Alignment of TMB measured on clinical samples: Phase 2B of the Friends of Cancer Research TMB Harmonization Project

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Disclosure of Relevant Financial Relationships

I have no relevant financial relationships to disclose
Tumor Mutational Burden (TMB)

• A measure of the number of somatic mutations per area of the tumor’s genome (mut/Mb)

• High TMB occurs in numerous tumor types and evidence is growing for the association of TMB with neoantigen load

• TMB is a predictive biomarker and has been shown to correlate with clinical benefit from cancer immunotherapies

• Methods of TMB estimation and reporting vary widely across clinical studies

Friends of Cancer Research
TMB Harmonization Project

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

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<td>Cells derived from human tumors</td>
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<td>Goals</td>
<td>Identify sources of variability between TMB calculated using whole exome sequencing (WES) &amp; various targeted panels used in the clinic</td>
<td>Agree upon creation of a universal reference standard using WES</td>
<td>Propose standards for defining clinical application of TMB and inform clinical use</td>
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Clinical Samples

- 25 tumor-normal matched FFPE clinical samples
- DNA extracted in reference lab
- Different cancer types (lung, bladder, gastric)

Phase 2B: Empirical Analysis - Clinical Samples

- Clinical samples WES
- Consortium agreed TMB algorithm
- Clinical samples WES-TMB score

- Clinical samples NGS panel
- Lab TMB algorithm
- Panel TMB score

Assessing empirical variability in WES TMB vs. panel TMB
Phase 2B: Empirical Analysis - Clinical Samples

25 tumor-normal matched FFPE clinical samples
DNA extracted in reference lab
Different cancer types (lung, bladder, gastric)

Clinical Samples

Clinical samples
WES

Clinical samples
NGS panel

Lab TMB
algorithm

Panel TMB
score

Assessing empirical variability in WES TMB vs. panel TMB

3 calibration methods

All TCGA WES vs. panel

Stratum 1 TCGA WES vs. panel

Cell line WES vs. panel

Apply calibration approaches to align across NGS panels

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Variability in estimated association between panel TMB and WES TMB across participating laboratories
Variability in estimated association between panel TMB and WES TMB across participating laboratories

All TCGA Samples (N=4065)

Stratum 1 TCGA Samples (N=1563)

Cell Line Samples (N=10)
Calibration approaches using TCGA & cell lines as reference standards

\[ y_0 = \text{observed panel TMB} \]

\[ x_0 = \text{estimated WES TMB calculated from calibration curve at observed } y_0 \]

\( (\text{CLL}_{95}(y_0), \text{CUL}_{95}(y_0)) = \text{interval of uncertainty around } x_0 \)
Calibration approaches using TCGA & cell lines as reference standards

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Application of Three Calibration Approaches to Lung Clinical Samples (N=10)

- Uncalibrated clinical samples
- Calibration with ALL TCGA
- Calibration with Stratum 1 TCGA
- Calibration with cell lines

![Graph showing TMB (mut/Mb) analysis for different samples and calibration approaches.](chart)

- Labels: WES, Lab.1, Lab.2, Lab.3, Lab.4, Lab.6, Lab.7, Lab.8, Lab.9, Lab.10, Lab.11, Lab.12, Lab.13, Lab.14, Lab.15, Lab.16
- Axes: TMB (mut/Mb) on the y-axis, Sample IDs on the x-axis

Legend:
-● WES
-○ Uncalibrated clinical samples
-△ Calibration with ALL TCGA
-◆ Calibration with Stratum 1 TCGA
-❖ Calibration with cell lines
Application of Three Calibration Approaches to Lung Clinical Samples (N=10)
Phase 2B: Summary of Findings

• Variability in the association between panel TMB and WES TMB across participating laboratories when testing FFPE-derived tumor samples was similar to that observed in TCGA samples and cell lines.

• Calibration approaches using TCGA data were more robust than those using a relatively small number of cell line samples, minimizing the spread in panel TMB values for clinical samples.

• Calibration methods using TCGA may be a viable approach to align across panel TMB scores.

• Ongoing work will explore whether TCGA calibration methods perform consistently in additional tumor samples, including TMB-high samples.
Convening stakeholders early in the development of complex diagnostics will help identify key challenges and streamline measures to solve them.

Collaborative approach is necessary to improve consistency and reliability of panel TMB estimates to be used in the clinic.

Scientific and regulatory approaches are necessary for alignment on optimal performance thresholds and standards development.
TMB Harmonization Consortium

**Government:** National Cancer Institute (NCI), U.S. Food and Drug Administration (FDA)  
**Academia:** Brigham & Women’s Hospital, College of American Pathologists (CAP), Columbia University, EORTC, Genomic Testing Cooperative, Hartwig Medical Foundation, Johns Hopkins University, Massachusetts General Hospital, MD Anderson Cancer Center, Memorial Sloan Kettering Cancer Center, Quality in Pathology (QuIP), University of Heidelberg  
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**Industry:** AstraZeneca, Bristol-Myers Squibb Company, EMD Serono, Inc., Genentech, Merck & Co., Inc., Pfizer, Inc., Regeneron Pharmaceuticals  
**Operational:** precisionFDA, SeraCare
Thank you!

For more information and updates, visit focr.org/tmb

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