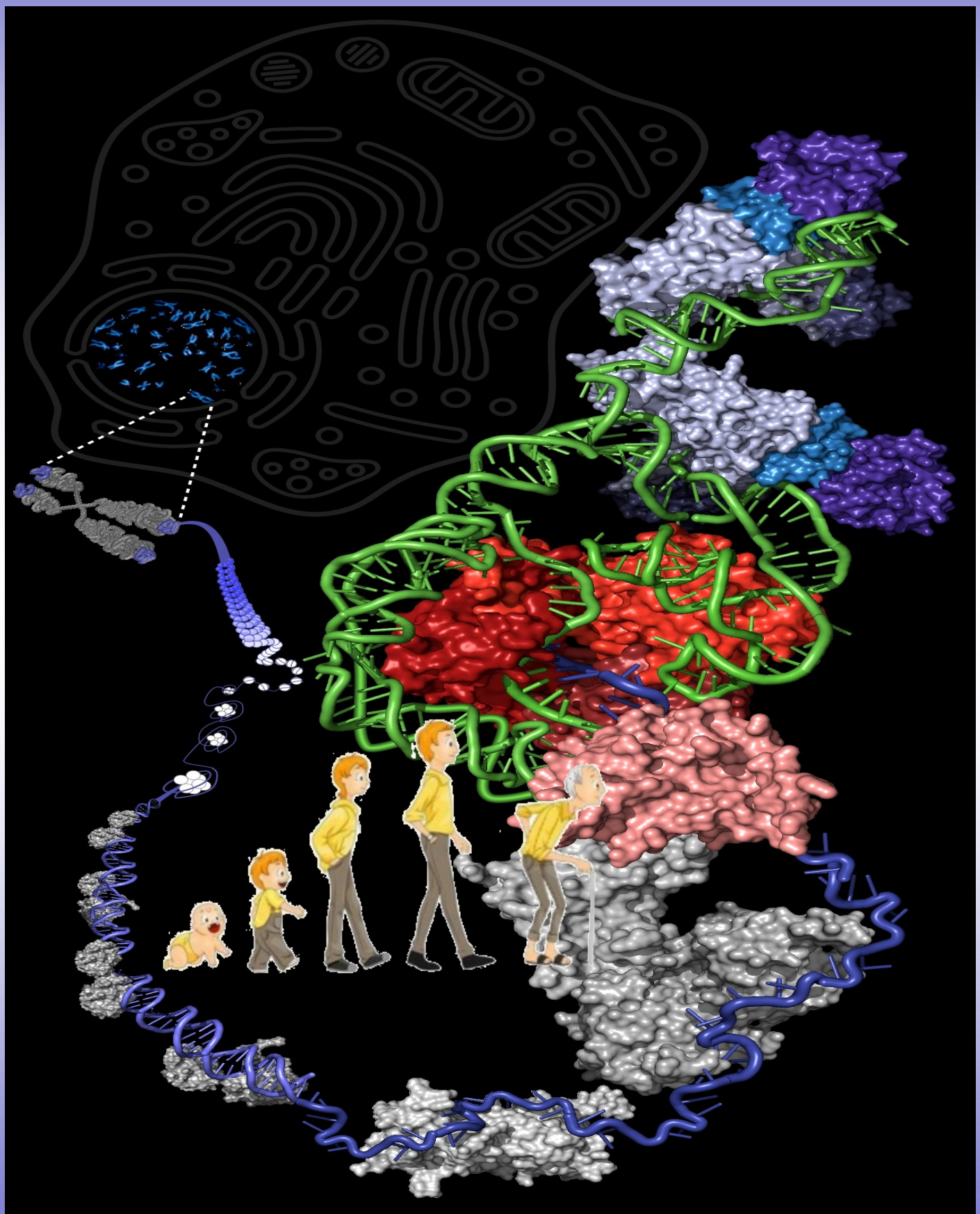
Kuwait Pharmacy Bulletin

DRUG INFORMATION FOR THE HEALTH PROFESSIONAL



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Telomeres and telomerase in cancer

This review discusses the basic structure of telomeres, the role of telomerase and their potential as targets for adjuvant chemotherapy.

Telomere structure

Telomeres are repetitive DNA sequences located at the ends of each chromosome. They consist of TTAGGG tandem repeats 5-20 kb, ending in a 300-500 nucleotide single stranded 3' overhang (Chai et al 2005). These guanine rich sequences have a role in stabilizing chromosomes, where two or three guanine tetrads stack on each other forming a g-quadruplex structure (Xu et al 2007).

This structure is stabilized by a monovalent cation, usually potassium with its high intracellular concentration, located at the center of the uni-molecular structure (Sen & Gilbert, 1988). G-quadruplexes may promote the formation of a telomere loop (T loop) at the 3' overhang, where the telomere curls back to form a long circle; at the end of this T loop the single stranded 3' overhang interacts with the double stranded telomeric DNA and displaces the hydrogen bonds to form a displacement D loop.

The telomere structure is stabilized and regulated by a protein complex called shelterin (or telosome), which is composed of six protein subunits (TRF1, TRF2, POT1, TPP1, TIN2 and Rap1) (Gomez et al 2012). Shelterin binds directly to the double stranded telomeric DNA through TRF1 and TRF2, whereas the POT1 connects to the single stranded telomeric DNA. TIN2 mediates TPP1 and POT1 binding to TRF1 and TRF2, bringing the complex together. Rap1, the sixth subunit, interacts only with TRF2 (Xin et al 2008).

General functions of telomeres and telosomes

During DNA replication, RNA primers used to initiate DNA synthesis are replaced with DNA base pairs, but the removal of the terminal RNA primer at the lagging strand results in a gap that cannot be filled, causing loss of the terminal sequences; this is referred to as the *'end replication problem*', which is resolved by the presence of telomeres, where they cap the end of the chromosomes to prevent the loss of DNA sequences.

In addition, telomeres prevent the linear chromosome ends from being recognized as DNA double-strand breaks (DSB), stopping the activation of DNA damage checkpoints and DSB repair pathways, thus protecting the chromosome ends from degradation and fusion (Aubert & Lansdorp, 2008). Though not all mechanisms regarding the suppression of DNA damage signaling and repair pathways are fully understood, one crucial mechanism involves the formation of t-loop structures, which are maintained by TRF2 subunit of the telosome. These structures make binding sites inaccessible for the DNA end-binding proteins, whose binding may initiate DNA damage signaling and repair pathways (c-NHEJ and ATM kinase pathway).

The suppression of ATR signaling by POT1 is thought to involve the protection of the single stranded DNA from the single stranded DNA binding protein, replication protein A (RPA), which functions as the sensor in the ATR pathway. The attachment of POT1 to the rest of the telosome complex by TPP1 is required for effective exclusion of RPA from telomeres (Xin et al., 2008).

Telomeres are transcribed to form a long non-coding RNAs called TERRA (telomeric repeat containing RNA). These molecules play critical roles in the regulation of telomerase activity and heterochromatin formation at chromosome ends. Pathologically, TERRA forms DNA-RNA hybrids at chromosome ends that can promote homologous recombination among telomeres, delaying cellular senescence and sustaining genome instability (Cusanelli & Chartrand, 2015).

Telomeres and cellular senescence

Each time the DNA replicates, a few nucleotides of the telomeres are inevitably lost to compensate for the end replication problem. With progressive loss of telomeres, shelterin subunits detach from telomere regions and this leads to destabilization of the t-loop structure. As a result, telomere ends get exposed and are recognised by the DNA repair signals as a DSB, promoting cellular senescence (Muraki et al 2012).

Cellular senescence is the progressive and irreversible loss of proliferative potential of the somatic cells. It does not only involve the inability to replicate but

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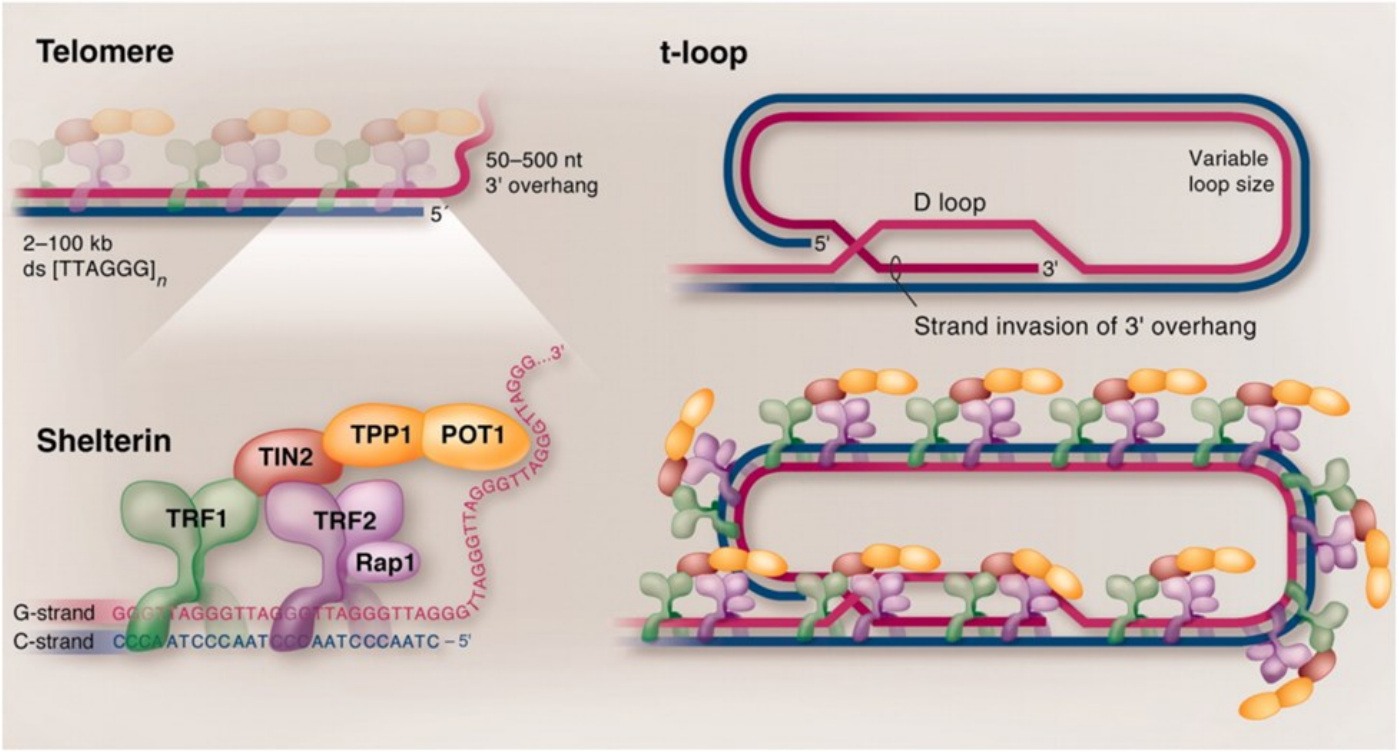


Fig 1. Telomere and shelterin structures (upper/lower left), and assembly and regulation of the T-loop (upper/lower right); de Lange (2009)

includes a number of major changes in cell morphology, gene expression, and metabolism. The number of times the DNA can replicate before exposing the telomeres and thus reaching cellular senescence or apoptosis is called the Hayflick limit, which is usually 40 -60 times in most human cells. This explains why telomeres work as a molecular clock that controls the replicative capacity of the cells and their entry into senescence (Victorelli & Passos, 2017).

Telomerase Structure

Telomerase, which is the main positive regulator of telomere length, is a ribonucleoprotein (RNP) that functions as a reverse transcriptase. It consists of two main components, telomere reverse transcriptase (hTERT) and telomere RNA component (hTERC or hTR) (Wai, 2004).

hTERT contains four major functional domains:

- TERT N-terminal domain (TEN), which interacts with the single-stranded telomeric DNA
- TERT RNA binding domain (TRBD), which binds multiple sites of hTR
- Reverse transcriptase domain (RT)
- C-terminal extension

The latter two bind the RNA/DNA hybrid and catalyse the addition of DNA base pairs to the 3' end (Zhang, et al 2011).

hTR structure varies widely among different species.

In humans, it contains three major structural and functional domains: the core domain and the conserved regions 4 and 5 (CR4/CR5 domain); these two are the catalytically active domains that bind independently to hTERT; the H/ACA small Cajal body-specific RNA (scaRNA) domain, which is located at the 3'end of the hTR and plays essential roles in the formation and regulation of telomerase holoenzyme, involving accumulation, 3'end processing, and localization of hTR. The H/ACA scaRNA domain also binds two sets of the four H/ACA RNP proteins (dyskerin, Gar1, Nop10, and Nhp2) (Cong et al 2002; Lewis & Wuttke, 2012).

Telomerase function and regulation

Telomerase catalyses addition of telomeric repeats onto the 3' overhang. This is a multistep process involving a series of molecular events including hTERT protein entering the nucleus, hTR and hTERT assembly with accessory proteins in the nucleus, recruitment to the telomeres during DNA replication, and involvement of hTERT in interactions with shelterin components to ensure correct positioning of telomerase at the 3' end of DNA for the appropriate synthesis of telomeric repeats (Dey & Chakrabarti, 2018).

In vitro, the minimal requirement for telomerase activity is the presence and activation of the hTR and hTERT; however, the presence of the aforementioned four H/ACA RNP proteins is essential for *in vivo* activity,

though this remains poorly understood (Masutomi et al., 2000). Other than maintaining the telomere length, telomerase is also involved in several functions including gene expression regulation, cell proliferation, apoptosis, some signaling pathways, MYC-driven oncogenesis, DNA damage response pathway, cell adhesion and migration, and epithelial–mesenchymal transition. All these activities mainly contribute to the process of oncogenesis (Jafri et al 2016).

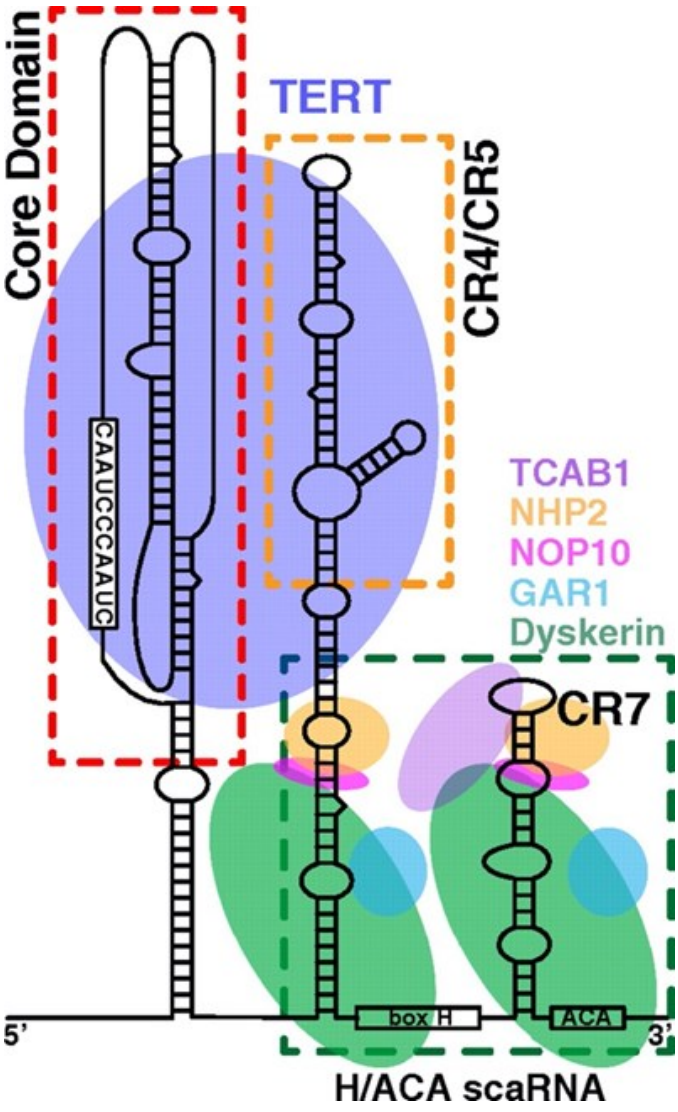


Fig 2. Telomerase structure. The three dotted boxes represent the three major domains of hTR, and the hTERT is shown in blue ellipse interacting with the two catalytic domains of hTR. The four H/ACA RNP proteins attaching to the H/ACA scaRNA domain are presented with different colors, corresponding to their molecules; Zhang et al (2011)

Telomerase activity regulation

In humans, a low level of telomerase activity has been noticed in mitotically active cells, including skin,

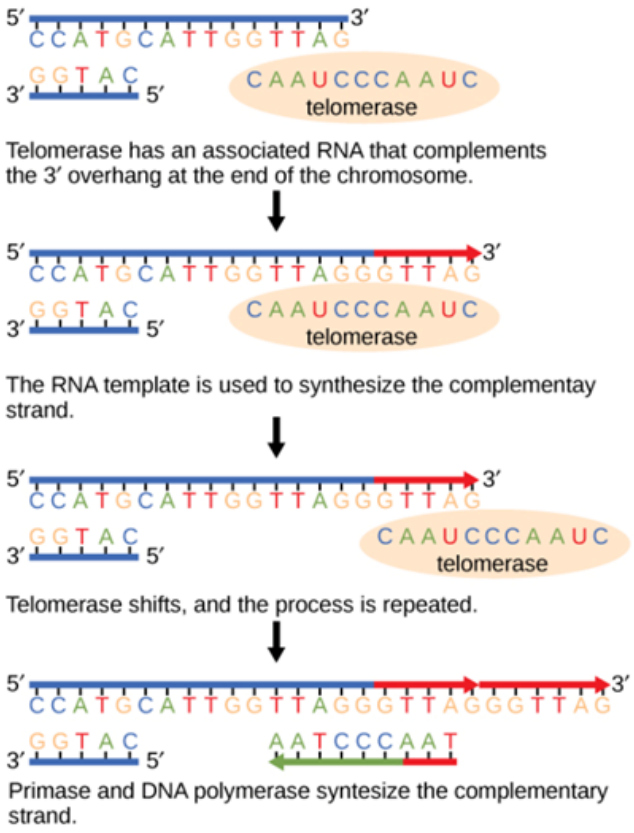


Fig 3. Reverse transcription of telomerase; Masutomi et al. (2000)

lymphocytes, and endometrium (Zvereva et al 2010). In these proliferating cells it is negatively regulated by growth. Stem cells and germ cells also express telomerase to maintain telomere length throughout their life cycle. In addition, about 85-90% of cancer cells have high levels of telomerase activity, even though they contain short telomeres (Schmidt & Cech, 2015).

Telomeres and telomerase in cancer

Cancer initiation pathway

Cellular senescence, or mortality stage 1 (M1), occurs physiologically when the telomeres shorten to a point where the ends become recognized by the DNA repair signals and checkpoints; however, in the presence of mutations in these checkpoints or the activation of telomerase, cells can bypass M1 pathway and continue to proliferate until they reach a crisis where the elomeres shorten significantly, causing chromosome end-to-end fusion. At this point, mortality stage 2 or M2 crisis pathway is activated and nearly all cells end in apoptosis. 1 in 100,000 to 1 in 10 million cells will escape M2 crisis pathway and become immortal by telomerase reactivation (85-90%) or following alternative lengthening of telomeres (ALT) pathways (10 -15%) (Shay & Wright, 2010).

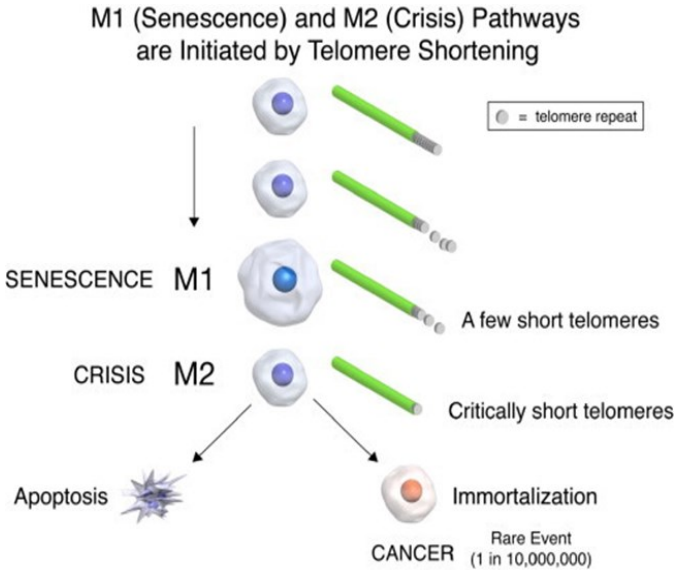


Fig 4. Cancer initiation pathway. Cancer cells acquire critically short telomeres resulting in genomic instability; Shay & Wright (2010

Telomere maintenance in cancer

Telomerase reactivation and ALT pathways are the two major controllers of telomere maintenance in cancer cells. Different genetic and epigenetic mechanisms are responsible for the reactivation of telomerase or provision of an alternative lengthening of telomeres:

Gene amplification of hTERT and hTR

In about 4% of observed cancer cases, DNA regions coding for hTERT and hTR were amplified (Barthel et al., 2017). This mechanism of maintaining telomeres is seen in several types of cancer; some are highly correlated with hTERT over-expression, such as lung adenocarcinoma and squamous cell carcinoma. Whereas, hTR up-regulation relates to lung squamous cell carcinoma. The amplification of both is seen in esophageal and ovarian cancers (Srinivas et al 2020).

Rearrangement of hTERT

hTERT may be upregulated in some types of tumors of the nervous system by genomic rearrangement, in which active enhancers are brought close to hTERT promoter region to increase hTERT expression (Leão et al 2018).

hTERT promoter mutations

This is a two-step mechanism that leads to tumorigenesis. The first step involves keeping cells proliferative through the stabilization of the extremely short telomeres. In the second step, genomic instability will result from these short telomeres, and telomerase will be upregulated in these cells (Chiba et al 2017). hTERT promoter mutations are observed in glioblastoma, melanoma, urothelial carcinoma, squamous cell carcinoma, medulloblastomas, and some aggressive subtypes of thyroid carcinoma (Srinivas et al 2020).

Epigenetic regulation of hTERT

Cancer cells show a disruption in the epigenetic DNA methylation process, in which the promoter regions of hTERT become hyper-methylated, resulting in the inability of the repressors to bind to these regions, allowing transcriptional activators to bind and upregulate hTERT. Histone acetylation is another epigenetic mechanism that affects hTERT transcription. Upon acetylation, histone becomes neutralized, causing a disruption in the electrostatic attraction with the DNA and upregulation of hTERT by allowing the transcriptional activators to bind to the promoter regions of the DNA. These two mechanisms are seen in pediatric brain tumors and prostate cancer (Sui et al 2013).

Alternative lengthening of telomeres

Cancer cells that follow this telomerase-independent mechanism for telomere maintenance show a heterogenous telomere length. In these cells, α-thalassemia/mental retardation syndrome X-linked protein (ATRX) and death domain-associated protein (DAXX) are inactivated, causing an up-regulation of TERRA, which promotes homologous recombination of the telomeres to sustain their length. This mechanism is observed in central and peripheral nervous system tumours and in sarcomas (Srinivas et al., 2020; Fig 5)

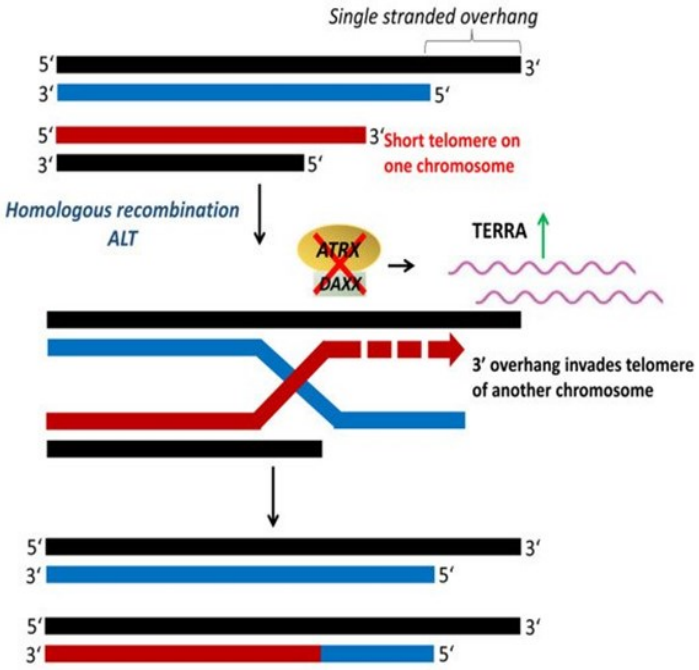


Fig 5. An overview of ALT pathway in maintaining telomere length in cancer; Srinivas et al (2020)

Clinical implications of telomere maintenance

Many epidemiological studies have shown an association between telomere length and cancer risk, where shorter

telomeres were related to head, neck, and renal cell cancers. Whereas long telomeres were associated with melanoma, basal cell carcinoma, glioma, lung cancers, and lymphoma (Srinivas et al., 2020). Since short telomeres lead to genomic instability and long ones predispose cells to mutations, the relationship between telomere length and cancer risk is described as paradoxical.

Diagnosis

Telomerase activity in tumors is determined using a telomere repeat amplification protocol (TRAP). Some tumor cells may contain TRAP assay inhibitors that may cause false-negative results, so immunoprecipitation with an antibody against hTERT is performed prior to the TRAP assay (Mender & Shay, 2015a).

Telomere restriction fragment (TRF) analysis is used to measure telomere length indirectly (Mender & Shay, 2015c).

Telomere dysfunction induced foci (TIF) analysis is a third method used to detect the damaged telomeric DNA. It involves using two antibodies, one against shelterin components that normally stabilise telomeres, and the other against DNA damage markers that recognize DSBs. TIF assay does not provide information about telomere length since it is based on localizsng the damaged telomeric DNA. This method is usually combined with TRAP or TRF assays to provide a cohesive analysis with regards to qualitative and quantitative measures (Mender & Shay, 2015b).

Prognosis

The main prognostic measures relating to telomere maintenance in cancer revolve around telomere length, telomerase activity, and the activation of ALT pathway.

i), ***Telomere length***: Bruneck cohort study that was done between 1995 and 2005 showed a correlation between shorter telomeres and worse prognostic outcomes Fig 6). This is explained by the genomic instability caused by critically short telomeres (Wenning et al 2005)

ii), ***Telomerase activity***: Studies associating telomerase activity to the overall clinical outcomes show conflicting results with regards to different cancer types. Though, colorectal cancer, pediatric high-grade gliomas, and non-small cell lung cancer were associated with consistent results that correlates higher telomerase activity with poor prognosis and overall survival (Reddel, 2014; Fernández-Marcelo et al., 2015).

Iii), ***ALT pathway:*** the presence of ALT was strongly linked to worse outcomes in liposarcomas and malignant fibrous histiocytomas while osteosarcomas and glioblastomas showed ALT activity correlated with better patient outcomes (Reddel, 2014).

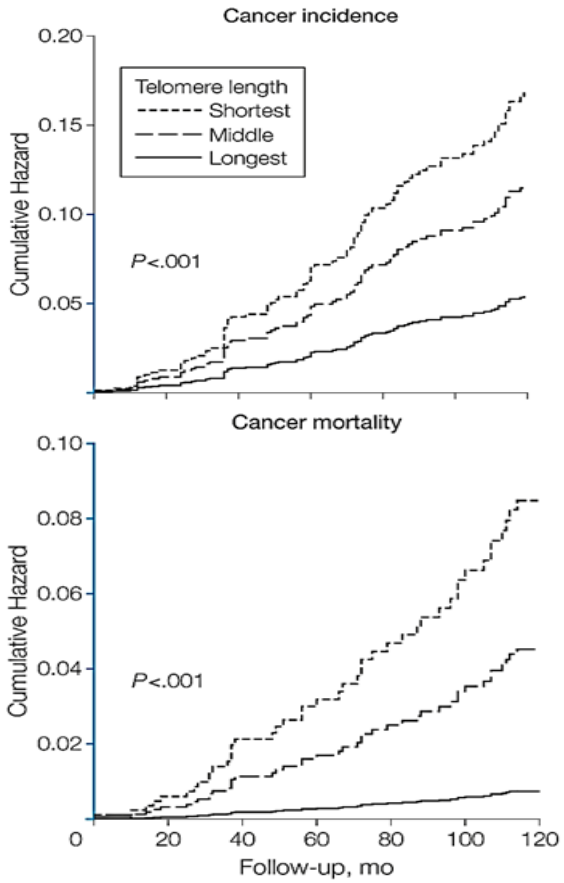


Fig 6. Bruneck cohort study (1995-2005). Shortest telomeres strongly correlated with higher cancer incidence & mortality; Wenning et al (2005).

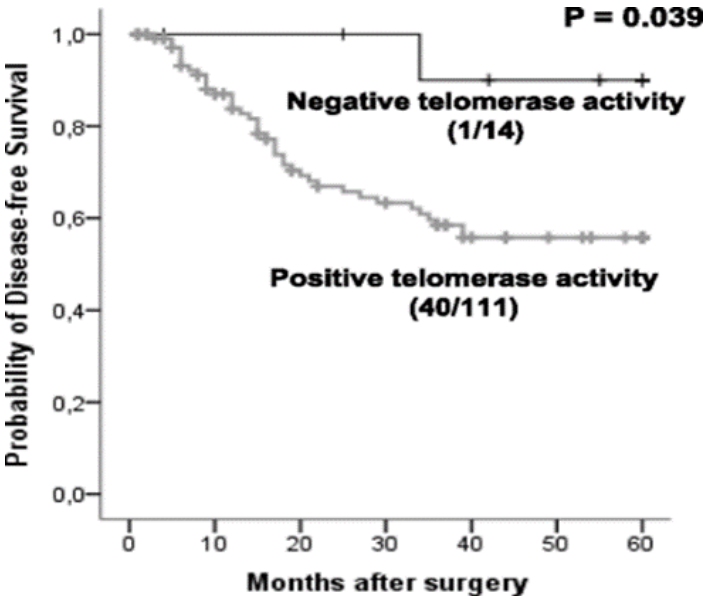


Fig 7. Kaplan-Meier disease-free survival curve in relation to telomerase activity in non-small cell lung cancer. The numbers between the parentheses represent cancer recurrence; Fernández-Marcelo et al., (2015).

Telomerase, ALT, and telomeres as targets for anti-cancer drug therapy

Targeting telomerase

As 85-90% of cancer casess are driven immortal by telomerase dependent mechanisms, telomerase is considered a good target for anticancer therapies. Telomere lengthening by telomerase mainly consists of binding of telomerase to its substrate, reverse transcribing the telomeric DNA, and continuing lengthening reaction of the extended substrate through translocation of telomerase.

Each of these steps can potentially be inhibited, where telomere lengthening can be prevented by either inhibiting hTR or hTERT component of the telomerase (Wai, 2004). In a detailed view of telomerase cycle in human cells, multiple steps may be potential targets:

a), hTERT gene is prone to alternative splicing, where the majority of the translated proteins from the spliced mRNAs are catalytically inactive; therefore, modifying the differential splicing of hTERT gene may be a useful clinical strategy.

b), Two proteins, NHP2 and NOP10, are involved in protein assembly. A mutation in one or both of these proteins is shown to shorten telomeres *in vivo,* suggesting a possible target for lowering telomerase.

c), TCAB1 and Cajal bodies participate in telomerase transport to telomeres. In humans, short telomere syndromes, which are clinically associated with higher incidences of systematic diseases, result from mutations in TCAB1. These alterations can be beneficial in decreasing telomerase activity in tumors and modulating other telomerase transport regulators may be seen as a potential therapeutic intervention.

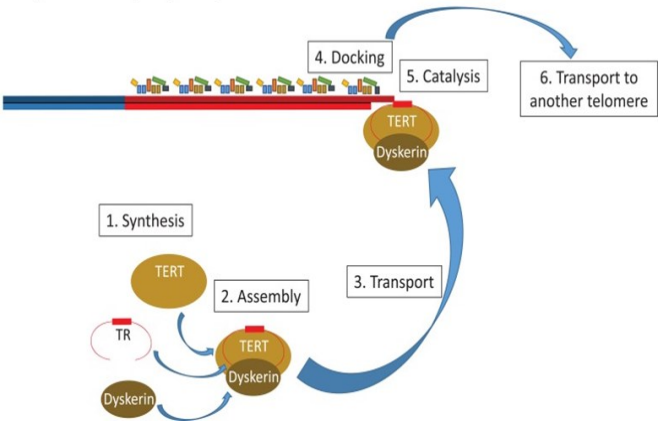


Fig 7. Telomerase cycle. Each step is a potential target for anti-cancer therapy; Reddel (2014).

d), Two main shelterin subunits are engaged in telomerase docking and attaching to the telomeric DNA, which are TPP1 that interacts with telomerase and TRF1 that prevents access of telomerase to telomeres. The inhibitory effect of TRF1 is silenced by tankyrase, which induces poly ADP-ribosylation of TRF1. Targeting tankyrase will allow TRF1 to play its inhibitory role and decrease telomerase activity.

e), Within a cell, telomerase numbers are less than telomeres, which implies that certain processes involved in undocking and transporting telomerase to another telomere can be studied as potential targets for lowering telomerase levels. (Reddel, 2014)

Main advantages of targeting telomerase

Telomerase is considered a good target for cancer herapy because most normal somatic cells have zero or very low-level telomerase activities. Therefore, selectively inactivating telomerase expression in cancer cells does not affect most healthy cells. Several therapeutic approaches for telomerase-based treatment of cancer have been developed or are under investigation Gomez, et al, 2016)

Main concerns about targeting telomerase in cancer therapy

Inhibiting telomerase to induce cellular senescence in cancer cells may affect the growth of telomerase-positive normal proliferating cells, such as skin cells, lymphocytes, endometrial and stem cells.

Cancer cells positively expressing hTERT can be eliminated by CD8+ cytotoxic T cells through the recognition of hTERT and major histocompatibility complex (MHC) class 1 on the cell surfaces. This immunotherapy is highly effective with rapid results; however, normal hTERT expressing cells would be affected.

Introducing a mutant hTR to the telomerase-dependent cancer cells will disrupt telomeres and induce apoptosis but telomerase-dependent normal cells would be affected as well (Wai, 2004).

Targeting ALT pathway

The ALT pathway involves homologous recombination and DNA synthesis which are vital in all normal cells, making this a highly toxic target; however, cancer cells that have lost p53 function are more prone to be inhibited than normal cells. Telomeres that are maintained by this pathway are shown to have variant sequences normally present at the centromeres, as well as replacement of some shelterin molecules by other binding

proteins such as nuclear receptors. This offers opportunities to improve ALT-specific therapies by targeting the abnormal telomeric sequences and binding proteins (Reddel, 2014)

Targeting telomeres

Stabilised g-quadruplexes inhibit telomerase function indirectly by blocking hTR from binding to telomeres. Intramolecular g-quadruplexes are formed spontaneously at telomere ends; beside promoting T-loop formation, these structures are found to inhibit telomeric replication by preventing telomerase and associated proteins from binding. Treatment with g-quadruplex stabilisers inhibit telomerase activity gradually, but this approach has shown moderate success in cancer patients so far.

Using guanine-rich oligonucleotides (GROs) homologous to the 3' telomere overhang sequence (T-oligos) is a new promising therapeutic approach in cancer. T-oligos is a specific 11-base oligonucleotide sequence (5'-dGTTAGGGTTAG-3') that is shown to induce DDR in cancer cells, resulting in cellular senescence and apoptosis with minimal to no effect on normal cells. The effect of this strategy is seen to mimic the physiological effects following shortening of telomeres in normal cells (Ivancich et al., 2017).

Strategies for anti-telomerase-based cancer therapies

Oligonucleotide inhibitors

Telomerase inhibition and telomere shortening induction were achieved by administering antisense oligonucleotides, with subsequent onset of cellular senescence/apoptosis in cancer cells. Targets include hTR, hTERT and other associated proteins. Imetelstat (GRN163L), a thiophosphoramidate oligonucleotide inhibitor, targets the RNA template by binding to hTERT, resulting in a duplex formation that prevents telomere lengthening by telomerase. And was successful for glioblastoma tumors. Combination therapies with well-established regimens for myeloproliferative neoplasms and acute myeloid leukemia are under investigation (Jäger & Walter, 2016).

Small-molecule telomerase inhibitors

BIBR1532 is a non-competitive inhibitor of TERT and hTR that reduces telomere length, inhibits cell proliferation, producing cell senescence as a final result *in vitro* (Gomez, 2016). BIBR1532 showed an increase in the inhibitory effects of telomerase in breast cancer when patients restricted their glucose intake, (Wardi et al., 2014). Moreover, The mTOR inhibitor rapamycin, was shown to inhibit telomerase activity in cancer (Jäger & Walter, 2016).

Immunotherapeutic approaches

Many studies have investigated hTERT immunotherapy in melanoma, acute myeloid leukemia, glioblastoma, prostate, renal, pancreatic, hepatocellular, and non-small-cell lung cancer. For example, hTERT-derived peptide was used in phase 1 clinical trial as a vaccine in hepatocellular carcinoma patients, with the majority of patients showing recurrence up to 24 weeks after vaccination (Jäger & Walter, 2016). Possible reasons for the limited success in many studies are the development of self-tolerance, the limited size of the precursor T-cell reservoir, negative effects of immunosuppressive tumor microenvironment on T cells, and other inter-individual differences. This therapy showed no significant decrease in immunoglobulins or increase in infections, but only showed a noticeable temporary autoimmune depletion of B cells as side effect.

Several points have been suggested for future studies to improve outcomes. These include:

i), Immunization with MHC class I and class II hTERT peptides to stimulate cooperation between CD8+ and CD4+ T cells, in order to increase and widen the persisting memory CD8+ T cells.

ii), Immunization with low affinity MHC I hTERT peptides mutants to limit development of immune tolerance, to increase efficacy of vaccination.

iii), Parallel immunisation with peptides from non-self-antigens to limit development of immune tolerance

iv), development of personalized strategies with a focus on patients with early stage diseases to prevent negative effects of immunosuppressive cancer microenvironments (Gomez et al., 2016).

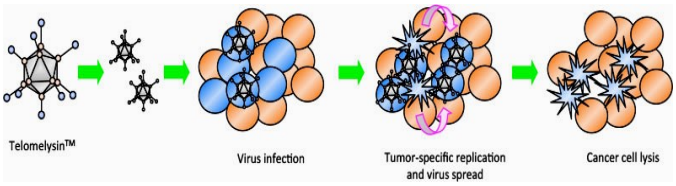
The basic mechanism of action of anti-telomerase immunotherapy is to sensitize immune cells to tumor cells expressing hTERT peptides as surface antigens via the human leukocyte antigen [HLA] class I pathway. Various hTERT peptides have been used to generate an anti-telomerase immune response, many of them showing promising preclinical and clinical results.

Telomerase-directed gene therapy

In cancer cells, the promoters for telomerase are targets for a tumor specific gene therapy that selectively kills cancer cells and leaves normal cells unharmed by expressing high concentrations of a therapeutic protein only in cancer cells. Both cytotoxic gene therapy and oncolytic virotherapy approaches have been used to selectively kill cells expressing telomerase.

Suicide gene therapy uses genetically modified viral vectors to encode a prodrug activating enzyme that will only replicate in hTERT-overexpressing cells, to produce cytotoxic metabolite (Gomez et al., 2016).

Telomelysin is an attenuated adenovirus-5 vector in which hTERT promoter element drives expression of E1A and B genes linked with and internal ribosome entry site. In this way, a virus-mediated lysis of cancer cells is induced after viral propagation in the hTERT-overexpressing cells. The drug is in the developmental stages to treat hepatocellular carcinoma and esophageal cancer (Jäger & Walter, 2016).



Phytochemicals

Allicin, an organophosphate derived from garlic; curcumin, a phenol present in turmeric; the flavonolignan silbinin; an organosulfur derived from cruciferous vegetables; epigallocatechin gallate (EGCG), a catechin in green tea have been investigated for inhibition of nuclear translocation of hTERT and decreased hTERT expression/activity (Jäger & Walter, 2016).

Other compounds that have been tested for telomerase inhibition include oleic acid, sesquiterpene lactone, helenalin and dictyodendrins (Jäger & Walter, 2016).

In conclusion, telomeres cap the two ends of each of the chromosomes to prevent the loss of DNA sequences and prevent chromosome ends from being recognized as DSBs. With progressive loss of telomeres, shelterin subunits detach from telomeric regions, and the t-loop is destabilized; telomere ends get exposed and become recognized by the DNA repair signals as a DSB, promoting cellular senescence. Telomerase catalyses addition of telomeric repeats onto the 3' overhang, but its activity is almost absent in normal somatic cells with a low level of activity in mitotically active cells, including skin, lymphocytes, and endometrium. When the telomeres become critically short and the cell manages to escape mortality stage 1 and mortality crisis, it becomes cancerous. Telomerase, ALT pathway, and telomeres are the main targets for anti-cancer therapies, with telomerase being widely studied. Different strategic anti-telomerase therapies are being developed or are under investigations for adjuvant telomerase targeted therapy.

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TEST YOUR KNOWLEDGE

Answers on back page

1) ***Which of the following is a standard method to determine telomerase activity in tumors?***

a) Microarray Immunoassay
b) ELISA
c) Four-point bioassay
d) TRAP

2) ***A thiophosphoramidate oligonucleotide inhibitor that targets the RNA template by binding to hTERT is***

a) Rosuvastatin
b) Botrostat
c) Imtelestat
d) Natalizumab

3) ***Which of the following is derived from garlic that inhibits telomerase activity?***

a) Curcumin
b) Allicin
c) Silbinin
d) Pinosylvin

Is there a problem?

A patient is given the prescription below to treat his acute back pain due to spasms. Is there any major error with the prescription?

BMX HOSPITAL

Patient Name: Ali Akbar Age: 38 years
Address: Street No: 453

Rx

Tolperisone tablet
150mg twice a day x 5 days
Send one packet

Dr. Suleiman
Signature Date: 15/3/21

Answer (Prescription Exercise)

The frequency is wrong. Usual oral doses of tolperisone are 50-150 mg three times daily.
(*Source: Micromedex, HSC Database*)

TOPICAL ISSUES AND CONTROVERSIES

Use of selenium to combat antibiotic resistance

Historically, many metals have been used over the years to treat infections; now there is resurgent interest in them for tackling the antibiotic resistance crisis.

Researchers at the University of Connecticut USA say they may have found a way forward, by looking back at how doctors treated infections before the advent of antibiotics.

Nosocomial infections contracted by patients during hospitalisation for other reasons are especially likely to be antibiotic resistant; these are often very hard to cure and can be fatal. Amongst the most common nosocomial bacteria is Acinetobacter baumannii.

This is primarily a nosocomial pathogen impacting especially those with compromised immune systems, the very young, the very old and burn victims. It is also reported in the wounds of combat soldiers. A. baumannii is adept at outsmarting antibiotics, with an array of mechanisms for evading successful treatment. Among these is its ability to form self-protective biofilms that facilitate travel to the lungs, sometimes causing pneumonia, and to the urinary tract. In biofilm form, it is also easier for the bacteria to spread to other patients.

After assessing a variety of metals and metalloids that doctors historically used to treat infections, the researchers settled on a metalloid, the essential mineral selenium (Se), as a promising candidate for treating A. baumannii. Antimicrobial selenium is a recognised dietary antioxidant, and the FDA recommend it for daily intake.

Other researchers have also found it to be a promising counter-agent to pathogens such as Staphylococcus aureus (S. aureus). Se is also an essential micronutrient

that helps the immune system function and aids nucleic acid synthesis.

Since A. baumannii is such an adaptable opponent, researchers adopted a strategy of disarming the bacteria rather than staging a full-on assault that would threaten its survival and provoke its defense mechanism. The researchers began by determining the minimum amount of Se required to inhibit the bacteria's virulence.

The researchers constructed a model matrix that simulated an infected wound environment containing cultured cells and wound fluids. They infected areas of their "wound" with A. baumannii and Se sufficient to inhibit virulence. They infected other samples with A. baumannii alone. The researchers examined the samples using scanning electron microscopy. They also performed DNA analysis to determine if the Se produced any genetic changes in the bacteria. In the Se treated samples, the biofilms produced by A. baumannii were severely degraded, diffuse, and structurally unsound. There is no clear information for how Se works but there seems to be toxicity against the outer membrane of the bacteria, and it might also cause damage the DNA, potentially affecting genes associated with biofilm creation.

Genetic analysis supported this suspicion, showing a reduction, or down-regulation, of genes responsible for biofilm production. Also, the bacteria treated with Se were no longer as good at sticking to and invading skin cells. The team has also looked at the use of Se for addressing other challenging infections, such as enterohemorrhagic Escherichia coli and Clostridium difficile. They advocate further exploration into the use of metals and metalloids as a way out of the antibiotic resistance dilemma, even as a stopgap, while researchers investigate and develop other treatments.

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Cancer drug shows promise in Parkinson's disease safety trial

According to the Parkinson's Foundation, about 60,000 people receive a diagnosis of Parkinson's per year in the United States, where nearly one million are living with the disease.

Parkinson's usually affects people after the age of 60y and is more common in males. It arises when cells in the corpus striatum region of the brain stop producing the inhibitory neurotransmitter dopamine, that helps to control movement. The disease gives rise to both motor, or movement-related, symptoms and also to non-motor symptoms, which include tremor, slowness, stiffness, and balance difficulties. Non-motor symptoms include depression, memory problems, emotional changes and constipation.

According to Parkinson's Foundation, around 60,000 people receive a diagnosis of Parkinson's per year in the United States, where nearly 1 million are living with the disease. Parkinson's usually affects people after the age of 60y and is more common in males. It arises when brain cells that produce dopamine, a neurotransmitter that helps to control movement, stop working and die. The disease gives rise to motor, or movement-related, symptoms and also non-motor symptoms. Motor symptoms include tremor, slowness, stiffness, and balance difficulties. Non-motor symptoms include depression, memory problems, emotional changes and constipation. Because Parkinson's is a lifelong, relentless, progressive disease, the symptoms gradually worsen over time. No two people will have exactly the same symptoms, and it is difficult to predict which symptoms will emerge and when and how rapidly they will progress in individuals. As the symptoms progress, they can interfere with daily living and the ability to lead an independent life. One of the biological hallmarks of Parkinson's disease is the accumulation of badly-folded alpha-synuclein protein in the affected areas of the brain. These clumps can be seen in post-mortem brain tissue.

Nilotinib, a drug that regulators have approved for the treatment of leukemia, has shown promise in a small clinical trial. The methods and findings were published in the JAMA Neurology journal. The primary goal of the trial was to assess the re-purposed drug's safety and tolerability and how it behaves in the body in people with moderately severe disease. A secondary goal was to investigate the impact of nilotinib on certain substances that could be useful biomarkers for tracking disease progress and the effectiveness of therapies.

These biomarkers include products of dopamine metabolism and levels of alpha-synuclein and tau - two proteins that build up in the brain in Parkinson's disease. The biomarkers can be measured by sampling cerebrospinal fluid through a lumbar puncture. The trial investi-

gators, from Georgetown University Medical Centre, also tracked changes in motor and - Parkinson's symptoms at various stages during the 15-month trial.

The team randomly assigned 75 participants of average age 68.4 y with moderately advanced Parkinson's disease to three groups. One received 150 mg of nilotinib per day, another received 300 mg per day and the third group received a placebo. These are lower doses than the twice-daily 300 mg dose that cancer patients receive. The participants took the daily oral dose of drug or placebo for 12 months. After this, they underwent a "washout" period with no nilotinib or placebo for 3 months. Neither the participants nor the administrators knew which individuals received the placebo and which received the active drug until the end of each participant's trial period. The purpose of this double-blinding is to prevent bias in reporting the results. The results showed that doses of 150 mg and 300 mg of nilotinib "were reasonably safe." However, people in the two nilotinib groups experienced more severe side effects than those in the placebo group.

Nilotinib blocks Abl tyrosine kinase, which is a protein essential for cell functioning. Because of this, the FDA require nilotinib to carry a black box warning about the risk of sudden death due to this effect. However, this warning relates to the higher doses in leukemia treatment and not to the lower doses that the investigators used in the Parkinson's disease trial. The results show that at these lower doses, nilotinib does not seem to cause Abl inhibition, suggesting it shouldn't have the same safety concerns that are potentially associated with Abl inhibition as might be the case at higher doses. When they examined the potential biomarkers, the team found that participants who took nilotinib had lower levels of alpha-synuclein and tau.

Individually, these are very important findings, but taken together, it means that the clearance of these neurotoxic proteins may not solely depend on Abl inhibition - other tyrosine kinases or alternate mechanisms may be involved. They also found higher levels of dopamine metabolites - typically more than 50% - in participants who took nilotinib. This would suggest that because the drug cleared away the toxic proteins, their brains were able to make better use of their own dopamine.

Other results suggest that nilotinib was able to slow the progression of - symptoms compared with the placebo. The symptoms became progressively worse over the study period in the placebo group. When they looked at effects on motor symptoms, the researchers found that all groups improved after 6 months of taking their doses. However, at the 12- and 15-month exam, those taking the 300 mg dose and placebo appeared to remain stable, whereas those on the 150 mg nilotinib dose improved over the 15 months. They saw overall improvements in motor symptoms in the participants who took nilotinib compared with the placebo group. The nilotinib groups also scored higher on quality of life measures during the trial.

However, researchers need to carry out more extensive studies in more diverse populations to confirm these results.

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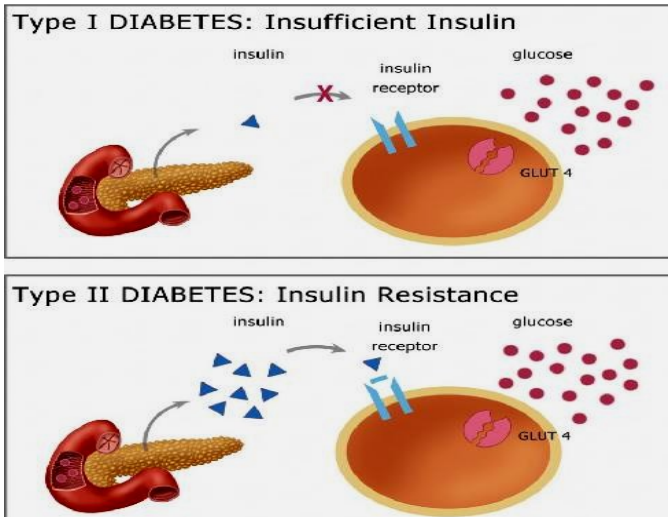
Cure of Type 1 diabetes in mice using stem cells

In type 1 diabetes, a faulty autoimmune response causes the immune system to attack and destroy insulin-producing beta cells within the pancreas. As many as 187,000 children and adolescents in the US were living with type 1 diabetes in 2018. An additional 1.4 million people aged over 20 years have the condition and manage it with insulin, according to the same statistics from the Centres for Disease Control and Prevention (CDC).

New research at Washington University School of Medicine in St. Louis uses an innovative technique to convert human stem cells into insulin-producing beta cells much more effectively. The insulin-producing cells



created rapidly cured type 1 diabetes in mice, and the benefits lasted for 9 months. Transplanting billions of such cells may soon cure type 1 diabetes.

Previous research has pointed to human pluripotent stem cells (hPSCs) as a potential therapeutic avenue for type 1 diabetes. Pluripotent stem cells are an attractive option for researchers from a therapeutic standpoint because they can self-renew in lab cultures and can differentiate into a variety of cell types. Researchers have previously used hPSCs to create insulin-producing beta cells. However, they were not able to do so effectively enough to cure type 1 diabetes.

A common problem when one tries to transform a human stem cell into an insulin-producing beta cell, or a neuron or a heart cell, is that other unwanted cells are also produced. For example, in the case of beta cells, we might get other types of pancreas cells or liver cells. While implanting these unnecessary or "off-target" cells does not cause any harm, creating more of them offsets the number of therapeutically useful cells.

A billion beta cells are needed to cure a person of diabetes. But if a quarter of the cells made are actually liver cells or other pancreas cells, instead of needing a billion cells, 1.25 billion cells are needed which makes curing the disease 25% more difficult. However, the new research used an innovative technique to bypass this problem.

The new stem cell technique targets the cytoskeleton, or inner "scaffolding", of the hPSC to direct their differentiation into pancreatic cells. The cytoskeleton is a structure that helps cells keep their shape and offers the mechanical support that allows cells to move, divide, and multiply. Targeting this structure allows the researchers to create fewer irrelevant cells and better functioning beta cells that helped control blood sugar.

The team transplanted the islet-sized aggregates of beta cells differentiated from hPSC into mice with type 1 diabetes. Pancreatic islets are groups of cells located in the pancreas. Some of these cells are insulin-producing beta cells. This transplantation procedure rapidly reversed severe pre-existing diabetes in mice. The reversal occurred at a rate similar to that of human islets, and normal blood sugar control was maintained for at least 9 months. These mice had very severe diabetes with blood sugar readings of more than 500 mg/dL of blood, levels that could be fatal for a person, and when they gave the mice the insulin-secreting cells, within 2 weeks their blood glucose levels had returned to normal and stayed that way for many months.

However, there are a few more steps to follow before this can help humans. First, researchers must test the cells in larger animals and then find a way to automate the new technique to produce the billions of cells required for the millions of people that have type 1 diabetes.

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How has clinical research fared in 2019?

Our diets influence our health

In 2019, the topic of how food choices influence our health has remained popular among researchers. One intriguing study, in Nature Metabolism in May, pointed out that protein shakes, which are popular among individuals who want to build muscle mass, may be a threat to health. Fitness protein powders, the study authors explain, contain mostly whey proteins, which have high levels of the essential amino acids leucine, valine, and isoleucine.

The research, in mice, suggested that a high intake of these amino acids led to overly low levels of serotonin in the brain, a key hormone that plays a central role in mood regulation, but which is also implicated in various metabolic processes. In mice, the heightened levels of leucine, valine, and isoleucine, which caused excessively low serotonin, led to obesity and a shorter life span.

So, if too much of certain types of protein can have such detrimental effects on health, what about fibre? Dietary fiber, present in fruit, vegetables, and legumes, is important in helping the body take up sugars little by little. But how much fiber should we consume? This is the question addressed in a study commissioned by the WHO, and appearing in The Lancet in January. The research took into account the findings of 185 observational studies and 58 clinical trials, covering almost 40 years. It concluded that to lower their death risk, as well as the incidence of coronary heart disease, stroke, type 2 diabetes, and colon cancer, a person should ideally consume 25–29 g of fibre per day. Fibre-rich whole foods that require chewing and retain much

of their structure in the gut increase satiety and help weight control and can favourably influence lipid and glucose levels.

On the other hand, several studies from this year draw attention to just how detrimental foods that are not 100% natural can be. A small trial, whose results came out in Cell Metabolism in May, showed that processed food leads to abrupt weight gain. The researchers blame this on the speed with which individuals end up eating processed foods, in particular. If one is eating very quickly, perhaps the gastrointestinal tract does not get enough time to signal to your brain that you're full. When this happens, one might easily over-eat.

And more research in mice, from Scientific Reports in January, found that emulsifiers, which are a common additive present in many products from mayonnaise to butter, could affect gut bacteria, leading to systemic inflammation.

Cardiovascular health

Many studies this year have also been concerned with cardiovascular health, re-visiting long held notions and holding them up to further scrutiny. For instance, a study, in the New England Journal of Medicine in July, which involved around 1.3 million people suggested that when it comes to predicting the state of a person's heart health, both blood pressure numbers are equally important.

Blood pressure assesses two different values. One is systolic blood pressure, which refers to the pressure the contracting heart puts on the arteries when it pumps blood to the rest of the body. The other is diastolic blood pressure, which refers to the pressure between heartbeats. So far, only elevated systolic blood pressure is taken into account as a risk factor for cardiovascular disease. However, the new study concluded that elevated systolic and diastolic blood pressure are both indicators of cardiovascular problems. Its authors emphasise that the large amount of data supports this notion.

When it comes to protecting heart health, 2019 studies have shown that diet likely plays an important role. Thus, research in the Journal of the American Heart Association in August, showed that people who adhered to plant-based diets had a 32% lower risk of death that researchers associate with cardiovascular disease than those who did not. People who ate plant-based foods also had a 25% lower risk of all-cause mortality, according to this study. And another study, from April in the journal Nutrients, warned that people who follow a ketogenic diet, which is high in fats and low in carbohydrates, and who decide to take a day off from this commitment every now and again, may experience blood vessel damage.

Medications: friend or foe?

Medication is meant to help fight off disease, and improve physical or mental well-being. But most drugs can sometimes cause side effects.

For instance, experts affiliated with the European Resuscitation Council, whose goal is to find the best ways to prevent and respond to cardiac arrest, found that a conventional drug used to treat hypertension and angina, may actually increase risk of cardiac arrest.

By analysing the data of more than 60,000 people, the researchers saw that a drug called nifedipine, which is often prescribed for cardiovascular problems, appeared to increase the risk of sudden cardiac arrest. So far, healthcare practitioners have considered nifedipine to be perfectly safe. The current findings, however, suggest that doctors may want to consider offering people an alternative.

Another study, appearing in JAMA Internal Medicine, found that anti-cholinergic drugs, which work by regulating muscle contraction and relaxation, may increase a person's risk of developing dementia. People may have to take anti-cholinergics if some of their muscles are not working correctly, such as bladder or gastrointestinal conditions. The research looked at the data of 58,769 people with and 225,574 people without dementia. It showed that older individuals, at least 55 years old, who were frequent users of anti-cholinergics were almost 50% more likely to develop dementia than those who had never used anticholinergics.

But, while common drugs that have been prescribed for years may come with hidden dangers, they are at least subject to trials and drug review initiatives. The same is not true for many other so-called health products that are readily available to consumers. Such findings highlight the importance of carrying out regular medication reviews.

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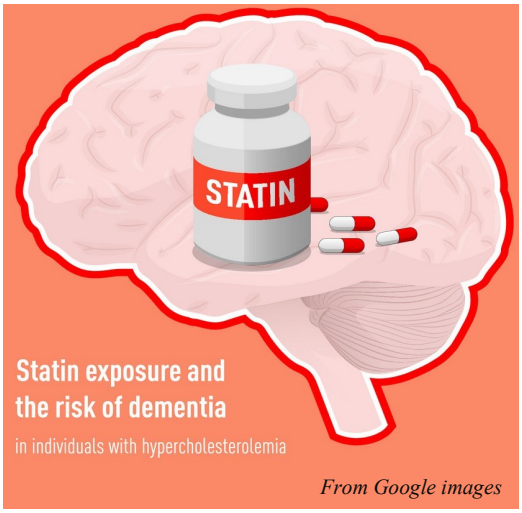
Heart drug combinations might lower dementia risk

It's long been known that keeping blood pressure and cholesterol under control is important for a healthy heart. A new study finds that certain combinations of cholesterol and blood pressure drugs may do more than help the heart. They might also lower a person's risk of dementia, which affects about 7 million in the US alone and that number is expected to increase to 12 million over the next two decades.

The drugs include two common types of blood pressure medications which are either ACE inhibitors orangiotensin II receptor blockers (ARBs), as well as cholesterol-lowering statins. In the study, published recently in the journal *PLOS One*, a team tracked 2007-2014 data from nearly 700,000 Medicare beneficiaries. The participants were aged 67y and older, and had used both a high blood pressure drug and a cholesterol -lowering statin drug for the two previous years. None had been diagnosed with dementia, and they had never taken any Alzheimer's disease-specific medications.

The use of the statins pravastatin and rosuvastatin, combined with ACE inhibitors or angiotensin II receptor blockers (ARBs) for high blood pressure, was associated with a reduced risk for dementia, compared to other combinations of drugs. One combination of pravastatin or rosuvastatin in combination with ARBs was especially good at lowering the risk, with men benefiting even more than women. The use of a combination of ARBs and pravastatin was associated with a 21% lower risk of dementia diagnosis over the seven years of the study, compared to other combinations of drugs, according to the study.

According to one of the study authors, there are currently no drugs that are proven to treat dementia, but even small delays in onset can dramatically reduce the burden on patients, caregivers, and the health system as a whole. If these findings are replicated in future research, they might lead to specific combinations of statins and high blood pressure drugs being recommended to reduce the risk of Alzheimer's disease and related dementias. The author also advises that



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besides using meds to improve heart health, people interested in keeping their brain healthy should consider eating a Mediterranean diet, doing aerobic exercise 30-45 minutes three to four days a week, maintaining healthy sleep habits and having community involvement.

Other experts believe that the finding makes sense, given the links between the two organs. They also state that the choice of medications make sense because not only do ARBs reduce blood pressure, but they have an anti-inflammatory effect, as do statins. Inflammation negatively affects blood vessel health in the brain. As we are moving into an era of precision medicine, the idea of targeted combination therapies for hypertension and cholesterol in patients over 67 years of age, translating to better vascular health in the brain and leading to a reduction of brain dysfunction is also exciting and warrants further research.

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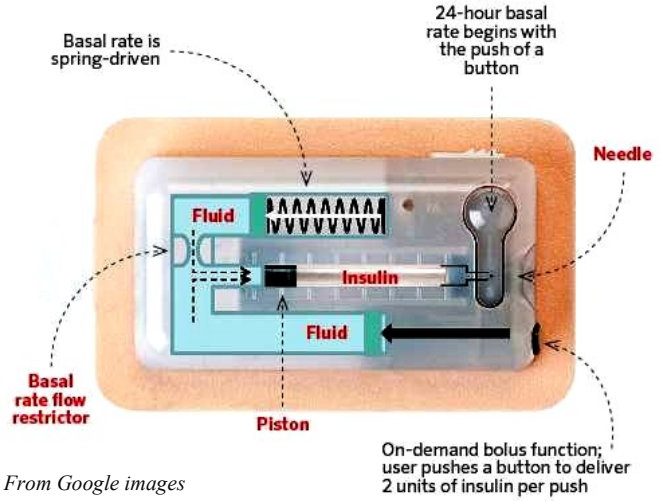
V-Go: Patch pump device for cheaper insulin delivery

Rising prices have grabbed headlines as people struggle to afford their lifesaving insulin, but new research may have found an alternative for people with type 2 diabetes. A study sponsored by Valeritas, Inc., the maker of V-Go, found that combining a wearable, patch-like insulin delivery device (called the V-Go) and an older, cheaper insulin could safely help people with type 2 diabetes achieve good blood sugar control.

For patients who need to take long-acting and mealtime insulins in which the cost is rising rapidly, insulin delivery devices such as the V-Go allow them to get both components from regular insulin with the same



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efficacy and safety, with the potential of improved costs.

When insulin pumps were first increasing in popularity, new types of insulin analogs, such as Humalog or NovoLog, had been introduced. Typically, people using an insulin pump might wear the tubing that delivers insulin under the skin for about three days. But older human insulin, also called regular human insulin (RHI), could clog the narrow tubing, stopping insulin delivery before three days.

Regular insulin was abandoned for use in pumps because there were good alternatives. But those alternatives are very expensive now. For people who have trouble affording insulin analogs, regular insulin may be the only affordable option. One issue with the older insulin is that it takes longer to begin working after it's injected under the skin. Another concern is that this type of insulin can be associated with a higher risk of low blood sugar (hypoglycemia).

The study authors thought that a device like V-Go might be able to use regular insulin. The device is worn for a day and then discarded. It's unlikely that any clogs blocking insulin delivery would form in that time frame. The patch-like device delivers a steady dose of background insulin and can also deliver additional insulin at mealtime. The researchers randomly assigned 54 patients to continue using the V-Go with rapid acting insulin (RAI), and another 59 patients were assigned to switch the insulin, used to fill V-Go, from RAI to RHI. Their average age was 61y. Volunteers were recruited from three medical centers in the US. They were randomly assigned to use either an analog insulin or regular insulin

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Answers to: Test your knowledge

Correct answers: 1-D; 2-C; 3-B

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