Q² Solutions®

The Importance of TMB Standardization for Diagnostic Assays

Tumor Mutational Burden (TMB) is a predictive biomarker for response to immune checkpoint inhibitor therapy. Laboratories routinely measure TMB using a variety of NGS panels, employing different chemistries and informatics algorithms. Standardization of TMB scores by using a common set of reference standards allows for a more direct comparison of results and straightforward interpretation from tests performed in different labs and platforms.

Highlights

- Estimates of TMB based on NGS panels show variability across laboratories and platforms.
- Friends of Cancer Research *(Friends)* has initiated the TMB Harmonization Consortium to drive development and interpretation of the TMB biomarker.
- The consortium comprises 30 organizations, including Q² Solutions (laboratories, companies, and manufacturers), working together to develop sustainable reference standard cell lines for use as a reliable alignment tool in measurement of TMB.
- TMB generated from panel-based and Whole Exome Sequencing (WES)-based will be compared for robustness of TMB.

The importance of standardization

It's important to better understand how to identify subsets of patients who are more likely to respond to immunotherapies. This has led, for example, to identifying PD-L1, TMB, and MSI-high as predictive biomarkers for immune checkpoint therapy.

PD-1/PD-L1 assays are used as companion diagnostics (CDx) for several different immune checkpoint inhibitors. These tests confirm the abundance of the checkpoint inhibitor protein these drugs are meant to inhibit. The identification of these various biomarkers in patients has real implications in terms of guiding treatment decision making for patients and physicians. There are multiple CDx tests for measuring PD-L1 levels, and while they measure the same underlying feature, their representation of levels are different. Ultimately, the differences among tests, due to different platforms, assays and cutoffs, select for slightly different patient characteristics and can become problematic for assessing and comparing efficacy of similar therapeutics.

Tumor Mutational Burden is following the same growth of biomarker interest PD-L1 had a decade ago. Drug manufacturers are already evaluating its utility as a CDx in dozens of therapeutic indications leveraging different technologies. Considering these independent pursuits, the biomarker thresholds identified will be variable across a therapeutic, molecular and (now) technological perspective, making their broad interpretation difficult. Again, this will make the outcomes incomparable.

Harmonization directs focus and can help alleviate this problem. It's important to make sure that the assays being used are accurate across labs and that there's strong evidence to support their use.

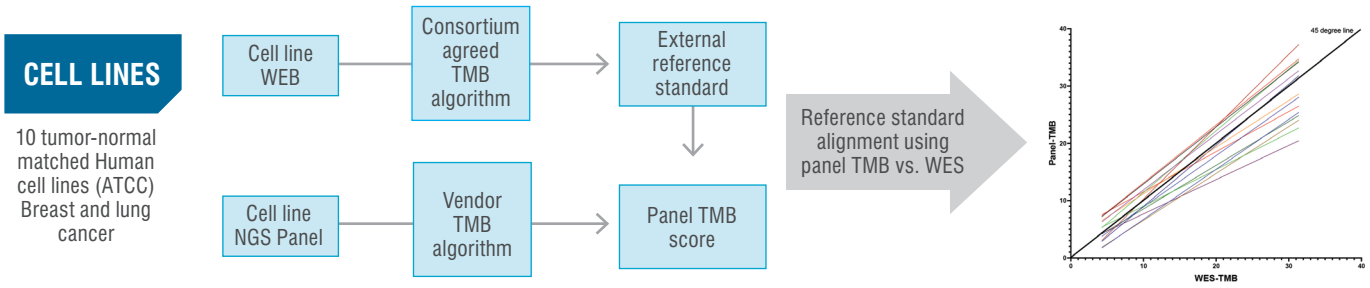
Being able to compare data across laboratories to known reference standards will reduce the likelihood of patients being prescribed treatments that are unsuccessful because they were tested on a different platform than was indicated.

It is very difficult for drug manufacturers to go back and harmonize their results once they've gone through the FDA approval process. It often requires an entirely new application to the agency, which can be time- and cost-restrictive. So how do these different tests relate to each other? And how can they be harmonized?

The TMB Harmonization Consortium

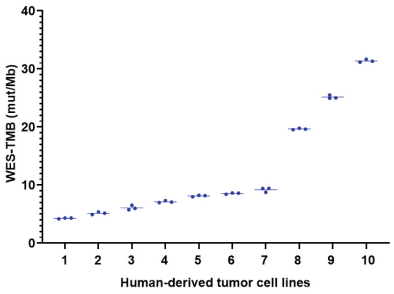
Friends of Cancer Research has initiated a TMB Harmonization Consortium to develop sustainable reference standard cell lines for use as a reliable alignment tool in measurement of TMB (Figure 1).

Figure 1: *Flowchart of the empirical phase of the Friends of Cancer Research TMB Harmonization Project*



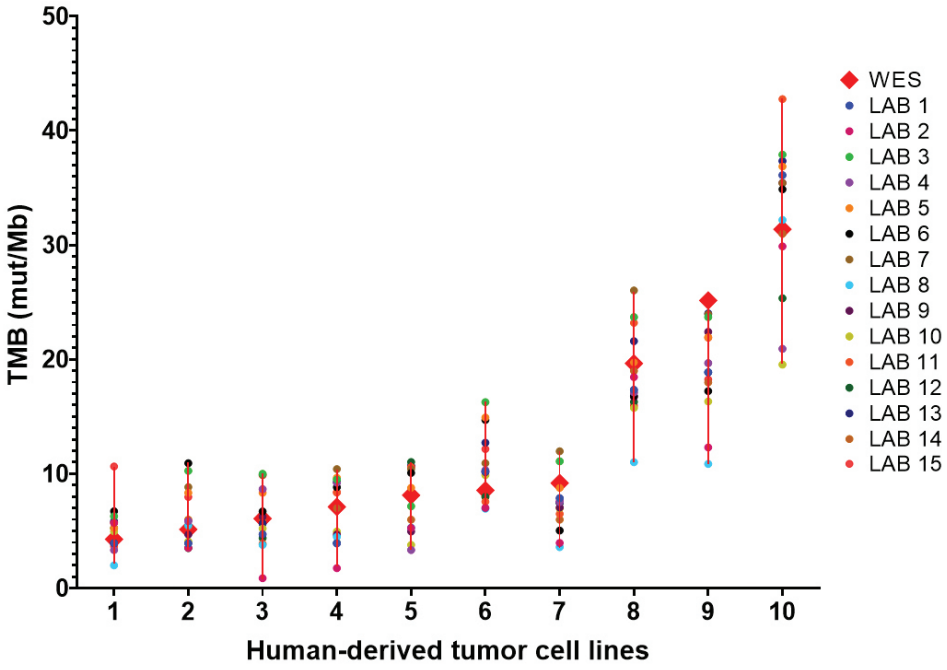
Source: *Friends* Poster

Figure 2: *WES-TMB values for the human-derived cell-line-based reference standard. Each cell line was run in triplicate and TMB was calculated using the TMB Harmonization Project uniform method*



Source: *Friends* Poster

Figure 3: *Variability in TMB estimates for each tumor cell line across all 15 participating laboratories*



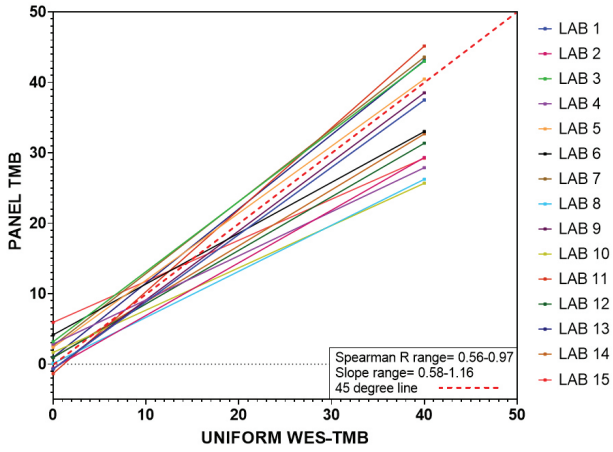
Source: *Friends* Poster

TMB is more complicated than an IHC assay for PD-1 in that it leverages Next Generation Sequencing (NGS) to generate a composite score from tens of thousands – or in some cases, millions – of bases of sequence. TMB summarizes a collection of somatic variants (specific type of mutations) in a singular value versus a more qualitative range of expression levels done by looking at proteins in a microscope.

The consortium has analyzed a set of 10 cell-line-based reference standards. Each cell line was analyzed for TMB using the same Whole Exome Sequencing protocol and bioinformatics pipeline (Figure 2).

These cell lines were analyzed by 15 different laboratories using targeted NGS panels in various stages of development. The data was similar but inconsistent. The variability was higher in cell lines that had higher levels of TMB by WES (Figure 3). Looking at this figure, one can see the variability of TMB scores across labs. Since numerical thresholds for treatment decisions are established in a trial, you can see the variability of that same threshold across technologies. Building a standard each lab can reference will serve to calibrate the variability and control for bias.

Figure 4: *Association between WES-TMB and panel-TMB for 15 participating laboratories using human-derived matched tumor-normal cell lines*



Source: *Friends* Poster

As a result, the different companies that are making these tests have a means to standardized values according to the reference standard. TMB measurements made on any platform will then be directly comparable to the results from these cell lines made on any other platform.

Important conclusions about the harmonization project:

1. Initiatives must begin early enough to impact the development of the diagnostic tests.
2. Transparency among members is critical.
3. Stakeholders must be present and willing to openly discuss data and issues with interpretation.
4. Investment into a universal TMB control will facilitate cross-laboratory calibration for this biomarker.

Ideally, this could lead to developing an overarching playbook that will guide harmonization of both TMB and emerging new complex biomarkers as they become available.

Q^2 Solutions has experience working with instrument vendors and diagnostic manufacturers who create clinical tests, along with pharmaceutical companies who leverage these tests in trials to determine drug efficacy by providing genomic testing, biomarker development and experimental design. We are pleased to leverage this experience in our partnership with Friends of Cancer Research. In particular, Q^2 Solutions processes samples and provides data from our comprehensive cancer panel to the consortia, along with advising and elaborating the Statistical Analysis plan used in Phase II.

Friends of Cancer Research (*Friends*) poster:

TMB Standardization by Alignment to Reference Standards
Phase 2 of the *Friends* TMB Harmonization Project

https://www.Q2LabSolutions.com/en/library/scientific-posters/tumor-mutational-burden-standardization

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