



Molly Pomerance. *Closeted Jackets*. Oil on canvas, 24" × 30".

*Understanding the telomere biology of hematopoietic cells will lead to improvements in the diagnosis, prognosis, and treatment of hematologic disease.*

# Telomere Biology of Human Hematopoietic Stem Cells

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**Background:** *Telomeres are protein DNA structures present at the ends of chromosomes and are essential for genetic stability and cell replication. Telomerase is the enzyme complex that maintains telomere integrity. Hematopoietic stem cells express telomerase and contain long telomeres, which become shorter as cells differentiate and mature. The extent of telomere shortening and the level of telomerase activity often correlate with the presence and severity of some hematopoietic diseases.*

**Methods:** *The fundamentals of telomeres and telomerase are reviewed, and the telomere biology of human hematopoietic cells is discussed.*

**Results:** *Telomere length and telomerase activity are important in the self-renewal of hematopoietic stem cells. Changes within these compartments affect both normal hematopoietic cells and the generation of hematopoietic disease. Telomere length provides information pertaining to the proliferative history and potential of a hematopoietic cell.*

**Conclusions:** *The role of telomerase and telomeres within the hematopoietic compartment needs further clarification. Advances in our knowledge in this field may improve clinical outcomes for the treatment of hematologic disease.*

## Introduction

Most circulating blood cells have a finite lifespan. To compensate for the continual loss of differentiated cells, it is estimated that an adult human needs to produce between  $10^{11}$  and  $10^{12}$  mature blood cells per day. In addition to normal cell turnover, the hematopoietic system must be able to respond rapidly to illness and trauma. All blood cells in the body are derived from a relatively rare population of hematopoietic stem cells (HSCs) and progenitor cells.<sup>1,2</sup> While fetal liver and umbilical cord blood are rich sources of HSCs, after birth the majority of HSCs reside in

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the bone marrow. These HSCs can be stimulated to migrate from the bone marrow into the peripheral blood following the administration of cytokines such as granulocyte colony-stimulating factor.<sup>3</sup> Whatever the source, by definition, an HSC must be able to generate all lineages of cells that comprise the hematopoietic compartment without losing its self-renewal capacity. In contrast, a progenitor cell will have the ability to differentiate into cells of various restricted lineages with an increased mitotic activity without maintaining a self-renewal ability. There is still much to be discovered regarding HSCs. An understanding of the mechanism that regulates self-renewal of stem cells is important since this ability is vital for the maintenance of hematopoiesis throughout life.<sup>4</sup> The self-renewal capacity of HSCs is most likely regulated at the molecular level and utilizes signaling pathways involving proteins such as Notch, Wnt, and HoxB4.<sup>5</sup> The activation of the enzyme telomerase that serves to maintain telomere length and preserve it is also thought to play an important role in the self-renewal of HSCs.<sup>6,7</sup> This review provides an overview of the function of telomeres and telomerase, focusing on the telomere biology of HSCs, progenitor cells, and their progeny both in normal and disease states.

## Telomere Structure and Function

Telomeres are the non-encoding regions of DNA capping the ends of chromosomes, in association with various proteins. The DNA that forms the telomere consists of the sequence (5'-TTAGGG-3')<sub>n</sub>, which is referred to as a "telomeric repeat" since it is repeated in tandem over 5 to 15 kilobases (kb).<sup>8</sup> In humans, each chromosome end contains approximately 1,000 to 2,000 telomeric repeats.<sup>9,10</sup> Broken ends of chromosomes that do not have telomeres are prone to recombination, often resulting in fusion with other broken ends. The presence of telomeres prevents the ends of intact chromosomes from appearing like DNA breaks to the DNA replication machinery. Thus, telomeres function to guard chromosomes against degradation, fusion, and rearrangements during DNA replication. They allow the complete replication of the 5' ends of chromosomal DNA without the loss of internal sequences and the genes these sequences may encode. In addition, telomeres function to correctly position the chromosomes within the nucleus for replication.<sup>11-13</sup> Beyond the double-stranded region of the telomere, the 3' end of the G-rich strand contains several hundred base pairs of a single-stranded extension,<sup>14-16</sup> which is known as the 3' overhang. This single-strand overhang folds back onto duplex telomeric DNA to form a duplex loop structure known as a t-loop,<sup>17</sup> and it hides the end of the telomere from normal DNA repair mechanisms.

The telomere-associated proteins are important for maintaining telomere stability and regulating telomere length. These proteins include TRF1 and TRF2,<sup>18-20</sup> Pot1,<sup>21,22</sup>

tankyrase,<sup>23,24</sup> Rap1,<sup>25</sup> and Ku.<sup>26,27</sup> TRF1 is thought to regulate telomere length by preventing the elongation of telomeres once they reach a critical size.<sup>28</sup> TRF2 appears to be important for stabilizing the chromosome ends by associating with the 3' overhang and suppressing end-to-end fusions between chromosomes.<sup>29,30</sup> Many additional proteins can bind indirectly to telomeres, often via TRF1 and TRF2, and together these proteins function to regulate telomere homeostasis.<sup>8</sup>

Most somatic human cells lose 50 to 100 base pairs of telomeric DNA with each successive round of cell division.<sup>10,31</sup> Hayflick and Moorhead<sup>32</sup> suggested more than 40 years ago that most normal cells are programmed for a given number of cell divisions and cannot divide indefinitely. This theory has been expanded by Harley et al<sup>33,34</sup> into the "telomere hypothesis of aging," which postulates that sufficient telomere loss on one or more chromosomes in normal somatic cells acts as a molecular "clock" that triggers cell senescence. In support of this notion, telomere attrition in human fibroblasts is prevented by upregulating telomerase activity, leading to immortalization of these cells.<sup>35,36</sup> Many studies now support the idea that telomere erosion limits the number of times most normal diploid cells can divide.<sup>37</sup> Recently, however, it has become clear that there are many complexities to this theory. Individual chromosome ends vary greatly in length, with chromosome 17p typically containing the smallest number of telomere repeats.<sup>38</sup> It is thought that recognition of the shortest individual telomere length within a cell, rather than the average telomere length, is important for cell viability and chromosomal stability.<sup>39</sup> Thus, the proliferative potential of cells may be limited to the chromosome with the shortest length of telomere repeats,<sup>6,9</sup> although experimental evidence suggests that short telomeres accumulate prior to senescence and that replicative senescence is not triggered by the first telomere to reach a critical minimal threshold length.<sup>40</sup> Furthermore, it has recently been shown that telomere shortening is proportional to the size of the G-rich telomeric 3' overhang such that cells with long overhangs lose more telomeric repeats with each cell division than those with shorter overhangs.<sup>41</sup> Finally, although not yet well understood, the *state* of the telomeric complex now appears to be a more informative indicator of aging and cell viability than telomere length alone.<sup>42-44</sup> For example, TRF2 has a protective effect on telomeres by inducing the t-loop conformation. The critically shortened telomeres in senescent human cells may no longer be able to bind enough TRF2 to achieve this protective state and thus replicative senescence is induced.<sup>45</sup>

The senescence and crisis induced in cells by telomere shortening act as checkpoint mechanisms to control unlimited proliferation.<sup>45</sup> Once a critical number of telomere repeats are lost, a DNA damage signal is produced, inducing cell-cycle arrest. Little is known regarding the mechanism by which telomere shortening induces a DNA

damage response.<sup>40,46,47</sup> It is thought that short telomeres may lead to activation of multiple signaling mechanisms.<sup>48</sup> Studies in yeast have indicated that the loss of telomeric DNA induces the same cellular processes (cell cycle arrest, DNA repair) as broken chromosomes.<sup>49</sup> Telomeres have a dynamic structure,<sup>50</sup> and it appears that the terminal 3' end signals downstream DNA damage pathways, often involving the ataxia-telangiectasia mutant gene<sup>51</sup> and p53.<sup>52,53</sup>

## Telomerase

In cells such as germ cells and stem cells, where telomere length must be conserved in order to avoid exit from the cell cycle and entry into senescence, expression of the enzyme telomerase is usually observed. Telomerase is a large ribonucleoprotein complex<sup>54,55</sup> comprised of the reverse transcriptase protein (hTERT) and an RNA template (hTR).<sup>8,46</sup> Both components are essential for telomerase activity. In humans, the RNA template is 445 nucleotides long with an 11 nucleotide template sequence (5'-CUAACCCUAAC-3') complementary to the human telomere sequence (TTAGGG)<sub>n</sub>.<sup>55,56</sup> In addition to its template function, hTR appears to be important at the enzyme active site.<sup>57</sup> Studies using mice and human cells have demonstrated that removal or downregulation of the RNA subunit leads to loss of telomerase activity, telomere attrition, and inhibition of cell growth.<sup>56,58-60</sup> Furthermore, in a rare autosomal dominant form of the disease dyskeratosis congenita, the disorder is caused by mutations in the hTR gene,<sup>61,62</sup> which is also implicated in a subset of patients with aplastic anemia (AA).<sup>63</sup>

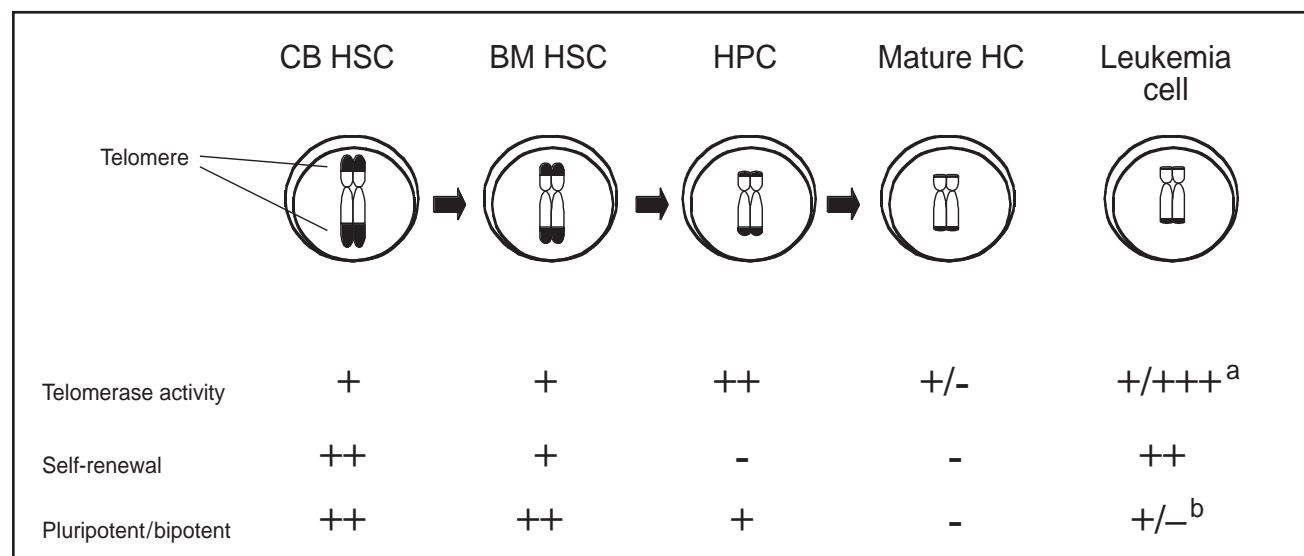
hTERT, the catalytic subunit of the telomerase holoenzyme, is a polypeptide of 1,132 amino acid residues.<sup>64,65</sup> Ectopic expression of hTERT extends the replicative life-

span of human fibroblasts, retinal pigment epithelial cells, and endothelial cells<sup>35,36,66,67</sup> without altering their karyotype, differentiation characteristics, or activation of known oncogenes.<sup>68-70</sup> While hTR is relatively ubiquitously expressed in embryonic and somatic tissues, expression of hTERT is tightly regulated and not detectable in most somatic cells.<sup>71</sup> It is therefore thought that expression of hTERT is the rate-limiting step in telomerase activity. Studies by many groups have now demonstrated that hTERT plays a fundamental role in telomere preservation and cell proliferation.<sup>46,72-75</sup>

## Telomeres in Hematopoietic Cells

Nearly 10 years ago, it was discovered that telomere length in HSCs decreases as a function of both ontogeny and age (Figure).<sup>6,76-78</sup> Telomere length measurements revealed a striking difference between fetal/neonatal and adult HSCs. Telomeres in stem/progenitor cells from human fetal liver and umbilical cord blood are approximately 4 kilobases (kb) longer than those in cells from adult bone marrow and peripheral blood.<sup>6,76,77</sup> These findings support the hypothesis that stem cells, although self-renewing throughout life, have a finite and limited replicative potential as we grow older.<sup>6</sup> In general, however, the replicative potential of human HSCs is unknown and could be anywhere between less than 100 population doublings to more than 5,000.<sup>79</sup>

Telomere loss in normal hematopoietic cells is rapid within the first year of life (equating to 15 to 30 stem cell divisions) and then continues at a slow decline until 50 to 60 years of age, after which the decline again accelerates.<sup>47,80</sup> Telomere attrition is even greater prior to birth (approximately 240 base pairs per week) and reflects the



Telomeres and telomerase activity in human hematopoietic cells (CB HSC = cord blood hematopoietic stem cells, BM HSC = bone marrow hematopoietic stem cells, HPC = hematopoietic progenitor cells, HC = hematopoietic cells). <sup>(a)</sup> The level of telomerase activity in leukemia varies depending on the type of leukemia and disease status.<sup>78</sup> <sup>(b)</sup> The pluripotent ability depends on the type of leukemia and response to chemical induction used for differentiation.

increased proliferation rate of progenitor cells at this time. Thus, the decline in mean telomere length in human hematopoietic cells follows a cubic function. Interestingly, this age-related decline is more rapid in lymphocytes than granulocytes, suggesting that telomere loss due to aging is more likely to compromise immune function than the function of HSCs.

There is a wide variability among individuals in mean telomere length in hematopoietic cells at any age,<sup>81,82</sup> and these differences appear to be genetically determined.<sup>83</sup> Furthermore, the chromosomes within an individual cell exhibit heterogeneity in the telomere length. However, it appears that a minimum number of repeats at each telomere is present during normal hematopoiesis.<sup>84</sup> It is most likely that the shortest telomere rather than the average telomere length within a cell dictates cell viability and chromosome stability.<sup>39</sup> According to the "telomere checkpoint" model,<sup>47,85</sup> telomeres in human HSCs play a pivotal role in signaling cell cycle progression and cell division. It is postulated that activation of a "telomere checkpoint" is prevented by the presence of a minimum number of telomeric repeats at each chromosome. Telomere length in granulocytes can be used as a surrogate marker for the telomere length in HSCs.<sup>47</sup> Under normal circumstances, the age-related decline in telomere length in granulocytes is slow enough that it is unlikely that HSCs enter senescence or undergo apoptosis due to telomere shortening. Even if the occasional HSC was lost due to telomere shortening, the large number of HSCs ensures that hematopoiesis is unlikely to be compromised. However, this may not be the case in hematologic disorders or in situations where the HSCs are placed under replicative stress.

## Telomeres and Hematopoietic Disease

Studies of telomere length have demonstrated that telomere attrition correlates with the presence and severity of some hematopoietic diseases, such as AA,<sup>86-91</sup> myelodysplastic syndrome (MDS)<sup>86,92-96</sup> and chronic myeloid leukemia (CML).<sup>87,94,97-102</sup> Hematopoietic cells from patients with AA bear shorter telomeres compared with those of age-matched control cells, with a strong correlation between telomere loss and disease duration. Telomere length appears to provide information regarding the disease status of patients; telomere length in AA patients who recover from immunosuppressive therapy is no different from controls. However, untreated patients and nonresponders to therapy have significant telomere shortening. Although the cause of AA is not known, recent data indicate that, at least in a subset of patients with AA, the disorder may be associated with a germline mutation in the RNA component of telomerase (hTR), directly inhibiting the telomere maintenance pathway.<sup>63</sup> Germline mutations in hTR are also observed in the autosomal dominant form of dyskeratosis congenita.<sup>61,62,103</sup> These studies provide

striking evidence that a failure to maintain a critical telomere length in HSCs results in bone marrow failure.

The ineffective hematopoiesis observed in MDS results from rapid hematopoietic cell division in the bone marrow. Hematopoietic cells from MDS patients do not usually express telomerase.<sup>93</sup> This means that the accelerated telomere erosion due to rapid cell division may not be prevented or restored in most of the MDS cells. This loss of telomere stability may induce further genetic changes such as gene amplification, loss of heterozygosity, or chromosomal rearrangement. In fact, MDS cells with short telomeres (<5 kb) frequently acquire complex cytogenetic abnormalities leading to other mutations and disease progression.<sup>93</sup> MDS is a preleukemic state, with approximately 25% of patients progressing into acute myeloid leukemia.<sup>104</sup> The incidence of leukemic transformation is significantly higher in patients with MDS with shortened telomeres compared to those with normal-range telomere length.<sup>93</sup> Thus, unstable telomeres without elevated telomerase activity may be a hallmark of MDS cells and is likely to be important in the pathogenesis of the disease.

Differences in telomere length have been documented between chronic phase and blastic phase of CML, a clonal myeloproliferative disease of stem cell origin.<sup>94,97,98,101</sup> In general, telomere length of cells in chronic phase is shorter, with the Philadelphia chromosome positive (Ph<sup>+</sup>) cells containing shorter telomeres than Ph<sup>-</sup> cells. Cells in the blastic phase of CML have even shorter telomeres than those observed in the chronic phase.<sup>87,97</sup>

The rate of telomere shortening in hematopoietic cells is increased at times of replicative stress, including the administration of chemotherapy.<sup>105</sup> Interestingly, the rate of telomere shortening in hematopoietic cells appears to be more pronounced in children who are treated for solid tumors compared with those undergoing therapy for leukemia.<sup>106</sup> This probably reflects the intense chemotherapy received for solid tumors.

Following allogeneic bone marrow transplant, the telomere length of donor hematopoietic cells preceding transplant is greater than that of the donor cells found in the recipient following transplant.<sup>107,108</sup> This change in telomere length is thought to be equivalent to approximately 15 years in aging<sup>109,110</sup> and perhaps even as great as 40 to 60 years in aging.<sup>111</sup> This difference is established within the first year posttransplant and does not appear to change after this time, rather following the normal rate of telomere attrition.<sup>111-115</sup> Studies from several groups suggest that the degree of telomere shortening does not appear to reach a level that compromises marrow function.<sup>114-116</sup> However, this circumstance could change with longer follow-up or in cases in which few donor stem cells are transplanted, where donor cells are from older individuals,<sup>108</sup> or where donor cells have unusually short telomeres.<sup>117</sup> As mentioned, support for a link between telomere dysfunction and marrow failure has been provided

by the data showing that haploinsufficiency for the telomerase RNA gene results in AA.<sup>63</sup> Furthermore, a recent study has described two cases in which telomere shortening following marrow transplantation appears to have played a role in poor graft function.<sup>118</sup> In the first of these cases, marrow from a 61-year-old donor was transplanted into a 7-year-old boy with acute lymphocytic leukemia. Despite successful engraftment of the donor cells, the patient experienced poor marrow function 25 months posttransplant, requiring cytokine support to maintain neutrophil and red cell counts. The telomere length of donor cells harvested from the patient was up to 2 kb shorter than that of the original donor cells, and the young patient had a hematopoietic system with telomere lengths significantly shorter than those of other children his age. In the second case study, a 13-year-old boy underwent transplantation with bone marrow from his 14-year-old sister for the treatment of severe AA. The transplanted cells engrafted well, and the patient had normal blood counts until 25 years posttransplantation, when abnormal counts revealed the patient's bone marrow to be hypocellular. When the patient developed pancytopenia, the telomere length in hematopoietic cells of the recipient was approximately 2.2 kb shorter than that of the donor. This suggests that even though the donor cells were normal, they were probably placed under extensive replicative stress in the patient, perhaps due to hematopoietic recovery, therapy, and disease.

More long-term follow-up data are required from patients who have had HSC transplants. Transplantation of limited number of stem cells place high demands on their replicative potential; long-term survivors of stem cell grafts may be at risk of stem cell exhaustion if extra demands are placed on the hematopoietic system, such as following a chronic infection.<sup>117</sup> In this respect, cord blood stem cells, with their longer telomeres and increased replicative ability, may have an advantage over bone marrow cells despite the limited number of cells usually available in a cord blood unit for transplant. Given that telomere length may provide information about both the proliferative *history* and *potential* of a particular cell, it is possible that measurement of telomere length in HSCs will be a useful indicator of proliferative and engraftment potential following transplant. Data from our laboratory examining the proliferative potential of cord blood stem cells as a function of telomere length suggest that this may indeed be the case (N.J.E., unpublished material, 2004).

## Telomerase in Hematopoietic Cells

Most cycling HSCs display telomerase activity. Following an initial increase, telomerase activity is downregulated as HSCs and progenitor cells proliferate and differentiate into more mature cells that display low to negligible levels of telomerase activity (Figure).<sup>7,119-127</sup> The *ex vivo* exposure

of HSCs and progenitor cells to cytokines transiently increases hTERT activity, but this is downregulated upon further proliferation and differentiation.<sup>121,124,128,129</sup> Analogously, in tumor cells with a high self-renewal potential, telomerase is constitutively expressed.<sup>130</sup> However, its activity decreases during differentiation in response to pharmacologic agents such as all-trans retinoic acid and dimethyl sulfoxide (DMSO).<sup>131-138</sup> These and other observations suggest that the level of telomerase activity may be associated with the self-renewal potential of a cell.<sup>7,92</sup> This raises the possibility that overexpression of hTERT, or even transient activation of telomerase, might enhance the self-renewal of primitive hematopoietic cells. This could result in expansion of HSCs and progenitors that could contribute to engraftment or increased numbers of their progeny, which could also be used for clinical and tissue engineering purposes.

There is some discrepancy in the literature regarding the relationship between cell cycle regulation and telomerase activity, although it does appear that cycling cells display increased telomerase activity. Cells in the G<sub>1</sub>, S, and G<sub>2</sub>+M phase of the cell cycle display telomerase activity, but this appears to be repressed upon growth factor deprivation or differentiation and subsequent entry into G<sub>0</sub>.<sup>124,139,140</sup> Thus, in a population of cells that display telomerase activity, the level of activity observed will most likely depend on the proportion of cells that are proliferating. Since primitive HSCs are predominantly quiescent, the population of cells will appear to have a low level of telomerase activity.

The presence of active telomerase does not always imply that telomere length will be stable and static. Telomerase activity is upregulated when stem cells are stimulated to generate progenitor cells<sup>121,124</sup> or when lymphocytes undergo clonal expansion.<sup>141</sup> However, this upregulation in telomerase activity is insufficient to prevent telomere attrition, but it may contribute to cell survival in the absence of proliferation. In a recent study, we used a retroviral vector to overexpress hTERT in HSCs/progenitor cells isolated from human cord.<sup>142</sup> These cells were maintained under *ex vivo* culture conditions. We did not observe elongation of telomere length or a prevention of telomere attrition compared with control cells. However, we observed an enhanced survival of cultured mature hematopoietic cells. Recent studies in T lymphocytes have demonstrated that while endogenous hTERT cannot prevent overall telomere shortening, it has a major influence on the longevity of human T cells.<sup>143</sup> It has traditionally been thought that the role of hTERT in HSCs is to maintain telomere length during cell division, thereby maintaining the chromosomal integrity and proliferative capacity of the stem cell pool. These observations suggest a more complex role for hTERT in hematopoietic cells than telomere elongation alone. Further evidence for a pro-survival action of hTERT independent of telomerase enzymatic activity has been found in human breast cancer

cells.<sup>144</sup> The mechanism for this pro-survival effect of hTERT in hematopoietic cells is not yet known, but it may have widespread implications for the importance of hTERT in normal hematopoiesis.

## Telomerase in Leukemia

Telomerase activity may be a prognostic factor in acute leukemias.<sup>78</sup> Telomerase activity is elevated in approximately 75% of cases of acute leukemia.<sup>145,146</sup> Compared to normal hematopoietic cells, acute myeloid leukemia and acute lymphocytic leukemia have more than a 10-fold increase in telomerase activity.<sup>92,94,119,122,146</sup> During remission, the level of telomerase activity usually decreases to normal levels but increases following relapse.<sup>94,106,146</sup> In general, the prognosis is poorer for patients with high levels of telomerase activity compared to those with low telomerase activity.<sup>146</sup> Since high telomerase activity and shortened telomeres are observed in most acute leukemias, especially high-grade lymphomas, it may be possible to monitor early relapse and blastic changes in care via the measurement of telomerase activity.<sup>78</sup> Although telomere maintenance may occur in a subset of leukemias via an alternative lengthening of telomeres,<sup>147-149</sup> telomere length and telomerase activity measurements may prove to be of significant benefit for the diagnosis and prognosis of hematologic disorders.

The ability to extend the replicative lifespan of primitive human hematopoietic cells has widespread clinical implications, including the ex vivo expansion of HSCs and more mature cells for improved transplantation procedures for the treatment of leukemia. Despite the efforts by many groups in examining the combinations of cytokines that may be useful for this purpose, cytokines and other environmental factors alone are unlikely to stimulate the ex vivo self-renewal of primitive hematopoietic cells.<sup>150</sup> The development of strategies that target the molecular regulation of primitive hematopoietic cells offers a more direct approach. Our recent studies (Elwood et al<sup>142</sup> and N.J.E., unpublished data, 2004) have identified an unexpected pro-survival role for hTERT in at least some progenitor cells, suggesting that, while the mechanism of this effect needs to be investigated, modulation of telomerase activity may be of benefit for the ex vivo expansion of hematopoietic cell populations.

## Conclusions

There is still much to learn about the telomere biology of human HSCs and progenitor cells. However, solving these mysteries should allow rapid translation of the findings into clinically useful outcomes for the treatment of hematologic disease. Since telomere length appears to reflect both the proliferative history and proliferative

potential of hematopoietic cells, there is a role for the measurement of telomere length for diagnostic and prognostic purposes in hematopoietic disorders. In the future, as we learn more about the complexities of the telomere and its binding proteins, such studies may also need to consider telomere state.

The presence of telomerase activity in hematopoietic stem/progenitor cells in comparison to the majority of human somatic cells implies an important function. While telomerase expression may serve to delay telomere shortening in these cells, telomere attrition is not prevented. It is beginning to emerge that telomerase may play an additional role to telomere maintenance in hematopoietic cell function. The expanding development of specific telomerase inhibitors for the treatment of cancer, including hematopoietic malignancies,<sup>151,152</sup> necessitates the urgent clarification of telomerase function in hematopoietic cells. It is crucial that the role of telomerase in both normal hematopoiesis and leukemia is understood, allowing us to determine the potential effects of clinical inhibition or activation of telomerase on HSC function.

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