

The Role of Telomerase and the Telomerase Reverse Transcriptase Subunit in Pediatric Cancers

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Abstract

Telomerase Reverse Transcriptase (TERT) is a subunit of telomerase, an enzyme that extends telomeres and delays replicative senescence. TERT mutations or upregulation commonly lead to cancer. Data on telomerase and TERT for pediatric cancer are relatively sparse. I aim to conduct a review on the current literature on telomerase and TERT, investigate whether TERT mutations affect pediatric cancers more severely than adult cancers, and establish a framework for future study using supporting bioinformatics analyses. I took TERT's FASTA sequence and used the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database, CLUSTAL Omega, Transmembrane Helix Hidden Markov Model (TMHMM), SnapGene, and National Center for Biotechnology Information (NCBI) to analyze proteins related to TERT, establish functional domains, and design a hypothetical CRISPR experiment. Brief analysis shows that TERT promoter mutation rates are lower in pediatric cancer than adult cancer, but still raise cancer risk in children. No significant result was found with TMHMM. CLUSTAL analysis indicates that TERT's Reverse Transcriptase (RT) and C-terminal extension (CTE) domains are much more conserved than other parts, while STRING shows several proteins that interact with TERT. A hypothetical CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) experiment shows that TERT knockout experiments are one way to further study the effects of TERT mutations on pediatric cancer. TERT promoter mutations are generally rarer in pediatric cancer than adult cancer. The relationship between telomerase/TERT expression and cancer severity and frequency is well established. In cancers where data were available, metastatic rates were higher in patients exhibiting telomerase than in those who did not. More data is needed to make a conclusion. I failed to find a significant association between telomerase/TERT and pediatric cancers specifically, since sample sizes were extremely small. However, relative risk statistics indicate that telomerase mutations are a promising area of investigation in pediatric cancer.

Keywords: telomerase, TERT (telomerase reverse transcriptase), pediatric cancer, telomere maintenance, TERT promoter mutations, alternative lengthening of telomeres (ALT), cancer metastasis, bioinformatics analysis



1. Introduction

Cancer is a group of diseases characterized by uncontrolled cell proliferation affecting both children and adults (National Cancer Institute [NCI], 2021). The causes of cancer are varied, but all known cancers involve genetic mutations. These mutations sometimes cause functional enhancements, such as telomerase enzymes increasing their activity in cancer cells. Up to 10% of cancers are hereditary (NCI, 2024). Most gene mutations are due to external factors, but are also influenced by internal factors such as cell division and replication. For example, mutations in genes called proto-oncogenes that regulate apoptosis or cell cycle functions can confer oncogenic (cancerous) properties (Chial, 2008). Specific oncogenes can vary depending on the type of cancer.

1.1. Hallmarks of Cancer

While cancers vary broadly, they share key common traits - the Hallmarks of Cancer. These hallmarks are sustained proliferative signaling, evasion of growth suppressors, metastasis, apoptotic resistance, angiogenesis, and replicative immortality (Hanahan & Weinberg, 2000). Replicative immortality, in particular, is closely linked to telomerase (Figure 1).

1.2. Telomerase Activity

Telomerase is studied in relation to cancer because it solves the “end replication” problem. Because DNA polymerase can only synthesize DNA in the 5' carbon to 3' carbon direction (referring to the carbons on DNA's sugar), the lagging strand of DNA needs to be synthesized in the opposite direction of the replication fork using primers. This leads to a major problem because primers cannot be placed past the ends of DNA, and polymerase cannot transcribe the last few nucleotides (Greider & Blackburn, 1989). This problem is solved by telomeres - 5-15,000 base pair-long noncoding repeats of the sequence 5'-TTAGGG-3'. Each “base pair” (bp) represents one rung of a DNA double helix. They reside on the ends of chromosomes and allow DNA polymerase to run past the coding region, allowing cells to divide without losing important coding regions. However, each cell division shortens telomeres by 30-150 bp (Vaziri et al., 1993; Harley et al., 1990; Allsopp et al., 1992) until they reach their Hayflick Limit, when telomeres are too degraded to protect against lagging-strand DNA loss (Hayflick & Moorhead, 1961). That is when a normal cell stops dividing. Cancer cells can bypass the Hayflick limit via telomerase activity, which continually lengthens telomeres and prevents DNA end degradation (Hanahan & Weinberg, 2000).

1.3. Study Aim

Because telomerase is known to be more active in children (since cells in children need to divide more times than in adults), telomerase inhibitors may provide a way to treat childhood cancer. Pediatric cancer patients also tend to be exposed to fewer lifestyle or environmental exposures, making trend examination easier.

Telomerase is a well-known contributor to both pediatric and adult cancers; however, studies on telomerase are mostly done in adults due to a relative lack of pediatric data. In this study, I aim to analyze the link between telomerase and pediatric cancer by focusing on the TERT gene and to determine whether telomerase's effect differs between pediatric and adult cancers. To do so, I will review literature on telomerase, the TERT gene, and the TERT subunit, including their expression



rates, protein structure, and conservation levels. I will also propose a hypothetical CRISPR (clustered regularly interspaced short palindromic repeats) gene-editing experiment that can serve as a framework for future experiments, such as TERT knockdown experiments.

2. Materials & Methods

2.1. Gathering Protein and Gene Information

NCBI (National Center for Biotechnology Information) is a research database containing DNA, RNA, and protein sequences (in FASTA format), as well as PubMed, a literature database. I retrieved protein and gene sequences via NCBI>Protein and NCBI>Gene. Additionally, most of the literature in this paper was found through NCBI. General literature searches used the keywords “TERT,” “telomerase,” “TERT promoter mutation,” and “cancer.” I searched using the keyword “pediatric” to obtain pediatric studies on TERT for comparison with adults. Search results were initially screened based on their titles, abstracts, and keywords to identify studies relevant to TERT and telomerase activity in cancer. Studies were selected based on their relevance to TERT activity in cancer. Further studies from external journals, such as *Nature*, were identified by examining the citations and references of relevant studies in the initial search.

2.2. Investigating Protein Properties

The TransMembrane Hidden Markov Model (TMHMM) was used to analyze amino acid sequences to determine whether they contain transmembrane domains based on residue hydrophobicity. Since hydrophobic amino acids usually remain inside lipid bilayers, consecutive hydrophobic amino acids indicate a transmembrane/intramembrane domain. I retrieved FASTA-formatted amino acid sequences via NCBI to perform TMHMM analysis.

CLUSTAL Omega compares protein sequences among different organisms to identify highly conserved amino acids/domains using the PAM250 scoring matrix, which compares the likelihood of different amino acid substitutions. Additionally, it shows the electrochemical properties of the amino acid sequences (e.g., hydrophobicity, charge) with color coding, providing insight into domain function.

2.3. CRISPR/Cas9 Gene Editing Design

I retrieved gene sequences via the NCBI Gene database, then used Ensembl, a genome database containing genetic information from various organisms – including gene sequences and their introns, exons, and isoforms. I annotated the exon and intron regions of the TERT gene by using this database.

SnapGene displays a gene or protein in its nucleic acid sequence and allows for various modifications, including annotations for primers and other notable DNA/RNA/protein domains. Using SnapGene, one can also perform PCR, predict primer annealing temperature, and add or delete nucleotides. I used SnapGene to design and visualize a CRISPR/Cas9 gene editing strategy.

CRISPR Finder provides CRISPR guide RNA candidates roughly 20 bp long, given a DNA sequence. I used CRISPR Finder to generate guide RNAs (gRNAs) for a hypothetical CRISPR/Cas9 knockout.



The Human Protein Atlas shows various data on specific proteins, such as predicted localization and molecular structure across cancer types, cell lines, and tissues. I used the Human Protein Atlas to obtain TERT expression rates for different cancers as well as predicted prognosis in glioma.

3. Telomerase in Cancer Progression

While telomerase upregulation (or alternative pathways, discussed later) is important throughout cancer progression, it is especially important in metastasis. For example, zebrafish melanoma exhibits spontaneous regression rather than metastasis when telomerase is deactivated (Lopes-Bastos et al., 2024). This trend is also present in pediatric cancers. In pediatric osteosarcoma, 75% of metastatic cancers exhibited telomerase, while only 11% of nonmetastatic cases showed telomerase activity, indicating a relative risk of 6.8. Similarly, in pediatric Ewing sarcoma, 100% of metastatic cancers showed telomerase expression, while only 12.5% of nonmetastatic tumors exhibited telomerase – a relative risk of 8. While data are limited for other pediatric cancer types, the association between telomerase activity and metastatic rate found in those two indicates that this is a promising area of future study (Table 1).

Table 1: Comparison of telomerase expression in different types of cancers

Pediatric cancer type	Telomerase % in primary tumor	Telomerase % in metastatic
Medulloblastoma	unknown/limited information	10.7%* (32.1% ALT)
Ependymoma	64% (23/36)	0% (0/57)
Osteosarcoma	11% (1/9)	75% (6/8)
Ewing sarcoma	12.5% (1/8)	100% (5/5)

Note. The leftmost column contains different types of pediatric cancers, while the two right columns show telomerase expression rates in primary and metastatic tumors, respectively. Data on medulloblastoma were obtained from Minasi et al. (2019). Data on ependymoma were found in Barszczyk et al. (2014). Data on osteosarcoma and Ewing sarcoma were found from Sotillo-Piñeiro et al. (2004).



4. Telomere Biology

Telomerase has been difficult to structurally resolve because of its relatively low abundance (Nguyen et al., 2019). However, it is known to be a reverse transcriptase holoenzyme, meaning it adds telomeric DNA repeats to the ends of chromosomes from an RNA template (Figure 3). It consists of several subunits, including Telomerase Reverse Transcriptase (TERT), the catalytic subunit, and human Telomerase RNA Component (hTERC), an RNA template for the TTAGGG repeats; see Figure 1 (Lee & Pellegrini, 2022). TERT consists of 16 exons encoding a 1134 amino-acid protein and 15 introns comprising 40,766 nucleotides.

The TERT protein does not contain any transmembrane domain. I ran a TMHMM analysis on TERT, which makes its best guess as to which domains are inside or outside of a lipid bilayer by applying the positive-inside rule (Heijne, 1986). A protein result of “all in,” as shown in Figure 2, does not narrow down a specific region in the cell, but does indicate it has no transmembrane helices (Krogh et al., 2001). TERT’s apparent lack of a transmembrane domain is consistent with the current consensus that TERT, as part of telomerase, is a nuclear protein.

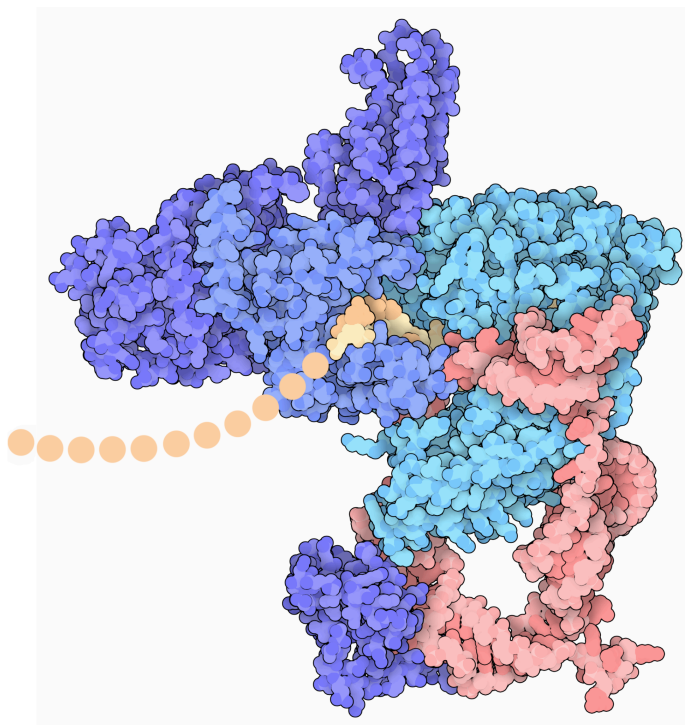


Figure 1: Molecular 3D structure of telomerase.

Note. The image shows the 3D structure of the catalytic TERT subunit (light blue) of telomerase as well as TERC (red), the RNA template for telomere synthesis. Telomerase adds a DNA strand, shown in yellow in the image (Goodsell, 2018).



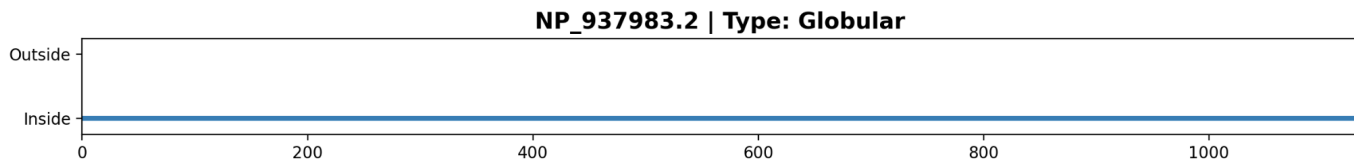


Figure 2: Transmembrane domain analysis of the TERT protein via the TMHMM algorithm.

Note. The graph shows TERT's predicted location relative to a membrane. The x-axis shows the residue position, while the y-axis indicates the likely location of that residue. The human TERT protein sequence (NP_937983.2) was obtained from the NCBI Protein database (NCBI, 2026) and analyzed using the TMHMM algorithm (Krogh et al., 2001).

4.1. Auxiliary Proteins and TERT-Interacting Proteins

It is also expressed in 85–95% of cancer cells (Shay & Bacchetti, 1997). While TERC is ubiquitously expressed in most cells (Liu et al., 2019), TERT is mainly expressed in germ cells (Leão et al., 2019). Many telomerase upregulators work by regulating TERT. Cancer often upregulates key TERT promoters; for example, a study by Dos Santos et al. found that in prostate cancer, upregulation of shelterin is associated with higher aggression and metastasis. Shelterin is an auxiliary complex composed of 6 proteins (TRF1, TRF2, POT1, RAP1, TIN2, and TPP1) responsible for protecting telomeres from DNA repair pathways. After telomere synthesis, the shelterin complex associates with telomeres to protect them from DNA repair pathway enzymes (de Lange, 2005). One shelterin function is to form D- and T-loops from the G-rich overhang of telomeres, hiding their 3' ends from exonucleases and other repair pathways. It is also a key regulator of telomerase; the POT1/TPP1 heterodimer promotes telomerase activity by binding to a region called the TEL patch and inhibiting ATR kinase (Mir et al., 2020). TIN2 causes conformational changes to TRF1 that inhibit telomerase access. TRF2 interacts with RAP1, preventing homologous recombination. The CST complex (originally thought to be unique to yeast) is a trimeric protein composed of Ctc1, Stn1, and Ten1 (Figure 3). It associates with the telomeric G-overhang and inhibits access of telomerase to the telomeres. While it can inhibit telomerase access by sequestering the G-overhang, it is also a critical regulator of telomere maintenance (Rice & Skordalakes, 2016). Together, shelterin proteins and the CST complex serve to protect telomeres from damage. Several methods of telomerase activation exist, such as promoter hypermethylation and point mutations (e.g., C228T or C250T) that create ETS (erythroblast-specific transcription factor) binding sites (Yuan et al., 2019). Multiple proteins induce TERT expression by attaching to the TEN domain and promoter; for example, c-Myc binds to E-boxes at +44 when bound to Max protein. TERT can, however, be downregulated by Mad1 (a suppressor of c-Myc) and histone deacetylases in most somatic cells (Khattar & Tergaonkar, 2017).

I also consulted the STRING database to identify other proteins that interact with TERT. STRING is a protein database that predicts protein-protein interactions. It does so via various methods, including text mining, consulting known protein databases, and computing interactions based on co-expression frequency. The proteins shown below are involved with the TERT subunit of telomerase in various ways (Figure 4). NHP2 binds to the H/ACA domain of telomeric RNA (Pogacik et al., 2000) and is involved in telomere maintenance, while WRAP53 binds to Cajal bodies and TERC and stimulates TERT activity. SMARCA4 is involved in chromatin remodeling; RUVBL2 possesses single-stranded DNA-stimulated ATPase and ATP-dependent DNA helicase (5' to 3') activity; TEP1 is another protein component of telomerase; DKC1 is a catalytic subunit



of the H/ACA small nucleolar ribonucleoprotein (H/ACA snoRNP) complex, which catalyzes pseudouridylation of ribosomal RNA; and CTNNB1 is involved in the WNT signaling pathway.

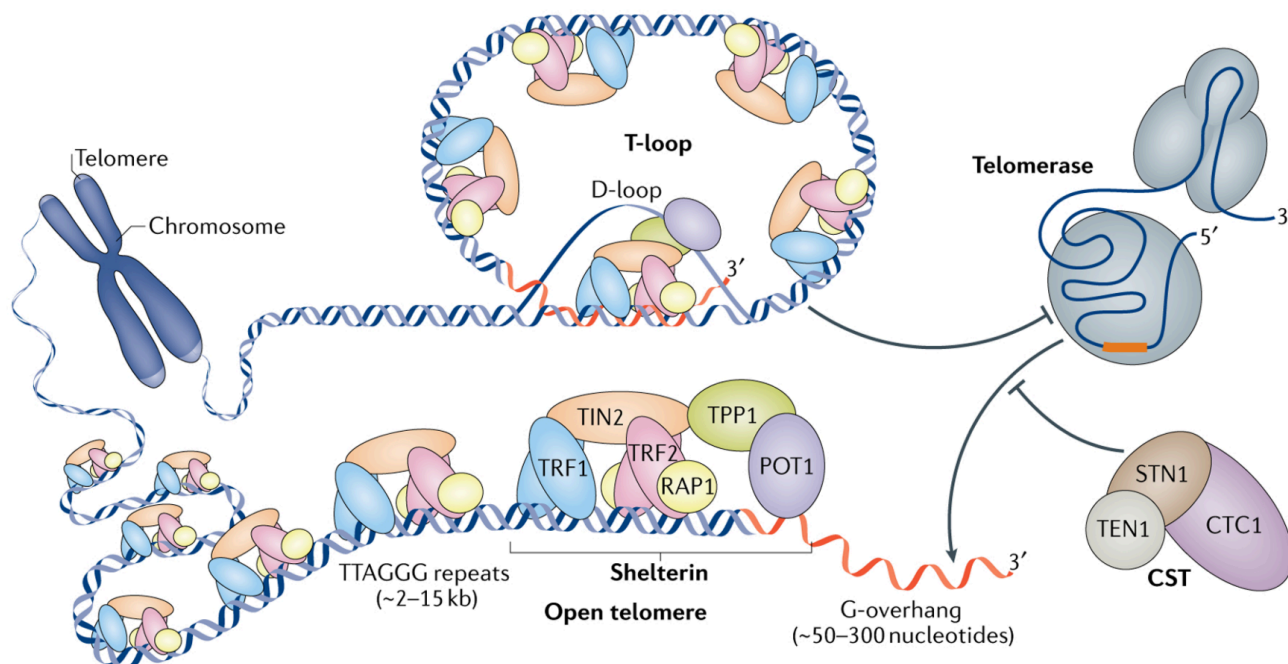


Figure 3: Telomere structure and synthesis.

Note. Telomerase synthesizes telomeres, repetitive sequences at the end of chromosomes. The shelterin complex promotes the formation of D-loops and T-loops, protecting telomeres from degradative enzymes, while the CST complex also contributes to telomere maintenance and regulation. (Lim & Cech, 2021)

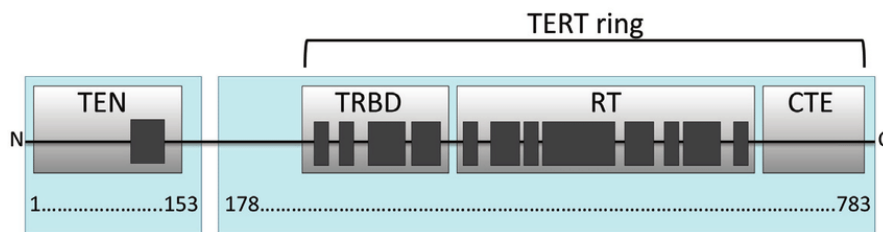


Figure 4: Protein-protein interaction map for the TERT protein.

Note. Lines indicate interactions between various proteins, each indicated by a node on the graph; each color indicates the type of evidence for interaction between two proteins. TERT has 8 experimentally determined interactions: NHP2, WRAP53, TEP1, RUVBL2, SMARCA4, DKC1, HSP90AA1, and CTNNB1. (STRING Consortium, n.d.).

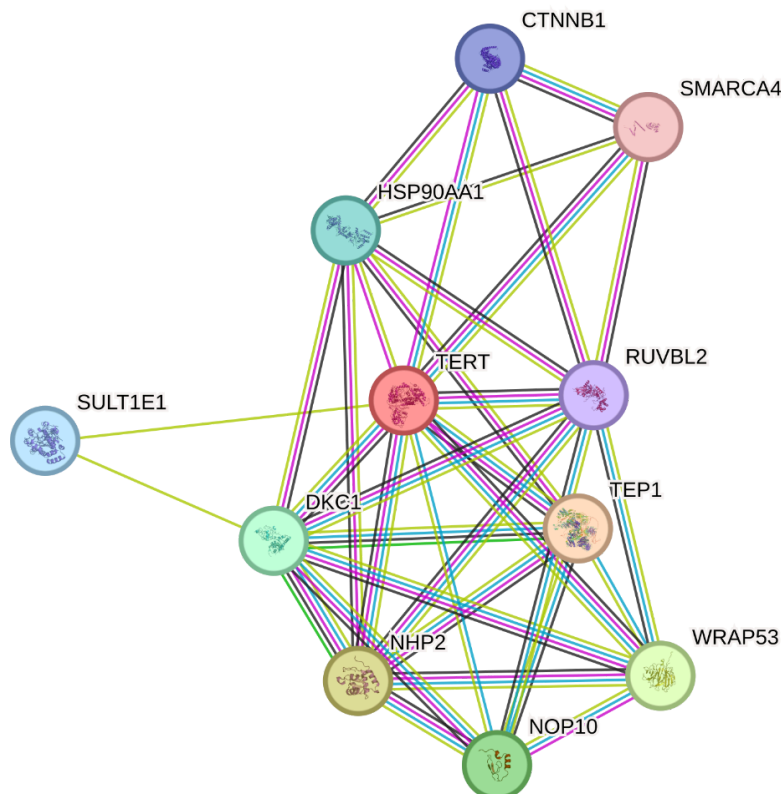


Figure 5: Protein subdomains of TERT.

Note. From the N- to C-terminus, TERT consists of TEN (TERT N-terminal domain), followed by TRBD (telomerase RNA binding domain), RT (reverse transcriptase), and C-terminal extension (CTE) (Petrova et al., 2018).

4.2. Alternative Lengthening of Telomeres

In 1997, Shay and Bacchetti first used the telomeric repeat amplification protocol (TRAP) to measure telomerase activity. They found that 82.4% of malignant tumors, 49.1% of premalignant tumors, and 23.6% of benign tumors expressed telomerase. Telomerase activity is associated with cancer malignancy; however, some tumors may utilize alternative lengthening of telomeres (ALT) pathways (Table 2). ALT utilizes proteins involved in homology-directed DNA repair (HDR) and DNA damage control to elongate telomeres, using existing chromosomal repeats as templates. There is no universal indicator of ALT activation, but typical biomarkers include heterogeneous telomere length (with some less than 8 kilobases (kb) and some greater than 50 kb) and extrachromosomal telomeric repeats (e.g., C-rich circular DNA ends) (MacKenzie et al., 2021). Expression is particularly common in mesenchymal neoplasms, with the highest rates of ALT activation being in

osteosarcoma (63%), leiomyosarcoma (60%), and undifferentiated pleomorphic sarcoma (59%). Several pediatric cancer types have a higher incidence of ALT activation than their adult counterparts (MacKenzie et al., 2021).

Table 2: Comparison of ALT activation and telomerase expression rates in pediatric cancers.

Pediatric cancer type	Telomerase positive % (cases/cohort study size)	ALT positive % (cases/cohort study size)
Acute lymphoblastic leukemia	54% (39/72)	0% (0/53)
Acute myeloid leukemia	55% (22/40)	0% (0/17)
Medulloblastoma	86% (6/7)	2% (3/137)
High-grade glioma	23% (5/22)	44% (14/32)
Low-grade glioma	0% (0/11)	2% (1/60)
Ependymoma	64% (23/36)	0% (0/95)
Neuroblastoma	38% (78/208)	15% (31/208)
Wilms tumor	72% (23/32)	35% (11/31)
Hepatoblastoma	31% (12/39)	N/A



Retinoblastoma	50% (17/34)	0% (0/4)
Osteosarcoma	32% (14/44)	80% (35/44)
Ewing Sarcoma	64% (9/14)	0% (0/14)
Embryonal rhabdomyosarcoma	50% (8/16)	38% (6/16)
Alveolar rhabdomyosarcoma	71 (10/14)	0 (0/15)

Note. This table shows various pediatric cancers and their relative telomerase (middle column) and ALT activation rates (right column). Telomerase activity and ALT status were not necessarily assessed in the same cohorts, and tumors may exhibit different or concurrent telomere-maintenance mechanisms. Percentages should not be interpreted as mutually exclusive and may not add up to 100%. All statistics were sourced from Ackermann & Fischer (2019).

5. Pediatric Cancer

Several aspects make pediatric cancer cases different from adult cancer cases. While breast, lung, and prostate cancers are the most common cancers in adults, dominant types in children are leukemia, brain and spinal cord tumors, and lymphoma (Ellicott, 2023). Pediatric neoplasms also respond better to chemotherapy. Because of this, children younger than 14 have a much higher 5-year survival rate (85%) than the general population (American Cancer Society, 2025). However, cancer is also the leading cause of disease-related death in children after infancy, with approximately 1600 children dying of cancer every year in the US alone, according to cancer.gov (Gröbner et al., 2018; Zhang et al., 2015; Bakhuizen et al., 2023). As childhood cancers are much earlier in life, there are far fewer external causes to control for, and thus, childhood cancers are much harder to prevent. Most cancers express telomerase (Hiyama et al., 2004). TERT promoter mutation rates in adult cancers can vary widely. Cancers with high promoter mutation rates ($\geq 15\%$) in adults include melanoma, liposarcoma, hepatocellular carcinoma, urothelial carcinoma, tongue squamous carcinoma, medulloblastoma, and adult glioma subtypes (including 83% of primary glioblastoma). Those with low promoter mutation rates (less than 15%) include leukemias (common in children), chordomas, synovial sarcomas, neuroblastomas, osteosarcomas (common in adults), and ependymomas (Killela et al., 2013; Ackermann & Fischer, 2019). While data on TERT promoter mutations are rare, limited studies find that TERT promoter mutation rates in children are much lower (Ackermann & Fischer, 2019).



5.1. Germline Mutations

As shown in Figure 6, testicular germ cell cancer has one of the highest TERT expression rates in the Human Protein Atlas data. As such, I decided to investigate the relationship between cancer and germline mutations. Germline mutations occur in sex cells, causing some or all cells in the body to inherit that mutation (depending on the developmental stage). Studies on pediatric soft-tissue sarcomas (e.g., Ewing, rhabdomyosarcoma) show 17% germline mutation rates in children (Yndestad et al., 2024), while diffuse midline glioma patients exhibited a 7.5% mutation rate (Mateos et al., 2025). Counterintuitively, results suggest there is no substantial difference between germline mutation rates in pediatric versus adult cancer; while the estimated contribution of germline mutations to pediatric cancer is 8-12% (Capasso et al., 2020), the attributable risk for cancer overall is 10% (NCI, 2024). However, germline mutations in TERT generally are associated with an increased risk of cancer - for example, in people with Lynch syndrome, an intronic G>A mutation increases cancer risk by 85% (Wiik et al., 2021).

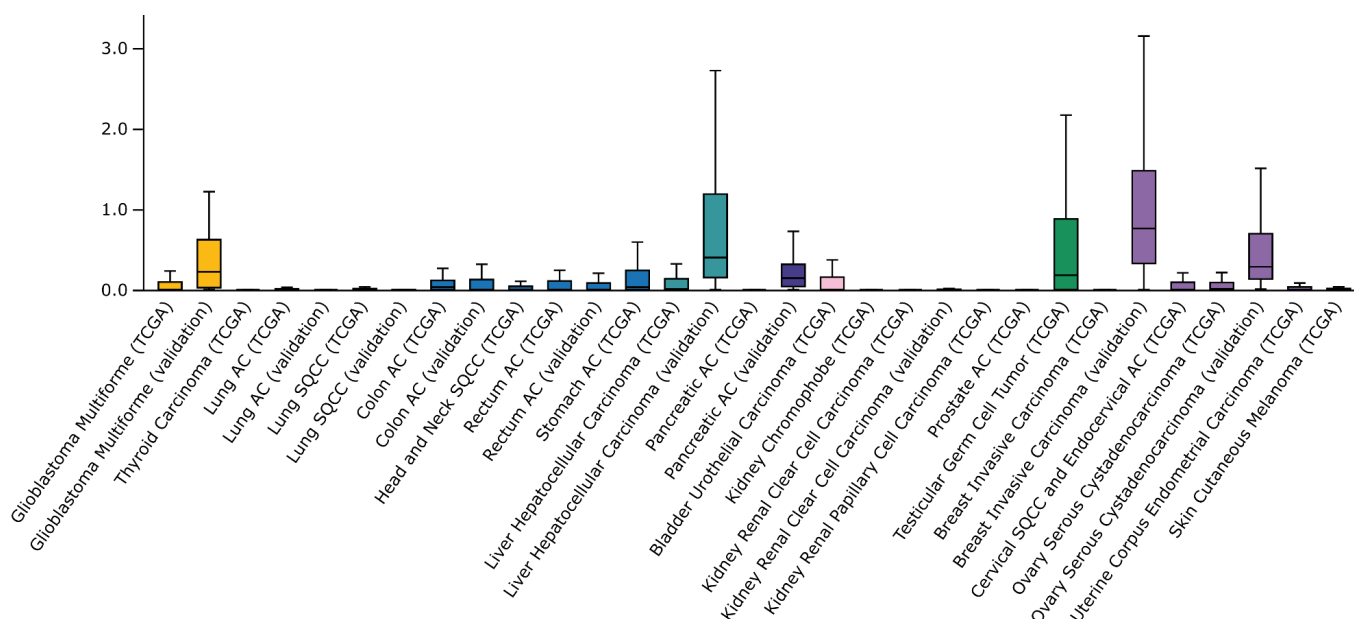


Figure 6: Median TERT expression rates of different types of cancers in protein-coding transcripts per million (pTPM).

Note. The y-axis shows TERT expression rates, while the x-axis stratifies by cancer type (The Human Protein Atlas, n.d.).

5.2. ALT Activation Rates

According to Table 2, pediatric tumors are overwhelmingly telomerase positive, similar to adult tumors. However, some cancers (osteosarcoma, Wilms tumor, neuroblastoma) exhibit ALT activation (telomerase negative), the reasons for which are still being investigated. The cancers with the highest telomerase expression rates are rhabdomyosarcoma, medulloblastoma, and Wilms tumor, while those with lower expression rates are high-grade glioma and osteosarcoma, which also have high



ALT activation rates. It is worth noting that the ALT activation rate is extremely low in low-grade glioma, so the ALT pathway could be used as a prognostic marker.

6. Hypothetical CRISPR Experiment

CRISPR is a gene editing technique originally discovered in bacterial antiviral systems that excises foreign DNA. It involves an endonuclease (usually Cas9) and a guide RNA, which attach to a target DNA sequence and cut at a recognized PAM (protospacer adjacent motif) sequence, usually 5'-NGG-3' for *S. pyogenes* Cas9. This cut is typically blunt, though Cas9 (typically derived from *Streptococcus pyogenes*) may occasionally produce short overhangs in vivo (Jasin, 2018). What happens next can vary. When attempting to change a nucleotide sequence, cells may use a template for HDR or utilize non-homologous end joining (NHEJ). This way, scientists could end up having mutations varying from single-nucleotide changes to large deletions or insertions. This has many uses; for example, one could create a double-strand break in a cell's DNA to generate mutations, analyze their effects, and thus infer the original function of a gene sequence. I decided to design a hypothetical CRISPR/Cas9 strategy for TERT to model how one would be done in a lab. Before doing so, I ensured that TERT's locus does not overlap with nearby genes, making it less likely that CRISPR experiments would disrupt other functions (Figure 7).

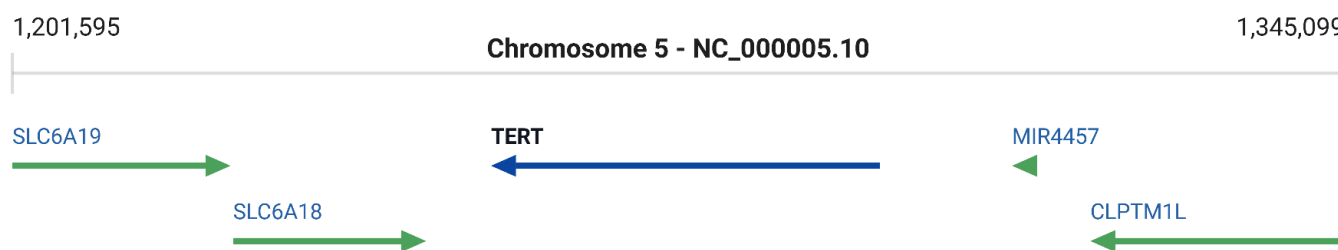


Figure 7: Chromosomal location of the TERT gene in humans.

Note. The figure shows a subsection of chromosome 5 from base pairs 1,201,595–1,345,099. A dark blue arrow indicates TERT's location and transcription direction on chromosome 5. Other human genes are marked in light green. No other genes overlap with TERT.

6.1. Reasoning

TERT is highly conserved. CLUSTAL analysis compares the amino acid sequences of a protein in different animals and indicates which residues are similar between animals using the PAM250 scoring matrix. If a region is highly similar between animals, it is conserved throughout species during evolution and could be an important functional region of the protein. When CLUSTAL analysis of TERT was done, I found that TERT's TRBD and CTE domains (Figure 5) were most strongly conserved (Figure 8). Additionally, there is a high concentration of red amino acids across all animal TERTs analyzed, meaning that TERT contains many positive residues (Figure 8). For visual ease, I also generated a heatmap of TERT residue conservation rates (Figure 9). The most highly conserved positions were residues 659–838 (Figure 9), roughly corresponding to the TRBD domain (National Center for Biotechnology Information NCBI, 2026), and residues 1016–1255 (Figure 9), roughly



corresponding to the CTE (C-terminal extension) domain (NCBI, 2026). The high conservation of these domains suggests that modifications to these regions could affect TERT's role in cancer. Since TERT upregulation and promoter mutations are linked with cancer, I wanted to design a potential CRISPR knockout experiment for others to use in telomerase inhibition studies.

chicken_NP_001026178.2	MERGAQPGVGVRRLRNVAEEPFPAVLGALRGCYAETPLFAFVRLQEGGTGEVEVLRG	60
mouse_sp 070372.1 TERT_MOUSE	-----	0
human_sp 014746.1 TERT_HUMAN	-----MPRAPRCRAVRSLRLSHYREVLPLATFVRLGPQGW---RLVQR	41
chimpanzee_PNI27662.1	-----MPRAPRCRAVRSLRLSHYREVLPLATFVRLGPQGW---RLVQR	41
chicken_NP_001026178.2	DDAQCYRTFVSQCVVCVPRGARAIPRPICFQQLSSQSEVITRIVQRLCEKKKNILAYGY	120
mouse_sp 070372.1 TERT_MOUSE	-----FHQVSSLKELVARVVQRLCERNERNVLAFGF	31
human_sp 014746.1 TERT_HUMAN	GDPAAFRALVAQCLVCPWDARPPPAAPSFQVSCLELVARVLQRLCERGAKNVLAFGF	101
chimpanzee_PNI27662.1	GDPAAFRALVAQCLVCPWDARPPPAAPSFQVSCLELVARVLQRLCERGAKNVLAFGF	101
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chicken_NP_001026178.2	SLLDENSCHFRVLPSSCIYSYLSNTVTETIRISGLWEILLSRIGDDVMMYLLHLCALFML	180
mouse_sp 070372.1 TERT_MOUSE	ELLNEARGGPPMAFTSSVRSYLPNTVIETLRVSGAWMLLLSRVGGDLLVYLLAHCALYLL	91
human_sp 014746.1 TERT_HUMAN	ALLDGARGGPPPEAFTTSVRSYLPNTVTDALRGSGAWGLLLRRVGGDDVLVHLLARCALFVL	161
chimpanzee_PNI27662.1	ALLDGARGGPPPEAFTTSVRSYLPNTVTDALRGSGAWGLLLRRVGGDDVLVHLLARCALFVL	161
	:: ::: * ** *::* ** * :** :*****::** :*****:	
chicken_NP_001026178.2	VPPSNCYQVCGQPIYELISRNVG-PSPGFVRRYSRFKHNSLLDYVRKRLVFHRYLSKS	239
mouse_sp 070372.1 TERT_MOUSE	VPPSCAYQVCGSPYQICATTDIWPVSASRYRPTRPVGRN---FTN--LRF----L-Q	139
human_sp 014746.1 TERT_HUMAN	VAPSCAYQVCGPPLYQLGAATQARPPPHASG-PRRRLGCE-----	200
chimpanzee_PNI27662.1	VAPSCAYQVCGPPLYQLGAATQARPPPHASG-PRRRLGCE-----	200
	* ** .***** *:*: : . . * . :	
chicken_NP_001026178.2	QWKKCRPRRRGRVSSRRKRSHRIQSLRSGYQPSAKVNFQAGRQISTVTARLEKQSCSSL	299
mouse_sp 070372.1 TERT_MOUSE	QIKSSSRQEA-----PKPL	153
human_sp 014746.1 TERT_HUMAN	RAWNHSVREA-----GVPL	214
chimpanzee_PNI27662.1	RAWNHSVREA-----GVPL	214
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chicken_NP_001026178.2      CLPARAPSLKRRKRDGEVEITAKRVKMEKEIEEQACSIPOVNOSSQRHGTSWHVAPR 359
mouse_sp|070372.1|TERT_MOUSE ALPSRG---TKRHLSLTS---TSVP---SAKKARCPVPVVEEGPH--RQVLP----- 195
human_sp|014746.1|TERT_HUMAN GLPAPG---ARRRGGASAS---RSLP---LPKRPRRGAAPERTPV--GQGSWAHPGR 261
chimpanzee_PNI27662.1      GLPAPG---ARRRGGASAS---RSLP---LPKRPRRGAAPERTPV--GQGSWAHPGR 261
          **:. : * : : : : : * : :

chicken_NP_001026178.2      AVGLIKEHYISERSNSEMSGSPVRRSHPGKRPVADKSSFPQGVQGNKRITGAEKRAES 419
mouse_sp|070372.1|TERT_MOUSE --TPSGK-----SW-VSPARSPPEVPTAEKDLSSKGKV----- 225
human_sp|014746.1|TERT_HUMAN TRGPSDR-----GFCVVSAPPAE-----EATSLEGAL----- 289
chimpanzee_PNI27662.1      TRGPSDR-----GFCVVSAPPAE-----EATSLEGAL----- 289
          . * : : : : : *

chicken_NP_001026178.2      NRRGIEMYINPIHKPNRRGIERRINPTHKPELNSVQTEPMEGASSGDRKQENPPAHLAKQ 479
mouse_sp|070372.1|TERT_MOUSE -----SDLSLS-GSVCKHKPSSTLSLSP 249
human_sp|014746.1|TERT_HUMAN -----SGTRHSHPSVGRQHAGPPSTSRPP 314
chimpanzee_PNI27662.1      -----SGTRHSHPSVGRQHAGPPSTSRPP 314
          . * : : : :

chicken_NP_001026178.2      LPNTFSRSTVYFEKKFLYSRSY-QEYFPKSFLSRLLGQCQAGRRLIETIFLSQNPLKE 538
mouse_sp|070372.1|TERT_MOUSE RQNAF-QLRPFIEIRHFLYSRGDQERLNPFSLLSNLQNLTGARRLVEIIFLGSRPRTS 308
human_sp|014746.1|TERT_HUMAN RPWDTPCPPVYAETKHFLYSSGDK-EQLRPSFLLSSLRPSLTGARRLVETIFLGSRPWMP 373
chimpanzee_PNI27662.1      RPWDTPCPPVYAETKHFLYSSGDK-EQLRPSFLLSSLRPSLTGARRLVETIFLGSRPWMP 373
          : * : : * * : * : * * : * : * * : * * : *

chicken_NP_001026178.2      QQNQSLPQQKWRKKRKPQYQMRIFQKLVKNHEKCPYLVFLRKNCPVLLSEACLKTE 598
mouse_sp|070372.1|TERT_MOUSE -----GPLCRTHRLSRRYWQMRPLFQQLLVNHAECQYVRLLRSHCRFRITANQOV--- 357
human_sp|014746.1|TERT_HUMAN -----GTPRRLPRLPQRYWQMRPLFLELLGNHAQCPYVLLKTHCPRLAAVTPAAG-- 424
chimpanzee_PNI27662.1      -----GTPRRLPRLPQRYWQMRPLFLELLGNHAQCPYVLLKTHCPRLAAVTPAAG-- 424
          * * : * * * * : * : * * : * : * : * :

chicken_NP_001026178.2      LTLQAALPGEAKVHKHTEHGKESTEGTAPNSFLAPPVLACGQPERGEQHPAEGSDPLL 658
mouse_sp|070372.1|TERT_MOUSE -----TDLANTSPPHLM 369
human_sp|014746.1|TERT_HUMAN -----VC-----AREKPQG-----SV-----AAPEEEDTDPRLRV 449
chimpanzee_PNI27662.1      -----VC-----AREKPQG-----SV-----AAPEEEDTDPRLRV 449
          . : . *

chicken_NP_001026178.2      ELLRQHSSHWQVYGFVRECLERVIPAEALWGSSHNKCRFFKNVKAFISMGYAKLSLQQLM 718
mouse_sp|070372.1|TERT_MOUSE DLLRLHSSPWQVYGFVRACLCKVVSASLWGTNRHNRFFKNLKKFISLGKYKLSLQELM 429
human_sp|014746.1|TERT_HUMAN QLLRQHSSPWQVYGFVRACLRLVPPGLWGSRHNRFFLRNTKKFISLGKHAKLSLQELT 509
chimpanzee_PNI27662.1      QLLRQHSSPWQVYGFVRACLRLVPPGLWGSRHNRFFLRNTKKFISLGKHAKLSLQELT 509
          : * * * * * : * : : * * : * : * : * : * : * : * : * : * : * : * : * : *

chicken_NP_001026178.2      WKMRVNDVCVWLRLAKGNHSPAYEHYREEILAKFLYWLMDSYVIELLKSFYITETMFQ 778
mouse_sp|070372.1|TERT_MOUSE WKMKVEDCHWLRSSPGKDRVPAAEHRLREIRLATFLFWMMDTYVVQLLRFFYITETTFQ 409
human_sp|014746.1|TERT_HUMAN WKMSVRDCAWLRSPGVCVPAAEHRLREILAKFLHMLMSVYVVELLRFFYVTTTFQ 569
chimpanzee_PNI27662.1      WKMSVRDCAWLRSPGVCVPAAEHRLREILAKFLHMLMSVYVVELLRFFYVTTTFQ 569
          * * * * * : * * * * * * * : * * : * : * : * : * : * : * : *

chicken_NP_001026178.2      KNMLFYRKFIWKLQNIQIRDHFAKVHLRALSSSEMEVIRQKQYFPIASRLRFIPKMNG 838
mouse_sp|070372.1|TERT_MOUSE KNRLFFYRKSVWSKLQSIGRQHLKRVQLRELSEAEVRQHREARPAALLTSRLRFIPKPDG 549
human_sp|014746.1|TERT_HUMAN KNRLFFYRKSVWSKLQSIGRQHLKRVQLRELSEAEVRQHREARPAALLTSRLRFIPKPDG 629
chimpanzee_PNI27662.1      KNRLFFYRKSVWSKLQSIGRQHLKRVQLRELSEAEVRQHREARPAALLTSRLRFIPKPDG 629
          * * * * * : * * * * * : * : * * : * : : * * * * * : *

chicken_NP_001026178.2      LRPVVRLSRVVGGKLSKESREKKIQRYNTQLKNLFSVLNVERTVNTSIIGSSVFGDDI 898
mouse_sp|070372.1|TERT_MOUSE LRPIVNMYSYMGTRALG---RRKQAHFTQRLKTLFSLNMYERTKHPHLMGSSVGLMNDI 606
human_sp|014746.1|TERT_HUMAN LRPIVNM DYVGARTFR---REKRAERLTSRVKALFSVLNMYERARRPGLLGASVGLDDI 686
chimpanzee_PNI27662.1      LRPIVNM DYVGARTFR---REKRAERLTSRVKALFSVLNMYERARRPGLLGASVGLDDI 686
          * * : * : : : * : : : : * * : * * : * : : * * : * : *

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chicken_NP_001026178.2	YRKWKFEVTKVFESGGEMPHFYFVKGDVSRADFDTIPHKKLVEVISQVLKPESTVYGI	958
mouse_sp 070372.1 TERT_MOUSE	YRTWRAFLRVRA-LDQTPRMVFVKADVTGAYDAIPQGLVEVVMIRH-SESTYCI	664
human_sp 014746.1 TERT_HUMAN	HRAWRTFVLRVRA-QDPPPELYFVKVDVTGAYDTIPQDRLTEVIASIK--PQNTYCV	743
chimpanzee_PNI27662.1	HRAWRTFVLRVRA-QDPPPELYFVKVDVTGAYDTIPQDRLTEVIASIK--PQNTYCV	743
	:* ** * : . * :***** ** :*::** :*::**:: : :..* *	
chicken_NP_001026178.2	YAVIMITPTGKARKLYKRHVSTFEDFIPDMKQFVSKLQER--TSLRNAIVVEQCLTF	1016
mouse_sp 070372.1 TERT_MOUSE	YAVVRRDSQGVHKSFRQVTTLSDLQPYMGQFLKHLQSDASALRNSVVEQSSIMNES	724
human_sp 014746.1 TERT_HUMAN	YAVVQKAAGHVRKAFKSHVSTLTDLQPYMRQFVAHLQET--SPLRDAVVEQSSSLNEA	801
chimpanzee_PNI27662.1	YAVVQKAAGHVRKAFKSHVSTLTDLQPYMRQFVAHLQET--SPLRDAVVEQSSSLNEA	801
	: *::* : :*:* * : * * ** : : *::**:: : :***	
chicken_NP_001026178.2	SSTLFTFFLQMLHNNILEIGHYYIQCSGIPQGSILSTLLCSLCYGDMMENKLLCGIK	1076
mouse_sp 070372.1 TERT_MOUSE	SSSLDFDFLHFLRHSVVKIGDRCYTQCQGIQGSILSTLLCSLCYGDMMENKLF	784
human_sp 014746.1 TERT_HUMAN	SSGLFDVFLRFMCHHAVIRGKSYVQCQGIQGSILSTLLCSLCYGDMMENKLF	861
chimpanzee_PNI27662.1	SSGLFDVFLRFVCRHAVIRGKSYVQCQGIQGSILSTLLCSLCYGDMMENKLF	861
	** ** ,*::* : . :* : * ** ,***** *****[*****] : :***	
chicken_NP_001026178.2	VLIRLIODFLLVTPHMQARTFLRTIAAGIPEYGFLLNAKKTVNFVDDI-PGCSKFKH	1135
mouse_sp 070372.1 TERT_MOUSE	LLLRFDVDFLLVTPHLDQAKTFLSTLVHGVPEYGCMLNKTQTVNFVPGTLGGAAPY	844
human_sp 014746.1 TERT_HUMAN	LLLRVDVDFLLVTPHLDQAKTFLSTLVHGVPEYGCVMNKTQTVNFVPGTLGGAAPY	921
chimpanzee_PNI27662.1	LLLRVDVDFLLVTPHLDQAKTFLSTLVHGVPEYGCVMNKTQTVNFVPGTLGGAAPY	921
	:*::***** : :*** * : . * :*** : :* :***** : * : :	
chicken_NP_001026178.2	LPDCRLISWCGLLLDVQTLLEVYCDYSSYAFTSIRSSLSFNSSRIAGNMCKLTAVL	1195
mouse_sp 070372.1 TERT_MOUSE	LPAHCLFPWCGLLLDVQTLLEVYCDYSSYAFTSIRSSLSFNSSRIAGNMCKLTAVL	904
human_sp 014746.1 TERT_HUMAN	MPAHGLFPWCGLLLDVQTLLEVYCDYSSYAFTSIRSSLSFNSSRIAGNMCKLTAVL	981
chimpanzee_PNI27662.1	LPAHGLFPWCGLLLDVQTLLEVYCDYSSYAFTSIRSSLSFNSSRIAGNMCKLTAVL	981
	:* ** ***** :*** ,*** ** *::*::* : ** :* : ** ,*::**	
chicken_NP_001026178.2	CHPLLDLKINSQTLVNIYKIFLLQAYRFHACVLQLPFNQKVRNNPDDFLRIISD	1255
mouse_sp 070372.1 TERT_MOUSE	CHGLFDLQVNSLQTVCTNIYKIFLLQAYRFHACVLQLPFDQVRKNLTFGLIISQ	964
human_sp 014746.1 TERT_HUMAN	CHSLFDLQVNSLQTVCTNIYKIFLLQAYRFHACVLQLPFHQVWKNPTFFLRVISD	1041
chimpanzee_PNI27662.1	CHSLFDLQVNSLQTVCTNIYKIFLLQAYRFHACVLQLPFHQVWKNPTFFLRVISD	1041
	** *::*::***** *****[*****]*** ,* : * ** :* : *	
chicken_NP_001026178.2	CCYFILKAKNPVSLGSKDASGMFPFEAAEWLCYHAFIVKLSNHKVIYKCLLKPL	1315
mouse_sp 070372.1 TERT_MOUSE	CCYAILKVNPGMTLKAS--GSFPPEAAHWLCYQAFLLKLAHSVYIKCLLGLPRTAQ	1021
human_sp 014746.1 TERT_HUMAN	LCYSILKAKNAGMSLGAKGAAGPLPSEAVQWLCHQAFLLKLTTRHRTYVPLLSLRTAQ	1101
chimpanzee_PNI27662.1	LCYSILKAKNAGMSLGAKGAAGPLPSEAVQWLCHQAFLLKLTTRHRTYVPLLSLRTAQ	1101
	** *** ** *::* : . * :* ** ,*** :*::*::* : * * * * * :* : :	
chicken_NP_001026178.2	HLFGKIPRDTMELLKTVTEPSLCQDFKTILD	1346
mouse_sp 070372.1 TERT_MOUSE	LLCRKLPEATMILKAAADPALSDQFQITILD	1052
human_sp 014746.1 TERT_HUMAN	QLSRKLPGTTLTALEAAANPALPSDFKTILD	1132
chimpanzee_PNI27662.1	QLSRKLPGTTLTALEAAANPALPSDFKTILD	1132
	* *:* * : *::*::* * :*****	

Figure 8: Multiple-protein alignment of the TERT protein in chicken, mouse, human, and chimpanzee.

Note. Each letter represents an amino acid, and colors correspond to chemical properties: red, positive charge; magenta, negative charge; blue, hydrophobicity; green, polarity; and pink, cysteine. Asterisks (*) indicate that an amino acid is fully conserved among the animals listed, colons (:) indicate that an amino acid is strongly conserved (PAM250 score >0.5), and a period (.) indicates weak conservation (PAM250 score between 0 and 0.5) (Sievers & Higgins, 2018). Human, chicken, and chimpanzee TERT isoforms were taken from NCBI>Protein, while chimpanzee TERT was taken from UniProt.

Heatmap of % of residues in each row (~60 residues per row) that are each symbol			
Residue position	Asterisk (fully conserved)	Colon (highly conserved)	Period (weakly conserved)
0-60	0	0	0
61-120	0.23	0.2	0.033
121-180	0.4	0.283	0.017
181-239	0.186	0.085	0.017
240-299	0.167	0.33	0.05
300-359	0.067	0.083	0.1
360-419	0.033	0.033	0.083
420-479	0.017	0.067	0.05
480-538	0.322	0.136	0.119
540-598	0.283	0.15	0.0033
600-658	0.017	0.167	0.033
659-718	0.583	0.2	0.0167
719-778	0.617	0.1	0.083
779-838	0.483	0.183	0.083



839-898	0.35	0.283	0.083
899-958	0.35	0.267	0.1
959-1016	0.345	0.345	0.034
1016-1076	0.567	0.167	0.083
1077-1135	0.525	0.254	0.017
1136-1195	0.55	0.167	0.083
1196-1255	0.7	0.13	0.033
1256-1315	0.433	0.183	0.067
1316-1346	0.419	0.258	0.032

Figure 9: Heatmap of TERT residue conservation frequency.

Note. This heatmap shows the proportion of each ~60-amino acid interval that was fully conserved (*), highly conserved (:), or weakly conserved (.) in the CLUSTAL alignment. These intervals are based on *Gallus gallus* TERT (NCBI, 2026).

6.2. gRNA Selection

Then, I gathered the TERT gene sequence from NCBI and annotated the exon and intron regions from Ensembl. I then selected a guide RNA from CRISPR Finder based on the off-target scores calculated for the allowed base mismatches of 0-4 bases, picking a guide (the upper image of Figure 10) that has no off-target binding sites with 0-3 allowed mismatches and 16 off-target binding sites with 4 allowed mismatches: the lowest of any available gRNA. SnapGene was used to visualize all the sequences and relevant regions (Figure 10). I also included potential PCR primers to show how the CRISPR results would be verified experimentally. Exon 1 is known to contain many promoter/regulatory sequences (Dratwa et al., 2020). The primers will be used to amplify the region of interest to test for potential CRISPR editing outcomes via a hypothetical polymerase chain reaction (PCR) and simulated DNA gel electrophoresis.





Figure 10: SnapGene view of CRISPR/Cas9 gene editing strategy for TERT.

Note. The upper image shows the guide RNA selected from CRISPR Finder based on its off-target score (Labun et al., 2019). The SnapGene view shows TERT exon 1 and intron 1 along with the guide RNA target sites, PAM sequence, potential cut site, and primers targeting an amplification region. Primers 1 and 2 amplify a 309-bp fragment.

6.3. Analysis of Hypothetical Results

PCR (polymerase chain reaction) is a technique used to rapidly amplify a DNA region, mimicking replication in vitro. In a hypothetical CRISPR experiment, PCR would be used to increase the quantity of DNA, making it more readily visible on an agarose gel. Gel electrophoresis could then be used to separate DNA fragments by size using an electric field. I simulated gel electrophoresis to show the hypothetical result of a CRISPR experiment (Figure 11). When interpreting the results, it's

important to note that fragments must be sequenced to resolve single-nucleotide changes, since gel electrophoresis only reveals sufficiently large DNA deletions or insertions.



Figure 11: DNA gel electrophoresis of a hypothetical CRISPR/Cas9 experiment on the TERT gene.

Note. Lane 0 contains a DNA fragment ladder with known sizes. Lane 1 contains wild-type TERT fragments. Lanes 4, 5, and 6 all contain single-nucleotide mutations, which cannot be distinguished on a gel. Lane 2 contains TERT with an ~80 bp deletion, while lane 3 contains TERT with a ~75 bp insertion.

7. Discussion

7.1. Limitations

Telomere and telomerase-related oncology studies, while common, are rarely specific to children. The few available studies had very small sample sizes—many cohorts comprised 10 to 20 children. Additionally, cancer is extremely heterogeneous; many cancers have vastly differing telomerase expression rates. Overall, there are very few comparative studies on telomerase and TERT in pediatric and adult cancers.

7.2. Telomerase and ALT

Telomerase activity was highly prevalent in both pediatric and adult cancers; however, one notable difference was a generally higher prevalence of ALT activation in some pediatric cancers. In Table 2, pediatric cancer telomerase expression rates



ranged from 0% in low-grade glioma to 86% in medulloblastoma, while ALT activation rates ranged from 0% in several cancers to nearly 80% in osteosarcoma. Current literature indicates that telomerase activity and TERT mutations are associated with cancer development and severity (Albadrani & Zakariyah, 2025), as well as lower average patient survival time; glioma is shown in Figure 12 as an example.

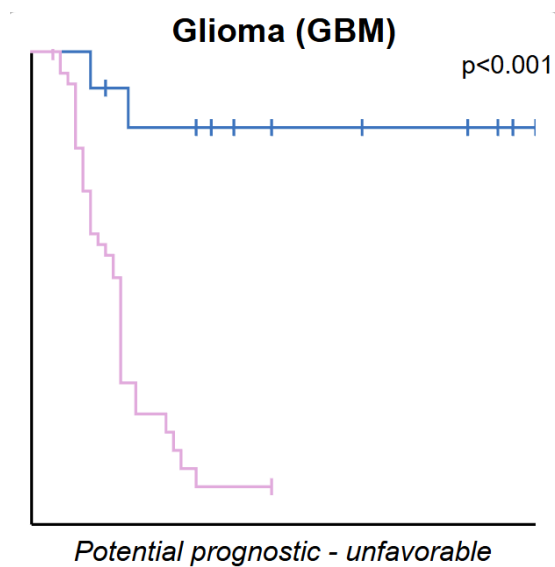


Figure 12: Prognosis of glioma.

Note. Kaplan-Meier survival curve comparing telomerase-negative (blue) and telomerase-positive (pink) glioma patients over time. Survival rates are significantly lower ($p < 0.001$) in telomerase-positive patients (The Human Protein Atlas, n.d.).

7.3. Potential Treatments and Future Research Strategies

In cancers that express it, telomerase inhibitors have demonstrated biological activity. Imetelstat, the only telomerase inhibitor approved by the U.S. Food & Drug Administration, is approved only for adults. At the time of review, it is undergoing phase 1 and 2 trials in children; a brief search of ClinicalTrials.gov reveals 3 studies, including one with published results. In that study, no objective tumor responses were recorded, but imetelstat successfully inhibited telomerase (Salloum et al., 2016).

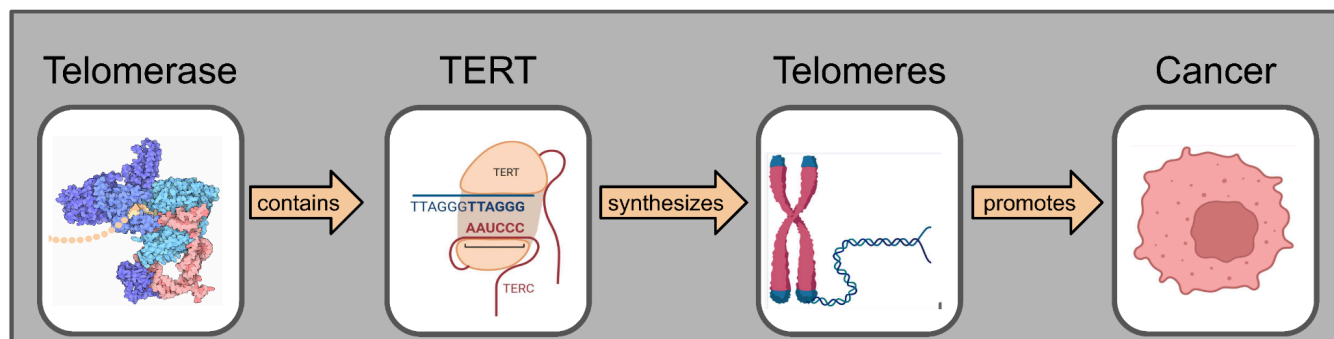
However, cancers can develop resistance to imetelstat. Another problem with telomerase inhibitors is their harmful side effects; for example, because it reduces the number of times cells can safely divide, imetelstat is known to cause temporary female infertility (Mayo Clinic, 2026), among other symptoms. By identifying associated proteins using STRING protein-protein interaction data (Figure 4), one could find alternative therapeutic targets that may avoid these limitations.

Meanwhile, CLUSTAL analysis can provide insight into telomerase functional domains to target. In TERT, targeting the more highly conserved RT and CTE domains with telomerase inhibitors could disrupt telomerase function. With supporting



bioinformatics analyses, CRISPR knockout experiments could provide a feasible approach to investigating the relationship between telomerase and cancer or potential treatment strategies.

Current Literature



Future Study Framework

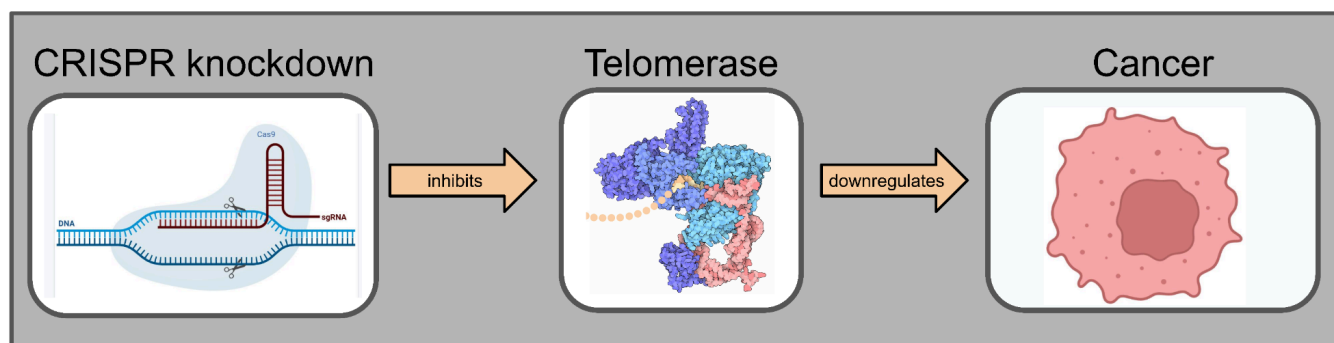


Figure 13: Graphical representation of a potential CRISPR experiment.

Note. This figure was created with BioRender.com.

8. Conclusion

Further studies are still needed. While current literature establishes an association between TERT and pediatric cancer, there is little data explicitly defined as non-pediatric for comparison. Thus, current literature does not establish a difference between telomerase and TERT's importance in pediatric cancer as opposed to adult cancer. However, several trends emerge. Telomerase and TERT are prevalent in both groups. Pediatric cancer, however, generally exhibits higher ALT activation and lower TERT promoter mutation rates. The available data on prognostic factors indicate that TERT and telomerase are associated with cancer progression in general; however, small sample sizes limit both the significance of these findings in

pediatric studies and comparison with adult cohorts. Studies that stratify pediatric and non-pediatric cohorts are needed to establish a link between TERT and pediatric cancer specifically. Such data, especially from cancers where telomerase assays are easily done, may provide valuable insights into how telomerase behaves in pediatric cancers. I established a CRISPR framework to analyze the TERT subunit of telomerase, which may be an effective way to elucidate the link between TERT and telomerase. With more data, this may prove to be a promising area of future study.

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Aaron Lim is a 17-year-old student at Canyon High School. Ever since he was a child, he has been fascinated by genetics



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After learning more about the role of enzymes in cancer during high school, Aaron was inspired to write about telomerase, in part due to the loss of his sister to cancer when he was younger. The relative rarity of pediatric TERT studies has led Aaron to appreciate the nuances and difficulties of discovering new knowledge. During undergraduate studies, he hopes to continue working in biomedical research and contribute to the advancement of the field.

When not doing research, he is also a passionate Science Olympiad competitor, competing in events spanning botany, entomology, anatomy, epidemiology, genetics, and protein design.

Mentor Contribution Statement

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