

Investigating the role Telomerase and TERT subunit in paediatric cancers



Abstract (summary)

Background TERT is a subunit of telomerase, an enzyme that extends telomeres and delays replicative senescence. Mutations or upregulation of it commonly lead to cancer.

Objectives: Data on telomerase and TERT for pediatric cancer are relatively sparse. I aim to investigate whether TERT mutations affect pediatric cancer more severely than adult cancer and establish a framework for future study.

Methods We took TERT's FASTA sequence and used the STRING protein database, CLUSTAL, TMHMM, SnapGene, and NCBI to analyze proteins related to TERT, establish functional domains, and perform a hypothetical CRISPR experiment.

Results: Brief analysis shows that TERT promoter mutation rates are lower in pediatric cancer than adult cancer, but still raise the risk of cancer. No significant result was found with TMHMM. CLUSTAL indicates that TERT's RT and CTE domains (catalytic and binding domains, respectively) are much more conserved than other parts, while STRING shows that many proteins are related to TERT. A hypothetical CRISPR experiment shows that TERT knockdowns are one way to further study the effects of TERT mutations on pediatric cancer.

Conclusion: TERT promoter mutations, are generally rarer in pediatric cancer than adult cancer, while increased telomerase expression overall has a significant effect on pediatric cancer. In cancers where data were available, metastatic rates were significantly higher in patients exhibiting telomerase than in those who did not. While sample sizes were extremely small, relative risk statistics indicate that telomerase mutations are a promising area of investigation in pediatric cancer.

Keywords: Telomerase, paediatric cancer, cancer, TERT gene

1. Introduction

Cancer is a group of diseases where cells rapidly multiply and can affect both children and adults (“What Is Cancer?” 2007). There are many types (usually named after the body part they affect), from leukemia and brain to prostate and breast cancer. The causes of cancers are equally varied, but all known cancers involve gene mutations. There are many genes that can be mutated in cancers and sometimes functional enhancements are observed such as telomerase enzymes increasing its activity in cancer cells. However, only 5-10% of cancers are hereditary (“Frequently Asked Questions about Hereditary Cancers | Memorial Sloan Kettering Cancer Center,” n.d.). Most gene mutations occur as a result of 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 turn genes oncogenic, or cancerous. Specific oncogenes can vary depending on the type of cancer. Some common oncogenes include p53, a tumor suppressor as a controller of cell division and an apoptotic inducer in the case of DNA damage at the G1/S and G2/M cell cycle checkpoints (Chen 2016)), as well as Ras, a protein family involved in cell differentiation and the MAPK/ERK pathway. (“Oncogene Ras - an Overview | ScienceDirect Topics,” n.d.) (Guo et al. 2020). For example, an R337H mutation in tp53 has led to a 15-fold increase in pediatric adrenocortical carcinoma in Paraná, Brazil (da Silva et al., n.d.).

Cancerous Hallmarks and Telomerase activity

While cancers vary broadly, they share key common traits - the Hallmarks of Cancer (Hanahan and Weinberg 2011). These hallmarks are sustained proliferative signaling, evasion of growth suppressors, metastasis, apoptotic resistance, angiogenesis, and replicative immortality. Cells require signalling factors to duplicate their DNA and eventually proliferate, however, these signalling factors are usually controlled by negative feedback mechanisms. Cancer cells can inhibit these proliferative signals,

produce their own growth factors, or inhibit negative feedback mechanisms. Metastasis, another criterion, is defined as the ability of cancer cells to spread to other organs. (“Definition of Metastasis - NCI Dictionary of Cancer Terms - NCI” 2011). Most cancers can metastasize, often as an escape mechanism from the immune system or lack of nutrients (WANG et al. 2015) to find better environments.

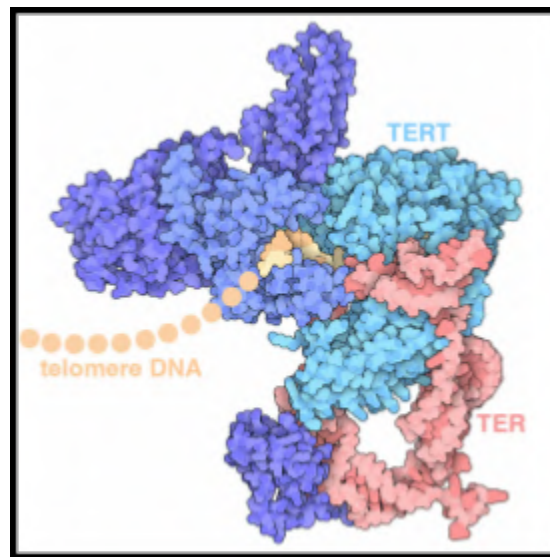


Figure 1: Molecular 3D structure of telomerase. The image shows the 3D structure of a TERT subunit of telomerase enzyme as well as TER/TERC (Telomerase RNA Component), an RNA template for telomere synthesis.

One of the hallmarks is replicative immortality, which is directly regulated by telomerase. (Figure 1). Telomerase is studied in relation to cancer as the end replication problem. Because DNA polymerase can only transcribe from the 5' to 3' direction, the lagging strand of DNA needs to be transcribed in the opposite direction of the replication fork using primers. This leads to a major problem, since primers can not be placed past the ends of DNA, and polymerase can not transcribe the last few nucleotides. This problem is solved by telomeres – 5-15000 base pair-long noncoding repeats of the sequence 5'-TTAGGG-3'. 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 30-150bp (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). That's 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. While telomerase upregulation (or alternative pathways, discussed later) is important throughout all parts of the cycle, it's especially important in metastasis. Experimental evidence shows that when telomerase is deactivated, melanoma exhibits spontaneous regression rather than metastasis (Lopes-Bastos et al. 2024). Telomerase activity correlates with pediatric metastasis. In osteosarcoma, 75% of metastatic cancers exhibited telomerase, while only 11% of nonmetastatic cases showed telomerase activity, indicating a relative risk of 6.8. In 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 is limited for other pediatric cancer types, the strong association between telomerase activity and metastatic rate found in those two indicates that this is a promising area of future study (Table 2).

Table 1. Comparison of telomerase expression in different types of cancers. The two leftmost columns contain different types of cancers (with the right column being more specific), while the middle column contains telomerase expression in all patients and the two right columns stratify telomerase expression rates by pediatric and adult. Medulloblastoma is a cerebellar cancer, ependymoma originates from ependymal cells, osteosarcoma develops in bone tissue around the knee, and Ewing sarcoma is similar to osteosarcoma, but often responsive to radiotherapy.

Pediatric cancer type	Telomerase % in primary tumor	Telomerase % in metastatic
Medulloblastoma	unknown/limited information	<u>10.7%*</u> (<u>32.1% alt</u>)
Ependymoma	<u>76%</u>	
Osteosarcoma	11% (1/9) ("Table 1 Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue Origin," n.d.)	75% (<u>6/8</u>) ("Table 1 Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue Origin," n.d.)
Ewing sarcoma	12.5% (<u>1/8</u>) ("Table 1	100% (<u>5/5</u>) ("Table 1

	Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue Origin," n.d.)	Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue Origin," n.d.)
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Due to its relatively low abundance (Nguyen et al. 2019), telomerase has been difficult to structurally resolve. However, it is known that it's a reverse transcriptase holoenzyme responsible for adding telomeres to the ends of chromosomes (Figure 2). It consists of several subunits, the two main ones being TERT (the actual enzyme) and hTERC (an RNA template for the TTAGGG repeats) (figure 1) (Lee and Pellegrini 2025a).

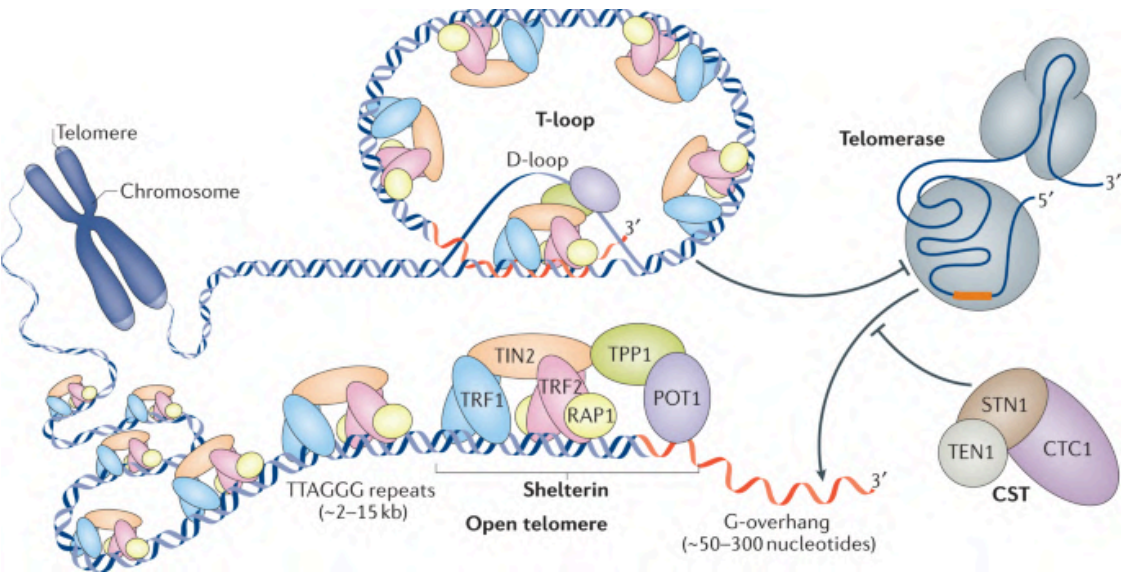


Figure 2. Telomere structure and synthesis. Telomeres are repetitive sequences at the end of chromosomes, synthesized by telomerase. Shelterin is a complex that plays several roles. After telomere synthesis, the shelterin complex wraps telomeres to protect them from enzymes involved in non-homologous end joining (NHEJ) (Lange 2005a). Shelterin's main function is to form D and T loops from the G-rich overhang of telomeres, hiding their 3' end from exonucleases and other repair pathways. It is also a key regulator of telomerase; the POT1/TPP1 heterodimer promotes telomerase activity via a binding region called the TEL patch (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. 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's also a critical upregulator (Rice and Skordalakes 2016).

TERT is functionally expressed in 85-95% of cancer cells, while being absent in most differentiated somatic cells. TERC is ubiquitously expressed in most cells, while TERT is only expressed in germ cells; most telomerase upregulators hinge on TERT expression. Cancer often upregulates key telomerase promoters; for example, a study by Santos et al. found that in prostate cancer, upregulation of shelterin is shown to correlate with higher aggression and metastasis (Liu et al. 2019). Shelterin is an auxiliary complex composed of 6 proteins (TRF1, TRF2, POT1, RAP1, TIN2, and TPP1) responsible for protecting telomeres from DNA repair pathways. In particular, catalytic activity in TPP1 and POT1 inhibits ATR kinase (a single strand break repair enzyme), while the rest also serves to inhibit ATM/ATR signalling. Together, shelterin proteins 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 transcription factor binding sites (Yuan et al. 2019). Therefore, in this study I wanted to investigate the telomerase and TERT subunit activity in pediatric cancers.

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 correlates with cancer malignancy; however, some tumors may utilize alternative lengthening of telomeres (ALT) pathways (Table 2). ALT utilizes proteins involved in homology-directed repair and DNA damage control to elongate telomeres, based on the existing repeats in chromosomes, which occurs independently of TERT upregulation. Because it uses a wide variety of DNA repair proteins, there is not a single indicator of ALT expression. Typical biomarkers include heterogeneous telomere length (with some less than 8kb and some greater than 50kb), and extrachromosomal telomeric repeats (e.g., C-rich circular DNA ends). Expression is

particularly common in mesenchymal neoplasms, with the highest rates of ALT expression being in osteosarcoma (63%), leiomyosarcoma (60%), and undifferentiated pleomorphic sarcoma (59%). Children's cancer has a higher incidence of ALT pathway (MacKenzie et al. 2021).

Table 2. Comparison of ALT pathway and telomerase activation rates in children cancers. This table shows various pediatric cancers and their relative telomerase expression rates (middle column) and ALT expression rates (right column). It's important to note that each column uses different cohorts and thus percentages may not add to 100%.

Pediatric cancer type ▾	Telomerase positive %	Alt positive %
ALL	54 (39/72)	0 (0/53)
AML	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)
Rhabdomyosarcoma (ERMS)	50 (8/16)	38 (6/16)
Rhabdomyosarcoma (ARMS)	71 (10/14)	0 (0/15)

The next hallmark is apoptosis (programmed cell death) resistance. Cells have many genes such as TP53 and Bcl2 that induce cell death when cells are damaged or have too many mutations, by doing this cancerous cell behavior can be recognized and suppressed. Cancers have various methods to resist apoptosis, including tumor gene suppression e.g, suppressing TP53 by overexpressing MDM2 or activating antiapoptotic

regulators, such as Bcl2 in lymphoma. Angiogenesis (blood vessel growth) is another main hallmark induced during hypoxia. Studies by Hanahan and Folkman of transgenic mice with islet cell carcinoma, dermal fibrosarcoma, and epidermal squamous cell carcinoma all found extensive vascularization. According to them, angiogenesis occurs when cancer cells activate an “angiogenic switch,” a discrete step occurring when the presence of pro-angiogenic factors (e.g. VEGF) outweighs the presence of anti-angiogenic factors (TSP-1) (Hanahan and Weinberg 2011).

Tumor cells also need to avoid growth suppressors. The human body has several genes (eg TP53 and RB) that phosphorylate in the presence of oxidative stress and other damages, stalling the life cycles of cancer cells (Lakin and Jackson 1999), (Zatulovskiy et al. 2020). Normally, the body inhibits cell growth via contact inhibition, where mitotic activity slows if cells detect other cells via cadherins. For example, Merlin protein couples cell-surface adhesion molecules and sequesters growth factors; because of this, it's known to inhibit neurofibromatosis II (Morrow et al. 2011). Cancerous cells must degrade these proteins to proliferate and form a tumour.

Pediatric Cancers and Telomerase

Several aspects make pediatric cancer cases different from adult cancer cases. The pediatric cancers are known to grow faster and one of the reasons for this could be that stem cells are more present and active in children vs adults. While breast, lung, and prostate cancers are the most common cancers in adults, dominant types in children are leukemia, lymphoma, brain and spinal cord cancers (“Six Ways Childhood Cancer Is Different to Adult Cancer | CCLG - The Children & Young People’s Cancer Association” 2023). Pediatric neoplasms also grow faster and respond better to chemotherapy. Because of this, children <14 have a much higher 5-year survival rate (85%) (“How Is Childhood Cancer Treated? | American Cancer Society,” n.d.) than the whole population ((Cancer Facts & Figures 2025 1930)). However, Cancer is also the leading cause of death in children after infancy, with approximately 1600 children dying of cancer every year in the US alone, according to cancer.gov (“Cancer in Children and Adolescents - NCI” 2024). 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). Cancers in adults can vary widely on TERT promoter mutation rates. Cancers with high promoter mutation rate ($\geq 15\%$) in adults include melanoma, liposarcoma, hepatocellular carcinoma, urothelial carcinoma, tongue squamous carcinoma, and glioma subtypes (83% of glioblastoma). Those with low promoter mutation rate ($< 15\%$) include Leukemias (common in children), various nervous cancers (eg gliomas), carcinomas, melanomas, and osteosarcomas (common in adults). While data on TERT promoter mutations is rare, limited studies find that TERT promoter mutation rates in children are much lower (Ackermann and Fischer 2019).

As shown in figure 11, Testicular germ cell cancer has one of the highest TERT expression rates in the data provided by the Human Protein Atlas. As such, I decided to investigate the relationship between cancer and germline mutations. Germline mutations are mutations that occur in sex cells, which could cause some or all cells in the body to inherit that mutation depending on the developmental stage at which they occurred. Studies on pediatric soft-tissue sarcomas (e.g. Ewing, rhabdomyosarcomas) show 17% germline mutation rates in children (Yndestad et al. 2024), while diffuse midline glioma patients exhibited 7.5% mutation rate (Mateos et al. 2025). Counterintuitively, there is no significant difference between germline mutation rates in pediatric vs adult cancer; while the estimated contribution of germline mutations to pediatric cancer is 8-12% ("Frontiers | Genetic Predisposition to Solid Pediatric Cancers," n.d.), this is very similar to the attributable risk for cancer overall, being 10% ("The Genetics of Cancer - NCI" 2015). 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).

According to table 2, pediatric tumors are overwhelmingly telomerase positive, similar to adult tumors. However, some cancers (osteosarcomas, wilms tumors, neuroblastomas) exhibit significant ALT pathway activation (telomerase negative) - the reasons behind

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

As a treatment perspective, telomerase inhibitors may provide a way to combat childhood cancer. Cells in children also need to divide more times than in adults - thus, telomerase is known to be more active in children. This, combined with the relative lack of confounding external factors in children, may help establish a clearer statistical trend between telomerase activity and cancer. TERT serves as the catalytic core of the telomerase holoenzyme. It consists of 16 exons (1134 amino acids), with 40766 amino acids interspersed as 15 introns. Multiple proteins induce TERT expression by attaching to the TEN domain and promoter; for example, cMyc binds to E-boxes at +44 when bound to Max protein. TERT can, however, be downregulated by Mad1 (a suppressor of Myc) and histone deacetylase in most somatic cells (Khattar and Tergaonkar 2017).

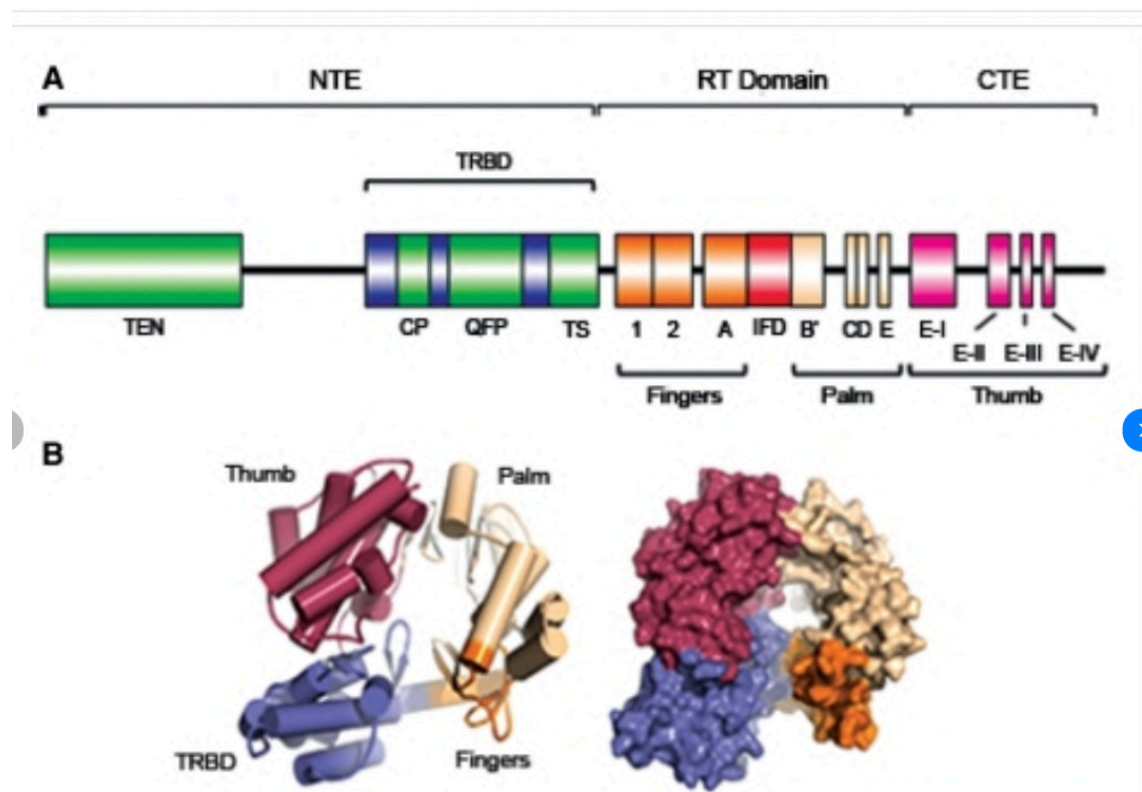


Figure 3: Protein subdomains of TERT. On the 5' end is TEN (TERT n terminal domain), which interacts with ssDNA telomere repeats, followed by TRBD, which binds to TERT. RT, the reverse transcriptase domain, and CTE, the C terminal domain, catalyze the addition of nucleotides to telomeres. As shown in figure 7, RT and CTE are the most conserved domains in TERT.

To study the telomerase and TERT in cancer mechanism, gene editing techniques can be utilized to generate a mutation screen across pediatric cancers. CRISPR is a technique used in gene editing, originally discovered in bacterial immune systems. CRISPR involves an endonuclease (Cas9) as well as a guide RNA, which attaches to a targeted DNA sequence and makes a cut at a recognized PAM (NGG) sequence. This cut is typically blunt, though Cas9 (typically derived from *Streptococcus Pyogenes*) may occasionally produce sticky ends in vivo (Jasin 2018a). What happens next can vary. When attempting to change a nucleotide sequence, a HDR mediating template can be used to insert a desired sequence via homology-directed repair; otherwise, the double-strand break is left to be repaired naturally via non-homologous end joining (NHEJ) or homologous end joining (HDR). As an outcome of these processes a cell can end up having altered DNA sequences (Redman et al. 2016).

Aim of the study:

Telomerase is a well-known carcinogenic factor for 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 determine whether telomerase's effect differs significantly compared to in adult cancer. To do so, I will gather literature knowledge on telomerase, one of the telomerase subunits TERT, expression rates, protein structure, conservation levels of different types of cancers affecting children vs

adults. I will also propose a hypothetical CRISPR/Cas9 gene editing design to generate a framework for future experiments that can be conducted to address the question experimentally.

2. Materials & Methods

I used publicly available databases and online tools to conduct my research about Telomerase and TERT:

Gathering protein and gene information:

NCBI (National Center for Biotechnology Information) is a research database containing a lot of information, including DNA, RNA, and protein sequences (in FASTA format) which is used in this study and it also holds pubmed which is a publication hub. I retrieved protein and gene sequences via NCBI>Protein and NCBI>Gene.

Investigating protein properties:

TMHMM is an algorithm that analyzes amino acid sequences to determine whether they contain transmembrane domains based on their amino acids' hydrophobicity. Since hydrophobic amino acids have to remain inside membrane layers, consecutive hydrophobic amino acids indicate a transmembrane/intramembrane domain. TMHMM uses the Hidden Markov model, explained in more detail below in results in results. I retrieved amino acid sequences via NCBI and submitted into TMHMM as a query.

CLUSTAL OMEGA is a software that compares protein sequences among different organisms to identify highly conserved amino acids/domains using the PAM 250 scoring matrix, which compares the likelihood of different amino acid substitutions. Additionally, it shows the electrochemical properties of the amino acid sequences (eg, hydrophobicity, charge) with color coding, providing insight into domain function.

CRISPR/Cas9 gene editing design:

I retrieved gene sequence via NCBI>Gene database then used **ENSEMBL**, which is a genome database containing genetic information from various organisms including gene sequences and their introns, exons, and isoforms. I annotated the exon and intron region 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 ~20bp CRISPR primers given a DNA sequence. I used CRISPR Finder to generate primers for a possible CRISPR/Cas9 knockdown/knockout.

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

3. Results

3.1 TERT protein does not contain any transmembrane/intramembrane domain

TERT's full sequence (in FASTA format) was taken from NCBI's protein database and then analyzed via TMHMM (Transmembrane Hidden Markov Model). TMHMM is a hidden Markov model, a model that relies on an unobservable initial state. Usual Hidden Markov Models do not understand initial conditions (like whether a given domain is "outside" or "inside" a domain), but track the changes between them (the transmembrane domains) and determine a path (Yoon 2009). TMHMM makes its best guess as to which domains are inside or outside of a membrane layer by applying the

positive-inside rule (Heijne 1986). That determines the probability of any given section of a protein being transmembrane or intramembrane. Since the cell membrane (a phospholipid bilayer) is hydrophobic while the cytosol is hydrophilic, a part of a protein that has a certain length of hydrophobic amino acids in an order is very likely to be a transmembrane domain, while hydrophilic regions are not. The presence of transmembrane domains defines the subcellular localisation of the protein, which could be linked to their functions or the phenotype that is observed in the case of dysfunction. TERT lacks any long hydrophobic amino acid chains; as a result, TMHMM analysis shows that it lacks transmembrane domains (Figure 4). A protein result of “all in” doesn't narrow down a specific region in the cell, but does indicate it has no membrane-bound position (“TMHMM 2.0 - DTU Health Tech - Bioinformatic Services,” n.d.).

RecName: Full=Telomerase reverse transcriptase; AltName: Full=HEST2; AltName: Full=Telomerase catalytic subunit; AltName: Full=Telomerase-associated protein 2; Short=TP2

UniProtKB/Swiss-Prot: O14746.1

[GenPept](#) [Identical Proteins](#) [Graphics](#)

>sp|O14746.1|TERT_HUMAN RecName: Full=Telomerase reverse transcriptase; AltName: Full=HEST2; AltName: Full=Telomerase catalytic subunit; AltName: Full=Telomerase-associated protein 2; Short=TP2

MPRAPRCRAVRSLRSHYREVLPLATFVRRLLGPQGWRLVQRGDPAAAFRALVAQCLVCPNDARPPPAAPS
FRQVSCLELVARVLQRLCERGAKNVLAFGFALLDGARGGPPEAF TTSVRSYLPNTVTDALRGSGAWGLL
LRRVGDDVLVHLLARCALFVLVAPSCAYQVCGPPLYQLGAATQARPPPHASGPRRRLGCERAWNHSVREA
GVPLGLPAPGARRRGGSASRSLPLPKRPRRGAAPERTPVGQGSWAHPGRTRGSPDRGFCVVSPARPAE
EATSLLEGALSGTRHSHPSVGRQHAGPPSTSRPPRPWDTPCPPVYAETKHFLYSSGDKQLRPSFLLSSL
RPSLTGARRLVETIFLGSRPWMPGTPRRLPRLPQRYWQMRPLFLELLGNHAQCPYGVLLKTHCPLRAAVT
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TKKFISLGKHAKLSLQELTWKMSVRDCAWLRRSPGVGCVPAAEHRLREEILAKFLHMLMSVYVVELLRSF
FYVTETTFQKNRLFFYRKSVMKLSQSIGIRQHLKRVQLRELSEAEVRQHREARPALTSRLRFIPKPDGL
RPIVNMIDYVVGARTFRREKRAERLTSRVKALFVSLNYERARRPGLLGASVLGLDDIHRANRTFVLRVRAQ
DPPPELYFVKVDVTGAYDITPQDRLTEVIASIIKPQNTYCVRRYAVVQKAAHGHVRKAFKSHVSTLTDLQ
PYMRQFVAHLQETSPLRDAVIEQSSSLNEASSGLFDVFLRFMCHHAVIRGKSYVQCQGIQGGIILSTL
LCSLCYGD MENKLFAGIRRDGLLLRLVDDFLLVTPHLTHAKTFLRTLVRGVPEYGCVVNLKTVVNFVPE
DEALGGTAFVQMPAHGLFPWCGLLLDTRTLEVQSDYSSYARTSIRASLTFRNGFKAGRNMRKLFGLVRL
KCHSLFLDLQVNSLQVTCNTIYKILLQAYRFHACVLQLPFHQQVWKNPTFFLRVISDTASLCYSILKAK
NAGMSLGAKGAAGPLPSEAVQNLCHQAFLLKLTRHRVTYVPLLGSLRTAQTLQSRKLP GTTTLTALEAAAN
PALPSDFKTIID

TMHMM result

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# sp|O14746.1|TERT_HUMAN Length: 1132
# sp|O14746.1|TERT_HUMAN Number of predicted TMHs: 0
# sp|O14746.1|TERT_HUMAN Exp number of AAs in TMHs: 0.5821799999999996
# sp|O14746.1|TERT_HUMAN Exp number, first 60 AAs: 0.01515
# sp|O14746.1|TERT_HUMAN Total prob of N-in: 0.02583
sp|O14746.1|TERT_HUMAN TMHMM2.0 outside 1 1132
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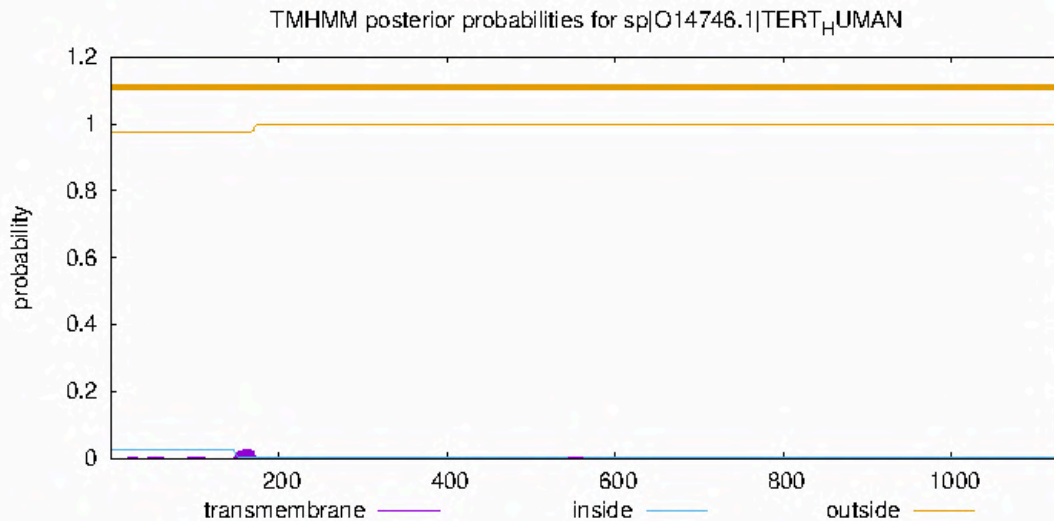


Figure 4. Transmembrane domain analysis of TERT protein via TMHMM algorithm.

The upper text shows the FASTA-formatted protein amino acid sequence of TERT taken from NCBI>Protein>FASTA. The bottom graph is the result of TMHMM analysis for TERT protein. The graph shows that TERT likely lies entirely outside any cell membranes (i.e. it lies in the cytosol). The x axis shows the amino acid sequence, while the y-axis indicates the probability calculation. A region being purple means it's likely to be transmembrane, yellow means it lies outside a cell membrane, and blue means it lies on the inner side of the membrane layer - this figure shows that most of TERT overwhelmingly lies outside. "Outside," in the context of TMHMM analysis, refers to the non-cytosolic side of a lipid bilayer; this fits current consensus that TERT (and telomerase in general) is primarily a nuclear protein.

3.2.TERT has 8 experimentally determined interactor proteins

Protein-protein interaction is an essential phenomenon in the human genome. By looking at the interactions between proteins, it's possible to infer the function of one protein based on the others it interacts with. 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. In this study, interactions found by STRING can serve as a starting point to find key upregulators and downregulators of telomerase and TERT, which can be further analyzed for cancer mechanisms. The proteins shown below are involved with TERT subunit of telomerase (Figure 5) in various ways. NHP2 binds to the H/ACA domain of telomeric RNA (Pogacić et al. 2001) and is involved in telomere maintenance, while WRAP53 binds to Cajal bodies and TERC, responsible for stimulating TERT catalytic 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 H/ACA small nucleolar ribonucleoprotein (H/ACA snoRNP) complex, which catalyzes pseudouridylation of rRNA, and CTNNB1 is involved in the WNT signalling pathway.

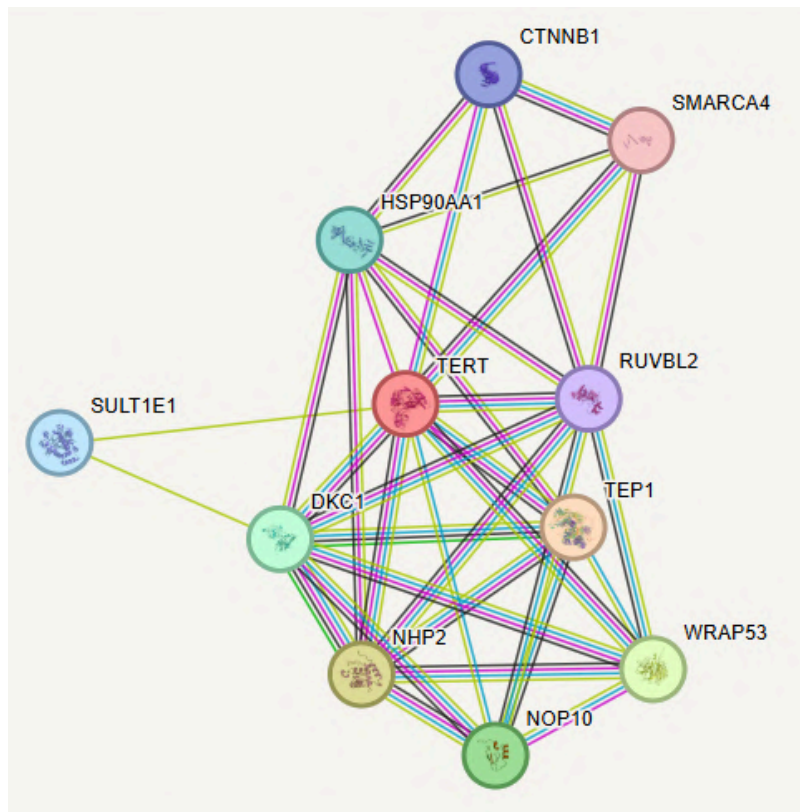


Figure 5. Protein-protein interaction map for the TERT protein. The image shows the predicted interactors of TERT as an outcome of STRING protein-protein interaction analysis. Lines (edges) show interaction depthness between various proteins, each indicated by a node on the graph. Pink and blue lines represent the strongest relationships (experimentally determined and database-sourced respectively), dark green lines represent relationships determined by gene proximity, and black and green lines indicate weak relationships (sourced from textmining and evidence of co-expression, respectively). TERT has 8 experimentally determined interactors. NHP2, WRAP53, TEP1, RUVBL2, SMARCA4, and CTNNB1.

3.3. TERT has highly conserved end domain

CLUSTAL Omega analysis compares the amino acid sequences of one protein among different animals and indicates which parts are similar between them using the PAM 250 scoring matrix. If a region is highly similar between animals, this indicates that it's preserved throughout genomes during evolution and could be very important or

essential amino acids for the function of the protein. When CLUSTAL analysis of TERT was done, I found that TERT's back end was most strongly conserved (Figure 7); this corresponds with the RT and CTE domains of TERT (Figure 3) in humans, which add nucleotides to telomeres. As can be seen by the graph, the back domain is much more highly conserved than the front. Additionally, there's a high concentration of red amino acids across all animal TERTs analyzed, meaning that TERT is strongly positive. I also generated a heatmap showing the conservation rates quantitatively (Figure 7). The most highly conserved area was amino acids 1140-1200, with 83% (50) amino acids being either highly or fully conserved. This highly conserved region roughly corresponds with the RT (reverse transcriptase) and CTE (c-terminal extension) domains, which catalyze telomere synthesis. While the first 50% of bases are significantly less conserved, the first 120 bases (the top two rows of the table) show relatively high conservation. As it corresponds to the N-terminal domain, it could be inferred that the N-terminal domain's function, while not as conserved as telomerase's catalytic domains (RT and CTE), is still very important to telomerase function. Other regions show very low conservation among organisms.

Species	Accession	Gene	Protein	Length
chicken	NP_001026178.2	MEG	MEG	60
mouse	sp 070372.1 TERT_MOUSE	MEG	MEG	0
human	sp 014746.1 TERT_HUMAN	MEG	MEG	41
chimpanzee	PNI27662.1	MEG	MEG	41
chicken	NP_001026178.2	DDA	DDA	120
mouse	sp 070372.1 TERT_MOUSE	DDA	DDA	31
human	sp 014746.1 TERT_HUMAN	DDA	DDA	101
chimpanzee	PNI27662.1	DDA	DDA	101
chicken	NP_001026178.2	SLL	SLL	180
mouse	sp 070372.1 TERT_MOUSE	SLL	SLL	91
human	sp 014746.1 TERT_HUMAN	SLL	SLL	161
chimpanzee	PNI27662.1	SLL	SLL	161
chicken	NP_001026178.2	VPP	VPP	239
mouse	sp 070372.1 TERT_MOUSE	VPP	VPP	139
human	sp 014746.1 TERT_HUMAN	VPP	VPP	200
chimpanzee	PNI27662.1	VPP	VPP	200
chicken	NP_001026178.2	QW	QW	299
mouse	sp 070372.1 TERT_MOUSE	QW	QW	152
chicken	NP_001026178.2	CL	CL	359
mouse	sp 070372.1 TERT_MOUSE	CL	CL	195
human	sp 014746.1 TERT_HUMAN	CL	CL	261
chimpanzee	PNI27662.1	CL	CL	261
chicken	NP_001026178.2	AV	AV	419
mouse	sp 070372.1 TERT_MOUSE	AV	AV	225
human	sp 014746.1 TERT_HUMAN	AV	AV	289
chimpanzee	PNI27662.1	AV	AV	289
chicken	NP_001026178.2	NRR	NRR	479
mouse	sp 070372.1 TERT_MOUSE	NRR	NRR	249
human	sp 014746.1 TERT_HUMAN	NRR	NRR	314
chimpanzee	PNI27662.1	NRR	NRR	314
chicken	NP_001026178.2	LP	LP	538
mouse	sp 070372.1 TERT_MOUSE	LP	LP	308
human	sp 014746.1 TERT_HUMAN	LP	LP	373
chimpanzee	PNI27662.1	LP	LP	373
chicken	NP_001026178.2	QQ	QQ	598
mouse	sp 070372.1 TERT_MOUSE	QQ	QQ	357
human	sp 014746.1 TERT_HUMAN	QQ	QQ	424
chimpanzee	PNI27662.1	QQ	QQ	424
chicken	NP_001026178.2	LTL	LTL	658
mouse	sp 070372.1 TERT_MOUSE	LTL	LTL	369
human	sp 014746.1 TERT_HUMAN	LTL	LTL	449
chimpanzee	PNI27662.1	LTL	LTL	449

Figure 6. Multiple protein alignment of TERT protein in multiple animals (chicken, mouse, human, chimpanzee). In the alignment each letter corresponds to a different amino acid while colors correspond to chemical properties - red indicates a positive charge, magenta indicates negative charge, blue indicates hydrophobicity, green indicates polarity, and pink indicates cysteine. CLUSTAL also displays colors for glycines, prolines, and aromatic amino acids, however, TERT lacks these. Meanwhile, symbols at the bottom indicate similarity between amino acids in different animals. Asterisks (*) indicate that an amino acid is fully conserved among animals listed (i.e. all bases match at that position), colons (:) indicate that an amino acid is strongly conserved (with a PAM 250 score >0.5), and a period (.) indicates weak conservation (PAM 250 score between 0 and 0.5).

Figure 7. Heatmap distribution of conservation frequency in TERT FASTA sequences. This map shows the proportion of each 60-amino acid interval that was denoted as * (fully conserved), : (highly conserved), or- (moderately conserved). (weakly conserved).

Heat map of % of amino acids in each row (~60 acids per row) that are each symbol			
asterisk (fully conserved)	colon (highly conserved)	period (weakly conserved)	
0	0	0	
0.23	0.2	0.033	
0.4	0.283	0.017	
0.186	0.085	0.017	
0.167	0.33	0.05	
0.067	0.083	0.1	
0.033	0.033	0.083	
0.017	0.067	0.05	
0.322	0.136	0.119	
0.283	0.15	0.0033	
0.017	0.167	0.033	
0.583	0.2	0.0167	
0.617	0.1	0.083	
0.483	0.183	0.083	
0.35	0.283	0.083	
0.35	0.267	0.1	
0.345	0.345	0.034	
0.567	0.167	0.083	
0.525	0.254	0.017	
0.55	0.167	0.083	
0.7	0.13	0.033	
0.433	0.183	0.067	

3.4. CRISPR/Cas9 gene editing strategy for TERT gene

CRISPR is a gene editing technique originally discovered in bacterial immune systems as an antiviral defense system that excises foreign DNA. It involves an endonuclease (Cas9) as well as a guide RNA, which attaches to a targeted DNA sequence and cuts at a recognized PAM (NGG) sequence. This cut is typically blunt, though Cas9 (typically

derived from *Streptococcus Pyogenes*) may occasionally produce sticky ends in vivo (Jasin 2018b). What happens next can vary. When attempting to change a nucleotide sequence, a template can be used to force HDR repair or NHEJ could be an option. This way scientists could end up having mutations varying from single nucleotide changes to big deletions or insertions. In other words, CRISPR allows scientists to generate precise mutations along DNA. This has many uses; for example, one could create a double strand break in a cell DNA to generate mutations to analyze its effects and infer the original function of a gene sequence, or assist in HDR via Cas9 (the main exonuclease).

I decided to design a CRISPR/Cas9 strategy for the TERT subunit of the telomerase. First I looked at the chromosomal location of the TERT gene (Figure 8) to understand whether the gene sequence is colliding with any other nearby genes.

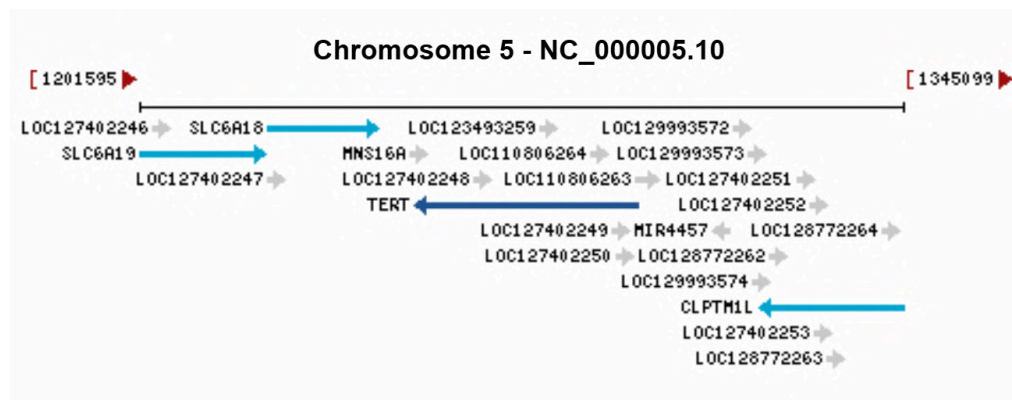


Figure 8. Chromosomal location of TERT gene. The above figure shows a subsection of chromosome 5, starting from base pair 1201595 (left) and ending at 1345099 (right). A dark blue arrow indicates TERT's location on chromosome 5 and its transcription direction. Other genes in humans are marked in light blue. It's important to note that none of them overlap with TERT. Thus, it's possible to CRISPR TERT without interfering with the function of other genes.

Then I gathered the TERT gene sequence from NCBI and annotated the exon and intron region from Ensembl annotations. Then via CRISPR Finder I selected a guide

RNA based on the off-target scores calculated for the allowed mismatches of 0, 1, 2, 3, 4. I picked a guide (the upper image of Figure 9) that has 0=1, 1=0, 2=0, 3 and 4 the lowest possible. I used Sanpgene to visualize all the sequences and label the required regions to assemble my strategy seen as in Figure 9. I also included potential PCR primers to hypothetically present possible outcomes of this CRISPR/Cas9 strategy.

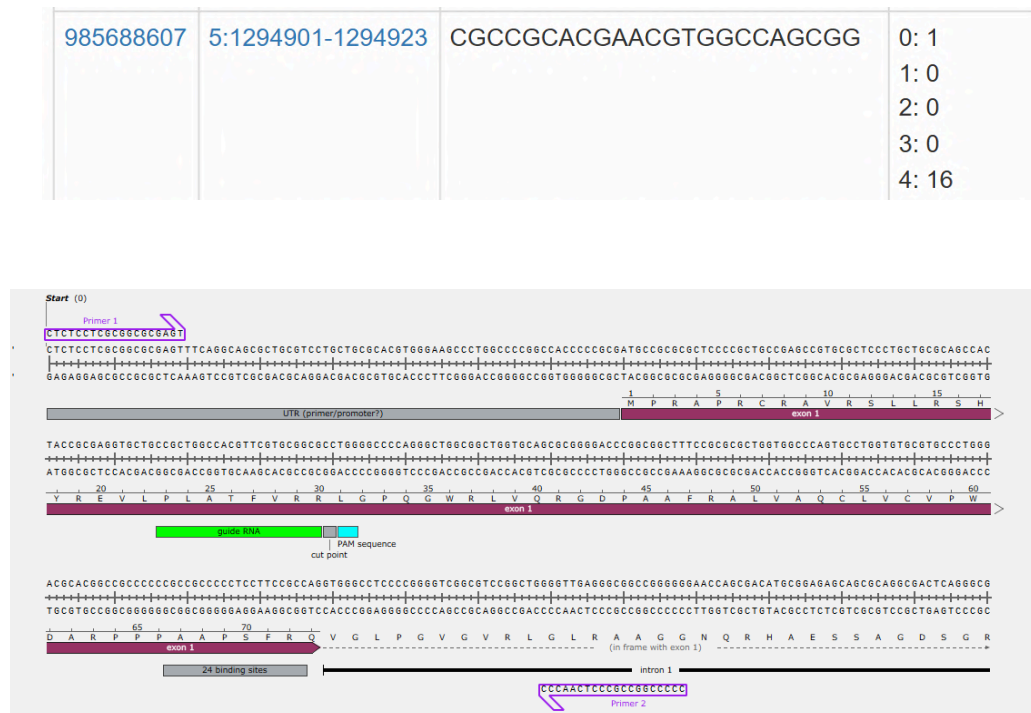


Figure 9. Snapgene view of CRISPR/Cas9 gene editing strategy for the TERT gene. The upper image shows the guide RNA selected from CRISPR Finder, as it had the best off-target score. The SnapGene view shows one part of the TERT gene (exon 1 and intron 1) along with the defined guide RNA locations, PAM sequence, a potential cut point, and primers targeting an amplification region. 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 covering the targetted CRISPR/Cas9 gene editing region in order to test for the potential outcomes of the editing via a hypothetical polymerase chain reaction (PCR) and simulated DNA gel electrophoresis. Primer 1 and primer 2 amplifies a fragment of length (309 bp).

PCR (polymerase chain reaction) is a technique to rapidly amplify a region of DNA mimicking the replication but in vitro. There are 3 steps: denaturation, annealing, and extension. In denaturation, the DNA strand is heated to ~95 C in order to split (denature) its hydrogen bonds, mimicking helicase activity to generate single strand DNA. Then, in annealing, DNA is cooled to a lower temperature (e.g., 70 C) to allow DNA primers to bind. A heat-resistant polymerase (for example, Taq) is then used to add nucleotides in the extension step, amplifying the DNA.

Gel electrophoresis is a lab technique that uses electricity to separate DNA fragments in a given sample by size. Each gel tank has a connection to a positive and a negative electrode that can be applied to the system. Samples containing DNA are loaded from the area of the tank that is closer to the negative electrode. The agarose made gel will have wells to load individual samples. Phosphate groups in DNA make the DNA molecule negatively charged, and thus once the electrical system starts running the negative electrons will push the DNA molecule from top of the gel towards the bottom of the gel. The smaller molecules move more quickly due to a lack of resistance in the gel and the larger ones will stick at the top area. Here I simulated a gel electrophoresis (Figure 10) for training purposes in order to present the hypothetical outcomes of the gene editing. Single nucleotide changes cannot be resolved in a gel therefore they are needed to be sequenced. All potential alterations must be confirmed with sequencing.



Figure 10. Hypothetical DNA gel electrophoresis for the expected outcomes of CRISPR/Cas9 gene editing for TERT gene. This gel electrophoresis shows a hypothetical aftermath of using CRISPR to generate TERT gene mutations. The leftmost lane contains a DNA fragment ladder or marker with known sizes, providing a reference for the fragment sizes in the other 6 lanes. Lane one (the second leftmost lane) contains unmodified TERT, which is slightly less than 350 bp in size as a wild type; lanes 4, 5, and 6 (single nucleotide deletion, insertion, and substitution, respectively) all have similar fragment lengths as this length of alteration cannot be resolved on a gel they will be same size as the wild type. However, TERT samples in lane 2 are significantly shorter, indicating a ~80 base pair deletion, while samples in lane 3 are significantly longer, indicating a ~75 base pair insertion.

Below TCGA expression data about TERT among different cancers shows that TERT expression can be used as a marker to identify differences among cancer types (Figure 11) however all this data does not say whether adult vs pediatric cases were compared or not. Therefore, all my investigation above will be providing knowledge about this gap in the future

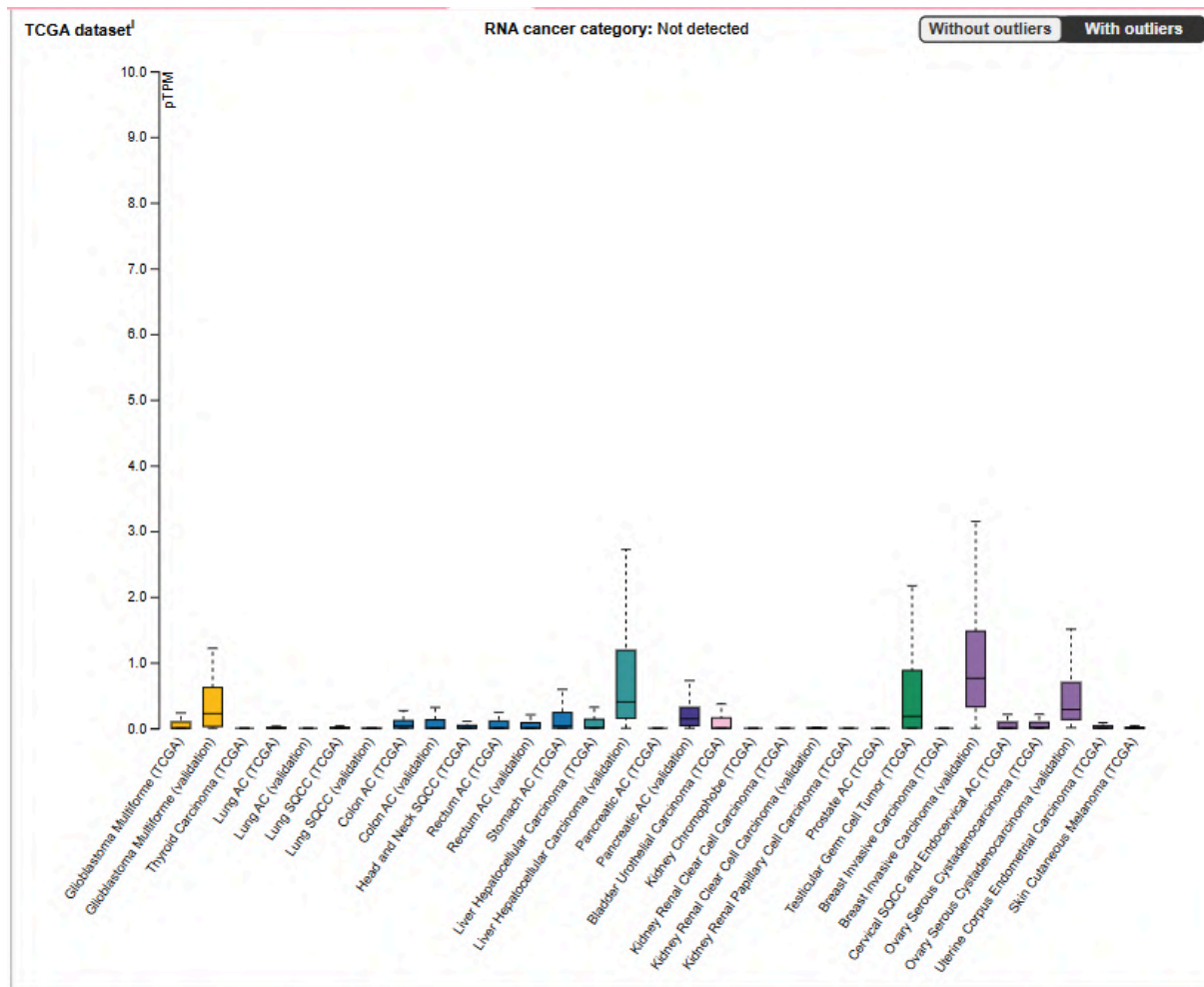


Figure 11. TERT expression rates in different types of cancers in protein transcripts per million. Breast invasive carcinoma has the highest median TERT expression rate (0.8), followed by Liver Hepatocellular carcinoma (0.4), ovary serous cystadenocarcinoma (0.3), testicular germ cell carcinoma (0.2), and glioblastoma multiforme (0.2). The graph is taken from the human protein atlas database.

4. Discussion

Telomere and telomerase-related oncology studies, while common, are rarely specific to children. The few studies available had very low sample sizes - many cohorts ranged from 10 to 20 children. Additionally, cancer is extremely heterogeneous; many cancers had vastly differing telomerase expression rates. Therefore I focused on telomerase and TERT in pediatric cancers and I tried to gather information to generate adult vs pediatric cancer comparison for the telomerase activity. I also wanted to establish a framework to study this question in the future. For this matter I analyzed the protein futures of the TERT subunit and designed CRISPR/Cas9 strategy.

Telomerase activity was highly prevalent in pediatric and adult cancer, however, one notable difference was a generally higher prevalence of the ALT pathway in children. In table 1, pediatric cancer telomerase expression rates ranged from 0% in low grade glioma to 86% in medulloblastoma, while ALT expression rates ranged from 0% in several cancers to nearly 80% in osteosarcoma. The results of the study show that telomerase activity and TERT mutations have a significant correlation with cancer severity and frequency (Albadrani and Zakariyah 2025). It is also known that when telomerase is positively marked among patients the survival ratio will be lower than the ones who have negatively marked telomerase activity (Figure 12).

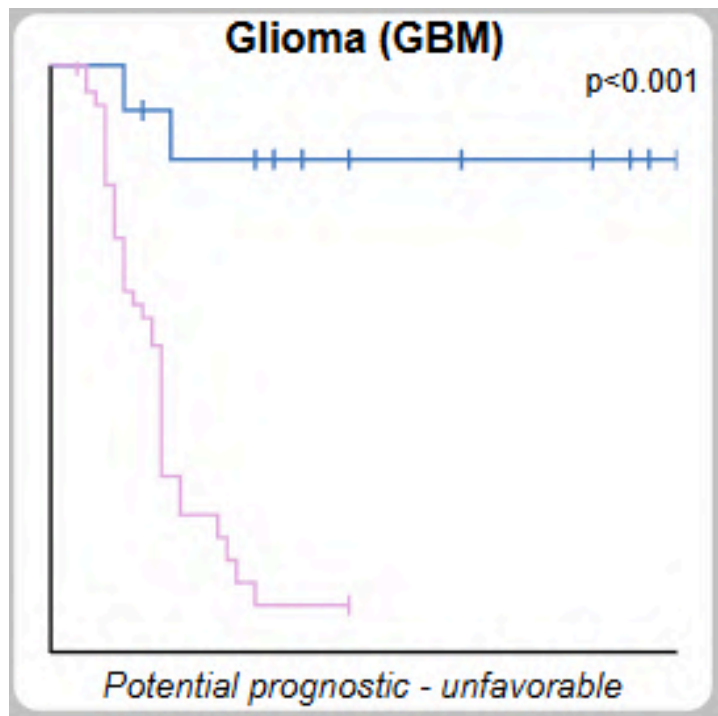


Figure 12. Prognosis of Glioma. Shows a Kaplan-Meier survivorship curve of telomerase-negative glioma patients (blue) and telomerase-positive glioma patients (pink) over time. Survival rates are significantly lower in patients expressing telomerase than in those who do not.

Telomerase is known to increase cancer rate, and in cancers that express it, telomerase inhibitors (e.g., imetelstat, rytelo) have been shown to be effective. However, cancers can develop resistance to imetelstat. Another problem with telomerase inhibitors is their lack of specificity; for example, because it reduces the number of times cells can safely divide, imetelstat is known to cause infertility (Mayo Clin., n.d.), among other symptoms. Interactions shown by STRING protein-protein interaction map (Figure 4) may lead to alternative methods of telomerase inhibition that don't have those shortfalls, for example, by discovering telomerase-related enzymes specific to cancers. STRING database may also provide further insight into telomerase and TERT-related pathways - proteins related to TERT can be affected by TERT mutations.

Meanwhile, CLUSTAL omega analysis, which shows that some regions of TERT are much more strongly conserved than others, can provide insight into telomerase functional domains to target. When certain sequences in a protein are similar across species, it may indicate that they're much more important to overall function than other sections - evolutionary variants that alter highly conserved domains sometimes die out. In TERT, targeting the more highly conserved RT and CTE domains with telomerase inhibitors could lead to stronger disruptions in cancer.

CRISPR knockout experiments appear feasible for investigating the relationship between telomerase and cancer. Chromosome location data from NCBI shows that no human genes overlap with TERT (Figure 8). This is important when performing CRISPR, as it means knocking out TERT will not affect other genes, ensuring there is no confounding. Meanwhile, Figure 7 shows examples of possible primers and PAM locations at annealing temperatures ~60 C. After attempting a CRISPR knockdown, one could verify their experiment worked by performing PCR to analyze the restriction fragments.

PCR could be used after CRISPR knockout to verify whether it has actually occurred. As shown in the hypothetical PCR result on a gel in Figure 10, DNA fragments of different lengths traverse different distances across the agarose gel - small fragments travel further downwards. As CRISPR knockouts and knockdowns have fewer DNA fragments than the original DNA, they necessarily travel further down an agarose gel than the DNA where knockouts did not happen. If the CRISPR knockout does not occur, one can redo the experiment using different guide RNAs..

No significant result was found via TMHMM analysis. The overwhelming majority of TERT protein localizes to "outside" (the noncytosolic side) of a lipid bilayer, indicating non-membranous localization.

Further studies are still needed. While I established a link between TERT and pediatric cancer, there is little data explicitly defined as nonpediatric for comparison. To establish the link between TERT and pediatric cancer specifically, studies with stratified pediatric and nonpediatric cohorts would benefit greatly. Such data from cancers where telomerase assays are easily available may provide valuable insights into how telomerase behave in pediatric cancers.

5. Conclusion

While the overall relationship between telomerase (or TERT promoter mutations specifically) and cancer is well established, data on telomerase/TERT in pediatric cancer is extremely sparse. The data available on prognostic factors indicates that TERT and telomerase are strong indicators of cancer progression, but small sample sizes limit both the significance of these results and comparison to adult cohorts. Here I established a framework related to TERT subunit of telomerase in order to study this in pediatric cancers in the future. As such, I could not determine whether TERT is more strongly involved in pediatric cancer than adult cancer. However, data in this paper indicate that this is a promising area of future study.

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Convergence Journal Review

Investigating the Role of Telomerase and TERT Subunit in Paediatric Cancers

I had the pleasure of reading and reviewing the paper titled “Investigating the Role of Telomerase and TERT Subunit in Paediatric Cancers.” The student chose a very important and clinically relevant topic — the role of telomerase and its TERT subunit in pediatric cancers — and examined it from multiple angles, including literature review, bioinformatics analysis, and a hypothetical CRISPR/Cas9 gene editing design. This is a strong paper that demonstrates a clear understanding of cancer biology, telomere maintenance, and the tools available for studying them. The figures are numerous and generally well-labeled, and the inclusion of tables comparing telomerase expression and ALT pathway rates across pediatric cancer types is useful as well. I particularly appreciated the student’s effort to design a CRISPR strategy for TERT, including primer selection, PAM site identification, and a simulated gel electrophoresis — this shows genuine engagement with experimental methods rather than just summarizing existing literature. The argument is well-organized and follows a logical progression from background biology to methods, results, and discussion. There are, however, some issues with the paper that should be addressed before publication.

The student cited a good amount of research and used appropriate bioinformatics tools (STRING, CLUSTAL, TMHMM, SnapGene). However, there are several issues that need attention. First, several citations appear to be incorrectly formatted or linked to the wrong sources — these need to be verified and corrected. Acronyms such as TERT should be expanded at first use, including in the abstract. The introduction, while thorough, is very long and spends considerable space explaining general cancer hallmarks (apoptosis resistance, angiogenesis, growth suppressor evasion) that, while relevant, take the focus away from telomerase and TERT for several pages. I would recommend condensing the hallmarks section and focusing more tightly on telomerase biology and its specific relevance to pediatric cancers. The TMHMM analysis, while correctly performed, yielded a negative result that does not contribute much to the paper’s central argument — the student acknowledges this, but it could be shortened. There is also a duplicate entry in the reference list (Jasin 2018a and 2018b appear to be the same paper). I would have liked to see more engagement with clinical data, for example searches from clinicaltrials.gov to identify ongoing trials targeting telomerase in pediatric populations, or data on imetelstat trials in children specifically. The student mentions imetelstat and its side effects but does not discuss whether any pediatric-specific trials exist or are planned. Additionally, a graphical abstract summarizing the relationship between TERT, telomerase, pediatric cancer, and the proposed CRISPR framework would help ground the reader in the paper’s core argument. The grammar and language are generally clean and professional, with only minor issues such as “statisictial” instead of “statistical” and a few instances of informal phrasing.

Based on all of this (a well-researched paper on an important topic with strong bioinformatics work and a creative CRISPR design component), I would recommend accepting this paper with minor revisions. These revisions should include: correcting the flagged citation errors and verifying all reference links, expanding acronyms at first use (especially TERT in the abstract), condensing the cancer hallmarks section in the introduction to keep the focus on telomerase and TERT, fixing the duplicate reference entry, adding a brief discussion of any existing or planned clinical trials targeting telomerase in pediatric cancers, and including a graphical abstract to help orient the reader. The student should also remove the redundant repetition of the CRISPR/Cas9 explanation, which appears in both the introduction and the results section with nearly identical wording.

This paper is a detailed and ambitious review about telomerase (an enzyme that helps protect chromosome ends) and a key part of it called TERT, focusing on childhood (pediatric) cancers. For a high school student, it shows great effort and curiosity. The author has tried to understand challenging topics like telomeres, CRISPR/Cas9 gene editing, how proteins interact, and how cancers develop in children. It's clear the student has read a lot of scientific sources and has attempted to use bioinformatics tools to support the review. The topic matters because pediatric cancer research is still growing, and the paper points out an important gap: we have less pediatric-specific telomerase data compared with adult cancers.

One of the biggest strengths of the paper is how much ground it covers. It links together the “hallmarks of cancer,” basic telomerase biology, the structure of TERT, other ways cells maintain telomeres (like ALT pathways), and modern gene-editing ideas. The use of tools such as TMHMM, STRING, CLUSTAL Omega, and SnapGene also shows the student went beyond what is normally expected at this level and learned independently. The section that proposes possible CRISPR approaches is especially interesting because it adds original thinking instead of only summarizing other people's work.

To make the paper stronger, it would help to revise the writing for clearer explanations, better organization, and consistent formatting. Some parts have very long sentences, repeated ideas, small spelling/typing mistakes, and mixed terms or citation styles. The scientific points are often good, but the way they are written can sometimes make them harder to follow. Editing the language and tightening the structure will make the key ideas stand out much more clearly.

Specific comments and suggestions (in simple terms):

- **Title:** The title is interesting, but you could shorten it. For example: “The Role of Telomerase (TERT) in Pediatric Cancers.”
- **Abstract:** The summary is good, but some sentences are very long. Shorter sentences will make it easier to read.
- **Word choice/accuracy:** A few biology terms need more precise wording. For example, apoptosis is “programmed cell death,” not a step in the cell cycle.
- **Introduction:** The background is strong, but some paragraphs pack in too many details at once. Breaking them into smaller paragraphs with clear transitions will help.
- **Clarify the paper type and central question:** At times the manuscript reads like a review, and at other times like a methods/results research report. Consider stating clearly in the Introduction/Abstract whether this is a *review with supporting bioinformatics analyses*. Then rewrite the aim as 1–2 precise questions (e.g., “What is known about TERT/telomerase alterations in paediatric cancers, and how do they compare with adults?”).

- **Keep each section tightly aligned to the aim:** Some background sections (general cancer causes, apoptosis, angiogenesis) become lengthy and drift away from telomerase/TERT. For a high school journal, it is better to shorten these parts and use the space to deepen telomerase-specific content (e.g., telomere biology, TERT regulation, paediatric tumour examples).
- **Citations:** The referencing style changes in different places. Choose one citation format and use it the same way throughout. Also make sure every figure/table clearly states its source.
- **Spelling style:** Pick either British or American spelling (e.g., “paediatric” vs “pediatric”) and keep it consistent across the whole paper.
- **Figures and captions:** Some figures/tables are helpful (especially CLUSTAL and STRING), but a few captions are too long or repeat information. Shorter captions will improve readability.
- **Be careful with strong claims:** When discussing how telomerase affects cancer, use cautious wording and mention that cancer is influenced by many factors, not just one.
- **Methods and acronyms:** The methods section is impressive. Just remember to spell out acronyms the first time you use them (for example: TMHMM, HDR, NHEJ, PAM).
- **Limitations:** It’s good that you mention limits like small sample sizes and limited pediatric data—this shows scientific maturity.
- **CRISPR section:** The CRISPR experiment idea is creative. Make it very clear it is a *theoretical plan* (a design), not something you actually carried out in a lab.
- **Proofreading:** The paper needs a careful proofread to fix grammar, repeated words, spacing, and typos.
- **Trim extra detail:** Some parts (like long explanations of PCR or gel electrophoresis) may be more detailed than needed for your main focus. Shortening them can improve flow.
- **Conclusion:** The ending does a good job summarizing why telomerase matters in pediatric cancer and why more research is needed.

Overall, this is a strong and ambitious project for a high school level. The author shows real curiosity, does a lot of background reading, and attempts advanced ideas and tools. With some careful rewriting for clarity, consistent citations, and a thorough proofread, this paper could be polished into an excellent submission for a student science journal or school research showcase.

Investigating the role Telomerase and TERT subunit in paediatric cancers

Commented [S1]: Avoid acronyms

[Author name redacted]

[School name redacted]

Abstract (summary)

Background TERT is a subunit of telomerase, an enzyme that extends telomeres and delays replicative senescence. Mutations or upregulation of it commonly lead to cancer.

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Objectives: Data on telomerase and TERT for pediatric cancer are relatively sparse. I aim to investigate whether TERT mutations affect pediatric cancer more severely than adult cancer and establish a framework for future study.

Methods We took TERT's FASTA sequence and used the STRING protein database, CLUSTAL, TMHMM, SnapGene, and NCBI to analyze proteins related to TERT, establish functional domains, and perform a hypothetical CRISPR experiment.

Results: Brief analysis shows that TERT promoter mutation rates are lower in pediatric cancer than adult cancer, but still raise the risk of cancer. No significant result was found with TMHMM. CLUSTAL indicates that TERT's RT and CTE domains (catalytic and binding domains, respectively) are much more conserved than other parts, while STRING shows that many proteins are related to TERT. A hypothetical CRISPR experiment shows that TERT knockdowns are one way to further study the effects of TERT mutations on pediatric cancer.

Conclusion: TERT promoter mutations, are generally rarer in pediatric cancer than adult cancer, while increased telomerase expression overall has a significant effect on pediatric cancer. In cancers where data were available, metastatic rates were significantly higher in patients exhibiting telomerase than in those who did not. While sample sizes were extremely small, relative risk statistics indicate that telomerase mutations are a promising area of investigation in pediatric cancer.

Keywords: Telomerase, paediatric cancer, cancer, TERT gene

1. Introduction

Cancer is a group of diseases where cells rapidly multiply and can affect both children and adults ("What Is Cancer?" 2007). There are many types (usually named after the body part they affect), from leukemia and brain to prostate and breast cancer. The causes of cancers are equally varied, but all known cancers involve gene mutations. There are many genes that can be mutated in cancers and sometimes functional enhancements are observed such as telomerase enzymes increasing its activity in cancer cells. However, only 5-10% of cancers are hereditary ("Frequently Asked Questions about Hereditary Cancers | Memorial Sloan Kettering Cancer Center," n.d.). Most gene mutations occur as a result of 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 turn genes oncogenic, or cancerous. Specific oncogenes can vary depending on the type of cancer. Some common oncogenes include p53, a tumor suppressor as a controller of cell division and an apoptotic inducer in the case of DNA damage at the G1/S and G2/M cell cycle checkpoints (Chen 2016)), as well as Ras, a protein family involved in cell differentiation and the MAPK/ERK pathway. ("Oncogene Ras - an Overview | ScienceDirect Topics," n.d.) (Guo et al. 2020). For example, an R337H mutation in tp53 has led to a 15-fold increase in pediatric adrenocortical carcinoma in Paraná, Brazil (da Silva et al., n.d.).

Cancerous Hallmarks and Telomerase activity

While cancers vary broadly, they share key common traits - the Hallmarks of Cancer (Hanahan and Weinberg 2011). These hallmarks are sustained proliferative signaling, evasion of growth suppressors, metastasis, apoptotic resistance, angiogenesis, and replicative immortality. Cells require signalling factors to duplicate their DNA and eventually proliferate, however, these signalling factors are usually controlled by negative feedback mechanisms. Cancer cells can inhibit these proliferative signals, produce their own growth factors, or inhibit negative feedback mechanisms. Metastasis, another criterion, is defined as the ability of cancer cells to spread to other organs. ("Definition of Metastasis - NCI Dictionary of Cancer Terms - NCI" 2011). Most cancers can metastasize,

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Commented [55]: Apoptosis is programmed cell death not cell cycle

Commented [56]: Spread to distant site not only to organs

often as an escape mechanism from the immune system or lack of nutrients (WANG et al. 2015) to find better environments.

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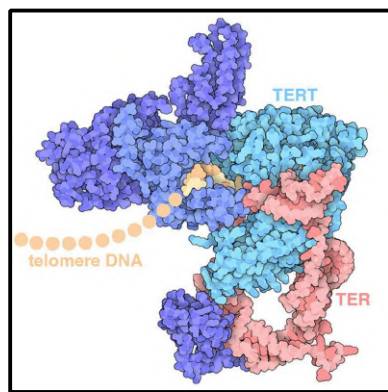


Figure 1: Molecular 3D structure of telomerase. The image shows the 3D structure of a TERT subunit of telomerase enzyme as well as TER/TERC (Telomerase RNA Component), an RNA template for telomere synthesis.

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One of the hallmarks is replicative immortality, which is directly regulated by telomerase. (Figure 1). Telomerase is studied in relation to cancer as the end replication problem. Because DNA polymerase can only transcribe from the 5' to 3' direction, the lagging strand of DNA needs to be transcribed in the opposite direction of the replication fork using primers. This leads to a major problem, since primers can not be placed past the ends of DNA, and polymerase can not transcribe the last few nucleotides. This problem is solved by telomeres – 5-15000 base pair-long noncoding repeats of the sequence 5'-TTAGGG-3'. 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 30-150bp (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). That's 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. While telomerase upregulation (or alternative pathways, discussed later) is important throughout all parts of the cycle, it's especially important in metastasis. Experimental evidence shows that when telomerase is deactivated, melanoma exhibits spontaneous regression rather than metastasis (Lopes-Bastos et al. 2024). Telomerase activity correlates with pediatric metastasis. In osteosarcoma, 75% of metastatic cancers exhibited telomerase, while only 11% of nonmetastatic cases showed telomerase activity, indicating a relative risk of 6.8. In 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 is limited for other pediatric cancer types, the strong association between telomerase activity and metastatic rate found in those two indicates that this is a promising area of future study (Table 2).

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Table 1. Comparison of telomerase expression in different types of cancers. The two leftmost columns contain different types of cancers (with the right column being more specific), while the middle column contains telomerase expression in all patients and the two right columns stratify telomerase expression rates by pediatric and adult. Medulloblastoma is a cerebellar cancer, ependymoma originates from ependymal cells, osteosarcoma develops in bone tissue around the knee, and Ewing sarcoma is similar to osteosarcoma, but often responsive to radiotherapy.

Pediatric cancer type	Telomerase % in primary tumor	Telomerase % in metastatic
Medulloblastoma	unknown/limited information	<u>10.7%*</u> (<u>32.1% alt</u>)
Ependymoma	<u>76%</u>	
Osteosarcoma	11% (1/9) ("Table 1 Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue Origin," n.d.)	75% (<u>6/8</u>) ("Table 1 Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue Origin," n.d.)
Ewing sarcoma	12.5% (<u>1/8</u>) ("Table 1 Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue Origin," n.d.)	100% (<u>5/5</u>) ("Table 1 Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue Origin," n.d.)

Due to its relatively low abundance (Nguyen et al. 2019), telomerase has been difficult to structurally resolve. However, it is known that it's a reverse transcriptase holoenzyme responsible for adding telomeres to the ends of chromosomes (Figure 2). It consists of several subunits, the two main ones being TERT (the actual enzyme) and hTERC (an RNA template for the TTAGGG repeats) (figure 1) (Lee and Pellegrini 2025a).

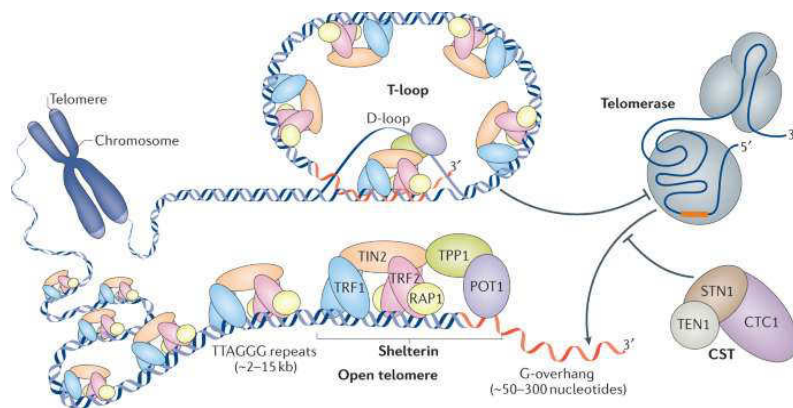


Figure 2. Telomere structure and synthesis. Telomeres are repetitive sequences at the end of chromosomes, synthesized by telomerase. Shelterin is a complex that plays several roles. After telomere synthesis, the shelterin complex wraps telomeres to protect them from enzymes involved in non-homologous end joining (NHEJ) (Lange 2005a). Shelterin's main function is to form D and T loops from the G-rich overhang of telomeres, hiding their 3' end from exonucleases and other repair pathways. It is also a key regulator of telomerase; the POT1/TPP1 heterodimer promotes telomerase activity via a binding region called the TEL patch (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. 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's also a critical upregulator (Rice and Skordalakes 2016).

TERT is functionally expressed in 85-95% of cancer cells, while being absent in most differentiated somatic cells. TERC is ubiquitously expressed in most cells, while TERT is only expressed in germ cells; most telomerase upregulators hinge on TERT expression. Cancer often upregulates key telomerase promoters; for example, a study by Santos et al. found that in prostate cancer, upregulation of shelterin is shown to correlate with higher aggression and metastasis (Liu et al. 2019). Shelterin is an auxiliary complex composed of 6 proteins (TRF1, TRF2, POT1, RAP1, TIN2, and TPP1) responsible for protecting telomeres from DNA repair pathways. In particular, catalytic activity in TPP1 and POT1 inhibits ATR kinase (a single strand break repair enzyme), while the rest also serves to inhibit ATM/ATR signalling. Together, shelterin proteins 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 transcription factor binding sites (Yuan et al. 2019). Therefore, in this study I wanted to investigate the telomerase and TERT subunit activity in pediatric cancers.

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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 correlates with cancer malignancy; however, some tumors may utilize alternative lengthening of telomeres (ALT) pathways (Table 2). ALT utilizes proteins involved in homology-directed repair and DNA damage control to elongate telomeres, based on the existing repeats in chromosomes, which occurs independently of TERT upregulation. Because it uses a wide variety of DNA repair proteins, there is not a single indicator of ALT expression. Typical biomarkers include heterogeneous telomere length (with some less than 8kb and some greater than 50kb), and extrachromosomal telomeric repeats (e.g., C-rich circular DNA ends). Expression is particularly common in mesenchymal neoplasms, with the highest rates of ALT expression being in osteosarcoma (63%), leiomyosarcoma (60%), and undifferentiated pleomorphic sarcoma (59%). Children's cancer has a higher incidence of ALT pathway (MacKenzie et al. 2021).

Table 2. Comparison of ALT pathway and telomerase activation rates in children cancers.

This table shows various pediatric cancers and their relative telomerase expression rates (middle column) and ALT expression rates (right column). It's important to note that each column uses different cohorts and thus percentages may not add to 100%.

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Pediatric cancer type TM	Telomerase positive %	Alt positive %
ALL	54 (39/72)	0 (0/53)
AML	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)
Rhabdomyosarcoma (ERMS)	50 (8/16)	38 (6/16)
Rhabdomyosarcoma (ARMS)	71 (10/14)	0 (0/15)

The next hallmark is apoptosis (programmed cell death) resistance. Cells have many genes such as TP53 and Bcl2 that induce cell death when cells are damaged or have too many mutations, by doing this cancerous cell behavior can be recognized and suppressed. Cancers have various methods to resist apoptosis, including tumor gene suppression e.g, suppressing TP53 by overexpressing MDM2 or activating antiapoptotic regulators, such as Bcl2 in lymphoma. Angiogenesis (blood vessel growth) is another main hallmark induced during hypoxia. Studies by Hanahan and Folkman of transgenic mice with islet cell carcinoma, dermal fibrosarcoma, and epidermal squamous cell carcinoma all found extensive vascularization. According to them, angiogenesis occurs when cancer cells activate an "angiogenic switch," a discrete step occurring when the

presence of pro-angiogenic factors (e.g. VEGF) outweighs the presence of anti-angiogenic factors (TSP-1) (Hanahan and Weinberg 2011).

Tumor cells also need to avoid growth suppressors. The human body has several genes (eg TP53 and RB) that phosphorylate in the presence of oxidative stress and other damages, stalling the life cycles of cancer cells (Lakin and Jackson 1999), (Zatulovskiy et al. 2020). Normally, the body inhibits cell growth via contact inhibition, where mitotic activity slows if cells detect other cells via cadherins. For example, Merlin protein couples cell-surface adhesion molecules and sequesters growth factors; because of this, it's known to inhibit neurofibromatosis II (Morrow et al. 2011). Cancerous cells must degrade these proteins to proliferate and form a tumour.

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Pediatric Cancers and Telomerase

Several aspects make pediatric cancer cases different from adult cancer cases. The pediatric cancers are known to grow faster and one of the reasons for this could be that stem cells are more present and active in children vs adults. While breast, lung, and prostate cancers are the most common cancers in adults, dominant types in children are leukemia, lymphoma, brain and spinal cord cancers ("Six Ways Childhood Cancer Is Different to Adult Cancer | CCLG - The Children & Young People's Cancer Association" 2023). Pediatric neoplasms also grow faster and respond better to chemotherapy. Because of this, children <14 have a much higher 5-year survival rate (85%) ("How Is Childhood Cancer Treated? | American Cancer Society," n.d.) than the whole population ((Cancer Facts & Figures 2025 1930)). However, Cancer is also the leading cause of death in children after infancy, with approximately 1600 children dying of cancer every year in the US alone, according to cancer.gov ("Cancer in Children and Adolescents - NCI" 2024). 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). Cancers in adults can vary widely on TERT promoter mutation rates. Cancers with high promoter mutation rate ($\geq 15\%$) in adults include melanoma, liposarcoma, hepatocellular carcinoma, urothelial carcinoma, tongue squamous carcinoma, and glioma subtypes (83% of glioblastoma). Those with

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Low promoter mutation rate (<15%) include Leukemias (common in children), various nervous cancers (eg gliomas), carcinomas, melanomas, and osteosarcomas (common in adults). While data on TERT promoter mutations is rare, limited studies find that TERT promoter mutation rates in children are much lower (Ackermann and Fischer 2019).

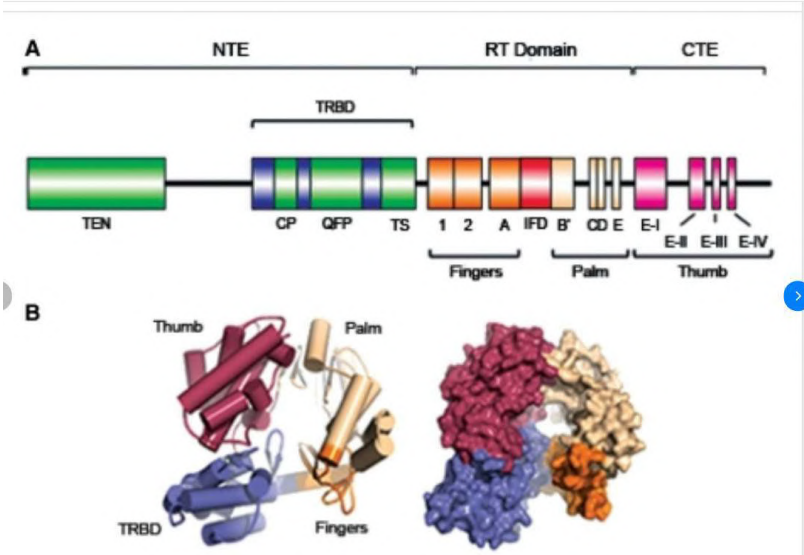
As shown in figure 11, Testicular germ cell cancer has one of the highest TERT expression rates in the data provided by the Human Protein Atlas. As such, I decided to investigate the relationship between cancer and germline mutations. Germline mutations are mutations that occur in sex cells, which could cause some or all cells in the body to inherit that mutation depending on the developmental stage at which they occurred. Studies on pediatric soft-tissue sarcomas (e.g. Ewing, rhabdomyosarcomas) show 17% germline mutation rates in children (Yndestad et al. 2024), while diffuse midline glioma patients exhibited 7.5% mutation rate (Mateos et al. 2025). Counterintuitively, there is no significant difference between germline mutation rates in pediatric vs adult cancer; while the estimated contribution of germline mutations to pediatric cancer is 8-12% ("Frontiers | Genetic Predisposition to Solid Pediatric Cancers," n.d.), this is very similar to the attributable risk for cancer overall, being 10% ("The Genetics of Cancer - NCI" 2015). 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).

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According to table 2, pediatric tumors are overwhelmingly telomerase positive, similar to adult tumors. However, some cancers (osteosarcomas, wilms tumors, neuroblastomas) exhibit significant ALT pathway activation (telomerase negative) - the reasons behind 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 rates. It's worth noting that ALT rate is extremely low in low grade glioma, so ALT pathway could be used as a prognostic marker.

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As a treatment perspective, telomerase inhibitors may provide a way to combat childhood cancer. Cells in children also need to divide more times than in adults - thus, telomerase is known to be more active in children. This, combined with the relative lack of confounding external factors in children, may help establish a clearer statistical trend between telomerase activity and cancer. TERT serves as the catalytic core of the telomerase holoenzyme. It consists of 16 exons (1134 amino acids), with 40766 amino acids interspersed as 15 introns. Multiple proteins induce TERT expression by attaching to the TEN domain and promoter; for example, cMyc binds to E-boxes at +44 when bound to Max protein. TERT can, however, be downregulated by Mad1 (a suppressor of Myc) and histone deacetylase in most somatic cells (Khattar and Tergaonkar 2017).

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Figure 3: Protein subdomains of TERT. On the 5' end is TEN (TERT n terminal domain), which interacts with ssDNA telomere repeats, followed by TRBD, which binds to TERT. RT, the reverse transcriptase domain, and CTE, the C terminal domain, catalyze the addition of nucleotides to telomeres. As shown in figure 7, RT and CTE are the most conserved domains in TERT.

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To study the telomerase and TERT in cancer mechanism, gene editing techniques can be utilized to generate a mutation screen across pediatric cancers. CRISPR is a technique used in gene editing, originally discovered in bacterial immune systems. CRISPR involves an endonuclease (Cas9) as well as a guide RNA, which attaches to a targeted DNA sequence and makes a cut at a recognized PAM (NGG) sequence. This cut is typically blunt, though Cas9 (typically derived from *Streptococcus Pyogenes*) may occasionally produce sticky ends in vivo (Jasin 2018a). What happens next can vary. When attempting to change a nucleotide sequence, a HDR mediating template can be used to insert a desired sequence via homology-directed repair; otherwise, the double-strand break is left to be repaired naturally via non-homologous end joining (NHEJ) or homologous end joining (HDR). As an outcome of these processes a cell can end up having altered DNA sequences (Redman et al. 2016).

Aim of the study:

Telomerase is a well-known carcinogenic factor for 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 determine whether telomerase's effect differs significantly compared to in adult cancer. To do so, I will gather literature knowledge on telomerase, one of the telomerase subunits TERT, expression rates, protein structure, conservation levels of different types of cancers affecting children vs adults. I will also propose a hypothetical CRISPR/Cas9 gene editing design to generate a framework for future experiments that can be conducted to address the question experimentally.

2. Materials & Methods

I used publicly available databases and online tools to conduct my research about Telomerase and TERT:

322

323 **Gathering protein and gene information:**

324 **NCBI** (National Center for Biotechnology Information) is a research database containing
325 a lot of information, including DNA, RNA, and protein sequences (in FASTA format) which
326 is used in this study and it also holds pubmed which is a publication hub. I retrieved protein
327 and gene sequences via NCBI>Protein and NCBI>Gene.

328

329 **Investigating protein properties:**

330 **TMHMM** is an algorithm that analyzes amino acid sequences to determine whether they
331 contain transmembrane domains based on their amino acids' hydrophobicity. Since
332 hydrophobic amino acids have to remain inside membrane layers, consecutive
333 hydrophobic amino acids indicate a transmembrane/intramembrane domain. TMHMM
334 uses the Hidden Markov model, explained in more detail below in results in results. I
335 retrieved amino acid sequences via NCBI and submitted into TMHMM as a query.

336

337 **CLUSTAL OMEGA** is a software that compares protein sequences among different
338 organisms to identify highly conserved amino acids/domains using the PAM 250 scoring
339 matrix, which compares the likelihood of different amino acid substitutions. Additionally,
340 it shows the electrochemical properties of the amino acid sequences (eg, hydrophobicity,
341 charge) with color coding, providing insight into domain function.

342

343 **CRISPR/Cas9 gene editing design:**

344 I retrieved gene sequence via NCBI>Gene database then used **ENSEMBL**, which is a
345 genome database containing genetic information from various organisms including gene
346 sequences and their introns, exons, and isoforms. I annotated the exon and intron region
347 of the TERT gene by using this database.

348

349 **SNAPGENE** displays a gene or protein in its nucleic acid sequence and allows for various
350 modifications, including annotations for primers and other notable DNA/RNA/protein
351 domains. Using SnapGene, one can also perform PCR, predict primer annealing

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temperature, and add or delete nucleotides. I used Snappene to design and visualize a CRISPR/Cas9 gene editing strategy.

CRISPR Finder provides ~20bp CRISPR primers given a DNA sequence. I used CRISPR Finder to generate primers for a possible CRISPR/Cas9 knockdown/knockout.

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

3. Results

3.1 TERT protein does not contain any transmembrane/intramembrane domain

TERT's full sequence (in FASTA format) was taken from NCBI's protein database and then analyzed via TMHMM (Transmembrane Hidden Markov Model). TMHMM is a hidden Markov model, a model that relies on an unobservable initial state. Usual Hidden Markov Models do not understand initial conditions (like whether a given domain is "outside" or "inside" a domain), but track the changes between them (the transmembrane domains) and determine a path (Yoon 2009). TMHMM makes its best guess as to which domains are inside or outside of a membrane layer by applying the positive-inside rule (Heijne 1986). That determines the probability of any given section of a protein being transmembrane or intramembrane. Since the cell membrane (a phospholipid bilayer) is hydrophobic while the cytosol is hydrophilic, a part of a protein that has a certain length of hydrophobic amino acids in an order is very likely to be a transmembrane domain, while hydrophilic regions are not. The presence of transmembrane domains defines the subcellular localisation of the protein, which could be linked to their functions or the phenotype that is observed in the case of dysfunction. TERT lacks any long hydrophobic amino acid chains; as a result, TMHMM analysis shows that it lacks transmembrane

383 domains (Figure 4). A protein result of “all in” doesn't narrow down a specific region in the
384 cell, but does indicate it has no membrane-bound position (“TMHMM 2.0 - DTU Health
385 Tech - Bioinformatic Services,” n.d.).
386

**RecName: Full=Telomerase reverse transcriptase; AltName: Full=HEST2;
AltName: Full=Telomerase catalytic subunit; AltName: Full=Telomerase-
associated protein 2; Short=TP2**

UniProtKB/Swiss-Prot: O14746.1
[GenPept](#) [Identical Proteins](#) [Graphics](#)
>sp|O14746.1|TERT_HUMAN RecName: Full=Telomerase reverse transcriptase; AltName:
Full=HEST2; AltName: Full=Telomerase catalytic subunit; AltName: Full=Telomerase-
associated protein 2; Short=TP2
MPRAPRCRAVRSLLRSHYREVLPLATFVRRLLGPQGWRLVQRGDPAAFRALVAQCLVCPMDARPPPAAPS
FRQVSCLELVARVLQRLCERGAKNVLAFGFALLDGARGGPPPEAFTTSVRSYLPNTVTDALRGSGAWGLL
LRRVGGDDVLVHLARCALFVLVAPSCAYQVCGPPLYQLGAATQARPPPHASGPRRLRGCEAMNHSVREA
GVPLGLPAPGARRRGGSASRSLPLPKRPRRGAPEPERTPVGGQSWAHPGRTRGPSDRGFCVVSAPRAE
EATSLGALSCTRHSHPVGRQHAGPPSTSRPPRPMDTPCPCPVYAETKHFLLYSGGDEQLRPSFLLSSL
RPSLTGARRLVETIFLGSRPWMPGTTPRRLPRLPQRYWQMRPLFLELLGNHAQCPYGVLLKTHCPLRAAVT
PAAGVCAREKPGGSVAAPPEEDTPRRLVQLLRQHSPPWQYGFVRACLRRLVPPGLWGSRHNERFRLN
TKKFISLGKHAKLSLQELTWKMSVRDCAWLRSPGVGCVPAAEHRLREEILAKFLHWLMSVYVVELLRSF
FYVTEITTFQKNRLFFYRKSVMSKLSIGIRQHLKRVQLRELSEAEVRQHREARPALTSRLRFIPKPDGL
RPINVMQYVVGARTFRREKRAERLTSRVKALFVNLNERARRPGLLGASVLGLDDIHRAWRTFVLRVRAQ
DPPPELYFVKVDVTGAYDTIPQDRLTEVIAASIKPQNTYCVRRYAVVQAAHGHVRKAFKSHVSTLTDLQ
PYMRQFVAHLQETSPLRDAVIEQSSSLNEASSGLFDVFLRFMCHHAVRIRGKSYVQCQIGIPQGSILSTL
LCSLCYGDMENKLFAGIRRDGLLLRLVDDFLLVTPHLTHAKTFLRTLVRGVPEYGCVVNLKTKTVNFVPE
DEALGGTAFVQMPAHGLFPWIGLLDTRTLEVQSDYSSYARTSIRASLTFNRGFKAGRNMRKLFGLRL
KCHSLFLDLQVNSLQTVCTNIYKILLQAYRFHACVLQLPFHQQVWKNPTFFLRVTSDTASLCYSILKAK
NAGMSLGAKGAAGPLPSEAVQNLCHQAFLLKLTRHRVTYVPLLGSLRTAQTQLSRKLPGTTLTALEAAAN
PALPSDFKTIID

387
388

TMHMM result

```
# sp|O14746.1|TERT_HUMAN Length: 1132
# sp|O14746.1|TERT_HUMAN Number of predicted TMHs: 0
# sp|O14746.1|TERT_HUMAN Exp number of AAs in TMHs: 0.5821799999999996
# sp|O14746.1|TERT_HUMAN Exp number, first 60 AAs: 0.01515
# sp|O14746.1|TERT_HUMAN Total prob of N-in: 0.02583
sp|O14746.1|TERT_HUMAN TMHMM12.0      outside      1 1132
```

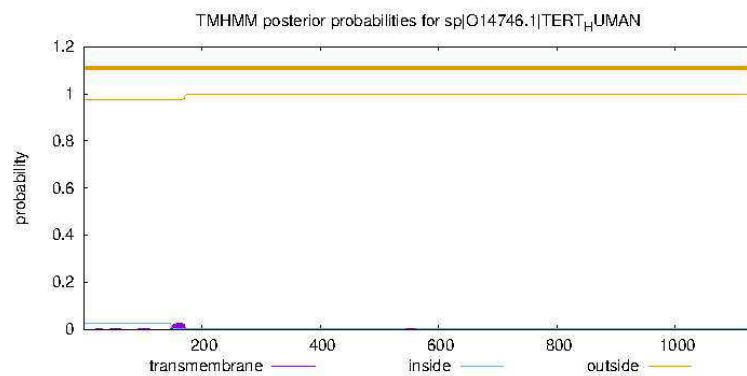


Figure 4. Transmembrane domain analysis of TERT protein via TMHMM algorithm.

The upper text shows the FASTA-formatted protein amino acid sequence of TERT taken from NCBI>Protein>FASTA. The bottom graph is the result of TMHMM analysis for TERT protein. The graph shows that TERT likely lies entirely outside any cell membranes (i.e. it lies in the cytosol). The x axis shows the amino acid sequence, while the y-axis indicates the probability calculation. A region being purple means it's likely to be transmembrane, yellow means it lies outside a cell membrane, and blue means it lies on the inner side of the membrane layer - this figure shows that most of TERT overwhelmingly lies outside. "Outside," in the context of TMHMM analysis, refers to the non-cytosolic side of a lipid bilayer; this fits current consensus that TERT (and telomerase in general) is primarily a nuclear protein.

404

405

406 **3.2.TERT has 8 experimentally determined interactor proteins**

407 Protein-protein interaction is an essential phenomenon in the human genome. By looking
408 at the interactions between proteins, it's possible to infer the function of one protein based
409 on the others it interacts with. STRING is a protein database that predicts protein-protein
410 interactions. It does so via various methods, including text mining, consulting known
411 protein databases, and computing interactions based on co-expression frequency. In this
412 study, interactions found by STRING can serve as a starting point to find key upregulators
413 and downregulators of telomerase and TERT, which can be further analyzed for cancer
414 mechanisms. The proteins shown below are involved with TERT subunit of telomerase
415 (Figure 5) in various ways. NHP2 binds to the H/ACA domain of telomeric RNA (Pogacić
416 et al. 2001) and is involved in telomere maintenance, while WRAP53 binds to Cajal bodies
417 and TERC, responsible for stimulating TERT catalytic activity. SMARCA4 is involved in
418 chromatin remodeling, RUVBL2 possesses single-stranded DNA-stimulated ATPase and
419 ATP- dependent DNA helicase (5' to 3') activity, TEP1 is another protein component of
420 telomerase, DKC1 is a Catalytic subunit of H/ACA small nucleolar ribonucleoprotein
421 (H/ACA snoRNP) complex, which catalyzes pseudouridylation of rRNA, and CTNNB1 is
422 involved in the WNT signalling pathway.

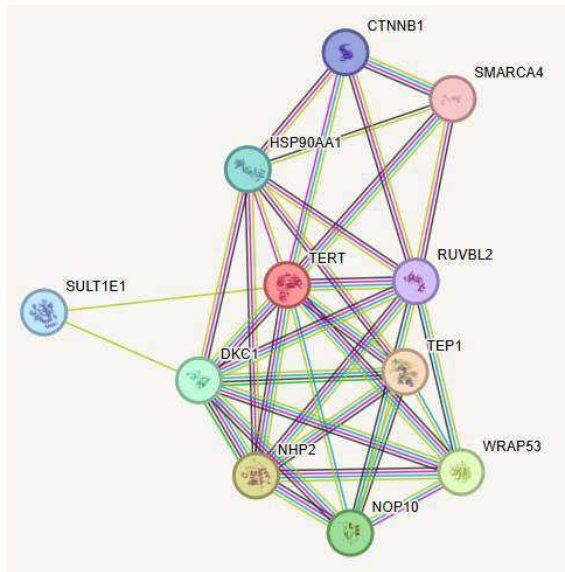


Figure 5. Protein-protein interaction map for the TERT protein. The image shows the predicted interactors of TERT as an outcome of STRING protein-protein interaction analysis. Lines (edges) show interaction depthness between various proteins, each indicated by a node on the graph. Pink and blue lines represent the strongest relationships (experimentally determined and database-sourced respectively), dark green lines represent relationships determined by gene proximity, and black and green lines indicate weak relationships (sourced from textmining and evidence of co-expression, respectively). TERT has 8 experimentally determined interactors. NHP2, WRAP53, TEP1, RUVBL2, SMARCA4, and CTNNB1.

Commented [SS21]: source

3.3. TERT has highly conserved end domain

CLUSTAL Omega analysis compares the amino acid sequences of one protein among different animals and indicates which parts are similar between them using the PAM 250 scoring matrix. If a region is highly similar between animals, this indicates that it's preserved throughout genomes during evolution and could be very important or essential

441 amino acids for the function of the protein. When CLUSTAL analysis of TERT was done,
442 I found that TERT's back end was most strongly conserved (Figure 7); this corresponds
443 with the RT and CTE domains of TERT (Figure 3) in humans, which add nucleotides to
444 telomeres. As can be seen by the graph, the back domain is much more highly conserved
445 than the front. Additionally, there's a high concentration of red amino acids across all
446 animal TERTs analyzed, meaning that TERT is strongly positive. I also generated a
447 heatmap showing the conservation rates quantitatively (Figure 7). The most highly
448 conserved area was amino acids 1140-1200, with 83% (50) amino acids being either
449 highly or fully conserved. This highly conserved region roughly corresponds with the RT
450 (reverse transcriptase) and CTE (c-terminal extension) domains, which catalyze telomere
451 synthesis. While the first 50% of bases are significantly less conserved, the first 120 bases
452 (the top two rows of the table) show relatively high conservation. As it corresponds to the
453 N-terminal domain, it could be inferred that the N-terminal domain's function, while not as
454 conserved as telomerase's catalytic domains (RT and CTE), is still very important to
455 telomerase function. Other regions show very low conservation among organisms.

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Figure 6. Multiple protein alignment of TERT protein in multiple animals (chicken, mouse, human, chimpanzee). In the alignment each letter corresponds to a different amino acid while colors correspond to chemical properties - red indicates a positive charge, magenta indicates negative charge, blue indicates hydrophobicity, green indicates polarity, and pink indicates cysteine. CLUSTAL also displays colors for glycines, prolines, and aromatic amino acids, however, TERT lacks these. Meanwhile, symbols at the bottom indicate similarity between amino acids in different animals. Asterisks (*) indicate that an amino acid is fully conserved among animals listed (i.e. all bases match at that position), colons (:) indicate that an amino acid is strongly conserved (with a PAM 250 score >0.5), and a period (.) indicates weak conservation (PAM 250 score between 0 and 0.5).

Figure 7. Heatmap distribution of conservation frequency in TERT FASTA sequences. This map shows the proportion of each 60-amino acid interval that was denoted as * (fully conserved), : (highly conserved), or- (moderately conserved). (weakly conserved).

Heat map of % of amino acids in each row (~60 acids per row) that are each symbol			
asterisk (fully conserved)	colon (highly conserved)	period (weakly conserved)	
0	0	0	0
0.23	0.2	0.033	
0.4	0.283	0.017	
0.186	0.085	0.017	
0.167	0.33	0.05	
0.067	0.083	0.1	
0.033	0.033	0.083	
0.017	0.067	0.05	
0.322	0.136	0.119	
0.283	0.15	0.0033	
0.017	0.167	0.033	
0.583	0.2	0.0167	
0.617	0.1	0.083	
0.483	0.183	0.083	
0.35	0.283	0.083	
0.35	0.267	0.1	
0.345	0.345	0.034	
0.567	0.167	0.083	
0.525	0.254	0.017	
0.55	0.167	0.083	
0.7	0.13	0.033	
0.433	0.183	0.067	

3.4. CRISPR/Cas9 gene editing strategy for TERT gene

CRISPR is a gene editing technique originally discovered in bacterial immune systems as an antiviral defense system that excises foreign DNA. It involves an endonuclease (Cas9) as well as a guide RNA, which attaches to a targeted DNA sequence and cuts at a recognized PAM (NGG) sequence. This cut is typically blunt, though Cas9 (typically

derived from *Streptococcus Pyogenes*) may occasionally produce sticky ends in vivo (Jasin 2018b). What happens next can vary. When attempting to change a nucleotide sequence, a template can be used to force HDR repair or NHEJ could be an option. This way scientists could end up having mutations varying from single nucleotide changes to big deletions or insertions. In other words, CRISPR allows scientists to generate precise mutations along DNA. This has many uses; for example, one could create a double strand break in a cell DNA to generate mutations to analyze its effects and infer the original function of a gene sequence, or assist in HDR via Cas9 (the main exonuclease). I decided to design a CRISPR/Cas9 strategy for the TERT subunit of the telomerase. First I looked at the chromosomal location of the TERT gene (Figure 8) to understand whether the gene sequence is colliding with any other nearby genes.

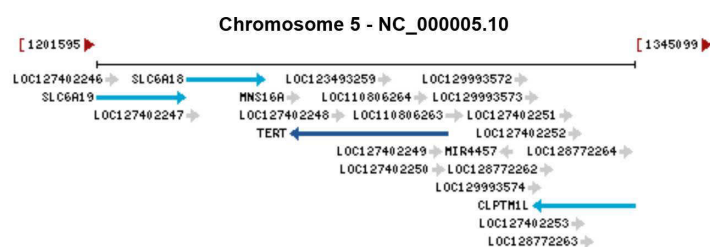


Figure 8. Chromosomal location of TERT gene. The above figure shows a subsection of chromosome 5, starting from base pair 1201595 (left) and ending at 1345099 (right). A dark blue arrow indicates TERT's location on chromosome 5 and its transcription direction. Other genes in humans are marked in light blue. It's important to note that none of them overlap with TERT. Thus, it's possible to CRISPR TERT without interfering with the function of other genes.

Then I gathered the TERT gene sequence from NCBI and annotated the exon and intron region from Ensembl annotations. Then via CRISPR Finder I selected a guide RNA based on the off-target scores calculated for the allowed mismatches of 0, 1, 2, 3, 4. I picked a

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its hydrogen bonds, mimicking helicase activity to generate single strand DNA. Then, in annealing, DNA is cooled to a lower temperature (e.g., 70 C) to allow DNA primers to bind. A heat-resistant polymerase (for example, Taq) is then used to add nucleotides in the extension step, amplifying the DNA.

Gel electrophoresis is a lab technique that uses electricity to separate DNA fragments in a given sample by size. Each gel tank has a connection to a positive and a negative electrode that can be applied to the system. Samples containing DNA are loaded from the area of the tank that is closer to the negative electrode. The agarose made gel will have wells to load individual samples. Phosphate groups in DNA make the DNA molecule negatively charged, and thus once the electrical system starts running the negative electrons will push the DNA molecule from top of the gel towards the bottom of the gel. The smaller molecules move more quickly due to a lack of resistance in the gel and the larger ones will stick at the top area. Here I simulated a gel electrophoresis (Figure 10) for training purposes in order to present the hypothetical outcomes of the gene editing. Single nucleotide changes cannot be resolved in a gel therefore they are needed to be sequenced. All potential alterations must be confirmed with sequencing.



Figure 10. Hypothetical DNA gel electrophoresis for the expected outcomes of CRISPR/Cas9 gene editing for TERT gene. This gel electrophoresis shows a hypothetical aftermath of using CRISPR to generate TERT gene mutations. The leftmost

lane contains a DNA fragment ladder or marker with known sizes, providing a reference for the fragment sizes in the other 6 lanes. Lane one (the second leftmost lane) contains unmodified TERT, which is slightly less than 350 bp in size as a wild type; lanes 4, 5, and 6 (single nucleotide deletion, insertion, and substitution, respectively) all have similar fragment lengths as this length of alteration cannot be resolved on a gel they will be same size as the wild type. However, TERT samples in lane 2 are significantly shorter, indicating a ~80 base pair deletion, while samples in lane 3 are significantly longer, indicating a ~75 base pair insertion.

Below TCGA expression data about TERT among different cancers shows that TERT expression can be used as a marker to identify differences among cancer types (Figure 11) however all this data does not say whether adult vs pediatric cases were compared or not. Therefore, all my investigation above will be providing knowledge about this gap in the future

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The role of Telomerase and the Telomerase Reverse Transcriptase subunit in paediatric cancers

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Abstract (summary)

Background: 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.

Objectives: 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.

Methods: We took TERT's FASTA sequence and used the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database, CLUSTAL, Transmembrane Helix Hidden Markov Model (TMHMM), SnapGene, and National Center for Biotechnology Information (NCBI) to analyze proteins related to TERT, establish functional domains, and perform a hypothetical CRISPR experiment.

Results: 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 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 knockdown experiments are one way to further study the effects of TERT mutations on pediatric cancer.

Conclusion: TERT promoter mutations are generally rarer in pediatric cancer than adult cancer, while increased telomerase expression overall has a significant effect

32 on pediatric cancer. In cancers where data were available, metastatic rates were higher
33 in patients exhibiting telomerase than in those who did not. While sample sizes were
34 extremely small, relative risk statistics indicate that telomerase mutations are a
35 promising area of investigation in pediatric cancer.

36 _____

37 **Keywords:** Telomerase, paediatric cancer, cancer, TERT gene

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39 1. Introduction

40 Cancer is a group of diseases characterized by uncontrolled cell proliferation, affecting
41 both children and adults (*What Is Cancer?*, 2007). The causes of cancers are varied, but
42 all known cancers involve genetic mutations. These mutations sometimes cause
43 functional enhancements, such as telomerase enzymes increasing their activity in
44 cancer cells. However, only 5-10% of cancers are hereditary (*Frequently Asked Questions*
45 *about Hereditary Cancers* | *Memorial Sloan Kettering Cancer Center*, n.d.) Most gene
46 mutations occur as a result of external factors, but are also influenced by internal factors
47 such as cell division and replication. For example, mutations in genes called
48 proto-oncogenes that regulate apoptosis or cell cycle functions can turn genes
49 oncogenic, or cancerous. Specific oncogenes can vary depending on the type of cancer.

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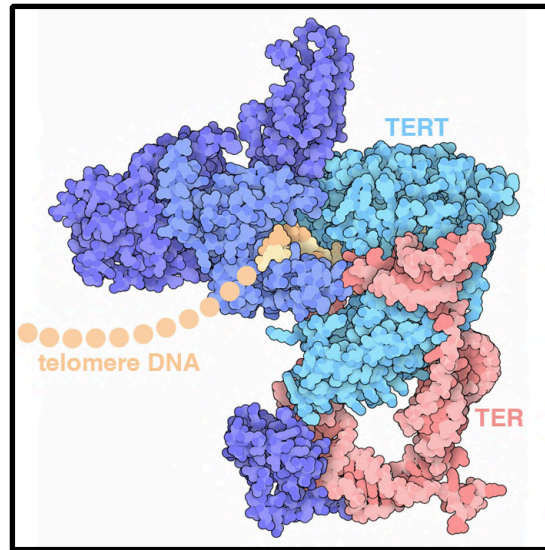
51 ***Cancerous Hallmarks and Telomerase Activity***

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57 **Figure 1: Molecular 3D structure of telomerase.** The image shows the 3D structure
 58 of a TERT subunit of telomerase enzyme, as well as Telomerase RNA Component
 59 (TERC), an RNA template for telomere synthesis. (*PDB101*, n.d.)

60

61 While cancers vary broadly, they share key common traits - the Hallmarks of Cancer
 62 (Hanahan and Weinberg 2011). These hallmarks are sustained proliferative signaling,
 63 evasion of growth suppressors, metastasis, apoptotic resistance, angiogenesis, and
 64 replicative immortality. Replicative immortality in particular is closely linked to
 65 telomerase (Figure 1).

66 Telomerase is studied in relation to cancer because it solves the end replication
 67 problem. Because DNA polymerase can only synthesize DNA in the 5' to 3' direction,
 68 the lagging strand of DNA needs to be synthesized in the opposite direction of the
 69 replication fork using primers. This leads to a major problem because primers can not
 70 be placed past the ends of DNA, and polymerase can not transcribe the last few
 71 nucleotides. This problem is solved by telomeres – 5-15000 base pair-long noncoding
 72 repeats of the sequence 5'-TTAGGG-3'. They reside on the ends of chromosomes and
 73 allow DNA polymerase to run past the coding region, allowing cells to divide without
 74 losing important coding regions. However, each cell division shortens telomeres by
 75 30-150bp (Vaziri et al., 1993, Harley et al., 1990; Allsopp et al., 1992) until they reach
 76 their Hayflick Limit (when telomeres are too degraded to protect against lagging-strand
 77 DNA loss). That's when a normal cell stops dividing. Cancer cells can bypass the

78 Hayflick limit via telomerase activity, which continually lengthens telomeres and
79 prevents DNA end degradation.

80 While telomerase upregulation (or alternative pathways, discussed later) is
81 important throughout cancer progression, it's especially important in metastasis.
82 Telomerase activity correlates with pediatric metastasis. For example, melanoma
83 exhibits spontaneous regression rather than metastasis when telomerase is deactivated
84 (Lopes-Bastos et al. 2024). In osteosarcoma, 75% of metastatic cancers exhibited
85 telomerase, while only 11% of nonmetastatic cases showed telomerase activity,
86 indicating a relative risk of 6.8. In Ewing sarcoma, 100% of metastatic cancers showed
87 telomerase expression, while only 12.5% of nonmetastatic tumors exhibited telomerase
88 - a relative risk of 8. While data is limited for other pediatric cancer types, the strong
89 association between telomerase activity and metastatic rate found in those two
90 indicates that this is a promising area of future study (Table 2).

91

92 **Table 1. Comparison of telomerase expression in different types of cancers.** The two
93 leftmost columns contain different types of cancers (with the right column being more specific),
94 while the middle column contains telomerase expression in all patients, and the two right
95 columns stratify telomerase expression rates by pediatric and adult.

Pediatric cancer type	Telomerase % in primary tumor	Telomerase % in metastatic
Medulloblastoma	unknown/limited information	<u>10.7%*</u> (32.1% ALT)
Ependymoma	<u>76%</u>	
Osteosarcoma	11% (1/9)	75% (<u>6/8</u>)
Ewing sarcoma	12.5% (<u>1/8</u>)	100% (<u>5/5</u>)

96

97 Data on medulloblastoma were found from (Minasi et al., 2019). Data on Ependymoma
98 were found from (*Telomerase as a Prognostic Marker and Therapeutic Target in Paediatric*
99 *Ependymoma*, n.d.). Data on osteosarcoma and Ewing Sarcoma were found from ("Table 1
100 Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue
101 Origin," n.d.)

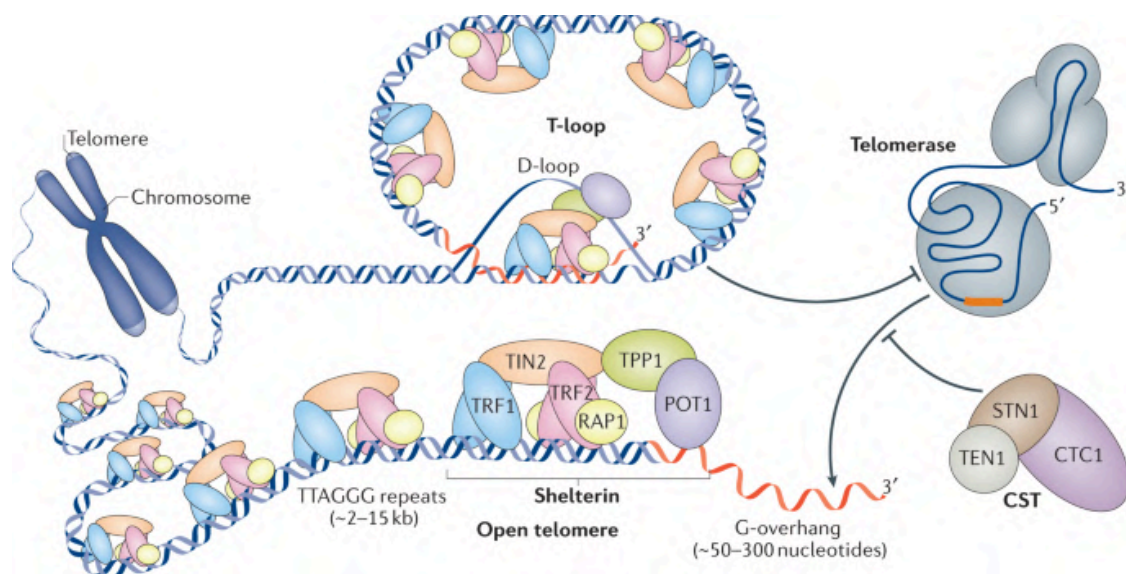
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105 Due to its relatively low abundance (Nguyen et al. 2019), telomerase has been
106 difficult to structurally resolve. However, it is known that it is a reverse transcriptase

107 holoenzyme responsible for adding telomeric repeats to the ends of chromosomes
 108 (Figure 2). It consists of several subunits, including TERT (the catalytic subunit) and
 109 hTERC, an RNA template for the TTAGGG repeats; see Figure 1 (Lee and Pellegrini
 110 2025a). TERT consists of 16 exons including 1134 amino acids, and 40766 nucleotides
 111 interspersed as 15 introns.



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113

114 **Figure 2. Telomere structure and synthesis.** Telomeres are repetitive sequences at the end of
 115 chromosomes, synthesized by telomerase. After synthesis, the shelterin complex promotes the
 116 formation of D-loops and T-loops, protecting telomeres from degradative enzymes. The CST
 117 complex also contributes to telomere maintenance and regulation. (Lim & Cech, 2021)
 118

119 It is also expressed in 85-95% of cancer cells. While TERC is ubiquitously expressed in
 120 most cells, TERT is mainly expressed in germ cells; most telomerase upregulators
 121 hinge on TERT expression. Cancer often upregulates key telomerase promoters; for
 122 example, a study by Santos et al. found that in prostate cancer, upregulation of shelterin
 123 correlates with higher aggression and metastasis (Liu et al. 2019). Shelterin is an
 124 auxiliary complex composed of 6 proteins (TRF1, TRF2, POT1, RAP1, TIN2, and TPP1)
 125 responsible for protecting telomeres from DNA repair pathways. After telomere
 126 synthesis, the shelterin complex wraps telomeres to protect them from enzymes
 127 involved in non-homologous end joining (NHEJ) (Lange 2005a). Shelterin's main

128 function is to form D and T loops from the G-rich overhang of telomeres, hiding their 3'
129 end from exonucleases and other repair pathways. It is also a key regulator of
130 telomerase; the POT1/TPP1 heterodimer promotes telomerase activity by binding to a
131 region called the TEL patch (Mir et al. 2020) and inhibiting ATR kinase. TIN2 causes
132 conformational changes to TRF1 that inhibit telomerase access. TRF2- interacts with
133 RAP1, preventing homologous recombination. The CST complex (originally thought to
134 be unique to yeast) is a trimeric protein composed of Ctc1, Stn1, and Ten1. It associates
135 with the telomeric G-overhang and inhibits access of telomerase to the telomeres. While
136 it can inhibit telomerase access by sequestering the g-overhang, it's also a critical
137 upregulator (Rice and Skordalakes 2016). Together, shelterin proteins serve to protect
138 telomeres from damage. Several methods of telomerase activation exist, such as
139 promoter hypermethylation and point mutations (e.g., C228T or C250T) that create ETS
140 (erythroblast-specific transcription factor) binding sites (Yuan et al. 2019). Multiple
141 proteins induce TERT expression by attaching to the TEN domain and promoter; for
142 example, cMyc binds to E-boxes at +44 when bound to Max protein. TERT can,
143 however, be downregulated by Mad1 (a suppressor of Myc) and histone deacetylase in
144 most somatic cells (Khattar and Tergaonkar 2017).

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148 In 1997, Shay and Bacchetti first used the telomeric repeat amplification protocol
149 (TRAP) to measure telomerase activity. They found that 82.4% of malignant tumors,
150 49.1% of premalignant tumors, and 23.6% of benign tumors expressed telomerase.
151 Telomerase activity correlates with cancer malignancy; however, some tumors may
152 utilize alternative lengthening of telomeres (ALT) pathways (Table 2). ALT utilizes
153 proteins involved in homology-directed repair (HDR) and DNA damage control to
154 elongate telomeres, based on the existing repeats in chromosomes, which occurs
155 independently of TERT upregulation. There is no universal indicator of ALT expression,
156 but typical biomarkers include heterogeneous telomere length (with some less than 8kb
157 and some greater than 50kb), and extrachromosomal telomeric repeats (e.g., C-rich
158 circular DNA ends). Expression is particularly common in mesenchymal neoplasms,

with the highest rates of ALT expression being in osteosarcoma (63%), leiomyosarcoma (60%), and undifferentiated pleomorphic sarcoma (59%). Pediatric cancers have a higher incidence of ALT pathway activation (MacKenzie et al. 2021).

Table 2. Comparison of ALT pathway and telomerase activation rates in children's cancers. This table shows various pediatric cancers and their relative telomerase expression rates (middle column) and ALT expression rates (right column). Each column uses different cohorts and thus percentages may not add to 100%. (Ackermann & Fischer, 2019)

Pediatric cancer type	Telomerase positive %	Alt positive %
ALL	54 (39/72)	0 (0/53)
AML	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)
Rhabdomyosarcoma (ERMS)	50 (8/16)	38 (6/16)
Rhabdomyosarcoma (ARMS)	71 (10/14)	0 (0/15)

Pediatric Cancers and Telomerase

Several aspects make pediatric cancer cases different from adult cancer cases. Pediatric cancers are known to grow faster, and one of the reasons for this could be that stem cells are more present and active in children than in adults. While breast, lung,

192 and prostate cancers are the most common cancers in adults, dominant types in
193 children are leukemia, lymphoma, brain and spinal cord cancers (“Six Ways Childhood
194 Cancer Is Different to Adult Cancer | CCLG - The Children & Young People’s Cancer
195 Association” 2023). Pediatric neoplasms also respond better to chemotherapy. Because
196 of this, children <14 have a much higher 5-year survival rate (85%) (*How Is Childhood
197 Cancer Treated?*, n.d.) than the whole population (Cancer Facts & Figures 2025 1930).
198 However, Cancer is also the leading cause of death in children after infancy, with
199 approximately 1600 children dying of cancer every year in the US alone, according to
200 cancer.gov (“Cancer in Children and Adolescents - NCI” 2024). As childhood cancers
201 are much earlier in life, there are far fewer external causes to control for, and thus,
202 childhood cancers are much harder to prevent. Most cancers express telomerase
203 (Hiyama et al. 2004). Cancers in adults can vary widely in TERT promoter mutation
204 rates. Cancers with high promoter mutation rate ($\geq 15\%$) in adults include melanoma,
205 liposarcoma, hepatocellular carcinoma, urothelial carcinoma, tongue squamous
206 carcinoma, and glioma subtypes (83% of glioblastoma). Those with Low promoter
207 mutation rate ($< 15\%$) include Leukemias (common in children), various nervous cancers
208 (e.g., gliomas), carcinomas, melanomas, and osteosarcomas (common in adults). While
209 data on TERT promoter mutations are rare, limited studies find that TERT promoter
210 mutation rates in children are much lower (Ackermann and Fischer 2019).

211

212 As shown in Figure 11, Testicular germ cell cancer has one of the highest TERT
213 expression rates in the data provided by the Human Protein Atlas. As such, I decided to
214 investigate the relationship between cancer and germline mutations. Germline
215 mutations are mutations that occur in sex cells, which could cause some or all cells in
216 the body to inherit that mutation, depending on the developmental stage at which they
217 occurred. Studies on pediatric soft-tissue sarcomas (e.g., Ewing, rhabdomyosarcomas)
218 show 17% germline mutation rates in children (Yndestad et al. 2024), while diffuse
219 midline glioma patients exhibited 7.5% mutation rate (Mateos et al. 2025).
220 Counterintuitively, there is no significant difference between germline mutation rates in
221 pediatric vs adult cancer; while the estimated contribution of germline mutations to
222 pediatric cancer is 8-12% (“Frontiers | Genetic Predisposition to Solid Pediatric

223 Cancers,” n.d.), this is very similar to the attributable risk for cancer overall, being 10%
224 (“The Genetics of Cancer - NCI” 2015). However, germline mutations in TERT generally
225 are associated with an increased risk of cancer - for example, in people with lynch
226 syndrome, an intronic G>A mutation increases cancer risk by 85% (Wiik et al. 2021).

227

228

229 According to Table 2, pediatric tumors are overwhelmingly telomerase positive, similar
230 to adult tumors. However, some cancers (osteosarcomas, Wilms tumors,
231 neuroblastomas) exhibit significant ALT pathway activation (telomerase negative) - the
232 reasons behind which are still being investigated. The cancers with the highest
233 telomerase expression rates are rhabdomyosarcoma, medulloblastoma, and Wilms
234 tumor, while those with lower expression rates are high-grade glioma and
235 osteosarcoma, which also have high ALT rates. It’s worth noting that the ALT rate is
236 extremely low in low-grade glioma, so the ALT pathway could be used as a prognostic
237 marker.

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240 From a treatment perspective, telomerase inhibitors may provide a way to combat
241 childhood cancer. Telomerase is known to be more active in children as cells in children
242 need to divide more times than in adults. This, combined with the relative lack of
243 confounding external factors in children, may help establish a clearer statistical trend
244 between telomerase activity and cancer.

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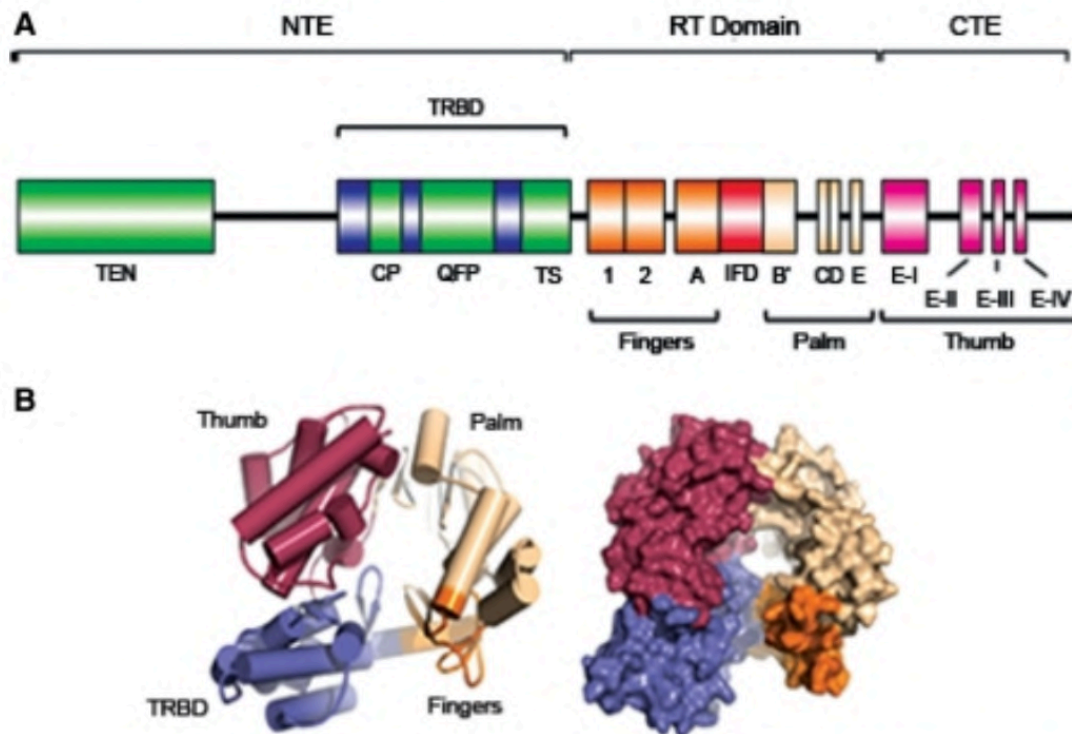


Figure 3: Protein subdomains of TERT. On the 5' end is TEN (TERT n-terminal domain), followed by TRBD (telomerase RNA binding domain) RT (reverse transcriptase), and C-terminal domain (CTE). As shown in Figure 7, RT and CTE are the most conserved domains in TERT. (Structural Organization of the TERT. (A) Predicted Linear Architecture... | Download Scientific Diagram, n.d.)

Aim of the study:

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 significantly compared to that in adult cancer. To do so, I will review literature on telomerase and the TERT subunit, including their expression rates, protein structure, and conservation levels of different types of cancers affecting children vs adults. I will also propose a hypothetical CRISPR/Cas9 gene editing design to generate a framework for future experiments that can be conducted to address the question experimentally.

2. Materials & Methods

I used publicly available databases and online tools to conduct research on telomerase and TERT:

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.

Investigating protein properties:

TMHMM was used to analyze amino acid sequences to determine whether they contain transmembrane domains based on their amino acids' hydrophobicity. Since hydrophobic amino acids usually remain inside membrane layers, consecutive hydrophobic amino acids indicate a transmembrane/intramembrane domain. I retrieved FASTA-formatted amino acid sequences via NCBI and submitted them to TMHMM as a query.

CLUSTAL Omega compares protein sequences among different organisms to identify highly conserved amino acids/domains using the PAM 250 scoring matrix, which compares the likelihood of different amino acid substitutions. Additionally, it shows the electrochemical properties of the amino acid sequences (eg, hydrophobicity, charge) with color coding, providing insight into domain function.

CRISPR/Cas9 gene editing design:

I retrieved gene sequences via 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

316 DNA/RNA/protein domains. Using SnapGene, one can also perform PCR, predict
317 primer annealing temperature, and add or delete nucleotides. I used SnapGene to
318 design and visualize a CRISPR/Cas9 gene editing strategy.

319

320 **CRISPR Finder** provides ~20bp CRISPR guide RNA candidates given a DNA
321 sequence. I used CRISPR Finder to generate gRNAs for a hypothetical CRISPR/Cas9
322 knockdown/knockout.

323

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

328

329

330 **3. Results**

331

332 **3.1 TERT protein does not contain any transmembrane/intramembrane domain**

333

334 TMHMM makes its best guess as to which domains are inside or outside of a
335 membrane layer by applying the positive-inside rule (Heijne 1986). The presence of
336 transmembrane domains narrows down the subcellular localization of the protein, which
337 could be linked to their functions or the phenotype that is observed in the case of
338 dysfunction. TERT lacks any long hydrophobic amino acid chains; as a result, TMHMM
339 analysis shows that it lacks transmembrane domains (Figure 4). A protein result of “all
340 in” doesn't narrow down a specific region in the cell, but does indicate it has no
341 membrane-bound position (“TMHMM 2.0 - DTU Health Tech - Bioinformatic Services,”
342 n.d.). TERT's apparent lack of a transmembrane domain is consistent with current
343 consensus that TERT is a nuclear protein.

RecName: Full=Telomerase reverse transcriptase; AltName: Full=HEST2; AltName: Full=Telomerase catalytic subunit; AltName: Full=Telomerase-associated protein 2; Short=TP2

UniProtKB/Swiss-Prot: O14746.1

[GenPept](#) [Identical Proteins](#) [Graphics](#)

>sp|O14746.1|TERT_HUMAN RecName: Full=Telomerase reverse transcriptase; AltName: Full=HEST2; AltName: Full=Telomerase catalytic subunit; AltName: Full=Telomerase-associated protein 2; Short=TP2

MPRAPRCRAVRSLLRSHYREVLPATFVRRRLGPQGWRLVQRGDPAAFRALVAQCLVCPWDARPPPAAPS
FRQVSCLELVARVLQRLCERGAKNVLAFGFALLDGARGGPPEAFTTSVRSYLPNTVTDALRGSGAWGLL
LRRVGDDVLVHLLARCALFVLVAPSCAYQVCGPPLYQLGAATQARPPPHASGPRRRRLGCERAWNHSVREA
GVPLGLPAPGARRRGGSASRSLPLPKRPRRGAAPPERTPVGGQSWAHPGTRGSPDRGFCVVSAPPAE
EATSLEGALSGRHSHPSVGRQHAGPPSTSRPPRPWDTPCPPVYAETKHFLLYSSGDKQLRPSFLLSSL
RPSLTGARRLVETIFLGSRPWMPGTPRRLPRLPQRYWQMRPLFLELLGNHAQCPYGVLLKTHCPLRAAVT
PAAGVCAREKPGQSVAAPEEEDTPRRLVQLLRQHSSPWQVYGFVRACLRLVPPGLWGSRHNERFLRN
TKKFISLGKHAKLSLQELTWKMSVRDCAWLRRSPGVGCVPAAEHRLREEILAKFLHWLMSVYVVELLRSF
FYVTETTFQKNRLLFFYRKSVWSKLSQSIGIRQHLKRVQLRELSEAEVRQHREARPALLTSRLRFIPKPDGL
RPINVMDYVVGARTFRREKRAERLTSRVKALFVSLNYERARRPGLLGASVLGLDDIHRAWRTFVLRVRAQ
DPPPELYFVKVDVTGAYDITPQDRLTEVIASIIKPNTYCVRRYAVVQKAAHGHVRKAFKSHVSTLTDLQ
PYMRQFVAHLQETSPLRDAVVIEQSSSLNEASSGLFDVFLRFMCHHAVRIRGKSYVQCQGIQGSILSTL
LCSLCYGD MENKLFAGIRRDGLLLRLVDDFLLVTPHLTHAKTFLRTLVRGVPEYGCVVNLKTVVNFVPE
DEALGGTAFVQMPAHGLFPWCGLLLDTRTLEVQSDYSSYARTSIRASLTFNRGFKAGRNMRKLFVGLRL
KCHSLFLDLQVNSLQTVCTNIYKILLQAYRFHACVLQLPFHQQVWKNPTFFLRVISDTASLCYSILKAK
NAGMSLGAKGAAGPLPSEAVQWLCHQAFLLKLTRHRVTYVPLLGSLRTAQTLRSRKLPGTTLTAAEAAAN
PALPSDFKTILD

344

345 **Figure 4. Transmembrane domain analysis of the TERT protein via TMHMM**
346 **algorithm.** The upper text shows the FASTA-formatted protein amino acid sequence of
347 TERT taken from NCBI>Protein>FASTA, while the lower graph shows that TERT likely
348 lies entirely outside any cell membranes (i.e., it lies in the cytosol).. The x-axis shows
349 the amino acid sequence, while the y-axis indicates the probability calculation.
350 “Outside,” in the context of TMHMM analysis, refers to the non-cytosolic side of a lipid
351 bilayer; this fits current consensus that TERT (and telomerase in general) is primarily a
352 nuclear protein.

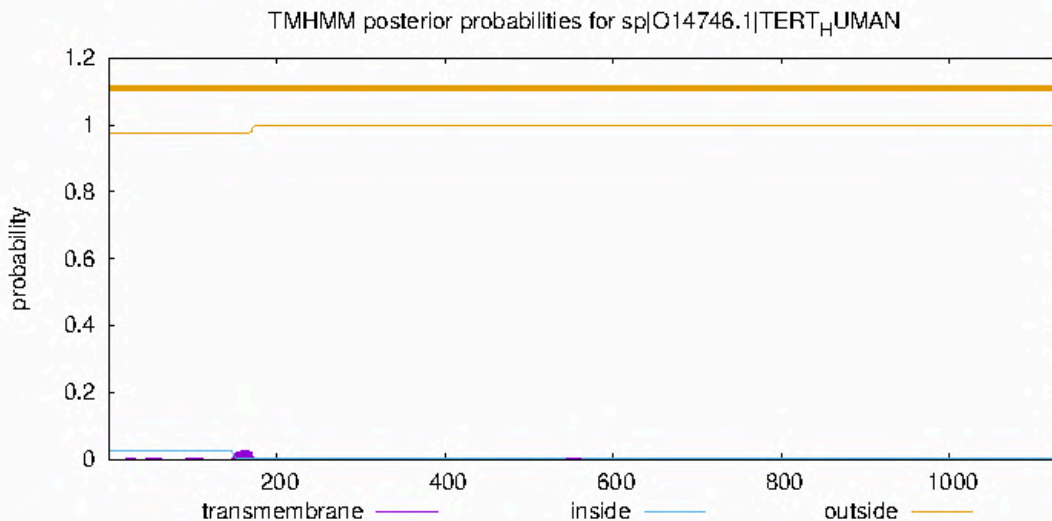
353

354

355

TMHMM result

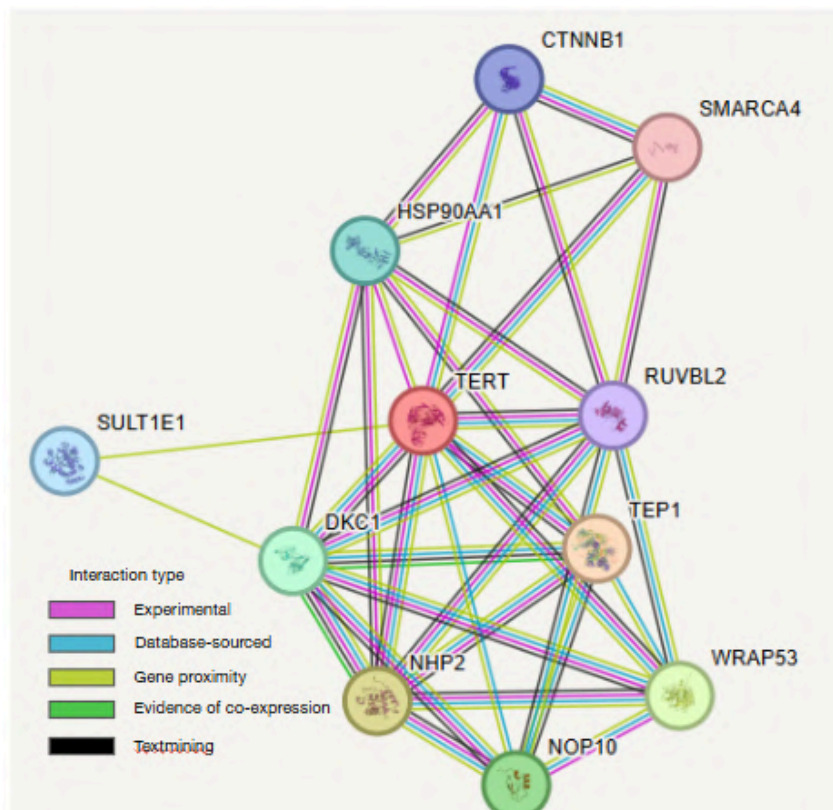
```
# sp|O14746.1|TERT_HUMAN Length: 1132
# sp|O14746.1|TERT_HUMAN Number of predicted TMHs: 0
# sp|O14746.1|TERT_HUMAN Exp number of AAs in TMHs: 0.5821799999999996
# sp|O14746.1|TERT_HUMAN Exp number, first 60 AAs: 0.01515
# sp|O14746.1|TERT_HUMAN Total prob of N-in: 0.02583
sp|O14746.1|TERT_HUMAN TMHMM2.0 outside 1 1132
```



3.2.TERT has 8 experimentally determined interactor proteins

Protein-protein interaction is essential for cellular function. By looking at the interactions between proteins, it's possible to infer the function of one protein based on the others it interacts with. 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. In this study, interactions found by STRING can serve as a starting point to find key upregulators and downregulators of telomerase and TERT, which can be further analyzed for cancer mechanisms. The proteins shown below are involved with the TERT subunit of telomerase (Figure 5) in various ways. NHP2 binds to the H/ACA domain of telomeric RNA (Pogacíc et al. 2001) and is involved in telomere maintenance, while WRAP53 binds to Cajal bodies and TERC, responsible for stimulating 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 H/ACA small nucleolar

371 ribonucleoprotein (H/ACA snoRNP) complex, which catalyzes pseudouridylation of
372 rRNA, and CTNNB1 is involved in the WNT signaling pathway.



373

374 **Figure 5. Protein-protein interaction map for the TERT protein.** Figure 5.
375 Protein-protein interaction map for the TERT protein. Lines show interaction between
376 various proteins, each indicated by a node on the graph - each color indicates the type
377 of evidence for interaction between two proteins. TERT has 8 experimentally
378 determined interactions: NHP2, WRAP53, TEP1, RUVBL2, SMARCA4, DKC1,
379 HSP90AA1, and CTNNB1. (*TERT Protein (Human) - STRING Interaction Network, n.d.*)

380

381

382 3.3. TERT has a highly conserved end domain

383

384 CLUSTAL Omega analysis compares the amino acid sequences of one protein among
385 different animals and indicates which parts are similar between them using the PAM 250
386 scoring matrix. If a region is highly similar between animals, this indicates that it's
387 conserved throughout species during evolution and could be an important region for the

388 function of the protein. When CLUSTAL analysis of TERT was done, I found that
389 TERT's RT and CTE domains (Figure 3) were most strongly conserved (Figure 7).
390 Additionally, there's a high concentration of red amino acids across all animal TERTs
391 analyzed, meaning that TERT contains many positive residues. I also generated a
392 heatmap showing the conservation rates quantitatively (Figure 7). The most highly
393 conserved area was amino acids 1140-1200, with 83% (50) amino acids being either
394 highly or fully conserved. This region roughly corresponds with the CTE (c-terminal
395 extension) domain. While the first 50% of the amino acids are significantly less
396 conserved, the first 120 residues (the top two rows of the table) show relatively high
397 conservation. As it corresponds to the N-terminal domain, it could be inferred that the
398 N-terminal domain's function, while not as conserved as telomerase's catalytic domains
399 (RT and CTE), is still very important to telomerase function. Other regions show very
400 low conservation among organisms.

401

402

403

404

Species	Accession	Gene	Protein	Length
chicken	NP_001026178.2	MEG	MEG	60
mouse	sp 070372.1 TERT_MOUSE	MEG	MEG	0
human	sp 014746.1 TERT_HUMAN	MEG	MEG	41
chimpanzee	PNI27662.1	MEG	MEG	41
chicken	NP_001026178.2	MEG	MEG	120
mouse	sp 070372.1 TERT_MOUSE	MEG	MEG	31
human	sp 014746.1 TERT_HUMAN	MEG	MEG	101
chimpanzee	PNI27662.1	MEG	MEG	101
chicken	NP_001026178.2	MEG	MEG	180
mouse	sp 070372.1 TERT_MOUSE	MEG	MEG	91
human	sp 014746.1 TERT_HUMAN	MEG	MEG	161
chimpanzee	PNI27662.1	MEG	MEG	161
chicken	NP_001026178.2	MEG	MEG	239
mouse	sp 070372.1 TERT_MOUSE	MEG	MEG	139
human	sp 014746.1 TERT_HUMAN	MEG	MEG	200
chimpanzee	PNI27662.1	MEG	MEG	200
chicken	NP_001026178.2	MEG	MEG	299
mouse	sp 070372.1 TERT_MOUSE	MEG	MEG	152
chicken	NP_001026178.2	MEG	MEG	359
mouse	sp 070372.1 TERT_MOUSE	MEG	MEG	195
human	sp 014746.1 TERT_HUMAN	MEG	MEG	261
chimpanzee	PNI27662.1	MEG	MEG	261
chicken	NP_001026178.2	MEG	MEG	419
mouse	sp 070372.1 TERT_MOUSE	MEG	MEG	225
human	sp 014746.1 TERT_HUMAN	MEG	MEG	289
chimpanzee	PNI27662.1	MEG	MEG	289
chicken	NP_001026178.2	MEG	MEG	479
mouse	sp 070372.1 TERT_MOUSE	MEG	MEG	249
human	sp 014746.1 TERT_HUMAN	MEG	MEG	314
chimpanzee	PNI27662.1	MEG	MEG	314
chicken	NP_001026178.2	MEG	MEG	538
mouse	sp 070372.1 TERT_MOUSE	MEG	MEG	308
human	sp 014746.1 TERT_HUMAN	MEG	MEG	373
chimpanzee	PNI27662.1	MEG	MEG	373
chicken	NP_001026178.2	MEG	MEG	598
mouse	sp 070372.1 TERT_MOUSE	MEG	MEG	357
human	sp 014746.1 TERT_HUMAN	MEG	MEG	424
chimpanzee	PNI27662.1	MEG	MEG	424
chicken	NP_001026178.2	MEG	MEG	658
mouse	sp 070372.1 TERT_MOUSE	MEG	MEG	369
human	sp 014746.1 TERT_HUMAN	MEG	MEG	449
chimpanzee	PNI27662.1	MEG	MEG	449

Figure 6. Multiple protein alignment of the TERT protein in multiple animals (chicken, mouse, human, chimpanzee). In the alignment, each letter corresponds to a different amino acid, while colors correspond to chemical properties - red indicates a positive charge, magenta indicates a negative charge, blue indicates hydrophobicity, green indicates polarity, and pink indicates cysteine. Symbols below each amino acid indicate similarities between animals. Asterisks (*) indicate that an amino acid is fully conserved among animals listed (i.e., all bases match at that position), colons (:) indicate that an amino acid is strongly conserved (with a PAM 250 score >0.5), and a period (.) indicates weak conservation (PAM 250 score between 0 and 0.5).

Figure 7. Heatmap distribution of conservation frequency in TERT FASTA sequences. This map shows the proportion of each 60-amino acid interval that was denoted as fully conserved (*), highly conserved (:), or weakly conserved (.) in the CLUSTAL alignment.

Heat map of % of amino acids in each row (~60 acids per row) that are each symbol			
asterisk	colon	period	
(fully conserved)	(highly conserved)	(weakly conserved)	
0	0	0	
0.23	0.2	0.033	
0.4	0.283	0.017	
0.186	0.085	0.017	
0.167	0.33	0.05	
0.067	0.083	0.1	
0.033	0.033	0.083	
0.017	0.067	0.05	
0.322	0.136	0.119	
0.283	0.15	0.0033	
0.017	0.167	0.033	
0.583	0.2	0.0167	
0.617	0.1	0.083	
0.483	0.183	0.083	
0.35	0.283	0.083	
0.35	0.267	0.1	
0.345	0.345	0.034	
0.567	0.167	0.083	
0.525	0.254	0.017	
0.55	0.167	0.083	
0.7	0.13	0.033	
0.433	0.183	0.067	

420

421 3.4. CRISPR/Cas9 gene editing strategy for the TERT gene

422

423 CRISPR is a gene editing technique originally discovered in bacterial immune systems
 424 as an antiviral defense system that excises foreign DNA. It involves an endonuclease
 425 (Cas9) as well as a guide RNA, which attaches to a targeted DNA sequence and cuts at
 426 a recognized PAM (NGG) sequence. This cut is typically blunt, though Cas9 (typically
 427 derived from *Streptococcus Pyogenes*) may occasionally produce sticky ends 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 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. First, I looked at the chromosomal location of the TERT gene (Figure 8) to understand whether the gene sequence overlaps with any other nearby genes. As shown in Figure 8, TERT's locus doesn't overlap with any other nearby genes, making it less likely for CRISPR experiments to disrupt other functions.

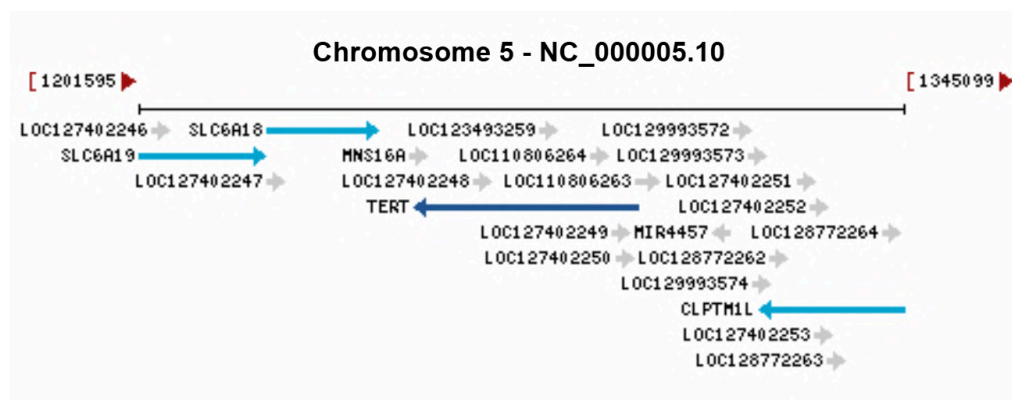


Figure 8. Chromosomal location of the TERT gene. Chromosomal location of the TERT gene. The figure shows a subsection of chromosome 5 from base pairs 1201595-1345099. A dark blue arrow indicates TERT's location and transcription direction on chromosome 5. Other genes in humans are marked in light blue. No other genes overlap with TERT.

Then, I gathered the TERT gene sequence from NCBI and annotated the exon and intron regions from Ensembl annotations. Via CRISPR Finder, I selected a guide RNA based on the off-target scores calculated for the allowed base mismatches of 0-4 bases.

I picked a guide (the upper image of Figure 9) that has no off-target binding site from 0-3 allowed mismatches and 16 off-target binding sites at 4 allowed mismatches - the lowest of any available gRNA. I used SnapGene to visualize all the sequences and label the required regions to assemble my strategy, as seen in Figure 9. I also included potential PCR primers to show how the CRISPR results would be verified experimentally.

985688607	5:1294901-1294923	CGCCGCACGAACGTGGCCAGCGG	0: 1 1: 0 2: 0 3: 0 4: 16
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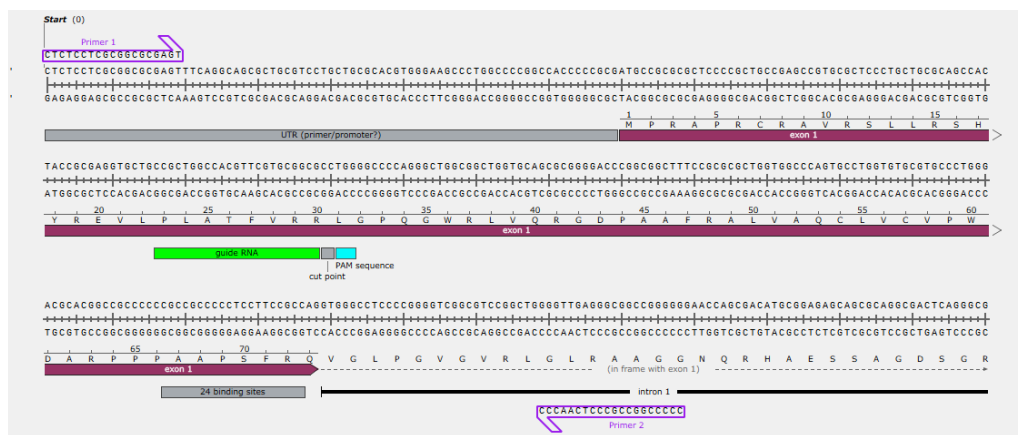
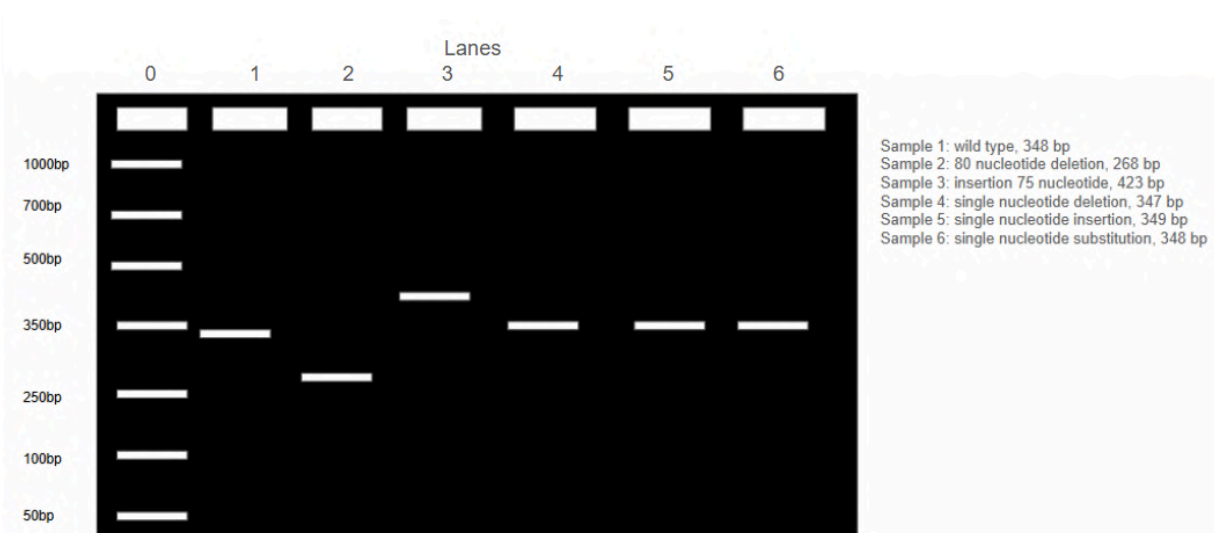


Figure 9. SnapGene view of CRISPR/Cas9 gene editing strategy for the TERT gene. The upper image shows the guide RNA selected from CRISPR Finder, as it had the best off-target score. The SnapGene view shows TERT exon 1 and intron 1 along with the defined guide RNA locations, PAM sequence, a potential cut point, and primers targeting an amplification region. Primers 1 and 2 amplify a fragment of length 309 bp. 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 in order to test for potential CRISPR editing outcomes via a hypothetical polymerase chain reaction (PCR) and simulated DNA gel electrophoresis.

473 PCR (polymerase chain reaction) is a technique to rapidly amplify a region of DNA
474 mimicking replication in vitro. In a hypothetical CRISPR experiment, PCR would be
475 used to visualize DNA on agarose gel.

476 Gel electrophoresis is a lab technique that uses electricity to separate DNA fragments in
477 a given sample by size. Here, I simulated a gel electrophoresis (Figure 10) for training
478 purposes in order to present the hypothetical outcomes of the gene editing.
479 Single-nucleotide changes cannot be resolved in a gel; therefore, they need to be
480 sequenced. All potential alterations must be confirmed with sequencing.

481



482]

483 **Figure 10. DNA gel electrophoresis showing possible outcomes of a hypothetical**
484 **CRISPR/Cas9 experiment on TERT.** Lane 0 contains a DNA fragment ladder with
485 known sizes, providing a reference for the fragment sizes in the other 6 lanes. Lane 1
486 contains wild-type TERT. Lanes 4, 5, and 6 all have similar fragment lengths as small
487 alterations cannot be resolved on a gel. TERT samples in lane 2 are significantly
488 shorter, indicating an ~80 base pair deletion, while samples in lane 3 are significantly
489 longer, indicating a ~75 base pair insertion.

490

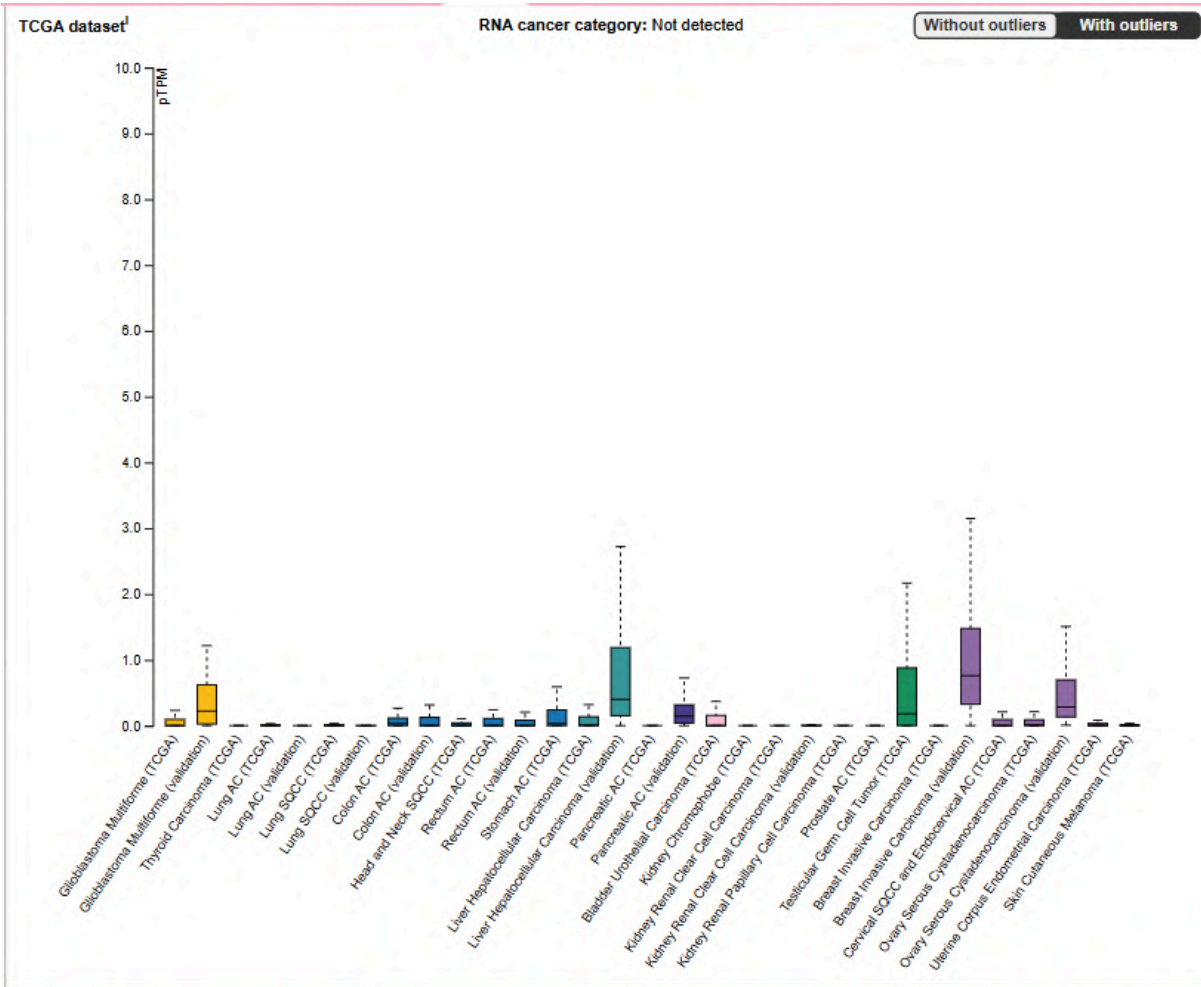
491 Below, TCGA expression data about TERT among different cancers shows that TERT
492 expression can be used as a marker to identify differences among cancer types
493 (Figure 11); however, all this data does not say whether adult vs pediatric cases
494 were compared or not. Therefore, all my investigation above will provide knowledge
495 about this gap in the future.

496

497

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499



500

501 **Figure 11.** TERT expression rates in different types of cancers in protein transcripts per million.
502 Breast invasive carcinoma has the highest median TERT expression rate (0.8), followed by
503 Liver Hepatocellular carcinoma (0.4), ovary serous cystadenocarcinoma (0.3), testicular germ
504 cell carcinoma (0.2), and glioblastoma multiforme (0.2). The graph is taken from the Human
505 Protein Atlas database.

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509

510 4. Discussion

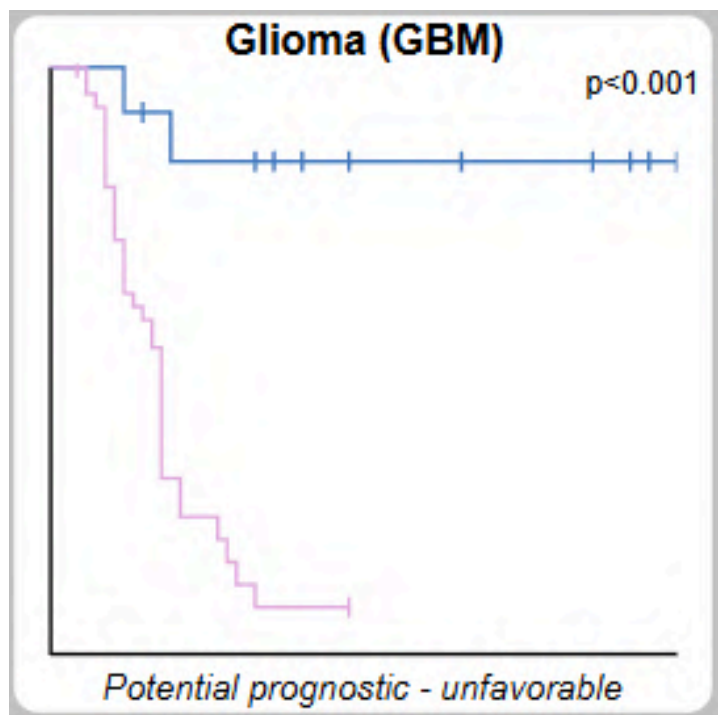
511

512 Telomere and telomerase-related oncology studies, while common, are rarely
513 specific to children. The few studies available had very low sample sizes - many cohorts

514 ranged from 10 to 20 children. Additionally, cancer is extremely heterogeneous; many
515 cancers have vastly differing telomerase expression rates. Therefore, I focused on
516 telomerase and TERT in pediatric cancers, trying to generate an adult vs pediatric
517 cancer comparison. I also wanted to establish a framework to study this question in the
518 future by analyzing a potential CRISPR/Cas9 strategy.

519

520 Telomerase activity was highly prevalent in pediatric and adult cancer; however,
521 one notable difference was a generally higher prevalence of the ALT pathway in
522 children. In Table 1, pediatric cancer telomerase expression rates ranged from 0% in
523 low-grade glioma to 86% in medulloblastoma, while ALT expression rates ranged from
524 0% in several cancers to nearly 80% in osteosarcoma. The results of the study show
525 that telomerase activity and TERT mutations have a significant correlation with cancer
526 severity and frequency (Albadrani and Zakariyah 2025), as well as lower average
527 patient survival time (Figure 12).



528

529 **Figure 12. Prognosis of Glioma.** Shows a Kaplan-Meier survivorship curve of
530 telomerase-negative glioma patients (blue) and telomerase-positive glioma patients (pink) over

time. Survival rates are significantly lower in patients expressing telomerase than in those who do not.

In cancers that express it, telomerase inhibitors have been shown to be effective. Imetelstat, the only FDA approved telomerase inhibitor, is only approved for adults. 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 lack of specificity; for example, because it reduces the number of times cells can safely divide, imetelstat is known to cause temporary female infertility (*Imetelstat (Intravenous Route) - Side Effects & Uses*, n.d.), among other symptoms. Interactions shown by the STRING protein-protein interaction map (Figure 4) may lead to alternative methods of telomerase inhibition that don't have those shortfalls, for example, by discovering telomerase-related enzymes specific to cancers. The STRING database may also provide further insight into telomerase and TERT-related pathways - proteins related to TERT can be affected by TERT mutations.

Meanwhile, CLUSTAL omega analysis, which shows that some regions of TERT are much more strongly conserved than others, can provide insight into telomerase functional domains to target. When certain sequences in a protein are similar across species, it may indicate that they're much more important to overall function than other sections - evolutionary variants that alter highly conserved domains sometimes die out. In TERT, targeting the more highly conserved RT and CTE domains with telomerase inhibitors could lead to stronger disruptions in telomerase function.

CRISPR knockout experiments appear feasible for investigating the relationship between telomerase and cancer. Chromosome location data from NCBI shows that no human genes overlap with TERT (Figure 8). This is important when performing

CRISPR, as it reduces the likelihood of TERT knockout experiments affecting other genes, thus reducing confounding. Meanwhile, Figure 9 shows examples of possible primers and PAM locations at annealing temperatures ~60 C. After attempting a CRISPR knockdown, one could verify their experiment worked by performing PCR to analyze the restriction fragments.

PCR could be used after CRISPR knockout to verify whether it has actually occurred. As shown in the hypothetical PCR result on a gel in Figure 10, DNA fragments of different lengths traverse different distances across the agarose gel. Thus, large indel mutations created by CRISPR can be compared to original DNA by analyzing how far fragments traveled compared to wild type TERT. If the CRISPR knockout does not occur, one can redo the experiment using different guide RNAs.

No significant result was found via TMHMM analysis. TERT was shown to likely have no transmembrane domains, indicating non-membranous localization.

Further studies are still needed. While I established a link between TERT and pediatric cancer, there is little data explicitly defined as nonpediatric for comparison. To establish the link between TERT and pediatric cancer specifically, studies with stratified pediatric and nonpediatric cohorts would benefit greatly. Such data from cancers where telomerase assays are easily available may provide valuable insights into how telomerase behaves in pediatric cancers.

5. Conclusion

While the overall relationship between telomerase, TERT, and cancer is well established, data on telomerase/TERT in pediatric cancer is extremely sparse. The data available on prognostic factors indicates that TERT and telomerase are strong indicators of cancer progression, but small sample sizes limit both the significance of these results and comparison to adult cohorts. Here, I established a framework related to the TERT subunit of telomerase in order to study this in pediatric cancers in the

future. As such, I could not determine whether TERT is more strongly involved in pediatric cancer than adult cancer. However, data in this paper indicate that this is a promising area of future study.

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The role of Telomerase and the Telomerase Reverse Transcriptase subunit in paediatric cancers

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Abstract (summary)

Background TERT (telomerase reverse transcriptase) is a subunit of telomerase, an enzyme that extends telomeres and delays replicative senescence. Mutations or upregulation of it commonly lead to cancer.

Objectives: Data on telomerase and TERT for pediatric cancer are relatively sparse. I aim to review 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.

Methods We took TERT's FASTA sequence and used the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database, CLUSTAL, TMHMM (Transmembrane Helix Hidden Markov Model), SnapGene, and NCBI (National Center for Biotechnology Information) to analyze proteins related to TERT, establish functional domains, and perform a hypothetical CRISPR experiment.

Results: Brief analysis shows that TERT promoter mutation rates are lower in pediatric cancer than adult cancer, but still raise the risk of cancer. No significant result was found with TMHMM. CLUSTAL indicates that TERT's RT (reverse transcriptase) and CTE domains (reverse transcriptase and C-terminal extension domains, respectively) are much more conserved than other parts, while STRING shows that many proteins are related to TERT. A hypothetical CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) experiment shows that TERT knockdowns are one way to further study the effects of TERT mutations on pediatric cancer.

Conclusion: TERT promoter mutations are generally rarer in pediatric cancer than adult cancer, while increased telomerase expression overall has a significant effect on pediatric cancer. In cancers where data were available, metastatic rates were significantly

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higher in patients exhibiting telomerase than in those who did not. While sample sizes were extremely small, relative risk statistics indicate that telomerase mutations are a promising area of investigation in pediatric cancer.

Keywords: Telomerase, paediatric cancer, cancer, TERT gene

1. Introduction

Cancer is a group of diseases ~~characterized by uncontrolled cell proliferation where cells rapidly multiply affecting and can affect~~ both children and adults ("What Is Cancer?" 2007). There are many types ~~(usually named after the body part they affect), from leukemia and brain cancer to prostate and breast cancer. The causes of cancers are equally varied, but all known cancers involve gene mutations.~~ Many genes can be mutated in cancers, and sometimes functional enhancements are observed, such as telomerase enzymes increasing their activity in cancer cells. However, only 5-10% of cancers are hereditary ("Frequently Asked Questions about Hereditary Cancers | Memorial Sloan Kettering Cancer Center," n.d.). Most gene mutations occur as a result of 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 turn genes oncogenic, or cancerous. Specific oncogenes can vary depending on the type of cancer. ~~Some common oncogenes include p53, a tumor suppressor as a controller of cell division and an apoptotic inducer in the case of DNA damage at the G1/S and G2/M cell cycle checkpoints (Chen 2016), as well as Ras, a protein family involved in cell differentiation and the MAPK/ERK pathway. ("Oncogene Ras — an Overview | ScienceDirect Topics," n.d.) (Guo et al. 2020). For example, an R337H mutation in tp53 has led to a 15-fold increase in pediatric adrenocortical carcinoma in Paraná, Brazil (da Silva et al., n.d.).~~

Cancerous Hallmarks and Telomerase Activity

While cancers vary broadly, they share key common traits - the Hallmarks of Cancer (Hanahan and Weinberg 2011). These hallmarks are sustained proliferative signaling, evasion of growth suppressors, metastasis, apoptotic resistance, angiogenesis, and replicative immortality. ~~Cells require signalling factors to duplicate their DNA and eventually proliferate; however, these signalling factors are usually controlled by negative feedback mechanisms. Cancer cells can inhibit these proliferative signals, produce their own growth factors, or inhibit negative feedback mechanisms. Metastasis, another criterion, is defined as the ability of cancer cells to spread to other organs. ("Definition of Metastasis - NCI Dictionary of Cancer Terms - NCI" 2011). Most cancers can metastasize, often as an escape mechanism from the immune system or lack of nutrients (WANG et al. 2015) to find better environments.~~

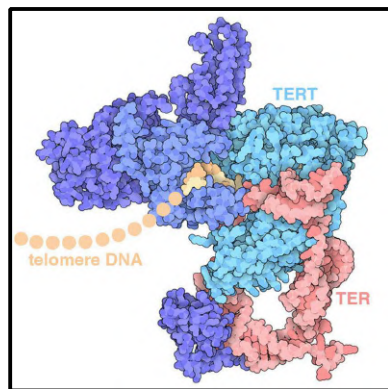


Figure 1: Molecular 3D structure of telomerase. The image shows the 3D structure of a TERT subunit of telomerase enzyme, as well as TER/TERC (Telomerase RNA Component), an RNA template for telomere synthesis. ~~((PDB101, n.d.))~~

One of the hallmarks is replicative immortality, which is ~~closely linked to~~ directly regulated by telomerase. (Figure 1).

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Telomerase is studied in relation to cancer ~~because it solves as~~ the end replication problem. Because DNA polymerase can only ~~synthesizetranscribe~~ from the 5' to 3' direction, the lagging strand of DNA needs to be ~~synthesizedtranscribed~~ in the opposite direction of the replication fork using primers. This leads to a major problem, since primers can not be placed past the ends of DNA, and polymerase can not transcribe the last few nucleotides. This problem is solved by telomeres – 5-15000 base pair-long noncoding repeats of the sequence 5'-TTAGGG-3'. 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 30-150bp (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). That's 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.

While telomerase upregulation (or alternative pathways, discussed later) is important throughout ~~cancer progression all parts of the cycle~~, it's especially important in metastasis. ~~Telomerase activity correlates with pediatric metastasis. For example, Experimental evidence shows that~~ when telomerase is deactivated, melanoma exhibits spontaneous regression rather than metastasis (Lopes-Bastos et al. 2024). ~~Telomerase activity correlates with pediatric metastasis.~~ In osteosarcoma, 75% of metastatic cancers exhibited telomerase, while only 11% of nonmetastatic cases showed telomerase activity, indicating a relative risk of 6.8. In 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 is limited for other pediatric cancer types, the strong association between telomerase activity and metastatic rate found in those two indicates that this is a promising area of future study (Table 2).

Table 1. Comparison of telomerase expression in different types of cancers. The two leftmost columns contain different types of cancers (with the right column being more specific), while the middle column contains telomerase expression in all patients, and the two right columns stratify telomerase expression rates by pediatric and adult. ~~Medulloblastoma is a cerebellar cancer, ependymoma originates from ependymal cells, osteosarcoma develops in bone tissue around the knee, and Ewing sarcoma is similar to osteosarcoma, but often responsive to radiotherapy.~~

Pediatric cancer type	Telomerase % in primary tumor	Telomerase % in metastatic
Medulloblastoma	unknown/limited information	<u>10.7%* (32.1% alt)</u>
Ependymoma	<u>76%</u>	
Osteosarcoma	11% (1/9) ("Table 1 Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue Origin," n.d.)	75% (<u>6/8</u>) ("Table 1 Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue Origin," n.d.)
Ewing sarcoma	12.5% (<u>1/8</u>) ("Table 1 Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue Origin," n.d.)	100% (<u>5/5</u>) ("Table 1 Telomerase Activity in Tissue Samples from Pediatric Bone Tumors in Relationship to Tissue Origin," n.d.)

Due to its relatively low abundance (Nguyen et al. 2019), telomerase has been difficult to structurally resolve. However, it is known that it's a reverse transcriptase holoenzyme responsible for adding telomeres to the ends of chromosomes (Figure 2). It consists of several subunits, including the two main ones being TERT (the catalytic subunit) and hTERC (an RNA template for the TTAGGG repeats) (figure 1) (Lee and Pellegrini 2025a). TERT consists of 16 exons (1134 amino acids), with 40766 amino acids interspersed as 15 introns

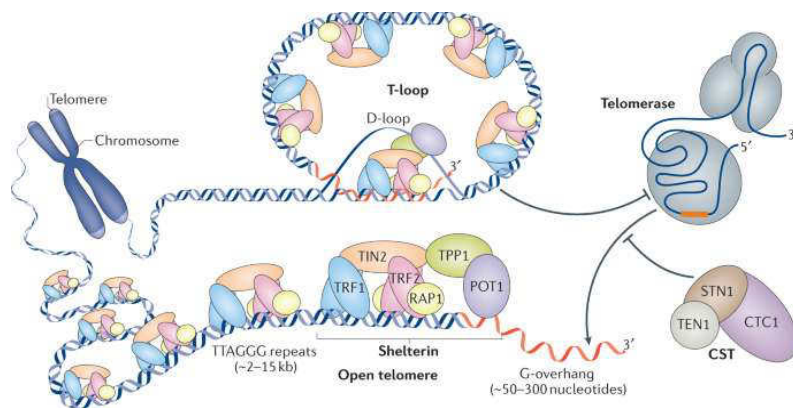


Figure 2. Telomere structure and synthesis. Telomeres are repetitive sequences at the end of chromosomes, synthesized by telomerase. After synthesis, the shelterin complex promotes the formation of D-loops and T-loops, protecting telomeres from degradative enzymes. The CST complex also contributes to telomere maintenance. Shelterin is a complex that plays several roles. After telomere synthesis, the shelterin complex wraps telomeres to protect them from enzymes involved in non-homologous end-joining (NHEJ) (Lange 2005a). Shelterin's main function is to form D and T loops from the G-rich overhang of telomeres, hiding their 3' end from exonucleases and other repair pathways. It is also a key regulator of telomerase; the POT1/TPP1 heterodimer promotes telomerase activity via a binding region called the TEL patch (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. 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's also a critical upregulator (Rice and Skordalakes 2016). (Lim & Cech, 2021)

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TERT is functionally expressed in 85-95% of cancer cells, while being absent in most differentiated somatic cells. While TERC is ubiquitously expressed in most cells, while TERT is mainly only expressed in germ cells; most telomerase upregulators hinge on TERT expression. Cancer often upregulates key telomerase promoters; for example, a study by Santos et al. found that in prostate cancer, upregulation of shelterin is shown to correlate with higher aggression and metastasis (Liu et al. 2019). Shelterin is an auxiliary

complex composed of 6 proteins (TRF1, TRF2, POT1, RAP1, TIN2, and TPP1) responsible for protecting telomeres from DNA repair pathways. In particular, catalytic activity in TPP1 and POT1 inhibits ATR kinase (a single-strand break repair enzyme), while the rest also serves to inhibit ATM/ATR signalling. Together, shelterin proteins 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). Therefore, in this study, I wanted to investigate the telomerase and TERT subunit activity in pediatric cancers.

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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 correlates with cancer malignancy; however, some tumors may utilize alternative lengthening of telomeres (ALT) pathways (Table 2). ALT utilizes proteins involved in homology-directed repair and DNA damage control to elongate telomeres, based on the existing repeats in chromosomes, which occurs independently of TERT upregulation. ~~Because it uses a wide variety of DNA repair proteins, there is not a single indicator of ALT expression. Typical~~ There is no universal indicator of ALT expression, but typical biomarkers include heterogeneous telomere length (with some less than 8kb and some greater than 50kb), and extrachromosomal telomeric repeats (e.g., C-rich circular DNA ends). Expression is particularly common in mesenchymal neoplasms, with the highest rates of ALT expression being in osteosarcoma (63%), leiomyosarcoma (60%), and undifferentiated pleomorphic sarcoma (59%). Children's cancer has a higher incidence of the ALT pathway (MacKenzie et al. 2021).

Table 2. Comparison of ALT pathway and telomerase activation rates in children's cancers. This table shows various pediatric cancers and their relative telomerase expression rates (middle column) and ALT expression rates (right column). It's important to note that each column uses different cohorts and thus percentages may not add to 100%.

Pediatric cancer type	Telomerase positive %	Alt positive %
ALL	54 (39/72)	0 (0/53)
AML	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)

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The next hallmark is apoptosis (programmed cell death) resistance. Cells have many genes, such as TP53 and Bcl2, that induce cell death when cells are damaged or have too many mutations. By doing this, cancerous cell behavior can be recognized and suppressed. Cancers have various methods to resist apoptosis, including tumor gene suppression, e.g., suppressing TP53 by overexpressing MDM2 or activating antiapoptotic regulators, such as Bcl2, in lymphoma. Angiogenesis (blood vessel growth) is another main hallmark induced during hypoxia. Studies by Hanahan and Folkman of transgenic mice with islet cell carcinoma, dermal fibrosarcoma, and epidermal squamous cell carcinoma all found extensive vascularization. According to them, angiogenesis occurs when cancer cells activate an “angiogenic switch,” a discrete step occurring when the presence of pro-angiogenic factors (e.g., vascular endothelial growth factor) outweighs the presence of anti-angiogenic factors (Thrombospondin-1) (Hanahan and Weinberg 2011).

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~~Tumor cells also need to avoid growth suppressors. The human body has several genes (e.g., TP53 and RB) that phosphorylate in the presence of oxidative stress and other damages, stalling the life cycles of cancer cells (Lakin and Jackson 1999), (Zatulovskiy et al. 2020). Normally, the body inhibits cell growth via contact inhibition, where mitotic activity slows if cells detect other cells via cadherins. For example, Merlin protein couples cell surface adhesion molecules and sequesters growth factors; because of this, it's known to inhibit neurofibromatosis II (Morrow et al. 2011). Cancerous cells must degrade these proteins to proliferate and form a tumor.~~

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Pediatric Cancers and Telomerase

Several aspects make pediatric cancer cases different from adult cancer cases.

~~Pediatric~~The pediatric cancers are known to grow faster, and one of the reasons for this could be that stem cells are more present and active in children than in adults. While breast, lung, and prostate cancers are the most common cancers in adults, dominant types in children are leukemia, lymphoma, brain and spinal cord cancers ("Six Ways Childhood Cancer Is Different to Adult Cancer | CCLG - The Children & Young People's Cancer Association" 2023). Pediatric neoplasms also grow faster and respond better to chemotherapy. Because of this, children <14 have a much higher 5-year survival rate (85%) ("How Is Childhood Cancer Treated? | American Cancer Society," n.d.) than the whole population (Cancer Facts & Figures 2025 1930). However, Cancer is also the leading cause of death in children after infancy, with approximately 1600 children dying of cancer every year in the US alone, according to cancer.gov ("Cancer in Children and Adolescents - NCI" 2024). 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). Cancers in adults can vary widely in TERT promoter mutation rates. Cancers with high promoter mutation rate (>=15%) in adults include melanoma, liposarcoma, hepatocellular carcinoma, urothelial carcinoma, tongue squamous carcinoma, and glioma subtypes (83% of glioblastoma). Those with Low promoter mutation rate (<15%) include Leukemias (common in children), various nervous cancers (e.g., gliomas), carcinomas, melanomas, and osteosarcomas (common in adults). While data on TERT promoter mutations are rare, limited studies find

that TERT promoter mutation rates in children are much lower (Ackermann and Fischer 2019).

As shown in Figure 11, Testicular germ cell cancer has one of the highest TERT expression rates in the data provided by the Human Protein Atlas. As such, I decided to investigate the relationship between cancer and germline mutations. Germline mutations are mutations that occur in sex cells, which could cause some or all cells in the body to inherit that mutation, depending on the developmental stage at which they occurred. Studies on pediatric soft-tissue sarcomas, like Ewing and rhabdomyosarcomas, show 17% germline mutation rates in children (Yndestad et al. 2024), while diffuse midline glioma patients exhibited 7.5% mutation rate (Mateos et al. 2025). Counterintuitively, there is no significant difference between germline mutation rates in pediatric vs adult cancer; while the estimated contribution of germline mutations to pediatric cancer is 8-12% ("Frontiers | Genetic Predisposition to Solid Pediatric Cancers," n.d.), this is very similar to the attributable risk for cancer overall, being 10% ("The Genetics of Cancer - NCI" 2015). 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).

According to Table 2, pediatric tumors are overwhelmingly telomerase positive, similar to adult tumors. However, some cancers (osteosarcomas, Wilms tumors, neuroblastomas) exhibit significant ALT pathway activation (telomerase negative) - the reasons behind 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 rates. It's worth noting that the ALT rate is extremely low in low-grade glioma, so the ALT pathway could be used as a prognostic marker.

From a treatment perspective, telomerase inhibitors may provide a way to combat childhood cancer. Cells in children also need to divide more times than in adults - thus, telomerase is known to be more active in children. This, combined with the relative lack of confounding external factors in children, may help establish a clearer statistical trend between telomerase activity and cancer. TERT serves as the catalytic core of the telomerase holoenzyme. It consists of 16 exons (1134 amino-acids), with 40766 amino acids interspersed as 15 introns. Multiple proteins induce TERT expression by attaching to the TEN domain and promoter; for example, cMyc binds to E-boxes at +44 when bound to Max protein. TERT can, however, be downregulated by Mad1 (a suppressor of Myc) and histone deacetylase in most somatic cells (Khattar and Tergaonkar 2017).

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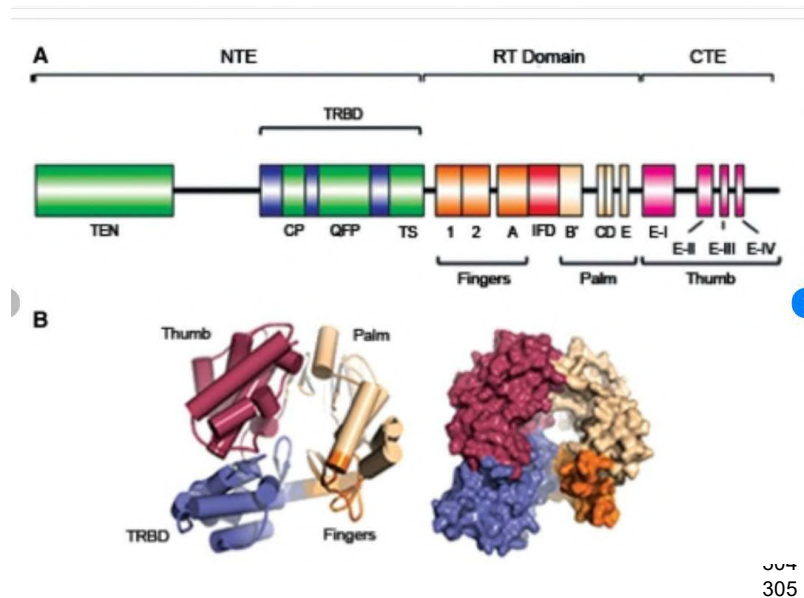


Figure 3: Protein subdomains of TERT. On the 5' end is TEN (TERT n-terminal domain), which interacts with ssDNA telomere repeats, followed by TRBD, which binds to TERT. RT, the reverse

transcriptase domain, and CTE, the C-terminal domain, catalyze the addition of nucleotides to telomeres. As shown in Figure 7, RT and CTE are the most conserved domains in TERT.

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~~To study telomerase and TERT in cancer mechanisms, gene editing techniques can be utilized to generate a mutation screen across pediatric cancers. CRISPR is a technique used in gene editing, originally discovered in bacterial immune systems. CRISPR involves an endonuclease (Cas9) as well as a guide RNA, which attaches to a targeted DNA sequence and makes a cut at a recognized Protospacer Adjacent Motif (PAM), usually a NGG sequence. This cut is typically blunt, though Cas9 (typically derived from Streptococcus Pyogenes) may occasionally produce sticky ends in vivo (Jasin 2018a). What happens next can vary. When attempting to change a nucleotide sequence, a Homology Directed Repair (HDR) mediating template can be used to insert a desired sequence via homology-directed repair; otherwise, the double-strand break is left to be repaired naturally via non-homologous end joining (NHEJ) or homologous end joining (HDR). As an outcome of these processes, a cell can end up having altered DNA sequences (Redman et al. 2016).~~

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Aim of the study:

Telomerase is a well-known ~~contributorearcinogenic factor for~~ 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 significantly compared to that in adult cancer. To do so, I will ~~gather literature knowledge on telomerase, one of the telomerase subunits, TERT, review literature on telomerase and the TERT subunit, including their~~ expression rates, protein structure, and conservation levels of different types of cancers affecting children vs adults. I will also propose a hypothetical CRISPR/Cas9 gene editing design to generate a framework for future experiments that can be conducted to address the question experimentally.

2. Materials & Methods

I used publicly available databases and online tools to conduct my research about Telomerase and TERT:

Gathering protein and gene information:

NCBI (National Center for Biotechnology Information) is a research database containing a lot of information, including DNA, RNA, and protein sequences (in FASTA format), which is used in this study, and it also holds as well as PubMed, which is a publication hub a literature database. I retrieved protein and gene sequences via NCBI>Protein and NCBI>Gene.

Investigating protein properties:

TMHMM is an algorithm that analyzes was used to analyze amino acid sequences to determine whether they contain transmembrane domains based on their amino acids' hydrophobicity. Since hydrophobic amino acids have to usually remain inside membrane layers, consecutive hydrophobic amino acids indicate a transmembrane/intramembrane domain. TMHMM uses the Hidden Markov model, explained in more detail below in the results. I retrieved amino acid sequences via NCBI and submitted them to TMHMM as a query.

CLUSTAL OMEGA is a software that compares protein sequences among different organisms to identify highly conserved amino acids/domains using the PAM 250 scoring matrix, which compares the likelihood of different amino acid substitutions. Additionally, it shows the electrochemical properties of the amino acid sequences (eg, hydrophobicity, charge) with color coding, providing insight into domain function.

CRISPR/Cas9 gene editing design:

I retrieved gene sequence via 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 region of the TERT gene by using this database. With SNAPGENE, C

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 ~20bp CRISPR ~~primers-guide RNA candidates~~ given a DNA sequence. I used CRISPR Finder to generate primers for a ~~possible- hypothetical~~ CRISPR/Cas9 knockdown/knockout.

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

3. Results

3.1 TERT protein does not contain any transmembrane/intramembrane domain

~~TERT's full sequence (in FASTA format) was taken from NCBI's protein database and then analyzed via TMHMM (Transmembrane Hidden Markov Model). TMHMM is a hidden Markov model, a model that relies on an unobservable initial state. Usual Hidden Markov Models do not understand initial conditions (like whether a given domain is "outside" or "inside" a domain), but track the changes between them (the transmembrane domains) and determine a path (Yoon 2009).~~ TMHMM makes its best guess as to which domains are inside or outside of a membrane layer by applying the positive-inside rule (Heijne 1986). ~~That determines the probability of any given section of a protein being transmembrane or intramembrane. Since the cell membrane (a phospholipid bilayer) is hydrophobic while the cytosol is hydrophilic, a part of a protein that has a certain length of hydrophobic amino acids in an order is very likely to be a transmembrane domain,~~

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406 ~~while hydrophilic regions are not.~~The presence of transmembrane domains ~~defines~~
407 narrows down the subcellular localisation of the protein, which could be linked to their
408 functions or the phenotype that is observed in the case of dysfunction. TERT lacks any
409 long hydrophobic amino acid chains; as a result, TMHMM analysis shows that it lacks
410 transmembrane domains (Figure 4). A protein result of “all in” doesn't narrow down a
411 specific region in the cell, but does indicate it has no membrane-bound position (“TMHMM
412 2.0 - DTU Health Tech - Bioinformatic Services,” n.d.). TERT's apparent lack of a
413 transmembrane domain is consistent with current consensus that TERT is a nuclear
414 protein.
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**RecName: Full=Telomerase reverse transcriptase; AltName: Full=HEST2;
AltName: Full=Telomerase catalytic subunit; AltName: Full=Telomerase-
associated protein 2; Short=TP2**

UniProtKB/Swiss-Prot: Q14746.1

[GenPept](#) [Identical Proteins](#) [Graphics](#)

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associated protein 2; Short=TP2

MPRAPRCRAVRSLRSHYREVLPATFVRRLLGPQGHRLVQRGDPAAFRALVAQCLVCPHDARPPPAAPS
FRQVSCLELVARVLQRLCERGAKNLAFGFALLDGARGGPEAFTTSVRSYLPNTVTDALRGSGAWGLL
LRRVGDDVLVHLARCALFVLVAPSCAYQVCGPPLYQLGAATQARPPPHASGPRRRLGCERAMNHSVREA
GVPLGLPAPGARRRRGSAASRLPLPKRPRRGAAPERTPVQGQSWAHPGTRGPSDRGFCVVSAPARPAE
EATSLLEGALSGTRHSHPSVGRQHAGPPSTSRPPRPWDTPCPPVYAETKHFLYSSGDKEQLRPSFLLSSL
RPSLTGARRLVETIFLGSRPWMPGTPRRLPRLPQRVWQMRPLFLELLGNHAQCPYGVLLKTHCPLRAAVT
PAAGVCAREKPGQSVAAPEEEDTPRRLVQLLRQHSSPWQVYGFVRACLRRLVPPGLWGSRHNERFRLRN
TKKFISLGKHAKLSLQELTWKMSVRDCAWLRRSPGVGCVPAAEHRLREEILAKFLHWLMSVYVVELLSRF
FYVTETTFQKNRFFRYKSVMSKLSQSIGIRQHLKRVQLRELSEAEVRQHREARPALTSRLRFIPKPDGL
RPIVNMDDYVVGARTFRREKRAERLTSRVKALFSVLNYERARRPGLLGASVGLDDITHRAWRTFVLRVRAQ
DPPPELYFVKVDVTGAYDTIPQDRLTEVIASIIKPQNTYCVRRYAVVQKAHGHVRKAFKSHVSTLDLQ
PYMRQFVAHLQETSPLRDAVIEQSSSLNEASSGLFDVFLRFWCHHAVRIRGKSYVQCQGITPQGSILSTL
LCSLCYGDMENKLFAGIRRDGLLLRLVDDFLLVTPHLTHAKTFLRTLVRGVPEYGCVVNLKRTVWVFPVE
DEALGGTAFVQMPAHGLFPWCGLLDTRTLEVQSDYSSYARTSIRASLTFRNGFKAGRNRRKLFGLRL
KCHSLFLDLQVNSLQTVCTNIYKILLQAYRFHACVLQLPFHQQVWKNPTFFLRVISTDASLCYSILKAK
NAGMSLGAKGAAGPLPSEAVQNLCHQAFLLKLTRHRVTYVPLLGSRLTAQTQLSRKLPGTTLTALEAAAN
PALPSDFKTIID

416

417

TMHMM result

```
# sp|O14746.1|TERT_HUMAN Length: 1132
# sp|O14746.1|TERT_HUMAN Number of predicted TMHs: 0
# sp|O14746.1|TERT_HUMAN Exp number of AAs in TMHs: 0.5821799999999996
# sp|O14746.1|TERT_HUMAN Exp number, first 60 AAs: 0.01515
# sp|O14746.1|TERT_HUMAN Total prob of N-in: 0.02583
sp|O14746.1|TERT_HUMAN TMHMM12.0 outside 1 1132
```

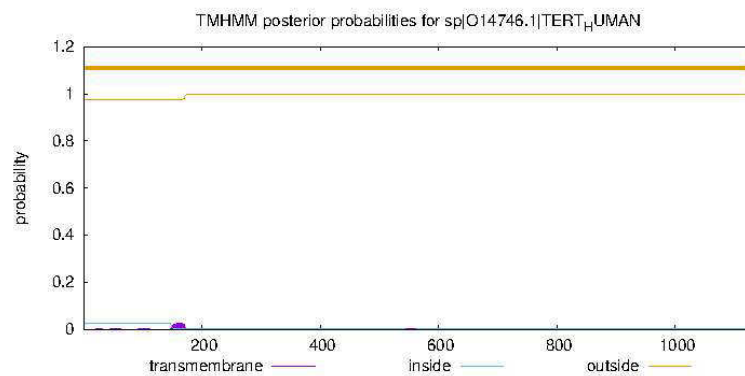


Figure 4. Transmembrane domain analysis of TERT protein via TMHMM algorithm.

The upper text shows the FASTA-formatted protein amino acid sequence of TERT taken from NCBI>Protein>FASTA. The bottom graph is the result of TMHMM analysis for the TERT protein. The graph shows that TERT likely lies entirely outside any cell membranes (i.e., it lies in the cytosol). The x-axis shows the amino acid sequence, while the y-axis indicates the probability calculation. A region being purple means it's likely to be transmembrane, yellow means it lies outside a cell membrane, and blue means it lies on the inner side of the membrane layer — this figure shows that most of TERT overwhelmingly lies outside. “Outside,” in the context of TMHMM analysis, refers to the non-cytosolic side of a lipid bilayer; this fits current consensus that TERT (and telomerase in general) is primarily a nuclear protein.

433

434

435 3.2.TERT has 8 experimentally determined interactor proteins

436 Protein-protein interaction is an essential phenomenon in the ~~human-genome~~ cellular
437 function. By looking at the interactions between proteins, it's possible to infer the function
438 of one protein based on the others it interacts with. STRING is a protein database that
439 predicts protein-protein interactions. It does so via various methods, including text mining,
440 consulting known protein databases, and computing interactions based on co-expression
441 frequency. In this study, interactions found by STRING can serve as a starting point to
442 find key upregulators and downregulators of telomerase and TERT, which can be further
443 analyzed for cancer mechanisms. The proteins shown below are involved with the TERT
444 subunit of telomerase (Figure 5) in various ways. NHP2 binds to the H/ACA domain of
445 telomeric RNA (Pogac   et al. 2001) and is involved in telomere maintenance, while
446 WRAP53 binds to Cajal bodies and TERC, responsible for stimulating TERT catalytic
447 activity. SMARCA4 is involved in chromatin remodeling, RUVBL2 possesses single-
448 stranded DNA-stimulated ATPase and ATP- dependent DNA helicase (5' to 3') activity,
449 TEP1 is another protein component of telomerase, DKC1 is a Catalytic subunit of H/ACA
450 small nucleolar ribonucleoprotein (H/ACA snoRNP) complex, which catalyzes
451 pseudouridylation of rRNA, and CTNNB1 is involved in the WNT signalling pathway.

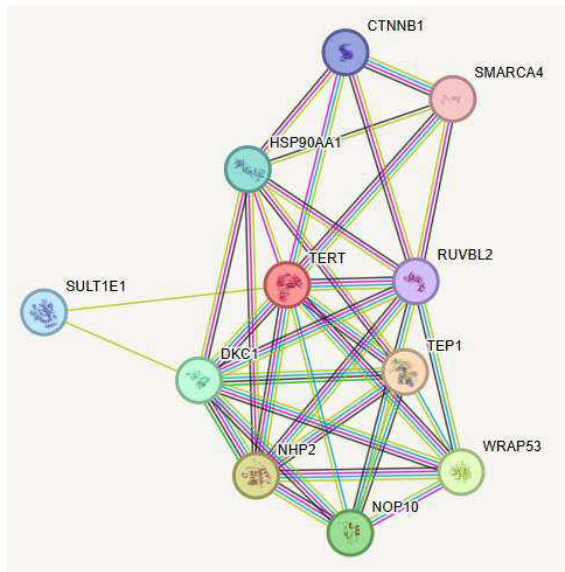


Figure 5. Protein-protein interaction map for the TERT protein. The image shows the predicted interactors of TERT as an outcome of STRING protein-protein interaction analysis. Lines (edges) show interaction depth between various proteins, each indicated by a node on the graph. Pink and blue lines represent the strongest relationships (experimentally determined and database-sourced, respectively), dark green lines represent relationships determined by gene proximity, and black and green lines indicate weak relationships (sourced from textmining and evidence of co-expression, respectively). TERT has 8 experimentally determined interactors. NHP2, WRAP53, TEP1, RUVBL2, SMARCA4, and CTNNB1.

3.3. TERT has a highly conserved end domain

CLUSTAL Omega analysis compares the amino acid sequences of one protein among different animals and indicates which parts are similar between them using the PAM 250 scoring matrix. If a region is highly similar between animals, this indicates that it's preserved throughout speciesgenomes during evolution and could be an essential region

470 ~~amino acid~~ for the function of the protein. When CLUSTAL analysis of TERT was done, I
471 found that TERT's RT and CTE domains (Figure 3) were most strongly conserved (Figure
472 7). ~~back end was most strongly conserved (Figure 7); this corresponds with the RT and~~
473 ~~CTE domains of TERT (Figure 3) in humans, which add nucleotides to telomeres. As can~~
474 ~~be seen by the graph, the back domain is much more highly conserved than the front.~~
475 Additionally, there's a high concentration of red amino acid residues across all animal
476 TERTs analyzed, meaning that TERT is strongly positive. I also generated a heatmap
477 showing the conservation rates quantitatively (Figure 7). The most highly conserved area
478 was amino acids 1140-1200, with 83% (50) amino acids being either highly or fully
479 conserved. This highly conserved region roughly corresponds with the ~~RT (reverse~~
480 ~~transcriptase) and CTE (c-terminal extension) domains, which catalyze telomere~~
481 ~~synthesis~~. While the first 50% of bases are significantly less conserved, the first 120 bases
482 (the top two rows of the table) show relatively high conservation. As it corresponds to the
483 N-terminal domain, it could be inferred that the N-terminal domain's function, while not as
484 conserved as telomerase's catalytic domains (RT and CTE), is still very important to
485 telomerase function. Other regions show very low conservation among organisms.

```
chicken_NP_001026178.2      MERGAQPGVGVRRLNVAREEPFAAVLGALRGYAEATPLEAFVRLQEGGTGEVVLRG 60
mouse_sp|070372.1|TERT_MOUSE  -----
human_sp|014746.1|TERT_HUMAN  -----MPRAPRCRAVRSLLRSHYREVLPLATFVRLGPQGW---RLVQR 41
chimpanzee_PNI27662.1        -----MPRAPRCRAVRSLLRSHYREVLPLATFVRLGPQGW---RLVQR 41

chicken_NP_001026178.2      DDAQCYRTFVSQCVVCPVPGARAIPRPICFQQLSSQSEVITRIVQRLCEKKKNILAYGY 120
mouse_sp|070372.1|TERT_MOUSE  -----FHQVSSLKELVARVVQRLCERNERNVLAFGF 31
human_sp|014746.1|TERT_HUMAN  GDPAAFRALVAQCLVCPWDARPPPAAPSFQVSCLELVARVLQRLCERGAKNVLAFGF 101
chimpanzee_PNI27662.1        GDPAAFRALVAQCLVCPWDARPPPAAPSFQVSCLELVARVLQRLCERGAKNVLAFGF 101
                               *::*. .:::*****: :*::*:

chicken_NP_001026178.2      SLLDENSCHFVLPSSCIYSYLSNTVTETIRISGLWEILLSRIGDDVMYLLHCAFLML 180
mouse_sp|070372.1|TERT_MOUSE  ELLNEARGGPPMAFTSSVRSYLPNTVITELRVSGAWMLLSRVGDDLLVYLLAHCALYLL 91
human_sp|014746.1|TERT_HUMAN  ALLDGARGGPEAFTTSVRSYLPNTVTDALRGSGAWGLLLRRVGDDVLVHLLARCALFVL 161
chimpanzee_PNI27662.1        ALLDGARGGPEAFTTSVRSYLPNTVTDALRGSGAWGLLLRRVGDDVLVHLLARCALFVL 161
                               **:      ::: *** ** ::: ** * :** *:::***:*** :***::*

chicken_NP_001026178.2      VPPSNCYQVCGQPIYELISRNVG-PSPGFVRRRYSRFKHNSLLDYVRKRLVFRHLYLSKS 239
mouse_sp|070372.1|TERT_MOUSE  VPPSCAYQVCGSPLYQTCATTDIWPVSASVYRPTRPVGRN---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  RAWNHVSREA-----GVPL 214
chimpanzee_PNI27662.1        RAWNHVSREA-----GVPL 214
                               : . :.
                               *

                               **: . :* .. : . . . * :.

chicken_NP_001026178.2      AVGLIKEHYISERSNSEMSGPSVVRSHPGKRPVADKSSFPQGVQGNKRIKTGAEKRAES 419

chicken_NP_001026178.2      LTLQALPGAEAKVHKHTEHGKESTEGTAPNSFLAPPSVLACGPQPERGEQHPAEGSDPLL 658
mouse_sp|070372.1|TERT_MOUSE  -----TDALNTSPPHLM 369
human_sp|014746.1|TERT_HUMAN  -----VC-----AREKPQG---SV-----AAPEEEDTPRRLV 449
chimpanzee_PNI27662.1        -----VC-----AREKPQG---SV-----AAPEEEDTPRRLV 449
                               . : . *

chicken_NP_001026178.2      ELLRQHSSHWQVYGFVRECLERVIPAELWGSNNKCRFFKNVKAFISMGYAKLSLQQLM 718
mouse_sp|070372.1|TERT_MOUSE  DLLRLHSSPWQVYGFRLRACLCKVVSASLWGRHNERFFKNLKKFISLGKYGLSLQELM 429
human_sp|014746.1|TERT_HUMAN  QLLRQHSSPWQVYGFVRACLRRLVPPGLWGRHNERFLRNTKKFISLGKHAHLSLQELT 509
chimpanzee_PNI27662.1        QLLRQHSSPWQVYGFVRACLRRLVPPGLWGRHNERFLRNTKKFISLGKHAHLSLQELT 509
                               :*** ** *****:* ** ::: ***: ** *:::* * ***:***:*****:*

chicken_NP_001026178.2      WKMRVNDVWLRLAKGNHSPVAYEHYREEILAKFLYWLMDSVYIELLSFFYITETMFQ 778
mouse_sp|070372.1|TERT_MOUSE  WKMKVEDCHWLRSPPGKDRVPAAEHLRERILATFLWMLDITYVQLLSFFYITESTFQ 489
human_sp|014746.1|TERT_HUMAN  WKMSVRDCAWLRRSPGVGCVAAEHLREEILAKFLHMLMSVYVELLSFFYVTTETTFQ 569
chimpanzee_PNI27662.1        WKMSVRDCAWLRRSPGVGCVAAEHLREEILAKFLHMLMSVYVELLSFFYVTTETTFQ 569
                               *** *.** ** : *      *** ** **_.**_.**_. **!*::*****:** **

chicken_NP_001026178.2      KNMLFYRKFIVGKLQINIGIRDHFAKVHLRALSSSEMEVIRQKKYFPIASRLRFIPKMNG 838
mouse_sp|070372.1|TERT_MOUSE  KNRLFFYRKSVWSKLQSIGVRQHLEVRVRLRELSQEEVRHQDTWLAMPICRLRFIPKPNG 549
human_sp|014746.1|TERT_HUMAN  KNRLFFYRKSVWSKLQSIGIRQHLEVRVRLRELSQEEVRHQEARPALLTSRLRFIPKPDG 629
chimpanzee_PNI27662.1        KNRLFFYRKSVWSKLQSIGIRQHLEVRVRLRELSQEEVRHQEARPALLTSRLRFIPKPDG 629
                               ** ***** :*.**_.**:*::: :*** **_. :. :. :***** :*

chicken_NP_001026178.2      LRPVRLSRVVEGQKLSKESREKKIQRYNTQLKNLFSVLNYERTVNTSIIGSSVFGRDDI 898
mouse_sp|070372.1|TERT_MOUSE  LRPVNMDSYMSGTRALG---RRKQAHFTQRLKTLFSLMYERTKPHMLGSSVLGNNDI 606
human_sp|014746.1|TERT_HUMAN  LRPVNMDSYVVGARTFR---REKRAERLTSRVKALFSVLNYERARRPGLGASVLGLDDI 686
chimpanzee_PNI27662.1        LRPVNMDSYVVGARTFR---REKRAERLTSRVKALFSVLNYERARRPGLGASVLGLDDI 686
                               ****.:. : : : *.*: :. :. :***:*****: . :*:***:***
```

Figure 6. Multiple protein alignment of the TERT protein in multiple animals (chicken, mouse, human, chimpanzee). In the alignment, each letter corresponds to a different amino acid, while colors correspond to chemical properties - red indicates a positive charge, magenta indicates a negative charge, blue indicates hydrophobicity, green indicates polarity, and pink indicates cysteine. CLUSTAL also displays colors for glycines, prolines, and aromatic amino acids; however, TERT lacks these. Meanwhile, symbols at the bottom indicate similarity between amino acids in different animals. Asterisks (*) indicate that an amino acid is fully conserved among animals listed (i.e., all bases match at that position), colons (:) indicate that an amino acid is strongly conserved (with a PAM 250 score >0.5), and a period (.) indicates weak conservation (PAM 250 score between 0 and 0.5).

Figure 7. Heatmap distribution of conservation frequency in TERT FASTA sequences. This map shows the proportion of each 60-amino acid interval that was denoted as * (fully conserved), : (highly conserved), or- (moderately conserved). (weakly conserved).



3.4. CRISPR/Cas9 gene editing strategy for the TERT gene

CRISPR is a gene editing technique originally discovered in bacterial immune systems as an antiviral defense system that excises foreign DNA. It involves an endonuclease (Cas9) as well as a guide RNA, which attaches to a targeted DNA sequence and cuts at a recognized PAM (NGG) sequence. This cut is typically blunt, though Cas9 (typically

derived from *Streptococcus Pyogenes*) may occasionally produce sticky ends in vivo (Jasin 2018b). What happens next can vary. When attempting to change a nucleotide sequence, ~~a template can be used to force cells can use a template for HDR repair, or utilize NHEJ could be an option.~~ This way, scientists could end up having mutations varying from single-nucleotide changes to big deletions or insertions. In other words, CRISPR allows scientists to generate precise mutations in DNA. This has many uses; for example, one could create a double-strand break in a cell's DNA to generate mutations, to analyze its effects and thus infer the original function of a gene sequence, ~~or assist in HDR via Cas9 (the main exonuclease).~~

I decided to design a hypothetical CRISPR/Cas9 strategy for ~~the TERT subunit of the telomerase to model how one would be done in a lab see how one would be conducted in real life.~~ First, I looked at the chromosomal location of the TERT gene (Figure 8) to understand whether the gene sequence ~~overlaps is colliding~~ with any other nearby genes. ~~As shown in Figure 8, TERT's locus doesn't overlap with any other nearby genes, making it less likely for CRISPR experiments to disrupt other functions.~~

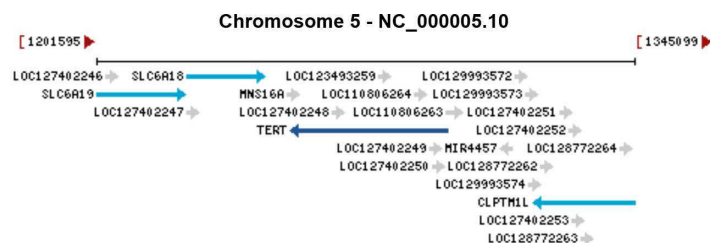


Figure 8. Chromosomal location of the TERT gene. The above figure shows a subsection of chromosome 5, starting from base pair 1201595 (left) and ending at 1345099 (right). A dark blue arrow indicates TERT's location on chromosome 5 and its transcription direction. Other genes in humans are marked in light blue. ~~No other genes overlap with TERT. It's important to note that none of them overlap with TERT. Thus, it's possible to CRISPR TERT without interfering with the function of other genes.~~

Then I gathered the TERT gene sequence from NCBI and annotated the exon and intron regions from Ensembl annotations. Then, via CRISPR Finder, I selected a guide RNA based on the off-target scores calculated for the allowed base mismatches of 0, 1, 2, 3, 4 0-4 bases. I picked a guide (the upper image of Figure 9) no off-target binding site from 0-3 allowed mismatches and 16 off-target binding sites at 4 allowed mismatches - the lowest of any available gRNA that has 0=1, 1=0, 2=0, 3, and 4, the lowest possible. I used Sanpgene to visualize all the sequences and label the required regions to assemble my strategy, as seen in Figure 9. I also included potential PCR primers to hypothetically present possible outcomes of this CRISPR/Cas9 strategy.

Commented [9]: edit for clarity?!

985688607	5:1294901-1294923	CGCCGCACGAACGTGGCCAGCGG	0: 1
			1: 0
			2: 0
			3: 0
			4: 16

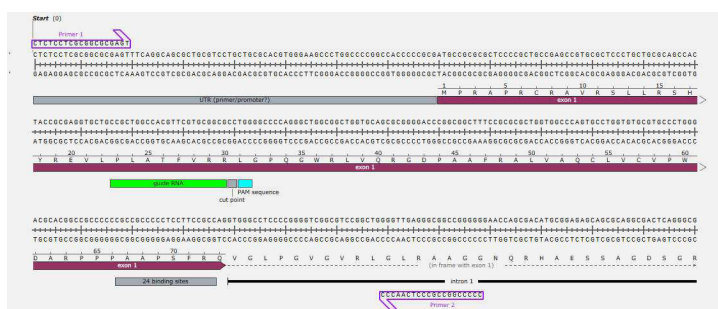


Figure 9. Snapgene view of CRISPR/Cas9 gene editing strategy for the TERT gene. The upper image shows the guide RNA selected from CRISPR Finder, as it had the best off-target score. The SnapGene view shows one part of the TERT gene (exon 1 and intron 1) along with the defined guide RNA locations, PAM sequence, a potential cut point, and primers targeting an amplification region. 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 covering the targeted CRISPR/Cas9 gene editing region in order to

test for the potential outcomes of the editing via a hypothetical polymerase chain reaction (PCR) and simulated DNA gel electrophoresis. Primer 1 and primer 2 amplify a fragment of length 309 bp.

PCR (polymerase chain reaction) is a technique to rapidly amplify a region of DNA mimicking the replication but in vitro. In a hypothetical CRISPR experiment, PCR would be used to visualize DNA on agarose gel. There are 3 steps: denaturation, annealing, and extension. In denaturation, the DNA strand is heated to ~95 C to split (denature) its hydrogen bonds, mimicking helicase activity to generate single-stranded DNA. Then, in annealing, DNA is cooled to a lower temperature (e.g., 70 C) to allow DNA primers to bind. A heat-resistant polymerase (for example, Taq) is then used to add nucleotides in the extension step, amplifying the DNA.

Gel electrophoresis is a lab technique that uses electricity to separate DNA fragments in a given sample by size. ~~Each gel tank has a connection to a positive and a negative electrode that can be applied to the system. Samples containing DNA are loaded from the area of the tank that is closer to the negative electrode. The agarose gel will have wells to load individual samples. Phosphate groups in DNA make the DNA molecule negatively charged. Once the electrical system starts running, the negative electrons will push the DNA molecule from the top of the gel towards the bottom of the gel. The smaller molecules move more quickly due to a lack of resistance in the gel, and the larger ones will stick to the top area.~~ Here, I simulated a gel electrophoresis (Figure 10) for training purposes in order to present the hypothetical outcomes of the gene editing. Single-nucleotide changes cannot be resolved in a gel; therefore, they need to be sequenced. All potential alterations must be confirmed with sequencing.

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<https://www.coolutils.com/TotalPDFConverter>



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Thank you for reviewing my paper! It really helped improve the clarity substantially. As far as I know, this is the list of your main critiques:

- 1) several citations appear to be incorrectly formatted or linked to the wrong sources — these need to be verified and corrected. I was able to catch the incorrect formatting errors (namely, some article citations use quotation marks instead of italicizations) but I failed to see which ones were linked to the wrong sources. If possible, could I please have a list of specific places where the sources are incorrect so I could double check the veracity or clarify why I cited those sources?
 - a) There is also a duplicate entry in the reference list (Jasin 2018a and 2018b appear to be the same paper). I ended up deleting the entire section that contained Jasin 2018b, so this should not be an issue anymore.
- 2) Acronyms such as TERT should be expanded at first use, including in the abstract. Most of the acronyms were expanded in the abstract; all of them were expanded upon first use except for when I listed off the shelterin proteins - most of them are commonly known by the abbreviated names any
- 3) The introduction, while thorough, is very long and spends considerable space explaining general cancer hallmarks (apoptosis resistance, angiogenesis, growth suppressor evasion) that, while relevant, take the focus away from telomerase and TERT for several pages. As seen in the track changes document, I deleted most of the elaborate explanations on hallmarks of cancer, except for the one on replicative immortality (which directly connects to telomerase). I hope it is more readable.
 - a) I would recommend condensing the hallmarks section and focusing more tightly on telomerase biology and its specific relevance to pediatric cancers.
- 4) The TMHMM analysis, while correctly performed, yielded a negative result that does not contribute much to the paper's central argument — the student acknowledges this, but it could be shortened. I deleted much of the section explaining how TMHMM works as it was less relevant to the paper. This already shortened the TMHMM section significantly, so I only made minor clarity/conciseness changes to the section on TMHMM interpretation. Please let me know if I can shorten it further.
- 5) I would have liked to see more engagement with clinical data, for example searches from clinicaltrials.gov to identify ongoing trials targeting telomerase in pediatric populations, or data on imetelstat trials in children specifically. There are very few studies on imetelstat in pediatric cancer; There were only 3 studies on clinicaltrials.gov (and few elsewhere), and of those 3, one was withdrawn before it started. However, I did add a brief 3-sentence discussion by the imetelstat section.
- 6) The student mentions imetelstat and its side effects but does not discuss whether any pediatric-specific trials exist or are planned.
- 7) Additionally, a graphical abstract summarizing the relationship between TERT, telomerase, pediatric cancer, and the proposed CRISPR framework would help ground the reader in the paper's core argument. I made a very simple graphical abstract using Biorender. The graphical abstracts I found online seemed to be relatively simple, so I also made a simple one in order to avoid obfuscating the overall message. Please feel free to tell me if I could make it look more visually appealing.
- 8) The grammar and language are generally clean and professional, with only minor issues such as “statisictial” instead of “statistical” and a few instances of informal phrasing.

Thank you for reviewing my paper! It really helped improve the clarity substantially. As far as I know, this is the list of your main critiques:

- 1) clearer explanations, better organization, and consistent formatting.
 - a) very long sentences, repeated ideas, small spelling/typing mistakes, and mixed terms or citation styles. The way they are written can sometimes make them harder to follow. Editing the language and tightening the structure will make the key ideas stand out much more clearly. **especially the abstract I don't really have a specific section that I made more or less changes to - see the "track changes" document where I recorded most of my changes in diction.**
 - b) Introduction should be broken down into smaller paragraphs with clear transitions **I broke the introduction down into smaller paragraphs but didn't add headings for each individual one. I already made it substantially shorter by trimming most of the cancer hallmarks section, so I believe it should be more readable.**
 - c) Title should be shortened (eg "The role of Telomerase (TERT) in Pediatric Cancers **see title**
 - d) A Few biology terms need more precise wording (eg apoptosis "programmed cell death")
- 2) **Citations:** The referencing style changes in different places. Choose one citation format and use it the same way throughout. Also make sure every figure/table clearly states its source.
- 3) **Clarify the paper type and central question:** At times the manuscript reads like a review, and at other times like a methods/results research report. Consider stating clearly in the Introduction/Abstract whether this is a *review with supporting bioinformatics analyses*. Then rewrite the aim as 1–2 precise questions (e.g., "What is known about TERT/telomerase alterations in paediatric cancers, and how do they compare with adults?"). **I clarified the paper type in the objectives section of the abstract. I believe my aim already sounds similar to the two example questions provided, so I did not change much there.**
- 4) **Keep each section tightly aligned to the aim:** Some background sections (general cancer causes, apoptosis, angiogenesis) become lengthy and drift away from telomerase/TERT. For a high school journal, it is better to shorten these parts and use the space to deepen telomerase-specific content (e.g., telomere biology, TERT regulation, paediatric tumour examples). **I deleted most of the cancer hallmarks section, except for a brief introduction to the framework and the**

explanation of replicative immortality (which directly ties into telomerase). I hope this made the paper more readable.

- 5) **Spelling style:** Pick either British or American spelling (e.g., “paediatric” vs “pediatric”) and keep it consistent across the whole paper. I switched everything to American spelling. Please let me know if I missed some words!
 - 6) **Shorter captions**
 - 7) Use cautious wording and mention that cancer is influenced by many factors, not just one. I did not emphasize the multifactorial nature of cancer much more than I originally did. While it is an important fact, I do mention it several times throughout the paper already (e.g. the second sentence of the introduction) and I don't believe I made strong claims that imply an exclusive or non-nuanced causal relationship between TERT and cancer. Please let me know if I made any claims that come off as too strong.
 - 8) **Methods and acronyms:** spell out acronyms the first time you use them (for example: TMHMM, HDR, NHEJ, PAM). I expanded most of the acronyms in the abstract .
 - 9) **CRISPR section:** Make it very clear it is a *theoretical plan* (a design), not something you actually carried out in a lab. I made sure to emphasize this in the methodology.
 - **Proofreading:** The paper needs a careful proofread to fix grammar, repeated words, spacing, and typos.
 - **Trim extra detail:** Some parts (like long explanations of PCR or gel electrophoresis) may be more detailed than needed for your main focus. Shortening them can improve flow. I cut out most of the detailed explanations of how the different bioinformatic tests work. Please tell me if I cut out too much.
- Thank you for your feedback.

Thank you for reviewing my paper! It really helped improve the clarity substantially. Many of the suggested edits were typographical corrections, so I may not cover all of them in great detail.

- 1) I expanded the acronym TERT in the title.
- 2) I expanded TERT again once in the abstract.
- 3) I checked the reference and I did indeed use that website to get the definition for the first sentence. As far as I know, the only thing wrong with it was the use of quotations rather than italicization for the article name, so I only changed that. Please let me know if there's more corrections I can do!
- 4) Same as the 3rd
- 5) For the incorrect terminology (apoptosis is not the cell cycle), I ended up deleting the entire section for clarity
- 6) Same as comment 5
- 7) Same as comment 5
- 8) Source was protein database. I put the citation in the figure legend..
- 9) I should have changed all of the spellings to American.
- 10) See #9.
- 11) I added the missing source for table 2 in the figure legend.
- 12) I used American spelling.
- 13) Like reference 3 and 4, I only changed the format of the citation rather than the source itself. Please let me know if there's anything I can clarify about that source in particular!
- 14) I removed the parentheses from Ewing and rhabdomyosarcoma
- 15) Capitalization done
- 16) I removed the parentheses in that specific instance, however, I feel like most of my use of brackets is hard to confuse for source references as usually I put long clauses in brackets.
- 17) See figure 3 legend for source
- 18) I expanded all acronyms in the abstract.
- 19) Deleted
- 20) I did not expand ENSEMBL as it is actually a portmanteau of "assembly" and "ensemble," not an acronym.
- 21) See Figure 5's legend for the source.

The revised manuscript is substantially improved, and the author has clearly engaged with the reviewers' comments in good faith. In particular, the introduction is more focused, much of the extraneous background material has been removed, the hypothetical nature of the CRISPR analysis is now clearer, and the manuscript more explicitly identifies itself as a review supported by bioinformatics analyses. The addition of pediatric clinical trial discussion is also helpful and much needed.

However, several minor issues remain before the paper is ready for acceptance. Most are relatively targeted, but a few are important because this is fundamentally a review paper, meaning that the accuracy and organization of the literature synthesis are central to the manuscript.

1. Clarify the paper's identity and organization as a review

The abstract now appropriately states that the author is conducting a review of the literature with supporting bioinformatics analyses. However, the manuscript still largely follows the structure of an original experimental paper, with Materials & Methods, Results, and then individual results subsections for TMHMM, STRING, CLUSTAL, and CRISPR, followed by Discussion.

I would recommend reorganizing the manuscript so that the literature review forms the main structure, with the computational analyses presented as supporting or exploratory analyses. For instance, sections could focus on telomerase biology, telomere-maintenance mechanisms in pediatric cancers, pediatric/adult differences, therapeutic implications, and then supporting bioinformatics analyses.

Relatedly, **the Methods section explains the computational tools in some detail, but does not explain how the literature review itself was conducted**. Even for a narrative review, the author should briefly indicate which databases were searched, the major search terms or themes, how pediatric studies were identified, and how studies were selected for discussion.

2. Conduct a complete citation and source audit

This remains the most important unresolved concern. The author's response indicates that citation formatting was corrected but that the author was unsure which references had been linked to incorrect sources. The revised manuscript still contains examples suggesting that the problem goes beyond formatting.

For instance, the text attributes a prostate-cancer shelterin finding to dos Santos et al., but cites Liu et al. (2019); the bibliography shows that the relevant prostate-cancer paper is dos Santos et al. (2024), whereas Liu et al. concerns TERC and inflammatory signaling. There are also citations appearing in the text that do not appear in the reference list and some malformed bibliography entries. Also, Figure 12 does not have a citation.

Because this is a review article, **I strongly recommend checking every substantive claim against the cited source**, rather than only standardizing citation formatting. You should do a full and careful audit of the sources.

3. Strengthen the pediatric-versus-adult synthesis

The central question of the paper is valuable, but the review could synthesize the available evidence more clearly. At present, the manuscript correctly concludes that the available literature is insufficient to determine whether TERT is more strongly involved in pediatric than adult cancer. Rather than treating this inability to reach a definitive answer as a weakness or limitation, the paper could make it one of its principal findings: The literature needs more studies on this. In particular, your review suggests that

- TERT promoter mutations are generally less common in pediatric cancers;
- telomerase activity nevertheless remains common;
- alternative lengthening of telomeres (ALT) may be particularly important in some pediatric tumors;
- pediatric sample sizes are often very small; and
- direct age-stratified pediatric/adult comparisons are uncommon.

Organizing the discussion around these points would make the review's contribution clearer and more coherent.

4. Use more cautious statistical and causal language

Some claims remain stronger than the evidence presented supports. For example, phrases such as TERT alterations “lead to” cancer, have a “significant effect,” or show a “significant correlation” should be reserved for cases where causality or statistical significance has actually been established. This is particularly important when discussing the metastatic osteosarcoma and Ewing sarcoma data, where the observed differences are large but the sample sizes are extremely small.

5. Further reduce or reposition analyses that contribute little to the review question

The TMHMM analysis has been shortened, which is an improvement, but it still occupies its own Results subsection despite producing a negative and relatively unsurprising result. For a review paper, this could be shortened further or folded into a brief supporting-analysis section.

The STRING, conservation, and hypothetical CRISPR analyses are more potentially relevant, but their relationship to the central questions on pediatric cancers should be made explicit. They should support the literature synthesis rather than appear to constitute the primary study.

6. Complete a final consistency and proofreading pass

The manuscript is cleaner than before, but several small issues remain. These include inconsistent use of “pediatric” and “paediatric” (you should use American English conventions), the typo “TERT” in the abstract, duplicated language in some figure captions, inconsistent acronym expansion, stray typographical characters, and some remaining awkward or informal wording.

Figure and table captions should also be checked systematically for concise wording, correct numbering, and complete source attribution.