Background: Tumor mutational burden (TMB) is a predictive biomarker of response to immune checkpoint inhibitors across multiple cancers. In Phase 1 of the TMB Harmonization Project, we demonstrated a robust correlation between TMB estimated using targeted NGS gene panels and whole exome sequencing (WES) applied to matched tumor vs. normal DNA. These findings demonstrated the potential for TMB to serve as a reliable alignment tool and had WES-TMB values spanning a clinically relevant range (4.3–21.4 mut/Mb).

Methods: Ten (2 breast, 8 lung cancer) publicly available human derived tumor cell lines were propagated to assess the reproducibility of TMB estimates across laboratories and to validate integration of increasing TMB values. Each laboratory used their own sequencing and bioinformatics pipelines to estimate TMB according to genes represented in their respective panels (panel-TMB). The association between WES-TMB and each panel-TMB was investigated using regression analyses. Variability in TMB estimates across panels was rigorously assessed. All analyses were blinded.

Conclusion: Preliminary findings demonstrate feasibility of using tumor derived matched tumor cell lines to assess the variability and promote alignment of TMB across different targeted NGS assays. Future studies aim to validate reference standards using a reliable alignment tool by using formalin-fixed paraffin-embedded human tumor samples.

Results: The set of reference standards spanned a clinically meaningful TMB range (4.3 to 31.4 mut/Mb). Data from 15 laboratories shows a good correlation generated from multiple assays.

Methods: Ten (2 breast, 8 lung cancer) publicly available human-derived matched tumor normal-cell lines were provided by Saracare and passed 2±x according to the culture methods provided.

DNA was extracted using the QIAGEN Gentra Puregene Kit. QC analysis of DNA was assessed fluorometrically.

Frederick National Laboratory for Cancer Research calculated WES-TMB using the previously described unified method (Dobrovitski et al., 2018) using 2 Novaseq S4 flowcells generating ~400M PE 150bp reads on tumor and ~135M reads on normal samples.

Median target coverage: Tumor >4000X; Normal >2000X

GATK based Senteion pipeline was used to call somatic variants from Sanger sequencing

Each participating laboratory ran the samples in duplicate/triplicate using their own sequencing platforms and pipelines.

A weighted least squares model was fit for each panel's data to account for heteroscedasticity in errors. Each model was fit using maximum likelihood implementation in the clinical setting.

Figure 1: Flowchart of the Empirical Phase of the Friends of Cancer Research TMB Harmonization Project

Figure 2: WES-TMB values for the human-derived cell line based WES-TMB values spanning a clinically relevant range (4.3–21.4 mut/Mb).

Figure 3: Variability in panel-TMB estimates was consistent across all laboratories and incorporating increasing with increasing TMB values.

Figure 4: Association between panel-TMB and WES-TMB varied across panels with slopes ranging between 1.07-1.16. Spearman's rank correlation coefficient ranged between 0.56-0.97.

Figure 5: Variability in TMB estimates for each tumor across the participating laboratories.

Figure 6: TMB standardization by alignment to reference standards

Phase 2 of the Friends of Cancer Research TMB Harmonization Project

TMB Standardization by Alignment to Reference Standards

Conclusions & Future Directions

There is variability in TMB estimates across laboratories.

Variability depends on panel specifications, in house algorithms, and absolute TMB values.

The non-constant variation across a spectrum of TMB values supports the need for alignment to a reference control. This approach can maximize consistency and resolve differences that arise from unique panel specifications and algorithms.

Future studies will focus on the use of human FFPE tumor samples to validate reference standards for use as a reliable alignment tool and implementation in the clinical setting.

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TMB Harmonization Consortium

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