Is telomerase a viable target in cancer?

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Abstract

The ideal cancer treatment would specifically target cancer cells yet have minimal or no adverse effects on normal somatic cells. Telomerase, the ribonucleoprotein reverse transcriptase that maintains the ends of human chromosome, is an attractive cancer therapeutic target for exactly this reason [1]. Telomerase is expressed in more than 85% of cancer cells, making it a nearly universal cancer marker, while the majority of normal somatic cells are telomerase negative. Telomerase activity confers limitless replicative potential to cancer cells, a hallmark of cancer which must be attained for the continued growth that characterizes almost all advanced neoplasms [2]. In this review we will summarize the role of telomeres and telomerase in cancer cells, and how properties of telomerase are being exploited to create targeted cancer therapies including telomerase inhibitors, telomerase-targeted immunotherapies and telomerase-driven virotherapies. A frank and balanced assessment of the current state of telomerase inhibitors with caveats and potential limitations will be included.

Keywords

Senescence; Telomeres; Virotherapy; Immunotherapy

1. Telomere structure and function

Human telomeres consist of tandem double stranded hexameric DNA repeats ($TTAGGG_n$) at the ends of each chromosome [3], with a 3′ G-rich single-stranded overhang. The ends of telomeres form a lariat-like structure called a t-loop [4], which is postulated to be formed by strand invasion of the 3′ single strand overhang into the preceding double stranded telomeric DNA that is then stabilized by bound telomeric proteins [5]. A six-protein complex known as "shelterin" has remarkable specificity for telomeres and some members of the complex specifically bind telomeric DNA. The shelterin component proteins function to maintain telomere length, promote t-loop formation, recruit telomerase to telomeric ends, and to protect the ends of chromosomes from being recognized as DNA damage [6–8]. In cells that lack telomerase, telomeres shorten with each cell division due to incomplete replication of the lagging strand, oxidative damage, and telomere processing events that are yet to be fully understood [9–11]. After a finite number of cell divisions, cells lacking telomerase enter a state of growth arrest called replicative senescence. Telomere attrition likely leads to cellular senescence because of a disruption of their ability to protect chromosome ends from DNA damage and repair mechanisms. Functional telomeres must evade both ATM and ATR checkpoint pathways, both of which respond to DNA double

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strand breaks at telomeres [12–15]. In normal cells that lack telomerase, telomere attrition leads to critically short telomeres which trigger a localized DNA damage response [12,16–19] and p53-mediated cell cycle arrest [12,16,18,20,21]. It is possible that telomeres reach a critically short length that is no longer competent to assemble into the t-loop structure, and that even a few "uncapped" telomeres in a cell is sufficient to initiate replicative senescence [17]. However, cells that have acquired an inactivation of common cell cycle checkpoint proteins in the p53 and p16/Rb pathways are able to continue dividing (termed extended lifespan) and continue to lose telomeric sequences with each division [19,22]. These cells ultimately reach a crisis stage [23,24] which is marked by genomic instability due to chromosome end fusions and increased aneuploidy, and leads to p53-independent apoptosis [25,26]. Cancer cells which are able to maintain their telomeres must escape this crisis stage in order to continue to proliferate without limits. Escape from crisis is a rare event in human cells and thus senescence (also called M1) and crisis (M2) likely serve a potent anti-cancer mechanisms in long-lived species such as humans (Fig. 1) [23,24,27]. While telomerase expression is the mechanism for telomere maintenance in the vast majority of cancers there is at least one other documented telomere maintenance mechanism termed ALT (alternative lengthening of telomeres), which permits cells to escape crisis by DNA recombination [28–32].

2. Telomerase

Telomerase activity is not detected in most somatic cells, with the exception of some adult pluripotent stem cells, proliferative cells of renewal tissues, and male germline cells [33]. However, telomerase activity is nearly universal in human cancer cell lines, and is found in about 85–90% of primary tumors [29]. Telomerase activity counteracts progressive telomere shortening during cellular replication by synthesizing new telomeric DNA repeats at the chromosomal termini [34]. The human telomerase holoenzyme core components are a catalytic reverse transcriptase called hTERT (human telomerase reverse transcriptase) [35,36], and an associated template RNA called hTR or hTERC (human Telomerase RNA) [36–39]. hTR is ubiquitously expressed in all human cells, and telomerase activity is limited by the expression of hTERT, which is only found in cells with detectable telomerase activity [40,41]. Telomerase extends telomeres by binding to the 3′ single stranded overhang at the telomere end, catalyzing the addition of a single telomere repeat, and translocating to the new terminus. This processive cycle continues until the holoenzyme dissociates from the telomere [42,43]. In addition to the two core components of the telomerase holoenzyme complex, there are many confirmed and putative telomerase-associating proteins. The size of the human telomerase holoenzyme is approximately 1.5 mDa, which is significantly larger than the expected mass of a single hTR and hTERT (approximately 280 kD) [44]. A recent purification strategy was designed to isolate only catalytically active telomerase, revealing that only one additional component, dyskerin, was required for *in vitro* telomerase activity [45]. While other telomerase-associated proteins may not be necessary for catalytic activity, they may play a role in holoenzyme stability, recruitment or regulation.

Telomerase levels must be limiting for cells to maintain normal telomere length homeostasis, demonstrated by the continued elongation of telomeres when both hTERT and hTR are overexpressed in human primary and cancer cell lines [46]. Telomerase levels are regulated at every level of protein and RNA processing, as well as at the level of complex assembly and subcellular localization [42,43]. Telomerase activity at the telomere is also regulated at the level of telomerase recruitment to the telomere. While the exact mechanism of telomerase recruitment is still not fully known, it is likely part of a negative feedback loop created by shelterin proteins bound at the telomere that serve as negative regulators of telomerase extension of telomeres (reviewed in [8,47]). The prevailing model of shelterin-mediated telomere length regulation is that longer telomeres recruit more shelterin complex,

which in turn limits future telomerase elongation. This negative feedback loop is thought to be responsible for the stable telomere length found in cancer cells, and is likely responsible for at least partially maintaining telomere length homeostasis in germ cells and other stem-like cells in which telomerase is active [8,48]. Thus, there is likely to be mechanisms regulating telomerase recruitment or processivity depending on whether cells are at equilibrium conditions versus non-equilibrium conditions.

Telomerase activity is detected and measured using a PCR-based assay called the Telomere Repeat Amplification Protocol, or TRAP [29,49,50]. The TRAP assay is very sensitive, and can detect telomerase activity from a wide range of sample sizes, from small tissue biopsies to a just a few cancer cells [51]. PCR-amplified telomerase reaction products from the TRAP assay can be semi-quantitated by normalizing the signal to an internal standard (ITAS) that accounts for variability in PCR efficiency. TRAP analysis of a large panel of primary normal versus tumor samples, as well as mortal versus immortalized cell lines, revealed that telomerase activity is restricted in normal human somatic cells but is activated in cancer and immortalized cells [29]. The approximately 10% of cancer cells that do not upregulate telomerase have been found either to be "mortal" tumor cells or to maintain their telomeres through a telomere recombination pathway called ALT (alternative lengthening of telomeres) [52]. The ability of cancer cells to replicate indefinitely through telomerase activation or ALT pathway activation has been called one of the hallmarks of cancer [2]. Indeed, tumors which have short telomeres and no detectable telomerase activity are self-limiting, and almost always regress spontaneously [53,54]. These results indicate that a telomere maintenance mechanism is necessary for tumor progression, and suggest that telomerase is an attractive target for selective cancer therapies. We will discuss three independent approaches to target telomerase expressing cells, their current status, and limitations to their use.

3. Telomerase inhibition

A fundamental quality of cancer cells is their capacity to replicate without limits, which is achieved by telomerase-mediated telomere maintenance in the majority of advanced tumors [19,55]. Thus, telomerase inhibitors have the potential to be used as a selective anti-cancer therapy which disrupts the replicative capacity of telomerase-positive cancer cells [2]. Normal somatic cells which do not utilize telomerase activity to maintain telomere length would be largely unaffected, as normal cells which are telomerase competent almost always have longer telomeres compared to telomerase-positive cancer cells [33]. This may provide a therapeutic window where tumor cells could be driven to apoptosis before normal telomerase competent cells are adversely affected. However, this generally indicates that inhibitors of telomerase will not be well suited without tumor debulking. The template region of the telomerase RNA (hTR) offers an accessible substrate for direct enzymatic inhibition using oligonucleotide-based small molecule inhibitors [56–71]. While much still needs to be learned regarding the conformation, composition and recruitment of the catalytically active telomerase holoenzyme, it is certain that the template region of hTR must be exposed and accessible in order to synthesize *de novo* telomere repeats. Thus, oligonucleotides that can hybridize to the 11-base hTR template region act as competitive telomerase inhibitors (not antisense targeting messenger RNA). One such compound that has been developed is GRN163L, currently known as Imetelstat, is a lipidated N3′–P5′ thio-phosphoramidate 13-mer. The thio-phosphoramidate backbone causes the oligonucleotide to be water soluble, acid stable, nuclease resistant and to form stable RNA duplexes [61,64,72–74]. The 5′ palmitoyl moiety of GRN163L causes the compound to be lipid soluble, allowing for cellular uptake without the use of lipophilic carriers and improving telomerase inhibition [64,65,68]. The sequence of GRN163L (5′-palmitate-TAGGGTTAGACAA-

NH_2-3′) targets a 13 nucleotide region of hTR, preventing it from forming an active complex with hTERT.

The effects of GRN163L have been investigated in a number of cancer cell lines and mouse xenograft models. Chronic exposure to GRN163L has been shown to inhibit telomerase and cause telomere shortening in cancer cell lines derived from diverse origins, including tumors of the brain, breast, bladder, liver, lung, prostate and stomach [56,61,66,68,75–78]. GRN163L-induced telomere shortening initiates cellular crisis caused by chromosomal fusions, anaphase bridges and subsequent apoptosis [61]. In mice with human tumor xenografts, GRN163L was well-tolerated and induced telomerase inhibition in doses ranging from 5 mg/kg to 1000 mg/kg [61]. Xenograft models showed that GRN163L works to inhibit tumor growth, prevent growth of metastases, and sensitizes tumors to conventional chemotherapy agents [61,68]. GRN163L also is able to cross the blood-brain barrier to target glioblastoma xenograft tumors, supporting the further study of using telomerase inhibition alone or in combination with conventional therapies in glioblastoma patients [77]. GRN163L has already completed several Phase I trials in patients with chronic lymphocytic leukemia and solid tumors such as breast cancer and non small cell lung cancer [79]. These Phase I trials showed that intravenously infused GRN163L has excellent bioavailability, pharmacokinetics and tolerability, and after dose-escalation studies, a dose of 9.4 mg/kg was chosen for Phase II clinical trials. Phase II trials of GRN163L alone, in combination with other chemotherapy and targeted drugs, or in a maintenance setting after standard chemotherapy are now being conducted for patients with NSCLC, advanced breast cancer, chronic leukemia, essential thrombocythemia and multiple myeloma (Table 1).

Because telomerase inhibitors may require a period of treatment to produce telomeres short enough to trigger cancer cell death, telomerase inhibition therapy may be most effective when used in conjunction with conventional chemotherapies, radiation or other targeted therapeutics such as angiogenic inhibitors. The long lag phase between initiating telomerase inhibition and induction of cancer cell death may allow for increasing the tumor mass, and is likely to be inefficient at reducing tumor bulk as an individual treatment. In addition, there may be some unknown off-target effects of Imetelstat. In contrast, conventional chemotherapy approaches result in immediate tumor mass reduction without effecting telomere length causing tumors to often develop treatment resistance and eventually there is recurrence of disease (Fig. 2). Using a telomerase inhibitor such as GRN163L in combination with conventional cancer therapies should cause progressive telomere shortening in cancer cells that are not initially susceptible to combination treatment, theoretically leading to a more durable response and decreased disease recurrence (Fig. 2). Telomerase inhibitor therapy may also be used as a maintenance therapy following conventional chemotherapy, radiation or surgery to extend survival with reduced side effects of long-term treatment (Fig. 2). Yet another potential use for telomerase inhibitors is as chemotherapy and/or a radiation sensitizing agent, enabling the efficient use of lower doses of chemotherapy drugs and radiation.

An important factor in whether telomerase inhibitors such as GRN163L are able to limit cancer recurrence and relapse is whether the drug is able to target residual stem-like cancer cells. A cancer stem cell is defined as one that can self-renew, initiate tumor formation and regenerate all of the cell types found in a tumor [80]. There is increasing evidence that rare cancer stem cell populations which are refractory to conventional treatments are responsible for initiation and recurrence in a variety of hematologic and solid tumors [81–83]. While conventional therapies may not target these cancer stem cells, progressive telomere shortening induced by combination or maintenance treatment with telomerase inhibitors would potentially impair their self-renewal properties. Recent studies have shown that telomerase inhibitors target cancer stem cell populations in multiple myeloma, prostate,

brain, breast and pancreatic cancer [77,78,84–86]. Cancer stem cell populations were reduced in size [78,85,86], had reduced proliferation [84], shortened telomeres [78,86], and impaired ability to form characteristic free-floating spherical colonies [77,85] after treatment with the GRN163L telomerase inhibitor. These studies indicate that telomerase inhibition does disrupt cancer stem cell self renewal, and supports the hypothesis that telomerase inhibition will be a viable maintenance treatment to decrease disease recurrence following other therapies.

4. Telomerase-targeted immunotherapy

Several telomerase-based immunotherapy strategies have been developed and many are in advanced clinical trials, making this a rapidly-progressing field of anti-telomerase cancer therapy [87–101]. Telomerase is an attractive target antigen for cancer immunotherapy because it is expressed almost universally in human cancers and is functionally required to sustain malignant tumor long-term growth [87]. In brief, anti-telomerase immunotherapy sensitizes immune cells to tumor cells expressing hTERT peptides as surface antigens via the human leukocyte antigen (HLA) class I pathway [102]. This causes an expansion of telomerase-specific CD8+ cytotoxic T lymphocytes (CTLs), directing the patient's own immune system to target and kill telomerase positive tumor cells [101,103]. The immune response can be induced by exposure to antigen presenting cells that either overexpress immunogenic hTERT fragments [92] or have been pulsed with immunogenic hTERT peptides. To date, 26 different hTERT peptides have been utilized to elicit an anti-telomerase immune response [88,97,101,102,104–111]. Vaccination studies using the I540–548 peptide have produced functional anti-tumor responses in prostate, breast and melanoma patients [89,96,112]. The hTERT E611–626 peptide is a promiscuous HLA class II epitope [91] that can be administered without HLA typing patients, making it a good candidate to stimulate CD4+ T lymphocytes and generate a general (perhaps universal) cancer vaccine. This peptide is available as an injectable vaccine, GV1001. GV1001 has been shown to induce hTERT-specific T cell responses in Phase I/II clinical trials for non-small cell lung cancer [91] and non-resectable pancreatic carcinoma [113]. GV1001 is currently in a randomized Phase III clinical trial in patients with locally advanced or metastatic pancreatic cancer (ClinicalTrials.gov Identifier: NCT00425360) with results expected in 2012. With objective responses in the randomized trial, GV1001 could become the first approved anti-telomerase based cancer therapy. Despite promising earlier trials with GV1001, a recent small trial with GV1001 did not show clinical efficacy. In this study none of six patients with cutaneous T cell lymphomas showed positive responses and one patient even had progressive disease leading the trial to end early (J Dermatological Science 62 (2011) 75–83). So as with all peptide immunotherapies, it is still too early to know if this will become an approved therapy for cancer.

Another anti-telomerase vaccine, GRNVAC1, utilizes autologous mobilized immature dendritic cells isolated from the patient's own circulating blood which are then transduced *ex vivo* with mRNAs encoding a near full-length hTERT protein. These primed dendritic cells display a multitude of hTERT fragments and are then matured *ex vivo* and returned back to patients to elicit a polyclonal anti-hTERT T cell response [92]. GRNVAC1 has completed Phase II clinical trials in patients with acute myelogenous leukemia and metastatic prostate cancer, and the vaccine has thus far proven to be well-tolerated and produce ahTERT-specific immune response in a significant fraction of patients [personal communications]. While the *ex vivo* immunotherapy trials may be more effective compared to peptide immunotherapies, they are quite expensive trials and require much more regulatory surveillance.

In summary, the current results of these anti-telomerase cancer immunotherapy clinical trials are encouraging. Multiple vaccine strategies are being developed and tested in patients with melanoma, lung, prostate, breast, and pancreatic cancers [89,91,92,96,99,112,113], and these trials have generally produced a specific immune response against hTERT positive tumor cells. While telomerase is expressed in some normal tissues [33,114,115], no patients have exhibited serious adverse effects (such as autoimmune disease or bone marrow depletion) indicative of an immune response against normal cells. One explanation is that normal cells express very low levels of hTERT [115], making them poor targets relative to tumor cells with high levels of hTERT expression. Another possibility is that hTERT peptides are differentially expressed on the surface of tumor and normal cells. Further studies are needed to better understand the regulation of the degradation, processing and presentation of hTERT antigens on tumor and normal cells. Nevertheless, current results indicate that induction of hTERT-specific immune responses is safe and well-tolerated and the use of anti-telomerase immunotherapies in cancer treatment regimens is promising. Additional studies are needed to address the processing, presentation and response elicited by hTERT epitopes, and future clinical studies are needed as well as the ones in progress that should help clarify the effect of telomerase-targeted immunotherapies on tumor regression.

5. Telomerase-directed viral therapy

While the RNA component of human telomerase is ubiquitously expressed in all human cells, the hTERT promoter is very tightly regulated and telomerase activity is generally not detected (e.g. the hTERT promoter is silent) in most normal cells [42,116,117]. Telomerase positive tumor cells can thus be directly targeted by introducing suicide genes or oncolytic viruses driven by the hTERT promoter. One approach that has been reported involves using the hTERT promoter to drive the expression of a pro-apoptotic "suicide" gene (such as tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [118] or caspase 6 [119]). The hTERT-driven suicide gene is then introduced to a tumor where expression of the suicide gene in telomerase-positive cells induces apoptosis. In an alternate version of this telomerase-driven gene therapy, tumors are provided with ahTERT-driven prodrug-activating enzyme [120–124], which results in apoptosis of telomerase-positive cells when administered in conjunction with a prodrug that is metabolized into a toxin.

A second strategy which utilizes the specificity of the hTERT promoter directs the replication of a lytic adenovirus in telomerase-positive cells [125]. The modified hTERT promoter driven adenovirus only expresses the viral proteins E1A and E1B, required for viral replication, when present in telomerase-positive cells [126–131]. Pre-clinical studies showed selective targeting and effective cytolysis of telomerase positive cells derived from a variety of human cancer cell lines, while normal telomerase-negative cells showed reduced viral replication and cytotoxicity. Furthermore, mouse xenograft studies showed that telomerase-specific viral therapy reduced the size of xenograft tumor, retarded tumor growth and extended survival [126–131]. Following direct injection of the adenoviral vector into the xenograft tumor, viral particles were found replicating in distant tumor tissues, suggesting that the viral particles can circulate in blood and lymph to target distant metastases [125,127]. These results encouraged the design of a Phase I clinical trial using the OBP-301 virus, also known as Telomelysin [132]. This study enrolled sixteen patients with a variety of advanced solid tumors, and determined that a single intratumoral injection of Telomelysin was well tolerated at all doses and generated anti-tumor activity [132]. Based on these results, Phase II trials of Telomelysin are expected, and second-generation viruses that combine telomerase targeted viral replication and suicide gene expression are being developed [133–135]. The use of telomerase-specific viral therapy in combination with irradiation and chemotherapy such as paclitaxel, cisplatin and gemcitabine is also being

examined [136–139]. These studies should determine if oncolytic viruses have potential as a broad-spectrum cancer therapy. However, it should be noted that most previously attempted adenoviral-based cancer therapies have failed to provide robust tumor responses.

6. Concluding remarks

Telomerase is considered an almost universal target for human cancers since telomerase-mediated telomere maintenance is the mechanism employed by a vast majority of cancer cells to enable limitless proliferation. In this review, we discussed three separate approaches to specifically target telomerase-expressing cancer cells, and clinical trials are currently underway to establish the utility of these strategies as cancer therapies (Table 1). Current and ongoing results suggest that telomerase-based therapies have an increasing potential to become an important component of future cancer treatment regimens.

Acknowledgments

Abbreviations

ALT	alternative lengthening of telomeres
hTERT	human telomerase reverse transcriptase
hTR	human telomerase RNA
TRAP	telomere repeat amplification protocol
CTL	cytotoxic T lymphocytes
NSCLC	non small cell lung cancer
HLA	human leukocyte antigen

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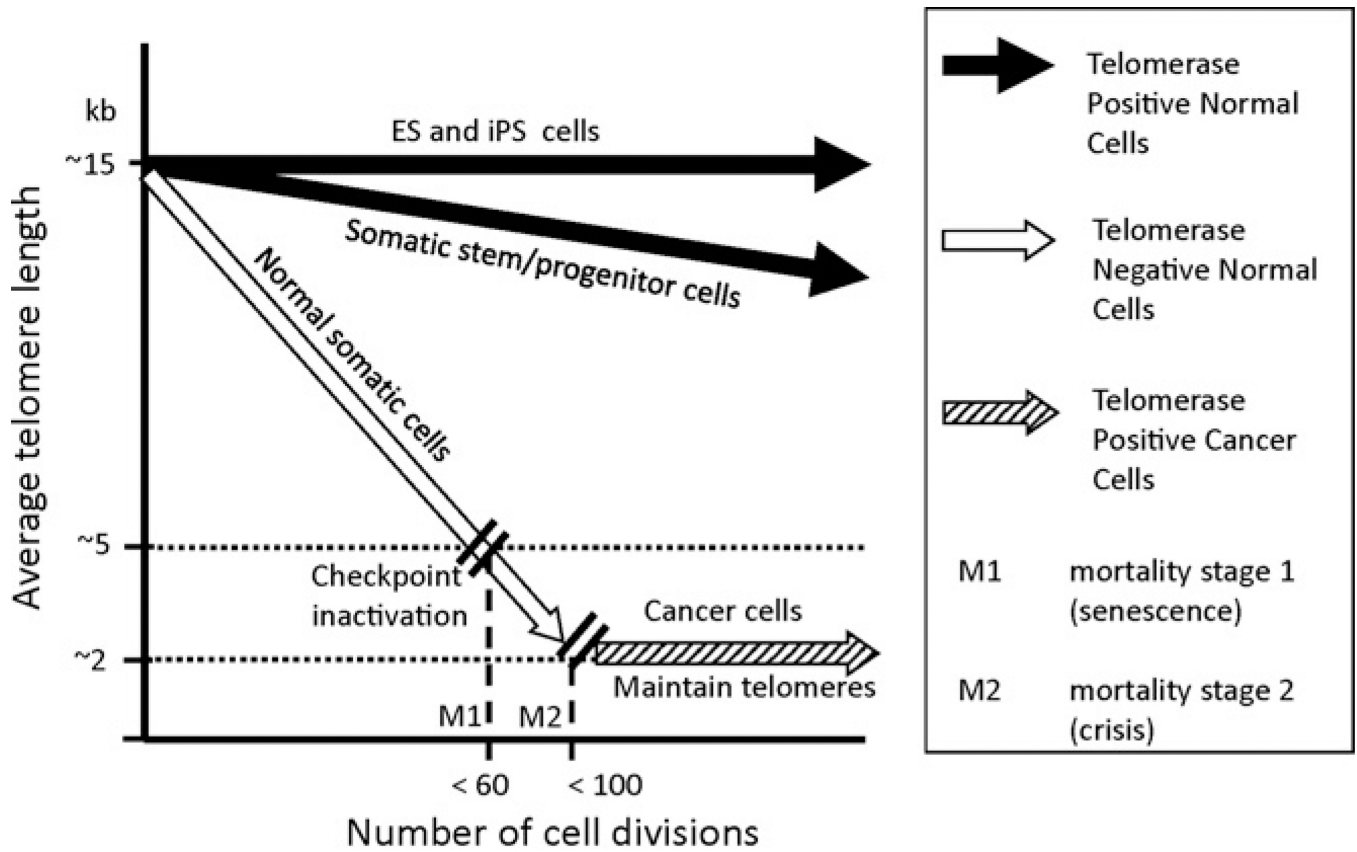
Fig. 1.
Comparison of telomere lengths to cell divisions: human embryonic stem cells (ES) and perhaps induced pluripotent stem cells (iPS) have robust telomerase activity and may completely maintain telomere lengths. Somatic stem cells/progenitors that transiently express telomerase or only express moderate amounts of telomerase partially, but not completely, maintain telomeres. With increased age and cell divisions, telomeres continue to shorten in these cells. Normal somatic cells which do not express telomerase have the greatest rate of telomere shortening with each cell division. Cancer cells which reactivate or upregulate telomerase fully maintain telomeres but generally at greatly reduced lengths.
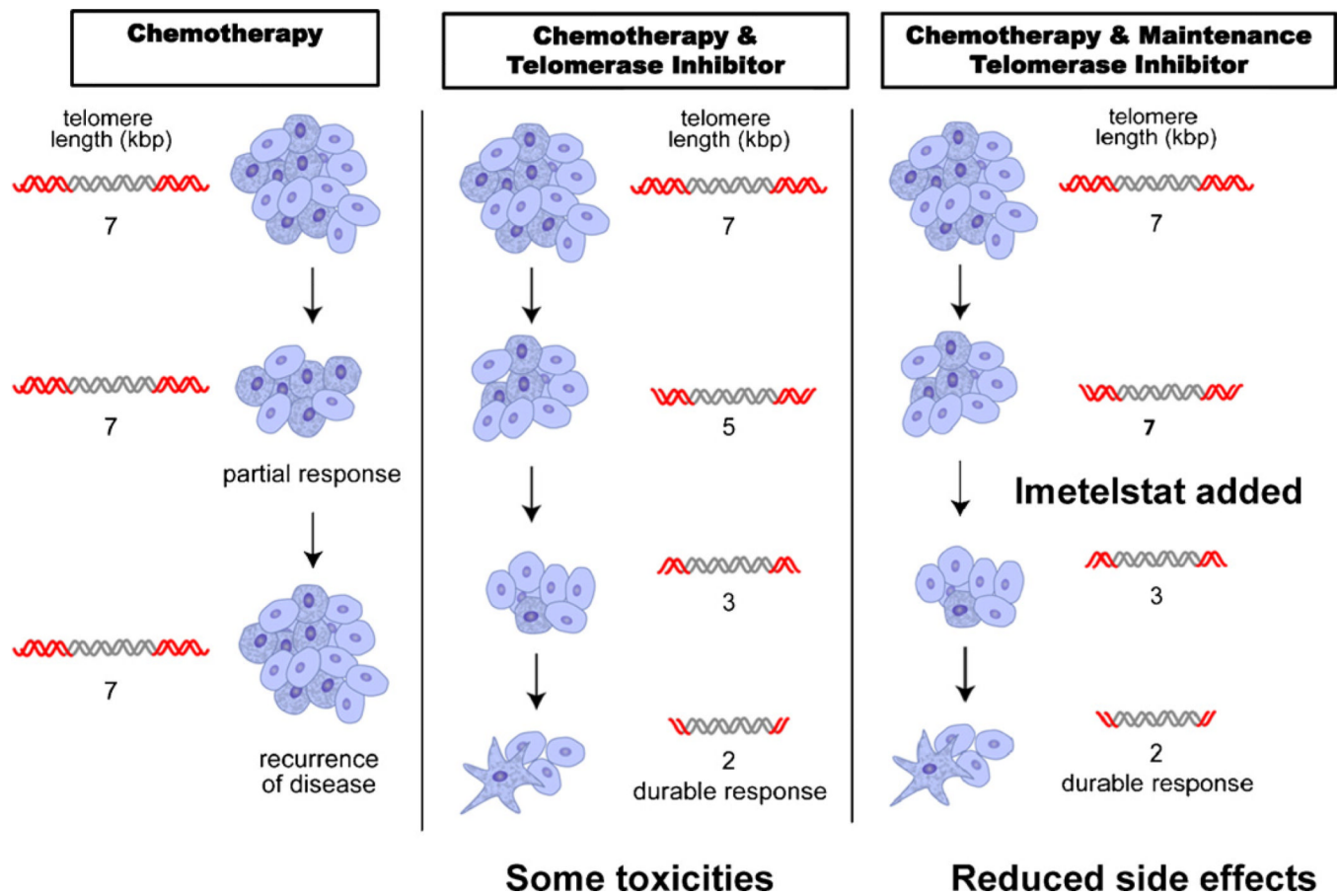
Fig. 2.
Comparisons of chemotherapy alone to combining chemotherapy with a telomerase inhibitor. Due to some toxicities in combining therapies in phase I clinical trials, a phase II trial is in progress using the telomerase inhibitor, Imetelstat, in a maintenance setting following standard chemotherapy.

Table 1

List of ongoing human clinical trials using a variety of approaches to targeting telomerase.

Trial identifier	Condition	Intervention	Phase
NCT00124189	Chronic lymphoproliferative diseases	Imetelstat (GRN163L)	I
NCT01242930	Multiple myeloma	Imetelstat (GRN163L)	II
NCT01256762	Locally recurrent or metastatic breast cancer	Imetelstat (GRN163L) + bevacizumab + paclitaxel	II
NCT01137968	Non-small cell lung cancer	Imetelstat (GRN163L) + bevacizumab	II
NCT01265927	Breast neoplasms	Imetelstat (GRN163L) + trastuzumab	I
NCT01243073	Essential thrombocythemia	Imetelstat (GRN163L)	II
NCT00021164	Melanoma adult solid tumor	Aldesleukin + incomplete Freund's adjuvant + telomerase 540–548 peptide vaccine	II
NCT00069940	Brain and central nervous system tumors; gastrointestinal stromal tumor; sarcoma	Telomerase 540–548 peptide vaccine + sargramostim	I
NCT00509457	Carcinoma, non-small-cell lung	GV 1001 telomerase peptide	
NCT01247623	Malignant melanoma	GV 1001 telomerase peptide + temozolomide	I, II
NCT00061035	Prostatic neoplasms	Anti-telomerase transgenic lymphocyte immunization vaccine (TLI)	I
NCT00197912	Advanced melanoma	Tumor antigen loaded autologous dendritic cells	I, II
NCT00925314	Stage III melanoma	Anti-telomerase transgenic lymphocyte immunization	II
NCT00079157	Breast cancer	Incomplete Freund's adjuvant + telomerase 540–548 peptide vaccine + sargramostim	I
NCT00425360	Pancreatic cancer	Sargramostim + telomerase peptide vaccine GV1001 + Capecitabine + gemcitabine	III
NCT00573495	Breast cancer	hTERT/survivin multi-peptide vaccine	I
NCT00197860	Advanced renal cell carcinoma	Tumor antigen loaded autologous dendritic cells	I, II
NCT00510133	Acute myelogenous leukemia	GRNVAC1	II
NCT01153113	Metastatic prostate cancer	GRNVAC1	I, II