



Review

Deciphering the Functions of Telomerase Reverse Transcriptase in Head and Neck Cancer

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Abstract: Head and neck cancers (HNCs) are among the ten leading malignancies worldwide. Despite significant progress in all therapeutic modalities, predictive biomarkers, and targeted therapies for HNCs are limited and the survival rate is unsatisfactory. The importance of telomere maintenance via telomerase reactivation in carcinogenesis has been demonstrated in recent decades. Several mechanisms could activate telomerase reverse transcriptase (TERT), the most common of which is promoter alternation. Two major hotspot TERT promoter mutations (C228T and C250T) have been reported in different malignancies such as melanoma, genitourinary cancers, CNS tumors, hepatocellular carcinoma, thyroid cancers, sarcomas, and HNCs. The frequencies of TERT promoter mutations vary widely across tumors and is quite high in HNCs (11.9–64.7%). These mutations have been reported to be more enriched in oral cavity SCCs and HPV-negative tumors. The association between TERT promoter mutations and poor survival has also been demonstrated. Till now, several therapeutic strategies targeting telomerase have been developed although only a few drugs have been used in clinical trials. Here, we briefly review and summarize our current understanding and evidence of TERT promoter mutations in HNC patients.

Keywords: head and neck cancer; telomerase reverse transcriptase (TERT); promoter mutations; prognosis



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1. Introduction

Head and neck cancers (HNCs) arising in the oral cavity, oropharynx, larynx, and hypopharynx were the seventh most common cancer worldwide in 2018 [1] and approximately seven to eight hundred thousand new cases are diagnosed each year worldwide [2]. The most common type, squamous cell carcinoma, is a highly lethal group of heterogeneous neoplasms often diagnosed at an advanced stage [3]. Tobacco and alcohol consumption are the main etiological factors [4]. Betel quid chewing and infection by oncogenic human papillomavirus (HPV) types 16 and 18 have emerged as important etiological factors for a subset of HNCs in the oral cavity and oropharynx, respectively [5–10]. HPV-positive malignancies represent 5–20% of all HNCs and 40–90% of those arising from the oropharynx [11].

The prevalence of HPV-driven HNCs has been dramatically increasing in developed countries, predominantly affecting middle-aged white men, non-smokers, non-drinkers, or mild-to-moderate drinkers with a higher socioeconomic status and better performance statuses than those with HPV-unrelated SCCs [11,12]. The treatment of HNC is generally multimodal, including surgery, chemotherapy, and radiotherapy, and differs according to disease stage, anatomical location, and surgical accessibility. However, despite significant progress in all therapeutic modalities, the 5-year overall survival (OS) rate of HNC patients remains unsatisfactory [13–15].

In the era of biomarker-driven personalized cancer therapy, several biomarkers have been proposed as prognostic and predictive factors in different cancers, such as KIT mutations in gastrointestinal stromal tumors, EGFR mutations in lung cancer, and HER2 overexpression in breast cancer [16]. However, unlike other cancer types, there are limited predictive biomarkers and targeted therapies for HNCs [4,16–18]. With the development of advanced technical approaches, genome, and exome analyses have provided a comprehensive view of genetic alterations in HNC and uncovered potential new therapeutic opportunities [17,19–24]. In addition to commonly mutated genes, such as *TP53*, *CDKN2A*, *CCND1*, *PIK3CA*, and *NOTCH1*, telomerase reverse transcriptase (*TERT*) promoter mutations have been detected in a significant proportion of HNC patients [3,4,12,13,15,25–29]. *TERT* is located on chromosome 5p15.33 in humans and is an integral and essential part of the telomerase holoenzyme, which plays a key role in cancer formation. Mostly, telomerase activity was increased by upregulation of *TERT* expression via several genetic and epigenetic alterations, and *TERT* promoter mutations are known as the most important [30]. However, the incidence of *TERT* promoter mutations varies in the head and neck subsites, and the association between *TERT* promoter mutations and outcomes is unclear. Therefore, in this review, we summarize our current understanding and evidence of *TERT* promoter mutations in HNC patients.

2. Telomeres and Telomerase in Normal Cells

Telomeres are the physical ends of eukaryotic linear chromosomes [31,32]. In human cells, telomeres are composed of TTAGGG nucleotide repeats with a 3' single-stranded overhang, and the variation ranges from 3 to 20 kilobase pairs [12,33]. They are bound by a six-member protein complex known as shelterin. Telomeres cover the coding DNA at the end to avoid loss of genetic information in linear DNA, act as a cap to prevent degradation by a nucleolytic attack, and prevent aberrant activation of a DNA damage response (DDR), which could lead to inappropriate processing of telomeres and as sites for double-strand break repair [31,34,35].

Telomerase, a specialized reverse transcriptase, is a large multi-subunit ribonucleoprotein complex that synthesizes telomeric DNA sequences and provides a molecular basis for unlimited proliferative potential [36]. Telomerase comprises two major components: the telomeric RNA component (also known as the telomerase RNA component: TERC or TR) and the telomerase reverse transcriptase (TERT), which is encoded by the *TERT* gene. The *TERT* is located in the human chromosome band 5p15.33, and the TERC is located at 3q26.3 [37]. The TERC serves as the template for telomere hexamer repeat additions onto the DNA, and the TERT is responsible for the reverse transcribing hexamer repeats onto the chromosomal ends [33,38–40]. *TERT* expression is silenced during development, unlike the TERC and other constitutively expressed telomerase components [35].

Telomerase is a key telomere length maintenance mechanism and is present in germline, hematopoietic, stem, and other rapidly renewing cells [41]. However, in most normal somatic cells, telomerase activity is extremely low or absent. Therefore, loss of telomeric repeats occurs at each round of DNA replication, after which the telomeres are reduced to a critical length [42]. Critical telomere attrition elicits a DDR that mediates cell cycle arrest and leads to replicative senescence or apoptosis via the p53 or Rb tumor suppressor pathways [35,43]. Telomere attrition acts as a barrier to replicative immortality, also called a “mitotic clock” that limits the cell cycle number and further triggers cellular senes-

cence [33,35]. Although rare, in the absence of telomerase, some cells employ another DNA recombination mechanism, termed alternative lengthening of telomeres (ALT), which reverses telomere attrition to bypass senescence [44].

3. Telomere and Telomerase in Cancer Cells

Telomere length and telomerase activity are crucial for cellular immortalization, tumorigenesis, and cancer progression. Telomere maintenance via telomerase reactivation is a nearly universal hallmark of cancer cells [35,45,46]. The vast majority of cancers overcome replicative senescence by upregulating TERT expression and telomerase activity [35]. Telomerase activity is upregulated in 80–90% of malignancies, enabling unlimited replication of cancer cells, similar to embryonic and stem cells [13,15,47]. For the remaining 10–15% of cancers, upregulation of telomerase activity is achieved through the ALT pathway [48].

There are several ways to upregulate telomerase activity and activate the normally silent human TERT (hTERT) gene. The mechanisms of hTERT activation include chromosomal rearrangements (i.e., duplications, amplifications, insertions, interchromosomal changes, inverted orientations, or deletions), TERT promoter somatic mutations, epigenetic modifications (i.e., DNA methylation, or post-transcriptional regulation by microRNAs), transcriptional activators or repressors, TERT gene polymorphism and alternative splicing (i.e., pre-mRNA alternative splicing of the TERT gene) [30,49–51]. In a pan-cancer genomics study, Barthel et al. detected TERT expression in 73% of the 6835 total tumor samples, which were associated with TERT point mutations, rearrangements, DNA amplifications, and transcript fusions. Among the TERT-expressing samples, there were 31% TERT promoter mutations, 3% TERT amplifications, 3% TERT structural variants, 5% TERT promoter structural variants, and 53% TERT promoter methylation [51]. Some of these mechanisms may interact with each other and have a synergistic effect on TERT expression [30].

Besides the canonical role of telomerase in telomere maintenance, there are also some non-canonical functions (telomere length-independent mechanisms) in tumorigenesis, such as the regulation of metabolic mechanisms, epigenetic regulation, and modulation of chromatin, oxidative stress protection, RNA silencing, signal transduction pathways (Wnt and c-MYC signaling pathways), enhanced mitochondrial function, cell adhesion, and migration [30,52–58].

4. Telomerase Reverse Transcriptase (TERT) Promoter Mutations

Among the several mechanisms of hTERT activation, TERT somatic promoter mutations are the most common non-coding driver mutations in cancer [30,59] and occurred at a high frequency in over 50 cancer types [60]. They have been reported in two major hotspots (mainly C > T transitions), which are located at –124 and –146 base pairs upstream of the transcriptional start site on chromosome 5 and are designated as C228T and C250T, respectively [13,25,32,61,62]. A less frequent hTERT promoter mutation –57 base pairs upstream of the transcriptional start site with an A > C transition (at position 1,295,161 on chromosome 5) has been found to be a disease-segregating germline mutation in a melanoma-prone family [63]. Other less frequent, yet recurrent, mutations on chromosome 5 have also been discovered in cancers at the following positions: 1,295,228 C > A, 1,295,248–1,295,243 CC > TT, and 1,295,161 A > C [64].

TERT somatic promoter mutations are predominantly heterozygous and lead to the generation of an 11 bp sequence, CCCGGAAGGGG, which is similar to the E26 transformation-specific (ETS) factor binding motif [60,65]. Then, ETS binding factors, such as GA-binding protein (GABP), are recruited. This recruitment resulted in direct transcriptional activation of hTERT expression and promoted an epigenetic shift from a repressed to active chromatin conformation [35,65–68]. These promoter mutations were proven to be associated with higher levels of TERT mRNA, TERT protein, telomerase enzymatic activity, and telomere length in a study of 23 human urothelial cancer cell lines [69].

Two TERT promoter hotspot mutations, C228T and C250T are the most common; however, their frequencies vary widely across tumors from different sites (Table 1). These mutations occur most frequently in cancers with low rates of self-renewal [25] and are rare in pediatric and young adult cancers [3,70]. The highest frequencies of TERT promoter mutations have been reported in melanoma, bladder cancer, urothelial carcinoma, CNS tumors, hepatocellular carcinoma, thyroid cancer, basal cell carcinoma, and cutaneous squamous cell carcinoma. Due to the variety of sarcoma subtypes, the prevalence of TERT promoter mutations varies widely, and the highest TERT promoter mutation rate is reported in myxoid liposarcoma (79.1%) [25].

Table 1. Frequency spectrum of hTERT promoter mutations across different cancer types.

Cancer Type	Mutation Frequency (%)	Reference
Malignant melanoma	17.0–85.0	[61,63,71,72]
Genitourinary cancers		
Bladder cancer	59.0–85.0	[25,61,73–76]
Urothelial carcinomas	29.5–64.5	[77,78]
Kidney cancers	0	[61]
Prostate Cancer	0	[79]
CNS tumors		
Glioblastoma	54.0–84.0	[61,70,73,78,80]
Other gliomas (ependymoma, astrocytoma, mixed glioma, oligodendroglioma)	2.7–78.0	[25,64,70,78]
Medulloblastoma	33.3–65.0	[70,78]
Hepatocellular carcinoma	31.4–59.0	[25,78,81–84]
Thyroid cancer (papillary, follicular, poorly differentiated, and anaplastic carcinomas)	3.4–46.3	[61,85–87]
Gastrointestinal stromal tumor	0–3.8	[61,88]
Malignant pleural mesothelioma	11.3	[89]
Atypical fibroxanthomas	93.0	[90]
Sarcomas (chondrosarcoma, fibrosarcoma, myxofibrosarcoma, myxoid liposarcoma, osteosarcoma, pleomorphic dermal sarcomas)	4.3–79.1	[25,90,91]
Basal cell carcinoma of the skin	73.8	[92]
Squamous cell carcinoma of the skin	20.0–74.0	[25,92,93]
Squamous cell carcinoma of esophageal	1.6	[94]
Squamous cell carcinoma of penile	48.6	[95]
Squamous cell carcinoma of the head and neck	11.9–64.7	[3,4,13,15,25–29,32,93,96,97]
Squamous cell carcinoma of the cervix	0–21.4	[25,26,93,96]
Breast cancer, colorectal cancer, ovarian cancer, esophageal adenocarcinoma, acute myeloid leukemia, chronic lymphoid leukemia, pancreatic cancer, and testicular carcinoma	0–5.0	[61,78]

5. TERT Promoter Mutations in Head and Neck Squamous Cell Carcinoma

5.1. The Frequency of TERT Promoter Mutations

For HNCs, the frequency of TERT promoter mutations varied significantly among previous studies. These differences could be explained by the tumor subsite, sample size, methodological sensitivity, risk factors, and population ethnicity (Table 2).

Table 2. Summary of studies evaluating the association between head and neck cancers with TERT promoter mutations.

Author, Country (Year)	Case Numbers	Cancer Sites	Prevalence of TERT Promoter Mutations	Special Findings	The Association with Survival
Killela, USA (2013) [25]	70	31 Oral cavity 23 Oropharynx 4 Supraglottic 12 Others	Total: 17.1% (12/70) C228T: 14.8% C250T: 2.8%	Highest frequency in tongues (47.8%, 11/23)	N/A
Schwaederle, USA (2018) [32]	28	28 HNC	Total: 28.6% (8/28)	N/A	Trend toward shorter survival
Cheng, USA (2015) [93]	12	12 HNSCC	Total: 16.67% (2/12) C228T: 16.67% C250T: 0%	No significant correlation was observed.	N/A
Barczak, USA (2017) [15]	61	25 Mouth 25 Voice box 5 Nose/sinuses 6 Throat	C250T homozygous T/T allele: 36% heterozygous C/T allele: 26%	Homozygous T/T mutation is associated with the grade of the tumor.	N/A
Yu, USA (2021) [29]	117	74 Oral cavity 24 Larynx 5 Hypopharynx 14 HPV (-) oropharynx	Total: 53.8% (63/117) C228T: 33.3% C250T: 9.4% C250T, C254T: 6% C228A: 4.3% CC434TT: 0.9%	Highest frequency in the oral cavity (81.1%, 60/74)	Increased risk of locoregional failure, but not distant failure or OS.
Morris, USA (2017) [97]	53	20 Oral cavity 18 Oropharynx 7 Larynx 2 Hypopharynx 6 Others (4 sinonasal cavity)	Total: 32.1% (17/53) C228T: 20.8% C250T: 5.7% C228A: 1.9%	TERT mutation and HPV infection may represent parallel mechanisms.	N/A
Boscolo-Rizzo, Italy (2020) [3]	101	27 Oral cavity 23 Oropharynx 15 Hypopharynx 36 Larynx	Total: 11.9% (12/101) C228T: 9.9% C250T: 2%	Highest frequency in the oral cavity (37%) TERT levels did not significantly differ according to the mutational status of TERT promoter.	No significant association between TERT promoter status and OS. Higher TERT levels, worse OS (43.6% vs. 60.1%)
Annunziata, Italy (2018) [96]	24	15 Oral cavity 9 Oropharynx	Total: 37.5% (9/24) C228T: 8.3% C250T: 12.5% Other: 16.7%	No mutation in oropharynx cancer. Mutations were independent of HPV status.	N/A
Yilmaz, Turkey (2020) [4]	189	102 Oral cavity 22 Oropharynx 6 Hypopharynx 59 Larynx	Total: 43.9% (83/189) C228T: 29.6% C250T: 11.6% C228A: 2.6%	Highest frequency in the oral cavity (75.5%, 77/102). TERT mutations are associated with younger age, female gender, and an inverse relationship to smoking and alcohol consumption.	No difference
Arantes, Brazil (2020) [13]	88	69 Oral cavity 11 Larynx 8 Pharynx	Total: 27.3% (24/88) C228T: 6.8% C250T: 20.5%	94.4% C250T were alcohol consumers. 66.7% C228T were not alcohol consumers	Decreased 5-year DFS and OS in C228T
Vinothkumar, India (2016) [26]	41	41 Oral cavity	Total: 31.7% (13/41) C228T: 21.9% C250T: 9.7%	No significant correlation was observed.	N/A
Chang, Taiwan (2017) [28]	201	201 Oral cavity	Total: 64.7% (130/201) C228T: 51.7% C250T: 12.9%	C228T mutation was associated with betel nut chewing.	No difference
Qu, China (2014) [27]	235	235 Laryngeal	Total: 27% (64/235) C250T: 23.8% C228T: 3.4%	Not significantly correlate with any clinicopathological variables	Poor survival, especially C250T mutation

Killela et al. surveyed 70 oral cavity cancers and identified TERT promoter mutations in 12 of the tumors (17.1%) [25]. Schwaederle et al. analyzed 423 cases of TERT promoter alterations using next-generation sequencing (NGS). Only 28 patients (6.6%) had HNCs. The incidence of TERT promoter alterations was 14.4% (61 of 423) in the overall population and 28.6% (8 of 28) in HNCs [32]. Cheng et al. collected 84 cases of SCC from different sites, including 12 HNC and C228T mutations, which were detected in 16.67% (2 of 12) [93].

Barczak et al. analyzed 61 HNC patients to determine the prevalence of the hTERT promoter C250T mutation. High-resolution melting mutation analysis was used to identify the C250T hTERT promoter mutation, followed by sequencing verification in 10% of the samples. The prevalence of the hTERT promoter C250T mutation was 36% [15]. Yu et al. identified TERT promoter mutations in 117 patients with SCC of the oral cavity (N = 74), larynx (N = 24), hypopharynx (N = 5), and HPV-negative SCC of the oropharynx (N = 14) using NGS. Overall, 63 patients (53.8%) had TERT promoter alterations, and the most common mutations were C228T and C250T [29]. Morris et al. collected 53 patients and 20 oral cavities, 18 oropharynges, 7 larynges, and 2 hypopharynges. Overall, the frequency of the TERT alteration was 32.1% (17 of 53), yet it was much higher in HPV-negative tumors (53% vs. 4.3%). Remarkably, 91% (10 of 11) of the HPV-negative tongue SCCs possessed TERT mutations [97].

In Italy, Boscolo-Rizzo et al. analyzed cancer tissue and adjacent mucosa specimens from 101 patients with HNCs and evaluated the prevalence of the TERT promoter mutations by Sanger sequencing. The tumor subsites in the HNCs included the oral cavity (N = 27), oropharynx (N = 23), hypopharynx (N = 15), and larynx (N = 36). The TERT promoter harbored mutations in 12 tumors (11.9%), with C228T and C250T, which accounted for 83.3% and 16.7%, respectively. They also evaluated the TERT mRNA level and found no significant difference between the TERT mRNA level and the mutational status of the TERT promoter [3]. Annunziata et al. analyzed tumor biopsies from 15 oral SCCs and nine oropharyngeal SCCs. The frequency of TERT promoter mutations was 60% (9 of 15) in oral SCCs and was absent in oropharyngeal SCCs. There were five hotspot mutations (three C228T and two C250T) and four other mutations. They also investigated the TERT mRNA levels and identified that the TERT mRNA levels were comparable to those detected in peri-tumor tissues. However, these data were from six oropharyngeal SCCs and illustrated that they all lacked mutations in the TERT promoter [96].

In Turkey, Yilmaz et al. collected a total of 189 patients with HNCs, including 102 oral cavities, 22 oropharynges, 6 hypopharynges, and 59 larynges. The TERT gene expression was examined by polymerase chain reaction (PCR)-based direct sequencing. TERT promoter mutations were detected in 43.9% (83 of 189) of the cases. Three TERT promoter region mutations were detected: C228T (56 of 83; 67.5%), C250T (22 of 83; 26.5%), and C228A (5 of 83; 6%). The frequency of the C228T mutation was almost twice that of the C250T and C228A mutations [4]. In Brazil, Arantes et al. collected 88 HNC patients and analyzed the TERT promoter mutations C228T and C250T using pyrosequencing. The overall prevalence of the TERT hotspot mutations is 27.3% (6.8% at locus C228T and 20.5% at C250T) [13]. In India, Vinothkumar et al. analyzed 181 primary tumors of the uterine cervix and oral cavity using PCR amplification and sequencing. A high frequency of TERT hotspot mutations was observed in both cervical (30 of 140, 21.4%) and oral (13 of 41, 31.7%) SCCs. Among the oral cancer samples, the TERT promoter hotspot mutations were frequent, while the C228T mutation (69.2%) was twice as frequent as the C250T (30.8%) [26].

In Taiwan, Chang et al. included 201 oral cavity SCC tumors and adjacent normal tissues to detect two TERT promoter mutations (C228T and C250T) using Sanger sequencing. Overall, the TERT hotspot promoter mutations occurred at a high frequency (64.7%) in patients with oral cavity SCCs. There were 52.5% (104 of 201) and 12.9% (26 of 201) oral cavity SCC tumor tissues containing that contained the C228T and C250T mutations, respectively [28].

In China, Qu et al. obtained 235 laryngeal cancer tissues using a pyrosequencing assay to detect the TERT promoter mutations C228T and C250T. The TERT promoter hotspot mutations were present in 27% (64 of 235) of the samples. The TERT C250T mutations were more common (56 of 235) than the C228T mutations (8 of 235) [27]. In Figure 1, we summarized the reported frequencies of the TERT promoter mutations in HNCs from the various studies mentioned above.

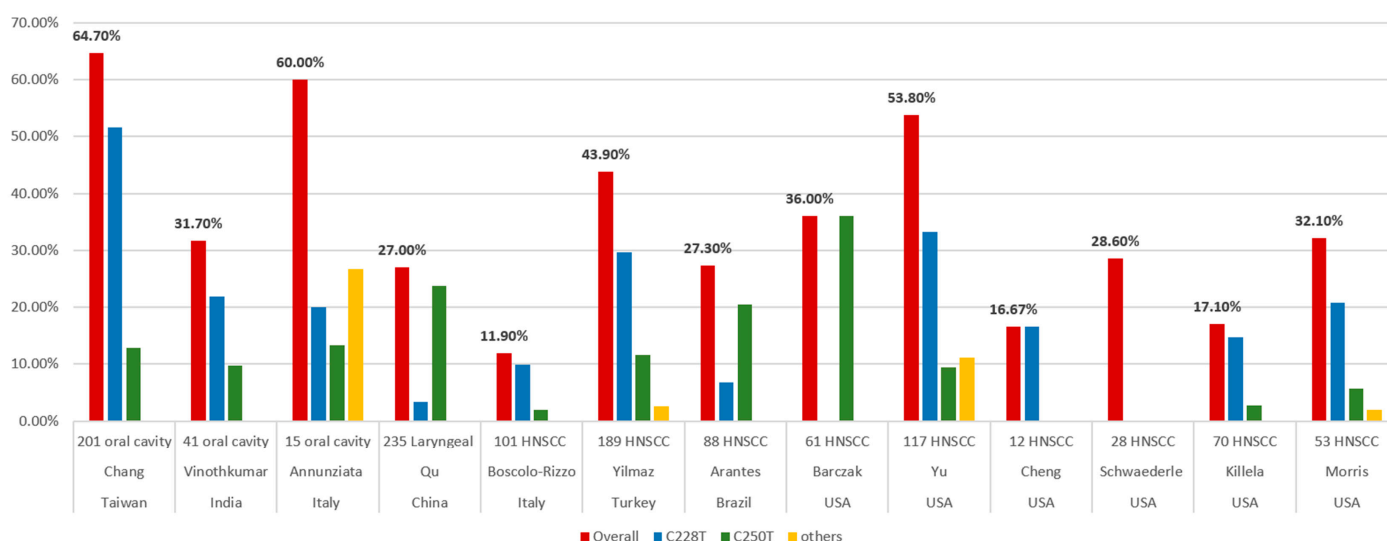


Figure 1. Frequencies of TERT promoter mutations in head and neck cancers from different studies.

5.2. TERT Promoter Mutations in Different Anatomic Distribution

HNC is a heterogeneous group of tumors involving distinct anatomical sites and subsites with varying etiological factors. Yu et al. showed that TERT promoter mutations were more abundant in oral cavity SCCs than in laryngopharyngeal cancers (81.1% vs. 7.0%) [29]. Boscolo-Rizzo et al. demonstrated that the prevalence of TERT hotspot promoter mutations is significantly higher in oral cavity SCCs (37%) [3]. Annunziata et al. also showed that TERT promoter mutations were predominant in oral SCCs (60%), yet absent in oropharyngeal SCCs [96]. Arantes et al. reported that 92% of the mutation cases were located in the oral cavity [13]. Finally, Yilmaz et al. showed that the frequency of the TERT promoter mutations in oral SCCs (75.5%) was significantly higher than in the other locations [4]. The anatomic distribution of cases is strongly associated with TERT promoter mutations, and the highest frequency is in oral cavity cancers.

As for the subsites in oral cavity SCCs, Arantes et al. noticed that 92% of the mutated cases were mainly in the tongue [13]. Killela et al. also revealed that 11 out of the 12 cancers with TERT promoter mutations were in the oral tongue, although only 23 of the 70 oral cavity cancers originated in the oral tongue [25]. However, Yilmaz et al. demonstrated that the highest rate was related to the buccal location and the lowest to the floor of the mouth (82.35% and 61.53%, respectively), although the difference was not statistically significant [4].

5.3. TERT Promoter Mutation and Human Papillomavirus Status

An association between HPV infection and oropharyngeal SCC has been proven. It was also clear that the molecular landscape and clinical pattern were different between HPV-positive and HPV-negative oropharyngeal cancers [10]. Only two studies have investigated the association between HPV status and TERT promoter mutations.

In a cohort of 53 patients with advanced HNCs, performed by Morris et al., a very high TERT alternation rate (53%, 16 of 30) was present in 30 HPV-negative tumors, however, there was only one TERT alternation (4.3%), which was a TERT amplification rather than a hotspot mutation, in 23 HPV-positive tumors. HPV-negative tongue SCCs showed the highest TERT mutation rate (91%). This demonstrated that TERT mutations and HPV infection may represent parallel mechanisms of telomerase activation in HNCs [97]. In another cohort study conducted by Annunziata et al., among the 9 patients with TERT promoter mutations in 15 oral SCC patients, 7 were HPV-negative and 2 were HPV-positive ($p = 0.486$). The frequency of TERT mutations was also independent of HPV tumor status in oral cancer [96].

5.4. TERT Promoter Mutation and Tobacco, Alcohol, and Betel Quid

Aside from HPV infection, tobacco smoking, alcohol consumption, and betel quid chewing are the other three main etiological factors of HNC [4,5]. Until now, the relationship between the TERT promoter mutations and these three factors remains inconclusive.

In a Brazilian cohort of 88 patients with HNC conducted by Arantes et al., the frequency of the C250T mutation appeared to be higher in alcohol consumers. Of the patients harboring the TERT promoter mutation C250T, 94.4% were alcohol consumers, and 66.7% of the patients harboring the TERT promoter mutation C228T did not consume alcohol [13]. In a Chinese cohort of 235 laryngeal cancer cases reported by Qu et al., hotspot mutations were not significantly correlated with any clinicopathological variables. However, TERT promoter mutations, particularly the C250T mutation, were more frequent in smoking patients (47 of 130) than in non-smoking patients (9 of 49), although no statistical significance was noted [27]. In a cohort of 201 patients with oral cavity SCC performed by Chang et al. in Taiwan, the C228T mutation was significantly associated with betel nut chewing [28]. In contrast, in a Turkish cohort of 189 HNC patients performed by Yilmaz et al., TERT promoter region mutations in HNC were inversely related to smoking and alcohol consumption [4].

5.5. TERT Promoter Mutation and Other Factors

Schwaederle et al. demonstrated that TERT promoter alterations are more frequent in men. They were also associated with brain cancers, skin/melanoma, head, and neck tumors, and increased median numbers of alterations in the univariate analysis. However, this association in head and neck tumors was not found in further multivariate analyses [32]. Yilmaz et al. reported that TERT promoter region mutations in HNCs are associated with younger age and female genders in a cohort from Turkey [4]. Barczak et al. demonstrated a significant association between the frequency of the homozygous C250T mutation and tumor grade (T1 = 27%, T2 = 36%, T3 = 35%, T4 = 46%, $p \leq 0.0001$) [15]. However, in a cohort of 41 patients with oral SCCs, performed by Vinothkumar et al. in India, no significant correlation was observed between any of the genotypes and the clinicopathological characteristics [26].

5.6. TERT Promoter Mutation and Survival

TERT promoter mutations in various reports of different cancers have been associated with aggressive characteristics, poor outcomes, and shorter survival [98–101]. In HNC, Qu et al. showed that TERT promoter mutations significantly affected the overall survival of laryngeal cancer patients, particularly those with the C250T mutation. TERT promoter mutations were significant predictors of poor prognosis in patients with laryngeal cancer, as an independent variable, with respect to age, tumor localization, TNM stage, tumor invasion, lymph node metastasis, and smoking history [27]. Schwaederle et al. also demonstrated a significantly shorter overall survival in patients harboring the TERT promoter alterations in the overall population in a univariate analysis. Subanalyses of the three tumor types with the highest prevalence of TERT alterations consistently showed a trend toward shorter survival for patients with altered TERT promoters in brain tumors, head, and neck cancers, and melanoma/skin tumors [32]. Arantes et al. demonstrated no statistically significant association between the presence of hotspot mutations (C228T and C250T) and survival. However, the presence of the C228T mutation impacted patient outcomes, with a significant decrease in 5-year disease-free survival (20.0 vs. 63.0%) and 5-year overall survival (16.7 vs. 45.1%) [13].

Similar results were reported by Yu et al. [29]. They reported that the TERT promoter mutations were associated with locoregional failure (LRF) in the overall cohort and in oral cavity SCCs. This increased risk for LRF is independent of the oral cavity primary site, TP53 mutation status, extracapsular extension, and positive surgical margins suggesting that the TERT promoter mutations are an independent biomarker of LRF rather than a surrogate for OSCCs, or other known prognostic markers. The cumulative incidence of LRF was similar between the two types of TERT promoter mutations (C250T and C228A/T groups), and

both were associated with a higher cumulative incidence of LRF compared to wildtype tumors. Overall, they demonstrated that TERT promoter mutations were associated with an increased risk of LRF, although not with distant failure or overall survival [29].

In contrast, Yilmaz et al. did not find a significant association between the presence of TERT mutations and OS, despite patients with HNCs harboring TERT mutations exhibiting a slightly shorter median OS [4]. Boscolo-Rizzo et al. showed no significant association between the TERT promoter status and overall survival, although the TERT mRNA level had an impact on clinical outcomes [3]. Chang et al. also reported that there was no significant difference in overall survival, disease-specific survival, and disease-free survival between TERT promoter mutations and the wildtype [28].

6. Anti-Telomerase Therapeutics

The unique feature of overexpression in most cancer cells, although absent or with low expression in somatic cells, makes telomerase and other telomere components a target for the development of therapeutics [30]. Several therapeutic strategies have been proposed to target telomerase, and some have already been evaluated in clinical trials against various cancer types [30,35,44,102]. However, the development of successful clinical therapies is hampered by significant challenges [35].

6.1. Direct Telomerase Inhibition

Direct telomerase inhibition by small molecules or oligonucleotides that directly bind to the TERT or TERC template region suppresses telomere extension.

The first-in-class modified oligonucleotide, GRN163L (Imetelstat), was developed in 2003 [103]. Imetelstat is a lipidated 13-mer thiophosphoramidate oligonucleotide complementary to the TERC template region, which competitively inhibits telomerase activity and suppresses cancer cell viability [103]. After showing activity and efficacy against multiple cancer cell lines and in mouse xenograft models, Imetelstat has moved to early clinical trials against solid tumor malignancies (such as breast cancer, non-small-cell lung cancer, brain tumor, and melanoma) and hematologic diseases (such as multiple myeloma, myelodysplastic syndrome, and myeloproliferative neoplasms) [35,44,102]. Although Imetelstat did not meet its efficacy endpoints in trials on non-small cell lung cancer and breast cancers [104,105], it showed robust response rates in patients with lower-risk myelodysplastic syndromes, myelofibrosis, and essential thrombocythemia [106–109], and further late-stage clinical trials are underway [110].

BIBR1532, 2-[[*(E)*-3-naphthalen-2-ylbut-2-enoyl]amino]benzoic acid, inhibits telomerase by non-competitively binding to the TERT active site [111]. BIBR1532 has generated promising preclinical results [112–118]. For example, it enhances the radiosensitivity of non-small cell lung cancer by increasing telomere dysfunction and ATM/CHK1 inhibition [117]. However, this has not yet progressed to clinical testing. Some natural compounds have also been reported to act as telomerase inhibitors, such as allicin (from garlic), curcumin (from turmeric), silibinin (from thistle), and epigallocatechin gallate (EGCG, from tea), and the EGCG's derivative, MST-312 [30,35,119].

6.2. G-Quadruplex Stabilizers

G-quadruplexes are tetrad planar structures formed in guanine-rich DNA or RNA sequences, including telomeres [120]. Compounds that would stabilize telomeric G-quadruplex secondary structures can disrupt telomere extension via telomerase, triggering a DNA damage response and cell death. Several G-quadruplex stabilizers, including telomestatin, BRACO-19, RHPS4, TMPyP4, CX-3543 (Quarfloxin), CX-5461 (Pidnarulex) and AS1411, have been tested in preclinical studies and some already progressed to clinical trials [30,102,121–130].

6.3. Nucleoside Analogues

Nucleoside analogs mimic the presence of uncapped telomeres and induce DNA damage response, apoptosis, and autophagy [102]. To date, several nucleotide analogs, including T-oligo, 6-thio-2'-deoxyguanosine (6-thio-dG), and 5-fluoro-2'-deoxyuridine (5-FdU) triphosphate, are under investigation, although have not yet advanced to clinical trials [131–134].

6.4. Telomerase-Based Cancer Vaccines

Telomerase-based therapeutic cancer vaccines aim to induce T cells that target a tumor antigen, leading to improved antitumor immune responses and cancer cell death [135]. TERT is an appropriate tumor-associated antigen. To date, telomerase vaccinations, including peptide vaccines (such as GV1001, GX301, UV1, and Vx-001), dendritic cell-based vaccines (such as GRNVAC1), and DNA vaccines (such as INVAC-1) have been evaluated in many clinical trials spanning almost two decades [35,44,102,135,136]. Clinical studies on hTERT have been applied to both solid tumors and hematologic malignancies, and some of them have already moved to the later stages of trials [136]. However, the efficacy of the TERT vaccines is insufficient [137,138]. Furthermore, therapeutic TERT-based vaccines can mediate specific T cell responses in a high proportion of cancer patients [35]. A more robust antitumor activity was observed when combining immune checkpoint blockade with TERT-based vaccines in preclinical research, proving the synergistic effect between these two drugs [139].

6.5. TERT or TERC Promoter-Driven Therapy

Owing to the hallmark role of TERT promoter mutation-induced TERT expression in tumorigenesis, correction of this mutation and reduction of TERT expression has become a therapeutic method, by using recently developed gene editing techniques, including oncolytic virus and suicide gene therapy [102]. Telomelysin (OBP-301), a telomerase-specific replication-component adenovirus with an hTERT promoter element, has shown strong antitumor effects in various human cancer cells, including HNCs [140,141]. Phase I trials for solid tumors [142] and advanced hepatocellular carcinoma [143] have already been completed. Further phase 2 trials on gastric/gastroesophageal junction cancers (NCT03921021), head and neck cancers (NCT04685499), and esophageal cancers (NCT03213054) are currently ongoing.

6.6. Other Therapeutics Strategies

In addition to the strategies mentioned above, there are other anti-telomerase therapies. For example, telomerase interference by altered TERC templates is introduced by lentiviral infection [144], CRISPR genome editing targeting TERT gene expression [145], inhibition of oncogenic signaling MAPK pathways that impinge on TERT transcription [35], epigenetic mechanisms using histone deacetylase [30,146], and Tankyrase inhibitors for telomere length regulation [102,147]. Furthermore, strategies relying on telomere attrition in the setting of adjuvant or maintenance therapies rather than frontline therapy are another consideration [35].

7. Conclusions

Telomeres shorten with each cell division and result in cellular senescence. Telomere maintenance via telomerase reactivation plays a critical role in tumorigenesis (Figure 2). Several mechanisms could increase telomerase activity and TERT promoter, C228T and C250T, mutations are the most well-known alternations, which have already been reported in several malignancies, including HNCs. The frequency of the TERT promoter mutations in HNCs is quite high, ranging from 11.9% to 64.7%, and is more enriched in oral cavity SCCs than in other subsites. In addition, several reports have demonstrated an association between TERT promoter mutations and poor survival. Over the past 10 to 20 years, several anti-telomerase therapeutic strategies have been developed. However, only a

few drugs have been used in clinical trials, and the results are only passable. Studies on immunotherapies targeting telomerase, such as cancer vaccines and oncolytic viruses, are very promising, however, trials are still ongoing. In addition, the potential synergy between TERT vaccines and immune checkpoint blockade may be another way to maximize anti-telomerase therapy.

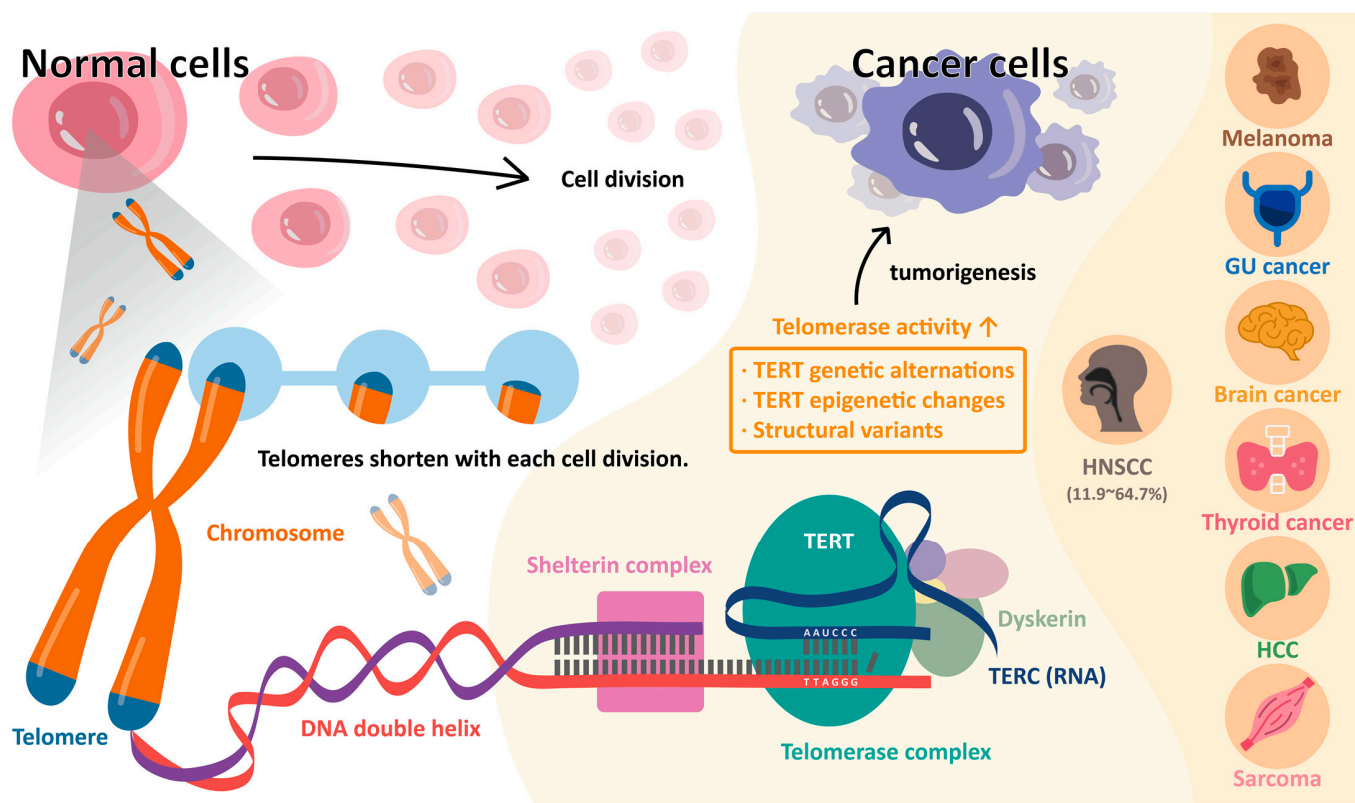


Figure 2. Telomere and telomerase play important roles in cellular biology and tumorigenesis. Telomeres are specialized structures that are located at the ends of chromosomes. They are composed of DNA repeating sequences (TTAGGG). Shelterin complexes are specific proteins known to protect chromosomes and regulate telomere length. Telomerase is a reverse transcriptase that synthesizes telomeric DNA sequences to maintain telomere length. Telomerase comprises two major components: the telomeric RNA component (TERC) and the telomerase reverse transcriptase (TERT). Other proteins, such as dyskerin, are also found in a complex with TERC. Telomeres shorten with each round of cell division and this mechanism limits the proliferation of cells to a finite number of cell divisions. Unlike normal cells, cancer cells are characterized by high telomerase activity, which could be achieved via mechanisms including TERT genetic alterations, TERT epigenetic change, or structural variants. TERT promoter mutations are the most common alternation and have been reported in several malignancies such as melanoma, genitourinary cancers, CNS tumors, hepatocellular carcinoma, thyroid cancers, sarcomas, and HNCs. In HNCs, the frequency of TERT promoter mutations is high (11.9–64.7% based on different studies).

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References

1. Siegel, R.L.; Miller, K.D.; Jemal, A. Cancer statistics, 2019. *CA Cancer J. Clin.* **2019**, *69*, 7–34. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **2018**, *68*, 394–424. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Boscolo-Rizzo, P.; Giunco, S.; Rampazzo, E.; Brutti, M.; Spinato, G.; Menegaldo, A.; Stellan, M.; Mantovani, M.; Bandolin, L.; Rossi, M.; et al. TERT promoter hotspot mutations and their relationship with TERT levels and telomere erosion in patients with head and neck squamous cell carcinoma. *J. Cancer Res. Clin. Oncol.* **2020**, *146*, 381–389. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Yilmaz, I.; Erkul, B.E.; Ozturk Sari, S.; Issin, G.; Tural, E.; Terzi Kaya Terzi, N.; Karatay, H.; Celik, M.; Ulasan, M.; Bilgic, B. Promoter region mutations of the telomerase reverse transcriptase (TERT) gene in head and neck squamous cell carcinoma. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.* **2020**, *130*, 63–70. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Chen, Y.J.; Chang, J.T.; Liao, C.T.; Wang, H.M.; Yen, T.C.; Chiu, C.C.; Lu, Y.C.; Li, H.F.; Cheng, A.J. Head and neck cancer in the betel quid chewing area: Recent advances in molecular carcinogenesis. *Cancer Sci.* **2008**, *99*, 1507–1514. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Gooi, Z.; Chan, J.Y.; Fakhry, C. The epidemiology of the human papillomavirus related to oropharyngeal head and neck cancer. *Laryngoscope* **2016**, *126*, 894–900. [\[CrossRef\]](#)
7. Mallen-St Clair, J.; Alani, M.; Wang, M.B.; Srivatsan, E.S. Human papillomavirus in oropharyngeal cancer: The changing face of a disease. *Biochim. Biophys. Acta* **2016**, *1866*, 141–150. [\[CrossRef\]](#)
8. Lee, Y.A.; Li, S.; Chen, Y.; Li, Q.; Chen, C.J.; Hsu, W.L.; Lou, P.J.; Zhu, C.; Pan, J.; Shen, H.; et al. Tobacco smoking, alcohol drinking, betel quid chewing, and the risk of head and neck cancer in an East Asian population. *Head Neck* **2019**, *41*, 92–102. [\[CrossRef\]](#)
9. Hashibe, M.; Brennan, P.; Benhamou, S.; Castellsague, X.; Chen, C.; Curado, M.P.; Dal Maso, L.; Daudt, A.W.; Fabianova, E.; Fernandez, L.; et al. Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: Pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *J. Natl. Cancer Inst.* **2007**, *99*, 777–789. [\[CrossRef\]](#)
10. Leemans, C.R.; Snijders, P.J.F.; Brakenhoff, R.H. The molecular landscape of head and neck cancer. *Nat. Rev. Cancer* **2018**, *18*, 269–282. [\[CrossRef\]](#)
11. Boscolo-Rizzo, P.; Del Mistro, A.; Bussu, F.; Lupato, V.; Baboci, L.; Almadori, G.; MC, D.A.M.; Paludetti, G. New insights into human papillomavirus-associated head and neck squamous cell carcinoma. *Acta Otorhinolaryngol. Ital. Organo Uff. Della Soc. Ital. Di Otorinolaringol. E Chir. Cervico-Facciale* **2013**, *33*, 77–87.
12. Boscolo-Rizzo, P.; Da Mosto, M.C.; Rampazzo, E.; Giunco, S.; Del Mistro, A.; Menegaldo, A.; Baboci, L.; Mantovani, M.; Tirelli, G.; De Rossi, A. Telomeres and telomerase in head and neck squamous cell carcinoma: From pathogenesis to clinical implications. *Cancer Metastasis Rev.* **2016**, *35*, 457–474. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Arantes, L.; Cruvinel-Carlioni, A.; de Carvalho, A.C.; Sorroche, B.P.; Carvalho, A.L.; Scapulatempo-Neto, C.; Reis, R.M. TERT Promoter Mutation C228T Increases Risk for Tumor Recurrence and Death in Head and Neck Cancer Patients. *Front. Oncol.* **2020**, *10*, 1275. [\[CrossRef\]](#)
14. Carvalho, A.L.; Nishimoto, I.N.; Califano, J.A.; Kowalski, L.P. Trends in incidence and prognosis for head and neck cancer in the United States: A site-specific analysis of the SEER database. *Int. J. Cancer* **2005**, *114*, 806–816. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Barczak, W.; Suchorska, W.M.; Sobocka, A.; Bednarowicz, K.; Machczynski, P.; Golusinski, P.; Rubis, B.; Masternak, M.M.; Golusinski, W. hTERT C250T promoter mutation and telomere length as a molecular markers of cancer progression in patients with head and neck cancer. *Mol. Med. Rep.* **2017**, *16*, 441–446. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Kang, H.; Kiess, A.; Chung, C.H. Emerging biomarkers in head and neck cancer in the era of genomics. *Nat. Rev. Clin. Oncol.* **2015**, *12*, 11–26. [\[CrossRef\]](#)
17. The Cancer Genome Atlas Network. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* **2015**, *517*, 576–582. [\[CrossRef\]](#)
18. Juodzbalsys, G.; Kasradze, D.; Cicciù, M.; Sudeikis, A.; Banyas, L.; Galindo-Moreno, P.; Guobis, Z. Modern molecular biomarkers of head and neck cancer. Part I. Epigenetic diagnostics and prognostics: Systematic review. *Cancer Biomark. Sect. A Dis. Markers* **2016**, *17*, 487–502. [\[CrossRef\]](#)

19. Ausoni, S.; Boscolo-Rizzo, P.; Singh, B.; Da Mosto, M.C.; Spinato, G.; Tirelli, G.; Spinato, R.; Azzarello, G. Targeting cellular and molecular drivers of head and neck squamous cell carcinoma: Current options and emerging perspectives. *Cancer Metastasis Rev.* **2016**, *35*, 413–426. [[CrossRef](#)]
20. Agrawal, N.; Frederick, M.J.; Pickering, C.R.; Bettegowda, C.; Chang, K.; Li, R.J.; Fakhry, C.; Xie, T.X.; Zhang, J.; Wang, J.; et al. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in NOTCH1. *Science* **2011**, *333*, 1154–1157. [[CrossRef](#)]
21. Stransky, N.; Egloff, A.M.; Tward, A.D.; Kostic, A.D.; Cibulskis, K.; Sivachenko, A.; Kryukov, G.V.; Lawrence, M.S.; Sougnez, C.; McKenna, A.; et al. The mutational landscape of head and neck squamous cell carcinoma. *Science* **2011**, *333*, 1157–1160. [[CrossRef](#)] [[PubMed](#)]
22. Parfenov, M.; Pedamallu, C.S.; Gehlenborg, N.; Freeman, S.S.; Danilova, L.; Bristow, C.A.; Lee, S.; Hadjipanayis, A.G.; Ivanova, E.V.; Wilkerson, M.D.; et al. Characterization of HPV and host genome interactions in primary head and neck cancers. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 15544–15549. [[CrossRef](#)]
23. Seiwert, T.Y.; Zuo, Z.; Keck, M.K.; Khattri, A.; Pedamallu, C.S.; Stricker, T.; Brown, C.; Pugh, T.J.; Stojanov, P.; Cho, J.; et al. Integrative and comparative genomic analysis of HPV-positive and HPV-negative head and neck squamous cell carcinomas. *Clin. Cancer Res.* **2015**, *21*, 632–641. [[CrossRef](#)] [[PubMed](#)]
24. Lechner, M.; Frampton, G.M.; Fenton, T.; Feber, A.; Palmer, G.; Jay, A.; Pillay, N.; Forster, M.; Cronin, M.T.; Lipson, D.; et al. Targeted next-generation sequencing of head and neck squamous cell carcinoma identifies novel genetic alterations in HPV+ and HPV-tumors. *Genome Med.* **2013**, *5*, 49. [[CrossRef](#)] [[PubMed](#)]
25. Killela, P.J.; Reitman, Z.J.; Jiao, Y.; Bettegowda, C.; Agrawal, N.; Diaz, L.A., Jr.; Friedman, A.H.; Friedman, H.; Gallia, G.L.; Giovannella, B.C.; et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 6021–6026. [[CrossRef](#)] [[PubMed](#)]
26. Vinothkumar, V.; Arunkumar, G.; Revathidevi, S.; Arun, K.; Manikandan, M.; Rao, A.K.; Rajkumar, K.S.; Ajay, C.; Rajaraman, R.; Ramani, R.; et al. TERT promoter hot spot mutations are frequent in Indian cervical and oral squamous cell carcinomas. *Tumour Biol. J. Int. Soc. Oncodevelopmental Biol. Med.* **2016**, *37*, 7907–7913. [[CrossRef](#)] [[PubMed](#)]
27. Qu, Y.; Dang, S.; Wu, K.; Shao, Y.; Yang, Q.; Ji, M.; Shi, B.; Hou, P. TERT promoter mutations predict worse survival in laryngeal cancer patients. *Int. J. Cancer* **2014**, *135*, 1008–1010. [[CrossRef](#)]
28. Chang, K.P.; Wang, C.I.; Pickering, C.R.; Huang, Y.; Tsai, C.N.; Tsang, N.M.; Kao, H.K.; Cheng, M.H.; Myers, J.N. Prevalence of promoter mutations in the TERT gene in oral cavity squamous cell carcinoma. *Head Neck* **2017**, *39*, 1131–1137. [[CrossRef](#)]
29. Yu, Y.; Fan, D.; Song, X.; Zakeri, K.; Chen, L.; Kang, J.; McBride, S.; Tsai, C.J.; Dunn, L.; Sherman, E.; et al. TERT Promoter Mutations Are Enriched in Oral Cavity Cancers and Associated With Locoregional Recurrence. *JCO Precis. Oncol.* **2021**, *5*, 1259–1269. [[CrossRef](#)]
30. Dratwa, M.; Wysoczańska, B.; Łacina, P.; Kubik, T.; Bogunia-Kubik, K. TERT-Regulation and Roles in Cancer Formation. *Front. Immunol.* **2020**, *11*, 589929. [[CrossRef](#)]
31. Giardini, M.A.; Segatto, M.; da Silva, M.S.; Nunes, V.S.; Cano, M.I. Telomere and telomerase biology. *Prog. Mol. Biol. Transl. Sci.* **2014**, *125*, 1–40. [[CrossRef](#)] [[PubMed](#)]
32. Schwaederle, M.; Krishnamurthy, N.; Daniels, G.A.; Piccioni, D.E.; Kesari, S.; Fanta, P.T.; Schwab, R.B.; Patel, S.P.; Parker, B.A.; Kurzrock, R. Telomerase reverse transcriptase promoter alterations across cancer types as detected by next-generation sequencing: A clinical and molecular analysis of 423 patients. *Cancer* **2018**, *124*, 1288–1296. [[CrossRef](#)] [[PubMed](#)]
33. Bhari, V.K.; Kumar, D.; Kumar, S.; Mishra, R. Shelterin complex gene: Prognosis and therapeutic vulnerability in cancer. *Biochem. Biophys. Rep.* **2021**, *26*, 100937. [[CrossRef](#)] [[PubMed](#)]
34. Rhodes, D.; Fairall, L.; Simonsson, T.; Court, R.; Chapman, L. Telomere architecture. *EMBO Rep.* **2002**, *3*, 1139–1145. [[CrossRef](#)]
35. Guterres, A.N.; Villanueva, J. Targeting telomerase for cancer therapy. *Oncogene* **2020**, *39*, 5811–5824. [[CrossRef](#)]
36. Cong, Y.S.; Wright, W.E.; Shay, J.W. Human telomerase and its regulation. *Microbiol. Mol. Biol. Rev. MMBR* **2002**, *66*, 407–425. [[CrossRef](#)]
37. Cao, Y.; Bryan, T.M.; Reddel, R.R. Increased copy number of the TERT and TERC telomerase subunit genes in cancer cells. *Cancer Sci.* **2008**, *99*, 1092–1099. [[CrossRef](#)]
38. Lu, W.; Zhang, Y.; Liu, D.; Songyang, Z.; Wan, M. Telomeres-structure, function, and regulation. *Exp. Cell Res.* **2013**, *319*, 133–141. [[CrossRef](#)]
39. McKelvey, B.A.; Umbricht, C.B.; Zeiger, M.A. Telomerase Reverse Transcriptase (TERT) Regulation in Thyroid Cancer: A Review. *Front. Endocrinol.* **2020**, *11*, 485. [[CrossRef](#)]
40. Greider, C.W.; Blackburn, E.H. The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. *Cell* **1987**, *51*, 887–898. [[CrossRef](#)]
41. Shammass, M.A. Telomeres, lifestyle, cancer, and aging. *Curr. Opin. Clin. Nutr. Metab. Care* **2011**, *14*, 28–34. [[CrossRef](#)] [[PubMed](#)]
42. Yuan, X.; Larsson, C.; Xu, D. Mechanisms underlying the activation of TERT transcription and telomerase activity in human cancer: Old actors and new players. *Oncogene* **2019**, *38*, 6172–6183. [[CrossRef](#)] [[PubMed](#)]
43. Jacobs, J.J.; de Lange, T. Significant role for p16INK4a in p53-independent telomere-directed senescence. *Curr. Biol. CB* **2004**, *14*, 2302–2308. [[CrossRef](#)] [[PubMed](#)]
44. Jafri, M.A.; Ansari, S.A.; Alqahtani, M.H.; Shay, J.W. Roles of telomeres and telomerase in cancer, and advances in telomerase-targeted therapies. *Genome Med.* **2016**, *8*, 69. [[CrossRef](#)]

45. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: The next generation. *Cell* **2011**, *144*, 646–674. [[CrossRef](#)]
46. Hanahan, D. Hallmarks of Cancer: New Dimensions. *Cancer Discov.* **2022**, *12*, 31–46. [[CrossRef](#)]
47. Chen, C.H.; Chen, R.J. Prevalence of telomerase activity in human cancer. *J. Formos. Med. Assoc.* **2011**, *110*, 275–289. [[CrossRef](#)]
48. Cesare, A.J.; Reddel, R.R. Alternative lengthening of telomeres: Models, mechanisms and implications. *Nat. Rev. Genet.* **2010**, *11*, 319–330. [[CrossRef](#)]
49. Shay, J.W. Are short telomeres predictive of advanced cancer? *Cancer Discov.* **2013**, *3*, 1096–1098. [[CrossRef](#)]
50. Leão, R.; Apolônio, J.D.; Lee, D.; Figueiredo, A.; Tabori, U.; Castelo-Branco, P. Mechanisms of human telomerase reverse transcriptase (hTERT) regulation: Clinical impacts in cancer. *J. Biomed. Sci.* **2018**, *25*, 22. [[CrossRef](#)]
51. Barthel, F.P.; Wei, W.; Tang, M.; Martinez-Ledesma, E.; Hu, X.; Amin, S.B.; Akdemir, K.C.; Seth, S.; Song, X.; Wang, Q.; et al. Systematic analysis of telomere length and somatic alterations in 31 cancer types. *Nat. Genet.* **2017**, *49*, 349–357. [[CrossRef](#)] [[PubMed](#)]
52. Masutomi, K.; Possemato, R.; Wong, J.M.; Currier, J.L.; Tothova, Z.; Manola, J.B.; Ganesan, S.; Lansdorp, P.M.; Collins, K.; Hahn, W.C. The telomerase reverse transcriptase regulates chromatin state and DNA damage responses. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 8222–8227. [[CrossRef](#)] [[PubMed](#)]
53. Nitta, E.; Yamashita, M.; Hosokawa, K.; Xian, M.; Takubo, K.; Arai, F.; Nakada, S.; Suda, T. Telomerase reverse transcriptase protects ATM-deficient hematopoietic stem cells from ROS-induced apoptosis through a telomere-independent mechanism. *Blood* **2011**, *117*, 4169–4180. [[CrossRef](#)] [[PubMed](#)]
54. Koh, C.M.; Khattar, E.; Leow, S.C.; Liu, C.Y.; Muller, J.; Ang, W.X.; Li, Y.; Franzoso, G.; Li, S.; Guccione, E.; et al. Telomerase regulates MYC-driven oncogenesis independent of its reverse transcriptase activity. *J. Clin. Investig.* **2015**, *125*, 2109–2122. [[CrossRef](#)]
55. Stewart, S.A.; Hahn, W.C.; O'Connor, B.F.; Banner, E.N.; Lundberg, A.S.; Modha, P.; Mizuno, H.; Brooks, M.W.; Fleming, M.; Zimonjic, D.B.; et al. Telomerase contributes to tumorigenesis by a telomere length-independent mechanism. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 12606–12611. [[CrossRef](#)]
56. Romaniuk, A.; Paszel-Jaworska, A.; Totoń, E.; Lisiak, N.; Hołysz, H.; Królak, A.; Grodecka-Gazdecka, S.; Rubiś, B. The non-canonical functions of telomerase: To turn off or not to turn off. *Mol. Biol. Rep.* **2019**, *46*, 1401–1411. [[CrossRef](#)]
57. Nassir, N.; Hyde, G.J.; Baskar, R. A telomerase with novel non-canonical roles: TERT controls cellular aggregation and tissue size in Dictyostelium. *PLoS Genet.* **2019**, *15*, e1008188. [[CrossRef](#)]
58. Saretzki, G. Extra-telomeric functions of human telomerase: Cancer, mitochondria and oxidative stress. *Curr. Pharm. Des.* **2014**, *20*, 6386–6403. [[CrossRef](#)]
59. Weinhold, N.; Jacobsen, A.; Schultz, N.; Sander, C.; Lee, W. Genome-wide analysis of noncoding regulatory mutations in cancer. *Nat. Genet.* **2014**, *46*, 1160–1165. [[CrossRef](#)]
60. Bell, R.J.; Rube, H.T.; Xavier-Magalhães, A.; Costa, B.M.; Mancini, A.; Song, J.S.; Costello, J.F. Understanding TERT Promoter Mutations: A Common Path to Immortality. *Mol. Cancer Res. MCR* **2016**, *14*, 315–323. [[CrossRef](#)]
61. Vinagre, J.; Almeida, A.; Pópulo, H.; Batista, R.; Lyra, J.; Pinto, V.; Coelho, R.; Celestino, R.; Prazeres, H.; Lima, L.; et al. Frequency of TERT promoter mutations in human cancers. *Nat. Commun.* **2013**, *4*, 2185. [[CrossRef](#)] [[PubMed](#)]
62. Huang, F.W.; Hodis, E.; Xu, M.J.; Kryukov, G.V.; Chin, L.; Garraway, L.A. Highly recurrent TERT promoter mutations in human melanoma. *Science* **2013**, *339*, 957–959. [[CrossRef](#)] [[PubMed](#)]
63. Horn, S.; Figl, A.; Rachakonda, P.S.; Fischer, C.; Sucker, A.; Gast, A.; Kadel, S.; Moll, I.; Nagore, E.; Hemminki, K. TERT promoter mutations in familial and sporadic melanoma. *Science* **2013**, *339*, 959–961. [[CrossRef](#)] [[PubMed](#)]
64. Heidenreich, B.; Rachakonda, P.S.; Hemminki, K.; Kumar, R. TERT promoter mutations in cancer development. *Curr. Opin. Genet. Dev.* **2014**, *24*, 30–37. [[CrossRef](#)]
65. Sharma, S.; Chowdhury, S. Emerging mechanisms of telomerase reactivation in cancer. *Trends Cancer* **2022**, *8*, 632–641. [[CrossRef](#)]
66. Min, J.; Shay, J.W. TERT Promoter Mutations Enhance Telomerase Activation by Long-Range Chromatin Interactions. *Cancer Discov.* **2016**, *6*, 1212–1214. [[CrossRef](#)]
67. Bell, R.J.; Rube, H.T.; Kreig, A.; Mancini, A.; Fouse, S.D.; Nagarajan, R.P.; Choi, S.; Hong, C.; He, D.; Pekmezci, M.; et al. Cancer. The transcription factor GABP selectively binds and activates the mutant TERT promoter in cancer. *Science* **2015**, *348*, 1036–1039. [[CrossRef](#)]
68. Stern, J.L.; Theodorescu, D.; Vogelstein, B.; Papadopoulos, N.; Cech, T.R. Mutation of the TERT promoter, switch to active chromatin, and monoallelic TERT expression in multiple cancers. *Genes Dev.* **2015**, *29*, 2219–2224. [[CrossRef](#)]
69. Borah, S.; Xi, L.; Zaug, A.J.; Powell, N.M.; Dancik, G.M.; Cohen, S.B.; Costello, J.C.; Theodorescu, D.; Cech, T.R. Cancer. TERT promoter mutations and telomerase reactivation in urothelial cancer. *Science* **2015**, *347*, 1006–1010. [[CrossRef](#)]
70. Koelsche, C.; Sahm, F.; Capper, D.; Reuss, D.; Sturm, D.; Jones, D.T.; Kool, M.; Northcott, P.A.; Wiestler, B.; Böhmer, K.; et al. Distribution of TERT promoter mutations in pediatric and adult tumors of the nervous system. *Acta Neuropathol.* **2013**, *126*, 907–915. [[CrossRef](#)]
71. Roh, M.R.; Park, K.H.; Chung, K.Y.; Shin, S.J.; Rha, S.Y.; Tsao, H. Telomerase reverse transcriptase (TERT) promoter mutations in Korean melanoma patients. *Am. J. Cancer Res.* **2017**, *7*, 134–138.
72. Gandini, S.; Zanna, I.; De Angelis, S.; Palli, D.; Raimondi, S.; Ribero, S.; Masala, G.; Suppa, M.; Bellerba, F.; Corso, F.; et al. TERT promoter mutations and melanoma survival: A comprehensive literature review and meta-analysis. *Crit. Rev. Oncol./Hematol.* **2021**, *160*, 103288. [[CrossRef](#)]

73. Liu, X.; Wu, G.; Shan, Y.; Hartmann, C.; von Deimling, A.; Xing, M. Highly prevalent *TERT* promoter mutations in bladder cancer and glioblastoma. *Cell Cycle* **2013**, *12*, 1637–1638. [[CrossRef](#)] [[PubMed](#)]
74. Hurst, C.D.; Platt, F.M.; Knowles, M.A. Comprehensive mutation analysis of the *TERT* promoter in bladder cancer and detection of mutations in voided urine. *Eur. Urol.* **2014**, *65*, 367–369. [[CrossRef](#)] [[PubMed](#)]
75. Allory, Y.; Beukers, W.; Sagrera, A.; Flández, M.; Marqués, M.; Márquez, M.; van der Keur, K.A.; Dyrskjot, L.; Lurkin, I.; Vermeij, M.; et al. Telomerase reverse transcriptase promoter mutations in bladder cancer: High frequency across stages, detection in urine, and lack of association with outcome. *Eur. Urol.* **2014**, *65*, 360–366. [[CrossRef](#)] [[PubMed](#)]
76. Rachakonda, P.S.; Hosen, I.; de Verdier, P.J.; Fallah, M.; Heidenreich, B.; Ryk, C.; Wiklund, N.P.; Steineck, G.; Schadendorf, D.; Hemminki, K.; et al. *TERT* promoter mutations in bladder cancer affect patient survival and disease recurrence through modification by a common polymorphism. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 17426–17431. [[CrossRef](#)] [[PubMed](#)]
77. Wang, K.; Liu, T.; Ge, N.; Liu, L.; Yuan, X.; Liu, J.; Kong, F.; Wang, C.; Ren, H.; Yan, K.; et al. *TERT* promoter mutations are associated with distant metastases in upper tract urothelial carcinomas and serve as urinary biomarkers detected by a sensitive castPCR. *Oncotarget* **2014**, *5*, 12428–12439. [[CrossRef](#)]
78. Huang, D.S.; Wang, Z.; He, X.J.; Diplas, B.H.; Yang, R.; Killela, P.J.; Meng, Q.; Ye, Z.Y.; Wang, W.; Jiang, X.T.; et al. Recurrent *TERT* promoter mutations identified in a large-scale study of multiple tumour types are associated with increased *TERT* expression and telomerase activation. *Eur. J. Cancer* **2015**, *51*, 969–976. [[CrossRef](#)]
79. Stoehr, R.; Taubert, H.; Zinnall, U.; Giedl, J.; Gaisa, N.T.; Burger, M.; Ruemmele, P.; Hurst, C.D.; Knowles, M.A.; Wullich, B.; et al. Frequency of *TERT* Promoter Mutations in Prostate Cancer. *Pathobiol. J. Immunopathol. Mol. Cell. Biol.* **2015**, *82*, 53–57. [[CrossRef](#)]
80. Nonoguchi, N.; Ohta, T.; Oh, J.E.; Kim, Y.H.; Kleihues, P.; Ohgaki, H. *TERT* promoter mutations in primary and secondary glioblastomas. *Acta Neuropathol.* **2013**, *126*, 931–937. [[CrossRef](#)]
81. Pezzuto, F.; Buonaguro, L.; Buonaguro, F.M.; Tornesello, M.L. Frequency and geographic distribution of *TERT* promoter mutations in primary hepatocellular carcinoma. *Infect. Agents Cancer* **2017**, *12*, 27. [[CrossRef](#)] [[PubMed](#)]
82. Lombardo, D.; Saitta, C.; Giosa, D.; Di Tocco, F.C.; Musolino, C.; Caminiti, G.; Chines, V.; Franzè, M.S.; Alibrandi, A.; Navarra, G.; et al. Frequency of somatic mutations in *TERT* promoter, TP53 and CTNNB1 genes in patients with hepatocellular carcinoma from Southern Italy. *Oncol. Lett.* **2020**, *19*, 2368–2374. [[CrossRef](#)] [[PubMed](#)]
83. Nault, J.C.; Mallet, M.; Pilati, C.; Calderaro, J.; Bioulac-Sage, P.; Laurent, C.; Laurent, A.; Cherqui, D.; Balabaud, C.; Zucman-Rossi, J. High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat. Commun.* **2013**, *4*, 2218. [[CrossRef](#)] [[PubMed](#)]
84. Cevik, D.; Yildiz, G.; Ozturk, M. Common telomerase reverse transcriptase promoter mutations in hepatocellular carcinomas from different geographical locations. *World J. Gastroenterol.* **2015**, *21*, 311–317. [[CrossRef](#)] [[PubMed](#)]
85. Liu, X.; Bishop, J.; Shan, Y.; Pai, S.; Liu, D.; Murugan, A.K.; Sun, H.; El-Naggar, A.K.; Xing, M. Highly prevalent *TERT* promoter mutations in aggressive thyroid cancers. *Endocr.-Relat. Cancer* **2013**, *20*, 603–610. [[CrossRef](#)]
86. Yang, H.; Park, H.; Ryu, H.J.; Heo, J.; Kim, J.S.; Oh, Y.L.; Choe, J.H.; Kim, J.H.; Kim, J.S.; Jang, H.W.; et al. Frequency of *TERT* Promoter Mutations in Real-World Analysis of 2,092 Thyroid Carcinoma Patients. *Endocrinol. Metab.* **2022**, *37*, 652–663. [[CrossRef](#)]
87. Alzahrani, A.S.; Alsaadi, R.; Murugan, A.K.; Sadiq, B.B. *TERT* Promoter Mutations in Thyroid Cancer. *Horm. Cancer* **2016**, *7*, 165–177. [[CrossRef](#)]
88. Campanella, N.C.; Celestino, R.; Pestana, A.; Scapulatempo-Neto, C.; de Oliveira, A.T.; Brito, M.J.; Gouveia, A.; Lopes, J.M.; Guimarães, D.P.; Soares, P.; et al. Low frequency of *TERT* promoter mutations in gastrointestinal stromal tumors (GISTs). *Eur. J. Hum. Genet. EJHG* **2015**, *23*, 877–879. [[CrossRef](#)]
89. Tallet, A.; Nault, J.C.; Renier, A.; Hysi, I.; Galateau-Sallé, F.; Cazes, A.; Copin, M.C.; Hofman, P.; Andujar, P.; Le Pimpec-Barthes, F.; et al. Overexpression and promoter mutation of the *TERT* gene in malignant pleural mesothelioma. *Oncogene* **2014**, *33*, 3748–3752. [[CrossRef](#)]
90. Griewank, K.G.; Schilling, B.; Murali, R.; Bielefeld, N.; Schwamborn, M.; Sucker, A.; Zimmer, L.; Hillen, U.; Schaller, J.; Brenn, T.; et al. *TERT* promoter mutations are frequent in atypical fibroxanthomas and pleomorphic dermal sarcomas. *Mod. Pathol.* **2014**, *27*, 502–508. [[CrossRef](#)]
91. Koelsche, C.; Renner, M.; Hartmann, W.; Brandt, R.; Lehner, B.; Waldburger, N.; Alldinger, I.; Schmitt, T.; Egerer, G.; Penzel, R.; et al. *TERT* promoter hotspot mutations are recurrent in myxoid liposarcomas but rare in other soft tissue sarcoma entities. *J. Exp. Clin. Cancer Res. CR* **2014**, *33*, 33. [[CrossRef](#)]
92. Scott, G.A.; Laughlin, T.S.; Rothberg, P.G. Mutations of the *TERT* promoter are common in basal cell carcinoma and squamous cell carcinoma. *Mod. Pathol.* **2014**, *27*, 516–523. [[CrossRef](#)]
93. Cheng, K.A.; Kurtis, B.; Babayeva, S.; Zhuge, J.; Tantchou, I.; Cai, D.; Lafaro, R.J.; Fallon, J.T.; Zhong, M. Heterogeneity of *TERT* promoter mutations status in squamous cell carcinomas of different anatomical sites. *Ann. Diagn. Pathol.* **2015**, *19*, 146–148. [[CrossRef](#)] [[PubMed](#)]
94. Zhao, Y.; Gao, Y.; Chen, Z.; Hu, X.; Zhou, F.; He, J. Low frequency of *TERT* promoter somatic mutation in 313 sporadic esophageal squamous cell carcinomas. *Int. J. Cancer* **2014**, *134*, 493–494. [[CrossRef](#)] [[PubMed](#)]
95. Kim, S.K.; Kim, J.H.; Han, J.H.; Cho, N.H.; Kim, S.J.; Kim, S.I.; Choo, S.H.; Kim, J.S.; Park, B.; Kwon, J.E. *TERT* promoter mutations in penile squamous cell carcinoma: High frequency in non-HPV-related type and association with favorable clinicopathologic features. *J. Cancer Res. Clin. Oncol.* **2021**, *147*, 1125–1135. [[CrossRef](#)] [[PubMed](#)]

96. Annunziata, C.; Pezzuto, F.; Greggi, S.; Ionna, F.; Losito, S.; Botti, G.; Buonaguro, L.; Buonaguro, F.M.; Tornesello, M.L. Distinct profiles of TERT promoter mutations and telomerase expression in head and neck cancer and cervical carcinoma. *Int. J. Cancer* **2018**, *143*, 1153–1161. [\[CrossRef\]](#)
97. Morris, L.G.T.; Chandramohan, R.; West, L.; Zehir, A.; Chakravarty, D.; Pfister, D.G.; Wong, R.J.; Lee, N.Y.; Sherman, E.J.; Baxi, S.S.; et al. The Molecular Landscape of Recurrent and Metastatic Head and Neck Cancers: Insights From a Precision Oncology Sequencing Platform. *JAMA Oncol.* **2017**, *3*, 244–255. [\[CrossRef\]](#)
98. Liu, R.; Xing, M. TERT promoter mutations in thyroid cancer. *Endocr.-Relat. Cancer* **2016**, *23*, R143–R155. [\[CrossRef\]](#)
99. Andrés-Lencina, J.J.; Rachakonda, S.; García-Casado, Z.; Srinivas, N.; Skorokhod, A.; Requena, C.; Soriano, V.; Kumar, R.; Nagore, E. TERT promoter mutation subtypes and survival in stage I and II melanoma patients. *Int. J. Cancer* **2019**, *144*, 1027–1036. [\[CrossRef\]](#)
100. Heidenreich, B.; Kumar, R. Altered TERT promoter and other genomic regulatory elements: Occurrence and impact. *Int. J. Cancer* **2017**, *141*, 867–876. [\[CrossRef\]](#)
101. Heidenreich, B.; Kumar, R. TERT promoter mutations in telomere biology. *Mutat. Res. Rev. Mutat. Res.* **2017**, *771*, 15–31. [\[CrossRef\]](#) [\[PubMed\]](#)
102. Xu, Y.; Goldkorn, A. Telomere and Telomerase Therapeutics in Cancer. *Genes* **2016**, *7*, 22. [\[CrossRef\]](#) [\[PubMed\]](#)
103. Asai, A.; Oshima, Y.; Yamamoto, Y.; Uochi, T.A.; Kusaka, H.; Akinaga, S.; Yamashita, Y.; Pongracz, K.; Pruzan, R.; Wunder, E.; et al. A novel telomerase template antagonist (GRN163) as a potential anticancer agent. *Cancer Res.* **2003**, *63*, 3931–3939. [\[PubMed\]](#)
104. Chiappori, A.A.; Kolevska, T.; Spigel, D.R.; Hager, S.; Rarick, M.; Gadgeel, S.; Blais, N.; Von Pawel, J.; Hart, L.; Reck, M.; et al. A randomized phase II study of the telomerase inhibitor imetelstat as maintenance therapy for advanced non-small-cell lung cancer. *Ann. Oncol.* **2015**, *26*, 354–362. [\[CrossRef\]](#)
105. Kozloff, M.; Sledge, G.; Benedetti, F.; Starr, A.; Wallace, J.; Stuart, M.; Gruver, D.; Miller, K. Phase I study of imetelstat (GRN163L) in combination with paclitaxel (P) and bevacizumab (B) in patients (pts) with locally recurrent or metastatic breast cancer (MBC). *J. Clin. Oncol.* **2010**, *28*, 2598. [\[CrossRef\]](#)
106. Tefferi, A.; Lasho, T.L.; Begna, K.H.; Patnaik, M.M.; Zblewski, D.L.; Finke, C.M.; Laborde, R.R.; Wassie, E.; Schimek, L.; Hanson, C.A.; et al. A Pilot Study of the Telomerase Inhibitor Imetelstat for Myelofibrosis. *N. Engl. J. Med.* **2015**, *373*, 908–919. [\[CrossRef\]](#)
107. Baerlocher, G.M.; Oppliger Leibundgut, E.; Ottmann, O.G.; Spitzer, G.; Odenike, O.; McDevitt, M.A.; Röth, A.; Daskalakis, M.; Burington, B.; Stuart, M.; et al. Telomerase Inhibitor Imetelstat in Patients with Essential Thrombocythemia. *N. Engl. J. Med.* **2015**, *373*, 920–928. [\[CrossRef\]](#)
108. Tefferi, A.; Begna, K.; Laborde, R.R.; Patnaik, M.M.; Lasho, T.L.; Zblewski, D.; Finke, C.; Schimek, L.; LaPlant, B.R.; Hanson, C.A. *Imetelstat, a Telomerase Inhibitor, Induces Morphologic and Molecular Remissions in Myelofibrosis and Reversal of Bone Marrow Fibrosis*; American Society of Hematology: Washington, DC, USA, 2013.
109. Steensma, D.P.; Fenaux, P.; Van Eygen, K.; Raza, A.; Santini, V.; Germing, U.; Font, P.; Diez-Campelo, M.; Thepot, S.; Vellenga, E.; et al. Imetelstat Achieves Meaningful and Durable Transfusion Independence in High Transfusion-Burden Patients With Lower-Risk Myelodysplastic Syndromes in a Phase II Study. *J. Clin. Oncol.* **2021**, *39*, 48–56. [\[CrossRef\]](#)
110. Mascarenhas, J.; Harrison, C.N.; Kiladjian, J.J.; Komrokji, R.S.; Koschmieder, S.; Vannucchi, A.M.; Berry, T.; Redding, D.; Sherman, L.; Dougherty, S.; et al. Imetelstat in intermediate-2 or high-risk myelofibrosis refractory to JAK inhibitor: IMPactMF phase III study design. *Future Oncol.* **2022**, *18*, 2393–2402. [\[CrossRef\]](#)
111. Damm, K.; Hemmann, U.; Garin-Chesa, P.; Huel, N.; Kauffmann, I.; Priepke, H.; Niestroj, C.; Daiber, C.; Enenkel, B.; Guilliard, B.; et al. A highly selective telomerase inhibitor limiting human cancer cell proliferation. *EMBO J.* **2001**, *20*, 6958–6968. [\[CrossRef\]](#)
112. Nasrollahzadeh, A.; Bashash, D.; Kabuli, M.; Zandi, Z.; Kashani, B.; Zaghali, A.; Mousavi, S.A.; Ghaffari, S.H. Arsenic trioxide and BIBR1532 synergistically inhibit breast cancer cell proliferation through attenuation of NF- κ B signaling pathway. *Life Sci.* **2020**, *257*, 118060. [\[CrossRef\]](#) [\[PubMed\]](#)
113. Tawfik, H.O.; El-Hamaky, A.A.; El-Bastawissy, E.A.; Shcherbakov, K.A.; Veselovsky, A.V.; Gladilina, Y.A.; Zhdanov, D.D.; El-Hamamsy, M.H. New Genetic Bomb Trigger: Design, Synthesis, Molecular Dynamics Simulation, and Biological Evaluation of Novel BIBR1532-Related Analogs Targeting Telomerase against Non-Small Cell Lung Cancer. *Pharmaceuticals* **2022**, *15*, 481. [\[CrossRef\]](#) [\[PubMed\]](#)
114. Doğan, F.; Özates, N.P.; Bağca, B.G.; Abbaszadeh, Z.; Söğütü, F.; Gasımlı, R.; Gündüz, C.; Biray Avcı, Ç. Investigation of the effect of telomerase inhibitor BIBR1532 on breast cancer and breast cancer stem cells. *J. Cell. Biochem.* **2019**, *120*, 1282–1293. [\[CrossRef\]](#) [\[PubMed\]](#)
115. Bashash, D.; Zareii, M.; Safaroghli-Azar, A.; Omrani, M.D.; Ghaffari, S.H. Inhibition of telomerase using BIBR1532 enhances doxorubicin-induced apoptosis in pre-B acute lymphoblastic leukemia cells. *Hematology* **2017**, *22*, 330–340. [\[CrossRef\]](#)
116. Shi, Y.; Sun, L.; Chen, G.; Zheng, D.; Li, L.; Wei, W. A combination of the telomerase inhibitor, BIBR1532, and paclitaxel synergistically inhibit cell proliferation in breast cancer cell lines. *Target. Oncol.* **2015**, *10*, 565–573. [\[CrossRef\]](#)
117. Ding, X.; Cheng, J.; Pang, Q.; Wei, X.; Zhang, X.; Wang, P.; Yuan, Z.; Qian, D. BIBR1532, a Selective Telomerase Inhibitor, Enhances Radiosensitivity of Non-Small Cell Lung Cancer Through Increasing Telomere Dysfunction and ATM/CHK1 Inhibition. *Int. J. Radiat. Oncol. Biol. Phys.* **2019**, *105*, 861–874. [\[CrossRef\]](#)
118. Altamura, G.; Degli Uberti, B.; Galiero, G.; De Luca, G.; Power, K.; Licenziato, L.; Maiolino, P.; Borzacchiello, G. The Small Molecule BIBR1532 Exerts Potential Anti-cancer Activities in Preclinical Models of Feline Oral Squamous Cell Carcinoma Through Inhibition of Telomerase Activity and Down-Regulation of TERT. *Front. Vet. Sci.* **2020**, *7*, 620776. [\[CrossRef\]](#)

119. Ameri, Z.; Ghiasi, S.; Farsinejad, A.; Hassanshahi, G.; Ehsan, M.; Fatemi, A. Telomerase inhibitor MST-312 induces apoptosis of multiple myeloma cells and down-regulation of anti-apoptotic, proliferative and inflammatory genes. *Life Sci.* **2019**, *228*, 66–71. [\[CrossRef\]](#)
120. Biffi, G.; Tannahill, D.; McCafferty, J.; Balasubramanian, S. Quantitative visualization of DNA G-quadruplex structures in human cells. *Nat. Chem.* **2013**, *5*, 182–186. [\[CrossRef\]](#)
121. Konieczna, N.; Romaniuk-Drapała, A.; Lisiak, N.; Totoń, E.; Paszel-Jaworska, A.; Kaczmarek, M.; Rubiś, B. Telomerase Inhibitor TMPyP4 Alters Adhesion and Migration of Breast-Cancer Cells MCF7 and MDA-MB-231. *Int. J. Mol. Sci.* **2019**, *20*, 2670. [\[CrossRef\]](#)
122. Leonetti, C.; Scarsella, M.; Riggio, G.; Rizzo, A.; Salvati, E.; D’Incalci, M.; Staszewsky, L.; Frapolli, R.; Stevens, M.F.; Stoppacciaro, A.; et al. G-quadruplex ligand RHPS4 potentiates the antitumor activity of camptothecins in preclinical models of solid tumors. *Clin. Cancer Res.* **2008**, *14*, 7284–7291. [\[CrossRef\]](#) [\[PubMed\]](#)
123. Fujimori, J.; Matsuo, T.; Shimose, S.; Kubo, T.; Ishikawa, M.; Yasunaga, Y.; Ochi, M. Antitumor effects of telomerase inhibitor TMPyP4 in osteosarcoma cell lines. *J. Orthop. Res.* **2011**, *29*, 1707–1711. [\[CrossRef\]](#)
124. Mikami-Terao, Y.; Akiyama, M.; Yuza, Y.; Yanagisawa, T.; Yamada, O.; Yamada, H. Antitumor activity of G-quadruplex-interactive agent TMPyP4 in K562 leukemic cells. *Cancer Lett.* **2008**, *261*, 226–234. [\[CrossRef\]](#) [\[PubMed\]](#)
125. Zhou, G.; Liu, X.; Li, Y.; Xu, S.; Ma, C.; Wu, X.; Cheng, Y.; Yu, Z.; Zhao, G.; Chen, Y. Telomere targeting with a novel G-quadruplex-interactive ligand BRACO-19 induces T-loop disassembly and telomerase displacement in human glioblastoma cells. *Oncotarget* **2016**, *7*, 14925–14939. [\[CrossRef\]](#) [\[PubMed\]](#)
126. Kim, M.Y.; Vankayalapati, H.; Shin-Ya, K.; Wierzba, K.; Hurley, L.H. Telomestatin, a potent telomerase inhibitor that interacts quite specifically with the human telomeric intramolecular g-quadruplex. *J. Am. Chem. Soc.* **2002**, *124*, 2098–2099. [\[CrossRef\]](#) [\[PubMed\]](#)
127. Hasegawa, D.; Okabe, S.; Okamoto, K.; Nakano, I.; Shin-ya, K.; Seimiya, H. G-quadruplex ligand-induced DNA damage response coupled with telomere dysfunction and replication stress in glioma stem cells. *Biochem. Biophys. Res. Commun.* **2016**, *471*, 75–81. [\[CrossRef\]](#) [\[PubMed\]](#)
128. Yao, Y.X.; Xu, B.H.; Zhang, Y. CX-3543 Promotes Cell Apoptosis through Downregulation of CCAT1 in Colon Cancer Cells. *BioMed Res. Int.* **2018**, *2018*, 9701957. [\[CrossRef\]](#) [\[PubMed\]](#)
129. Xu, H.; Di Antonio, M.; McKinney, S.; Mathew, V.; Ho, B.; O’Neil, N.J.; Santos, N.D.; Silvester, J.; Wei, V.; Garcia, J.; et al. CX-5461 is a DNA G-quadruplex stabilizer with selective lethality in BRCA1/2 deficient tumours. *Nat. Commun.* **2017**, *8*, 14432. [\[CrossRef\]](#)
130. Carvalho, J.; Mergny, J.L.; Salgado, G.F.; Queiroz, J.A.; Cruz, C. G-quadruplex, Friend or Foe: The Role of the G-quartet in Anticancer Strategies. *Trends Mol. Med.* **2020**, *26*, 848–861. [\[CrossRef\]](#)
131. Mender, I.; Gryaznov, S.; Dikmen, Z.G.; Wright, W.E.; Shay, J.W. Induction of telomere dysfunction mediated by the telomerase substrate precursor 6-thio-2'-deoxyguanosine. *Cancer Discov.* **2015**, *5*, 82–95. [\[CrossRef\]](#)
132. Zeng, X.; Hernandez-Sanchez, W.; Xu, M.; Whited, T.L.; Baus, D.; Zhang, J.; Berdis, A.J.; Taylor, D.J. Administration of a Nucleoside Analog Promotes Cancer Cell Death in a Telomerase-Dependent Manner. *Cell Rep.* **2018**, *23*, 3031–3041. [\[CrossRef\]](#) [\[PubMed\]](#)
133. Sarkar, S.; Faller, D.V. T-oligos inhibit growth and induce apoptosis in human ovarian cancer cells. *Oligonucleotides* **2011**, *21*, 47–53. [\[CrossRef\]](#) [\[PubMed\]](#)
134. Pitman, R.T.; Wojdyla, L.; Puri, N. Mechanism of DNA damage responses induced by exposure to an oligonucleotide homologous to the telomere overhang in melanoma. *Oncotarget* **2013**, *4*, 761–771. [\[CrossRef\]](#) [\[PubMed\]](#)
135. Ellingsen, E.B.; Mangsbo, S.M.; Hovig, E.; Gaudernack, G. Telomerase as a Target for Therapeutic Cancer Vaccines and Considerations for Optimizing Their Clinical Potential. *Front. Immunol.* **2021**, *12*, 682492. [\[CrossRef\]](#)
136. Negrini, S.; De Palma, R.; Filaci, G. Anti-cancer Immunotherapies Targeting Telomerase. *Cancers* **2020**, *12*, 2260. [\[CrossRef\]](#)
137. Middleton, G.; Silcocks, P.; Cox, T.; Valle, J.; Wadsley, J.; Propper, D.; Coxon, F.; Ross, P.; Madhusudan, S.; Roques, T.; et al. Gemcitabine and capecitabine with or without telomerase peptide vaccine GV1001 in patients with locally advanced or metastatic pancreatic cancer (TeloVac): An open-label, randomised, phase 3 trial. *Lancet. Oncol.* **2014**, *15*, 829–840. [\[CrossRef\]](#)
138. Zanetti, M. A second chance for telomerase reverse transcriptase in anticancer immunotherapy. *Nat. Rev. Clin. Oncol.* **2017**, *14*, 115–128. [\[CrossRef\]](#)
139. Duperret, E.K.; Wise, M.C.; Trautz, A.; Villarreal, D.O.; Ferraro, B.; Walters, J.; Yan, J.; Khan, A.; Masteller, E.; Humeau, L.; et al. Synergy of Immune Checkpoint Blockade with a Novel Synthetic Consensus DNA Vaccine Targeting TERT. *Mol. Ther.* **2018**, *26*, 435–445. [\[CrossRef\]](#)
140. Fujita, K.; Kimura, M.; Kondo, N.; Sakakibara, A.; Sano, D.; Ishiguro, Y.; Tsukuda, M. Anti-tumor effects of telomelysin for head and neck squamous cell carcinoma. *Oncol. Rep.* **2008**, *20*, 1363–1368. [\[CrossRef\]](#)
141. Kondo, N.; Tsukuda, M.; Kimura, M.; Fujita, K.; Sakakibara, A.; Takahashi, H.; Ishiguro, Y.; Toth, G.; Matsuda, H. Antitumor effects of telomelysin in combination with paclitaxel or cisplatin on head and neck squamous cell carcinoma. *Oncol. Rep.* **2010**, *23*, 355–363. [\[CrossRef\]](#)
142. Nemunaitis, J.; Tong, A.W.; Nemunaitis, M.; Senzer, N.; Phadke, A.P.; Bedell, C.; Adams, N.; Zhang, Y.A.; Maples, P.B.; Chen, S.; et al. A phase I study of telomerase-specific replication competent oncolytic adenovirus (telomelysin) for various solid tumors. *Mol. Ther.* **2010**, *18*, 429–434. [\[CrossRef\]](#) [\[PubMed\]](#)

143. Heo, J.; Liang, J.-D.; Kim, C.W.; Woo, H.Y.; Shih, I.-L.; Su, T.-H.; Lin, Z.-Z.; Chang, S.; Urata, Y.; Chen, P.-J. *Safety and Dose-Escalation Study of a Targeted Oncolytic Adenovirus, Suratadenoturev (OBP-301), in Patients with Refractory Advanced Liver Cancer: Phase I Clinical Trial*; American Society of Clinical Oncology: Alexandria, Virginia, 2022.
144. Li, S.; Rosenberg, J.E.; Donjacour, A.A.; Botchkina, I.L.; Hom, Y.K.; Cunha, G.R.; Blackburn, E.H. Rapid inhibition of cancer cell growth induced by lentiviral delivery and expression of mutant-template telomerase RNA and anti-telomerase short-interfering RNA. *Cancer Res.* **2004**, *64*, 4833–4840. [[CrossRef](#)] [[PubMed](#)]
145. Akincilar, S.C.; Khattar, E.; Boon, P.L.; Unal, B.; Fullwood, M.J.; Tergaonkar, V. Long-Range Chromatin Interactions Drive Mutant TERT Promoter Activation. *Cancer Discov.* **2016**, *6*, 1276–1291. [[CrossRef](#)] [[PubMed](#)]
146. Wu, Y.F.; Ou, C.C.; Chien, P.J.; Chang, H.Y.; Ko, J.L.; Wang, B.Y. Chidamide-induced ROS accumulation and miR-129-3p-dependent cell cycle arrest in non-small lung cancer cells. *Phytomedicine* **2019**, *56*, 94–102. [[CrossRef](#)]
147. Kim, M.K. Novel insight into the function of tankyrase. *Oncol. Lett.* **2018**, *16*, 6895–6902. [[CrossRef](#)] [[PubMed](#)]

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