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Production by "The Team"

Cellular senescence and telomerase

Cells of nearly every complex organism do not have an unlimited ability to divide. This phenomenon was described by Leonard Hayflick in 1961 (Hayflick 1965). The number of potential divisions – about fifty – was dubbed the "Hayflick Limit" and sometimes is called cellular senescence. For the first time, it was appreciated that cells could be mortal (normal cells) or immortal (tumor cells). This distinction underpins much of modern cancer research.

The mechanism accounting for the Hayflick Limit remained an enigma for many years to come. The first major clue occurred in 1986 when Cooke and Smith observed that the ends (telomeric regions) of human sex chromosomes were substantially shorter in non-germline cells than in germline cells (Cooke and Smith 1986). Like scientists of their time, they appreciated that in the absence of an active mechanism, some telomeric DNA would otherwise be lost during each replication cycle. Less than a year earlier, Greider and Blackburn provided evidence for an enzyme-mediated mechanism to maintain telomeres: they discovered that the ends of the linear chromosomes in *Tetrahymena*, a ciliated protozoan, were maintained by an enzyme, **telomerase**, which added new terminal DNA sequences (Greider and Blackburn 1985). In their paper, Cooke and Smith hypothesized that telomerase might not be active in somatic cells (non-germline cells). Furthermore, they provocatively proposed that sustained loss of telomeric DNA could eventually limit the ability of somatic cells to divide. Eventually a mechanism to explain the phenomenon emerged, two and a half decades after discovery of the Hayflick Limit.

The hypothesis that telomere loss eventually limits replication of human cells was not universally accepted. Critics cited apparent exceptions: telomeres in mouse cells are much longer than in human cells, but mouse cells don't have significantly more proliferative potential (Kipling and Cooke 1990); and senescent human cells still have telomeres. On the other hand, many observations supported the causal relationship between telomerase, telomeres and cell proliferation. For example, human cells with shorter telomeres could not replicate as many times as cells with longer telomeres (Allsopp *et al.* 1992), and telomerase activity was detected in immortal or tumor cells but not in normal cells (Kim *et al.* 1994). While the evidence favored a causal relationship, the evidence was circumstantial or correlative.

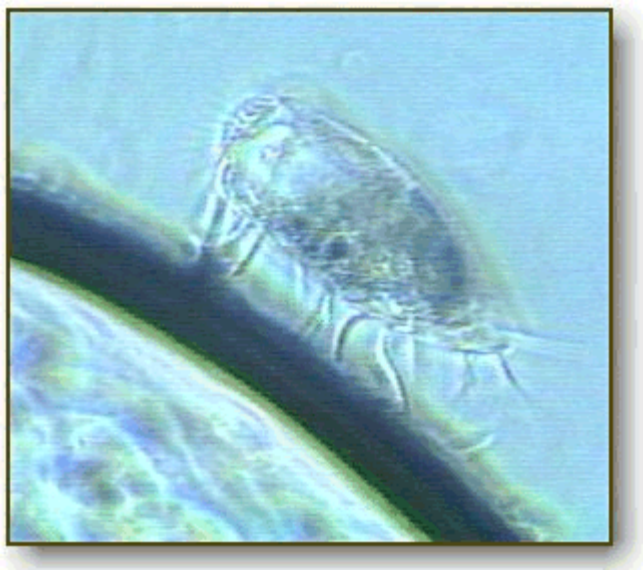
Definitive proof that shortened telomeres are responsible for cellular senescence finally emerged in 1998. Bodnar et al. forced expression of telomerase in normal human cells by transfection of retinal pigment epithelial cells and foreskin fibroblasts with a vector encoding the human telomerase enzyme. Remarkably, these cells exhibited elongated telomeres, "divided vigorously", and proliferated at least 20 doublings beyond their normal life-span; in contrast, the control cells showed shortening of telomeres and senescence (Bodner *et al.*
1998). Thus, in typical somatic cells, human telomeres normally undergo shortening at each cell division, and when several kilobases of telomeric DNA is gone, cell division halts and senescence manifests.

Unlimited proliferative potential of cells is not necessarily advantageous to organisms. It may seem that an unlimited capacity to replicate would be a good thing – the body could undergo self-repair in response to disease or trauma. Yet, replication is not a risk-free event – mutations can accumulate, chromosomes can break or incompletely separate, etc. If multiple mutations are required for tumorigenesis, then fewer replications favor maintenance of a normal phenotype. Thus, one consequence of the Hayflick Limit appears to be beneficial to the organism, acting as part of a tumor suppressor mechanism.

Telomerase gene structure and expression

The elusive telomerase gene was long sought after. Candidates from a number of organisms came and went until investigators finally turned their attention to *Euplotes*, a hypotrichous ciliate. These ciliated protozoans contain two types of nuclei: micronuclei and macronuclei. The macronucleus of hypotrichous ciliates contains at least 10 million small DNA molecules (about 1800 – 2500 bp long). Moreover, each of the DNA molecules have telomeric sequences capping their ends. (Hoffman *et al.* 1995)

Fig. 1. Euplotes on a droplet of water.



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The ribonucleoprotein containing telomerase was purified from *Euplotes* macronuclei by affinity chromatography with antisense 2'-O-methyl oligonucleotides (Lingner and Cech 1996). The active complex contained two proteins, a 123 kD and 43 kD protein, along with an RNA subunit. The larger protein proved to be the catalytic subunit - analysis of the predicted translation of the gene sequence revealed characteristic reverse transcriptase (RTase) motifs (Lingner *et al.* 1997). The requirement of the RTase motifs for telomerase activity was shown using Est2p, a yeast homolog. Conserved amino acids in the RTase motifs of Est2p were changed by site-directed mutagenesis, and the mutants transformed into yeast lacking the *est2* gene. Expression of the mutant proteins led to senescence and shortened telomeric tracts (Lingner *et al.* 1997). This provided the best evidence that the 123 kD protein from *Euplotes* and Est2p were the catalytic subunit of telomerase.

Despite knowledge of the DNA and protein sequences of two telomerases, direct cloning of the gene for human telomerase was not possible. Generally, using sequence from one species to find the homolog in an evolutionarily distant second species is most successful when used to probe cDNA libraries. Because humans have only 46 chromosomes (92 telomeres), very little telomerase protein - and very low amounts of RNA encoding telomerase - is expected, even in immortalized cells. Fortunately, in the late 1990s, large scale EST (expressed sequence tags) projects were underway, both in industry and in academia. A BLAST search of EST databases with the *Euplotes* telomerase sequence as the query sequence identified a single EST as a significant match (Kilian *et al.* 1997; Meyerson *et al.* 1997d; Nakamura *et al.* 1997). The EST sequence was then used to identify cDNA clones in libraries constructed from transformed human cell lines. Three research groups used this approach to nearly simultaneously isolate a human telomerase cDNA (Kilian *et al.* 1997; Meyerson *et al.* 1997c; Nakamura *et al.* 1997). The importance of this discovery was underscored by numerous write-ups in mainstream news organizations, such as the New York Times, the Washington Post, and Associated Press.

Subsequently, telomerase gene sequences have been cloned from a plethora of other organisms. The organisms include other mammals (e.g., macaque, mouse, rat, cattle, pig, dog), birds, amphibians (e.g., *Xenopus laevis*), echinoderms (e.g., sea urchin), insects (e.g., bee, silkworm, drosophila), *Caenorhabditis elegans*, *Trypanosoma brucei*, plants (e.g., corn, barley), and fungi (e.g., *Aspergillus*, *Cryptococcus*).

Telomerase has reverse transcriptase motifs

The human telomerase (hTERT) sequence identified by all three groups has an open reading frame of 1132 amino acids predicted to encode a 127 kDa protein. The identification of hTERT as a member of the telomerase family was based on the presence of motifs characteristic of the telomerase and reverse transcriptase (RT) family of enzymes. In particular, hTERT contains six conserved motif sequences, shown below aligned to homologous motifs in other telomerase sequences and to RT motifs in HIV-1 RT (because the boundaries of these motifs are based on similarity and identity with other telomerase sequences, the functional boundary of each motif may be different.). Consensus telomerase motif sequence is shown above and consensus RT motif sequence is shown below the alignments. Notably, the invariant aspartic acid residues implicated in RT catalysis are conserved in the hTERT sequence.

Fig. 2. Alignment of RT sequence motifs of telomerase proteins.

	Motif 1	Motif 2	Motif A
TRT con	hRhIPKK p	FRhI h h	PcLYFh hDh CYD I hhK K
hTERT	SRLRFIPKPDG-	LRPIVNMDYVVG	PPPELYFVKVDVTGAYDTIPQDRLTEVIASIIKP
Ea_p123	GKLRLIPKKTT-	FRPIMTFNKKIV	GQPKLFFATMDIEKCYDSVNREKLSTFLKTTKLL
Sc_Est2p	SKMRIIPKKSNN	FRIIAIPCRGAD	VLPELYFMKFDVKSCYDSIPRMECMRILKDALKN
Sp_Trt1p	AVIRLLPKKNT-	FRLITNLRKRFL	FGRKKYFVRIDIKSCYDRIKQDLMFRIVKKKLKD
HIV-1	TPVFAIKKKDST	WRKLVDFRELNK	LKKKKSVTVLDVGDAYFSVPLDEDFRKYTAFTIP
RT con	p hh h K	hR h K	h hDh GY h

	Motif B	Motif C	Motif D
TRT con	K Y Q GIPQGS LS hL h Y DL F	LLRL DDFLhIT	p N cK
hTERT	KSYVQCQGIPQGSILSTLLCSLCYGDMENKLFAGI	LLRLVDDFLLVT	YGCVVNLRKTVV
Ea_p123	KFYKQTKGIPQGLCVSSILSSFYYATLEESSLGFL	LMRLTDDYLLIT	NGFKFNMKKLQT
Sc_Est2p	KCYIREDGLFQGSSLSAPIVDLVYDDLLEFYSEFK	ILKLADDFLIIS	YNAKANRDKILA
Sp_Trt1p	SQYLQKVGIPQGSILSSFLCHFYMEDLIDEYLSFT	LLRVVDDFLFIT	HNFSTSLEKTVI
HIV-1	GIRYQYNVLPQGWKGSPAIFQSSMTKILEPFKKQN	IYQYMDDLYVGS	WGLTTPDKKHQK
RT con	hPQG pP hh h	h Y DDhhh	Gh h cK h

For historical reasons, the motifs are called “1”, “2”, “A’”, “B”, “C”, and “D”.

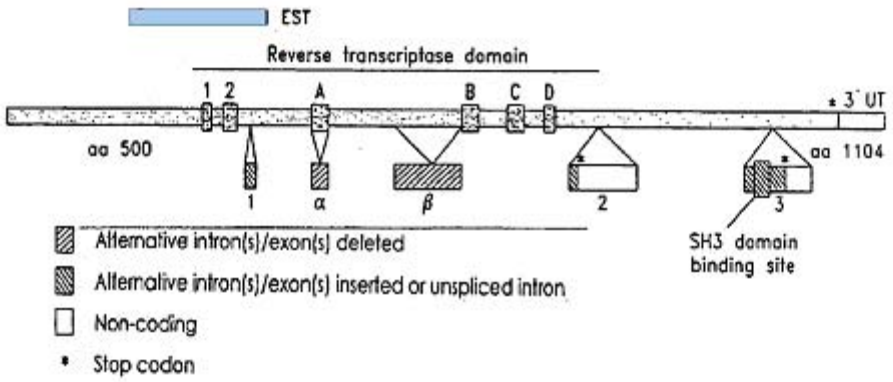
Although the overall homology among the telomerase proteins is relatively low (approximately 40% similarity in all pair-wise combinations), the overall structure of the protein seems to be well conserved. Four major domains: N-terminal, basic, reverse transcriptase (RT) and C-terminal are present in all telomerase proteins. The regions of most sequence similarity are within the RT domain and in a motif located N-terminal to motif 1 (Nakamura *et al.* 1997; Meyerson *et al.* 1997b). This first motif, called motif T, is not found in RTs or other proteins, suggesting that it may be specific to the telomerase family.

Structure of RNA splice variants

In addition to the cDNA sequence encoding the 1132 amino acid telomerase (referred to as “reference” or “wild-type” telomerase), cDNAs were found that encoding differing lengths of telomerase. In fact, the initial cDNA clones characterized by Nakamura et al. and Meyerson et al. (Nakamura *et al.* 1997; Meyerson *et al.* 1997a) had a 182 bp deletion that resulted in a shortened telomerase due to presence of a premature stop codon.

As it turns out, transcription of the single-copy gene produces a number of variant telomerase transcripts. While assaying for expression of telomerase by amplification of cDNA synthesized from normal cells, immortalized cells and tumor cells RNA, Kilian et al. noticed multiple amplification products (Kilian *et al.* 1997). The amplification products were isolated, and their DNA sequence determined. Some of the products contained insertion of sequence and others deletion of sequence relative to the reference sequence. One of the deletions (β – 182 bp) corresponded to the deletion found by Nakamura et al. and Meyerson et al.

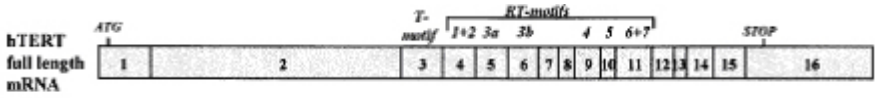
Fig. 3. Alternative sequences used in splicing of hTERT transcript.



Based on cDNA analysis, at least six different alternative sequences appear to be retained in mRNAs (see Figure 3, which shows 5 of the 6 alternatives). The sixth and 5'-most alternative sequence (not shown in Fig. 3) has an unknown length. The nucleotides that are inserted or deleted relative to the reference telomerase are called “alternative sequences”. Because these sequences are derived from partial or entire exons and introns, “alternative sequences” is chosen as a neutral term.

The human telomerase gene is composed of 16 exons and 15 introns spanning approximately 40 kb of chromosome 5 (Wick *et al.* 1999; Leem *et al.* 2002). The size of the exons ranges from 62 to 1354 bp in length. The reference hTERT mRNA (Fig. 4) contains all 16 exons.

Fig. 4. Reference hTERT mRNA structure.



For each of the known RNA splice variants, the effect of the presence/absence and location of each alternative sequence is presented on the assumption that it is the only alteration. It will be appreciated that a particular alternative sequence may alter the sequence of the translated product, regardless of whether other alternative sequences are spliced in or out. For example, the presence of alternative sequence 1 results in a frameshift and truncated protein, regardless of whether alternative sequences a, b, 2 or 3 are spliced in or out.

Alternative sequence X is derived from intron 3. Alternative sequence X was found inserted between bp 1769 / 1770, but was of unknown length (Kilian and Bowtell – WO 99/01560). Intron 3 is 2089 bp, thus sequence X could be that long. Because of stop codons present in all three reading frames of alternative sequence X, hTERT that contains X would result in a truncated protein that contains approximately 600 N-terminal amino acids and lacks all of the RTase motifs.

Alternative sequence 1 (Kilian and Bowtell – WO 99/01560), inserted at nucleotide 1950/1951, contains the first 38 bp of intron 4, which is 687 bp long. Its presence in mRNA causes a frame-shift and ultimate translation of a truncated protein due to a stop codon at nt 1973. This truncated protein contains only RTase domains 1 and 2.

Alternative sequence a (Kilian and Bowtell – WO 99/01560), located at bp 2131-2166 in the reference hTERT, is frequently observed spliced out of telomerase mRNA. Because these 36 bp are only part of exon 6, presumably the in-frame deletion of this variant results from an alternative 3'-splice acceptor sequence in exon 6. A protein translated from such an RNA is deleted for 12 amino acids, removing nearly all of RTase motif A. This motif appears to be critical for RT function; a single amino acid mutation within this domain in the yeast EST2 protein results in a protein that functions as a dominant negative and results in cellular senescence and telomere shortening.

Another RNA splice variant is deleted for alternative sequence β (Kilian and Bowtell – WO 99/01560). The deletion encompasses all of exons 7 and 8 -- bp 2286-2468 – and encodes a truncated protein, due to a reading frameshift at bp 2287 leading to a termination codon at bp 2605. This variant protein has RTase domains 1, 2, A, B, and part of C, but lacks a motif (AVRIRGKS SEQ. ID NO:90) identified in the b sequence. The motif matches a P-loop motif consensus AXXXXGK(S) found in a large number of protein families including kinases, bacterial dnaA, recA, recF, mutS and ATP-binding helicases (Saraste *et al.* 1990). The importance of the P-loop in hTERT remains to be investigated.

Alternative sequence 2 – unknown length, derived from intron 11, which is 3801 bp – is inserted between bp 2843 / 2844 (Kilian and Bowtell – WO 99/01560). The sequence contains an in-frame termination codon at its extreme 5-end, resulting in a truncated protein of 948 amino acids, which has the entire RTase domain region, but lacks the C-terminus. Mutations constructed in the C-terminus region (from about aa 926 to 1132) revealed that regions E-I to E-IV (located from 926-1100) are essential for catalytic and biologic function of hTERT, while mutations at the extreme C-terminus (aa 1127) generated a catalytically active but functionally dead protein (Banik *et al.* 2002). Cells expressing the +1127 mutant failed to immortalize due to shortened telomeres. Whatever the exact mechanism that causes this phenotype, it is clear that the C-terminus plays a critical regulatory role in humans, and that the product of the RNA splice variant containing alternative sequence 2 lacks the C-terminus.

In addition to the variant above, the product of a second splice variant also lacks the reference C-terminal domain (Kilian and Bowtell – WO 99/01560). In this splice variant, alternative sequence 3 (derived from the 3'-most 159 bp of the 781 bp intron 14) is inserted at bp 2157/2158. An in-frame stop codon within alternative sequence 3 results in a protein having an alternative C-terminal domain. Furthermore, the coding region donated by alternative sequence 3 contains a potential SH3 binding site, SGQPEMEPPRRPSGCVG, which matches the consensus c-Abl SH3 binding peptide (PXXXXPXXP) found in proteins such as ataxia telangiectasia mutated (ATM). Curiously, this motif is also found near the N-terminus of hTERT (HAGPPSTSRPPRPWDTP, amino acids 304-320).

A transcript lacking all of exon 11 (189 bp spanning nucleotides 2655 to 2843) was found in hepatocellular carcinoma cell lines (Hisatomi *et al.* 2003). The protein encoded by this variant doesn't have a reading frameshift and is 63 amino acids shorter than the reference protein. Exon 11 contains RT motifs D and E, suggesting that the splice variant is missing residues from the catalytic site of the protein.

Finally, an RNA splice variant was found to contain 600 bp of intron 14 fused directly to exon 16 (Wick *et al.* 1999). An in-frame stop codon close to the 5'-end of intron 14 causes a prematurely terminated hTERT protein.

Expression of RNA splice variants

The critical issue is the importance and specific role of hTERT splicing variants in regulation of telomerase activity and as a potential marker of health status and survival. Despite the provocative predictions for activities of products of splice variants, little direct evidence has accumulated.

Of particular interest however, the splice variant deleted for alternative sequence a is a dominant-negative inhibitor of hTERT activity in cell lines (Colgin *et al.* 2000; Saraste *et al.* 1990; Yi *et al.* 2000). The variant hTERT that causes these effects lacks a mere 12 amino acids, although these 12 aa contain conserved RT motif A. When over expressed in immortalized fibroblasts and carcinoma cells, the variant inhibited telomerase activity. Furthermore, these cells also exhibited progressively shortening telomeres and eventually apoptotic cell death or a senescence-like state. Importantly, these data suggest that telomerase activity is controlled in part by post-transcriptional events.

Most of the data regarding telomerase splice variants is descriptive, mainly observations of the quality and quantity of splicing variants of hTERT in normal, immortal, and cancer cells. Although some correlations are observed (e.g., in kidney, different splice variants appear dependent on the level of telomerase activity (Ulaner *et al.* 1998) a number of different variants are expressed in cancer cells (Barclay *et al.* 2005; Fujiwara-Akita *et al.* 2005; Fujiwara *et al.* 2004; Hisatomi *et al.* 2003; Nagao *et al.* 2004; Ulaner *et al.* 2000; Yokoyama *et al.*
2001) without apparent commonality. The complexity of the system and low expression and activity levels in some cells add to the difficultness of deciphering the intricacies of telomerase regulation.

A word or two about nomenclature

"Telomerase" is used in this landscape paper to mean the catalytic subunit (i.e., protein with enzymatic activity) of the telomerase complex. Some use the term "telomerase" to mean the whole ribonucleoprotein complex, of which the enzyme is but one component. Thus, when reading articles or patents about telomerase, the reader is cautioned to confirm how the term is used in the context of the document.

Furthermore, in early papers and patent applications, the human protein and gene name of the catalytic subunit was variously designated as hTRT, hEST2, and hTCS1. While hTRT is still sometimes seen, the consensus term is hTERT. In this report, hTERT is used regardless of the nomenclature in the original document.

The purpose of this patent landscape analysis is to inform the reader of the major patents and players in the area of telomerase. It is intended as a source of scientific information or to facilitate risk management by uncovering patented technology that may need to be licensed or avoided in order to make and use a product.

Strategy for finding patent documents

Searches were performed using keywords (e.g., telomerase, splice variants), company names (e.g., Geron, Bayer), and inventors' names (e.g., Shay). Both Patent Lens and PatBase (www.patbase.com) database were queried. The general approach to finding the most relevant patent documents is a scan of titles, abstracts, and lead claim when available. Generally, scanning titles for relevance is the quickest and easiest way to comb through many documents; when a title or abstract was suggestive, but inconclusive about the claimed subject matter, the lead claim was reviewed. In this way, a manageable list of about 100 documents was obtained. A second review of this list entailed a review of the independent claims. Ultimately, a final list of documents was selected.

Decisions were made to exclude certain documents. In particular, if the only document in the patent family were a Japanese patent or patent application, the document was omitted from analysis. The only substantive

information from these documents was a translation of the abstract, which may not accurately reflect the claims. In addition, for areas that contained U.S. patents, U.S. applications were not analyzed. Updates of this report will consider any that have been granted in the interim.

Limitations of patent searches

Two main types of limitations may affect the outcome of a search: those that are inherent in the data and those resulting from the search process. While every effort is made to minimize the limitations and their effects, inevitably there will be some. These limitations and their effect are discussed in this section to promote appropriate and informed reliance on the patent data and analyses.

Data are not error free. Common types of data problems include misspellings, alternate spellings especially of names and assignees, inconsistency of patent examiners in classifying patent subject matter, translation errors (especially for Japanese documents, but also for European patent documents). Some of these errors can be overcome by using wildcards in formulating the search or broadening the scope of the search; however, other errors inherent in the data cannot be neutralized.

Another source of possible problems arises from the INPADOC data, which includes patent information of more than 70 countries. Because countries use a variety of different update schedules (two weeks to one year) and report different types of information (e.g., not all report legal status), it is not always possible to verify filing or legal status of a patent application in national patent offices.

Furthermore, the search process may engender additional consequences. Although using a combination of key term and classification criteria will usually capture all the key patents and applications in a field, the downside is that too many documents to realistically further screen may be found. When there is a substantial number of documents recovered (more than a few hundred), or the primary document is not in English, the only practical means of identifying potential key documents is on the basis of the title and abstract, which may or may not represent an accurate picture of the claimed subject matter.

As well, patents are dynamic. On a steady basis, new patent applications are filed and published, new patents are granted, patents are abandoned, and the law changes too. Therefore, this report is but a snapshot of the landscape as it appeared in September 2006.

Claim construction rules

The interpretation of a claim is primarily based on the plain language of the claim and on definitions, explicit or implicit, in the text of the patent document. Of course, claims in patent applications have not been examined and may well be different when granted (if granted at all). For granted claims, a more precise interpretation requires a reading of the prosecution history – the written record of the examination process between a patent office and the patent owner – for instances of the patent owner stating what the claims mean or giving up claim scope.

Patent claims are intended to provide notice to others of the scope of the protected invention. Unfortunately, more often than not, reality falls short of the ideal. Part of the challenge, when reading a claim, is to set aside your own biases about what words mean and question the definition of each term. While technical terms beg definition and thus often become the subject of dispute, common words may also be contested. Recently, patent claim construction in litigation has sometimes centered on disputes regarding definitions of words as “about” and “adjacent”, among others.

Claims often use terms, especially technical terms, that require a bit of detective work to understand their meaning. The steps of investigation include identifying terms that need defining (generally, words other than common English words), looking in the text of the patent document for an explicit definition or use of the term, look in the prosecution history for definition or use of the term, and consulting a dictionary or expert in the field, if available. Pursuit of some of these steps is not within the scope of this report; the order that the steps are performed and the weight given to each is currently the subject of hotly contested litigation, but for our purposes starting with the plain language of the claim and incorporating definitions from the text of the patent will provide a useful working interpretation.

While reading claims, a very important rule of claim construction is useful to keep in mind: when the term “comprising” is used, a device that has more elements than the claimed device falls within the scope of the claim. Thus, if the claim recites “an antibody comprising A, B, and C” and there is an antibody made up of A, B, C, and D, the second antibody falls within the claim. Conversely, an antibody made up of A and B does not

fall within the claim.

Other useful rules:

- the term “a” or “an” means *one or more*;
- a patentee can write her own definitions of terms;
- claims are interpreted by the current law, not the law at the time of grant.

Note that in addition to issued patents, patent applications are included in this report. Sometimes, the claims of patent applications are written so broadly that it is not readily apparent what claim language is likely to be granted, if there is a grant.

Content of survey

In the following section of this report, an overview of the patent landscape is presented. Attention is drawn to the key patents – those that appear to have the broadest claims. For a number of reasons, the report analyses mainly patents sought or granted in the United States: the largest key player, Geron, is based in the United States, U.S. patents are readily obtained, the law regarding claim interpretation is most well developed. In contrast, because patents granted by the European Patent Office are interpreted according to each country’s laws, the body of patent law isn’t homogeneous.

The documents discussed in this report are a mix of patent and patent applications. For patent applications, caution is warranted when evaluating the claim scope because the claims have not been examined and may differ if and when granted.

The format of the survey generally contains a summary of the patent description, a listing of the most relevant independent claim(s) and discussion of the claim meaning.

DISCLAIMER

The analyses presented herein are informational ONLY. They are not to be construed as specific legal advice. As advice on infringement depends upon the specific circumstances of each party and the laws of the country where the allegedly infringing activities take place, nothing provided herein should be used as a substitute for advice of counsel on the particular matter at issue.

Patent landscape of telomerase

The patent documents discussed in this report are divided into four categories depending on the protected subject matter:

- Wild-type telomerase
- Variants of telomerase
- Diagnostics
- Stem cells

The selected patents and patent applications were chosen as those with the broadest claims in the area.

Wild-type telomerase

This section presents patents that claim aspects of nucleic acid sequences encoding wild-type[1] telomerase. What is “wild-type” telomerase? For this landscape analysis it is defined as the catalytic protein containing 1132 amino acids in human and homologues in other species. This definition, prevalent in scientific literature and in patents, has been arrived at somewhat arbitrarily. It came about from the first publications describing the cloning of human telomerase (Meyerson *et al.* 1997e; Nakamura *et al.* 1997) in which two sequences of telomerase were found, but only one of which had an open reading frame encoding the known motifs of telomerase.

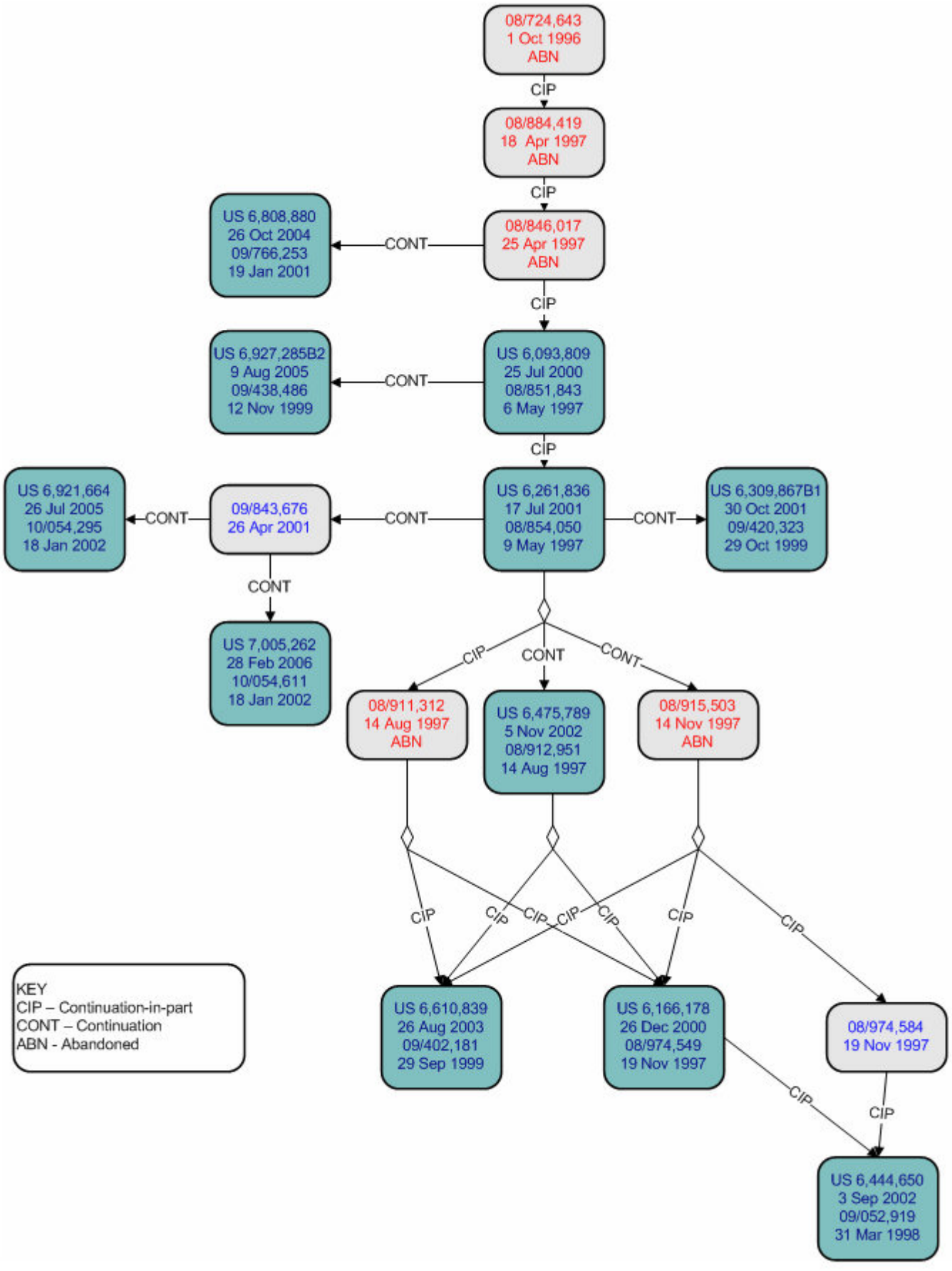
[1] In some publications, the term “reference telomerase” is used synonymously with “wild-type telomerase”.

Human gene sequences

Not surprisingly, the co-assignees Geron Corp. and University of Colorado (current assignee of University Technology Corp., the former Colorado University organization that handled licensing for all CU campuses) dominate this area, in sheer numbers of patents as well as in scope. A minimum of 16 U.S. patent applications claim priority from an initial filing in October 1996 (which did not contain any human-derived nucleotide sequences). At least 10 of these applications have resulted in granted U.S. patents, some of which are directed to human telomerase and others to homologues from other species. In addition, numerous patent applications (up to about 55) were filed in countries other than the United States. Many of these have been granted.

Before launching into a discussion of the Geron / University of Colorado patents, a few comments are warranted on the likely disposition of a patent application filed by Whitehead Institute (Boston, MA). A series of U.S. provisional patent applications were filed between February and October 1997, which were based on the work of the Robert Weinberg lab (Meyerson *et al.* 1997e). A PCT application (WO 98/37181) was also filed, but never converted into national applications in the non-U.S. designated countries. Because U.S. patent procedures were conducted in secret until 2000, nothing is known about the status of any U.S. patent applications. Because no patent has issued to date, any U.S. applications most likely have either been abandoned or are in interference[1] with one or more Geron / UC patents.

Fig 5. Brief history of Geron / University of Colorado selected patent application filings



Following a series of rapid succession of filings of patent applications on 18 April, 25 April, and 6 May 1997, containing incomplete sequences of human telomerase, on 9 May 1997, the Geron / University of Colorado groups filed a patent application with the full-length sequence. US 6,261,836 B1 was granted on 17 July 2001 from this filing; its claims are directed to "human telomerase reverse transcriptase protein".

With respect only to the disclosure related to human telomerase, the application details the cloning of hTERT. Briefly, partial homologous sequences were identified in a BLAST search of publicly available EST sequences queried against the *Euplotes* and *Schizosaccharomyces* telomerase sequences. A match was scored for an EST sequence derived from a partial cDNA clone (GenBank accession AA281296). When the open reading frame of this clone was aligned to the query sequences, the presence of signature motifs

strongly suggested that this was the long-sought after human telomerase. Using standard techniques, additional 5' sequence information was obtained as well as a lambda vector clone (l25-1.1) that had complementary sequence that was sub-cloned into a plasmid (pGRN121 – ATCC accession no. 209016). The sequence of the insert revealed the entire open reading frame encoding the human telomerase protein. Interestingly, pGRN121 contained an insertion of 182 nucleotides relative to the GenBank accession AA281296. The open reading frame of pGRN121 encoded a protein of 1132 amino acids having a molecular weight of approximately 127,000 daltons. SEQ ID No: 224 presents the nucleotide and amino acid sequences of hTERT.

The independent claims are directed to nucleic acid sequences encoding the hTERT protein, hTERT protein encoded by the sequences, cells comprising the nucleic acid sequence, and a method of preparing the telomerase complex using the claimed hTERT protein and a telomerase RNA component.

Claim 1 asserts a "synthetic or recombinant human telomerase reverse transcriptase" (hTERT)

- protein;
- variant of the protein;
  - variant is encoded by a polynucleotide that hybridizes under stringent conditions to a complement of SEQ ID NO: 224 (hTERT); or
- fragment of the protein;

AND

- the protein, variant or fragment has telomerase catalytic activity when complexed with a telomerase RNA component.

Literally read, the claim terms, "hTERT protein", "variant of the protein", and "fragment of the protein" extend beyond the disclosed amino acid sequence. The term "hTERT protein" has no limitation of specific sequence, as would have been expected. Its only limitation is the requirement of catalytic activity (discussed below). But what exactly falls within the scope of "hTERT protein"?

Reading the claim literally, *any* human TERT protein that has catalytic activity would be encompassed. Any hTERT protein could arguably include polymorphisms, RNA splice variants, deletions, etc. It's unclear whether or not the modifier "human" means that the sequence has to be a natural sequence. If the telomerase is not a natural sequence found in humans, then what makes a telomerase a "human" telomerase? Another reason to believe that only natural sequences, e.g., the amino acid sequence of SEQ ID NO: 225 and polymorphisms, are encompassed is the recitation of "variant of the protein" as an alternative element. If the term "protein" also included non-natural sequences such as those encoded by hybridizing polynucleotides, then there wouldn't have been any need to recite "variants" in the claim.

Reading the claim in light of the specification, however, an argument can be made that the term only encompasses the specific amino acid sequence set forth in SEQ ID NO: 225, because that is the only amino acid sequence shown. (The Federal Circuit Court applies a strict written description requirement to biotechnology inventions, which usually limits the claims to the precise sequences disclosed in the patent application.) This interpretation however presumes that a court would find it so. As it stands in 2006, though, most patent lawyers lament the lack of certainty of claim interpretation.

While by no means certain, the term hTERT protein probably means just SEQ ID NO: 225. This conclusion is supported by the presumed meaning of "fragment thereof", which refers to sequences found *within* SEQ ID NO: 225 – see below for more discussion. If hTERT refers just to SEQ ID NO: 225, then polymorphisms of hTERT and RNA splice variants are excluded.

"Fragments" of hTERT are contemplated to be "of various lengths. In one embodiment, the portion of polypeptide comprises fragments of lengths greater than 10 amino acids. However, the present invention also contemplates polypeptide sequences of various lengths, the sequences of which are of which are included within SEQ ID NOS: …225, from 5 to 1100 amino acids (as appropriate, based on the length of (SEQ ID NOS: ….. 225)." So, from this description, is the minimal length of a fragment 5 amino acids? 10 amino acids? Or some other length? Because of the requirement that the fragment have catalytic activity, the minimum length of a fragment is not terribly relevant. To have catalytic activity, a fragment of hTERT would need to be substantially longer than 5 or 10 amino acids. What we can infer from the description however is that the fragment sequence is found within SEQ ID NO: 225. So, fragments of polymorphic hTERT or RNA splice variant hTERT do not appear to be encompassed by this element of the claim.

On the other hand, "variant of the protein" carries a definition in the claim itself – a protein encoded by a hybridizing nucleic acid, which hybridizes under "stringent conditions". According to the patent application, "stringency" typically occurs in a range from about 5° C to about 20° C to 25° C below Tm of the probe. From the point of view of hybridization kinetics, this is not much of a limitation. While it is often stated that the maximum divergence of sequences that hybridize at about 20-25° C below Tm is 25% mismatch, it is the stringency of the wash conditions that generally controls the amount of mismatch (or conversely, the amount of identity) observed.[2] The common hybridization condition of 20-25° C below Tm is a consequence of experiments begun in the 1970s by Wetmur and Davidson, who determined that this temperature resulted in maximal hybridization efficiency, not necessarily maximal hybridization specificity. Even though it is an erroneous belief, many, or most, scientists would consider the stated stringent hybridization conditions to yield nucleic acid molecules having at least 75-80% identity to the probing sequence. The various RNA splice variants would hybridize under these conditions, as would many related sequences. Because of the activity requirement, at least some of the splice variants would necessarily be excluded from the claimed telomerase molecules.

Any protein / variant / fragment of hTERT has a major limitation in that it *must* have "telomerase catalytic activity" when complexed with a telomerase RNA. While this term is not explicitly defined, the most probable meaning is that the catalytic activity is using a "portion of its internal RNA moiety as a template for telomere repeat DNA synthesis" and more specifically, "extend[ing] the G strand of telomeric DNA." (col. 3, lines 17-30) Example 4 provides the method for assaying telomerase activity; no other assay appears to be described. The assay is for extension of dGTP on an oligonucleotide substrate; assay conditions do not mention the inclusion of a telomerase RNA component, however.

US 6,921,664
has identical disclosure to US '836. The claims however recite a recombinant expression vector comprising an encoding region for telomerase protein, a variant or a fragment. As for the claims of US '836, the protein, variant or fragment must have telomerase catalytic activity when complexed with a telomerase RNA. Similarly, the nucleic acid encoding telomerase hybridizes to the complement of SEQ ID NO: 224 under stringent conditions.

Because of how the claim is written, it appears that a nucleic acid sequence that incorporates enough codon differences (taking advantage of codon degeneracy) wouldn't fall within the scope of this claim because it couldn't hybridize to the complement of SEQ ID NO: 224.

US 6,927,285
has a disclosure written prior to establishing full-length telomerase sequence. Only a partial sequence, replete with sequence errors, was presented. To overcome that deficiency, Geron / University of Colorado made a biologic deposit of the plasmid pGRN121, which contained an insert encoding the full-length telomerase protein. Under U.S. patent law a biological deposit can satisfy the enablement - and written description - requirements. The deposit was therefore a wise decision.

This patent has six independent claims to the telomerase sequence. With reference to pGRN121, both claim 1 and claim 5 are directed to an isolated cDNA encoding hTERT, wherein the cDNA is contained in pGRN121 (claim 1) or wherein the cDNA hybridizes to the insert of pGRN121 (claim 5). The cDNA of Claim 2 has the restriction map of pGRN121.

Claims 3 and 6 claim an isolated nucleic acid encoding a "naturally occurring" hTERT or variant thereof (claim 3) and an isolated cDNA encoding hTERT (claim 6), wherein the nucleic acid or the cDNA hybridize to the incomplete sequence presented as SEQ ID NO: 173. Claim 4 recites SEQ ID NO:173 with a 5'-Met codon.

Usually claims to a deposited sequence would be considered to be relatively narrow. Essentially such claims are equivalent to a claim to SEQ ID NO: N. In a twist however, claims in this patent recite hybridizing sequences, effectively broadening the scope. The requirement exists though that the nucleic acids must encode human telomerase reverse transcriptase protein. As discussed for other patents in this section, a plausible interpretation is that the claim encompasses the "wild-type" or full-length telomerase and possibly polymorphisms.

A continuation-in-part of US 6,261,836, the claims of **US 6,475,789** are directed to mammalian cells that contain a recombinant nucleic acid sequence that encodes hTERT. The disclosure of US '789 first describes the consensus motifs that are characteristic of TERT proteins and nucleic acids identified in species such as *Oxytrichia*, *Schizosaccharomyces*, *Euplotes*, and human. Most of the remaining disclosure provides pages of detail about the human gene and protein, including how to clone the gene, recombinant expression of the protein, purification of the protein, and raising antibodies to the protein. Multiple activities of the protein are discussed along with descriptions of assays for the activities. The activities include reverse transcriptase

activity, telomere binding, dNTP binding, and telomerase RNA binding.

Uses for the nucleic acids and proteins comprise the remainder of the disclosure. The uses include treatment of cancer and other diseases and conditions, vaccine production, and increasing the proliferative capacity and production of immortalized cells and animals. As well, diagnostic assays for the presence of telomerase are provided. These assays are proposed for diagnosis and prognosis of cancer and other conditions and monitoring cells in culture, among other uses.

Although the disclosure is hefty, because of U.S. patent law, claims are limited to one invention – as determined by the U.S. Patent Office.[3] In this case, the eight claims are directed to:

A mammalian cell that contains

· a nucleic acid sequence that encodes TERT protein, variant or fragment having telomerase catalytic activity;

o the TERT sequence hybridizes to the hTERT sequence (ID NO: 1) using stringent conditions.

The terms and hybridization conditions have all been discussed above for US '836. Whereas the claims of US '836 claim a TERT protein (or variant or fragment), the claims here are directed to nucleic acid sequences. Because of how the claim is written, it appears that a nucleic acid sequence that incorporates enough codon differences (taking advantage of codon degeneracy) wouldn't fall within the scope of this claim because it couldn't hybridize to SEQ ID NO: 1.

The title of US 6,444,650 is "Antisense compositions for detecting and inhibiting telomerase reverse transcriptase." This very slim patent is directed to antisense oligonucleotides that specifically anneal to hTERT nucleic acid molecules. These include not only sequences encoding hTERT, but also any upstream, flanking, noncoding, and transcriptional control elements, hTERT pre-mRNA, mRNA, cDNA, and the like. Antisense is considered to be at least 7 nt up to about 100 nt, but is often in the range from 10-50 nt. The inventors prefer approximately 30 nt long antisense.

Like their other patent applications, "specific binding" or "specific hybridization" is that annealing to a target polynucleotide that occurs under stringent conditions. In this patent, "stringent conditions" are also defined as from about 5 to about 20 to 25° C below Tm of the target sequence in 1 M NaCl. (See discussion about this definition under US 6,261,836.) Other definitions of terms are broader in this disclosure than in earlier disclosures – e.g., hTERT activity now refers to one or more of activities found in naturally-occurring full-length hTERT protein.

The lead claim recites antisense oligonucleotides that

· hybridize to SEQ ID NO: 1 under stringent conditions; and

· inhibits expression of hTERT mRNA.

Although the text of the patent discusses antisense oligonucleotides that anneal to other than hTERT coding sequences, the claim limits them to annealing to SEQ ID NO: 1. This sequence contains approximately 50 bases of 5'-noncoding sequence, the entire hTERT coding sequence, and approximately 575 bases of 3-noncoding sequence. According to the claim, the antisense could have a different complementary sequence than found in SEQ ID NO: 1 as long as the antisense anneals under the stated conditions. As is well known, hybridization kinetics and duplex stability is not the same for oligonucleotides as it is for polynucleotides over about 600 bases long. Base mismatches in short duplexes can significantly affect thermal stability. With these considerations, this claim is unlikely to encompass widely divergent oligonucleotide sequences; a prediction of the likely boundaries is outside the scope of this landscape.

Rounding out the group of Geron patents, US 6,610,839 claims hTERT promoter sequences. A human genomic DNA library was screened to identify a clone containing hTERT coding sequences. One isolated clone (lGF5) contained approximately 13 kb of DNA upstream of the start site of the cDNA sequence. Subfragments of this region were tested for promoter activity by linking them to a reporter gene sequence. Claims were granted to the deposited lambda phage clone, hybridizing nucleic acid sequences, and specific sequences, along with 80% identical sequences.

Patent Data	Title and relevant claims	Family Data**
US 6,261,836	Telomerase	See Appendix 1 for

Assignee:

Geron Corp.

University Technology Corp.

Earliest priority:

01 Oct 1996

Filed:

09 May 1997

Granted:

17 Jul 2001

Expiry date:

01 Oct 2016 *

Claim 1: A synthetic or recombinant human telomerase reverse transcriptase (hTRT) protein, or a variant thereof, or a fragment thereof, wherein said variant is encoded by a polynucleotide that hybridizes under stringent conditions to a polynucleotide having a sequence complementary to SEQ ID NO: 224, and wherein said hTRT protein, variant, or fragment has telomerase catalytic activity when complexed with a telomerase RNA.

See Appendix 1 for complete list of family members.

Patents:

AU 734089

GB 2317891

EP 841396

Patent applications:

CA 2267664

EP 0954585

EP 1333094

US 6,444,650

Antisense compositions for detecting and inhibiting telomerase reverse tran-scriptase

Assignee:

Geron Corp.

University Technology Corp.

Earliest priority:

01 Oct 1997

Filed:

31 Mar 1998

Granted:

03 Sep 2002

Expiry date:

01 Oct 2017 *

Claim 1: An isolated antisense oligo-nucleotide that hybridizes to a target DNA having the nucleotide sequence of SEQ. ID NO:1 at 5° C. to 25° C. below Tm in aqueous solution at 1 M NaCl; wherein Tm is the melting temperature of a complementary oligonucleotide hybridized to the target DNA in aqueous solution at 1 M NaCl, wherein the complementary oligonucleotide is exactly complementary to SEQ. ID NO:1 and the same length as the antisense oligonucleotide; and wherein hybridization of the antisense oligonucleotide to an mRNA encoding hTRT (SEQ. ID NO:1) inhibits expression of the mRNA.

See Appendix 1 for complete list of family members.

Patents:

EP 841396

Patent applications:

CA 2267664

EP 0954585

US 6,475,789

Human telomerase catalytic subunit: diagnostic and therapeutic methods

Assignee:

University Technology Corp.

Geron Corp.

Earliest priority:

01 Oct 1996

Filed:

14 Aug 1997

Granted:

Claim 1: A mammalian cell that contains a recombinant polynucleotide comprising a nucleic acid sequence that encodes a telomerase reverse transcriptase protein, variant, or fragment having telomerase catalytic activity when complexed with a telomerase RNA, wherein said recombinant polynucleotide hybridizes to a DNA having a sequence complementary to SEQ ID NO: 1 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl, wherein T_m is the melting temperature of a complementary polynucleotide hybridized to said DNA in aqueous

See Appendix 1 for complete list of family members.

Patents:

AU 734089

GB 2317891

EP 841396

Patent applications:

CA 2267664

EP 0954585

05 Nov 2002 **Expiry date:** 01 Oct 2016 *	solution at 1M NaCl, wherein the complementary polynucleotide is exactly complementary to SEQ ID NO: 1 and is the same length as the recombinant polynucleotide.	
US 6,610,839 **Assignee:** Geron Corp. **Earliest priority:** 14 Aug 1997 **Filed:** 01 Oct 1997 **Granted:** 26 Aug 2003 **Expiry date:** 14 Aug 2017	**Promoter for telomerase reverse transcriptase** Claim 1: An isolated nucleic acid comprising a promoter sequence that either: a) is contained in lambda phage GΦ5 deposited as ATCC Accession No. 98505; or b) hybridizes to the DNA of lambda phage GΦ5 at 5 to 25° C. below the melting temperature (T_m) of a double-stranded DNA having the sequence of lambda phage GΦ5 in aqueous solution at 1 M NaCl; wherein the promoter sequence promotes transcription in cells endogenously expressing human telomerase reverse transcriptase (hTRT).	See Appendix 1 for complete list of family members. Patents: GB 2321642
US 6,921,664 **Assignee:** University of Colorado Geron Corp. **Earliest priority:** 18 Apr 1997 **Filed:** 18 Jan 2002 **Granted:** 26 Jul 2005 **Expiry date:** 18 Apr 2017 *	**Telomerase** Claim 1: A recombinant expression vector containing a polynucleotide that comprises an encoding region for a telomerase reverse transcriptase protein, variant, or fragment, wherein the protein, variant or fragment has telomerase catalytic activity when complexed with a telomerase RNA, and wherein a single-stranded DNA consisting of said encoding region hybridizes to a second single-stranded DNA at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl, wherein said second DNA is exactly complementary to SEQ. ID NO:224, and T_m is the melting temperature under the same reaction conditions of double-stranded DNA having the sequence of SEQ. ID NO:224.	See Appendix 1 for complete list of family members. Patents: EP 841396 Patent applications: CA 2267664
US 6,927,285 **Assignee:** Geron Corp. University Technology Corp. **Earliest priority:** 18 Apr 1997	**Genes for human telomerase reverse transcriptase and telomerase variants** Claim 1: An isolated cDNA encoding human telomerase protein, wherein said cDNA is contained in plasmid pGRN121 having ATCC Deposit Accession No. 209016. Claim 3: An isolated nucleic acid encoding a naturally occurring human telomerase reverse transcriptase protein	See Appendix 1 for complete list of family members. Patents: AU 734089 GB 2317891 EP 841396 Patent applications:

Filed: 12 Nov 1999 **Granted:** 09 Aug 2005 **Expiry date:** 18 Apr 2017 *	or variant thereof, wherein the polynucleotide hybridizes to a nucleic acid having the sequence in SEQ ID NO:173 at 5C to 25C below T_m in aqueous solution at 1M NaCl.	CA 2267664 EP 0954585 EP 1333094

** Patents and applications that are published in English and that have claims similar to the listed United States patent are specifically mentioned. The claims needed to be quite similar before it was included. Even if not mentioned, a non-U.S. patent could have claims that encompass similar subject matter. For example, a patent claiming a cell having an introduced nucleic acid encoding telomerase would encompass a cell containing an expression vector expressing telomerase, but is not listed as an "equivalent".

Homologous telomerase gene sequences

This group of patents – three of which are owned by Geron and University of Colorado (individually or jointly), one of which is co-owned by Geron and Albert Einstein College of Medicine, and one of which is owned by Research & Development Institute in Montana – concern nucleic acid sequences and gene products of telomerase from other species. By no means does this group exhaust the species from which telomerase gene has been cloned; telomerase from other species can be found in the scientific literature.

The species represented in this group are:

- *Candida albicans* (US 6,541,202);
- *Euplotes* (US 6,093,809 and US 6,166,178);
- *Schizosaccharomyces pombe* (US 6,309,867);
- *Mus musculus* (US 6,767,719).

Patent Data	Title and relevant claims	Family Data
US 6,093,809 **Assignee:** University Technology Corp. Geron Corp. **Earliest priority:** 01 Oct 1996 **Filed:** 06 May 1997 **Granted:** 25 Jul 2000 **Expiry date:** 01 Oct 2016	**Telomerase** Claim 1: An isolated polynucleotide consisting of the nucleic acid sequence shown in SEQ. ID. No. 1. No other claims.	No functionally related patents or applications – published in English

US 6,166,178 **Assignee:** University Technology Corp. Geron Corp. **Earliest priority:** 01 Oct 1996 **Filed:** 19 Nov 1997 **Granted:** 26 Dec 2000 **Expiry date:** 01 Oct 2016	**Telomerase catalytic subunit** Claim 1: An isolated polypeptide consisting of the amino acid sequence shown in SEQ ID. NO. 110. No other claims.	No functionally related patents or applications – published in English
US 6,309,867 **Assignee:** University Technology Corp. **Earliest priority:** 01 Oct 1996 **Filed:** 29 Oct 1999 **Granted:** 30 Oct 2001 **Expiry date:** 01 Oct 2016	**Telomerase** Claim 1: An isolated polypeptide consisting of the amino acid sequence shown in SEQ. ID. NO. 69. No other claims	No functionally related patents or applications – published in English
US 6,541,202 **Assignee:** Research & Development Institute **Filed:** 13 Oct 1999 **Granted:** 01 Apr 2003 **Expiry date:** 13 Oct 2019	**Telomerase reverse transcriptase (TERT) genes from *Candida albicans*** Claim 1: An isolated nucleic acid molecule having at least 80% identity to a polynucleotide molecule that encodes the amino acid sequence of SEQ ID NO:2 or SEQ ID NO: 4, wherein said nucleic acid molecule encodes an amino acid sequence having telomerase reverse transcriptase activity.	AU 2000/80023 A5 US 2003/134275 A1 W0 01/27287 A1

US 6,767,719 **Assignee:** Geron Corp. Albert Einstein College of Medicine **Earliest priority:** 26 Nov 1997 **Filed:** 16 Mar 1998 **Granted:** 27 Jul 2004 **Expiry date:** 26 Nov 2017 *	**Mouse telomerase reverse transcriptase** Claim 1: An isolated, purified or recombinant polynucleotide encoding a telomerase reverse transcriptase protein, wherein said protein: (i) has at least 90% sequence identity to SEQ. ID NO:2; (ii) has telomerase catalytic activity when associated with telomerase RNA component; and (iii) contains at least one of the following amino acid motifs; Motif T: W-X12 -FFY-X1 -TE-X11 -R-X3 -W; Motif 1: LR-X1 -IPK; Motif 2: R-X1 -I-X15 -K; Motif A: P-X3 -F-X3 -D-X4 -YD; Motif B: Y-X4 -G-X2 -QG-X3 -S; Motif C: DD-X1 -L; or Motif D: A-X2 -F-X18 -K; wherein Xn is a sequence of unspecified amino acids of length "n".	See Appendix 1 for complete list. No applications or patents filed outside of U.S. with claims to mouse TERT

* - A terminal disclaimer was filed for this patent. Without obtaining the full prosecution history of this patent to reveal which patent it was disclaimed in favor of, certainty of the expiry date is not possible.

[1] An interference is a procedure conducted by the United States Patent and Trademark Office when multiple applications have identical claims. The purpose of an interference is to determine who was the first-in-time inventor and so deserves the patent.

[2] For a review of hybridization, see www.roche-applied-science.com/PROD_INF/MANUALS/InSitu/pdf/ISH_33-37.pdf.

[3] Geron has many pending patent applications on telomerase, some of which may contain claims directed to disclosure that isn't claimed in this patent.

Concluding remarks

With only a single exception, all the patents directed to wild-type telomerase are owned at least in part by Geron Corp. Its dominance came about by "winning the race" to clone human telomerase. The patents discussed above include claims for telomerase protein and fragments, nucleic acids encoding telomerase, expression vectors that encode telomerase, mammalian cells containing a nucleic acid that encodes telomerase, anti-sense oligonucleotides, and promoter sequence of the gene encoding telomerase.

Although only one telomerase sequence was disclosed, Geron's claims encompass enzymatically active variants and fragments of human telomerase protein and nucleic acid sequences. The range of the variant sequences is determined by hybridization conditions – conditions that are called "stringent". Such conditions generally result in related sequences that are at least 75% identical. Although by no means certain, a plausible interpretation of these claims is that the variants of the claims are full-length telomerase molecules.

In addition, Geron has protected many related concepts including: cells transfected with and expression telomerase, expression vectors encoding telomerase, anti-sense oligonucleotides, antibodies that specifically bind telomerase, and telomerase promoter sequences.

Outside the United States, Geron has actively pursued protection in at least European countries, Australia, Brazil, Canada, China, Israel, Japan, Republic of Korea, New Zealand and Singapore. (Geron may have filed in other countries that do not report or reliably report patent application data.) The scope of protection varies among these countries in part due to what is protectable subject matter in each country and also to differences in application of patent laws. In countries where there isn't any patent protection of telomerase, it is safe for anyone to practice the invention(s) providing that the output isn't being exported to a country where the invention is protected. Where telomerase has been protected and you want to use one or more of the protected telomerase inventions, we suggest you obtain legal advice directed to your particular circumstance.

Variants of telomerase

This section presents patents directed to variants (alternate forms) of wild-type human telomerase. Variants come in many different flavors, including: mutations, insertions, deletions, polymorphisms, and RNA splice variants. The first set of patents presented in this section is about RNA splice variants. Two groups initially described RNA splice variants: CAMBIA[1] and Bayer. In the United States, CAMBIA obtained two granted patents, while Bayer appears to have largely abandoned their effort to obtain protection for splice variants, possibly because their disclosure of splice variants was later in time than CAMBIA's. Both CAMBIA and Bayer have been granted Australian patents however.

US 6,846,662 and **US 6,916,642**, which are owned by CAMBIA, have identical disclosures.[2] The disclosure presents a number of RNA splice variants of telomerase. Specifically, seven alternative "introns/exons" were identified, which theoretically could yield 128 different RNAs. Because of frame-shifts or stop codons introduced by some of the alternative "introns/exons", the number of different telomerase variant polypeptides would be much smaller. Now that the genomic structure of the telomerase gene is known, these alternative sequences can be mapped.

Strikingly, most of the alternative sequences do not correspond to entire exons or introns, and as such, the RNA splice variants disclosed would not have been predicted by the genomic sequence.

The claims of the two patents are directed to proteins (US '662) and to nucleic acids (US '642). The protein patent claims telomerase proteins having:

- specific sequences of Figure 11 (claim 1);
- proteins that are 90% identical to the specific sequences and that have either catalytic activity or binds telomerase RNA – and is not wild-type telomerase (claim 4);
- individual alternative "introns/exons" (claim 2);
- a splice variant lacking 13 amino acids from residues 711-722 (inclusive) and proteins having 90% identical amino acids as well as same requirements of claim 4 (claim 3);
- a sequence encoded by a nucleic acid having at least one of the disclosed alternative introns/exons excluding wild-type telomerase (claim 6).

The lead claim of the nucleic acid patent recites a molecule encoding a splice variant in which the variant has at least one of six alternative introns/exons, which would yield 64 different proteins. Other claims are directed to specific DNA sequences encoding variant telomerases presented in Figure 11 and sequences encoding a protein having at least 95% identity to the variants. Individual nucleic acid sequences of the alternative introns/exons are also claimed. There are also claims to nucleic acid probes and amplification primers for detecting and replicating RNA splice variants. Methods for establishing patterns of expression of the variants, e.g., for detection or prognosis of cancer, form many of the remaining claims. While not claiming *all* RNA splice variants, these patents encompass nearly every splice variant identified to date.

Australian patent **AU 748442** is related to the two CAMBIA U.S. patents and has essentially identical disclosure. The claims in AU '442 are broader however.

The lead claim encompasses a "nucleic acid molecule encoding a splice variant" of wild-type telomerase. Moreover, the wild-type telomerase reference protein is not limited to humans, but includes related telomerases that are encoded by nucleic acid molecules that hybridize under conditions of low stringency to the region containing reverse transcriptase motifs. By limiting the hybridization region to the most conserved part of telomerase and setting the hybridization conditions to low stringency, the reference telomerases will likely include many vertebrate species. Low stringency hybridization may include molecules with as little as 50% identity and possibly less. Thus, the claim covers splice variants of telomerase from a variety of vertebrate species.

In contrast to the broad claims of CAMBIA's Australian patent, the claims in the Bayer patents **AU 742489** and **AU 745420** recite specific DNA sequences of splice variants. Although the earliest priority date of AU '420 is before the earliest priority date of AU '442, splice variants weren't disclosed in the earliest priority document for AU '420. Thus, CAMBIA filed disclosure of splice variants prior to Bayer. The lead claim in AU '420 recites four different variants encoded by nucleic acid sequences that are:

- deleted for 182 bp (b region);
- deleted for 36 bp (a region);

- deleted for both the 182 bp and 36 bp; or
- deleted for about 1800 bp at the 5' end and has an alternate 3' end.

The first three variants were described in AU '442 and appear to be encompassed by its claims, or they are identical claims (and probably not valid as a result). The fourth variant is a fragment, and whether it is also covered by the claims of AU '442 requirse a more precise inquiry into how Australian patent law would interpret the claim of AU '420.

The second Bayer patent – AU '489 – claims isolated intron sequences or fragments of these sequences which have a regulatory effect. In the intron sequences, mainly in intron 2, the inventors have identified potential binding sequences for DNA-binding proteins – regulatory proteins. The regulatory proteins include C/EBP, CRE.2, Sp1, GRE, CREB, c-Myc, CCAAT site, and Rb site. In addition to the numerous candidate binding sites in intron 2, one potential Sp1 binding site was found in intron 1 and one potential c-Myc binding site was found in the 5'-untranslated region.

These introns, intron 1 and 2, have not been found in RNA splice variants of telomerase to date. Moreover, each intron sequence that is found in splice variants corresponds only to a portion of the intron. Unless the portion specifically has a regulatory effect, and none have been identified so far, then the claims in Bayer's patent AU '498 do not cover individual alternate intron/exon sequences.

Patent Data	Title and relevant claims	Family Data
US 6,846,662 **Assignee:** CAMBIA **Earliest priority:** 01 Jul 1997 **Filed:** 11 Feb 2000 **Granted:** 25 Jan 2005 **Expiry date:** 01 Jul 2017	**Vertebrate telomerase genes and proteins and uses thereof** Claim1: An isolated protein, wherein the protein comprises one of SEQ ID Nos. 37, 39, 42, 44, 46, 48, 50, 56-58, 60-62, 64-66, 68-70, 72-74, 76-78, 80-82, 84-86.	AU 748442 B2 BR PI9810643 A CA 2294782 AA CN 1270634 A EP 0917579 A1 EP 1571215 A2 JP 2002/514928 T2 NZ 501962 A US 2005/176022 A1 US 6,916,642 B1 WO 99/01560 A1
US 6,916,642 **Assignee:** CAMBIA **Earliest priority:** 01 Jul 1997 **Filed:** 11 Feb 2000 **Granted:** 12 Jul 2005 **Expiry date:**	**Vertebrate telomerase genes and proteins and uses thereof** Claim 1: An isolated nucleic acid molecule encoding a splice variant of a gene sequence capable of being spliced to result in a reference human telomerase encoding SEQ ID No: 2, wherein the splice variant has at least one of the following insertions or deletions: (a) an insertion of sequences X (comprising SEQ ID No: 32) at nucleotide 1766 of SEQ ID No: 1; (b) an insertion of nucleic acid sequence encoding sequence 1 (SEQ ID No: 24) at nucleotide 1950 of SEQ ID No: 1;	AU 748442 B2 BR PI9810643 A CA 2294782 AA CN 1270634 A EP 0917579 A1 EP 1571215 A2 JP 2002514928 T2 NZ 501962 A US 2005/176022 AA US 6,846,662 B1

01 Jul 2017	(c) a deletion of nucleotides 2131 through 2166 of SEQ ID No: 1; (d) a deletion of nucleotides 2287 through 2468 of SEQ ID No: 1; (e) an insertion of sequence 2 comprising SEQ ID No: 29 at nucleotide 2843 of SEQ ID No: 1; and (f) an insertion of nucleic acid sequence encoding sequence 3 (SEQ ID No: 31) at nucleotide 3157 of SEQ ID No: 1, and wherein the splice variant does not encode SEQ ID No: 2.	WO 99/01560 A1
AU 742489 **Assignee:** Bayer **Earliest priority:** 24 Dec 1997 **Filed:** 22 Dec 1998 **Granted:** 01 Jan 2002 **Expiry date:** 22 Dec 2018	**Regulatory DNA sequences of the human catalytic telomerase sub-unit gene, diagnostic and therapeutic use thereof** Claim 1: Isolated DNA characterized in that the sequences are intron sequences in accordance with SEQ ID NO 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and/or 20 or fragments of these sequences which have a regulatory effect.	CA 2316282 AA DE 19757984 A1 EP 1040195 A2 JP 2003519462 T2 US 2005/032094 A1 WO 99/33998 A3
AU 745420 **Assignee:** Bayer **Earliest priority:** 20 Jun 1997 **Filed:** 09 Jun 1998 **Granted:** 21 Mar 2002 **Expiry date:** 09 Jun 2018	**Human catalytic telomerase sub-unit and its diagnostic and therapeutic use** Claim 1: Functional equivalents, variants and catalytically active fragments of the catalytically active human telomerase subunit in isolated or purified form, comprising the amino acid sequence depicted in Fig. 2, characterized in that they comprise an amino acid sequence encoded by (1) the DNA sequence depicted in Fig. 1 with a deletion of 182 bp in length extending from nucleotide 2345 to 2526. (2) the DNA sequence depicted in Fig. 1 with a deletion of 36 bp in length extending from nucleotide 2184 to 2219. (3) the DNA sequence depicted in Fig. 1 with a deletion of 36 bp in length extending from nucleotide 2184 to 2219 and a deletion of 182 bp in length extending from nucleotide 2345 to 2526	CA 2294646 AA DE 19816496 A1 EP 0990037 A2 JP 2002508662 T2 WO 98/59040 A3

or.

(4) The DNA sequence depicted in Fig. 14.

AU 748442	**Vertebrate telomerase genes and proteins and uses thereof**	BR PI9810643 A
Assignee:		CA 2294782 AA
CAMBIA	Claim 1: An isolated nucleic acid molecule comprising a sequence corresponding to a nucleic acid molecule encoding a splice variant of a reference sequence of a catalytic subunit of a vertebrate telomerase,	CN 1270634 A
Earliest priority:		EP 0917579 A1
01 Jul 1997		EP 1571215 A2
Filed:	Wherein the reference sequence has the nucleic sequence presented in Figure 1 or the reference sequence hybridizes under conditions of low stringency to the complement of nucleic acid sequence encoding amino acids 5605-915 of Figure 1.	JP 2002514928 T2
01 Jul 1998		NZ 501962 A
Granted:		US 2005/176022 A1
06 Jun 2002		US 6,846,662 B1
Expiry date:		US 6,916,642 B1
01 Jul 2018		WO 9/901560 A1

The second set of patents in this section is directed to telomerases deleted for specific regions of sequence. All of these patents were granted to Geron. The claims in one are directed to specific deletions that do not affect the activity of telomerase, while the claims in the other recite deletions that abolish telomerase activity. According to the patent, a variant telomerase has “activity” if it has at least 40% of the activity of a wild-type telomerase. “Lack of activity” means that the variant has less than 1% of “wild-type” activity; “intermediate activity” is used to describe a telomerase with between 1% and 40% activity.

The inventors discovered that amino acid residues 192-323 or residues 415-450 can be deleted and the resulting telomerase retains catalytic activity. Some decrease was observed of the binding of the telomerase RNA component to these variants. More interesting however, are the variants that are deleted for one or more of residues 192-450, 637-660, 638-660, 748-766, 748-764 and 1055-1071. These variants not only lack telomerase catalytic activity but appear to inhibit the activity of wild-type telomerase – “dominant negative variants”. For reference, the region from residues approximately 620-902 contains the telomerase-specific and reverse transcriptase motifs, and the splice variant that is dominant negative - Da - is deleted for residues 708-722.

The claims of **US 6,337,200** are relatively straightforward. Claim 1 recites a polynucleotide encoding an hTERT variant, which is deleted for at least 10 amino acids of residues 192-323 or 415-450. Moreover, the variant has catalytic activity. Other independent claims are directed to using the variants to increase the proliferative capacity of a human cell in vitro by expressing the variant in the cell and to producing the variant protein by expression in a host cell or in a cell-free expression system.

The claims of **US 7,091,021** are directed to polypeptide variants and methods of using the variants to inhibit telomerase catalytic activity. The simplest claim (claim 3) for polypeptide variants recites a telomerase deleted for one or more of residues 326-415, 560-565, 637-660, 748-766, 930-934, 1055-1071, or 1084-1116. An unusual aspect of this claim is the recitation that the deletions “consist[ing] essentially of” the residues already mentioned. “Consisting essentially of” is a patent term that “limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention (MPEP 2111.03, 8th ed.; emphasis in original). It is the understanding of the author that “consisting essentially of” is rarely, if ever, used in biotechnology claims, and that the meaning of this term for biotechnology claims is uncertain.

Claim 2 is relatively simple and is directed to a variant lacking telomerase activity, in which the variant is wild-type telomerase deleted for one or more of the following:

- at least 10 consecutive amino acids between 326-415, 637-660, 748-766, 1055-1071, 1084-1116;

- amino acids 560-565; 930-934.

Claim 1 is similar to claim 2 except that "wild-type telomerase" is replaced by "a polypeptide encoded by DNA that hybridizes to" the complement of the sequence encoding wild-type telomerase. Hybridization conditions are the same as for other Geron patents and have been discussed in detail elsewhere in this landscape. An additional requirement is that the variants inhibit telomerase enzyme activity *in situ*. This requirement doesn't impose an additional limitation relative to the other claims; it is intended to exclude polypeptides that have the recited deletions but are not dominant negative variants.

Patent Data	Title and relevant claims	Family Data
US 6,337,200 **Assignee:** Geron Corp. **Earliest priority:** 31 Mar 1998 **Filed:** 03 Aug 1998 **Granted:** 08 Jan 2002 **Expiry date:** 31 Mar 2018	**Human telomerase catalytic subunit variants** Claim 1: A polynucleotide encoding a variant of human telomerase reverse transcriptase (hTRT), said variant having processive catalytic activity and comprising a deletion of at least 10 amino acids from region 192-323 or 415-450 of SEQ. ID NO:2.	AU 3375299 A1 US 2002/102686 A1 US 2006/275267 A1 US 7,091,021 B2 WO 99/50386 A3
US 7,091,021 **Assignee:** Geron Corp. **Earliest priority:** 31 Mar 1998 **Filed:** 21 Nov 2002 **Granted:** 15 Aug 2006 **Expiry date:** 31 Mar 2018	**Inactive variants of the human telomerase catalytic subunit** Claim 1: A polypeptide encoded by DNA that hybridizes to the sequence complementary to SEQ. ID NO:1 at 5.degree. C. to 25.degree. C. below Tm in aqueous solution at 1 M NaCl, wherein Tm is the melting temperature of double-stranded DNA having the sequence of SEQ. ID NO:1 under the same reaction conditions; wherein said polypeptide has one or more of the following deletions: a) residues 560 565, b) residues 930 934, c) at least 10 consecutive amino acids from residues 326 415, d) at least 10 consecutive amino acids from residues 637 660, e) at least 10 consecutive amino acids from residues 748 766, f) at least 10 consecutive amino acids from residues 1055 1071, or g) at least 10 consecutive amino acids from residues 1084 1116 of SEQ. ID NO:2; and wherein said polypeptide inhibits telomerase enzyme activity when introduced into a cell expressing human telomerase reverse transcriptase (hTRT) (SEQ. ID NO:2).	Continuation of '200 and has the same family history

[1] The original assignees were Cambia Biosystems LLC and Peter MacCallum Cancer Research Institute.

[2] In the United States, claim sets are often subject to "restriction", meaning that the Patent Office believes that the claims are drawn

to multiple inventions. Because only one invention can be claimed in a patent, the applicant chooses an invention for examination; the remaining inventions can be examined in separate applications. The separate applications have identical disclosures and are called "divisional" applications.

Concluding remarks

Are the claimed variants dominated by claims to wild-type telomerase? The question is moot for the constructed deletion variants of the second set because they are also owned by Geron. The claimed splice variants are not owned by Geron however, and so the answer to the question is of paramount importance. This question can't be definitively answered here; an answer requires additional documents and considerable legal analysis. But, an educated guess can be hazarded.

As discussed in the section on wild-type telomerase, some of the claims at first blush appear to read on certain RNA splice variant sequences (e.g., hybridizing molecules will encompass polymorphisms, small base changes, some insertions and deletions). Under current United States patent law, sequences must be fully described and enabled. Recent case law indicates that the threshold amount and type of disclosure to satisfy the written description (especially) and enablement requirements is exacting. For the most part, claims to undisclosed sequences aren't patentable. Geron disclosed wild-type telomerase and did not disclose splice variants. Moreover, Geron's disclosures for wild-type telomerase are directed to full-length molecules. Analysis above of the key claims for wild-type telomerase preliminarily concluded that hybridizing molecules were directed to full-length telomerase; in addition, fragments of wild-type and related telomerases aren't likely to encompass or contemplate splice variants. Furthermore, the key claims for wild-type telomerase require catalytic activity. Therefore, the splice variants that do not have catalytic activity should fall outside these claims. Taken all together, a credible interpretation can conclude that claims to splice variants of telomerase are not dominated by Geron's claims to wild-type telomerase and related molecules.

The impact of this conclusion, if correct, will be most keenly felt by CAMBIA[1] and any of its licensees who will be able to practice the invention without needing permission or a license from Geron.

[1] The author of this patent landscape has substantial connections with CAMBIA and thus is not without some bias.

Diagnostic assays based on telomerase

Long before the gene encoding telomerase catalytic protein was isolated, telomerase was recognized as a key regulator of the replicative lifespan of cells. About the time that recognition of its importance was coalescing, scientists at Geron obtained data indicating that impeding telomeres from shortening could be used to control proliferative diseases such as cancer (see WO 93/23572). As part of its drug discovery program for inhibitors, Geron scientists developed an assay for telomerase activity, which was a modification of the method of Morin (Cell 59: 521, 1989).

As disclosed in WO 93/23572, one method for determining telomerase activity is "by measuring the rate of elongation of an appropriate repetitive sequence, having 2 or more, usually 3 or more, repeats of the telomere unit sequence, TTAGGG." In this assay, radiolabeled nucleotides are incorporated into an elongated oligonucleotide substrate containing the TTAGGG repeats; the reaction products are resolved by gel electrophoresis and visualized. Because the telomerase enzyme stalls and can release the substrate after adding the first G in the repeat, the pattern obtained is a six nucleotide ladder of extended substrates.

Assays for telomerase activity

Following on this initial assay, Geron scientists concocted a new assay, which is disclosed and claimed in **US 5,629,154**. As claimed, the new assay comprised:

- preparing an extract from a cell sample;
- mixing an aliquot of the extract with (i) a telomerase substrate that lacks a repeat sequence, and (ii) an oligonucleotide primer complementary to a telomeric repeat sequence; and
- detecting a double-stranded DNA reaction product, one strand of which is an extended telomerase substrate containing repeat sequences and the other strand of which is extended primer.

The conditions for extension of the substrate and for extension of the primer are not specified in the claims and as such, the conditions are not limiting. For example, the extension of the primer can be effected by a DNA polymerase, a template-dependent DNA ligase, or other suitable enzyme and the reaction product may

be labeled by incorporation of radio-labeled dNTPs or by a 5' label or by some other means.

A further improvement of this basic strategy at Geron resulted in the widely used TRAP (telomeric repeat amplification protocol) assay (Kim *et al.* 1994). The claimed method of **US 5,863,726** comprises:

- incubating a cell sample with an exogenous telomerase substrate under conditions such that telomerase can catalyze extension of the substrate by addition of telomeric repeat sequences;
- replicating (e.g., by amplification such as PCR) the extended substrate; and
- correlating telomerase activity with presence of extended substrate and lack of activity with absence of extended substrate.

Although the claims don't limit many of the elements, such as the nature of the substrate (e.g., lacking a telomeric repeat sequence), the method of replication, means for detecting the extended substrate, in practice most scientists perform TRAP using PCR and visualizing the amplified product by detection of radioactive-labeled nucleotides after the products are separated by electrophoresis. As is typical of patents, dependent claims in the patent recite these specific elements as well as a variety of substitutes for the elements. Other claims are directed to kits for detecting telomerase activity having (i) a telomerase substrate, and (ii) a primer that is complementary to a telomeric repeat sequence.

Further consolidating Geron's position on assays for telomerase activity, **US 5,804,380** claims using the TRAP assay for screening candidate modulators of telomerase activity (claim 1). In addition, Geron claims a kit for detecting telomerase activity similar to the one in US '726 except that the substrate is labeled and instructions are included. This kit claim actually falls within the scope of the claim in US '726, but is directed to what is probably a more likely format for commercial kits.

Geron has not left a lot of room for others in this area, as indicated by the dearth of other pending or issued patents. CTRC Research Foundation in Texas however has a patent, **US 5,856,096**, that claims a ligation-style assay for telomerase activity. In their method, activity is detected in a sample by:

- incubating a sample with a telomerase substrate and dNTPs to form an extended substrate; then
- adding two oligonucleotides to the extended substrate, in which the two oligonucleotides are adjacent to each other when annealed to the extended substrate;
- adding a ligase that will ligate together the two oligonucleotides; and
- detecting the ligated product.

Other claims are directed to use of assay to screen inhibitors of telomerase activity and to distinguish between processive and nonprocessive activities of telomerase.

Patent Data	Title and relevant claims	Family Data
US 5,629,154	**Telomerase activity assays**	Patents:
Assignee: Geron Corp.	Claim 1: A method for determining whether a cell sample contains telomerase activity, said method comprising the steps of: (a) preparing a cell extract from said cell sample; (b) placing an aliquot of said cell extract in a reaction mixture comprising a telomerase substrate lacking a telomeric repeat sequence and a buffer in which telomerase can catalyze extension of said telomerase substrate by addition of telomeric repeat sequences; (c) adding to said reaction mixture a primer comprising a sequence sufficiently complementary to a telomeric repeat to hybridize specifically thereto under conditions such that if an extended telomerase substrate is present in said	AU 682082 B2 AU 688262 B2 CA 2173872 C
Earliest priority: 12 Nov 1993		
Filed: 28 Sep 1994		
Granted: 13 May 1997		
Expiry date: 13 May 2014		

	reaction mixture, said primer will hybridize to said extended telomerase substrate and extend to form a complementary copy of said extended telomerase substrate, thereby forming duplex DNA molecules comprising an extended telomerase substrate bound to an extended primer; and (d) correlating presence of telomerase activity in said cell sample with presence of duplex DNA molecules comprising an extended telomerase substrate bound to an extended primer and absence of telomerase activity in said cell sample with absence of said duplex DNA molecules.	
US 5,804,380 **Assignee:** Geron Corp. **Earliest priority:** 12 Nov 1993 **Filed:** 15 Apr 1996 **Granted:** 08 Sep 1998 **Expiry date:** 13 May 2014	**Telomerase activity assays** Claim 19: A kit for detecting telomerase activity, said kit comprising: (a) a labeled telomerase substrate; and (b) a primer comprising a sequence complementary to a telomeric repeat sequence; and (c) instructions.	See Appendix 2 for complete list of related family members. Patents: AU 723767 B2 CA 2173872 C EP 0728207 B1
US 5,856,096 **Assignee:** CTRC Research Foundation **Filed:** 21 Sep 1995 **Granted:** 05 Jan 1999 **Expiry date:** 21 Sep 2015	**Rapid and sensitive assays for detecting and distinguishing between processive and non-processive telomerase activities** Claim 1: A method for detecting telomerase activity in a sample comprising the steps of: (i) obtaining said sample; (ii) contacting said sample with a telomerase primer and dNTPs under conditions permitting formation of a telomerase product; (iii) contacting the telomerase product of step (ii) with a first oligonucleotide and a second oligonucleotide under conditions permitting hybridization, wherein said first and said second oligonucleotides hybridize to said telomerase product such that no single-stranded region intervenes between said first and said second oligonucleotides to form a hybridized product; (iv) contacting the hybridized product and oligonucleotides with a ligase; and (v) detecting the ligated form of said first and said second oligonucleotides.	AU 7115496 A1 WO 97/11198 A1

US 5,863,726	**Telomerase activity assays**	See Appendix 2 for complete list of family members.
Assignee: Geron Corp.	Claim 1: A method for determining whether a cell sample contains telomerase activity, said method comprising the steps of: (a) collecting a cell sample; (b) incubating said cell sample in a reaction mixture comprising an exogenous telomerase substrate under conditions such that telomerase can catalyze extension of said telomerase substrate by addition of telomeric repeat sequences; (c) replicating said extended telomerase substrate; and (d) correlating presence of telomerase activity in said cell sample with presence of said extended telomerase substrate and absence of telomerase activity in said cell sample with absence of said extended telomerase substrate.	Patents: AU 723767 B2 CA 2173872 C
Earliest priority: 12 Nov 1993		
Filed: 12 Apr 1996		
Granted: 26 Jan 1999		
Expiry date: 13 May 2014		

Assays for nucleic acid sequences encoding telomerase

As you might expect, the groups that first cloned genes encoding telomerase have the dominant position(s) in this area. In this regard, we are not disappointed.

Geron has the main position effected by two U.S. patents (discussed here) and their related family counterparts. One of the patents **US 6,808,880** claims detecting nucleic acid sequences encoding at least a portion of telomerase by identifying a signature amino acid sequence. The motif is W-X_{12}-FFY-X-TE, found in motif T, a motif unique to telomerases (Nakamura *et al.* 1997). Certainly the easier and more common methods to detect a nucleic acid are by hybridization or by amplification; both of these assay types are claimed in **US 7,005,262**.

The main claim directed to hybridization (claim 1) recites a method comprising:

- combining sample nucleic acids with a probe that anneals specifically to a nucleic acid sequence encoding hTERT (human telomerase) or a fragment of hTERT; and
- detecting hybridization.

The conditions for specific hybridization are detailed in the claim. The conditions (5-25°C below T_m in a 1M NaCl solution) aren't very stringent. Most assuredly the probe will hybridize to related sequences. The limitation on the probe however is that the probe does not hybridize to the EST sequence derived from hTERT (SEQ ID No: 62) and originally found by scientists at Merck. Other independent claims (claims 22 and 27) are similar except that the probe hybridizes specifically to the EST sequence.

Claim 11 is broadly directed to an amplification (PCR) assay to detect sequences encoding human telomerase. In this claim:

- a sample is combined with a primer pair that amplifies nucleic acid encoding hTERT or a fragment of hTERT; and
- detecting amplified product.

Each of the primers contain at least 15 consecutive nucleotides (or complementary nucleotides) found in the sequence encoding hTERT as long as the primers are not derived from the EST sequence. In a related independent claim (claim 37) the primers are derived from the EST sequence. Other claims to amplification assays recite only "a polynucleotide primer" that either doesn't hybridize (claim 10) or does hybridize (claim 32) to the EST sequence. In patent claim language, the indefinite article "a" means "one or more". Thus, the claims reciting "a" primer are not limited to single primer amplification; instead, the amplification reaction can proceed using one, two, three, etc. primers. A plain reading of the claim however, limits *one* of the primers to not being derived from the EST sequence (claim 10) or to being derived from the EST sequence (claim 32), without limitation on the other primer(s).

Methods of detecting splice variants of human telomerase are claimed in **US 6,916,642** owned by CAMBIA. In particular, claims 27 and 28 recite ways to determine a pattern of expression of the splice variants. The method:

- amplifies cDNA using primers that amplify a splice variant; and
- hybridizes the amplified product with a probe derived from one or more of the alternative listed "exon" sequences (claim 27) or derived from two or more of the specified sequences (claim 28).

Patent Data	Title and relevant claims	Family Data
US 6,808,880 **Assignee:** Geron Corp. University of Colorado **Earliest priority:** 01 Oct 1996 **Filed:** 19 Jan 2001 **Granted:** 26 Oct 2004 **Expiry date:** 01 Oct 2016	**Method for detecting polynucleotides encoding telomerase** Claim 1: A method for detecting the presence of polynucleotide sequences encoding at least a portion of telomerase in a biological sample, comprising the steps of: a) obtaining an amino acid sequence encoded in a polynucleotide contained in a biological sample; b) comparing the amino acid sequence with the telomerase amino acid motif W-X^{12} -FFY-X^{1} -TE, Wherein X is any amino acid; and then c) determining that the sample contains a polynucleotide encoding at least a portion of telomerase if the sequence obtained in step a) contains said telomerase amino acid motif.	See Appendix 1
US 6,916,642 **Assignee:** CAMBIA **Earliest priority:** 01 Jul 1997 **Filed:** 11 Feb 2000 **Granted:** 12 Jul 2005 **Expiry date:** 01 Jul 2017	**Vertebrate telomerase genes and proteins and uses thereof** Claim 28: A method of determining a pattern of telomerase RNA expression in cells, comprising, (a) preparing cDNA from mRNA isolated from the cells, (b) amplifying the cDNA using primers that amplify a splice variant of nucleic acid encoding human telomerase to form an amplified product and (c) hybridizing the amplified product with two or more of the following: all or at least 15 contiguous nucleotides of the sequence of region 1 (SEQ ID No: 23), all or at least 15 contiguous nucleotides of the sequence of region Î± (SEQ ID No: 25), all or at least 15 contiguous nucleotides of the sequence of region Î² (SEQ ID No: 27), all or at least 15 contiguous nucleotides of the sequence of region 2 (SEQ ID No: 29), all or at least 15 contiguous nucleotides of the sequence of region 3 (SEQ ID No: 30),	AU 748442 B2 BR PI9810643 A CA 2294782 AA CN 1270634 A EP 0917579 A1 EP 1571215 A2 JP 2002514928 T2 NZ 501962 A US 2005/176022 A1 US 6,846,662 B1 WO 99/01560 A1

	all or at least 15 contiguous nucleotides of the sequence of region X (SEQ ID No: 32) or all or at least 15 contiguous nucleotides of the sequence of region Y (SEQ ID No: 18); or a complement thereof; and (d) detecting hybridization; therefrom determining the pattern of telomerase RNA expression.	
US 7,005,262 **Assignee:** Geron Corp. University of Colorado **Earliest priority:** 18 Apr 1997 **Filed:** 18 Jan 2002 **Granted:** 28 Feb 2006 **Expiry date:** 18 Apr 2017	**Methods for detecting nucleic acids encoding human telomerase reverse transcriptase** Claim 1: A method of identifying a nucleic acid that encodes human telomerase reverse transcriptase (hTRT) or fragment thereof in a sample, comprising: a) combining the sample with a polynucleotide probe such that the probe hybridizes specifically to the nucleic acid if the nucleic acid encodes hTRT or fragment thereof: b) detecting any hybrid formed as a result of a); and c) identifying the nucleic acid as encoding hTRT or fragment thereof if the hybrid is detected; wherein the probe hybridizes specifically to a DNA having the sequence of the hTRT encoding region of SEQ. ID NO:224 at 5° C. to 25° C. below T_m in aqueous solution at 1 M NaCl but does not hybridize to a DNA having the sequence of SEQ. ID NO:62 under the same reaction conditions; wherein T_m is the melting temperature of double-stranded DNA having the sequence of said encoding region under the same reaction conditions. Claim 11: A method of detecting a nucleic acid encoding hTRT or fragment thereof in a sample, comprising: a) combining the sample with polynucleotide primers so as to prime amplification of nucleic acid encoding hTRT or fragment thereof if present in the sample; b) detecting any amplified product formed as a result of a); and c) identifying the nucleic acid as encoding hTRT or fragment thereof if the	See Appendix 1

	amplification product is detected: wherein each of said primers consists essentially of a sequence identical or complementary to 15 or more consecutive nucleotides from the hTRT encoding region of SEQ. ID NO:224, but at least one of the primers does not consist sequence identical or complementary to 15 or more consecutive nucleotides from SEQ. ID NO:62.

Diagnostic assay for cancer

The group of patents falling in this category is more numerous than the other groups. To the extent that any of the assays for detecting cancerous conditions use one of the assays for telomerase activity or for nucleic acids encoding telomerase, the claims of these patents are dominated by the patents in the two groups discussed above.

One of the leaders in this area is the University of Texas. It owns the first three patents in the table, **US 5,639,613**; **US 5,648,215**; and **US 5,693,474**. In patents US '613 and US '474, the lead claims are both directed to a method for making a prognosis of cancer; in the '613 patent the method is practiced for humans, whereas the '474 patent doesn't have a limitation for the type of organism. Both methods comprise:

- analyzing a sample for telomerase activity; and
- correlating high activity with an unfavorable prognosis and low activity with a favorable prognosis.

Dependent claims recite additional steps for the analyzing step, such as extending a telomerase substrate and amplifying the extended substrate. Other dependent claims recite a variety of tumor types.

The US '215 patent claims a method for detecting cancerous cells in a human breast, prostate, colon or lung tissue sample (claim 1) as well as a method for determining prognosis of a cancer patient by detecting cancerous cells in a tissue sample. The steps of the method for each of these claims comprises:

- incubating an aliquot of a cell extract with a telomerase substrate;
- determining whether the substrate has been extended; and
- correlating addition of repeat sequences to the substrate with the presence of cancerous cells.

Patents **US 5,989,807** and **US 6,391,554** claim a method for diagnosis of a cancerous or precancerous condition through detection of an elevated level of telomerase activity in cells (US '807) or mammalian cells (US '554) that are classified as normal by pathology (US '807). Dependent claims recite a laundry list of cancers and particulars about the assay method.

Patent Data	Title and relevant claims	Family Data
US 5,639,613 **Assignee:** University of Texas **Earliest priority:** 13 May 1993 **Filed:** 18 Apr 1995	**Methods for cancer diagnosis and prognosis** Claim 1: A method for prognosing cancer in humans, said method comprising: (a) collecting a sample suspected of containing tumor cells; (b) analyzing said sample for telomerase activity; (c) correlating said activity with a standard level of telomerase activity; and (d) correlating a high telomerase activity with an indication of unfavorable prognosis and a low telomerase activity	See Appendix 2

	with a favorable prognosis.	
Granted: 17 June 1997 **Expiry date:** 17 Jun 2014		
US 5,648,215 **Assignee:** University of Texas **Earliest priority:** 13 May 1992 **Filed:** 28 Sep 1994 **Granted:** 15 Jul 1997 **Expiry date:** 06 Feb 2013	**Telomerase diagnostic methods** Claim 1: A method for detecting whether a human breast, prostate, colon, or lung tissue sample contains cancerous cells, said method comprising (a) preparing a cell extract from said tissue sample; (b) incubating an aliquot of said cell extract in a reaction mixture comprising a telomerase substrate and a buffer in which telomerase can catalyze the extension of said telomerase substrate; (c) determining whether said telomerase substrate has been extended in step (b) by addition of telomeric repeat sequences; and (d) correlating presence of cancerous cells in said sample with the addition of telomeric repeat sequences to said telomerase substrate and absence of cancerous cells in said sample with no addition of telomeric repeat sequences to said telomerase substrate.	See Appendix 2
US 5,693,474 **Assignee:** University of Texas **Earliest priority:** 13 May 1992 **Filed:** 07 Jun 1995 **Granted:** 02 Dec 1997 **Expiry date:** 02 Dec 2014	**Methods for cancer diagnosis and prognosis** Claim 1: A method for prognosing cancer, said method comprising: (a) collecting a sample suspected of containing cancer cells; (b) analyzing said sample for telomerase activity; (c) correlating said activity with a standard level of telomerase activity; and (d) correlating a high telomerase activity with an indication of unfavorable prognosis and a low telomerase activity with a favorable prognosis.	See Appendix 2
US 5,989,807 **Assignee:** Geron Corp. and Board of Regents University of Texas **Earliest priority:**	**Detecting cancerous conditions by assaying for telomerase activity** Claim 3: Method for detection of a cancerous or precancerous condition in a patient comprising assaying for telomerase activity in cells classified as non-cancerous by pathology, wherein a detectable level of telomerase activity is indicative of said condition.	See Appendix 2

13 May 1992

Filed:

07 Jun 1994

Granted:

23 Nov 1999

Expiry date:

23 Nov 2016 *

US 6,391,554 **Assignee:** Geron Corp. and Board of Regents University of Texas **Earliest priority:** 13 May 1992 **Filed:** 23 Nov 1999 **Granted:** 21 May 2002 **Expiry date:** 13 May 2012 *	**Detecting cancerous conditions by assaying for telomerase activity** Claim 3: A method for detection of a cancerous or precancerous condition in a mammal, the method comprising assaying for telomerase activity, wherein a detectable level of telomerase activity is indicative of a cancerous or precancerous condition.	See Appendix 2

The next group of patents discussed claim methods that detect nucleic acids encoding telomerase.

US 6,664,046, owned by Roche Molecular Systems, determines the quantity of hTERT mRNA in a test sample compared to a normal control and concludes that cancerous cells are present if the amount of hTERT mRNA is greater in the test sample than in the normal control. The method used to quantify hTERT mRNA is amplification using a primer pair in which one primer hybridizes within exon 8 and the other primer hybridizes either upstream of exon 7 or downstream of exon 8. The b region, which is present in wild-type telomerase is encoded by exons 7 and 8. Therefore, this method detects the presence of the b region.

The rest of the U.S. patents and applications contain claims that are specific for the source of the nucleic acids encoding telomerase and relatively non-specific as to the particulars of assay. **US 6,821,726** amplifies mRNA coding for telomerase from tumor cells that are isolated by centrifugation of a body fluid sample layered over a cell separation medium of 1.060-1.065 g/mL and removal of cells at the interface. This is how lymphocytes are commonly isolated from a blood sample. The patent applications **US 2004/0132019** and **US 2006/0204956** claim analyses of RNAs encoding telomerase that are present in blood plasma or serum. In its lead claim, US '956 further specifies analysis by amplification.

Patent Data	Title and relevant claims	Family Data
US 6,664,046 **Assignee:** Roche Molecular Systems	**Quantitation of hTERT mRNA expression** Claim 1: A method for identifying the presence of cancerous cells in a human sample wherein said method comprises: (a)	EP 1108789 A3 JP 2001204483 A2

Filed: 16 Dec 1999 **Granted:** 16 Dec 2003 **Expiry date:** 16 Dec 2019	determining the quantity of hTERT mRNA comprising β-region coding sequence in said sample and in a control sample of non cancerous cells by: (1) contacting RNA from said sample and said control sample with a pair of primers, wherein said pair of primers consists of a first primer which hybridizes within exon 8 of the hTERT gene and a second primer which hybridizes upstream of exon 7 or downstream of exon 8 of the hTERT gene; (3) measuring the generation of amplification products; (4) determining the quantity of hTERT mRNA comprising β-region coding sequence in said sample from the results obtained in step (3); and (b) identifying the presence of cancerous cells in said sample if the quantity of hTERT mRNA comprising β-region coding sequence in said sample is greater than the quantity of hTERT mRNA comprising β-region coding sequence in said control sample.	
US 6,821,726 **Assignee:** Inventors - Dahm, M Phelps, R Brockmeyer, C **Earliest priority:** 04 Feb 1998 **Filed:** 04 Aug 2000 **Granted:** 23 Nov 2004 **Expiry date:** 04 Feb 2018	**Method for quantitatively analyzing tumor cells in a body fluid and test kits suited therefore** Claim 1: A method for the quantification of tumor cells in a body fluid, comprising: (a) concentrating tumor cells in a sample of a body fluid by covering a cell separation medium with a density in the range of from 1.060-1.065 g/ml with a layer of the body fluid, centrifuging the cell separation medium covered with the body fluid and collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid; (b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase; (c) quantitatively determining the amount of amplified nucleic acid; and (d) correlating the amount of amplified nucleic acid with the number of tumor cells in the body fluid.	AU 756814 B2 BR PI9910193 A CA 2319709 AA DE 19804372 A1 EP 1051522 A2 JP 2002503454 T2 WO 99/40221 A3
US 2004/132019 **Assignee:** Inventors - Chen, X Stroun, M Anker, P **Earliest priority:**	**Cancer diagnosis method** Claim 1: Method for diagnosis and/or follow-up of the development of cancers comprising analysis of the RNAs of the telomerase enzyme present in the blood plasma or serum, characterized in that the telomerase RNAs analyzed are the hTR RNA matrix, the catalytic part of the hTERT enzyme or the TEP1 RNA coding for the associated protein, and in that the RNAs are analyzed in relation to a reference RNA corresponding to the expression of a	AU 200155021 A5 EP 1158055 B1 EP 1283907 A1 WO 0190409 A1

	coding gene.	
26 May 2000		
Filed:		
16 May 2001		
US 2006/204956	**Method for detection of hTR and hTERT telomerase-associated RNA in plasma or serum**	AU 200185157 A5 AU 200188442 A5 AU 722476 B2 CA 2250118 AA CA 2421007 AA EP 0938320 A4 EP 1354060 A2 US 2002/106684 A1 US 2002/155469 A1 US 2003/036068 A1 US 2004/014079 A1 US 2005/003440 A1 US 2005/069906 A1 US 2005/260594 A1 US 2006/166229 A1 US 2006/204989 A1 US 2006/228729 A1 US 2006/228732 A1 US 2006/286578 A1 US 6,329,179 B1 US 6,607,898 B1 US 6,759,217 B2 US 6,794,135 B1 US 6,916,634 B2 US 6,939,671 B2 WO 02/18645 A3 WO 02/18652 A3 WO 03/028531 A3 WO 97/35589 A1
Assignee:	Claim 1: A method for detecting extracellular human telomerase reverse transcriptase protein (hTERT) RNA in blood plasma or serum for detecting, diagnosing, monitoring, treating or evaluating neoplastic disease comprising cells that express hTERT, the method comprising the steps of: a) extracting mammalian, heterogeneous extracellular RNA from human blood plasma or serum; b) amplifying a portion of the extracted extracellular RNA or cDNA produced therefrom wherein said portion comprises hTERT RNA to produce an amplified product, and wherein amplification is performed qualitatively or quantitatively using primers or probes that target hTERT RNA or the corresponding cDNA; and c) detecting qualitatively or quantitatively the amplified product produced from hTERT RNA or corresponding cDNA.	
OncoMEDx Inc.		
Earliest priority:		
22 Sep 1998		
Filed:		
09 Jun 2003		

Two additional patent applications, exemplified by the PCT filing, claim diagnosis of bladder tumor in a urine sample (**WO 01/86288**) by detecting mRNA for hTERT, b-actin (to verify the quality of the RNA), and either a cytokeratin family member or a marker indicative of inflammatory cells. Finally, **WO 02/15770** claims a method for detection of cell transformation by measuring total and mature hTERT mRNA expression levels and diagnosing hyperplasia or dysplasia if the ration is from 3-10. Mature hTERT encodes wild-type telomerase; total hTERT mRNA encodes both wild-type telomerase and splice variant telomerase.

Patent Data	Title and relevant claims	Family Data

WO 01/86288 **Assignee:** Macrochip S.R.L. **Earliest priority:** 08 May 2000 **Filed:** 04 May 2001	**Method and apparatus for early diagnosis of bladder tumor in urine samples** Claim 1. A method for early diagnosis of bladder tumor in a urine sample, characterized in that it comprises the determination, on the RNA extracted from the cells present in the urine, of: --a marker for the messenger RNA of the catalytic component of telomerase (hTRT) and a marker for B-actin, to demonstrate RNA accessibility and as standard for quantitative estimation, in association with at least one additional molecular marker chosen from the group that comprises: --a marker for a protein of the cytokeratin family; --a lymphocyte marker which is suitable to detect inflammatory cells associated with neoplastic infiltration.	AU 200170505 A5 EP 1281087 A2 IT 1318504 B1
WO 02/15770 **Assignee:** Digene Corporation **Earliest priority:** 18 Aug 2000 **Filed:** 17 Aug 2001	**Methods of cancer prognosis and assessment** A method of detecting cell transformation in a patient comprising the steps of: - measuring total hTERT mRINA expression level in a sample collected from the patient; - measuring mature hTERT mRNA expression level in the sample; and - determining a ratio of total hTERT mRNA expression level to m[1]i.ture hTERT expression level, wherein a ratio of between about 10 and about 3 is indicative of hyperplasia and/or dysplasia or an increased risk thereof.	AU 200187192 A5

Diagnostic assays for non-cancerous conditions

Three patents are categorized in this group. Briefly, **US 5,695,932** claims a method for detecting the presence of a eukaryotic pathogen by detection of the telomerase activity of the pathogen. Examples of pathogens include *Plasmodium*, *Sporothrix, Coccidioides, Histoplasma, Blastomyces, Paracoccidioides, Cryptococcus, Aspergillus, Mucor, Rizopus, Candida, Kluyveromyces,* and *Saccharomyces*.

The claims of **US 6,391,554** are silent with respect to the condition and broadly claim a method for diagnosis of a condition associated with an elevated level of telomerase activity within mammalian cells. The assay for detection is also an assay for telomerase activity. The claims of **US 6,551,774** are directed also to diagnosis of a condition that is associated with an elevated level of telomerase activity, except that the determining step does not specify the type of assay; a dependent claim recites measuring the rate of elongation of a primer having two or more telomeric repeat sequences.

Patent Data	Title and relevant claims	Family Data
US 5,695,932 **Assignee:** University of Texas	**Telomerase activity assays for diagnosing pathogenic infections** Claim 1: A nucleic acid method for detecting the presence of a eukaryotic	See Appendix 2 for complete list of family members

University of California **Earliest priority:** 13 May 1992 **Filed:** 13 May 1993 **Granted:** 09 Dec 1997 **Expiry date:** 09 Dec 2014	pathogen in a patient wherein presence of said eukaryotic pathogen is detected by their telomerase activity within a somatic cell population or tissue, comprising the steps of: obtaining a sample of somatic tissue or cells from said patient; determining whether telomerase activity is present within said sample in said patient; and correlating presence of telomerase activity with presence of said eukaryotic pathogen.	
US 6,391,554 **Assignee:** Geron Corp. and Board of Regents University of Texas **Earliest priority:** 13 May 1992 **Filed:** 23 Nov 1999 **Granted:** 21 May 2002 **Expiry date:** 13 May 2012 *	**Detecting cancerous conditions by assaying for telomerase activity** Claim 1: A method for diagnosis of a condition associated with an elevated level of telomerase activity within mammalian cells comprising: determining the presence or amount of telomerase activity within said cells; and correlating the presence or amount of telomerase activity with a condition associated with an elevated level of telomerase activity.	See Appendix 2 for complete list of family members. Patents: AU 735840 B2 CA 2245461 C EP 0728207 B1
US 6,551,774 **Assignee:** University of Texas University of California **Earliest priority:** 13 May 1992 **Filed:** 20 Aug 1999 **Granted:** 22 Apr 2003 **Expiry date:**	**Diagnostic methods for conditions associated with elevated cellular levels of telomerase activity** Claim 1: Method for diagnosis of a condition in a patient associated with an elevated level of telomerase activity within a mammalian cell comprising the steps of: determining the presence or amount of telomerase within a sample obtained from said patient, and correlating the presence or amount of telomerase with a condition associated with an elevated level of telomerase activity.	See Appendix 2 for complete list of family members.

13 May 2012 *

Concluding remarks

One of the purposes of granting patents is to stimulate innovation. Suppose that obtaining a license for one of the claimed assays is too costly. The alternatives include moving operations to a country without the patent, ignoring the patent and hope to not be sued for infringement, use a method or composition that is in the public domain, and design around the patent. Designing around a patent is innovating. Some of the organizations above no doubt invented new assays to circumvent patented assays. Total freedom to operate in this area will be difficult to obtain however if an assay depends upon using nucleic acids encoding telomerase. In such case, the precise nucleic acids used in the assay should be evaluated vis a vis the patents to wild-type telomerase, related variants, and RNA splice variants. Unfortunately, a general conclusion cannot be stated as each specific candidate sequence needs to be evaluated.

Stem cells and telomerase

Stem cell biology is both a fascinating area of research and a controversial one. When hearing the term "stem cell", most people think of embryonic stem cells, cells that are immortal and also have the potential to differentiate into any of the specialized cells that make up the body. For this reason, it is hypothesized that stem cells may become the basis for treating a variety of diseases, including Parkinson's disease, traumatic spinal cord injury, Alzheimer's disease, heart disease, diabetes, and muscular dystrophy.

Embryonic stem cells are isolated from embryos that develop after *in vitro* fertilization. At four to five days post-fertilization, the embryo has formed a hollow ball of cells called the blastocyst. The blastocyst includes three structures: the trophoblast, a layer of cells that forms the exterior surface; the blastocoel, the hollow cavity in the blastocyst, and the inner cell mass, which is approximately 30 cells clustered at one end of the blastocoel. The cells of inner cell mass are the embryonic stem cells. These cells are removed and are cultured without differentiating.

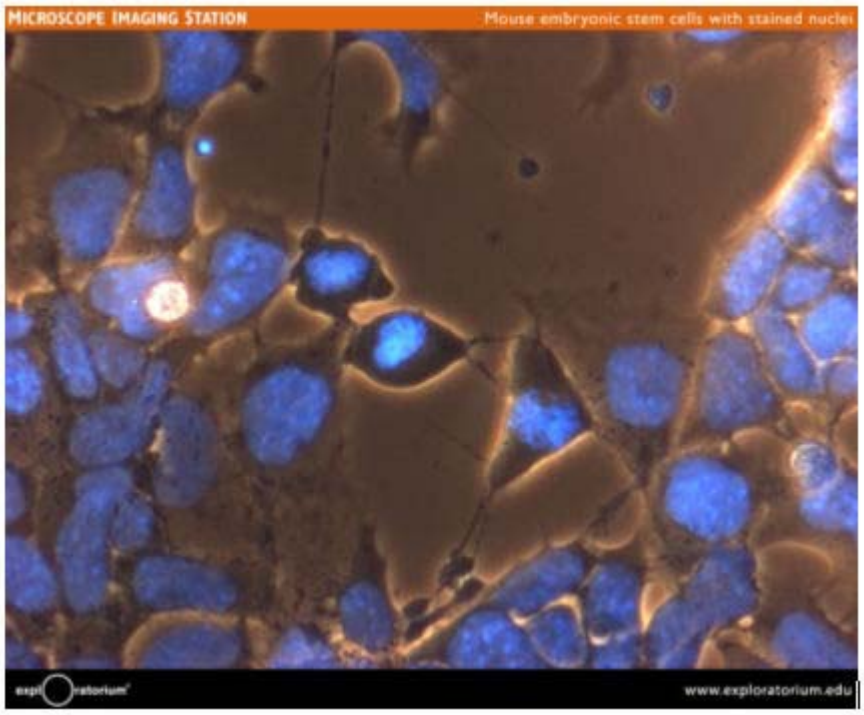
In 1998, Thomson et al. (Thomson *et al.* 1998) at the University of Wisconsin developed a number of human embryonic stem cell lines. Furthermore, a number of U.S. patents were granted to Dr Thomson in this area. (see, e.g., US 5,843,780; US 6,200,806; US 7,005,252; US 7,029,913)

In September 2006 the United States Patent and Trademark Office granted requests for re-examination of patents held by the Wisconsin Alumni Research Foundation (WARF) on human embryonic stem cells. The requests were filed by the Public Patent Foundation (PUBPAT) on behalf of the Foundation for Taxpayer and Consumer Rights (FTCR) a nationally recognized not-for-profit consumer organization on the grounds that the patents are invalid and their existence is causing significant public harm. In the press, various representatives of PUBPAT and FTCR have stated that they consider the patents overly broad, impede scientific progress and are driving stem cell research overseas. After the time period which allows WARF the opportunity to file a statement and PUBPAT and FTCR to file a statement in response, the Patent and Trademark Office will proceed to determine whether the patents are invalid in light of the issues raised.

Because embryos are sacrificed to obtain the stem cells, some people object to the procedure. In the United States, on 9 August 2001, federal policy restricting research using embryonic stem cells was implemented. The policy restricts federal funding to research using lines derived before 9 August 2001, leaving private firms untouched to develop and use new stem cell lines.

One of the hallmarks of embryonic stem cells is the high expression of telomerase. The expression of telomerase permits embryonic stem cells to escape senescence (Thomson *et al.* 1998). As discussed in the Background of this paper, very few normal cell types express telomerase in sufficient quantity to overcome the Hayflick limit to proliferation. Once the telomerase sequence became available, introduction of nucleic acids encoding and constitutively expressing telomerase was quickly adopted as a means to immortalize cells. For example, ectopic expression of hTERT has been shown to immortalize human skin keratinocytes, dermal fibroblasts, muscle satellite (stem), and vascular endothelial, myometrial, retinal-pigmented, and breast epithelial cells (see review by (Shay and Wright 2005). In some experiments, expression of hTERT increased the proliferative capacity of cells but did not lead to immortalization (Migliaccio *et al.* 2000; Di Donna *et al.* 2003).

Fig 6. Mouse embryonic stem cells with stained nuclei



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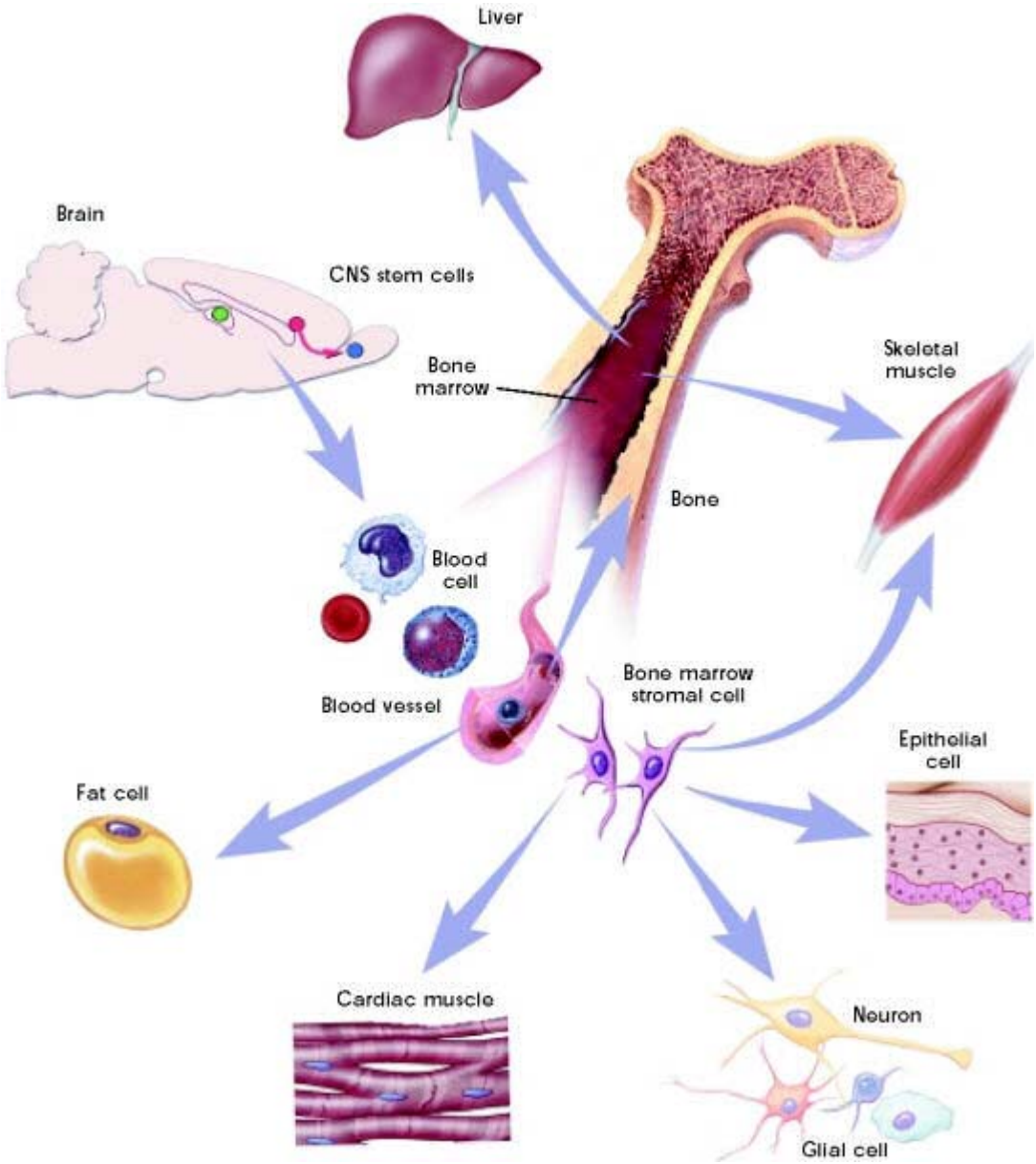
Embryonic stem cells are pluripotent. That is, they can give rise to cells of all three embryonic germ layers – *ectoderm*, which differentiates into brain, spinal cord, nerve cells, skin, teeth, etc.; *mesoderm*, which differentiates into muscles, blood, blood vessels, connective tissues, and the heart; and *endoderm*, which differentiates into the gut, lungs, bladder and germ cells.

Other stem cells are found in somatic tissues and are called either somatic stem cells or adult stem cells. These stem cells are multipotent; they are committed to give rise to cells that have a particular function. Examples of somatic stem cells include hematopoietic stem cells, which give rise to all blood cells; mesenchymal stem cells, which give rise to tissue such as bone, tendon, muscle, cartilage, nerve tissue; stromal stem cells, which generate bone, cartilage, fat, connective tissue; brain stem cells, which can generate both non-neuronal and neuronal cells in the brain (please see figure 7 below). Most, if not all, tissues and organs contain a small number of undifferentiated cells that can differentiate into the major cell types of the tissue or organ.

Fig 7. Common functional cells that are derrived from central nervous system (CNS) stem cells and somatic stem cells



Stem Cell Basics: What are adult stem cells? . In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2006 [cited Monday, January 29, 2007] Available at <http://stemcells.nih.gov/info/basics/basics4>

Even when somatic stem cells can be identified, a downside to using them in therapeutic procedures is the difficulty in growing them in cell culture and manipulating them to generate the desired specific cell types. Hematopoietic stem cells on the other hand have been exploited since the 1970s in bone marrow transplants. Rarely are the stem cells isolated, instead whole bone marrow is transplanted. In fact, only recently has it been possible to identify and isolate hematopoietic stem cells (see Chapter 2 in (2006).

Some progress in growing somatic stem cells has transpired from immortalizing the cells by introduction and expression of exogenous telomerase. The most spectacular success occurred in lineage-restricted progenitors of the central nervous system – the neuronal-restricted progenitor cells derived from human fetal spinal cord were immortalized by overexpression of exogenous telomerase, and furthermore they matured into neurons upon xenograft to rats (Roy *et al.* 2004). In other reports, immortalization of human hematopoietic progenitor cells (Akimov *et al.* 2005) and immortalization of mesenchymal progenitor cells (Zhang *et al.* 2006), differentiation capacity of the cells was either not thoroughly investigated or was lost.

The substantial interest in and research on stem cells and their potential therapeutic benefits is also reflected by numerous filings of patent applications.

Alternative expression of endogenous telomerase

Because of the direct correlation between length of telomeres and life-span of cells, increasing or causing constitutive expression of telomerase should increase proliferative capacity of cells and bypass or delay the normal onset of senescence. As you might imagine, many mechanisms and pathways can be exploited to affect telomerase activity. Maybe for this reason, the claims in this group of patent documents aren't limited to any particular cell or cell type. For the patent applications, it remains to be seen if such broad claims will

be granted.

The only granted patent in this group – **US 5,686,306** assigned to University of Texas – is directed to increasing the length of telomeres in normal cells by providing the cells with an oligonucleotide substrate for telomerase. Due to the typical approach of filing patent applications early in the research process, often before dissecting biological underpinnings, the claims in patents such as this one may be based on little more than preliminary experiments. While some scientific evidence is presented both in the patent application and in a peer-reviewed journal article (Wright *et al.* 96 A.D.), judging by the few number of journal articles citing the EMBO Journal paper, it does not appear to have resulted in a quantum leap for therapy.

The remaining patent documents in this area are all applications. Claims in patent applications will almost always be written broader than what ultimately is granted. For this reason, these applications will be discussed in general terms; the thrust of the disclosure will be presented in broad brush strokes.

US 2006/0052324, assigned to Stanford University, is directed to conditionally increasing transcription of either TERT or a telomerase RNA component (TERC) in a cell, such that the cell is activated to a proliferative state. An "agent" is used to increase transcription. Many different types of agents, e.g., small molecules, are disclosed in the text. The Examples describe experiments performed using a gene construct in which sequences encoding TERT is under control of a *tet* promoter. Treatment with doxycycline turns on transcription. Much of the Example section is devoted to hair follicle stem cells, leading the reader to surmise that it's claims to this subject matter that will ultimately be granted.

The remaining document in this area – **WO 2006/066247**, assigned to Exvivo Technologies – regards technology to enhance hTERT expression and increase processivity of telomerase. The inventors contemplate a variety of methods to enhance hTERT expression, including treating with hypoxic conditions, up-regulate hypoxia inducible factor, treatment with histone deacetylator inhibitors. Processivity may be increased by modulation of a "gene responsible for repressing hTERT expression or a gene that is associated with telomerase processivity". By "processivity" the applicant means that the telomerase is not prevented or inhibited from extending a telomere. No data are presented.

As an alternative to treating cells in order to increase endogenous telomerase, one invention, disclosed in patent application **US 2006/0160732**, provides compositions and methods for inhibiting cell senescence. In particular, the therapeutic agent is a fusion protein or covalently attached protein comprising telomerase and a tumor suppressor protein (e.g., p53, retinoblastoma protein). A transport agent, such as an arginine-rich peptide, is also present to ensure that the therapeutic agent is taken up by cells.

Patent Data	Title and relevant claims	Family Data
US 5,686,306 **Assignee:** University of Texas **Earliest priority:** 13 May 1992 **Filed:** 10 Nov 1994 **Granted:** 11 Nov 1997 **Expiry date:** 7 Jun 2015	**Methods and reagents for lengthening telomeres** Claim 1: A method for increasing the proliferative capacity of normal cells having telomerase activity, which method comprises culturing or cultivating said cells in the presence of an oligonucleotide substrate for telomerase under conditions such that said oligonucleotide substrate enters said cells and acts to lengthen telomeric DNA of said cells and the proliferative capacity of said cells is increased.	See Appendix 2
US 2006/052324 **Assignee:**	**Methods and compositions for cell activation**	WO 06/031313 A2

Leland Stanford Junior University **Earliest priority:** 05 Aug 2004 **Filed:** 04 Aug 2005	A method for activating a cell comprising: conditionally increasing transcription of a coding sequence of only one of: (a) a telomerase reverse transcriptase (TERT), or (b) a telomerase RNA component (TERC); in said cell in a manner sufficient to activate said cell	
US 2006/160732 **Assignee:** Inventor – Aronson, G **Earliest priority:** 10 Oct 2003 **Filed:** 08 Oct 2004	**Compositions and methods for inhibiting cell senescence and hyperproliferative disorders** Claim 1: A composition for inhibiting cell senescence comprising a transport agent and a therapeutic agent, wherein the therapeutic agent comprises a first region having telomerase activity and a second region having tumor suppressor activity.	EP 1673099 A1 WO 05/037297 A1
WO 2006/066247 **Assignee:** Exvivo Technologies **Earliest priority:** 17 Dec 2004 **Filed:** 19 Dec 2005	**Methods and compositions for extending telomere length and cell lifespan** Claim 1: A method of elongating a telomere of a cell, the method comprising: enhancing liTERT expression in the cell; and increasing telomerase processivity.	No family members

Stem cells engineered to express telomerase

With the isolation of the gene encoding the catalytic subunit telomerase, scientists could now transform cells with an expression vector comprising sequences encoding telomerase. In turn, they expected that the transformed cells, which expressed increased levels of telomerase, would be immortalized or at least have a longer lifespan. What a boon it would be for the numerous cell types that are difficult to grow in vitro.

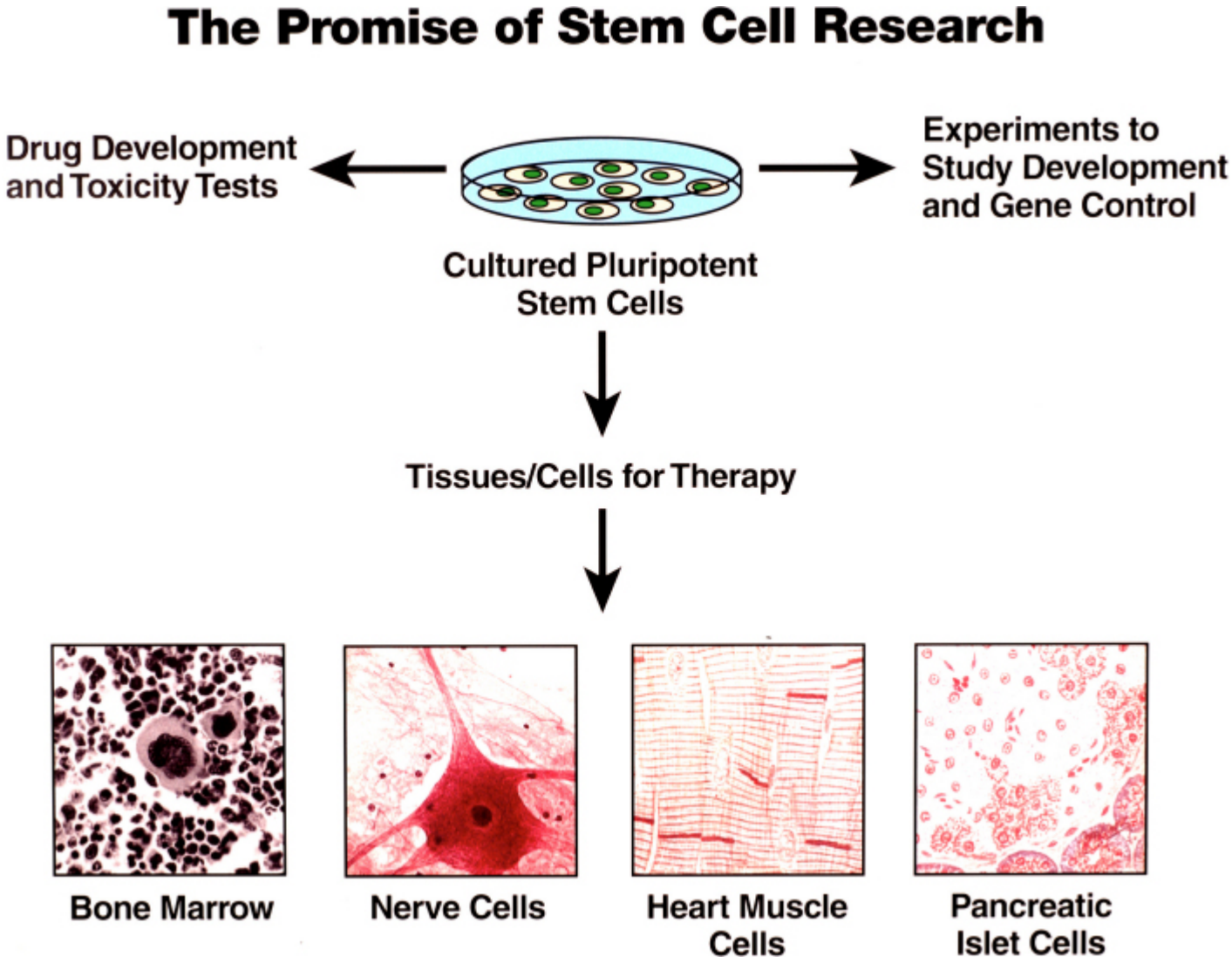
Given that human telomerase was cloned in 1997, it is a bit surprising (to the author) that there don't appear to be any issued U.S. patents in the field of transformed stem cells. Moreover, there aren't a huge number of pending patents. Maybe some of the paucity can be explained by the huge number of U.S. patents issued on aspects of stem cells. For example, the web site www.stemcellpatents.com lists over 1000 granted U.S. patents. Within the "minefield" of stem cell-related patents you may well find at least a few patents with claims that would dominate a claim to stem cells (of any sort) that are transformed with a sequence encoding telomerase. For instance, the WARF patents, referred to above, claim a "purified preparation of pluripotent human embryonic stem cells" albeit with specific characteristics. U.S. Patent 4,714,680 (now expired) claimed "A suspension of human cells comprising pluripotent lympho-hematopoietic stem cells substantially free of mature lymphoid and myeloid cells."

Against this backdrop, we present 8 patent applications and 1 patent directed to compositions and methods for inhibiting cell senescence or increasing proliferative potential of cells and for isolating stem cells. The inventions disclosed in each of these applications uses telomerase in achieving these results.

Pluripotent stem cells

In US 2005/0079608, the inventors propose a method to isolate adult stem cells. A cell population containing committed progenitor cells is transformed with a construct comprising a telomerase promoter operably linked to a selectable marker. Because stem cells express telomerase, the introduced telomerase promoter is expected to be active. By growing the transformed cells under selecting conditions, an enriched population of adult stem cells can be obtained.

Mesenchymal stem cells from bone marrow are the subject of a series of patent documents. Mesenchymal stem cells have been reported to differentiate into bone cells, chondrocytes, adipocytes, myocytes, tendon cells, and cardiomyocytes. Clearly, if mesenchymal stem cells can be propagated in culture without losing pluripotency, the stem cells would be a valuable resource for regenerative medicine.



In *Stem Cell Information* [World Wide Web site]. Bethesda, MD: National Institutes of Health, U.S. Department of Health and Human Services, 2007 [cited Monday, January 29, 2007] Available at <http://stemcells.nih.gov/info/media/defaultpage>

US 6,645,763 B2 claims a specific, immortalized bone marrow mesenchymal stem cell line. The line was made by transforming human adult bone marrow mesenchymal cells, which were commercially obtained, with a retroviral vector that contained hTERT-encoding sequences inserted between a pair of site-specific recombination sequences (e.g., LoxP). The cell line was demonstrated to differentiate into osteoblasts and into adipocytes.

Patent applications, US 2005/0084959 and EP 1 669 441 A1, filed by a Japanese group, are directed to immortalized mesenchymal stem cells and a use for them, respectively. In US '959, immortalized mesenchymal stem cells are obtained by immortalization using a telomerase gene, a "gene derived from telomerase" or a gene that regulates the expression of telomerase. In EP '441, the applicants claim a method for differentiating a mesenchymal stem cell into a hepatocyte. One of the sources for the stem cell is a mesenchymal stem cell that has been transformed with a construct expressing hTERT.

One of the very difficult cell types to propagate in culture is neural cells. Moreover, damaged brain or spinal cells are largely incapable of significant self-repair. The plight of Christopher Reeve dramatically underscored this problem. One impediment to scientific and medical advances is the scarcity of adult neuronal precursor cells. Other clinically-important progenitor cells in the brain, such as progenitors for oligodendrocytes, spinal cord motor neurons, midbrain dopaminergic and cholinergic neurons, are also resistant to propagation in vitro. In early 2004, Roy and Goldman published a break-through set of experiments in this area (Roy *et al.* 2004). Lineage-restricted progenitors of the central nervous system (CNS) in fetal spinal cord were transformed with a retroviral vector that over-expressed hTERT. The immortalized cells gave rise to phenotypically restricted subpopulations of either glia or neurons.
Importantly, the cells differentiated into neurons when implanted in xenografts. The method used to generate the immortalized neural progenitor cells is claimed in **US 7,150,989**.

Cartilage degradation is a feature of osteoarthritis and rheumatoid arthritis. Degradation results in joint pain and impairs mobility. Sufferers of these diseases may even need a wheelchair to get around. Some limited progress has been made in propagating and differentiating chondrocyte precursors. Unfortunately, the sources of these cells are limited. The patent application **US 2003/0109038** addresses this problem by disclosing a method to obtain human chondrocytes by differentiating them from embryonic stem cells. The embryonic stem cells are induced to undergo general differentiation and then coaxed along the chondrocyte differentiation pathway by culturing them in the presence of factors. Further, the cells can be immortalized by transformation with an expression vector containing telomerase-encoding sequence.

Obesity is a disease of "epidemic" proportions and one of the most significant health problems in industrialized countries. Consequently, more scientific investigation has been directed to all aspects of adipocytes biology, including adipogenesis. While there is plenty of fat tissue in obese people, adipocytes make up about a third of the cells, the other two-thirds comprise blood vessels, nerve tissue, fibroblasts and preadipocytes in various stages of differentiation. Preadipocytes are not easily distinguished from fibroblasts. Even when preadipocytes are isolated and cultured, they senesce and also lose adipogenic potential. **US 2005/0008621** is directed to methods for generating primary preadipocyte strains that have increased replicative potential and also maintain adipogenic capacity. One of the methods that is claimed and demonstrated in the application is to transform primary adipocytes with sequences encoding hTERT and select for those that express hTERT.

Many other patent applications, not discussed in detail here, disclose methods for obtaining pluripotent stem cells and, once obtained, increasing their lifespan by transformation with hTERT-encoding sequences.

Patent Data	Title and relevant claims	Family Data
EP 1 669 441 **Assignee:** Renomedix Institute **Earliest priority:** 27 Aug 2003 **Filed:** 27 Feb 2004	**Method of differentiating mesenchymal stem cell into liver cell and artificial human liver cell** Claim 1: A method for differentiating a mesenchymal stem cell, a mesenchymal progenitor cell, or a mesenchymal cell into a hepatocyte using liver tissue that has experienced chronic livery injury.	CA 2536934 AA KR 2006065712 A WO 05/024004 A1
US 6,645,763 **Assignee:** Inventors - Kobayashi, N Leboulch, P Tanaka, N	**Immortalized bone marrow mesenchymal stem cell** Claim 1: An immortalized bone marrow mesenchymal stem cell line deposited as FERM BP-8197.	JP 2003174870A2

Fujiwara, T

Filed:

12 Oct 2001

Granted:

11 Nov 2003

Expiry date:

27 Nov 2021

US 7,150,989 **Assignee:** Cornell Research Foundation Inc. **Earliest priority:** 10 Aug 2001 **Filed:** 09 Aug 2002 **Granted:** 19 Dec 2006 **Expiry date:** 05 Nov 2022	**Telomerase immortalized neural progenitor cells** Claim 1: A method of immortalizing neural progenitor cells comprising: providing a population of neural progenitor cells and immortalizing the population of neural progenitor cells by introduction of a nucleic acid sequence encoding telomerase reverse transcriptase operably linked to a promoter, either before or after the neural progenitor cells are enriched or purified wherein said telomerase reverse transcriptase is produced in sufficient quantity to immortalize said neural progenitor cells.	AU 2002327433 A1 WO 03/014320 A3
US 2003/109038 **Assignee:** Geron Corporation **Earliest priority:** 07 Dec 2001 **Filed:** 06 Dec 2002	**Chondrocyte precursors derived from human embryonic stem cells** Claim 1: A cell population obtained by differentiating primate pluripotent stem (pPS) cells, in which at least 5% of the cells synthesize either Type II collagen or aggrecan from an endogenous gene.	AU 2002366602 AA CA 2468335 AA CN 1599793 A EP 1463800 A4 GB 2399348 A1 JP 2005511083 T2 KR 2005044733 A US 2006148077 A1 WO 03/050250 B1
US 2005/008621 **Assignee:** Boston Medical Centre Corporation **Earliest priority:** 06 Oct 2001 **Filed:** 07 Oct 2002	**Preadipocyte cell strains and uses therefore** Claim 1: A primary preadipocyte strain, wherein said strain expresses telomerase reverse transcriptase (TERT) such that said strain maintains replicative potential and adipogenic capacity.	AU 2002332028 A1 WO 03/031640 A3

US 2005/079608 **Assignee:** Rappaport Family Institute **Earliest priority:** 05 Feb 2002 **Filed:** 04 Aug 2004	**Lineage committed stem cells selected for telomerase promoter activity** Claim 1: An enriched population of adult stem cells derived from stem cells comprising a plurality of committed progenitor cells stably transfected with a polynucleotide construct comprising a telomerase promoter element operably linked to a sequence encoding a selectable marker, wherein the progenitor cells express telomerase promoter activity.	AU 2003208580 A1 EP1472340 A4 WO 03/066839 A1
US 2005/084959 **Assignee:** Renomedix Institute **Earliest priority:** 31 Oct 2001 **Filed:** 31 Oct 2002	**Immortalized mesenchymal cells and utilization thereof** Claim 1: An immortalized mesenchymal system-related cell, which is a mesenchymal system-related cell that is selected from mesenchymal stem cells, mesenchymal precursor cells, mesenchymal cells and cells derived from mesenchymal cells, and is immortalized by high expression or activation of an immortalizing gene.	EP 1447443 A4 WO 03/038076 A1

Concluding remarks

It is perhaps surprising that only one patent has been granted in the United States in this area. Identification and isolation of stem cells, such as hematopoietic, embryonic, and tissue-specific stem cells, has been a fertile area of scientific pursuit for many years. Moreover, many patents have been granted for markers of stem cells, methods of isolation, methods of propagating, and uses of stem cells. With the cloning of telomerase in 1997 – 10 years ago – and the discovery that telomerase is active in stem cells, it would seem a natural progression to attempt to increase the longevity of stem cells in culture by transforming the cells with an exogenous telomerase gene.

While it might appear that freedom-to-operate is readily available in this area, caution is warranted. Besides any patents that claim some aspect of stem cells, use of the sequences encoding wild-type telomerase, related molecules and splice variants is governed by the patents granted on this subject matter. Geron's major position could be seen as a barrier to entry for commercial entities, unless Geron licenses its intellectual property. On the other hand, patents claiming splice variants of telomerase is in the hands of CAMBIA, an organization that historically has licensed its intellectual property. The splice variants exhibit a range of activities, including addition of nucleotides to telomeres and inhibition of endogenous telomerase activity. For those seeking to develop a commercial product in which human telomerase is used, a careful choice of the telomerase sequence along with a license to use and a legal opinion regarding its use is a necessary avenue of pursuit.

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Appendix 1

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