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60 DEVELOPMENT AND VALIDATION OF BLOOD TUMOR MUTATIONAL BURDEN REFERENCE STANDARDS

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Background Tumor mutational burden (TMB), as measured by exome or panel sequencing of tumor tissue (tTMB) or blood (bTMB), has been identified as a potential predictive biomarker for treatment benefit in patients with various cancer types receiving immunotherapy targeting checkpoint inhibitors (e.g. PD-1, PD-L1, CTLA-4). However, significant variability in TMB measurement has been reported due to differences in pre-analytical and laboratory methods, panel size, number of genes covered and bioinformatics pipelines. Reference standards have been proposed and evaluated for tTMB analysis by the Friends of Cancer Research (FoCR) to enable harmonization and standardization across different tTMB panel providers. Reference standards for bTMB are likely to be even more important given the unique challenges and higher sensitivity required for bTMB assays.

Methods Contrived bTMB reference materials with 0.5% and 2% tumor content were developed using DNA from tumor cell lines and donor-matched lymphoblastoid cell lines fragmented and size-selected to mimic cell-free DNA with TMB scores of 7, 9, 20 and 26 mut/Mb. Mutation coverage, mutant allele frequency (MAF) and bTMB scores were assessed using the PredicineATLAS and GuardantOMNI next-generation sequencing (NGS) platforms.

Results The DNA fragment size for the contrived samples was similar to naturally occurring circulating cell-free tumor DNA and mutation patterns were aligned with those from parental tumor lines. As anticipated, low frequency artefactual MAF variants were observed, requiring removal by bioinformatic filtration. For samples with 2% tumor content, standards for 7, 20 and 26 mut/Mb were found to have as-expected bTMB scores across both evaluation platforms, with good reproducibility, following removal of low frequency MAFs. Results for 0.5% tumour content were also promising, although with greater variability in post-filtration bTMB scores observed.

Conclusions The findings demonstrate it is feasible to produce bTMB reference standards across a range of bTMB levels. The data highlight the importance of data filtration to account for underlying low MAFs in such cell-line derived samples and that this reference material can control for variant sensitivity though not variant specificity. bTMB reference standards reported here could support the calibration and validation of bTMB platforms and help harmonization between panels and laboratories, thus improving the accuracy of testing to aid treatment decisions in oncology.

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61 TUMOR MUTATIONAL BURDEN ASSESSMENTS BY TWO COMMERCIAL TARGETED SEQUENCING ASSAYS

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Background Tumor mutational burden (TMB) is a key biomarker for immune checkpoint inhibitor across several cancer types. While TMB as calculated from whole exome sequencing of the tumor tissue is still the gold standard, enabling TMB in clinical labs requires targeted sequencing panels for faster turnaround time and low input requirements. Herein, we assess two commercially available, targeted sequencing (research-use-only) TMB assays for the possibility of offering in the Medgenome labs.

Methods Two assays, OncoPrint Tumor Mutation Load Assay (or OncoPrint) by Thermo Fisher Scientific and QIAseq Tumor Mutational Burden Panel (or QIAseq) by Qiagen, were studied. One negative control (NA12878), five positive control (A549, lung; T47D, breast; SKMEL2, skin; HCT-15, large intestine; HCT116, large intestine) cell lines, and 18 FFPE (13 colon, 1 lung, 1 testicular, and 1 oral cancer; 2 healthy) samples were ran on both assays. Sample QC was performed through measuring DNA fragmentation on TapeStation and concentration on Qubit. Failure rates on FFPE samples were investigated. TMB values by both assays were compared on all samples, as well as with expected TMB on cell line samples. Expected TMB on the negative control was considered zero; expected TMB for positive cell lines was calculated by restricting somatic mutations (from cBioPortal.com) to each panel, normalizing by panel size, and averaging. TMB values of 3 samples with known MSI were evaluated and signature patterns of relatively high TMB samples were studied.

Results On cell line samples, high correlation ($r^2 = 0.9994$) was observed between TMB values by both assays. TMB values were consistently zero on negative control by both assays. Both assays estimated lower than expected TMB on positive control samples. 6/18 FFPE samples failed on both assays, with OncoPrint's error mode was high deamination (i.e., number of C:G>T:A mutations at low allelic frequency) and QIAseq's was low confidence (i.e., < 0.9 Mb sequenced panel). All 6 failed samples showed either low DNA integrity (DIN<2) or low concentration (<6 ng/μl). A combined analysis of all QC pass samples showed high correlation ($r^2 = 0.97$) between two assays. TMB values on two MSI cell lines was > 50 by both assays, but 14 by QIAseq and 33 by OncoPrint on one MSI FFPE sample. Four out of five FFPE samples with > 25 TMB by both assays displayed MSI signature patterns from COSMIC or incorporated a pathogenic mutation in MLH1 gene.

Conclusions Preliminary analyses showed comparative accuracy and failure rates on FFPE samples. Future analyses will aim at comparison with WES based TMB on reference cell line and FFPE material.

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