s immune cell repertoire. Yet, even these biomarkers are inexact proxies for gauging T-cell recognition of tumor cells. “So far, the T-cell recognition assays have been what’s lacking in clinical development,” says Kellie Smith, PhD, of Johns Hopkins University in Baltimore, MD, who recently developed an assay, dubbed MANAFEST, for quantifying mutation-associated, neoantigen-specific T-cell responses in blood and tumor tissue (*Cancer Immunol Res* 2018;6:888-99).

Attempting to capture aspects of both tumor and immune biology on a common platform, many teams are now turning to multiplex IHC assays that involve serial staining to simultaneously visualize the position and interaction of various immune checkpoint molecules alongside different types of immune cells—including cytotoxic T cells, regulatory T cells, and macrophages. “You get essentially the cartographic coordinates of every cell when you do a multiplex,” says Bernard Fox, PhD, of the Earle A. Chiles Research Institute in Portland, OR.

However, this approach takes several days to weeks to read out, at which point the results may no longer accurately reflect the dynamic nature of tumor immunity. That’s a weakness of nearly all proposed biomarkers for immunotherapy, says Taha Merghoub, PhD, of Memorial Sloan Kettering Cancer Center (MSKCC) in New York, NY. “The immune landscape is dynamic. Cells are coming in and out,” he says. “We need to probe the tumor in real time.” Preliminary data on the use of PET imaging for cytotoxic T cells shows that same-day results are possible (*J Clin Oncol* 36, 2018 [suppl; abstr e24160]).

Real-time imaging can still capture only one biomarker at a time, and a truly predictive test for immunotherapy responsiveness will likely involve “multiple variables with complex interactions,” says MSKCC’s Vinod Balachandran, MD. That’s why he’d like to see more trials in which researchers gather reams of data on every biomarker imaginable. “It really has to be a broad net,” Balachandran says. “We need to better understand what is happening in patients so we can tailor treatments for them” —*Elie Dolgin* ■

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