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WHOLE-BODY INTERDICTION OF LENGTHENING OF TELOMERES: A PROPOSAL FOR CANCER PREVENTION

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1. ABSTRACT

The intrinsic genetic instability of cancer cells makes age-related cancers more difficult to postpone or treat than any other age-related disease. Any treatment that a cancer can resist by activating or inactivating specific genes is unlikely to succeed over the long term, because pre-existing cancer cells with the necessary gene expression pattern will withstand the therapy and proliferate. "Whole-body Interdiction of Lengthening of Telomeres" (WILT) is a proposal to pre-empt this problem by deleting from as many cells as possible, the genes required for telomere elongation. Cancers lacking these genes can never reach a life-threatening stage by altering gene expression, only by acquiring new genes, which is far more unlikely. Continuously-renewing tissues can be maintained by periodic reseeding with telomere elongation-incompetent stem cells that have had their telomeres lengthened *in vitro* with exogenous telomerase. Here, I describe why WILT might prove to be an exceptionally powerful anti-cancer modality.

2. INTRODUCTION

Though some success has been achieved in suppresing tumour growth with immunotherapy (1-3), the very long-term management of cancer may well prove impossible in most cases. A tumour must grow to around 10^{12} cells before becoming clinically relevant. Many of these are stromal and inflammatory cells (4), but a large proportion are genuine tumour cells, all of which exhibit extreme genomic instability. The continuous emergence of additional mutations, epimutations, chromosomal rearrangements and aneuploidy is, more often than not, deleterious for individual cancer cells. However, in a minority of cases it provides those cells with new ways to escape inhibitory therapeutic strategies for proliferation that the clinician might utilize. One example of this may be presence of cells expressing markers normally seen in stem cells, such as CD34 (5). The discovery of such "cancer stem cells" has aroused considerable interest in the possibility that targeting them might be sufficient for elimination of the entire tumour. However, this may be

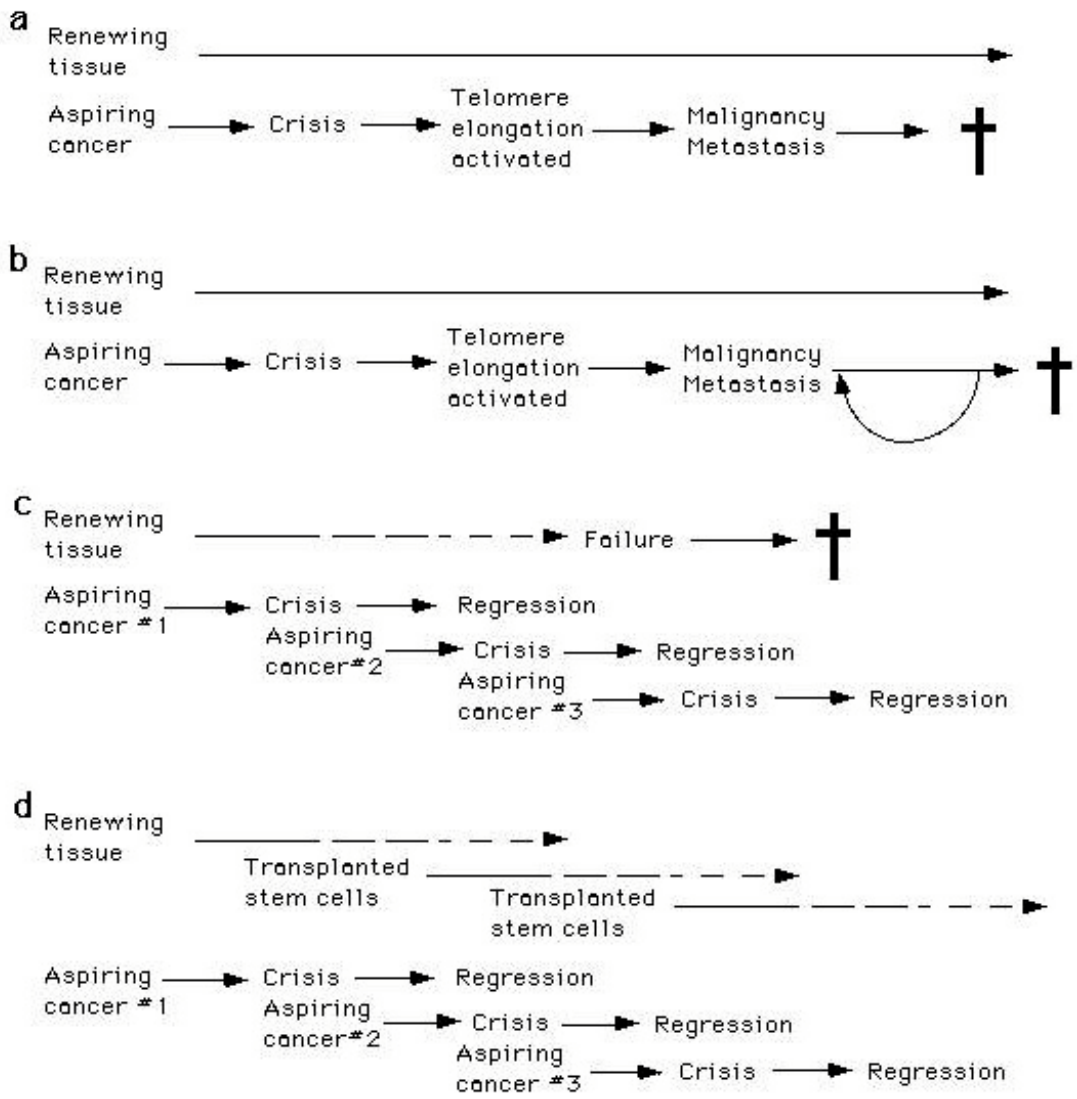

Figure 1. The WILT concept. Metastatic cancers kill a typical untreated patient (a) rapidly and a beneficiary of contemporary treatments (b) only slightly less rapidly, on account of the genomic instability and inventiveness of such tumours. Uncompensated deletion of telomerase genes (c) prevents this but kills much sooner by preventing the maintenance of continuously renewing tissues such as the blood. In WILT, deletion of telomerase genes is combined with periodic stem cell therapy to maintain such tissues indefinitely, so that death from either cause is avoided (d).

overoptimistic if (as seems inevitable) tumour stem cells are continuously generated by mutation-driven dedifferentiation processes.

Here I describe and update a proposal first published in 2004 (6) that seeks to overcome the aforementioned innate ingenuity which cancers exhibit. Termed WILT, for "Whole-body Interdiction of Lengthening of Telomeres", it seeks to block cancer growth in a manner that cannot be evaded by the changes of gene expression which genomic instability causes, but only (in principle) by the creation of totally new genes. This is something that evolution does all the time but cancers, possessing "only" a trillion cells and existing for only a few decades, are vanishingly unlikely to achieve. The genes in question are those that extend the cell's telomeres and thereby compensate for the inherent telomere shortening that accompanies cell division. The multitudinous side-effects of this therapy are proposed to be counteracted by periodic stem cell reseeding to maintain rapidly-proliferating tissues even though their individual stem cells are no longer capable of indefinite division (figure 1). At present, a therapy as complex and ambitious as WILT would have no place in the clinic, given the availability of simpler therapies that modestly postpone cancer and somewhat better ones likely to arrive soon. However, we may need to accelerate our progress in postponing cancer in order to reduce mortality from it, as progress in postponing other age-related killers may accelerate sharply in the next few decades (7-9). In this review I discuss the practicalities of implementing the deletion of telomere-elongation genes, the various stem cell therapies that would be needed in

compensation, and a variety of related issues bearing on the eventual feasibility of this prospective anti-cancer modality.

3. TELOMERE ELONGATION IN NORMAL AND CANCEROUS CELLS

3.1. Telomere structure and shortening

The nuclear genome of all mammalian species consists of a set of linear chromosomes. Since chromosomes are frequently subject to damage, including outright double-strand breakage, machinery exists to repair such breaks. This presents an obvious problem: that machinery must be able to distinguish between DNA ends that have resulted from a double-strand break and ones that are simply the ends of chromosomes, in order to ligate the former but not the latter. This is achieved by capping chromosome ends (telomeres) with special sequences that are bound by numerous sequence-specific proteins and thereby protected from being processed by the double-strand break repair machinery. In mammals, these sequences consist of thousands of repeats of the hexamer TTAGGG (10).

In the early 1970s, long before anything was known about telomere sequence or structure, Olovnikov (11) and Watson (12) independently realised that the process of DNA replication of linear chromosomes faces a severe problem. The replication machinery only goes in one direction on a given strand, and it cannot start at the absolute tip. At the end of a chromosome, therefore, one strand can be replicated right up to the tip by a polymerase that begins some distance away, but the other strand will not be replicated completely – the portion between the tip and the nearest replication initiation site will not be replicated. This "end-replication problem" will preclude the indefinite division of a cell, because eventually the chromosome end will be misinterpreted as a double-strand break and will be joined to the end of another chromosome, to form what is known as a Robertsonian fusion (13). Such two-centromere molecules cannot be handles properly by the cell division machinery, so cell division either stops immediately or is rapidly prevented by the insupportable degree of aneuploidy resulting from chromosomal mis-segregation and breakage/fusion/bridge cycles. In fact, the rate of chromosome shortening per cell division is much greater than would be predicted purely from the end-replication problem, because telomeres are sequestered from the double-strand break machinery by the formation of a "t-loop". This is a structure in which the tip of the telomere consists of a single-stranded overhang that is intercalated into the double-stranded sequence to form a triple helix bound by various proteins (14). This overhang is always on the G-rich strand and is presumed to be formed by the action of an as yet unidentified exonuclease.

3.2. Compensating for telomere shortening with telomerase and ALT

The progressive shortening of telomeres is theoretically tolerable in all somatic tissues, because the finite duration of life places a limit on how many divisions such cells ever need to perform. This is not so, however, for the germ line, in which cell division progresses through organismal generations and is thus indefinite. It has therefore been necessary for species with linear chromosomes to evolve a way to nullify the end-replication problem. In almost all species (the known exceptions all being insects (15,16)), the method used is to extend the telomeres with a specific enzyme, telomerase, which adds copies of the simple sequence described above to "top up" the telomere. Telomerase has also been used in the soma of many organisms, including mammals, to allow the development of tissues that are continuously replenished by stem cells which divide considerably more often in an organismal lifetime than would be possible without telomerase. Telomerase is a ribonucleoprotein consisting of an RNA subunit, TERC, that provides the template sequence, and a catalytic subunit, TERT, that reverse-transcribes the template to extend the telomere (17).

Indefinite division, uncontrolled by the factors that regulate division of stem cells, is the defining feature of cancer. It is thus no surprise that over 90% of human cancers are found to express telomerase (18): if they did not, they could not have divided often enough to reach a clinically relevant size, since that takes at least 200-300 divisions (19). (Note that this does not follow simply from the number of cells in a clinically relevant tumour. One must also take into account the rate of cell death within the developing tumour and the fact that multiple mutational events, each happening in just one of the many descendents of the cell that experienced the previous event, are required to achieve malignancy.) Those that do not express telomerase are found to possess an alternative mechanism for maintaining telomere length during cell division. This mechanism is termed ALT, for "Alternative Lengthening of Telomeres" and is thus far only superficially characterised (20).

3.3. Consequences of uncompensated telomere shortening

Human cells that do not divide often enough in a normal lifetime to need telomerase are found to have its expression powerfully suppressed, presumably as an anti-cancer mechanism. An example of such a cell is the fibroblast, which was famously discovered by Hayflick to undergo "replicative senescence" after around 50 divisions (the "Hayflick limit") in cell culture (21). For many years this phenomenon was widely held, possibly as a result of the name Hayflick gave to it, to be likely to contribute to mammalian aging, overlooking the fact that fibroblasts are vanishingly unlikely to divide that often in a normal human lifetime. Since cells that do need to divide more often than the Hayflick limit duly express telomerase (22), the likelihood that replicative senescence truly underlies much of mammalian aging must be considered low on current evidence (23). (The possibility remains, however, that cells which enter a state very similar to replicative senescence as a result of double-strand breaks may indeed contribute to mammalian aging or cancer (24,25).) The activation of telomerase seems generally to occur late in the development of a tumour (e.g. 26). This may be caused by a period of especially elevated genomic instability known as "crisis" that is actually brought on by the telomere

shortening and consequent Robertsonian fusions, chromosome breakage and aneuploidy which occurred during the prior growth of the tumour. It should be noted that stem cells express much lower mean levels of telomerase than typical tumours, possibly by activating it only during mitosis (27). The systems that prevent telomerase expression from rising to a tumour-compatible level are poorly understood at this point and are probably less effective than those which totally suppress telomerase in mesenchymal cells. This has led to the belief that most cancers derive from stem cells (28).

Further illumination of the above points has emerged from study of laboratory mice. Mice express telomerase in many tissues, including cell types such as fibroblasts that divide quite rarely, and their telomeres also contain many more TTAGGG repeats than those of humans (29). This does not challenge the above logic, because mice are so small that telomerase suppression would probably not prevent death from cancer even with telomeres as short as possible compatible with t-loop formation. In particular, primary tumours generally kill mice without metastasising (e.g. 30). Telomerase-negative mice duly die of cancer with at least the same frequency as normal mice, even after being inbred for a few generations so that their telomeres are unnaturally short at birth (31).

Absence of telomere elongation is never found in humans, presumably because it would cause prenatal lethality. Impaired elongation is seen, however, in a condition termed dyskeratosis congenita (DC) (32). DC is caused by mutations in various loci, of which two have been identified thus far. One is the RNA component of telomerase and causes autosomal dominant inheritance of DC, and the other is in a telomere maintenance protein named dyskerin causing X-linked DC (33). Autosomal recessive inheritance is seen in about half of DC sufferers but the affected loci are not yet known. DC sufferers exhibit failure of stem cell-derived tissues, especially the blood, skin and gut. They also show elevated incidence of cancer: this is consistent with the observations outlined above, namely telomere shortening causing crisis and consequent activation of hitherto robustly suppressed telomere elongation mechanisms. Unfortunately, the rarity of DC has so far prevented the determination of whether DC sufferers' tumours mainly use telomerase or ALT for telomere elongation.

4. A UNIQUE FEATURE OF TELOMERE ELONGATION: ITS SHORT-TERM DISPENSABILITY IN ALL SOMATIC CELL TYPES

The adage that cancer cells are very hard to kill is of course a simplification: in fact they are easy to kill, but not without killing a great many non-cancer cells (and hence, maybe, the patient) at the same time. The intrinsic phenotypic similarity of cancer cells to the normal cells from which they arose is the main reason for the modest therapeutic index of most cancer therapies. One of the few features that can reliably distinguish them is that cancer cells divide more rapidly, and this is the basis for many if not most cancer treatments, from chemotherapy on. However, it is only a difference of degree, so any therapy that specifically targets dividing cells is bound to inflict collateral damage on normal mitotic cells, including stem cells.

Thus, to make ourselves much more resistant to death from cancer than at present, it seems that we must alter normal cells pre-emptively, before they have become cancerous, so that they cannot acquire the genetic changes necessary to allow hundreds of cell divisions. At first this seems clearly impossible, given the aforementioned requirement for such proliferative potential in stem cells. WILT (Whole-body Interdiction of Lengthening of Telomeres) is an attempt to resolve this conundrum (6). Since stem cells only require telomerase to maintain their telomere length above a threshold, they would not be expected to malfunction for at least a few dozen cell divisions even if they had no telomerase activity at all. Thus, elimination of telomerase activity from all cells in the body of an adult might in principle be tolerable if stem cells of all rapidly-renewing tissues were replenished sufficiently often with new stem cells, also lacking telomerase activity but with initially long telomeres. If the elimination of telomerase activity were accomplished by the actual deletion of the genes encoding one or both of the core telomerase subunits, such individuals would be constitutionally unable to suffer from telomerase-positive cancers. The only theoretical way out would be the implausible acquisition of telomerase activity out of nothing by the creation of a new telomerase gene. A corresponding deletion of essential components of the ALT pathway might also be conceivable, depending on how it is eventually found to function.

The remainder of this article discusses the reasons why the above proposal, though ambitious, may be feasible in the foreseeable future. I survey the practicalities of deleting telomerase genes from all cell types, and the plausibility of replenishing stem cell pools frequently enough to maintain function of all stem-cell-derived tissues. I then discuss our current knowledge concerning whether ALT is likely to be amenable to this approach too, and reasons why WILT can be a truly comprehensive anti-cancer therapy even if implemented incompletely. I conclude with a brief discussion of why it is not too soon to be exploring the components of such a futuristic therapy.

5. DELETING TELOMERASE GENES FROM ADULT TISSUES

Our cells can be considered as falling into three broad categories: mitotically active, mitotically competent but quiescent, and postmitotic (mitotically incompetent). Genetic modification of mitotically incompetent cells to delete telomerase genes would be harmless but is not obviously necessary for efficacy of this approach. Given the inevitable incidence of disruption of vital genes by random integration of engineered DNA it seems that somatic gene therapy targeting postmitotic cell types is not a priority as part of this therapy. I will thus focus below on mitotically active cells and quiescent cells.

5.1. Mitotically active cells

The technology to introduce new stem cells into a continuously renewing tissue has existed for many years, in the form of the bone marrow transplant (BMT). BMT is a standard component of treatments for numerous haematological problems, whether genetic (such as aplastic anaemia) or induced as a side-effect of other treatments (particularly radiotherapy or chemotherapy for cancer) (34). There have recently been dramatic advances in our ability to expand haematopoietic stem cells (HSCs) *in vitro* while maintaining their long-term repopulating potential (35,36). This means that it is now practical to isolate bone marrow from a patient, isolate HSCs from it, make genetic alterations to them, select cells that have undergone the desired modification, and expand them to therapeutic numbers before reintroducing them into the patient. Thus, the blood is a tissue into which telomerase-knockout stem cells can in principle be introduced easily, even though gene targeting (homologous recombination-based modification of a specific chromosomal sequence) is currently achievable only at very low efficiency (37). Stem cells introduced by BMT do not readily occupy the bone marrow niches that support long-term survival, but engraftment can be stimulated by first depleting the resident bone marrow with chemotherapy (38). (I will return to this last point below.)

The other major tissues that are maintained by stem cells are the skin, the gut and the lung. In each case, even though the procedure is not as routine as for the blood, replacement of stem cells by ones that have been genetically modified *in vitro* is foreseeable. The skin is furthest advanced: burns therapy and cosmetic surgery have driven research in this area and it is now possible to introduce new epidermal stem cells into skin that has been peeled down to the dermal layer, with fully functional reconstitution of the epidermis (39,40). Similar therapy for the lung is being explored in the context of cystic fibrosis therapy (41). In the case of the gut, initial work in mice showed that disaggregated cells isolated from the intestine of a mouse could be plated onto a surgically denuded portion of the intestinal lining of a genetically distinguishable mouse and fully reconstitute the gut wall (42). Problems of fibrosis were encountered when attempting to extend this approach to pigs (unpublished data), but encouraging progress has recently been reported by another group in a canine setting (43). (Non-surgical, e.g. endoscopy-based, approaches are clearly required if this is to be a practical component of WILT.)

5.2. Quiescent cells

The large majority of human cancers derive from haematopoietic or epithelial lineages that are maintained by stem cells, but a substantial minority, such as sarcomas and gliomas, derive from quiescent, typically mesenchymal cell types. Deleting telomerase from these cells seems sure to require *in situ* gene targeting. In view of the low efficiency of *in vitro* gene targeting, this is a daunting problem, since in contrast to the *in vitro* setting there is the issue of excessive random integration of constructs if a high titre of vector is administered. However, progress is being made on a number of fronts. Perhaps the most promising is the use of bacteriophage integrases as gene therapy vectors (44). The bacteriophage phiC31 is a double-stranded, circular DNA molecule that inserts into its host bacterial genome by the action of an integrase, an enzyme that performs a recombination between a specific site on the phage and another on the bacterial chromosome. These sites are not identical, so they are both destroyed by the integration event; thus, the phage cannot be excised by the same enzyme but instead does so using a distinct "excisionase". It has been shown that animal genomes, including those of mouse and human, possess a few sites with just sufficient sequence similarity to the bacterial site targeted by phiC31 integrase that it can insert into the genome at those sites but does not insert elsewhere (45,46). Furthermore, initial studies indicate the possibility of *in vitro* evolution of the integrase gene to alter the sequences that it recognises (47). By this means it may in due course be possible to engineer reliable insertion of phiC31 into a chosen genomic location of a high proportion of cells via somatic gene therapy, with only a minimal incidence of random integration.

6. REQUIRED FREQUENCY OF RESEEDING

6.1. Blood and skin stem cells

It is not straightforward to determine how frequently the ultimate stem cells of a tissue divide. In the case of the blood, however, reasonably accurate numbers have been obtained, and the consensus is that division occurs only every few months (48). This is heartening, because it means that a stem cell with initially normal-length telomeres but no telomerase genes should be able to function for about a decade before undergoing differentiation (or possibly apoptosis) as a result of critically short telomeres. Similar numbers have been calculated for the skin (49). There will be telomere shortening after transplantation, as the stem cells expand to occupy their niches, but they can have their telomeres extended before transplantation (with exogenous telomerase) to a length that compensates for this.

6.2. Gut stem cells

In the gut, however, the situation is generally thought to be very different. Potten has calculated that intestinal stem cells divide as often as once per day in mice and once per week in humans (50). If true, this would mean that reseeding of gut stem cells would need to occur at least annually. However, it is now clear that the assumptions underlying Potten's calculations are not entirely correct. In particular, analysis of spontaneous nuclear or mitochondrial mutations reveals that intestinal crypts are generally monoclonal (51,52), whereas in Potten's model there are 4-8 stem cells dividing independently.

In order to resolve this issue it is instructive to consider the models of impaired telomere elongation mentioned earlier. In both DC humans and telomerase-knockout mice, the gut is an affected tissue – but it does not generally exhibit dysfunction any sooner than the blood or the skin (53,54). In DC one may object that this could be because other aspects of the telomere maintenance machinery are up-regulated to compensate for the increased cell division rate; this might suffice, given that DC

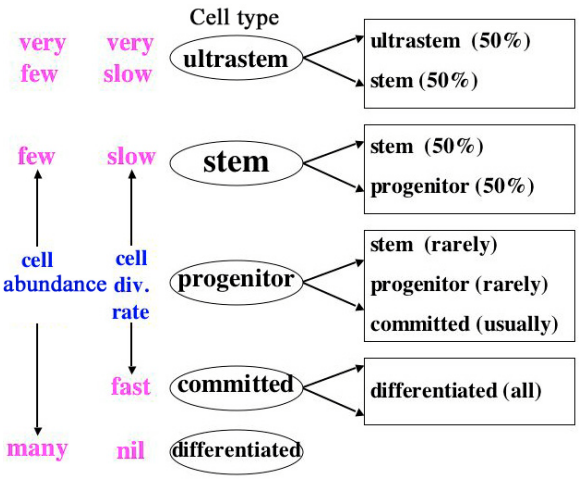

Figure 2. A possible mechanism to explain why the gut lining survives telomerase dysfunction as well as the blood and skin. In this scheme, most gut stem cells divide much more often than typical blood or skin stem cells, but a few "ultra-stem" cells (not necessarily even resident in the gut stem cell niche at or near the base of the crypt) divide much more slowly and, when doing so, produce a new stem cell as well as an "ultra-stem" cell. Division of ultra-stem cells need only be rapid enough to replace stem cells that die or differentiate.

sufferers do retain residual telomere elongation capacity. But in telomerase knockout mice it seems inescapable that the ultimate gut stem cells must be dividing only about as often as HSCs or skin stem cells. Various models can be imagined that would account for this, including the possibility that crypts are periodically replenished by circulating stem cells originating in the bone marrow (figure 2).

6.3. T and B lymphocytes

Another cell type that must sometimes divide many times is the lymphocyte. Both T cells and B cells undergo massive expansion in response to stimulation when the body is exposed to their cognate antigen, and loss or diminution of this capacity would presumably be severely life-threatening. Again, however, a careful analysis of the available data indicates that telomere elongation should not be needed. Expansion of naïve cells in response to an infection is of a scale that should be achievable by cells with normal-length telomeres, since it should only require a few dozen doublings as judged by the rate of telomere shortening in telomerase-knockout mice (55). What would be prevented, however, is re-expansion of memory cells on re-exposure to the same antigen. But in considering the impact of this loss of function we must recall that WILT is only proposed as an anti-cancer therapy in the context of an array of medical treatments that jointly postpone, by a large amount, all other aspects of age-related decline. Memory cells are essential for middle-aged and elderly adults only because their advancing age renders them more likely to succumb more quickly to a given infection than a young adult would, and thus reliant on memory cell expansion to eliminate the infection quickly. Young people do not have memory cells for many antigens, because they have not been exposed to many, so they rely on their overall youthfulness to maintain function while the slower naïve cell response builds up. Yet, young adults are more resistant to death from infectious diseases than older people. Moreover, memory T and B cells can in principle be isolated and subjected to exogenous telomere lengthening before reintroduction into the circulation. The number of circulating memory cells reactive to each antigen that the individual has experienced is in the region of 10^7 (56), ample to allow all such clones to be represented in a small blood sample. *Ex vivo* expansion of these cells in concert with their telomere elongation can thus allow a reasonably comprehensive restoration of the memory cell arsenal to an expansion-competent state.

6.4. Other tissues

Various other epithelial tissues will need similar periodic reseeding. Examples include the lung and the uterus. There is no evidence, however, of any specific obstacles to be overcome in reseeding these tissues as frequently as necessary, as for the tissues discussed above. As mentioned above, stem cell therepy for the lung is already being explored (41).

7. ALT (ALTERNATIVE LENGTHENING OF TELOMERES)

Roughly 10% of human cancers do not exhibit detectable telomerase activity. They do not tolerate telomere shortening, however; rather, they possess an alternative telomere-elongation mechanism termed ALT. ALT was first identified in the mid-1990s (57) but has only recently attracted the attention of more than a handful of researchers. Accordingly, it is perhaps no surprise that progress in determining its genetic basis has been slow. It is presently the focus of increasingly widespread interest, however, so this may change soon.

In order to eliminate cancers' ability to maintain telomeres using ALT as effectively as for telomerase, it would seem necessary to identify an essential protein component of ALT and delete its gene. This may well turn out to be possible, as ALT involves recombination (58), a process for which we possess many genes that are needed only in meiosis. (A mechanism of telomere elongation involving neither telomerase nor recombination was recently reported in yeast (59), but the evidence to date is that human ALT cells always use recombination in some way.) Another alternative is that it uses genes only otherwise involved in the V(D)J recombination pathway: this is hinted at by the remarkable finding that immunising late-generation telomerase knockout mice causes a sharp lengthening of their lymphocyte telomeres (55). Moreover, all ALT cell lines appear to share various distinctive characteristics, such as promyelocytic leukaemia (PML) bodies (60). This suggests that the ALT phenomenon is a single pathway rather than a family of recombination-based mechanisms possibly not sharing any one essential gene. However, until ALT is well characterised genetically and a wide range of ALT cell lines tested to determine their genetic basis, incorporation of a detailed anti-ALT

procedure into the WILT proposal cannot occur. Furthermore, even if all existing ALT cell lines are found to employ the same mechanism of telomere elongation, cancer cells might still evolve a hitherto unobserved mechanism when the selective pressure to do so was created by the removal of the telomerase and "standard ALT" options. As will be explained in the next section, this is not a particular cause for pessimism concerning WILT's potential. However, further characterisation of ALT is a very high priority.

8. CANCERS THAT WILT DOES NOT DIRECTLY PREVENT

It is evident that WILT consists of doing a number of things to the body that we are unlikely ever to be able to do with 100% efficiency. Foremost among these are the elimination of the telomerase and (hypothetical) ALT genes from quiescent cells and the identification of genes essential for all forms of telomerase-independent telomere elongation but not for other essential metabolic processes. These failings do not, however, mean that WILT cannot be as effective as if we could do these things perfectly.

8.1. Chemoresistant telomerase-knockout cells

First, let us return to the fact that WILT involves the reseeding of stem cell niches with cells engineered *ex vivo*, something that is inefficient unless niches are vacated by the death of resident stem cells. When this reseeding is being done for the second and subsequent times this difficulty may be neglected, because stem cells will be disappearing spontaneously as their telomeres shorten to critical lengths. But the first reseeding is harder. In order to achieve it, high-dose chemotherapy may be required, killing a large proportion of the resident stem cells to make way for the engineered replacements. It will therefore be beneficial if those replacements are engineered to be not only telomerase-negative but also genetically resistant to certain chemotherapeutic agents. If so, they can be introduced prior to the chemotherapy and are thus poised to occupy vacated niches as soon as they become available. Fortuitously, such genetic chemoresistance is already the subject of extensive study, since it has obvious value independently of WILT in the protection of cancer patients from chemotherapy-induced anaemia. MGMT (also known as ATase), the protein that removes alkyl adducts from guanine in DNA, is not an enzyme in the strict sense of being a catalyst, but rather a "one-shot" protein that is degraded after transferring the alkyl group to itself (61). For this reason it can readily be depleted by the introduction of pseudosubstrates, leading to the death of cells that are acquiring such adducts at an appreciable rate (due, for example, to the coadministration of a chemotherapeutic agent that forms them, such as temozolomide). A mutant form is known, however, which retains the physiological activity but is completely unreactive with the pseudosubstrate benzylguanine. Introduction of this mutant form of the MGMT gene into HSCs that are provided via BMT in advance is thus a highly promising adjunct to high-dose chemotherapy (62).

This will provide a very useful backup to eliminate cancers that arise in residual, telomerase-positive cells even after most cells have been rendered telomerase-knockout. Such cells, and thus the cancers arising from them, will necessarily also possess only the wild-type MGMT gene; hence, they will be amenable to treatment by the same chemotherapy that allowed the initial engraftment of the engineered stem cells. This concept can clearly be elaborated by the incorporation of chemoresistance-conferring variants of other genes as and when they become available.

8.2. Stem cell death as pre-emption of any hypothetical "variant ALT"

Second, the need to replenish all stem cell compartments every decade or so may appear to be a major drawback of WILT but is in fact a notable plus. Part of the reason stem cells are likely to be the source of most cancers is that their telomerase expression is not fully suppressed, but another reason is simply that they divide so often, since DNA replication is the source of most mutations. Thus, the death of the stem cell after only a decade may be a powerful defence against the initiation of cancer, giving insufficient time for a stem cell to accumulate all the necessary mutations to become fully tumour-forming. Importantly, this logic applies especially forcefully to the putative "variant ALT" mechanisms alluded to above, as the fact that they are not normally seen implies that they would require more mutational events than standard ALT or telomerase activation. It only works well if the stem cells are broadly mutation-free when introduced, but this can be checked with arbitrary thoroughness late in the procedure (after genetic modifications are complete and the stem cells expanded).

9. A BONUS: HSC ENGINEERING AS AN IMMUNOREJUVENATION STRATEGY

Immunosenescence is a major and growing research field within biogerontology, and with good reason: the immune system is among the most complex parts of the body and its decline with age is severely life-threatening (63). Evidence is mounting that a major contributor to reduced immune function in the elderly is the excessive expansion of T cell clones reactive to certain viruses, especially cytomegalovirus, which seem to occupy the "immune space" and inhibit expansion of other T cells (64). Young memory cells are initially able to up-regulate telomerase, but cytotoxic T cells in particular lose this ability after repeated exposure to antigen and descend into a state known as anergy, in which they are unable to support an immune response (65). The HSC-reseeding aspect of WILT provides an opportunity to prevent this, by altering the engineered HSCs to promote apoptosis of lymphocytes when they approach this state.

10. PERSPECTIVE

WILT is a highly ambitious proposed therapy that might at first sight appear too futuristic to be worth pursuing at this time. Indeed, in today's biomedical environment, where several other age-related conditions are comparably major killers, the case for administering a treatment as radical as WILT is highly debatable. This is because simpler therapies show considerable promise in

postponing cancer by a further decade or two in the foreseeable future, and that would reduce the proportion of people who die of cancer by a large factor, since most of them would die of something else first. In actuality, however, progress will surely also be made against these other major age-related diseases too. Progress in those other areas is likely to accelerate sharply in the next few decades as we develop ways to reverse (not merely retard) the accumulation of the underlying molecular and cellular damage that eventually gives rise to these diseases (7-9). No similar acceleration in the postponement of cancer is foreseeable unless we develop therapies with considerably more anti-cancer potential than anything presently being explored. Thus, we have good reason to begin now to explore much more radical alternatives such as WILT, in order to reduce the risk that cancer will become the dominant cause of death in the developed world a few decades hence.

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