Accelerated Telomere Shortening Precedes Development of Therapy-Related Myelodysplasia or Acute Myelogenous Leukemia After Autologous Transplantation for Lymphoma

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The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose

Therapy-related myelodysplasia or acute myelogenous leukemia (t-MDS/AML) is a lethal complication of autologous hematopoietic stem-cell transplantation (aHCT) for Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL). Here, we investigated the hypothesis that accelerated telomere shortening after aHCT could contribute to the development of t-MDS/AML.

Patients and Methods

A prospective longitudinal cohort was constructed to investigate the sequence of cellular and molecular abnormalities leading to development of t-MDS/AML after aHCT for HL/NHL. This cohort formed the sampling frame for a nested case-control study to compare changes in telomere length in serial blood samples from patients who developed t-MDS/AML with matched controls who did not develop t-MDS/AML.

Results

An initial increase in telomere length at day 100 after aHCT was followed by an accelerated telomere shortening in t-MDS/AML patients when compared with controls. These telomere alterations preceded the onset of t-MDS and were independent of other known risk factors associated with development of t-MDS/AML on multivariate analysis. Additionally, we observed reduced generation of committed progenitors in patients who developed t-MDS/AML, indicating that these telomere alterations were associated with reduced regenerative capacity of hematopoietic stem cells.

Conclusion

The development of t-MDS/AML after aHCT is associated with and preceded by markedly altered telomere dynamics in hematopoietic cells. Accelerated telomere loss in patients developing t-MDS/AML may reflect increased clonal proliferation and/or altered telomere regulation in premalignant cells. Genetic instability associated with shortened telomeres may contribute to leukemic transformation in t-MDS/AML.

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INTRODUCTION

Therapy-related myelodysplasia or acute myelogenous leukemia (t-MDS/AML) is a lethal complication of conventional treatment for Hodgkin's lymphoma (HL) and non-Hodgkin's lymphoma (NHL), with an incidence of 0.8% to 6.3% at 20 years and a median time to occurrence of 3 to 5 years from diagnosis of HL or NHL.¹⁻¹³ The risk of t-MDS/AML is increased among HL and NHL patients receiving autologous hematopoietic stem-cell transplantation (aHCT; cumulative probability: 1.1% to 24.3% at 4 years, with median time to development of 12 to 24 months).¹⁴⁻²⁵ Epidemiologic studies indicate that t-MDS/AML results from damage to hematopoietic stem cells (HSCs)

from genotoxic cancer treatment, such as alkylating agents, topoisomerase II inhibitors, and radiation^{14,18,19,21,22,26}; older age^{14,20,25} and use of peripheral-blood stem cells (PBSCs) rather than bone marrow (BM) grafts^{14,17,18} are contributing factors. However, the sequential cellular and molecular changes leading to t-MDS/AML are not known.

Telomeres are noncoding repeated sequences at chromosome ends that function to stabilize chromosomes and prevent chromosomal aberrations. Each somatic cell division is associated with loss of telomere length.²⁷ Cumulative telomere shortening imposes a limit on cell divisions and leads to cell senescence.²⁸ Telomere shortening may be compensated by a telomerase complex that maintains telomere length.²⁹ In hematopoietic tissues, there is

progressive shortening of telomere length through life.²⁷ Increased replicative demand on HSC is associated with accelerated shortening of telomere length in the early post-aHCT period.^{27,30-34} Telomere shortening may be accentuated after aHCT because of shorter telomere length in transplanted cells from prior therapy, older age, and increased replicative demand because a smaller number of cells may be transplanted.^{35,36} Reduction in telomere length is observed in a subset of de novo MDS patients with more aggressive disease and complex karyotypic abnormalities.³⁷⁻⁴⁰ Mutations in telomere-related genes are observed in some patients with BM failure syndromes.⁴¹⁻⁴⁴ Telomerase dysfunction is a feature of dyskeratosis congenita, which is an inherited BM failure syndrome with predisposition to AML.⁴⁵ These observations support further investigation of the role of telomere shortening in patients who develop t-MDS/AML after aHCT.

We conducted a prospective study of patients undergoing aHCT for HL and NHL to investigate the pathogenesis of t-MDS/AML. The known nature and timing of genotoxic exposures and the ability to follow these patients longitudinally provided a unique opportunity to study sequential molecular and cellular changes in this high-risk population. Patients were observed longitudinally from before aHCT to 5 years after aHCT, with serial banking of peripheral blood (PB) and BM samples. We used a nested case-control design to compare serial changes in telomere lengths in myeloid and lymphoid cells in patients who developed t-MDS/AML (patient cases) and matched controls without t-MDS/AML from within this cohort.

PATIENTS AND METHODS

Patients and Sample Processing

Serial PB and BM samples were obtained from 287 patients who were 18 years or older and undergoing aHCT for NHL or HL at the City of Hope National Medical Center. Samples were collected prospectively at the following predetermined time points: before aHCT; at day 100, 6 months, and 1 year after aHCT; and annually thereafter to 5 years or until development of t-MDS/AML. An aliquot of PBSC was obtained at the time of stem-cell collection. This study was approved by the institutional review board in accordance with an assurance filed with and approved by the Department of Health and Human Services and met all requirements of the Declaration of Helsinki.

Clinical data obtained from medical records included disease characteristics, pre-aHCT cumulative therapeutic exposures, conditioning regimens, priming with growth factors and/or chemotherapy for PBSC collection, number of PBSC collections, dose of CD34⁺ cells infused, recovery of WBC counts, vital status, and disease status after aHCT. Nucleated cells were isolated from PB using gravity sedimentation of RBCs. BM mononuclear cells were isolated using Ficol-Hypaque density gradient separation.⁴⁶ Samples were aliquoted and cryopreserved.

Nine patients who developed t-MDS/AML in this cohort and for whom sufficient samples were available were studied. Only samples obtained before the development of t-MDS/AML were included in this analysis. A nested case-control study design was used. The rest of the 287 study participants formed the sampling frame for control selection. The matching criteria included for control selection were underlying disease (HL or NHL), age at aHCT, race/ethnicity, and length of follow-up after aHCT (period of follow-up for the controls exceeded that of the patient cases under consideration). The nine patients with t-MDS/AML were matched to 24 control patients (median number of controls per patient case, three controls; range, two to three controls). Fifteen additional patients from the cohort developed t-MDS/AML but were not included because of insufficient sampling or because t-MDS/AML was diagnosed after the current study was completed. Patients with t-MDS/AML included in the study did not significantly differ from other t-MDS/AML patients within the cohort by primary diagnosis, sex, race/ethnicity, age at aHCT, or age at diagnosis of t-MDS/AML.

Cell Lines

The 1301 cell line was a gift from Dr Beth Jamieson at University of California, Los Angeles (Los Angeles, CA).⁴⁷ An hTERT-expressing 293T cell line was generated by transfection of 293T cells with the pBABE-hygro-hTERT expression vector (obtained from Dr Robert Weinberg).

Assessment of Relative Telomere Length by Flow Cytometry

Flow cytometric assessment of telomere length was performed using the Telomere PNA Kit/FITC (DakoCytomation, Carpinteria, CA) following the manufacturers' instructions. Advantages of the flow fluorescent in situ hybridization technique over Southern blotting include reduced time, labor, and cell requirements and ability to analyze different cell subsets from the same sample. The 1301 cell line, which is near tetraploid with long telomeres (> 25 kb), was used as an internal control, allowing for acquisition of more reproducible data.⁴⁸⁻⁵¹ PB and 1301 cells were mixed and incubated with or without the fluorescein isothiocyanate-conjugated peptide nucleic acid telomere probe. Cells were counterstained with propidium iodide. Fluorescein isothiocyanate mean channel fluorescence, which is representative of relative telomere length (RTL), was measured by flow cytometry (BD FACSCalibur; BD Biosciences, San Jose, CA). RTL was also separately measured in lymphoid and myeloid populations identified by their scatter properties. RTL values were expressed as percent ratio of telomere signal between each PB mononuclear cell sample and 1301 cells with correction for the DNA index of G_{0/1} cells. Samples were analyzed in duplicate. The average variation in fluorescence intensity between duplicate analyses was 4.9% ± 0.4% for PB cells and 3.3% ± 0.3% for 1301 cells. Analysis of the same sample on 3 different days indicated that the intra-assay coefficient of variability was 1.3%.

Telomere Restriction Fragment Length Analysis by Southern Blot

Genomic DNA was extracted from PB cells (ZR Genomic DNA II kit; Zymo Research, Orange CA). Southern blotting to measure telomere restriction fragment lengths was performed using the TeloTAGGG Telomere Length Assay kit (Roche Applied Science, Indianapolis, IN). Signal intensities were measured using ImageQuant software (GE Healthcare Life Sciences, Piscataway, NJ), and the telomere restriction fragment mean sizes were calculated.⁵²

Progenitor Assays

Committed progenitor or colony-forming cell (CFC) and primitive progenitor or long-term culture-initiating cell (LTCIC) assays were performed using BM or PBSC mononuclear cells, and results were expressed as progenitor frequency per 10⁶ mononuclear cells, as previously described.⁴⁶

Telomerase Assay

Telomerase activity was quantitated using the SYBR Green RQ-TRAP assay kit (Allied Biotech Inc, Ijamsville, MD) following the manufacturer's instructions. Cell extracts from PB mononuclear cells were studied. Standard curves were generated from serial dilutions of telomerase standard and reference templates and from telomerase-positive 293T-hTERT cell extracts. Samples were analyzed in triplicate, and telomerase activity was expressed relative to 293T-hTERT cells.⁵³

Statistical Methods

Demographic and clinical characteristics were compared between patient cases and controls using standard parametric and nonparametric tests. Cumulative incidence of t-MDS/AML was calculated using the method proposed by Gray.⁵⁴ Although controls were selected using pre-defined matching criteria, all subsequent analyses were unmatched, and all variables used in the matching were included in the statistical model. Comparison of telomere length from total, lymphoid, and myeloid cells between patient cases and controls was conducted using the generalized linear model (least squares means) with adjustment for the following variables: age at aHCT, primary diagnosis, race/ethnicity, sex, disease status, CD34 counts, and cumulative therapeutic exposures (alkylating agent scores: cyclophosphamide: 0 mg/m² = 0, < 4,500 mg/m² = 1, and ≥ 4,500 mg/m² = 2; ifosfamide: 0 mg/m² = 0, < 8,000 mg/m² = 1, and ≥ 8,000 mg/m² = 2; nitrogen mustard: 0 mg/m² = 0 and any exposure = 1; dacarbazine: 0 mg/m² = 0 and any exposure = 1; procarbazine: 0 mg/m² = 0 and any exposure = 1; topoisomerase II inhibitor scores:

anthracyclines: 0 mg/m² = 0, < 300 mg/m² = 1, and ≥ 300 mg/m² = 2; etoposide: 0 mg/m² = 0, < 600 mg/m² = 1, and ≥ 600 mg/m² = 2; mitoxantrone: 0 mg/m² = 0 and any exposure = 1; platinum compounds: 0 mg/m² = 0 and any exposure = 1). Chemotherapy scores were summarized for each patient and categorized for each class of agents into low (alkylating agent score ≤ 3 and topoisomerase II inhibitor score ≤ 3) versus high exposure (alkylating agent score > 3 and topoisomerase II inhibitor score > 3). A summary of both classes of agents was calculated

and classified as low (total chemotherapy score ≤ 4) versus high exposure (total chemotherapy score > 4). Linear mixed effect models (SAS Proc Mixed procedure; SAS Institute, Cary, NC) were used to investigate the telomere length change after aHCT (from day 100 to 3 years after aHCT), after adjusting for potential confounders (age at aHCT, primary diagnosis, race/ethnicity, sex, disease status, CD34 counts, and therapeutic exposures). The mixed model allows multiple sources of variation to be addressed while analyzing longitudinal data. The time variable for the linear

Table 1. Demographic, Diagnosis, and Treatment Characteristic Among Patient Cases and Controls

Characteristic	Patient Cases (n = 9)		Controls (n = 24)		P
	No.	%	No.	%	
Sex					.44*
Female	4	44	7	29	
Male	5	56	17	71	
Race/ethnicity					1.00*
Non-Hispanic white	6	67	17	71	
Hispanic white	2	22	4	17	
Black	1	11	3	12	
Primary diagnosis					1.00*
Non-Hodgkin's lymphoma	8	89	22	92	
Hodgkin's lymphoma	1	11	2	8	
Age at diagnosis of primary disease, years					.73
Mean	49		46		
SD	8.84		9.78		
Age at aHCT, years					.77
Mean	50		49		
SD	9.13		9.85		
Disease status at aHCT					1.00*
In remission	2	22	5	21	
Not in remission	7	78	19	79	
Priming for stem-cell mobilization with cyclophosphamide					.44*
No	5	56	17	70	
Yes	4	44	7	30	
Dose of cyclophosphamide, g/m ² †					.75
Median	2		2		
Range	1.5-2.5		1.5-2.0		
Priming for stem-cell mobilization with etoposide					.48*
No	8	89	23	96	
Yes	1	11	1	4	
Dose of etoposide, mg/kg	60		60		
CD34 counts, 10 ⁶ kg					.75
Mean	4.7		5.17		
Range	2.59-17.44		2.11-33.5		
Priming for stem-cell mobilization with filgrastim					
No	0		0		
Yes	9		24		
Dose of filgrastim, μg/kg					.49
Median	10		10		
Range	5-16		5-10		
Alkylating agent score‡					1.00*
Low	6	67	16	67	
High	3	33	8	33	
Topoisomerase II inhibitor score‡					.70*
Low	6	67	13	54	
High	3	33	11	46	
Combined alkylating agent and topoisomerase II inhibitor score‡					1.00*
Low	5	56	12	48	
High	4	44	12	52	

Abbreviations: SD, standard deviation; aHCT, autologous hematopoietic stem-cell transplantation.

*Fisher's exact P values.

†Cyclophosphamide dose was not known for one patient case.

mixed effect model was coded as days after HCT (eg, day 100 = 100, 6 months = 180, and so on). All *P* values were two sided, and *P* < .05 was considered statistically significant.

RESULTS

PB samples from nine patients who developed t-MDS/AML after aHCT and 24 matched controls who did not develop t-MDS/AML were analyzed. Only samples obtained before the development of t-MDS/AML were studied. The demographic and clinical characteristics of patient cases and controls are listed in Table 1. Appendix Table A1 (online only) lists cytogenetic, BM, and PB characteristics of patients with t-MDS/AML. There was no difference in sex, age at primary disease diagnosis and at aHCT, disease status at aHCT, CD34 counts, priming regimen, or cumulative exposures to therapeutic agents between patient cases and controls. PB samples obtained before aHCT (nine patient cases and 24 controls) and after aHCT at day 100 (seven patient cases and 21 controls), 6 months (eight patient cases and 19 controls), 1 year (five patient cases and 17 controls), 2 years (four patient cases and 14 controls), and 3 years (three patient cases and seven controls) were analyzed for telomere length. Cumulative incidence of t-MDS/AML was 6.3% (95% CI, 3.0% to 9.7%) at 6 years.

RTLs were measured in total cells and in lymphoid and myeloid subpopulations. Significant differences in the pattern of telomere length change were observed between patient cases with t-MDS/AML and controls as they were observed longitudinally after aHCT (Figs 1, 2). Telomere length was increased in cells from t-MDS/AML patients compared with controls at day 100 after aHCT (Figs 1, 2). Subsequently, a progressive reduction in telomere length in myeloid cells was seen in patients who subsequently developed t-MDS/AML compared with controls. No difference in telomere length was seen between patient cases and controls for lymphoid cells at the same time points. In contrast to t-MDS/AML patient cases, controls showed an initial decrease in telomere length at day 100 after aHCT followed by subsequent stabilization (Fig 2). Southern blot analysis of genomic

DNA from PB cells from t-MDS/AML patients (*n* = 5) and matched controls (*n* = 5) confirmed increased telomere length in t-MDS/AML patients at day 100 compared with before aHCT and reduced telomere length before t-MDS/AML compared with day 100 (Appendix Fig A1, online only).

Thus, in the patient cases with t-MDS/AML, a distinct pattern was observed in telomere length over time; there was an increase in telomere length between the pre-aHCT time point and day 100, followed by a decline after day 100 over the subsequent time points. Therefore, our subsequent analysis examined the two directions of telomere length change separately.

Before aHCT to Day 100 After aHCT

After adjustment for age at aHCT, race/ethnicity, sex, primary diagnosis, disease status, CD34 counts, and therapeutic exposures, a significant change in telomere length between the pre-aHCT time point and day 100 (increase in t-MDS/AML patient cases; decrease in controls) was confirmed in all cell types, but it was most pronounced in myeloid cells (*P* < .001; Table 2).

Day 100 to 3 Years After aHCT

A linear mixed effect model was used to analyze telomere shortening at time points after day 100, after adjusting for age at aHCT, primary diagnosis, race/ethnicity, sex, disease status, CD34 counts, and therapeutic exposures. A significant association was observed between t-MDS/AML and the rate of telomere shortening from day 100 to 3 years in total cells (*P* = .008) and myeloid cells (*P* = .002; Table 3). A significantly faster rate of telomere shortening was seen in t-MDS/AML patient cases (−1.59 per 100 days in patient cases *v* −0.17 in controls for total cells; −2.15 per 100 days in patient cases *v* −0.03 per 100 days in controls for myeloid cells; and −0.81 per 100 days in patient cases *v* −0.07 in controls for lymphoid cells; Fig 3).

Because telomere length can be modulated by telomerase activity, we used the RQ-TRAP assay to measure telomerase activity in PB

Fig 1. Representative flow cytometry data showing FITC fluorescence histograms. Gold and blue lines represent FITC fluorescence signal with and without labeling with FITC-telomere probe, respectively. Telomere length is proportional to the difference in fluorescence between labeled and unlabeled samples.

Fig 2. Relative telomere lengths in total peripheral blood (PB) cells and myeloid and lymphoid populations after autologous hematopoietic stem-cell transplantation (aHCT). Relative telomere lengths were measured in (A) total cells and (B) myeloid cells and (C) lymphoid subpopulations in serial samples from patients who developed therapy-related myelodysplasia or acute myeloid leukemia (t-MDS/AML) and matched controls using flow cytometry after hybridization with a fluorescein isothiocyanate (FITC)-conjugated peptide nucleic acid telomere probe (before aHCT: nine patient cases and 24 controls; day 100: seven patient cases and 21 controls; 6 months: eight patient cases and 19 controls; 1 year: five patient cases and 17 controls; 2 years: four patient cases and 14 controls; and 3 years: three patient cases and seven controls). Data shown represent the mean with or without the standard error of the mean of multiple samples. Significance of differences in telomere length in patients who developed t-MDS/AML compared with controls is shown. (*) $P < .05$; (**) $P < .01$; (†) $P = .07$; (††) $P = .06$.

cells from t-MDS/AML patient cases and controls.⁵³ Telomerase activity levels were minimal or undetectable in PB cells from patient cases and controls (Appendix Table A2, online only). The low levels of telomerase activity may reflect the differentiated nature of PB cells.

To determine whether telomere shortening was associated with altered proliferative potential of hematopoietic progenitors, we evaluated the frequency of LTCIC (primitive progenitors) and CFC (com-

Table 2. Difference in the Least Squares Mean of Telomere Length Between Pre-aHCT Time Point and Day 100 After aHCT: Comparison Between Patient Cases and Controls

Cell Type	Patient Cases		Controls		P
	Mean	SE	Mean	SE	
Total peripheral cells	12.22	6.3	-7.36	3.6	.02
Lymphoid cells	10.80	7.1	-6.74	4.0	.05
Myeloid cells	18.04	5.5	-8.23	3.2	< .001

NOTE. Least squares mean was adjusted for age at aHCT, primary diagnosis, race/ethnicity, sex, disease status, CD34 counts, and cumulative therapeutic exposures.

Abbreviation: aHCT, autologous hematopoietic stem-cell transplantation.

mitted progenitors) in BM samples from t-MDS/AML patient cases (PBSC, $n = 7$; day 100, $n = 6$; 1 year, $n = 6$) and matched controls (PBSC, $n = 17$; day 100, $n = 17$; 1 year, $n = 18$; Fig 4). CFC frequency was lower but not significantly reduced in the PBSC and day 100 samples from t-MDS/AML patient cases compared with controls. CFC frequency was further reduced at 1 year after aHCT in t-MDS/AML patient cases and was significantly lower than controls. LTCIC frequency was markedly reduced after aHCT in both patient cases and controls, with no significant difference between the two groups. The CFC-to-LTCIC ratio (ratio of committed to primitive progenitors—an indirect measure of the proliferative capacity of primitive progenitors) was significantly reduced in t-MDS/AML patient cases compared with controls at 1 year after aHCT.

DISCUSSION

aHCT for NHL and HL is associated with a high risk of t-MDS/AML. We observed a cohort of 287 patients with NHL or HL who were

Table 3. Change in Telomere Length Over Time From Day 100 to 3 Years After aHCT

Cell Type	Estimate of Change	P
Total peripheral blood cells		
Intercept	19.70	.003
Case-control status	7.07	.008
Days after BMT (per 100 days)	-0.17	.54
Case-control status $\times$ days after aHCT	-1.42	.008
Lymphoid cells		
Intercept	18.13	.01
Case-control status	5.41	.007
Days after BMT (per 100 days)	-0.074	.72
Case-control status $\times$ days after aHCT	-0.74	.07
Myeloid cells		
Intercept	28.82	.02
Case-control status	10.82	.002
Days after BMT (per 100 days)	-0.03	.93
Case-control status $\times$ days after aHCT	-2.12	.002

NOTE. Estimates were adjusted for age at aHCT, primary diagnosis, race, sex, disease status, CD34 counts, and cumulative therapeutic exposures.

Abbreviations: aHCT, autologous hematopoietic stem-cell transplantation; BMT, bone marrow transplantation.

Fig 3. Change in telomere length over time from day 100 to 3 years after autologous hematopoietic stem-cell transplantation (aHCT). A linear mixed effect model was used to analyze telomere shortening at time points after day 100, after adjusting for age at aHCT, primary diagnosis, race, sex, disease status, CD34 counts, and cumulative therapeutic exposures. The line represents the calculated rates for telomere shortening after day 100 for therapy-related myelodysplasia or acute myelogenous leukemia (t-MDS/AML) patient cases and controls. A significantly faster rate of telomere shortening in myeloid cells was seen in patients developing t-MDS/AML (-2.15 per 100 days in patient cases *v* -0.03 per 100 days in controls; $P = .002$).

undergoing aHCT to investigate the sequence of cellular and molecular abnormalities that occur in the course of development of t-MDS/AML. This report focuses on alterations in telomere length in patients from within this cohort who developed t-MDS/AML compared with matched controls from within the cohort who did not develop t-MDS/AML. We observed a significant alteration in telomere dynamics in myeloid cells from patients who developed t-MDS/AML after aHCT compared with controls that consisted of initial increase in telomere length from before HCT to day 100 after aHCT, followed by accelerated telomere shortening before the development of t-MDS/AML; this finding was statistically significantly different from that observed in the matched controls.

Telomere length in mature hematopoietic cells reflects the telomere length of the HSCs from which the cells are derived, the number of cell divisions occurring during hematopoietic maturation, and reg-

ulation of telomere shortening during cell division.²⁷ The telomere length of marrow cells from allogeneic transplantation recipients is considerably shorter than telomere length of donor cells, likely reflecting increased replicative demand after aHCT.^{27,31-34,55} Accelerated telomere shortening has also been observed after aHCT and has been reported to persist long term.⁵⁵⁻⁵⁷ In this study, we also observed a reduction in telomere length at day 100 after aHCT compared with before aHCT in control patients. The increase in telomere length at day 100 after aHCT in hematopoietic cells from t-MDS/AML patient cases was unexpected. Increased telomere length may be explained by reduced proliferative potential of premalignant HSCs in patients at risk for t-MDS/AML, which would lead to a requirement for enhanced activation and recruitment of dormant HSCs to achieve hematopoietic regeneration after aHCT. The newly recruited HSCs are expected to have longer telomeres, explaining the increased telomere length seen soon after hematopoietic recovery.

Significantly accelerated telomere shortening was seen in myeloid (but not lymphoid) cells from t-MDS/AML patient cases. In contrast, in patients who did not develop t-MDS/AML, telomere length stabilized after achievement of hematopoietic regeneration after aHCT. This is consistent with a prior analysis in which we observed telomere shortening in marrow cells early after aHCT, with subsequent restoration to pre-aHCT levels, but poor telomere recovery after aHCT in a subset of patients who developed t-MDS/AML.⁴⁶ Accelerated telomeric shortening in patients developing t-MDS/AML may reflect increased cell cycling of premalignant myeloid cells, resulting from cell cycle dysregulation or ineffective hematopoiesis.^{41,43-45,58} Alteration in telomere regulatory genes could also play a role in accelerated telomere shortening in the course of development of t-MDS/AML.

Although telomere length is reduced in a subset of primary MDS patients, it is not clear whether telomere shortening contributes to the development of MDS.³⁷⁻⁴⁰ The results of the current study clearly demonstrate that accelerated telomere shortening precedes development of t-MDS/AML and is an independent risk factor on multivariate analysis. Our ability to detect this association was possible because of the unique study design that we used, which allowed patients to be observed from time of genotoxic insult through the period of risk of development of t-MDS/AML with serial banking of biologic samples

Fig 4. Committed and primitive progenitor frequencies after autologous hematopoietic stem-cell transplantation (aHCT). The frequencies of colony-forming cells (CFCs; committed progenitors) and long-term culture-initiating cells (LTCICs; primitive progenitors) in bone marrow samples from patients who developed therapy-related myelodysplasia or acute myelogenous leukemia (t-MDS/AML; peripheral-blood stem cells [PBSCs], $n = 7$; day 100, $n = 6$; and 1 year, $n = 6$) and matched controls (PBSCs, $n = 17$; day 100, $n = 17$; and 1 year, $n = 18$) were evaluated. The CFC-to-LTCIC ratio (ratio of committed to primitive progenitors) was further analyzed as a measure of the proliferative capacity of primitive progenitors. Data shown represent the mean $\pm$ SEM of multiple samples. Significant differences in progenitor frequency in patients who developed t-MDS/AML compared with controls are shown: (*) $P < .05$ and (**) $P < .01$.

at frequent predetermined time points. Our results support an important pathogenetic role for telomere dysfunction in the development of t-MDS/AML after aHCT.

Telomere length in hematopoietic cells correlates with their proliferative potential *in vitro*.⁵⁹ Telomere shortening in patients developing t-MDS/AML was associated with reduced proliferative capacity of primitive hematopoietic progenitors, as evidenced by reduced frequency of committed progenitors and reduced ratio of committed to primitive progenitors in the BM at 1 year after aHCT. Telomere shortening may predispose towards development of t-MDS/AML by triggering genetic instability.^{60,61} Cells with critically short telomeres may fail to form protective telomere structures (T-loop and D-loop) and are prone to end-to-end fusion, propagating a breakage-fusion-bridge cycle resulting in either gene deletion or gene amplification.⁶⁰ As discussed by Lansdorp,⁶² loss of HSCs via telomere attrition may support selection for abnormal cells with genome instability. One third of patients with aplastic anemia have short telomeres, which may be associated with *TERT* or *TERC* mutations. Progressive telomere shortening may contribute to predisposition towards developing the MDS seen in these patients.^{63,64}

In conclusion, results of the current study indicate significant alteration in telomere dynamics in hematopoietic cells preceding the development of t-MDS/AML. Telomere shortening may reflect increased clonal proliferation or altered telomere regulation in premalignant cells, and the resultant genetic instability associated

with shortened telomeres may contribute to leukemic transformation in t-MDS/AML.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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