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The future of telomere length in personalized medicine

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1. ABSTRACT

Telomere length has been subject of studies for many decades, aiming to elucidate its role in physiological processes, in process of aging and in diverse pathologies. Yet today, there is still no "big title" discovery that would lead to a practical use of telomeres as a reliable biomarker or target for a new drug. However, therapies for chronic disease patients are being tested and companies are already offering commercial tests for telomere length measurement. The strong genetic heritability of telomeres is opening the place for pharmacogenomics researches that could promote the personalized treatment of diverse diseases. In this article, we present the recent knowledge of telomeres genetic determination obtained by genome-wide association studies (GWAS), important biomarkers related to telomere length and review the possibilities of telomere's practical implementation in the medical treatment of diverse

diseases and as a potential biomarker in personalized medicine. Furthermore, we summarise commercial offers of telomere length measurements available and we discuss the actions that should be taken to make steps forward into final application of the accumulated knowledge into practical use.

2. INTRODUCTION

2.1. Telomere structure

Telomeres are terminal nucleoprotein structures, composed of 5,000-12,000 base pairs of repetitive sequences of TTAGGG (3), playing a key role in the protection against genome instability-promoting events (4). During cell division, DNA polymerase cannot fully replicate the 3' end of linear DNA, resulting in the loss of 30-200 nucleotides from telomeric sequence by each cell division (5). Moreover, other mechanisms such as nuclease activation, oxidative damage and DNA replication stress may as well contribute to telomere shortening (6). After a critical degree of shortening, telomeres become dysfunctional and activate a DNA damage response, the p53 or the p16INK4a signaling pathway, which leads to senescence and finally to cell death, apoptosis (7). Because of their progressive shortening with age and sensitivity to oxidative stress, telomeres have been suggested as a biomarker of aging (6, 8) and age-related diseases (9).

2.2. Telomere function

Telomeres are preventing the end of the linear chromosomal DNA from being recognized by DNA reparative machinery as a broken end (6). They prevent distinct biochemical processes such as degradation of the terminal regions of chromosomes, fusion of a telomere, either with another telomere or with a broken DNA end and homologous recombination between the telomeric regions (4, 10). Apart from DNA end protection, the telomeres are also involved in the regulation of gene expression through transcriptional silencing of genes located close to the telomeres (11).

2.3. Telomere-binding proteins

Proteins binding to telomeres have a critical and complex role in regulating telomere function (12) and higher-order DNA conformations are necessary for normal telomere function (11). The structure of telomeres is determined by a complex of six telomere-specific proteins named shelterin (13). Telomeres have long G (guanine)-rich overhangs, called 3′-overhang or G-tail, overhanging 130-200 bases in length that result from degradation of both telomere ends (14). Shelterin affects the structure of G-tails by forming the so called "T-loop", which provides a general mechanism for protection of telomeres by masking the chromosome end from being recognized as DNA double-strand breaks (15). Additional protein-protein interactions mediate the communication of telomeres with structural components of the cell nucleus (16). Because of its crucial role in chromosome protection, it has been recently suggested that shorter G-tail length is more directly associated with age and higher disease risk factors than total telomere length (TL) (17), suggesting G-tail as a new potential biomarker, alternative to TL *per se* (18). Besides shelterin, G-quadruplexes are other higher-order structures, protecting the 3' overhang of telomeres from being accessed by the enzyme telomerase (19).

2.4. Telomere lengthening

Some normal human cells, immortalised cells and cancer cells are capable of preventing the shortening of telomeres with a specialised cellular ribonucleoprotein reverse transcriptase (10), which consists of the catalytic subunit *hTERT* (human Telomerase Reverse Transcriptase) and RNA component *hTERC* (human Telomerase RNA Component), a template for the elongation of the telomeric sequences by hTERT (20). Although *hTERT* and *hTERC* are sufficient to generate enzyme activity *in vitro*, telomerase relies on additional proteins to enable its complete functioning, within which, the best characterized is the dyskerin protein (*DKC1*) (21). By copying a template sequence, telomerase synthesizes one strand of the telomeric DNA running 5' to 3' towards the distal end of the chromosome and therefore extends the single-stranded overhang, G-tail (10). The telomerase activity in normal human cells appears during development of fetus and disappears in most somatic cells during embryonic differentiation, except in highly proliferative cells with a need for telomerase to maintain TL and genetic stability (22). This includes male germ cells, activated lymphocytes and certain types of stem cell populations (23).

Capped telomeres are inaccessible for telomerase; therefore, cells containing active telomerase can regulate the length of telomeres within well-defined limits by switching telomeres from capped to uncapped state with the higher-order telomeres DNA-protein complex (16). Capped state preserves the physical integrity of the telomere and enables cell division to proceed. However, whether telomere exists in capped or uncapped state depends also on its length; longer telomeres are more likely to form protective capped structure than shorter telomeres (24). Some immortalized human cells and some tumours maintain their telomeres in the absence of any detectable telomerase activity by a mechanism, named alternative lengthening of telomeres (ALT) (25). ALT is based on the homologous recombination, a common method of DNA repair, where a double-strand break of one chromosome gets repaired by DNA polymerase using template DNA, taken from a matching sister chromatid (26). During cancer development, cells mostly use the

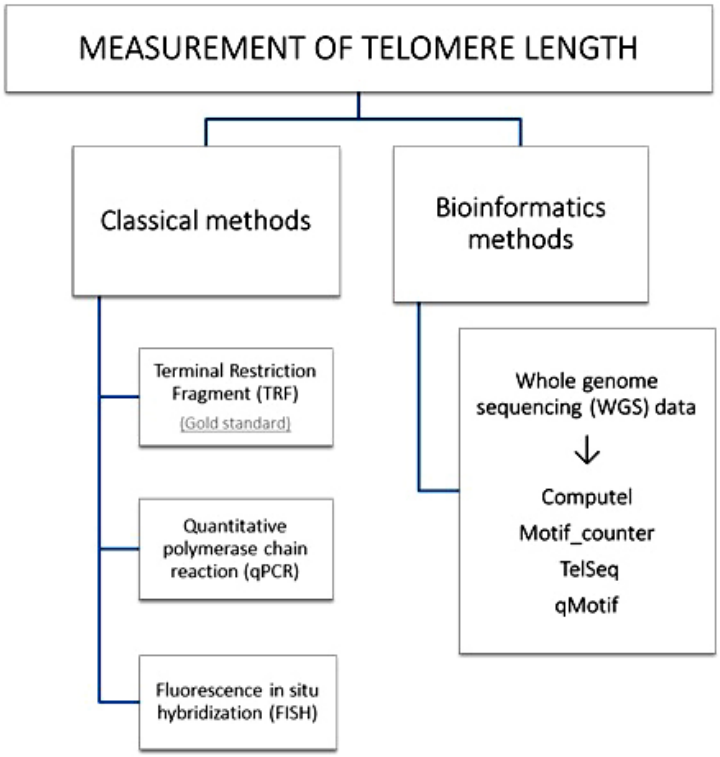

Figure 1. Presentation of methods used for TL measurement

telomerase-based telomere elongation, but also ALT process to acquire the ability to divide indefinitely (27).

3. THE TELOMERE SYNDROMES AND TREATMENT

Telomerase reconstruction for therapeutic use was firstly considered in diseases with distorted enzymatic activity of telomerase, the telomere syndromes (1). These are premature aging disorders with common defect of short telomeres, caused by the mutations in the genes that encode the essential telomerase components – *TERT, TERC* and *DKC1*. The most known and first discovered was dyskeratosis congenita (DC), a disease with childhood-onset, classically defined by mucocutaneous triad of skin hyperpigmentation, nail dystrophy and oral leukoplakia. All of the mutant genes identified in DC encode telomerase or telomere protein components. Mutations *DKC1* and *TIN2* gene are the most commonly identifiable (28). DC is associated with increased morbidity, often caused by bone-marrow failure, pulmonary fibrosis and/or severe immunodeficiency (21). Other disorders with onset in infancy are Hoyeraal–Hreidarsson syndrome and Revesz syndrome, both with severe telomere dysfunction.

More commonly, telomere disorders manifest in adulthood as a consequence of germline *hTERT* or *hTERC* mutations. The most common is idiopathic pulmonary fibrosis (IPF), followed by aplastic anemia. Among patients, 3-5% carry mutant *hTERT* and *hTERC* genes (28). Strategies of telomerase actie vation are raising safety concerns due to the close association with most cancers. Cycloastragenol, a molecule with a product name TA-65®, for example, has been shown to activate *hTERT* through activation of mitogenic pathways that lead to the activation of the oncogene c-myc and thus may drive cancer. For this reason, more targeted therapies are needed to ensure the safety. The most promising new strategy is hTERT therapy with gene therapy vectors (29), *demonstrated to improve blood counts and increase TL in the treatment of aplastic anemia, produced by short telomeres resulting from TERT deletion in mice (30),* that could be used particularly for the treatment of the human telomere syndromes (29). So far, no such study has been done in human population.

4. LEUKOCYTE TL AND METHODS OF MESUREMENTS

TL can be measured with different methods in different tissues. There are three methods that are currently used in laboratories. Telomere Restriction Fragment (TRF) (31) is a modification of Southern blot, considered as the “gold standard” of TL measurement. Quantitative real-time PCR (qPCR) (32) is the fastest and recently most used technique. The third method, fluorescence *in situ* hybridization (FISH) method (33), is using flow cytometry and defines the

length of the telomeres on individual chromosomes (Figure 1). Whole genome sequencing (WGS) - based TL measurement techniques (34) are new promising tools that use WGS data to calculate TL (Figure 1). Some studies have already calculated TL with bioinformatics tools such as Computel, Motif_counter, TelSeq and qMotif (34-40). These methods had a high level of concordance with q-PCR measurements (34). As a result, already existing WGS data could be used in the research of TL and could extend the range of possible studies with no further analytical cost.

Mean leukocyte TL (LTL) reflects systematic influence on telomere maintenance in other tissues and therefore, leukocytes are the most commonly used cell group for TL measurement (6). TL varies across somatic tissues in proportion to their replicative activity, but there is a strong correlation in TL across somatic tissues. Furthermore, it has been proven that within an adult, both highly and minimally proliferative tissues appear to display similar rate of age-dependent telomere shortening, suggesting that differences between TL in tissues were largely established at younger age (41).

5. DETERMINANTS OF TL

Since the discovery of DNA replication process, scientists pointed out the “end replication problem”(42, 43) and suggested that most cultured cells could survive only a limited number of cell divisions, commonly termed as the “Hayflick limit“, which might explain why physiologic function breaks down as organisms age (44). Thus, telomeres were since their discovery in 1970s an interesting subject of research because of their special nature, which is labelling them as the “aging biomarker“. Since then, hundreds of studies have been done, proving their inevitable correlation to age (8, 45-48). However, chronological age accounts for less than 10% of the variance in human TL (6). Besides age, gender (49) and ethnicity (50, 51) have also been considered as consistent predictors of TL, associating female gender and African and Hispanic origin with predisposition to longer telomeres in comparison to males and European origin. Human telomeres are relatively short in comparison to other mammals. From an evolutionary point of view, the cell division limit could be developed as a mechanism for tumor suppression (1). The presence of relatively shorter LTL in Europeans could attenuate the risk of melanoma in individuals with light skin pigmentation (50).

Environmental factors, such as smoking and physical activity, have been shown to be significantly correlated to shortening of TL (52-54), as well as traumatic events or certain psychiatric illnesses (55-58). In addition, studies on diseases’ risk factors, such as obesity, lipid profiles and hypertension have shown some inconsistent results. However, new evidences of correlation of these factors with TL are arising (59-61). The determination of epigenetic regulation of TL homeostasis with or without life style risk factors (eg obesity and smoking) remains an interesting area to be explored in telomeres biology (62-64), especially since genetic and epigenetic alterations seem important for a normal cell to suppress the telomere attrition and become malignant (65).

5.1. Biomarkers related to TL

A number of biomarkers related to different physiological processes are strongly associated with an acceleration of TL shortening, even though the mechanistic details and the molecular pathways of these connections have not been uncovered yet (66, 67).

Therefore, the blood or urine detection of protein biomarkers, produced as a consequence of telomere dysfunction in a disease state in any tissue, can provide a valuable clue towards the diagnosis of telomere-associated disorders (Table 1). Different physiological conditions, such as inflammation, oxidative stress and angiogenesis, play significant roles in determining an individual’s TL, both in health and disease (Figure 2) (61).

Chronic inflammation contributes significantly to the pathogenesis of multiple age-related diseases including cancer, atherosclerosis, autoimmune disorders, obesity, chronic obstructive pulmonary disease, diabetes, hematological disorders and neurodegenerative diseases (68-74), which in turn, are strongly associated with telomere shortening (66). Accumulating evidence from different epidemiological studies (Table 1) suggests a strong association of increased systemic inflammation with decreased TL (75, 76). The most significant inflammatory biomarkers include tumor necrosis factor alpha (77), C-reactive protein (76-82), serum amyloid A (76) and interleukin-6 (77, 78, 83). Recent studies have demonstrated that the inflammation is closely interconnected with angiogenesis, whereby the pathological angiogenesis exacerbates the chronic inflammation by stimulating the recruitment of inflammatory cells that release pro-angiogenic cytokines and growth factors (84). Various studies have shown an association between different angiogenic biomarkers with TL. Vascular endothelial growth factor-A (VEGF-A or VEGF) is a potent and cell-specific angiogenic factor (85), which has also been shown to be associated with various inflammatory markers (86). VEGF positively regulates telomerase activity in both *in vivo* and *in vitro* models (87, 88). However, some epidemiological studies have shown that plasma VEGF is negatively or not correlated with TL (76, 89). In addition, hepatocyte growth factor and granulocyte colony-stimulating factor, which contribute significantly both in physiological and pathological angiogenesis, were also found to be negatively correlated with TL in knee osteoarthritis patients (Table 1) (89).

Table 1. Association of TL with the biomarkers of inflammation, angiogenesis and oxidative stress in different population studies

Population	Study	Measurement method	Biomarkers	References
Inflammatory biomarkers				
1,517 Caucasian female twins, aged 18–79 years	TwinsUK	Southern blot	Higher CRP in postmenopausal than premenopausal women CRP levels inversely correlated with LTL in premenopausal but not postmenopausal women	(79)
419 men and women, aged 65–93 years	Cardiovascular Health Study (CHS)	Southern blot	Negative correlation of LTL with IL-6 and CRP levels	(78)
2,160 healthy Caucasian women twins, aged 18–79 years	TwinsUK	Southern blot	Positive correlation between high serum vitamin D levels and LTL Negative association of CRP levels with LTL and vitamin D concentrations	(81)
1,319 healthy Caucasian twins, aged 18–81 years	TwinsUK	Southern blot	Negative correlation between LTL and high CRP levels	(80)
136 patients with COPD, aged (mean±SD) 62.9±6.6, 113 age-and sex-matched smokers, aged (mean±SD) 62.2±7.7 and 42 nonsmokers, aged (mean±SD) 61.4±6.1 with normal lung function	Case-control study	Real time-qPCR	Negative correlation between IL-6 and LTL COPD patients	(83)
1,962 well-functioning men and women, aged 70–79 years	Health, Aging and Body Composition (Health ABC) Study	Real time-qPCR	High IL-6 and TNF-α levels associated with short LTL	(77)
87 male subjects over 18 years (mean±SD) 41.5±13 exposed to environments with high levels of occupational fine particulate matter (PM2.5)	The Harvard Boilermakers Longitudinal Study	Real time-qPCR	No change in TNF-α and IL-1β, IL-2, IL-6, IL-8, and IL-10 levels Negative correlation of plasma SAA and CRP levels with LTL	(76)
274 women volunteers: 150 with PCOS and 124 healthy women constituting the control group, aged 13-45 years	Case-control study	Real time-qPCR	High CRP levels negatively correlated with LTL in PCOS group	(82)
Angiogenic biomarkers				
87 male subjects over 18 years (mean±SD) 41.5±13, exposed to environments with high levels of occupational fine particulate matter (PM2.5)	The Harvard Boilermakers Longitudinal Study	Real time-qPCR	No change in VEGF levels Insignificant negative correlation with sICAM-1 and sVCAM-1	(76)
80 knee osteoarthritis patients (63 females and 17 males), aged 49–84 years and 60 healthy controls (34 females and 26 males); aged 50–80 years	Case-control study	Real time-qPCR	Negative correlation between RTL and plasma levels of VEGF, HGF, G-CSF	(89)
Oxidative stress biomarkers				
327 Caucasian men, aged 40–89 years	Offspring cohort of the Framingham Heart Study	Southern blot	Inverse correlation between LTL and 8-epi-PGF2α	(99)
34 T1D patients (21 men and 13 women), aged (mean±SD) 26.32±6.46; 62 T2D patients (35 men and 27 women), aged (mean±SD) 50.15±12.17; 40 non-diabetic controls (21 men and 19 women), aged (mean±SD) 32.25±9.74	Case-control study in Chinese Han participants	Real time-qPCR	Negative association of higher 8-OHdG levels and LTL in both T1D and T2D patients	(100)
88 (36 men and 52 women) metabolic syndrome subjects	LIPGENE cohort	Real time-qPCR	Elevated plasma protein carbonyl and urinary F2-isoprostanes levels in subjects with the shortest RTL	(98)
71 patients with newly diagnosed T2D (40 men and 31 women), aged (mean±SD) 54.55±8.37, with (n = 17) or without (n = 54) depression; 52 normal glycemic control subjects (30 men and 22 women), aged (mean±SD) 51.27±7.66; with (n = 6) or without (n = 46) depression	Case-control study	Real time-qPCR	8-OHdG levels contribute to TL shortening and depression development in newly diagnosed type 2 diabetic patients	(101)

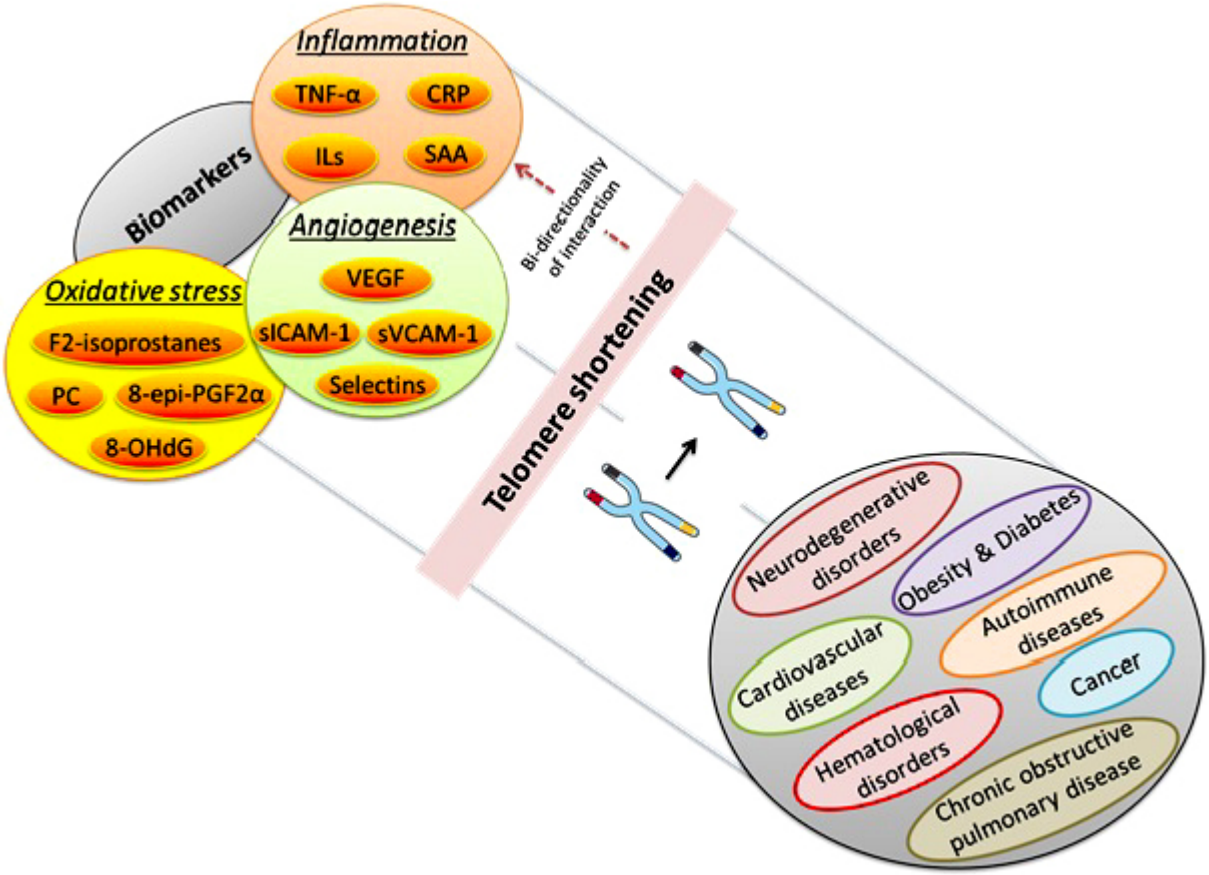

Figure 2. Inflammation, angiogenesis and oxidative stress biomarkers related to telomere shortening and increased risk of various age-related diseases.

Moreover, the relation between inflammation and angiogenesis is further bridged by the oxidative stress (84), which is implicated in aging and various age-related diseases including cancer, cardiovascular and neurodegenerative disorders (90-92). Oxidative stress plays an important role in telomere attrition (93), mainly because of the high content of guanines in telomeric sequences, which renders them highly prone to damage by free radicals (94-96). Another mechanism of telomere shortening by reactive oxygen species includes the single-strand breaks caused by hydroxyl radicals as well as the inefficient repair system for these lesions in the telomeric DNA (97). Elevated levels of urinary F2-isoprostanes and plasma protein carbonyl (produced through the oxidation of protein backbones) have been associated with the shortest relative TL in a population of 88 subjects with metabolic syndrome (98). Moreover, the LTL was also shown to be inversely correlated with another biomarker of oxidative stress, 8-epi-$PGF_{2\alpha}$, the product from isoprostane pathway (99). In addition, increased levels of 8-hydroxy-desoxyguanosine (8-OHdG), a marker of oxidative DNA damage, have also been negatively correlated with TL in type 1 and type 2 diabetes patients (Table 1) (100, 101).

To summarize, the cross-connections of these biomarkers with one another as well as with the telomerase and telomere-associated proteins could emerge as an excited area of research to develop the therapeutic approaches targeting specific pathological conditions. However, most of the studies linking TL to inflammatory biomarkers were based on cross-sectional design, and therefore, cannot explain the bi-directionality that exists between TL shortening and inflammation. It is, therefore, highly desirable to use data from longitudinal studies with large sample sizes in order to determine the evolution of various biomarkers at multiple time points, which will allow to better explore the telomere dynamics in relation with various chronic diseases (76, 89, 102, 103).

6 HERITABILITY AND GENETIC VARIANTS ASSOCIATED WITH RELATIVE TL

Previous studies have shown that TL is a highly heritable trait (36-86 % in different family and twin studies) (104-109). Whereas TL has a high heritability, the inheritance patterns require further investigations, as some studies suggested stronger paternal inheritance, indicating the possible modification by

parental age at conception and provided evidence that newborns with older fathers had significantly longer telomeres (109, 110). However, in some other studies, maternal TL inheritance was found to be more important (111).

Genome-wide association studies (GWAS) in large populations have been used to investigate associations between genetic variants and complex clinical conditions and phenotypic traits (112).

A search that we performed using the term "telomere" in GWAS Catalog (https://www.ebi.ac.uk/gwas/) and HuGE Navigator – GWAS Integrator (https://www.cdc.gov/genomics/hugenet/hugenavigator.htm) identified GWAS associated with TL. Selected articles concern those where only TL was reported as a trait and were excluded the diseases/traits with indirect connection to TL, such as sickle cell anaemia, testicular germ cell cancer or glioma. Eleven publications matched our searching criteria and are presented in Table 2. The presented studies identified common genetic variants associated with relative TL, where single nucleotide polymorphisms (SNPs) from different genes were proposed to explain the inter-individual variability of TL (113-123). In Table 2, we present only SNPs that have reached the threshold for genome-wide significance ($p \leq 5\times 10^{-8}$) or have shown supportive evidence for association ($p \leq 5x\ 10^{-5}$) (117). In case, where more SNPs had been identified for the same locus, the SNP with the strongest association is presented. Furthermore, the roles of the genes on loci with significantly associated variants are also described. The variance of TL explained by discovered SNPs is not higher than 1% for single identified locus, which indicates that until now, only a small part of genetic variability of TL has been identified (113-123) and further investigations are needed for the discovery of causal genetic biomarkers.

Most of SNPs that reached genome-wide significance threshold harbor genes that encode proteins with known functions in telomere biology (*i.e.* genes, directly involved in telomere maintenance). Among them, the most significantly associated locus is *TERC* on 3q26, reported and replicated in several studies, encoding telomerase RNA component. Furthermore, 5p15.3.3 locus harbors *TERT*, a gene encoding the reverse transcriptase subunit of the telomerase enzyme. The mutations of these genes are causing rare monogenic diseases that are associated with short TL (*e.g.* dysteratosis congenita) and are linked to several types of cancers (115). Gene *OBFC1* (oligonucleotide/oligosaccharide-binding fold containing 1) on 10q24.3.3 is specifically involved in the replication and capping of telomeres; along with *CTC1* and *TEN1* genes it encodes a component of the telomere-binding CST complex, which binds and protects telomeres *via* association with the shelterin complex. Shelterin complex consists of six other proteins (TRF1, TRF2, TIN2, TPP1, RAP1 and POT1). Zinc finger proteins can bind to G-quadruplex DNA and stabilize it; *ZNF676* and *ZNF208* are supposed to modify TL by direct binding to DNA and altering the expression of genes involved in telomere maintenance or through interaction with RNA to affect the post-translational signaling of these genes (113, 117). *MYNN* is located on 3q26.2. and encodes myoneurin, a member of the BTB/POZ and zinc finger domain-containing protein family, is controlling gene expression (124). Furthermore, gene *NAF1* (nuclear assembly factor 1) on 4q32.2. locus is involved in the formation of the telomerase enzyme and *RTEL1* on 20q13.3. is the regulator of telomere elongation helicase 1 involved in setting TL.

However, some significant loci do not harbor obvious genes related to telomere biology. In the second general category of genes associated with TL, we find genes that impact the turnover rate of hematopoietic stem cells (115). The 2p16.2. locus, for instance, contains the gene Acylphosphatase 2, muscle type (*ACYP2*) that has a specific role in muscle differentiation and stress induced apoptosis. Another gene, unlikely to be involved in telomere biology, is *PXK* gene, which codes for a serine/threonine kinase and is involved in regulation of electrical excitability and synaptic transmission. *DKK2* gene plays a role in embryonic development and may be involved in bone diseases, cancer and Alzheimer's disease in adults. The *PAPSS1* gene encodes a trypsinogen. Mutations on this gene are associated with hereditary pancreatitis. Finally, *CSNK2A2* encodes an enzyme, casein kinase II subunit alpha, which phosphorylates a large number of substrates and regulates numerous cellular processes, such as cell cycle progression, apoptosis and transcription. It is affiliated with the members of the shelterin complex involved in chromosome end protection, TL regulation and maintenance (113-123).

7 TELOMERES AS A BIOMARKER

By the definition of the Surrogate Endpoint Working Group, a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (125). Short telomeres are associated with increased risk of early death and measurement of TL has been mentioned as a possible biomarker of aging and many age-related diseases, also because of (*i*) its tight correlation with oxidative stress and cellular senescence, two important regulators of human aging (9), (*ii*) its relation to basic biological mechanisms, (*iii*) record of past cell divisions, (*iv*) its high correlation across tissues and (*v*) association with lifestyle factors (126). A lot of effort has been done to evaluate whether clinical practice could benefit from TL measurement;

Table 2. Common genetic variants associated with relative TL

Number of individuals	Methods for TL analysis	SNP	p-value	Chromosomal Region	Reported gene(s)	Reference
1625 1165 (replication)	Southern blot	rs2162440	3×10^{-6}	18q12.2	BRUNOL4, PIKC3C	(118)
2917 9492 (replication)	Quantitative PCR	rs12696304	4×10^{-14}	3q26.2	TERC	(122)
3417 1893 (replication) + 2876 (in silico)	Southern blot	rs4387287 rs1975174 rs4452212 rs2736428	2×10^{-11} 2×10^{-6} 2×10^{-6} 3×10^{-6}	10q24.33 19p12 2q22.1 6p21.33	OBFC1 ZNF676 CXCR4 SLC44A4	(115)
459 890 (replication)	Quantitative PCR	rs6028466 rs621559 rs398652 rs654128	3×10^{-7} 2×10^{-6} 2×10^{-6} 3×10^{-6}	20q11.22 1p34.2 14q21 6q22.1	DHX35 WDR65 PELI2 KPNA5	(114)
3554 2460 (replication)	Quantitative PCR	rs12696304	2×10^{-14}	3q26.2	TERC	(120)
9190 2226 (replication)	Southern blot	rs9419958 rs1317082 rs412658 rs3027234	9×10^{-11} 1×10^{-8} 1×10^{-8} 2×10^{-8}	10q24.33 3q26.2 19p12 17p13.1	OBFC1 TERC ZNF676 CTC1	(117)
37 684 10 739 (replication)	Quantitative PCR	rs10936599 rs2736100 rs7675998 rs9420907 rs8105767 rs755017 rs11125529	3×10^{-31} 4×10^{-19} 4×10^{-16} 7×10^{-11} 1×10^{-9} 7×10^{-9} 7.5×10^{-10}	3q26.2 5p15.33 4q32.2 10q24.33 19p12 20q13.3 2p16.2	TERC TERT NAF1 OBFC1 ZNF208 RTEL1 ACYP2	(113)
2240 15 065 (replication) + 11 024 (replication)	Quantitative PCR	rs1317082 rs7726159 rs2487999 rs6772228 rs9257445 rs6060627	1×10^{-19} 5×10^{-17} 4×10^{-14} 4×10^{-10} 1×10^{-7} 6.5×10^{-7}	3q26.2 5p15.3 10q24.3 3p14.4 6p22.1 20q11.2	*TERC* *TERT* *OBFC1* *PXK* *ZNF311* *BCL2L1*	(119)
4289	Quantitative PCR	rs7680468 rs12638862 rs1317082 rs7100920 rs11787341 rs10904887 rs16859140 rs34596385 rs10466239 rs73394838 rs4902100	5×10^{-8} $<5 \times 10^{-8}$ $<5 \times 10^{-8}$ $<5 \times 10^{-8}$ 9×10^{-7} 4×10^{-6} 5×10^{-6} 6×10^{-6} 7×10^{-6} 9×10^{-6} 4×10^{-6}	4q25 3q26.2 3q26.2 10q24.33 8p21.3 10p13 3q13.2 6q24.1 10q11.21 22q12.2 14q23.2	DKK2, PAPSS1 TERC MYNN OBFC1 LOC100128993 TRDMT1 TMPRSS7 AK097143 FXYD4, RASGEF1A ASCC2 SYT16	(123)
2632 3917 (replication) + 696 (replication)	Quantitative PCR	rs2736100 rs17653722	2×10^{-5} 7×10^{-6}	5p15.33 12q13.13	TERT KRT80	(116)
1616 2397 (replication) + 2952 (replication)	Quantitative PCR	rs74019828 rs2098713 rs78869517	5×10^{-8} 3×10^{-6} 3×10^{-5}	16q21 5p13.2 5q21.3	CSNK2A2 C5orf42 FER	(121)

from determination of biological age of individual in order to predict mortality, to stratification of patients into different risk groups of precise medical conditions.

Recent discoveries showed that DNA can be damaged also with normal TL, if G-tail structure is disrupted, suggesting, that G-tail length contributes more to pathologies than TL itself (18). It has been associated with age and vascular risk factors (17) and methods for the examination of G-tail length with a high-throughput platform using genomic DNA or cell lysate are faster than classical methods for TL determination, giving G-tail priority as a biomarker choice in the future studies (127).

7.1. Telomeres as aging biomarker

Wide inter-individual differences among the subject of the same age-group suggest that chronological age is not a relative measure of person's health status, therefore, research of biomarkers has arisen to provide more information about an individual's biologi-

cal health, to point out those at high risk of age-related conditions, and to evaluate the progression of interventions, designed to delay the onset of age-related conditions (128). Aging Research proposed four criteria for aging biomarkers: 1) it must predict the rate of aging better than chronological age and tell exactly where a person is in their total life span, 2) it must monitor a basic process that underlies the aging process, not the effects of disease, 3) it must be able to be tested repeatedly without harming the person and 4) it must be something that works in humans and in laboratory animals in order to be tested in lab animals before being validated in humans (129).

Because it is difficult to separate the effects of chronic diseases from normal aging, there has been no such marker that would fulfill all the requested criteria. It has been suggested that, if TL is a biomarker of aging, it is a weak biomarker with poor predictive accuracy (9). However, looking wider at the perspective, TL can be considered as "a marker that predicts biological age of organism" and could be an ideal candidate for life course analysis, the study of long-term effects of physical and social exposures on chronic disease risk in different life stages, from childhood to late adulthood (9). Telomere shortening rates are not constant, but are influenced by positive or negative regulators (8). With systematic testing, factor such as TL can represent etiology of diseases more accurately and might thus be very advantageous.

Another perspective of epidemiological research of aging is studying TL in people who age slowly and exhibit "longevity potential" or "healthy aging". To do so, CHARGE Consortium (the Cohorts for Heart and Ageing Research in the Genomic Epidemiology) created longevity phenotype for GWAS (9). TL has been therefore investigated within the research of healthy aging and longevity biomarkers. However, it is hard to distinguish the changes causal to aging and longevity from those that are a consequence of normal aging and such marker, which could well explain the longevity potential of individual has not yet been discovered (130, 131).

7.2. Telomeres as a biomarker in age-related diseases

As telomeres are involved in the etiology of diverse diseases, new possible progress in diagnosis was seen in potential use of telomeres as a biomarker. Consequently, it was necessary first to elucidate their role in disease development, confirm the causality and separate their effect from any other disease risk factors. These tasks are complex and the conclusions obtained from the studies are not always clear. TL is therefore still not present in everyday clinical practice. Nevertheless, a lot of progress has been done and ongoing and prospective studies are getting closer to find the solutions for better and faster diagnosis and treatments.

7.3. Telomeres as a biomarker in cancer prognostic

Diverse studies have reported association of short TL in bladder (132), breast (133), colorectal (134), leukemia (135), lung (136), ovarian (137), pancreatic cancer (138) and others. Even though most of the studies reported higher risk of cancer in patients with shorter telomeres, these results are not consistent for all cancer types. Increasing evidence shows that mutations, which appear to lengthen telomeres, are linked to increased risk of cancer as well (139). Longer telomeres were associated with increased melanoma (140), glioma (141), endometrial cancer (142) and prostate cancer (143, 144). What are the reasons for the discrepancy of these results?

There are two different mechanisms in etiology of cancer, both related to telomeres. Short telomeres are promoting the events that lead cell to senescence or apoptosis, which causes accumulated mutations, genetic lesions and inactivated tumor suppressor checkpoints that can result in cancer. On the other hand, cancer cells are maintaining their capacity for infinitive division with the elongation of telomeres; elevated levels of various telomerase-stimulating factors may increase telomerase activity and contribute to longer TL (145). Second reason for discrepancy of results might be the study design. Different studies in TL differ in the use of measuring method, technique of sample collection, statistical analysis and other parameters (145). Only with additional studies, the hypothesis scientists try to confirm will be clarified and will open the way to new challenges. New insights into mechanisms of TL maintenance and telomerase expression and their transcriptional, post-transcriptional and epigenetic regulation can help to set telomeres as a new biomarker for early detection and prognosis of disease (146).

7.4. Telomeres as a biomarker in cardiovascular diseases (CVD)

As already discussed, reduced TL is associated with many intermediate phenotypes, namely chronic inflammation, hypertension, obesity and unhealthy lifestyle, all of which are risk factors for CVDs besides age, which is the major risk factor. Furthermore, several CVD manifestations such as coronary heart disease, carotid atherosclerosis, stroke, and abdominal aortic aneurysm have showed association with short telomeres, which might be shortened because of the interaction with common intermediate phenotypes of CVD (147). The mechanisms of causality still need to be revealed, however, growing understanding of cardiovascular

aging and telomere biology is enabling novel possible interventions and new therapies; maintenance of TL and telomerase activity regulation are already being tested for treatment of CVD (148). Moreover, some medications showed beneficial effects on TL maintenance. Statins and pioglitazone were associated with increased telomerase activity and protection of telomere by up-regulation of *TRF2,* and angiotensin II with reduction of oxidative stress (149-152).

In order to understand whether TL represents a unique biomarker for CVD, is it a marker of one primary risk factor such as inflammation, or even multiple risk factors, a study of TL in relation to 17 biomarkers of CVD risk was performed (153). The findings showed moderate association with multiple important CVD risk factors, such as BMI, waist circumference, percentage of body fat, HDL, TGs, pulse rate, CRP and cysteine C, probably by increased tissue inflammation and oxidative stress (148). However, there was no association with measures of overall CVD risk (the metabolic syndrome), suggesting that TL could be a biomarker of cardiovascular aging through established physiological mechanisms. TL is related to biomarkers of multiple regulatory systems that indicate risk for CVD; it could therefore serve as a cellular based indicator of systemic allostatic load, the "wear and tear on the body" (153).

Among traditional risk factors for CVD, decreased regenerative capacity, normally estimated by circulating levels of progenitor cells (PCs), was believed to be another major link to TL. However, a study of patients with coronary artery disease (CAD) showed that shorter TL is associated with lower levels of PCs, but they predicted adverse cardiovascular outcomes independently and additively one of another (154). The role of telomeres in CAD has been investigated in many studies (155-161), suggesting that TL is involved in the pathology of the disease, associating it with artery stiffness (156) and atherosclerosis (161). Furthermore, TL was found to be significantly shorter in young healthy adults from families with higher incidence of CAD (159). These results are suggesting the causal rather than consequential relation of telomeres and CAD. In favor of this hypothesis is also the association of genetic variants affecting TL with the risk of CAD (148); the polymorphism $^{-1327}$T/C of *hTERT* was found to be significantly associated with susceptibility to CAD among CAD patients in comparison to healthy controls. Moreover, in a large GWAS of TL in association with age-associated diseases, a sample of 22,233 coronary artery disease cases and 64,762 controls showed association of the alleles related to shorter TL with increased risk of CAD (113). It is therefore indisputable that TL has a big prognostic value in patients with CAD and could import additional support in preliminary CAD diagnostic.

Besides CAD, decreased TL was significantly correlated with heart failure (162) and peripheral arterial disease (163). Causality of TL in health outcomes was investigated with two-approach analysis also in other CVD and risk factors, using GWAS data and individual data of 3,734 individuals. However, examination of risk factors including diabetes, hypertension and obesity did not confirm any causal relationship with shorter telomeres, but showed decreased risk of stroke and increased risk of heart disease (164). Finally, studies of TL in stroke have shown very inconsistent results; another study investigating telomere-related SNPs in relation to predisposition for ischemic stroke in a case-control study suggested that variants of the *hTERC* and *hTERT* genes could have shortened LTL and lead to increased possibility of having ischemic stroke (165).

7.5. Telomeres as a biomarker in psychiatric disorders

Many psychiatric disorders, such as major depressive disorder (MDD), bipolar disorder (BD), post-traumatic stress disorder and schizophrenia have shown correlation to shorter leukocyte TL (40, 55-58, 166-168). A recent meta-analysis confirmed these findings in patients with MDD (169), a syndrome of "premature aging". Patients suffering from this disorder have an accelerated cellular aging that can reduce life expectancy up to 10 years (57). Telomere shortening may thus increase the vulnerability of psychiatric patients to premature death (167). Furthermore, rs10936599 SNP for T-carriers, located upstream of *hTERC* gene was found to predict a small, but significant increase in the risk of childhood-onset recurrent MDD (170). These findings suggest an important role of LTL in this disorder (169) and the telomerase enzyme might thus represent an important drug target for the prevention of early-onset MDD (170). Some pre-clinical studies suggested that the use of antidepressants, selective serotonin reuptake inhibitors (*SSRIs*) and lithium can increase telomerase activity; fluoxetine increased telomerase activity in hippocampus in mice (171) and continuous administration of lithium for 6 weeks significantly increased TERT expression and telomerase activity in hippocampus (172). Patients suffering from BD showed significantly increased LTL after receiving treatment with lithium. Thus, it might exert a protective effect against telomere shortening *via* telomerase activation (57).

However, it is still unknown which pathway underlies the association of shorter TL and psychiatric disorders; inflammation, metabolic alterations and smoking are important mediators in tight connection to psychiatric diseases. Therefore, additional studies should evaluate the role of lifestyle and telomerase in the evolution of mental illness in order to elucidate the complex mechanisms and to consider telomeres as a potential treatment target.

Table 3. Basic approaches of telomerase-based anti-cancer therapy

Direct enzyme inhibition (active site inhibitors, nucleotide substrates and allosteric inhibitors) by targeting TERT or TERC unit of enzyme
Direct enzyme inhibition (active site inhibitors, nucleotide substrates and allosteric inhibitors) by targeting TERT or TERC unit of enzyme
Active immunotherapy (antigen-presenting cells) by activation of native or memory TERT-specific T cells, which cooperate to kill tumour cells with displayed TERT peptides, through classical major histocompatibility complex (MHC) presentation
Telomere-disrupting agents by altering the structure of the telomere, leading to inability of telomerase to access the telomere, or to a TL-independent damage signal causing immediate cell arrest or death
TERT promoter-driven suicide gene therapy by delivering suicide genes with promoter regions (TERT and TERC) that tumour cells transcription factors activate
Blocking telomerase expression or biogenesis by down-regulating the amount of functionally active telomerase in a tumour cell, based on the growing understanding of how the telomerase holoenzyme is made, from transcription to post-translational modification, assembly and transport

7.6. Telomeres as a biomarker in Alzheimer's disease

Alzheimer's disease (AD) is considered an age-related pathology for which there is no causal therapy because of the still unknown mechanisms of neuronal cell death. However, it is known that telomere shortening plays an important role in cognitive function in AD. Evidences from studies suggest that AD patients have shorter LTL than age-matched controls (173-177) and that shortening is linked with the pathogenesis of AD *via* oxidative stress and inflammation (174, 178). A variant of the gene *APOE*, which encodes apolipoprotein E, is associated with an increased susceptibility to AD. One study (176) demonstrated the possible association between LTL and the *ApoE4/4* phenotype. Investigation of genetic variants of *hTERT* and *hTERC* genes in relation with AD susceptibility has shown that *hTERT* and *hTERC* genotypes which have been previously associated with reduced *hTERT* expression or shorter LTL, are implicated in AD development. This indicates that telomerase can be directly involved in the pathogenesis of AD (179). Therefore, it seems that TL therapy holds a big potential to become a next attractive drug target for the disease (174), but for this, fundamental knowledge of the role of telomeres in AD still has to be revealed.

8. TELOMERES IN ANTI-CANCER TREATMENT

Telomeres play an important role in cancer development since their length and telomerase activity are crucial for initiation and the survival of tumors, giving to cells the capability of faster and infinitive division. On the other hand, however, critically short telomeres can lead to chromosomal instability and as a result provoke a tumor growth in the affected cell. New insights into molecular regulation of tumorigenesis have significantly improved our understanding of the role of telomerase in cancer progression and provided the opportunities for the development of new diagnostic tools and effective anticancer molecules.

8.1. Telomerase inhibitors

Once the problem of "how the chromosomes can be copied in a complete way during cell divisions and how they are protected against degradation" was solved and awarded the 2009 medicine Nobel prize to three scientists for "adding a new dimension to our understanding of the cell, shedding light on disease mechanisms, and stimulating the development of potential new therapies" (180), extensive research was performed for the development of specific inhibitors of telomerase for targeted anti-cancer drugs. Studies demonstrated that 80-95% of all malignancies showed increased telomerase activity, enabling cancer cells a fast and continued replication and promoting cellular immortality, whereas in normal cells, a very low or almost undetectable telomerase activity was present (181). Therefore, telomerase upregulation is considered to be a critical step in cell tumorigenesis and a major hallmark of cancer (146). Up-regulation is achieved by activation of the normally silent *hTERT* gene, which later creates complexes with other proteins and *hTERC* component. The proposed mechanisms of this activation include mutations in *hTERT* promotor, *hTERT* alternative splicing, gene amplification and epigenetic changes (65). In 10-15% of tumors, the senescence is halted by the ALT mechanism, although telomerase maintains TL in the majority of cancer cells (11). Inhibition of telomerase may therefore lead to a decrease in TL, resulting in cell senescence and apoptosis in telomerase positive tumors, while having a minimal impact on normal cells (19). In this aspect, telomerase seems an excellent target for targeted anti-cancer treatment.

Currently, many anti-telomerase therapeutics are being evaluated for treatment of a variety of cancer types, using different approaches, including the nucleosides, oligonucleotides, small-molecule inhibitors, immunotherapeutic molecules, G-quadruplex stabilizers and gene therapy constructs to inhibit telomerase function (Table 3)(182).

8.1.1. Telomerase inhibitor Imelstat (GRN163L)

The most promising group of new agents seems to be the first category that led to the discovery of the so far most promising drug Imetelstat, a specific oligonucleotide telomerase inhibitor. GRN163L was taken into clinical trials after numerous preclinical studies provided evidence of its good pharmacokinetics and pharmacodynamics properties, sufficient potential, efficacy and safety (2). To date, Imetelstat has been the only telomerase-based inhibitor that was sufficiently developed to advance to late-phase clinical trials (183). Its activity was clinically demonstrated in patients with myeloproliferative neoplasms and with solid tumors but caused thrombocytopenia, which is considered as a common side-effect of the treatment (184). Imetelstat is now in the last phase of clinical trials for patients with lower risk of myelodysplastic syndromes and relapsed or refractory myelofibrosis (185).

8.1.2. G-quadruplex stabilisers

Another promising group of widely studied inhibitors are G-quadruplex stabilisers. Telomeres G-strand DNA can form a G4 DNA or G-quadruplex structures, a highly-order structure that is required for a proper regulation of TL *in vivo* by direct inhibition of telomerase binding to long telomere sequences (186). By stabilizing the G-quadruplex with small molecule ligands, the telomeres 3' overhang can be locked in place, thus blocking telomerase from accessing telomeres (19). G4s have become important drug targets that may regulate gene expression and telomere maintenance. Researchers are focusing on new methods to study the ligand binding affinities and selectivity (187) and to propose new bioactive chemotypes, identified after combined ligand-based virtual and experimental screening (188).

8.1.3. Natural products

Many studies have shown that natural phytochemicals can have inhibitory effects on telomerase activity through affecting its subunits and components (189). In traditional Chinese medicine, diverse ingredients with anti-aging effects, acting on different pathways including telomeres and telomerase, are being studied. Natural derivatives, such as astragalosides, polysacharides, flavonoids and others have shown to influence telomerase activity and represent a great potential for further research of anti-cancer drugs in the future (190). Chinese herbal medicine Tianshengyuan-1 (TSY-1) has been recently shown to increase telomerase activity in normal peripheral blood mononuclear cells and CD34+ hematopoietic stem cells with innately low telomerase activity but to decrease telomerase activity in human promyelocytic leukemia (HL60) cells with high intrinsic telomerase activity. Genome screening analysis identified TERT as potential target gene associated with the TSY-1, possibly via the methylation of TERT promoter (191).

8.2. Future perspectives and other therapies

Despite all the progress that has been achieved, the research has shown limited success with only one candidate drug waiting to enter the market (183). Telomerase targeting molecules present a long gap between the administration time and the appearance of a visible effect of producing critically short telomeres in cancer cells (182). This is why most teste ed therapeutics have shown to be more effective when combined with traditional therapies such as chemotherapy or radiotherapy, resulting in telomere shortening, tumor mass shrinkage and preventing resistance to single agent therapy (19). However, overall benefit is rather limited. One of the reasons might be the regulation of telomerase homeostasis, which is controlled by a complex network of genes having inter-connected signals (191). Nowadays, other targets than telomerase and its regulation are becoming the subject of research, such as ESCRT system, a multi-protein complex, required for the formation of transport vesicles within the cell, critical for safeguarding proper TL maintenance. A fully functional ESCRT system is required for proper telomere homeostasis; mutations in any of the genes encoding the complex could therefore cause telomere shortening. Deletion of ESCRT-0 gene seems to protect the cells from uncapped telomeres, making it suitable as a new anti-cancer drug target (192). Finally, even though an approved therapy based on telomerase inhibition has not yet been proposed, it is still one of the best targets to point out in oncology (182).

9. TELOMERES IN TREATMENT OF OTHER DISEASES

Telomere shortening has been associated with the occurrence of many psychiatric disorders, common aging morbidities, including diabetes, cancer, dys-regulated immune function and multiple aspects of cardiovascular diseases (CVD) as well as with rare monogenetic diseases. It is not surprising that fighting against telomere's shortening became a new considered strategy for prevention of these pathologies. In contrary to most cancers, the therapy that could serve for the treatment of these diseases relies on telomerase as a target for regenerative medicine. The week point, however, are complicated pathways of many diseases and the role of telomeres in it. "Are short telomeres the reason, or the consequence of aging and related diseases?". That is the question that still needs to be answered.

One of the isolated cases of medications in market is TA-65®, a small molecule activator of

Table 4. Basic approaches of telomerase-based anti-cancer therapy

Classical gene therapy (by creation of viral vectors) *can be used for tissue engineering, optimization of stem cell transplantation in donor cells with short telomeres and, in principle, also for the treatment of chronic diseases in the whole organism.*
Re-expression of silenced telomerase *(by various molecules and mechanisms such as histone deacetylase inhibitors and estrogen receptor agonists) can upregulate hTERT expression and/or activity.*
Activation of residual enzymatic activity *(by direct interaction with the telomerase holoenzyme or the telomerase activating signaling pathways) is an option for cells with residual telomerase activity to activate the telomerase activity itself.*
Modulation of the intracellular location *(by translocation of telomerase between the nucleus and the cytosol) can regulate telomerase activity.*

telomerase, discovered from screening of natural products from traditional Chinese medicine. Studies of the product showed improvements in biomarkers of aging, including immune, cardiovascular, metabolic, bone, and inflammatory markers and did not show any significant signs of toxicity. A purified compound from *Astragalus membranaceus* plant has been approved as a dietary supplement and is available commercially since 2007. Growing evidences are showing its beneficial role in diverse diseases. Further use of this molecule in disease treatment still calls for long-term prospective studies with larger samples that would reveal its positive effects or possible diverse side-effects (Table 4)(193-195).

10. COMMERCIAL OFFER OF TELOMERE TESTING

Several commercial laboratories over the world are nowadays promoting the TL testing; some of them in purely medical and diagnostic purposes (196), whereas others appeal on tests to reveal the biological aging of human body (197-202). The Canadian company RepeatDx, using Flow-FISH technology to assess TL of blood cells of different type, is offering licensed and validated clinical testing service since 2005 and promoting: *i)* check of possible telomere disorders, such as dyskeratosis congenita, *ii)* bone marrow donors screening to prevent the transplantation of hematopoietic cells with short telomeres, *iii)* testing the patients with pulmonary fibrosis to aid in disease course prediction and risk assessment and finally, *iv)* testing for its use as a biomarker for monitoring other diseases or family history risk. Commercial testing is possible only with the supervision of a practicing licensed physician (196); in the view of the founders of the company RepeatDx, testing outside the context of research studies and diseases related to telomeres is premature for wider public (203).

Other laboratories, such as Telomere Diagnostics, Inc., founded by a Nobel prize winner, Dr. Elizabeth Blackburn, are offering the assessment of age in "TeloYears", which is promoted as an indicator of a healthy lifestyle rather than a tool for screening, diagnosing, treating or preventing diseases or medical conditions (201, 204). Similar is the Spanish company Life Length; for them, telomere testing is a "valuable emerging diagnostic tool within the area of functional and preventive/personalized medicine with a number of clinical applications", such as being global biomarker of health, early detector of age-related diseases, determinant of prognosis and risk stratification (200). The mission of the company Titanovo is to promote longevity science and personalized medicine. Their aim is to "impact longevity research by making important correlations between TL, genotypes, and lifestyle choices". They are offering insight into key biomarkers in combination with longevity genotype (*ACE*, *APOC3*, *APOE*, *FOXO3A*). The aim of the company is also to encourage public to join a research, which "may impact the future of medicine" (202). Furthermore, Veritas, the genome company founded by Dr. George Church is offering telomere testing within the whole genome sequencing insight, where the traits related to disease risk or healthy lifestyle are investigated (197).

TL might easily be widely used as predictor of the biological age of an individual. But the important fact that there is still no reliable solution to repair or stop the process of telomere attrition is diminishing the utility of this testing. Diagnostic laboratories give some advices for individuals with short telomeres, such as proposing "healthy lifestyle and Mediterranean diet" (Cleveland HearthLab) (198) or appealing on the nutritional supplements (quality and balanced multivitamin) and pharmacologic treatments (ACEI, ARB, statins, Aspirin *etc.*) with positive effects on TL (SpectraCell Laboratories) (199). Combination of these advices may help to reduce risk for heart and other chronic diseases or for age-related ailments for which those individuals might be critically susceptible. With the strong scientific teams, which stand behind those companies and other laboratories around the world, it might not be too optimistic to expect that in some time, there will be a new value added to these tests - better understanding of the biomarker and a step towards the solution of premature aging.

11. CONCLUSIONS AND NEW PERSPECTIVES

Years of research have made a big improvement in our knowledge on telomeres biology. The influences of a complex mixture of genetics and environment are combined within the simple

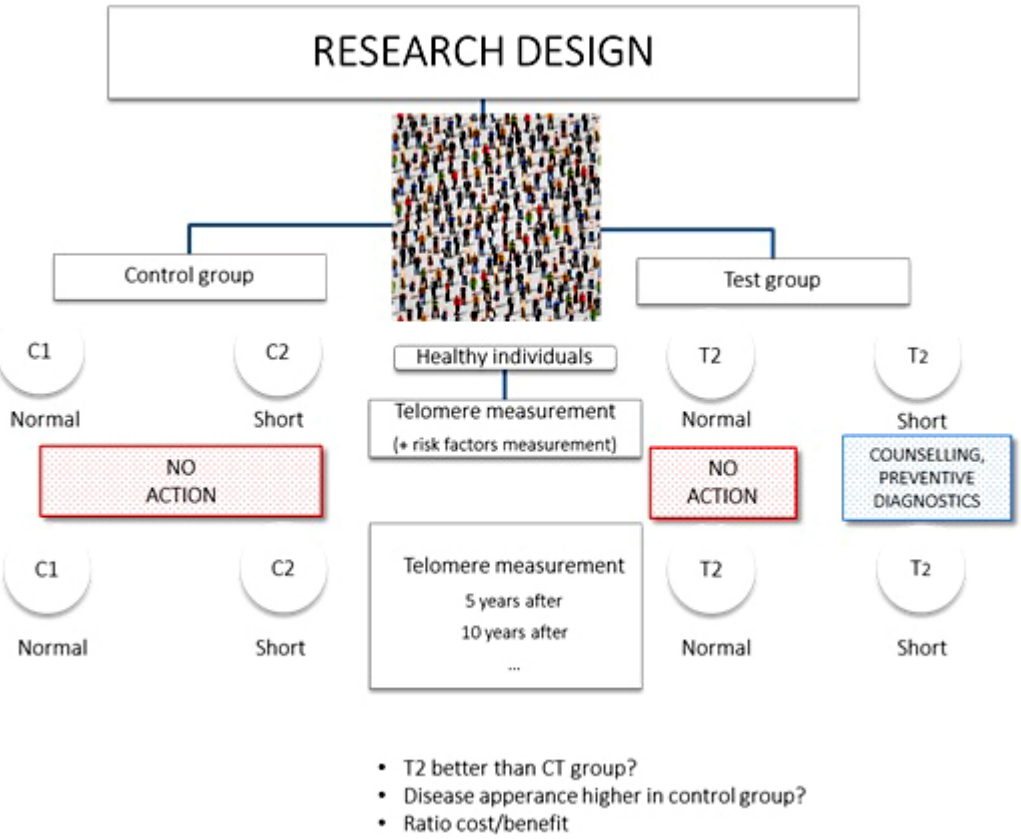

Figure 3 Presentation of the study design aiming to evaluate the potential benefit of TL measurement.

repeated structure TTAGGG, which is lively reacting to diverse factors of biology and society. Hundreds of studies have confirmed the correlation of telomeres with diseases, genetics and lifestyle. But how can we finally take the advantage of all this knowledge and put it into a successful clinical practice? The use of telomeres as a treatment target for diverse diseases is slowly making its progress. Even though there has not yet been an EMA or FDA approved medication that would act on telomeres, we might expect first results in following years, most probably in anticancer therapy, where tested drugs are approaching to the final steps of validation before their release in the market. Dietary supplements that are already being used as natural stimulators of the telomerase activity, such as TA-65®, still have to be further assessed for their beneficial effects and potential adverse effects. Moreover, gene therapy is showing a high potential; bigger progress in this field will open new possibilities also in treatment of telomere-related genes that are being involved in the onset of many chronological diseases.

GWAS studies gave a new insight in genetics and several SNPs were found to be correlated with TL, mostly within genes coding for telomere-related proteins. Rare variants of these genes can provoke diseases called “telomere syndrome”. Telomere measurement or genotyping are one of the most reliable methods of diagnosing these diseases. Furthermore, several candidate genes with function in telomere biology showed association with other age-related diseases. Further studies of ‘omics’ regulation of telomeres will contribute to better understanding of regulatory pathways which is crucial for successful drug design.

“Is TL a biomarker of … aging? Coronary artery disease? Breast cancer?” are the common questions that scientists tried to answer. Despite all hard work, so far, TL has not fulfilled all of the conditions for being considered as a “biomarker” for diagnostics of disease conditions or in predicting the “biological age” of individual. The reasons for this are many individual factors that are influencing TL and are preventing the identification of a clear casual relation between condition itself and telomeres. Next, there is an inconsistence of measuring methods of TL determination, used by different laboratories, another weak point of biomarker that we cannot neglect. However, the connection between TL and many diseases and risk factors has been proved in many different studies, suggesting a beneficial indicative ability of telomeres in disease prognostic.

Now it is time for new perspectives that will accelerate the development of diagnostic methods with TL measurement.

To summarize, TL should not be tested as a biomarker of the single disease condition, but as a general state of organism that reveals potential susceptibility for all-disease risk. Measuring of TL could present a follow-up of the patient's health and create an alert of possible disease setup, if patient would be classified in the lowest (or in some cases highest) quartile of TL of his age-group or would expose faster rate of telomere shorting than usual. The patients at risk would have further testing for particular diseases and additional counseling about the risk factors that might threaten their health from their personal doctor. As we know that changes in lifestyle may significantly decrease TL attrition and improve general health of individual, this might prevent or delay disease onset and ameliorate medical conditions. If the progress of telomere-related drugs will evaluate, a premature treatment will also be possible. In order to make a step further in application of telomeres into a personalized medicine, large prospective studies should be done, assessing the ratio between the cost-benefit of measuring TL in everyday clinical practice (Figure 3). Furthermore, with the advancements in the field of bioinformatics, WGS will become another future diagnostics tool. With WGS, TL determination would be possible without "classical methods" but with computational calculation only. This could become a new reference method for TL determination. Further research in this field, however, is essential and the establishment of unique, well performative technique is indispensable for faster progress in telomere research.

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Abbreviations: LTL: Leukocyte telomere length; TNF-α: Tumor necrosis factor α; CRP: C-reactive protein; COPD: Chronic obstructive pulmonary disease; sICAM-1: Serum intercellular adhesion molecule-1; sVCAM-1: Serum vascular cell adhesion molecule-1; SAA: Serum amyloid A; PCOS: Polycystic ovary syndrome; VEGF: Vascular endothelial growth factor; HGF: Hepatocyte Growth Factor; G-CSF: Granulocyte colony-stimulating factor; IL: Interleukin; T1D: type 1 diabetes; T2D: type 2 diabetes; 8-OHdG: 8-hydroxy-desoxyguanosine

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