Harmonization of Tumor Mutation Burden estimation: comparison of different TMB testing

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Introduction

The advent of immune checkpoint inhibitors (ICIs) has revolutionized cancer therapy, but reliable biomarkers for patients' selection patients are still lacking. Tumor Mutational Burden (TMB) has emerged as a potential biomarker for ICIs, including non-small cell lung cancer (NSCLC) [1]. TMB is the number of mutations per Megabase in the coding area of the genome. In the clinical trial leading to the approval of TMB as biomarker, the FoundationOne CDx (F1CDx) (Foundation Medicine Inc.) panel was used, with a cut-off of 10 muts/Mb to define a high TMB value [2,3]. Different target sequencing tests, formalin fixation and bioinformatics platforms can affect TMB estimation. Establishing validated cut-off values is crucial for patient selection. Many laboratories report TMB values without clinical interpretation, highlighting the need for validation data and standardized cut-off values.

Aim

The aim of this study is to perform a comparison of TMB testing with three commercially available NGS panels to create a reference table for mapping TMB scores between tests.

Methods

Genomic DNA (gDNA) was isolated from FFPE tissue sections. TMB was calculated using four different methods. Oncomine Comprehensive Plus Assay (OCA) from Thermo Fisher Scientific covers 1.50M total bases, including 1.06M exonic bases of 500+ cancer-associated genes. Libraries were prepared using Ion AmpliSeqTM Library Kit Plus from 20ng of gDNA and sequenced on Ion S5TM XL sequencer. TruSight Oncology 500 Assay (TSO500) from Illumina, analyzes 523 genes in a 1.3 Mb coding region. gDNA (80 ng) were fragmented using M-220 Focused-ultrasonicator. QIAseq® Multimodal Panel (QIA) from Qiagen, targets relevant mutations in tumor-related genes, covering 1.44 Mb of DNA with Single Primer Extension technology. Both panels were sequenced on NextSeq® 500 using High Output reagents. FoundationOne CDx (F1CDx) is the reference standard method analyzing exonic regions of 324 cancer-related genes and selected introns from 51 genes. Analysis was conducted at Roche FMI based on KEYNOTE 158 trial using two 10µm slides per sample.

Statistical analyses were performed on the complete case dataset, removing missing samples for one or more NGS targeted panels. TMB measures for each panel were described with mean and standard deviation (SD) and median value with interquartile range (IQR) to better evaluate the different distributions. Spearman's R correlation values were calculated, and scatterplots were created to assess linearity of the relationship between each pair of panel. Measurements of agreement between F1CDx, as gold standard, and the other panels were evaluated using the concordance correlation coefficient (CCC) with the relative 95% confidence interval (95% CI). Furthermore, the bias between two panels (F1CDx as gold-standard) was evaluated with a Bland and Altman plot. The complete case samples were categorized according to F1CDx TMB value ≥10 mut/Mb and further investigated. The ability discriminatory of each panel, with respect to F1CDx method as reference, was studied using the area under the receiver operating characteristic curve (ROC), with relative 95% CI. The decision thresholds were displayed on the ROC plot and, using the Youden Index, a cut-point was estimated for each method. The sensitivity, specificity, positive and negative predictive values, and accuracy were estimated to characterize the different cut-off points. Statistical analysis was performed using the R statistical software R version 4.3.0.

Results

Sixty NSCLC FFPE samples were tested with four different panels: OCA, TSO500, QIA and F1CDx. The success rate of TMB assessment was 91.7%, 100%, 96.7% and 100% for F1CDx, TSO500, OCA and QIA respectively. The test failures led us to reduce the analysis to 53 samples with available TMB values for the four tests, due to 2 analyses failed with OCA evaluation and 5 with F1CDx. The TMB measured with F1CDx had a mean value of 10.1 (SD 8.6) muts/MB and a median value of 7.6 muts/MB (IQR 10). TSO500, OCA and QIA TMB showed a mean value of 10.7 (SD 9), 9.7 (SD 5.6) and 11.6 (SD 8) muts/MB, and a median value of 8.6 (IQR 7.8), 8.5 (IQR 5.7) and 9.6 (IQR 8.1) muts/MB, respectively. All the measurements had a skewed distribution. CCC showed that there was a higher concordance correlation between F1CDx and TSO500 TMB values (0.95, 95%CI 0.91 – 0.97) than in the other two comparisons (0.76, 95%CI 0.66 – 0.84 for OCA and 0.86, 95%CI 0.78 – 0.92 for QIA, respectively versus F1CDx).

Categorizing the samples by the cut-off value defined by F1CDx, the analyzed cohort of samples consisted of 31 patients (58.5%) with TMB <10 muts/Mb and 22 (41.5%) patients with a TMB ≥10. The Area Under the Curve (AUC) (with 95% CI) for TSO500 was 0.96 (95%CI 0.91-0.99), for OCA 0.83 (95%CI 0.71-0.94) and for QIA 0.88 (95%CI 0.78-0.96). TSO500 shows a higher AUC, and this difference is statistically significant (bootstrap test for two correlated ROC curves: p-value = 0.01 vs OCA; p-value = 0.03 vs QIA) (Figure). The Youden Index calculation allowed to extrapolate a TMB threshold value with higher sensitivity and specificity for TSO500, OCA and QIA measurements. This value was 10.19 for TSO, 10.4 for OCA and 12.37 for QIA, respectively. Considering these cut-offs and compared with the gold-standard of F1CDx, the TSO500 panel showed the best accuracy measures in terms of sensitivity (86%), specificity (94%), accuracy (91%), PPV (90%) and NPV (91%) if compared with OCA and QIA.

Conclusions

Overall, TSO500 demonstrated higher sensitivity, specificity, accuracy, and predictive values compared to the other panels, while OCA and QIA showed similar results. These findings may contribute to the uptake of TMB as a possible biomarker for the selection of patients who have a better chance of benefiting immunotherapy.

Bibliografia

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Figure - ROC curve and relative Area Under the Curve for each TMB test used in the assessment versus F1CDx.