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% tumor 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 methods 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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