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Real-world applications of tumor mutation burden (TMB) analysis using ctDNA and FFPE samples in various cancer types of Turkish population

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Abstract: Tumor mutation burden (TMB) has become one of the most popular approaches in the last decade as a molecular genetic testing strategy for cancer therapeutics that represents the somatic variations per Mbase in coding regions of the genome and which can be performed via comprehensive genomic profiling (CGP) by next generation sequencing (NGS). TMB is most commonly used to stratify the patients for immunotherapy as well as the actionable variant detection for possible other therapeutics. In this context, within this study, we share our results of the TMB score distribution of cancer patients together with distinctive diagnoses and specimens. The study was conducted from a total of 278 samples. One hundred seventy six (176) of them were formalin-fixed paraffin-embedded (FFPE) tissue samples and 102 liquid biopsy samples. Samples were sequenced using a multi-gene NGS panel consisting of 486 cancer-related genes (Illumina-NextSeq500/550). Bioinformatics analyzes were performed using an optimized in-house bioinformatics pipeline. As a result, the studies of 91.7% (n=255) among all samples were successfully performed in which total of 21 different cancer types were included. The lung cancer group was the most frequent (n=43 patients), followed by 31 colorectal cancer and 22 ovarian cancer patients. The classification of TMB scoring was very high (>50), high (20-50), moderate (5-20) and low (<5). The shared data of this study represents a cancer genome atlas-like data set for TMBs of Turkish cancer patients in relation to various cancer types and specimens in comparison with The Cancer Genome Atlas (TCGA) data.

Introduction

Tumor mutation burden (TMB) is one of the most popular biomarkers in cancer that indicates sensitivity to immunotherapies and have started to be used routinely for immune checkpoint blockade (ICB) response across all cancer types (Lawlor et al., 2021; McGrail et al., 2022). High tumor mutational burden (TMB-H) has shown promise as an indicator for PD-1 inhibitor therapies, such as pembrolizumab that FDA had approved in the cases (Strickler et al., 2021). Even though there are differences in oncological points of view and the clinical approaches in terms of effectivity and utilization in cancer patients, no specific cut-offs have been identified, neither differential specimens nor subtypes of cancers.

Next-generation-sequencing-based comprehensive genomic profiling (CGP) of cancer-related genes has become the most commonly used approach since tumor whole exome sequencing studies were first used to identify TMB scores in cancer. In addition to the identification of TMB scores, CGP also provides to detect the actionable somatic genetic alterations in relation to possible targeted treatment modalities. Even though there are different algorithms in literature to analyze TMB scoring, there are no large cohort studies evaluating the various specimens, such as formalin fixed paraffin embedded tissues and/or liquid biopsies in different cancer types of distinctive populations, to enlighten the fundamentals and utilization.

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Thus, this aim consisted of two components: presenting the cumulative tumor mutation burden data of 278 samples with 21 different cancer types and discussing the findings in comparison with TMB cut-off values presented in the literature.

Material and Methods

Cukurova University's institutional ethical committee approved this work. The Declaration of Helsinki and any subsequent revisions were followed in conducting the study.

Sampling

The samples used in this study were collected from the biobank of the Genetic Diseases Diagnosis Center that the written informed consent had been obtained priorly to the biobanking.

FFPE and ctDNA samples were obtained from 278 patients between 2016-2019. Genomic and circulating tumor DNA was extracted by the methodology previously optimized in our centre (Sonmezler et al., 2020; Boga et al., 2020). Quality controls of genomic materials were assessed using Qubit 4 (Thermo Scientific, USA). Samples with inadequate concentrations were excluded from the study.

NGS Methodology

Sufficient samples went under next-generation sequencing work flow consisting of fragmentation, adapter ligation, target enrichment, library generation and sequencing. A CGP panel (1.3 Mbase) consisting of 486 cancer related genes (*PRKCZ, TNFRSF14, TP73, TNFRSF9, ERRFI1, MTOR, SPEN, SDHB, CNKSR1, ARID1A, RPA2, PSMB2, MYCL, MPL, MUTYH, CDKN2C, NRD1, JUN, JAK1, GADD45A, MSH4, FUBP1, PSMA5, NRAS, VTCN1, NOTCH2, MCL1, FAM46C, CTSS, PSMD4, PSMB4, RIT1, NTRK1, CD48, SDHC, DDR2, TNFSF18, TNFSF4, ABL2, MR1, PTGS2, CDC73, PIK3C2B, MDM4, IKBKE, IRF6, H3F3A, PARP1, FH, EXO1, AKT3, MYCN, DNMT3A, ASXL2, ALK, SOS1, EPCAM, MSH2, MSH6, PSME4, REL, XPO1, POLE4, ERCC3, MCM6, LRP1B, PSMD14, PDK1, NFE2L2, ITGAV, PMS1, SF3B1, CASP8, IDH1, ERBB4, BARD1, XRCC5, CUL3, DNER, PSMD1, RAD18, FANCD2, VHL, RAF1, TGFBR2, MLH1, MYD88, CTNNB1, SETD2, TREX1, RHOA, BAP1, PBRM1, PSMD6, MITF, FOXP1, EPHA3, CD200, CD80, GSK3B, CD86, MCM2, GATA2, EPHB1, PIK3CB, FOXL2, ATR, TERC, PRKCI, PIK3CA, SOX2, PSMD2, RFC4, BCL6, FGFR3, FGFBP1, RFC1, PDGFRA, KIT, KDR, EPHA5, TET2, INPP4B, FBXW7, FAT1, SDHA, TERT, IL7R, RICTOR, MAP3K1, PIK3R1, RAD17, MSH3, RASA1, ERAP1, ERAP2, LNPEP, APC, RAD50, CTNNA1, CSF1R, PDGFRB, GABRA6, NPM1, FGFR4, NSD1, CANX, FLT4, IRF4, PSMG4, HIST1H3B, HLA-F, HLA-G, HLA-A, HLA-E, HLA-C, HLA-B, MICA, MICB, TNF, MSH5, NOTCH4, TAP2, PSMB8, TAP1, PSMB9, TAPBP, DAXX, FANCE, CDKN1A, PIM1, CCND3, VEGFA, MCM3, EPHA7, PRDM1, ROS1, MYB, TNFAIP3, ESR1, ARID1B, IGF2R, PARK2, QKI, PSMB1, PSMG3, CARD11, PMS2, RAC1, RPA3, FKBP9, PSMA2, POLD2, IKZF1, EGFR, RFC2, HGF, CDK6, TRRAP, MCM7, MUC17, CUX1, PSMC2, PIK3CG, MET, SMO, BRAF, SSBP1, KEL, EZH2, RHEB, KMT2C, TNKS, GATA4, CTSB, FGFR1, KAT6A, POLB, PRKDC, MCM4, SOX17, NBN, RUNX1T1, RAD21, MYC, JAK2, CD274, PDCD1LG2, PTPRD, CDKN2A, CDKN2B, FANCG, PAX5, GNAQ, NTRK2, CTSL, SYK, FANCC, PTCH1, PSMD5, PSMB7, ABL1, TSC1, COL5A1, NOTCH1, GATA3, RET, ARID5B, SIRT1, C10orf54, PTEN, FAS, IDE, SUFU, SMC3, TCF7L2, FGFR2, PSMD13, HRAS, IGF2, LMO1, WEE1, PSMA1, FANCF, WT1, PSMC3, MEN1, POLD4, CCND1, FGF19, FGF4, FGF3, POLD3, EED, MRE11A, ATM, SDHD, KMT2A, CBL, CHEK1, KDM5A, CCND2, TAPBPL, CHD4, ETV6, CDKN1B, KRAS, ARID2, KMT2D, ACVR1B, ERBB3, GLI1, CDK4, MDM2, TDG, TCP11L2, PTPN11, TBX3, RFC5, HNF1A, PSMD9, ABCB9, POLE, CDK8, FLT3, FLT1, HMGB1, BRCA2, RFC3, RB1, DIS3, TPP2, ERCC5, IRS2, PSMB5, PSMB11, PSME1, PSME2, PSMA6, NFKBIA, NKX2-1, FOXA1, PSMC6, PSMA3, MLH3, TSHR, PSMC1, LGMN, DICER1, HSP90AA1, AKT1, RAD51, TP53BP1, PDIA3, B2M, HERC1, MAP2K1, SMAD3, CD276, PSMA4, NTRK3, IDH2, BLM, IGF1R, AXIN1, TSC2, CREBBP, GRIN2A, SOCS1, ERCC4, PALB2, CYLD, CBFB, CTCF, PSMB10, CDH1, ZFHX3, PSMD7, PLCG2, FANCA, RPA1, PSMB6, TP53, AURKB, MAP2K4, MYOCD, NCOR1, FLCN, LGALS9, NF1, SUZ12, PSMD11, LIG3, PSMB3, CDK12, ERBB2, PSMD3, RARA, STAT3, PSME3, BRCA1, CDC27, ITGB3, NPEPPS, SPOP, RNF43, RAD51C, BRIP1, PSMC5, CD79B, GNA13, AXIN2, PSMD12, PRKAR1A, SOX9, RPTOR, PSMG2, GATA6, PSMA8, SMAD2, SMAD4, ALPK2, BCL2, STK11, DOT1L, GNA11, MAP2K2, TNFSF9, CD70, TNFSF14, KEAP1, SMARCA4, RNASEH2A, CALR, NOTCH3, BRD4, JAK3, PIK3R2, IFI30, MEF2B, LPAR2, CCNE1, CEBPA, PSMD8, PSMC4, AKT2, AXL, CD79A, CIC, ERCC2, ERCC1, LIG1, POLD1, PPP2R1A, PRKCG, PSMF1, PCNA, BCL2L1, ASXL1, SRC, TOP1, CD40, ZNF217, AURKA, GNAS, PSMA7, RUNX1, ERG, PSMG1, HMGN1, U2AF1, ICOSLG, CRKL, LZTR1, SMARCB1,*

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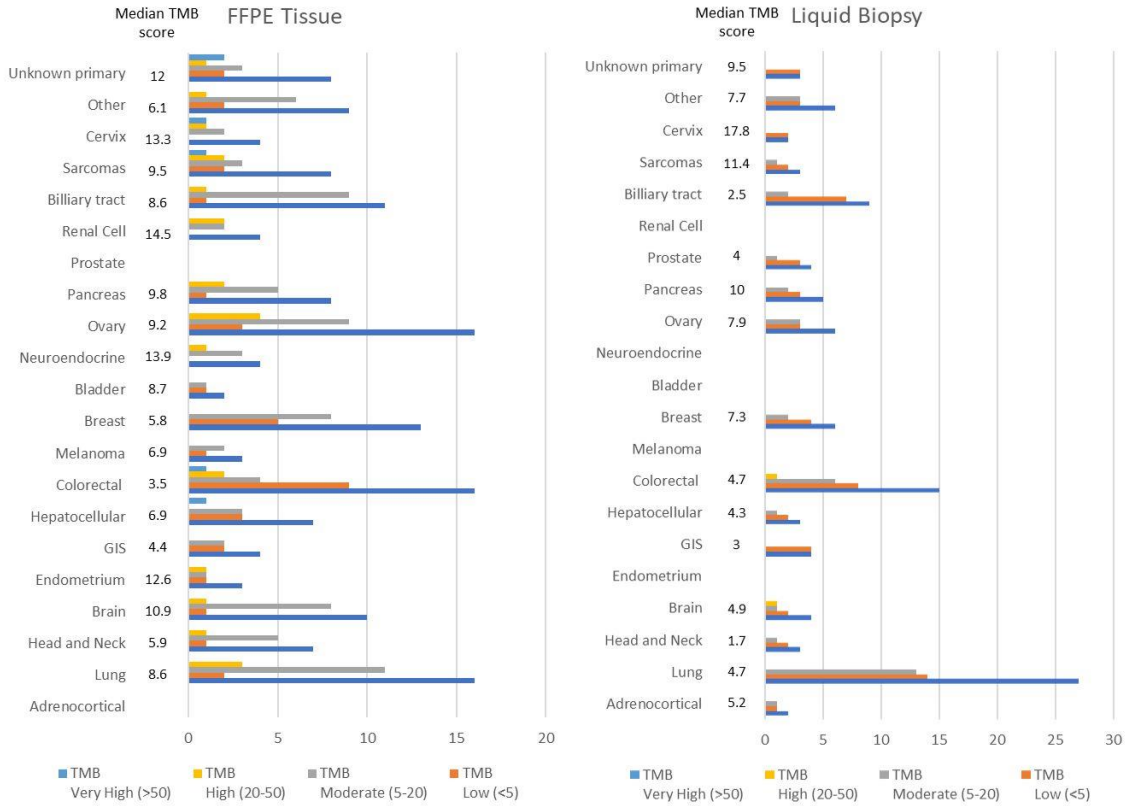

Figure 1. The median mutation burden plots for each cancer type were represented.

CHEK2, EWSR1, NF2, MCM5, SOX10, EP300, CRLF2, FIGF, ACE2, DMD, BCOR, DDX3X, KDM6A, RBM10, ARAF, GATA1, KDM5C, SMC1A, AMER1, AR, MED12, ATRX, RPA4, BTK, MORC4, PSMD10, CUL4B, STAG2, BCORL1, PHF6, CD40LG, DHS-6600Z, Qiaseq Targeted DNA Panel, Qiagen, Germany) was used for target enrichment. Sample libraries were sequenced by Illumına Next Seq 500/550 System (Illumina, USA).

Bioinformatics and TMB scoring

The quality control assessments of output were performed via CLC Genomics Workbench version 20.4.0. A TMB-specific in-house pipeline was developed to determine TMB scores from both FFPE and ctDNA specimens using GRCh38 data sets, and mapped reads were evaluated in terms of TMB score and actionable somatic alterations. Control and reference materials of different TMB scores were used for pipeline optimization (Seraseq® FFPE TMB RM Score 7, Seraseq® FFPE TMB RM Score 13, Seraseq® FFPE TMB RM Score 21 and Seraseq® gDNA TMB Reference, Sera Care, UK). Cancer subtypes were classified based on their median TMB scores as very high (>50), high (20-50), moderate (5-20) and low (<5).

Result

The study was conducted from different specimens of 278 cancer patients. Among them, while 176 were FFPE tissue samples, and another 102 were liquid biopsy. The success rate of sequencing together with TMB analysis was 91.7% (n=255) among all samples. Total of 21 different cancer types was included, in which lung cancers were the most frequent group of 43 cases, followed by 31 cases of colorectal cancer and 22 of ovarian cancer.

We demonstrated that measurements of TMB via CGP as the strongest reflective of measurements and a model for that considered depending upon cancer type. We found that a variety of TMB scores differs between 0 and 221.9. The median TMB ranged widely from 3.5 muts/Mbase in colorectal cancer to 14.5 muts/Mbase in renal cancer. The highest TMB score in all FFPE tissue studies was 221.9, while the minimum score was 0.2 in which the lowest median was in colorectal cancer, and the highest median score was in renal cancer. However, among the ctDNA studies, TMB scores differentiated between 0 to 24 that the lowest ratio was in sarcomas and the highest ratio was in the head and neck cancer group.

The median TMB values of 21 cancer types were evaluated, while 14 had unmatched FFPE and liquid biopsy specimens. Among them, eight (8) of these including lung, head and neck, brain, gastrointestinal stromal, hepatocellular, ovary, biliary tract and primary unknown cancers had higher median TMB scores than the score of ctDNA (in which gastrointestinal stromal cancer median value was <5, other types' median scores

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were 5-20). On the other hand, six (6) cancer types (colorectal, breast, pancreas, sarcoma, cervix and others) showed higher median TMB scores in ctDNA than FFPE tissue (in which colorectal cancer was low, and others' median TMB values were moderate).

Among the rest, melanoma, endometrium, bladder, neuroendocrine and renal cancers were the subgroups that only had FFPE tissues with low TMB scores (<5), while adrenocortical and prostate cancer groups were only consisted of liquid biopsy specimens, which had low and moderate (5-20) TMB scores respectively.

The landscape of TMB across all cancer types of specimens in Turkish cancer patients was given in detail in Figure 1.

Moreover, the identified TMB scores of different cancer types of FFPE samples were compared together with the TCGA datasets to clarify the changes in scoring, as shown in figure 2 (Chalmers et al., 2017). The comparison was not performed between our ctDNA data and TCGA datasets because the TCGA did not include any information on ctDNA specimens but only FFPE samples.

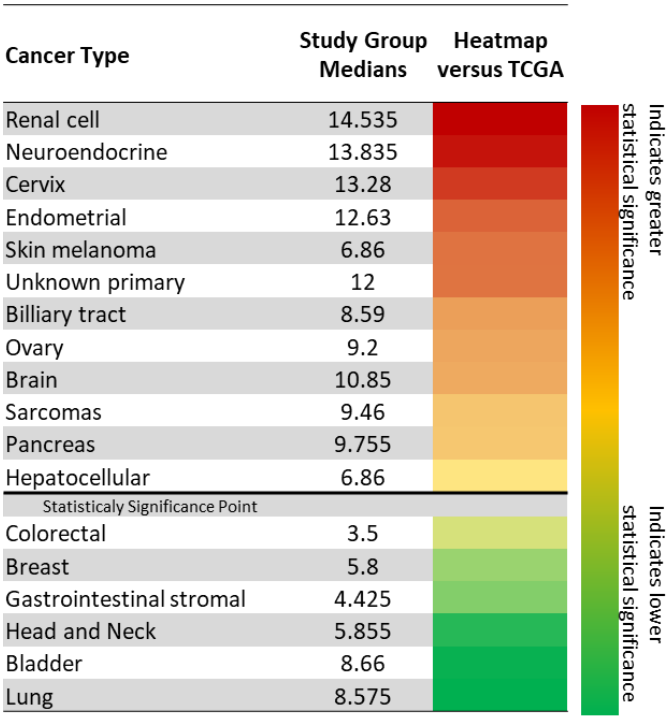

Figure 2. TMB scoring identified in our study groups of FFPE samples was listed. Comparing these scores with TCGA datasets was given as a heat map in which the statistically significant point was set on the graphic.

Conclusion

In the present study, the in-house developed algorithm which utilizes the mutational burden have been used and the distribution of these scoring in both FFPE and liquid biopsy samples has been compared. Such panels can likely be used instead of whole exome or whole genome studies to identify the patients for possible immunotherapy regimens. However, there shall be cut-off values but in regard to whether the specimen was FFPE or ctDNA, whereas the scoring showed different median numbers depending on the cancer types. Thus, further cut-off values should be optimised with a large-scale sampling.

The limitation of our study was that we did not analyze the performance of the patients who underwent immunotherapy. Thus, we do not know whether the scoring cut-off values work in all cancer types.

In summary, a simplified analysis with higher depth and coverage makes this targeted panel an attractive alternative to tumor whole exome sequencing (WES) for routine use. Even though the size of the panel influences the precision of TMB measurement that previous studies showed that too small and the measurement is clinically sub-optimal for patient stratification, the panel used in this study at 1.3 Mbase delivers accurate TMB estimation cost-effectively.

Even though many cancers share common driver and passenger mutations, all cancers are also molecularly distinct. As the NGS multi-gene panels used in this study allow the clinical laboratories to perform pan-cancer analysis, these studies have significantly contributed to our understanding of variations and more across many cancer types. But there is still a need to optimise tumor mutation burden analysis due to the differential and heterogeneous data collected. Although a limited retrospective analysis has shown the predictivity of TMB score for a better response to immune checkpoint inhibitors, there is still no optimal cut point to define high and low for each cancer types. The other important limitation of the studies in the literature, as well as ours is that the number of patients of human entities was relatively small, which might lack power to discover significant differences and fail to establish the predictive functional TMB scoring.

The present study has one of the most important data when compared with The Cancer Genome Atlas (TCGA). Although TCGA provides us with high-quality data and the included confounding factors, our study presents the first study that might project the importance of ethnicity and the impact of unaccounted confounders (such as treatment information, age, etc.)

In conclusion, we found that TMB has divergent scoring in different cancer types, thus, further validation from prospective studies is still needed.

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Conflicts of interest

None

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