BIOCENTURY
11:52 AM, MAR 15, 2018

SHARED BURDEN

BY KAREN TKACH TUZMAN, ASSOCIATE EDITOR

Having learned their lesson with PD-L1, companies are collaborating on assay standards for tumor mutation burden -- a likely contender for the next major biomarker in immuno-oncology. A Friends of Cancer Research-led consortium of pharmas and diagnostics companies is driving the charge and plans to publish a white paper with its recommendations this year.

The coalition has been gathering members and momentum since it began meeting in September to create a framework for comparing tests on tumor mutation burden (TMB).

So far, discussions have included representatives from Friends, FDA, six test developers and six pharmas -- AstraZeneca plc, Bristol-Myers Squibb Co., the Genentech Inc. unit of Roche, the EMD Serono Inc. subsidiary of Merck KGaA, Merck & Co. Inc. and Pfizer Inc.

The partners are aiming to avoid the problems created for drug developers by the lack of harmony over assays for PD-L1. Four immunohistochemistry (IHC) assays, each with its own scoring algorithm and method of measuring cells, were used to support trials for at least five Phase III mAbs against PD-1 or PD-L1. That has made it nearly impossible to compare results across the class and determine what level of PD-L1 expression predicts patient benefit.

In 2015, a group of 10 companies, regulatory agencies and cancer associations launched the Blueprint Project to compare PD-L1 assays, standardize scoring and recommend best practices. The project published the first phase of its analysis last year in the *Journal of Thoracic Oncology*.

Since TMB could upstage PD-1/PD-L1 for stratifying patients in cancer immunology, stakeholders are pooling heads now to define common assay parameters for the new biomarker.

“We saw this play out with the PD-L1 IHC story, and we wanted to get ahead of the problem,” said David Fabrizio, leader of cancer immunotherapy at Foundation Medicine Inc., a member of the consortium.

Evidence has been growing in the literature that TMB, a sign of how immunogenic a tumor is, could be a better predictor than PD-L1 of how a patient will respond to an immunotherapy.

BMS acted on that evidence, opting to switch from PD-L1 status to TMB level for a subset of patients in its Phase III CheckMate -227 trial. On Feb. 5, BMS announced Opdivo nivolumab plus Yervoy ipilimumab met the progression-free survival (PFS) endpoint vs. chemotherapy in first-line non-small cell lung cancer (NSCLC) patients with high TMB.

The decision followed BMS’s 2016 high profile Phase III miss in CheckMate -026. In that trial, Opdivo monotherapy missed the endpoint of improving PFS vs. chemotherapy in first-line NSCLC patients whose tumors expressed PD-L1 at ≥5%. Post-hoc whole exome sequencing suggested the compound would have met in TMB-high patients.

TMB is a measure of the number of somatic mutations in a tumor genome, which correlates with the number of neoantigens on tumor cell surfaces. Because T cells recognize neoantigens, which are not found in normal cells, the more neoantigens are present, the higher the likelihood and potential potency of a T cell-mediated antitumor response.

"I think the jury is still out from a prospective perspective, but there is enough data now from us and others that suggests it may be a reasonable biomarker to identify patients who derive benefit from this class of agents," said Priti Hegde, director and senior scientist for oncology biomarker development at Genentech. The company is including a prospectively defined TMB-high cohort in a Phase II/III study of its anti-PD-L1 mAb Tecentriq atezolizumab.

Unlike IHC, TMB assays aren't tied to the subjectivity of a pathologist's eye. But the science is still in flux on questions such as how to weigh different mutations and how to extrapolate mutation counts between gene panels and whole exome sequencing.

The coalition's goal is to create a centralized understanding of best practices and make it easier to compare results across assays. “We want to generate alignment against a universal set of standards,” said Fabrizio.

"We saw this play out with the PD-L1 IHC story, and we wanted to

get ahead of the problem."

David Fabrizio, Foundation Medicine

COMMON GROUND

Friends of Cancer Research President and CEO Jeff Allen told BioCentury the TMB coalition emerged from a forum on analytical performance standards for next-generation sequencing (NGS) testing co-sponsored by Friends and Alexandria Real Estate Equities Inc.

Allen said companies have been very willing to work with competitors to enable comparison across assays. "There's a recognition that the way that PD-L1 expression was moving forward, with multiple tests and multiple drugs, was becoming quite complicated and probably not advantageous to anyone."

"The idea is to try to get out in front of it this time," said Eric Rubin, SVP of Oncology Early Development at Merck & Co. "The Blueprint Project arose when folks had already filed. In this case it's a little bit earlier, so there's an opportunity for some alignment before labels get out there."

Allen said the coalition hopes to formalize protocols that will allow companies and academics to determine the degree to which they'd like to participate.

The goal is to "unify the approach to measuring" TMB. "It's not that any one approach is the right way; we just want to better understand how they relate to one another," said Allen. "I hope this analytical collaboration will allow different test manufacturers to be able to say, here's how our test relates to diagnostic company A. That may make the drugs and the testing available to more patients."

To enable comparison across tests, the partners hope to generate shared reference standards based on whole exome sequencing from common cell lines or databases.

They also want consensus on the relationship between the frequency of mutations per megabase as calculated via targeted sequencing panels, and the total number of mutations in the exome, said John Simmons, director of translational science and diagnostics at Personal Genome Diagnostics Inc. (PGDx). He said 10 mutations per megabase roughly corresponds to 200 distinct mutations in an exome.

The coalition also hopes to align companies on protocol issues such as determining which mutations to include.

"In some instances, different mutations may be weighed more heavily than others," said Allen. "We're trying to allow that type of flexibility as the understanding behind TMB continues to evolve."

He said agreeing on cutoffs for different cancers is outside the scope of the coalition for now, although a feasible aim is to "get to the point where we're able to discuss different common ranges, in terms of what constitutes high levels of mutational burden, versus intermediate and low."

"It's not that any one approach is the right way; we just want to better understand how they relate to one another."

Jeff Allen, Friends of Cancer Research

MEASURING MUTATIONS

Even before launching the consortium, consensus had been emerging across assay developers that sampling a few hundred genes could replace sequencing the whole exome.

TMB calculations from whole exome sequencing correlate well with Foundation's 324-gene panel (R2 = 0.95), according to Fabrizio, and with Illumina Inc.'s TruSight Tumor 170 assay (R2 = 0.91), according to the product datasheet.

At least eight diagnostic companies and one non-profit organization are commercializing NGS-based tests to measure TMB. All are commercializing tests using panels of 170 to 592 genes (see "Weighing the Burden").

TABLE: WEIGHING THE BURDEN

At least eight companies and one not-for-profit organization are developing or marketing next-generation sequencing (NGS) assays to measure tumor mutation burden (TMB). Most are including TMB analysis as part of a larger profile of tumor tissue or circulating tumor DNA. Some, like FoundationOne CDx from Foundation Medicine Inc. (NASDAQ:FMI) and MSK-IMPACT from Memorial Sloan Kettering Cancer Center (MSKCC), are FDA-approved

as in vitro diagnostics, but most are currently for research use only. (A) MSKCC and five companies -- Foundation Medicine, Illumina Inc. (NASDAQ:ILMN), Personal Genome Diagnostics Inc., Qiagen N.V. (Xetra:QIA; NYSE:QGEN) and Thermo Fisher Scientific Inc. (NYSE:TMO) -- are participating in a coalition led by Friends of Cancer Research to harmonize assay standards. Source: ClinicalTrials.gov; company websites

Company	Test name	Test description
Caris Life Sciences Inc.	Caris Molecular Intelligence CGP+	Assay profiling mutations in tumor tissue using a 592-gene panel
Foundation Medicine Inc. (NASDAQ:FMI) (A)	FoundationOne CDx	Assay profiling mutations in tumor tissue using a 324-gene panel
Foundation Medicine Inc.; Roche (SIX:ROG; OTCQX:RHHBY)	bTMB assay	Assay profiling mutations in cell-free DNA in plasma using a 394-gene panel
Illumina Inc. (NASDAQ:ILMN) (A)	TruSight Tumor 170	Assay profiling mutations in tumor tissue using a 170-gene panel
KEW Group Inc.	Cancerplex	Assay profiling mutations in tumor tissue using a >400-gene panel
Memorial Sloan Kettering Cancer Center (A)	MSK-IMPACT	Assay profiling mutations in tumor tissue using a 468-gene panel
NeoGenomics Inc. (NASDAQ:NEO)	NeoTYPE Discovery Profile	Assay combining NGS testing of 315 molecular markers and Tumor Mutation Burden (TMB) analysis
Personal Genome Diagnostics Inc. (A)	Unnamed panel	Assay profiling mutations in tumor tissue using a >500 gene panel
Qiagen N.V. (Xetra:QIA; NYSE:QGEN) (A)	GeneRead DNAseq Mix-n-Match Panels	Customizable assay profiling mutations using 570 primer sets
Thermo Fisher Scientific Inc. (NYSE:TMO) (A)	Ion Torrent Oncomine Tumor Mutational Load Assay	Assay profiling mutations in tumor tissue using a 409-gene panel

Simmons said PGDx shifted from whole exome sequencing to targeted sequencing panels because they are more economical, scalable and accessible to patients. In addition, the panels are better able to identify chromosomal rearrangements, and have a clearer regulatory path, he said.

"We know that for a lot of the thresholds that are in range, you can make a smaller panel to deliver that," he said.

The relationship between panel size and sensitivity for detecting low rates of TMB is also important, said Fabrizio. While panel sizes are limited by the cost of sequencing at depths required to maintain accuracy, "a smaller panel will only have accuracy down to a high TMB cutoff."

According to Simmons, PGDx performed retrospective studies with undisclosed pharmas and discovered TMB thresholds as low as six or eight mutations per megabase might be needed to identify patients likely to benefit from certain combinations or specific monotherapies.

"We needed an assay that had at least a megabase of coding sequence included to have accuracy across that dynamic range," he said.

The companies differ on whether and how they define high TMB status.

For example, Caris Life Sciences Inc. defines high TMB status for its assay as 17 mutations per megabase or more; Foundation Medicine and PGDx leave that decision to the pharmas.

"They're the ones with the clinical data and the statistical power to decide those cutoffs," which will vary by cancer type and line of therapy, said Simmons.

Steven Averbuch, head of precision medicine at BMS, said the pharma plans to publish how it selected a prospective cutoff of 10 mutations per megabase for CheckMate -227 based on retrospective analyses. At this year's meeting of the American Association for Cancer Research (AACR), BMS will present data on identification of a TMB cutoff from CheckMate -568, a Phase II trial on Opdivo plus Yervoy in stage IV NSCLC.

The pharma employed Foundation's 324-gene FoundationOne CDx assay to calculate TMB for the trial. FoundationOne CDx is FDA approved as a companion diagnostic test for 17 targeted therapies used to treat solid tumors. In February, the company told BioCentury it plans to use data from CheckMate - 227 to support addition

of TMB to its label.

"It's very plausible that the quality of the neoantigens will be very important, especially in tumors where there is low TMB."

Steven Averbuch, BMS

POPULATING PANELS

Companies are designing panels of genes that serve two primary purposes: acting as surrogates for neoantigen load, and matching patients with targeted therapies.

But the analysis methods for the two purposes are different, said Simmons. For TMB, he said PGDx and others de-prioritize resistance mutations that are enriched after treatment with tumor-targeted therapies, and "hotspot" regions of the genome that are prone to mutations in many patients.

"Hotspot mutations don't differentiate between a high mutation burden cancer and low mutation burden cancer," said Simmons. "It doesn't really capture the biology of neoantigens that are really what's driving the T cell response."

Fabrizio said Foundation's panel includes genes in the DNA damage response pathway whose mutant forms are likely to drive accumulation of additional mutations.

The panels also often include genes important for cancer biology, that don't yet have a clear link to drug response or resistance.

"There aren't 500 clinically relevant genes," said Simmons. His company looks at pathways commonly involved in cancer and drug resistance, and at how prevalent alterations are across the exome, "to make sure that we're covering the cancer genome."

Another consideration is whether to use reference genomes to calculate TMB, or to match tumor sequences to normal tissue from the same patient.

Foundation and PGDx are using the former. "We want to make sure we're making a test that is deployable and has all the efficiencies needed to enter routine clinical practice," said Simmons.

Fabrizio acknowledged public reference genomes don't fully account for patient diversity. Foundation supplements them with private databases of rare germline variants and computational methods to find out whether mutations are germline or somatic; the latter is more likely to be specific to the tumor.

FUTURE BURDEN

BMS's Averbuch believes TMB will be most effective when combined with other biomarkers, such as markers of host inflammation or resistance.

"Probably composite biomarker approaches will more precisely define opportunities for response, especially when you're thinking about combination immunotherapies with different mechanisms of action," he said.

He thinks TMB and PD-L1 are "very complementary" biomarkers. "They don't correlate with one another; they're telling us about different aspects about the tumor-host environment and the immunobiology."

But combining different diagnostic technologies like IHC and NGS-based panels with rapid turnaround for patient decision-making will be challenging, said Averbuch. "We put a lot of effort into collaborations with the diagnostic industry to help us sort through that."

BMS is also exploring the predictive value of additional host, tumor and microbial factors, both internally and through a five-year collaboration with Johns Hopkins University launched in 2016.

An open question is whether directly counting neoantigens will enable better predictions than TMB.

"It's very plausible that the quality of the neoantigens will be very important, especially in tumors where there is low TMB," Averbuch said. BMS is investigating this as well, internally and through collaborations.

Merck's Rubin said that while the link between TMB and neoantigen load is the leading explanation for TMB's predictive value, there are competing theories he thinks are viable.

"There are competing theories that have more to do with other changes that happen in the cell in response to high mutation rates. For example, there are sensor pathways like STING that recognize disturbed DNA," Rubin said. "We're still sorting through that."

COMPANIES AND INSTITUTIONS MENTIONED

American Association for Cancer Research (AACR), Philadelphia, Pa.
AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Bristol-Myers Squibb Co. (NYSE:BMY), New York, N.Y.
Caris Life Sciences Inc., Irving, Texas
Foundation Medicine Inc. (NASDAQ: FMI), Cambridge, Mass.
Friends of Cancer Research, Washington, D.C.
Genentech Inc., South San Francisco, Calif.
Illumina Inc. (NASDAQ: ILMN), San Diego, Calif.
Johns Hopkins University, Baltimore, Md.
Merck & Co. Inc. (NYSE:MRK), Kenilworth, N.J.
Merck KGaA (Xetra:MRK), Darmstadt, Germany
Personal Genome Diagnostics Inc., Baltimore, Md.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Roche (SIX:ROG; OTCQX:RHHBY), Basel, Switzerland
U.S. Food and Drug Administration (FDA), Silver Spring, Md.

TARGETS AND COMPOUNDS

PD-1 (PDCD1; CD279) - Programmed cell death 1
PD-L1 (B7-H1; CD274) - Programmed cell death 1 ligand 1
STING (TMEM173) - Transmembrane protein 173

REFERENCES

Hirsch, F., et al. **"PD-L1 immunohistochemistry assays for lung cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project."** *Journal of Thoracic Oncology* (2017)
McCallister, E. **"Necessary adjustment."** *BioCentury* (2018)
Hansen, S. **"The devil is in the assay."** *BioCentury* (2016)
Tkach, K. **"Unmarked territory."** *BioCentury Innovations* (2016)
Tuzman, K. **"Quality over quantity."** *BioCentury Innovations* (2018)