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

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Summary

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 TCGA data. These findings demonstrated the theoretical variability in TMB estimates across panels. Phase 2 employs sustainable TMB reference standard materials to assess the empirical variability in TMB estimates. The goal of this Project is to harmonize TMB estimates through alignment to reference standards in order to improve consistency across panels, for the sake of optimizing clinical application and facilitating integration of datasets generated from multiple assays.

Methods: Fifteen laboratories with targeted panels at different stages of development participated. We identified a set of reference standards consisting of 10 well-characterized human-derived lung and breast tumor-normal matched cell lines. WES was performed using a uniform bioinformatics pipeline agreed upon by all team members (WES-TMB). 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 were rigorously assessed. All analyses were blinded.

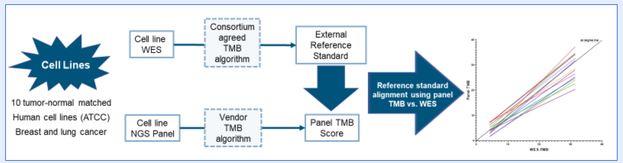

Figure 1: Flowchart of the Empirical Phase of the Friends of Cancer Research TMB Harmonization Project

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 between panel-TMB and WES-TMB in this empirical analysis. Across laboratories, Spearman R values range 0.56-0.97 with slopes ranging 0.58-1.16. We observed the variability across laboratories tended to increase with increasing WES-TMB value. Some laboratories had consistently over- or underestimated TMB values, while other laboratories only overestimated at low WES-TMB values (5-15 mut/Mb).

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

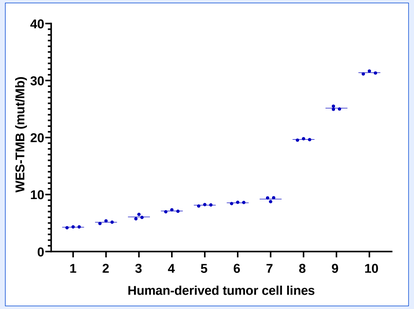

Figure 2: WES-TMB values for the human-derived cell line based reference standard. Each cell line was run in triplicate and TMB was calculated using the TMB Harmonization Project uniform method

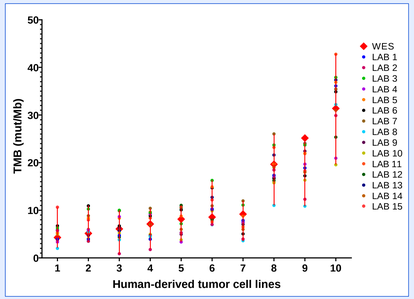

Figure 3: Variability in TMB estimates for each tumor cell line across all 15 participating laboratories

Results

- A set of cell line-based reference standards was identified to help with panel-TMB alignment and had WES-TMB values spanning a clinically relevant range (4.3-31.4 mut/Mb). **(Figure 2)**
- Variability in panel-TMB estimates was consistent across all laboratories and increased with increasing TMB value. **(Figure 3)**
- Absolute mean difference values between panel-TMB and WES-TMB ranged between 1.51 and 4.13 for all the cell lines assessed across laboratories.
- The mean and range of absolute difference values were ±1.53 (0.3-6.4) at 4.3 mut/Mb; ± 2.6 (0.3-5.6) at 9.2 mut/Mb; and ± 5.12 (0.1-11.8) at 31.4 mut/Mb.
- Correlation between panel-TMB and WES-TMB varied across panels with slopes ranging between 0.58-1.16. Spearman's rank correlation coefficient ranged between 0.56-0.97. **(Figure 4)**

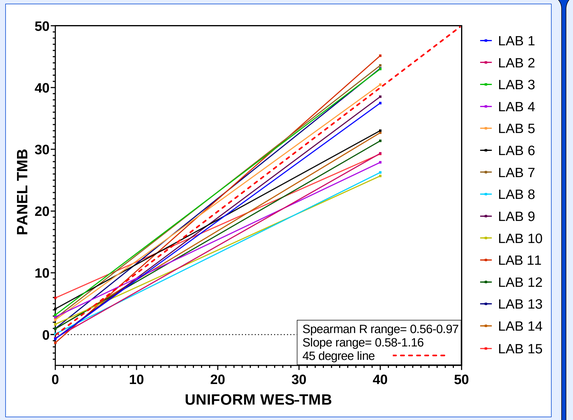

Figure 4: Association between WES-TMB and panel-TMB for 15 participating laboratories using human-derived matched tumor-normal cell lines

- Eight laboratories consistently either over- or underestimated TMB values (Labs 1,2,3,5,7,8,9, and 13).
- For all remaining laboratories, the relationship between panel-TMB and WES-TMB changed at different TMB values, with most panels overestimating at lower TMB values (WES-TMB<15 mut/Mb) and underestimating at higher TMB values (>15 mut/Mb) showing that the association between panel-TMB and WES-TMB is not constant across a relevant spectrum of TMB values.

Methods

- Ten (2 breast, 8 lung cancer) publicly available human-derived matched tumor-normal cell lines were provided by SeraCare and passaged 2-5x according to the culture methods provided.
- DNA was extracted using the QIAGEN Gentra Puregene Kit. QC analysis and quantification was assessed fluorometrically.
- Frederick National Laboratory for Cancer Research calculated WES-TMB using the previously described uniform method *(Fabrizio 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 >400X; Normal >200X
- GATK based Sentieon pipeline was used to call somatic variants *(https://github.com/FNL-MoCha/nextgenseq_pipeline)*
- Each participating laboratory ran the samples in duplicate/triplicate using their own sequencing platforms and panels.
- 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 with a linear mean structure and power variance structure.
- Descriptive analyses plotted Panel-TMB vs WES-TMB value for replicates and with median value. Mean of WES-TMB was used for the rest of the analyses.

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.

Acknowledgements & References

TMB Harmonization Consortium

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Fabrizio et al., 2018 J Immunother Cancer. 2018;6(Suppl 2):1–13