TERT mutations correlate with higher TMB value and unique tumor microenvironment and may be a potential biomarker for anti-CTLA4 treatment

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Abstract

Immune checkpoint inhibitors (ICIs) have recently changed therapeutic paradigms for patients across multiple cancer types. However, current biomarkers cannot accurately predict responses to ICIs. Telomerase reverse transcriptase (TERT) mutations lead to an aberrant upregulation of TERT expression, and ultimately allow telomere maintenance, thus supporting immortalization of cancer cells. This study aimed to investigate whether the TERT mutation is a potential predictor of ICI treatment across all cancer types. TERT mutations positively correlated with a higher tumor mutational burden (TMB) value, neoantigen load, and tumor purity. Lymphocyte infiltration, macrophage regulation, interferon-gamma (IFN-γ) response, and transforming growth factor-β (TGF-β) response which was representative immune-expression signatures, all had higher signature scores in the TERT mutation group. Activated CD4 T cell, naive B cell, activated dendritic cell, M0 macrophage, M1 macrophage, neutrophil, resting NK cell, and plasma cells all had relatively higher immune scores in the TERT mutation group, whereas Th series cells, memory B cell, resting mast cells, monocytes, and activated NK cells had lower immune scores. Notably, in the subgroup analysis of monotherapy and combination ICI treatment, only in the anti-cytotoxic-T-lymphocyte-associated antigen 4 (anti-CTLA4) group, patients with TERT mutations had a better prognosis, especially for melanoma. Therefore, TERT mutations were closely related to a higher TMB value and unique tumor microenvironment, which may be the reason that TERT mutations may be a potential biomarker for anti-CTLA4 treatment.

Keywords

anti-CTLA4 treatment, immune cells infiltration, melanoma, TERT mutation, TMB
Immune checkpoint inhibitors (ICIs), such as anti-cytotoxic-T-lymphocyte-associated antigen 4 (anti-CTLA4) (ipilimumab), anti-programmed cell death 1 (anti-PD1) (nivolumab, pembrolizumab, and cemiplimab) and anti-programmed cell death ligand 1 (anti-PD-L1) (atezolizumab, avelumab, and durvalumab), have recently changed the therapeutic paradigm for patients across multiple cancer types. Currently, ICIs have been approved by the Food and Drug Administration for the treatment of numerous cancers, because of their significant and durable clinical response in a subset of patients with certain types of cancer. However, there are also dilemmas, such as low response rates of checkpoint inhibitor monotherapy, significantly higher toxicity of combination treatment, and high treatment costs. Therefore, early identification of potential beneficiaries from ICIs would be a greatly important step forward. Ideal biomarkers would be able to stratify patients who are more sensitive to immunotherapy and monitor the treatment response in real-time.

Programmed cell death-L1 (PD-L1) expression has been identified as one of the biomarkers used in response to ICIs. The percentage of PD-L1 expression in the tumor tissue can be used as a predictor of the efficacy of pembrolizumab in non-small cell lung cancer (NSCLC). However, because of inaccurate quantification, lack of standardization across platforms, and inconsistent scoring systems, PD-L1 expression cannot accurately predict responses to ICIs in some types of cancer. The Tert mutational burden (TMB) has also recently gained popularity as a predictive biomarker associated with ICI responses. Patients with high TMB have obtained a higher overall response rate and median progression-free survival, and therefore have better efficacy in immunotherapy for NSCLC. Moreover, cancer DNA mismatch repair gene mutations could also be used as clinically applicable biomarkers. However, the non-uniform calculation, various sequencing approaches, and exorbitant expenses have also rendered them as not optimal indicators of ICI responses. Therefore, new biomarkers are urgently needed for the identification of patients who will most likely benefit from ICIs, and even for treatment monitoring.

The telomerase reverse transcriptase (TERT) gene encodes the catalytic subunit of the telomerase complex, which maintains chromosomal ends, thus supporting the immortalization of cancer cells. TERT mutations include multiple cancer-specific genetic alterations, such as TERT promoter mutations, TERT amplification, TERT rearrangements, and TERT transcriptional activation. TERT mutations may cause aberrant up-regulation of TERT expression. In addition, the increase in TERT expression may lead to the unlimited proliferative capacity of tumor cells, which is an important factor in tumorigenesis. TERT mutations are also outlined as markers of tumor aggressiveness and poor prognosis in several human cancer types. A recent study revealed that in bladder cancer, TERT promoter mutation appeared to be a potential predictive marker of response to Bacillus Calmette-Guérin treatment which was regarded as one of the first and most successful oncological immunotherapy. Therefore, TERT mutations may be used as a predictive marker for ICI responses.

In this study, using public databases, we analyzed the TERT mutation spectrum to elucidate the correlation between TERT mutation and TMB and immune cell infiltration. Notably, TERT mutant patients may benefit from anti-CTLA4 treatment, especially for melanoma. Together, TERT mutation could be a promising potential prognostic biomarker for anti-CTLA4 responses. This may be related to the high TMB value and unique tumor microenvironment (TME).

2 | MATERIALS AND METHODS

2.1 | Data source and processing

All data in this study were selected from the cBioPortal database (https://www.cbioportal.org). The MSK-IMPACT Clinical Sequencing Cohort including 10,336 patients or 10,945 samples was selected. All mutations with copy number alteration (CNA) data including fusion, amplification, deep deletion, and multiple alterations of TERT were considered. We also obtained information on the TMB score, neoantigen load, and tumor purity related to TERT mutations through public databases, eliminating information with unclear TERT mutation data.

In the relationship analysis between TERT mutation and immunotherapy, we also selected the TMB and immunotherapy cohort, which consisted of 1,661 patients with various cancer types with available genomic, TMB, survival, and immunotherapy information. This study was mainly based on the public database and personal privacy information was not involved, so informed consent was not required.

2.2 | Tumor immune estimation resource analysis

The tumor immune estimation resource (TIMER) algorithm database (https://cistrome.shinyapps.io/timer/) is used to comprehensively investigate the molecular characterization of tumor–immune interactions. TIMER provides a module “DiffExp” to explore target genes expressed in tumors and adjacent normal tissues. The “DiffExp” module is used to study the differential expression between tumor and adjacent normal tissues for target genes across all the cancer genome atlas tumors. Distributions of gene expression levels
are displayed using box plots, with statistical significance of differential expression evaluated using the Wilcoxon test.

### 2.3 Identification of neoantigens

We downloaded supplementary materials from the literature (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5982584/), and carefully analyzed information on neoantigen load related to TERT mutations. The specific steps for determining neoantigens load in the literature were as follows: potential neoantigenic peptides were identified using NetMHCpan 3.0, based on human lymphocyte antigen (HLA) types derived from RNA-seq using OptiType (https://portal.gdc.cancer.gov/). First, all pairs of major histocompatibility complex (MHC) and minimal mutant peptide were input into NetMHCpan v3.0 by the HLA calls of each sample from OptiType. Second, peptides containing amino acid mutations were identified as potential antigens, based on predicted binding to autologous MHC (IC50 < 500 nmol/L) and detectable gene expression meeting an empirically determined threshold of 1.6 transcripts per million. Specifically, somatic nonsynonymous coding single nucleotide variants and indel variants were extracted from the MC3 variant file (mc3.v0.2.8.CONTROLLED.maf) with filters.32,34

### 2.4 Identification of the immune-expression signature

We also applied the supplementary materials in this literature and then identified the immune-expression signature related to TERT mutations. The iterative binary bi-clustering of gene sets (iBBiG) algorithm (http://www.bioconductor.org/packages/release/bioc/html/iBBiG.html) was used for meta-gene set analysis of large numbers of gene expression datasets in the literature.35 The iterative algorithm extracted groups of phenotypes from multiple studies that were associated with similar gene sets and identified similarity blocks within the matrix of signature scores. The five identified representative signatures were as follows: lymphocyte infiltration, macrophage Regulation, interferon-gamma (IFN-γ) response, transforming growth factor-β (TGF-β) response, and wound healing.

### 2.5 Quantification of immune cell infiltration

Using supplementary materials downloaded from the literature (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5982584/), we quantified immune cell infiltration in the TERT mutation and wild type group. The relative fraction of immune cell types within the leukocyte compartment was estimated using CIBERSORT (https://cibersort.stanford.edu/). The proportion of these cells was multiplied to yield corresponding estimates in terms of the overall fraction in tissue. Moreover, the numerical values were aggregated in various combinations to produce abundant comprehensive cell categories. CIBERSORT uses a set of immune cell reference profiles to derive a base (signature) matrix that could be applied to mixed samples to determine the relative proportions of immune cells.36

### 2.6 TISIDB analysis

The TISIDB database (http://cis.hku.hk/TISIDB) integrated multiple types of data resources in oncoimmunology and reported 988 genes related to anti-tumor immunity.37 TISIDB integrated five types of data resources to annotate each gene with 10 categories of knowledge. The “Drug” tab of the database enabled the analyses of the related drugs targeting the gene, which was helpful in designing a combinatory treatment with immunotherapy.

### 2.7 Statistical analysis

The Kaplan-Meier method was used to calculate the survival probability and the log-rank test was used to compare the survival curves. Data between the two groups were compared using the two-tailed unpaired t test or Wilcoxon rank-sum test. All reported P values are two-tailed, and for all analyses, a P < .05 is considered statistically significant.

### 3 RESULT

#### 3.1 Characteristic of TERT mutation spectrum and relationship between TERT mutation and TMB

Tumor mutational burden is related to the number of gene mutations.14 To understand the relationship between gene mutations and total number of gene mutations. We divided the number of gene mutations into four groups (0-2; 2-4; 4-7; 7-455). Figure 1A demonstrates that tumor protein p53 (TP53), TERT, and KRAS were ranked in the top three mutations. Among them, the mutation frequencies of TERT in the four groups were as follows: A, 4.02%, B, 10.71%, C, 14.29%, and D, 24.33%. We further analyzed the waterfall chart of TERT mutation and CNA change. Figure 1B shows that sex cord-stromal tumor, bladder cancer, and glioma were the top three TERT mutation frequencies, while thymic tumor, histiocytosis, and mature T and NK neoplasms were the last three. Figure 1C shows the mutant
genes that co-occur with TERT mutations in 1564 TERT mutation samples. Compared with TERT wild type, TERT mutations co-mutated with most genes. The blue dots indicate statistically significant co-expressed genes (P < .05).

We analyzed the relationship between TERT mutation and TMB score. The results showed that the TMB score of the TERT mutation group (n = 521) was significantly higher than that of the wild-type group (n = 1140) (Figure 1D, 19.45 ± 26.02 vs 8.54 ± 12.71, P < .001) (mean ± SD). We further tested the relationship between TERT mutation and neoantigen load. Figure 1E shows that patients with TERT mutation (n = 386) had higher neoantigen load than wild-type patients (n = 6055) (Figure 1E, 264.60 ± 665.50 vs 185.70 ± 585.10, P = .010) (mean ± SD). The relationship between TERT mutation and tumor purity (mutations = 521, wild type = 1140, 51.92 ± 21.88 vs 44.48 ± 22.18, P < .001) (mean ± SD).

3.2 | Relationship between TERT mutation and immune cell infiltration and its prognostic value

To examine TERT expression in various tumors, we measured its expression in different types of tumors using the TIMER database. Figure 2A has demonstrated that TERT expression was higher in a variety of tumors than adjacent normal tissue. Using supplementary materials downloaded from the literature, we collected 9504 patients, including 551 patients with TERT mutations. We first analyzed the correlation between TERT mutation and immune-expression signatures. Lymphocyte infiltration, macrophage regulation, IFN-γ response, and TGF-β response which were representative immune-expression signatures, all had higher gene signature score in the TERT mutation group (Figure 2B-E, 0.1386, −2.9184 to 3.1753 vs −0.0452, −3.4861 to 4.1743, P = .002; 0.0501, −2.5456 to 2.0633 vs −0.0448, −2.8156 to 2.3769, P = .004; 0.2394, −2.2508 to 2.5762 vs 0.0333, −3.0325 to 3.0649, P < .001; 0.1403, −1.6772 to 1.3826, P < .001) (mean, minimum–maximum).

We further analyzed the relationship between TERT mutations and immune cells. Among Th series cells, including Th1, Th2, and Th17, the immune cell score of the TERT mutation group was lower than that of the wild-type group (Figure 2F, −2.9184 to 3.1753 vs −0.0452, −3.4861 to 4.1743, P = .002; −2.5456 to 2.0633 vs −0.0448, −2.8156 to 2.3769, P = .004; −2.2508 to 2.5762 vs 0.0333, −3.0325 to 3.0649, P < .001; −1.6772 to 1.3826, P < .001) (mean ± SEM). It is similar, in B series cells, which contained memory and naïve B cells. As shown in Figure 2G, the immune cell score of memory B cells in the TERT mutation group was also lower (0.0185 ± 0.0014 vs 0.0235 ± 0.0005,
The immune cell score of naïve B cells in the TERT mutation group was higher (0.0447 ± 0.0023 vs 0.0378 ± 0.0006, P = .004) (mean ± SEM). Subsequently, we analyzed the distribution of T cells in the TERT mutation and wild-type group, such as activated CD4 memory T cells, resting CD4 memory T cells, naïve CD4 T cells, CD8 T cells, follicular helper T cells, gamma delta T cells, and regulatory T cells. Only the immune score of activated CD4 memory T cells in the TERT mutation group was higher than that in the wild-type group, and there was a significant difference (Figure 2H, 0.0070 ± 0.0007 vs 0.0054 ± 0.0002, P = .032). Finally, we analyzed the relationship between TERT mutation and other immune cell types. It was found that there were differences in nine types of immune cells (Figure 2I), such as activated dendritic cells (0.0232 ± 0.0018 vs 0.0134 ± 0.0003, P < .001) (mean ± SEM), M0 macrophages (0.0864 ± 0.0047 vs 0.0759 ± 0.0011, P = .025) (mean ± SEM), M1 macrophages (0.0518 ± 0.0020 vs 0.0468 ± 0.0005, P = .011) (mean ± SEM), resting mast cells (0.0313 ± 0.0021 vs 0.0468 ± 0.0007, P < .001) (mean ± SEM), monocytes (0.0299 ± 0.0016 vs 0.0384 ± 0.0005, P < .001) (mean ± SEM), neutrophils (0.0076 ± 0.0008 vs 0.0055 ± 0.0002, P = .002) (mean ± SEM), activated NK cells (0.0312 ± 0.0015 vs 0.0357 ± 0.0004, P = .004) (mean ± SEM), resting NK cells (0.0164 ± 0.0011 vs 0.0134 ± 0.0003, P = .007) (mean ± SEM), and plasma cells (0.0551 ± 0.0030 vs 0.0431 ± 0.0006, P < .001) (mean ± SEM).

In analyzing the association between TERT mutation and overall survival (OS) in the cBioPortal database. Kaplan-Meier survival analysis showed that patients with TERT mutation (n = 1166) showed a significantly shorter median OS than the wild-type population (n = 6369) (Figure 2J, 22.58 months vs 26.56 months, P = .005).

### 3.3 Relationship between TERT mutations and ICIs

From the abovementioned results, we found that patients with TERT mutations had a worse prognosis. However, in patients who received ICIs, what was the relationship between TERT mutation and OS? Surprisingly, patients with TERT mutations (n = 521) in the ICI treatment cohort showed a significantly longer median OS than the wild-type population (n = 1140) (Figure 3A, 22.00 months vs 16.00 months, P = .002).

To understand whether patients with TERT mutations can benefit from monotherapy or combination treatment, we conducted subgroup analysis in the monotherapy and combination treatment groups. Notably, only in the anti-CTLA4 group, patients with TERT mutation (n = 43) had longer OS than those with wild type (n = 56) (Figure 3C, NA vs 17.00 months, P < .001). However, there were no statistical differences in the PD1/PDL1 blockade and combination treatment groups (Figure 3B, P = .312; Figure 3D, P = .956). We also found 99 patients who received anti-CTLA4 treatment, including 75 patients with melanoma (43 with TERT mutations and 32 with TERT wild type), 20 patients with breast cancer (all with TERT wild type), and four patients with other cancer types (also TERT wild type). Therefore, we further explored the predictive value of TERT mutation on the efficacy of anti-CTLA4 treatment in the melanoma group. The results showed that patients with melanoma with TERT mutations (n = 43) had longer OS than those with wild type (n = 32) (Figure 3E, P < .001). Currently, drugs targeting TERT have been under investigation and development. For instance, DB12747, DB00495, and DB05036 have been developed (Figure 3F). DB12747 (tertomotide) is an immunotherapy drug with mere targeting of TERT.

### 4 DISCUSSION

In our study, we first demonstrated a specific cancer spectrum of TERT mutation and CNA change in 10,336 patients or 10,945 samples to date. Strikingly, TERT mutation was common and found to be frequent in many malignancies, such as sex cord-stromal tumor, bladder cancer, and glioma. Other surveys on different tumor types confirmed the high prevalence of TERT mutations in bladder cancer, and glioma, although they developed with different frequencies. However, TERT mutation is not universal and has been absent, or rarely observed, in some cancer types such as breast cancer, prostate cancer, thymic tumor, histiocytosis, and mature T and NK neoplasms. Consistent results have also been obtained in breast cancer and prostate cancer with a lower mutation frequency. In addition, we found that TERT co-mutated with most genes. Therefore, we speculated that TERT mutation might play a role in the majority of cancers.

Tumor mutational burden, as a biomarker of response to ICIs, is closely related to the number of gene mutations and neoantigens. Our results demonstrated that TERT mutation was ranked second in the top three mutation frequencies. Moreover, we mainly explored the correlation between TERT mutation and TMB score or neoantigens load in multiple cancer types. TERT mutation was significantly associated with high TMB score and neoantigen load in all cancer types. Tumor heterogeneity is the key to determining the tumor's immune response, and tumors with high heterogeneity can suppress the immune response. Our results showed that the tumor purity of the TERT mutation group was higher, indicating lower tumor heterogeneity. These findings highlight that TERT mutation may be related to the tumor's immune response.

To maintain immortal characteristics, malignant tumor cells constantly induce TERT mutations to aberrantly upregulate TERT expression, and ultimately enable telomere...
maintenance, which is tightly regulated in normal somatic cells.45–17,44 In the present study, we first measured TERT expression in different types of tumors using the TIMER database. We found that the expression level of TERT was generally higher in various tumors, than in the adjacent normal tissue, which is consistent with previous studies. Therefore, TERT is frequently activated in many malignant tumors and closely related to cancer progression.

The TME comprises immune cells, mesenchymal cells, endothelial cells, and inflammatory mediators.45,46 Immunocompetence, to some extent, partially reflects the microenvironment in which the tumor is involved. Previous studies have also provided an elegant analysis on how the activation of tumor-intrinsic genes shapes TME.47–49 We first analyzed the correlation between TERT mutation and immune-expression signatures. Lymphocyte infiltration, macrophage regulation, IFN-γ response, and TGF-β response which were representative immune-expression signatures, all had higher gene signature scores in the TERT mutation group. Lymphocytes are cells characterized by high telomerase activity to maintain telomere length.50 Interestingly, the signature score of macrophage regulation, IFN-γ response, and TGF-β response in the TERT mutation group was also relatively higher. It is all known that both tumor-associated macrophages and TGF-β promote key processes in immunosuppression via effects on the TME.51,52 IFN-γ can also induce M2 macrophage differentiation, which plays a suppressive role in immune function.53 We speculated that the TERT mutation may cause an immunosuppressive TME.

The infiltrating immune cell, an integral component of TME, is usually a heterogeneous mixture of immune cells, including cell types associated with activity and inhibition.45,54 We further analyzed the relationship between TERT mutations and immune cells. Among the Th series cells, including Th1, Th2, and Th17 cells, we found that the immune cell score of the TERT mutation group was lower. Similarly, the immune cell score of memory B cells in the TERT mutation group was also lower, while the immune cell score of naïve B cells was higher. Subsequently, we analyzed the distribution of T series cells in the TERT mutation group. Only the immune score of activated memory CD4 memory T cell in the TERT mutation group was higher, and there was a significant difference. Subsequently, we also found significant differences in the nine types of immune cells. Activated dendritic cells, M0 macrophages, M1 macrophages, neutrophils, resting NK cells, and plasma cells had relatively higher immune scores in the TERT mutation group, while resting mast cells, monocytes, and activated NK cells had lower immune scores. The abovementioned results suggested that TERT mutation might play an important role in immunologic dysfunction and unique TME. Moreover, we also found that TERT mutation was related to worse prognosis in all cancer types. However, in patients who received ICIs, what was the relationship between TERT mutations and OS?

Finally, we investigated the relationship between TERT mutation and ICIs. Surprisingly, patients with TERT mutations in the ICI treatment cohort showed a significantly longer OS than the wild-type population. Notably, in the subgroup analysis of monotherapy and combination treatment, only in the anti-CTLA4 group, patients with TERT mutations had a better prognosis. We further explored the predictive value of TERT mutations on the efficacy of anti-CTLA4 treatment in certain cancer types.
cancer types. However, the results showed that only patients with melanoma with TERT mutation could more likely benefit from anti-CTLA4 treatment. Therefore, TERT mutant patients may benefit from anti-CTLA4 treatment, especially for melanoma.

Our study may have some clinical relevance. Immunotherapeutic approaches targeting TERT have been evaluated in many clinical trials. For instance, tertomotide, a peptide vaccine that can activate the immune system to kill cancer cells, is under investigation in a clinical trial NCT01223209. Further immunologic targeting of TERT may represent a promising new aspect in cancer treatment.

This study also has certain limitations. First, our study was only a bioinformatic and pan-cancer analysis of anti-CTLA4 treatment. The next step is to confirm whether TERT mutation is an immune predictive marker for a specific tumor or certain types of tumors through prospective or retrospective studies. Second, there were few studies on anti-CTLA4 treatment and no other data on anti-CTLA4 treatment were currently collected. We cannot use another cohort to verify our findings.

In conclusion, we have identified that TERT mutations are unevenly distributed in different cancer types which may lead to aberrant upregulation of TERT expression in various tumors. TERT mutations were significantly associated with higher TMB value and neoantigen load and may lead to an immunosuppressive microenvironment. TERT mutation was related to worse prognosis in the cBioPortal database and better prognosis in the anti-CTLA4 treatment cohort. Therefore, our study confirmed for the first time that TERT mutation could be used as a predictive marker for anti-CTLA4 treatment, especially for melanoma. Based on these data, further clinical trials are necessary to confirm whether TERT mutation, which is a potential predictor for anti-CTLA4 treatment and TERT-targeted therapy combined with immunotherapy, has better benefits for TERT mutant patients.

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CONFLICT OF INTEREST
All authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTIONS
Haiyong Wang designed the project and proposed the idea; Huahua Li wrote the manuscript; Jia Li carried out data
download and literature collection; Chenxue Zhang conducted bioinformatics analysis; Chenxing Zhang conducted chart and statistical processing.

**ETHICAL APPROVAL**
This study was approved by the Ethics Committee of the Shandong Cancer Hospital.

**CONSENT FOR PUBLICATION**
All authors agree to publish.

**DATA AVAILABILITY STATEMENT**
We declared that materials described in the manuscript, including all relevant raw data, will be freely available to any scientist wishing to use them for non-commercial purposes, without breaching participant confidentiality.

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