# IN SILICO ASSESSMENT OF VARIATION IN TMB QUANTIFICATION ACROSS DIAGNOSTIC PLATFORMS: PHASE 1 OF THE FRIENDS OF CANCER RESEARCH HARMONIZATION PROJECT

FRIENDS of CANCER RESEARCH

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## 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 (ICI) 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 partners 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 relationship 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 effect of biologic factors (e.g. specimen type, cancer type, the impact of variation in 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.

| | Analytical Validation | | Clinical Validation → |
|---|---|---|---|
| **Workflow** | **Phase 1: In silico analysis** | **Phase 2: Empirical analysis** | **Phase 3: Clinical analysis** |
| **Samples** | Publicly available TCGA data | Cells derived from human tumors | Clinical Samples |
| **Goals** | Identify sources of variability between TMB calculated using whole exome sequencing (WES) & various targeted panels used in the clinic | Agree upon creation of a universal reference standard using WES<br>Identify sources of variability after alignment of TMB scores from targeted panels to the reference standard | Propose standards for defining clinical application of TMB and inform clinical use |
| **Timeframe** | **May 2018** | **Fall 2018** | **Winter 2018/Spring 2019** |

www.focr.org/tmb

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

**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

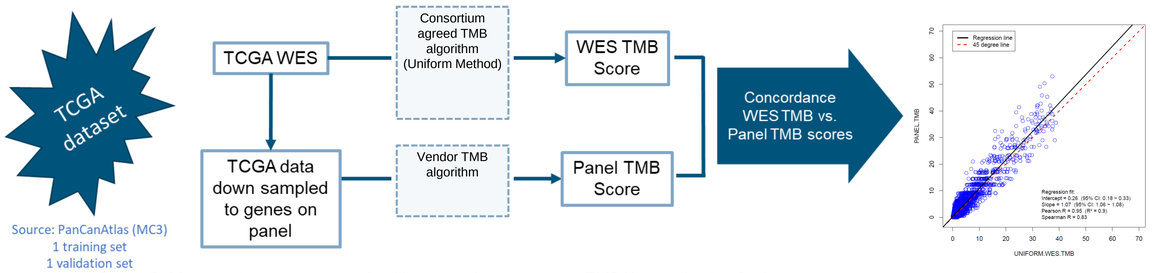


**Figure 2:** Methods flowchart for the in-silico analysis phase of the TMB Harmonization Project

### TMB Harmonization Team Uniform Method

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

## 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.

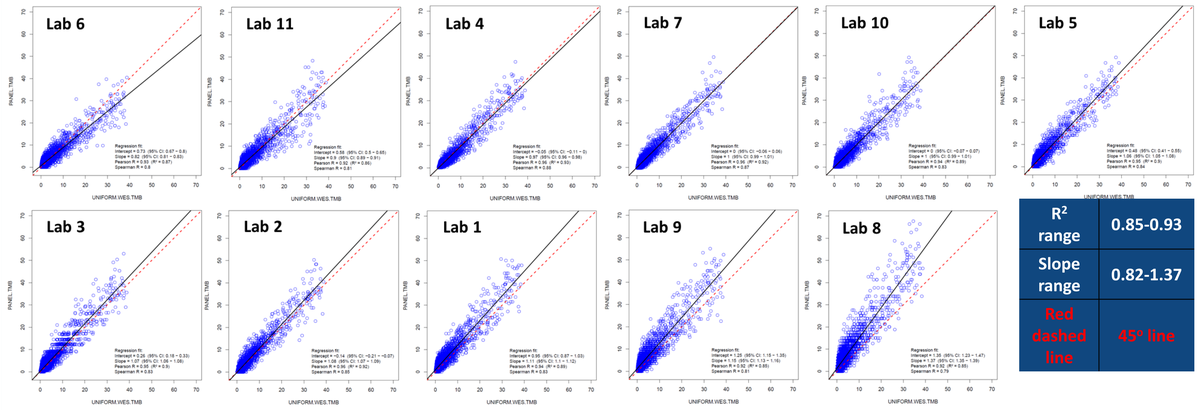


**Figure 3:** Correlation plots for WES-TMB and Panel-TMB values for each of the 11 participating panels. Plots ordered by slope value. Solid black line represents the regression line. Red dashed line represents 45° line

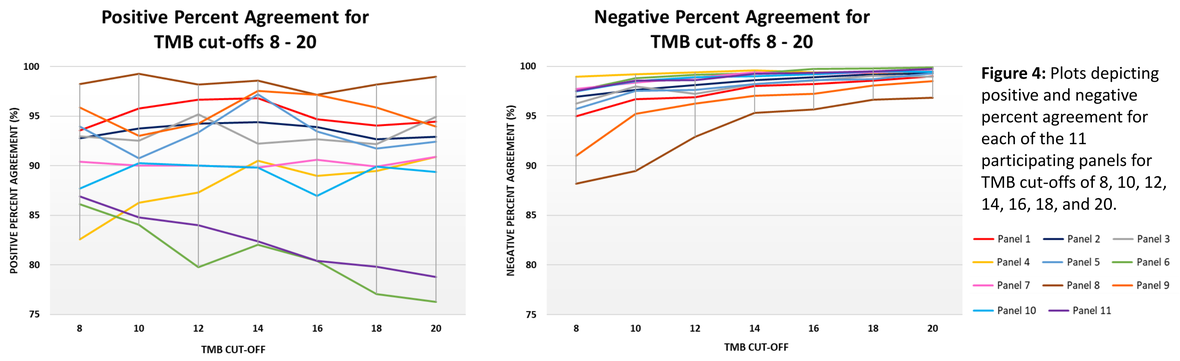


**Figure 4:** Plots depicting positive and negative percent agreement for each of the 11 participating panels for TMB cut-offs of 8, 10, 12, 14, 16, 18, and 20.

Positive (PPA) and negative (NPA) percent agreement were calculated for TMB cut-offs ranging from 8-20 **(Figure 4)**.

Agreement values varied by panel, but positive agreement was observed highest at TMB 10 (84.0%-99.3%), with negative agreement ranging from 89.5% to 99.2% at this cut-off value.

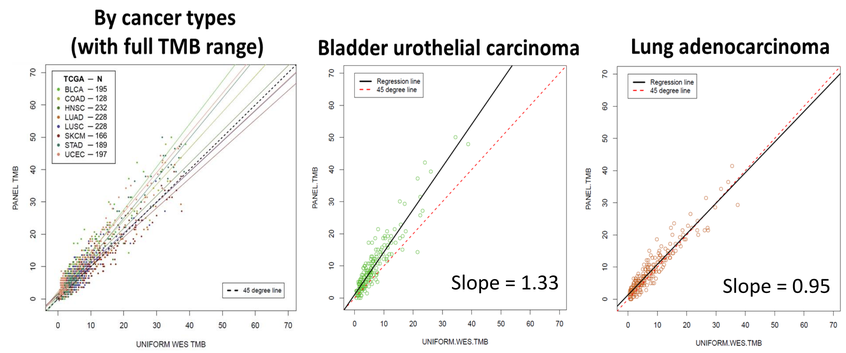


**Figure 5:** Correlation plots for WES-TMB and Panel-TMB values by cancer type. Only cancer types with samples across TMB range of 0-40 were depicted in (A). Bladder urothelial carcinoma (B) and lung adenocarcinoma (C) were selected as representative examples of cancer types for which immune checkpoint inhibitors are indicated

The associations between WES-TMB and panel-TMB appear to differ by cancer type. The TCGA MC3 dataset consisted of 32 different datasets. Only eight of these cancer types had more than 10 samples whose WES-TMB values spanned a clinically meaningful TMB range (TMB 0-40, **Figure 5**). Exploratory investigation of the association between WES-TMB and panel-TMB by cancer type suggested that regression slopes may differ by cancer type and across panel used.

## CONCLUSIONS & FUTURE DIRECTIONS

- Panel-TMB was strongly correlated to WES-TMB in TCGA samples
- Associations between panel-TMB and WES-TMB were observed to differ by cancer type
- Theoretical variation in TMB quantification across panel-based diagnostic platforms exists and warrants empirical alignment with reference standard
- 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:**
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