Tumor mutational burden (TMB) is a measure of the number of somatic mutations and a predictive biomarker of response to immune checkpoint inhibitors (ICIs) across several cancers. TMB can be estimated using targeted next-generation sequencing (NGS), but differences in quantification can arise based on platform differences, testing panel size and composition, and bioinformatic algorithms. Harmonization of methods to quantify TMB will facilitate biomarker development and optimize clinical utilization and treatment decision-making. Friends of Cancer Research (Friends) convened a group of leading diagnostic experts to assess and identify sources of TMB variability and determine best practices for harmonizing TMB estimation to ensure consistent clinical interpretation in the future.

Methods: Eleven diagnostic members of the Friends TMB Harmonization Team used whole exome sequencing (WES) data from The Cancer Genome Atlas (TCGA) MC3 samples, comprising 32 cancer types. Each diagnostic partner calculated TMB from the subset of the exome restricted to the genes covered by their targeted panel and using their own bioinformatics pipeline (panel-derived TMB). A "gold-standard" TMB estimate was calculated from the entire exome using a uniform bioinformatics pipeline that all members agreed upon (WES-derived TMB). Linear regression analyses were performed to investigate relationships between WES-derived TMB and each panel-derived TMB. Exploratory analyses by cancer type were also performed. Bias and variability in TMB estimates across panel-derived TMB values relative to WES-derived TMB were assessed.

Results: In silico quantification of TMB is relatively consistent between panels across a wide range of TMB values (0-40 mut/Mb). Panel-derived TMB strongly correlated with WES-derived TMB (regression R^2 values range across panels 0.85-0.93, with slopes ranging from 0.82-1.37). Variation in TMB quantification was attributable to unique composition and technical specifications of each panel, as well as differences in bioinformatics algorithms and approaches to counting somatic mutations. Exploratory analyses suggested possible cancer type dependence for the relationship of panel vs WES-derived TMB, meriting further investigation.

Conclusions: In this in silico analysis, panel-derived TMB was strongly associated to WES-derived TMB. Some variation in TMB quantification across panel-based diagnostic platforms exists. Identifying factors that contribute to variation will facilitate harmonization and help ensure appropriate use and implementation of tests results in the clinic. Subsequent steps will assess the impact of biologic factors (e.g. specimen type, cancer type) on TMB estimation on TMB's association to clinical outcomes, align standards, and define best practices for TMB quantification.

Friends of Cancer Research TMB Harmonization Project

Multi-stakeholder working group to align on and publish universal best practices for defining TMB, analytic validation, and alignment against reference standards.

Academia: Columbia University, Johns Hopkins University, Memorial Sloan Kettering Cancer Center; Diagnostics: ACT Genomics, Caris Life Sciences, Foundation Medicine, Guardant Health, Illumina, NeoGenomics Laboratories, OmniSeq, Personal Genome Diagnostics, QIAGEN, Thermo Fisher Scientific; Government: U.S. FDA, NCI, Pharma: AstraZeneca, Bristol-Myers Squibb, Genentech, EMD Serono, Merck, Pfizer, Regeneron Pharmaceuticals

METHODS

1. TMB Harmanization Team Uniform Method
   - Types of mutations counted: missense, nonsense, in-frame insertions & deletions, and frame-shift insertions & deletions
   - Sample QC metric: Discard sample when ≥ 50% of total variants don’t meet PASS filter
   - Variant allele frequency (VAF) ≥ 0.05
   - Tumor depth (coverage) ≥ 25
   - Minimum variant count ≥ 3
   - Denominator (stop minus start) = 32.10 Mb

2. TCGA data
3. WES TMB Score
4. Panel TMB Score
5. Concordance WES TMB vs Panel TMB scores

Source: PanCancerAtlas (McShane et al.) 2018 (modified)

ABSTRACT

Background: Tumor mutational burden (TMB) is a measure of the number of somatic mutations and a predictive biomarker of response to immune checkpoint inhibitors (ICIs) across several cancers. TMB can be estimated using targeted next-generation sequencing (NGS), but differences in quantification can arise based on platform differences, testing panel size and composition, and bioinformatic algorithms. Harmonization of methods to quantify TMB will facilitate biomarker development and optimize clinical utilization and treatment decision-making. Friends of Cancer Research (Friends) convened a group of leading diagnostic experts to assess and identify sources of TMB variability and determine best practices for harmonizing TMB estimation to ensure consistent clinical interpretation in the future.

Methods: Eleven diagnostic members of the Friends TMB Harmonization Team used whole exome sequencing (WES) data from The Cancer Genome Atlas (TCGA) MC3 samples, comprising 32 cancer types. Each diagnostic partner calculated TMB from the subset of the exome restricted to the genes covered by their targeted panel and using their own bioinformatics pipeline (panel-derived TMB). A “gold-standard” TMB estimate was calculated from the entire exome using a uniform bioinformatics pipeline that all members agreed upon (WES-derived TMB). Linear regression analyses were performed to investigate relationships between WES-derived TMB and each panel-derived TMB. Exploratory analyses by cancer type were also performed. Bias and variability in TMB estimates across panel-derived TMB values relative to WES-derived TMB were assessed.

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Conclusions: In this in silico analysis, panel-derived TMB was strongly associated to WES-derived TMB. Some variation in TMB quantification across panel-based diagnostic platforms exists. Identifying factors that contribute to variation will facilitate harmonization and help ensure appropriate use and implementation of tests results in the clinic. Subsequent steps will assess the impact of biologic factors (e.g. specimen type, cancer type) on TMB estimation on TMB’s association to clinical outcomes, align standards, and define best practices for TMB quantification.

RESULTS

A strong association was observed between WES-TMB and Panel-TMB values, yet some variability exists across panels (Figure 3). Variability in TMB estimation could be attributed to each panel's unique composition and technical specifications, as well as differences in bioinformatics algorithms and types of mutations counted. These unique sources of variability point to the need for alignment against a reference standard.

Figure 1: Overview of the Friends of Cancer Research TMB Harmonization Project

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METHODS

1. Panel-TMB was strongly correlated to WES-TMB in TCGA samples
2. Associations between panel-TMB and WES-TMB were observed to differ by cancer type
3. Theoretical variation in TMB quantification across panel-based diagnostic platforms exists and warrants empirical alignment with reference standard
4. Subsequent steps will include:
   - Assessing the influence of biologic factors (e.g. specimen type, cancer type) on panel-TMB measures
   - Investigating impact of variability in panel-TMB measures and TMB's association to clinical outcomes
   - Defining best practices and standards for alignment of panel-TMB measures

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TMB Harmonization Team:
ACT Genomics, AstraZeneca, BMS, Caris Life Sciences, Columbia University, DNAnexus, EMD Serono, Foundation Medicine, Genentech, Guardant Health, Illumina, Johns Hopkins University, Memorial Sloan Kettering Cancer Center, Merck, National Cancer Institute, NeoGenomics Laboratories, OmniSeq, Pfizer, PGDs, precisionFDA, QIAGEN, Regeneron Pharmaceuticals, SeraCare, Thermo Fisher Scientific, U.S. FDA