Original Article

Myeloablative Doses of Yttrium-90-Ibritumomab Tiuxetan and the Risk of Secondary Myelodysplasia/Acute Myelogenous Leukemia

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BACKGROUND: Because the long-term toxicity of myeloablative radioimmunotherapy remains a matter of concern, the authors evaluated the hematopoietic damage and incidence of secondary myelodysplastic syndrome and acute myelogenous leukemia (sMDS/AML) in patients who received myeloablative doses of the radiolabeled antibody yttrium-90 (^{90}Y)-ibritumomab tiuxetan. **METHODS:** The occurrence of sMDS/AML was investigated prospectively in 53 elderly patients with non-Hodgkin lymphoma (NHL) who underwent an autograft after high-dose radioimmunotherapy (HD-RIT) myeloablative conditioning with ^{90}Y-ibritumomab tiuxetan. Bone marrow (BM) hematopoietic progenitors and telomere length (TL) also were investigated. **RESULTS:** At a median follow-up of 49 months, 4 patients developed sMDS/AML at 6 months, 12 months, 27 months, and 36 months after HD-RIT, and the 5-year cumulative incidence of sMDS/AML was 8.29%. A significant but transient decrease in BM granulocyte-macrophage progenitors was observed; whereas multilineage, erythroid, and fibroblast progenitors were unaffected. A significant and persistent shortening of BM TL also was detected. A matched-pair analysis comparing the study patients with 55 NHL patients who underwent autografts after chemotherapy-based myeloablative conditioning demonstrated a 8.05% 5-year cumulative incidence of sMDS/AML. **CONCLUSIONS:** HD-RIT for patients with NHL was associated with 1) limited toxicity on hematopoietic progenitors, 2) accelerated TL shortening, and 3) non-negligible incidence of sMDS/AML, which nevertheless was comparable to the incidence observed in a matched group of patients who received chemotherapy-based conditioning. Thus, in the current series of elderly patients with NHL, the development of sMDS/AML was not influenced substantially by HD-RIT. ***Cancer* 2011;000:000–000.** © *2011 American Cancer Society.*

KEYWORDS: non-Hodgkin lymphoma, secondary myelodysplastic syndrome and acute myelogenous leukemia, high-dose radioimmunotherapy, yttrium-90-ibritumomab tiuxetan, telomere length.

Rituximab-based high-dose (HD) sequential chemotherapy (HDS-CT) followed by myeloablative conditioning and autologous stem cell transplantation (ASCT) is an effective and widely used treatment strategy for patients with relapsed or prognostically unfavorable non-Hodgkin lymphoma (NHL).[1,2] Pretransplantation conditioning regimens usually include myeloablative chemotherapy with or without total body irradiation (TBI).[3] However, novel regimens currently are being investigated to reduce hematopoietic and nonhematopoietic toxicity and to overcome age-related and comorbidity-related restrictions, thereby ultimately enhancing the efficacy and feasibility of ASCT.

The efficacy and good toxicity profile of radiolabeled antibodies prompted the inclusion of these drugs into myeloablative regimens.[4-10] Radioimmunoconjugate-containing conditioning combined with chemotherapy has demonstrated short-term toxicity comparable to that observed after chemotherapy-based conditioning.[11-13] Recently, we reported that myeloablative doses (1.2 mCi/kg) of yttrium-90 (^{90}Y)-ibritumomab tiuxetan (Zevalin) combined with tandem stem cell

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support represent a feasible pretransplantation conditioning regimen with mild-to-moderate, short-term hematologic and nonhematologic toxicity.[14]

Secondary myelodysplastic syndrome (sMDS) and acute leukemia (sMDS/AML) are the predominant types of secondary malignancies observed after HDS-CT and ASCT, and the estimated 5-year cumulative probability of sMDS/AML ranges from 1% to 24% in different series.[15-20] The risk of sMDS/AML includes age and exposure to alkylating agents and likely is caused by prolonged or repetitive exposure to cytotoxic drugs as well as the relatively long natural history of underlying disease.[21,22] Whether bone marrow (BM) exposure to high radiation doses because of radiolabeled antibodies may further increase hematopoietic damage, thereby increasing the risk of sMDS/AML, remains a matter of concern. Witzig et al reported on 5 patients with sMDS/AML among 349 patients who had relapsed or refractory NHL treated with standard-dose (0.4 mCi/kg) ^{90}Y-ibritumomab tiuxetan.[6] More recently, Czuczman et al performed an extensive evaluation of 746 patients who received standard-dose ^{90}Y-ibritumomab tiuxetan and reported a 2.5% incidence of sMDS/AML after a median follow-up of 4.4 years.[23] Likewise, a 5-year cumulative incidence rate for sMDS/AML of 6.3% was observed in patients with recurrent disease who received standard-dose ^{131}I-tositumomab.[24] A similar incidence of sMDS/AML was observed in patients who received myeloablative doses of either iodine-131 (^{131}I)-tositumomab or chemotherapy-based conditioning for recurrent follicular NHL.[12]

An analysis of hematopoietic reconstitution after ASCT has demonstrated that there was permanent damage of hematopoietic and stromal progenitor cells.[25-27] Moreover, hematopoietic regeneration after ASCT has been associated with accelerated telomeric shortening because of increased replicative proliferation during hematopoietic recovery and exposure to HD cytotoxic drugs.[28-31] Recently, Bhatia et al reported that, after chemotherapy-based conditioning and ASCT, patients who developed sMDS/AML had a significantly reduced recovery of committed progenitors and telomere length (TL) compared with autografted patients who did not develop sMDS/AML.[32]

Because the long-term toxicity of ^{90}Y-ibritumomab tiuxetan at myeloablative doses remains unknown, in this longitudinal study, we prospectively investigated a cohort of high-risk patients with NHL who received a rituximab-based HDS-CT program followed by myeloablative conditioning with ^{90}Y-ibritumomab tiuxetan and autograft with peripheral blood stem cells (PBSCs). The occurrence of sMDS/AML was monitored along with the incidence of BM hematopoietic and stromal progenitors and telomere analysis. Moreover, the cumulative incidence of sMDS/AML in the study group was compared in a matched-pair analysis with the incidence observed in a historic group of patients who were underwent autograft after receiving chemotherapy-based myeloablative conditioning.

MATERIALS AND METHODS

Patients and Treatment Plan

Between July 2004 and December 2007, 65 consecutive patients with refractory, recurrent, or de novo high-risk NHL received HD radioimmunotherapy (RIT), based on a single administration of ^{90}Y-ibritumomab tiuxetan given at 1.2 mCi/kg body weight.[33] Fifty-three of 65 patients who had at least a 6-month, continuous complete remission after transplantation were enrolled in this study. One patient who developed secondary acute myeloid leukemia with inversion of chromosome 16 4 months after transplantation was excluded from the current analysis, because the cytogenetic abnormality was detected retrospectively in the leukapheresis collected before HD-RIT, thus ruling out any causative role of ^{90}Y-ibritumomab tiuxetan.[34]

This study was approved by the institutional ethical committee, and written informed consent was obtained from each patient. Patients were followed until occurrence of death, relapse, or development of sMDS/AML. The histologic diagnosis in the 53 patients was diffuse large B-cell lymphoma (n = 19), Richter syndrome (n = 2), follicular cell lymphoma (n = 20), mantle cell lymphoma (n = 8), marginal zone lymphoma (n = 3), and lymphoplasmacytic lymphoma (n = 1). Before they received HD-RIT, all patients received 5 chemotherapy courses, including 3 cycles of anthracycline-containing or platinum-containing regimens, 1 course of HD cyclophosphamide (4-7 g/m^2), and 1 cycle of HD cytarabine (12-24 g/m^2). Mobilized PBSCs were harvested either after HD cyclophosphamide (n = 9), HD cytarabine (n = 36), or both (n = 8).[35] The ^{90}Y-ibritumomab tiuxetan dose of 1.2 mCi/kg is myeloablative; therefore, an autograft was required after HD-RIT. To overcome both short-term and long-term hematologic toxicity, the autograft schedule included a "tandem" reinfusion of autologous

Table 1. Clinical Characteristics of Study Patients and Historic Controls

Characteristic	No. of Patients (%)	
	Study Patients	Controls
No. of patients	53	55
Age, y		
Median	64	62
Range	26-76	59-69
>65	24 (45)	15 (27)
Sex: Women/men	33/20	30/25
Diagnosis		
High grade: DLBCL, Richter syndrome	21 (43)	24 (44)
Low grade: FCL, MZL, lymphoplasmacytic lymphoma	24 (41)	18 (33)
Mantle cell lymphoma	8 (15)	13 (24)
Disease status		
Diagnosis	17 (32)	13 (24)
Refractory/relapsed	36 (68)	42 (76)
Ann Arbor stage at diagnosis		
I-II	14 (26)	3 (5)
III-IV	39 (74)	52 (95)
No. of previous chemotherapy regimens		
Median	2	1
Range	1-5	1-4
Prior autologous SCT	3 (6)	0 (0)
Previous radiotherapy	9 (17)	2 (4)
Extended field radiotherapy	3 (6)	0 (0)
Bone marrow involvement		
No	21 (40)	20 (36)
Yes	32 (60)	35 (64)
Type of grafted PBSCs		
After HD cyclophosphamide	9 (17)	10 (18)
After HD cytarabine	44 (83)	45 (82)

Abbreviations: DLBCL, diffuse large B-cell lymphoma; FCL, follicular cell lymphoma; HD, high dose; MZL, marginal zone lymphoma; PBSCs, peripheral blood stem cells; SCT, stem cell transplantation.

PBSCs on Days +7 and +14 after HD-RIT.[14] Clinical characteristics of the patients who received HD-RIT are summarized in Table 1. The series included elderly patients; indeed, the median age at transplantation was 64 years, and 45% of patients were aged >65 years. Most patients had received previously treatment; thus, the median time from diagnosis to HDS-CT with HD-RIT was 6 years.

Samples for Biologic Monitoring

BM samples were collected before HD-RIT, every 6 months for 2 years, and annually thereafter. In total, 240 BM samples were evaluated, including 51 pretransplantation samples (96% of assessable patients) and 189 post-transplantation samples. Overall, 60% to 100% of planned samples actually were harvested and analyzed at each post-transplantation time point. Reasons for incomplete collection of samples included inadequate amount of material to perform all tests and patient refusal to undergo planned examinations. Harvested samples were analyzed for incidence of hematopoietic and stromal progenitors, TL, and cytogenetics. In addition, complete blood counts and disease evaluations with computed tomography and/or positron emission tomography imaging studies were performed.

Cytogenetic Analysis and sMDS/AML Assessment

Chromosomal analysis and G-banding were performed using standard techniques on at least 20 BM metaphases. The diagnosis of MDS was assessed according to World Health Organization criteria.[36]

Multilineage Colony-Forming Unit, Erythroid Burst-Forming Unit, and Granulocyte-Macrophage Colony-Forming Unit Assay

The assay for committed colony-forming cells (CFCs), including multilineage colony-forming units (CFU-Mix), erythroid burst-forming units (BFU-E), and granulocyte-macrophage CFUs (CFU-GM), was carried out as previously described.[37] Briefly, from 1×10^4 to 5×10^4 mononuclear cells were plated in 35-mm Petri dishes in cytokine-supplemented, methylcellulose-based medium (Methocult GF H4034; Stem Cell Technologies, Vancouver, British Columbia, Canada). Progenitor cell growth was evaluated according to previously published criteria.

Fibroblast CFU Assay

Fibroblast CFUs (CFU-F) were assayed according to a previously described technique.[37] Briefly, mononuclear cells (5×10^4/mL resuspended in alpha-medium supplemented with fetal bovine serum; 12.5%, volume/volume), horse serum (12.5%, volume/volume), and freshly dissolved hydrocortisone (10^{-6} M) were plated onto 60-mm Petri dishes. Fibroblastoid cell aggregates of >50 cells were scored as CFU-F after staining with crystal violet.

TL Analysis

The TL of BM mononuclear cells was assessed by Southern blot analysis, as previously described.[30,31] Briefly, DNA fragments that were obtained after digestion with *Hinf I* and *Rsa I* (Roche Diagnostic, Mannheim, Germany) were separated by agarose gel electrophoresis and then transferred onto a nylon membrane. The TeloTAGGG TL Assay Kit (Roche Diagnostic) was used for the hybridization phase according to manufacturer's instructions. For each telomere smear, mean terminal restriction fragment (TRF) lengths and the point of maximum signal intensity defining the highest concentration of telomere repeats were calculated using Quantity 1 software (Bio-Rad Laboratories, Inc., Herts, United Kingdom).

Historic Cohort of Matched Pairs

The matched-pair analysis was executed by using a 3-step strategy. First, patients who received an HDS-CT program followed by a chemotherapy-based myeloablative conditioning regimen and ASCT were identified as a historic control group from a large lymphoma database.[38] Second, patients in the 2 groups were matched for the following risk factors: age, sex, histologic subtype, disease status at transplantation, and type of grafted PBSCs. Third and finally, 1 randomly chosen patient from the historic control group was matched with each HD-RIT study patient. The comparison of main clinical parameters between the HD-RIT study group and the historic control group are detailed in Table 1.

Statistical Analysis

Longitudinal measurements of CFU-Mix, BFU-E, CFU-GM, CFU-F, and TL were analyzed using linear mixed-effect models to account for the correlation between measurements within the same category. Time was modeled as the fixed-effect factor variable. Model assumptions were verified by examining the residual plots. Two-sided *P* values below the 5% conventional threshold were considered significant. The cumulative incidence of sMDS/AML was determined using the Fine and Gray competing risk regression model[39] to identify the effect of risk factors on the cumulative incidence function for competing risks data. The Gray test was used to compare the cumulative incidence curves in the presence of a competing risk (always defined as death from any cause other than sMDS). Event times were computed from the date of autograft, either after HD-RIT or after HD-CT, to the date of last follow-up assessment for event-free patients. Data were analyzed as of June 2010 by using SAS statistical software (SAS Institute Inc., Cary, NC) and R 2.11.1 (package cmprsk; The R Project for Statistical Computing, Austria, Vienna).

RESULTS

Engraftment and Response to Therapy

After HD-RIT with ^{90}Y-ibritumomab tiuxetan, all patients received 2 PBSC reinfusions with a median of 2×10^6 CD34-positive cells/kg (range, $1\text{-}3.7 \times 10^6$ CD34-positive cells/kg) and 9.3×10^6 CD34-positive cells/kg (range, 6.6-26.8 CD34-positive cells/kg) transplanted on Days +7 and +14, respectively. Neutrophil counts $\geq$500/μL and platelet counts $\geq$20,000/μL were achieved at a median of 6 days (range, 2-10 days) and 12 days (range, 7-17 days) after the second PBSC reinfusion, respectively. Pretransplantation values of hemoglobin, neutrophils, and platelet counts were within the normal ranges (Fig. 1). Six months after ^{90}Y-ibritumomab tiuxetan, both neutrophil and platelet counts were reduced significantly compared with baseline counts, but complete and sustained recoveries to baseline values were observed

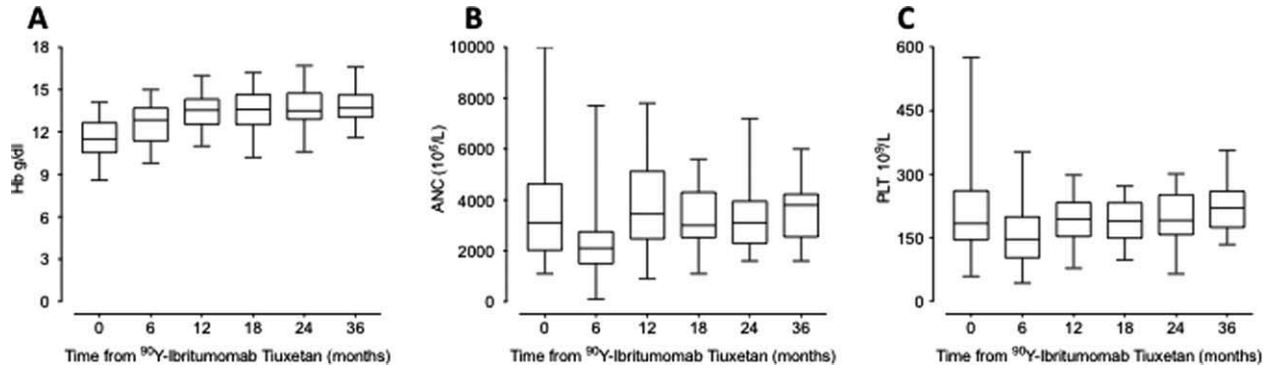

Figure 1. (A) Hemoglobin (Hb) values, (B) absolute neutrophil counts (ANC), and (C) platelet (PLT) counts are illustrated at baseline and at 6 months, 12 months, 18 months, 24 months, and 36 months after high-dose radioimmunotherapy. Boxes extend from the 25th percentile to the 75th percentile, the middle lines indicate median values, and the whiskers indicate the range of values.

Month +12 after transplantation. As of June 2010, at a median follow-up of 49 months (range, 32-74 months), 50 of 53 patients who had received ^{90}Y-ibritumomab tiuxetan with stem cell rescue remained alive. The 5-year overall and event-free survival rates were 94% and 68%, respectively. Disease status at last follow-up was as follows: Thirty-nine patients (73%) remained alive in complete remission, 8 patients (15%) remained with relapsed disease, and 3 patients remained alive with sMDS/AML. To date, 3 patients have died of pulmonary infection, sMDS/AML, and disease relapse, respectively.

Occurrence and Characterization of sMDS/AML in HD-RIT-Treated Patients

Although secondary solid tumors were not observed in study patients, BM cytogenetic abnormalities were detected in 4 of 53 patients at 6 months, 12 months, 27 months, and 36 months after ^{90}Y-ibritumomab tiuxetan and at 124 months, 19 months, 176 months, and 48 months after diagnosis, respectively. At the time cytogenetic abnormalities initially were detected, 3 patients had unilinear cytopenia without morphologic evidence of BM blasts, whereas 10% BM blasts were detected in 1 patient (Table 2). According to World Health Organization criteria,[36] refractory anemia with excess of blasts 1 (RAEB-1) was diagnosed in 1 patient (Unique Patient Number [UPN] 48), whereas refractory cytopenia with unilineage dysplasia (RCUD) was diagnosed in the other 3 patients, with acute leukemia transformation observed in 2 patients (UPN 5 and UPN 34) 12 months and 18 months after the initial detection of abnormal cytogenetics, respectively. The median dose of CD34-positive cells (7.4×10^6 cells; range, 7.2 to 10.0×10^6 cells) reinfused per kilogram of body weight in the 4 patients who developed sMDS/AML did not differ significantly compared with the dose received by the remaining patients. All patients who developed sMDS/AML were reinfused with PBSCs that were harvested after HD-cytarabine. Three of 4 patients had received alkylating agents as part of the different chemotherapy regimens administered before transplantation. In addition, 3 of 4 patients were aged >65 years, and 2 of 4 patients received ^{90}Y-ibritumomab tiuxetan treatment several years after their initial diagnosis of lymphoma. In all instances, cytogenetic abnormalities involved chromosomes 5 and/or chromosome 7 (Table 2).

BM Hematopoietic Progenitors

Before patients received ^{90}Y-ibritumomab tiuxetan treatment, blood values and BM morphology were normal, and no patient had BM fibrosis. Compared with the control group (n = 57), study patients had a statistically significant reduction in the mean number (±standard error of the mean [SEM]) of BM CFU-Mix values (5.4 ± 0.6 vs 1.6 ± 0.3; $P \leq .05$) and CFU-GM values (200 ± 11 vs 69 ± 6; $P \leq .05$) but not BFU-E values (68 ± 5 vs 59 ± 7; $P > .05$) (Fig. 2A,B). Compared with pretransplantation values, an analysis using the mixed-effects models detected a transient but significant ($P = .002$) decrease in CFU-GM growth 6 months after transplantation followed by a sustained recovery to baseline values at Month +12 after transplantation. CFU-mix and BFU-E measurements did not vary significantly over time ($P = .138$ and $P = .066$, respectively). An analysis of BM hematopoietic progenitors in the 4 patients with sMDS/AML demonstrated baseline and post-transplantation frequencies of CFU-Mix, BFU-E, and CFU-GM similar to those detected in patients who did not develop sMDS/AML. As observed in 2 cases (UPN 34 and UPN 50), expansion of the leukemic clone was associated with a

progressive reduction in committed progenitor cell frequency (Table 3).

BM Mesenchymal Progenitors

Compared with normal individuals from the control group (n = 41), study patients had a statistically significant reduction in the mean (±SEM) CFU-F values (56 ± 7 vs 14 ± 2; $P \leq .05$) (Fig. 2C). Compared with pretransplantation values, an analysis of CFU-F growth at different time points indicated a trend toward reduction that was more evident 12 months after transplantation but that in no instance reached the level of statistical significance in the mixed-effects model ($P = .211$).

TL Analysis

Compared with baseline values, BM TL had a marked and progressive decrease after transplantation. Telomere loss was evident at the 12-month assessment; however, further loss occurred later on, as observed at 18 months and even at 24 months after transplantation (Fig. 2D). Although few BM samples were available for 2 patients (UPN 5 and UPN 48) who developed sMDS/AML, a marked reduction in TL was detected on a 12-month BM sample in a third patient (UPN 50), who then developed sMDS/AML; a fourth patient (UPN 34) had an early sMDS/AML at 6 months after ASCT, preventing any possible correlation with postgraft TL reduction.

Comparison of the Cumulative Incidence of sMDS/AML in Patients who Received HD-RIT and Historic Controls

The 5-year cumulative incidence of sMDS/AML in patients who received HD-RIT was 8.29%, as indicated in Figure 3. Fifty-five patients were identified as a control group (Table 1); at 5 years of follow-up, the cumulative incidence of sMDS/AML in this control group was 8.05%, and 5 of those 55 patients developed sMDS/AML (Fig. 3). There was no statistical differences in the cumulative incidence of sMDS/AML between the HD-RIT patients and the historic control group ($P = .655$).

DISCUSSION

The use of radiolabeled antibody as part of pretransplantation conditioning is an attractive approach to increase the feasibility and efficacy of ASCT. However, the theoretical risk of increasing sMDS/AML by myeloablative radioimmunotherapy remains a matter of concern. To address this issue, 53 patients with relapsed/refractory or de novo, high-risk NHL and a median age of 64 years were evaluated prospectively by clinical and biologic analyses after a treatment program that included myeloablative doses of ^{90}Y-ibritumomab tiuxetan and tandem stem cell support. The main observation was a 8.29% 5-year cumulative incidence of sMDS/AML, suggesting an increased risk compared with recently reported series of younger patients with lymphoma who received HD therapy and autograft.[15,19,38,40] However, when the sMDS/AML incidence was analyzed in a pair-matched group of NHL patients who received analogous treatment without HD-RIT, a comparable 8.05% 5-year cumulative incidence of sMDS/AML was observed.

Although comparing single-armed study groups with "matched" historic control groups is associated with limitations inherent to retrospective comparisons, our observation strongly suggests that ^{90}Y-ibritumomab tiuxetan has limited influence on the development of sMDS/AML; whereas other factors, including advanced age, extensive pretreatment, and type of PBSCs harvested and reinfused during the autograft procedure, are likely to be involved in sMDS/AML development after RIT-based and chemotherapy-based autograft treatments.

The current prospective study included serial karyotype monitoring. This allowed the early identification of cytogenetic abnormalities in 4 of 53 HD-RIT-treated patients. These abnormalities were associated with morphologic signs of unilinear dysplasia in the absence of BM blasts as well as CFC growth impairment, suggesting that conventional morphologic and cytogenetic analysis, but not CFC assays, are critical for identifying patients with sMDS/AML. It is noteworthy that mild but persistent neutropenia and/or thrombocytopenia were early markers of sMDS/AML that could be detected at the time of, or even before, cytogenetic abnormalities. Overall, these findings suggest that the occurrence of cytopenia in patients who receive myeloablative doses of ^{90}Y-ibritumomab tiuxetan requires careful examination, including morphologic and cytogenetic BM analysis.

The most common cytogenetic abnormalities detected in sMDS/AML involve either loss of a whole chromosome 5 and/or chromosome 7 or deletion of the long arm of these chromosomes,[15,41] and no clear relation has been identified between primary treatment modalities, ie, chemotherapy or radiotherapy, and distinct chromosomal abnormalities.[41] The 4 patients who developed sMDS/AML in our study had complex chromosomal abnormalities that usually are associated with exposure to

Table 2. Clinical and Cytogenetic Characteristics of Patients Who Developed Secondary Myelodysplastic Syndrome and Acute Myelogenous Leukemia at the Time of First Detection of Cytogenetic Abnormalities

UPN	Diagnosis	Age, y	Sex	Interval From Diagnosis to ^{90}Y-Ibritumomab Tiuxetan, mo	Interval From ^{90}Y-Ibritumomab Tiuxetan to sMDS/AML, m	MDS/AL Subtype	Cytogenetics	Relapsed/ Refractory	Previous Therapy (No. of Cycles)	Interval From Last Treatment to ^{90}Y-Ibritumomab Tiuxetan, mo
5	MCL	67	Woman	12	36	RCUD	46,XX,del(7)q(11)[19]/45, XX,−7[4]	Yes	R-CHOP (6)	5
34	FL	55	Man	118	6	RCUD	46,XY[2]/45-46,XY, del(5)(q22q35), der(7)del(7)(q11)r (7)(p22q11)[9],−7[3],inc	Yes	Autologous SCT, R-FND (4)	54
50	MCL	76	Man	7	12	RCUD	46,XY,der(7)t(1;7)(q10; p10)[7]	No	—	—
48	FL	68	Woman	149	27	RAEB-1	46,XX[7]/45,X-X,-4, del(5)(q12q33), add(6p),add(13q), add(16q),+mar[10]	Yes	Leukeran	120

Abbreviations: AL, acute leukemia; AML, acute myelogenous leukemia; FL, follicular lymphoma; MCL, mantle cell lymphoma; MDS, myelodysplasia; NHL, non-Hodgkin lymphoma; RAEB-1, refractory anemia with excess blasts-1; R-CHOP, rituximab, cyclophosphamide, vincristine, and prednisone; RCUD, refractory cytopenia with unilineage dysplasia; R-FND, rituximab, fludarabine, mitoxantrone, and prednisone; SCT, stem cell transplantation; sMDS, secondary myelodysplasia; UPN, unique patient number; ^{90}Y, yttrium-90.

alkylating agents, including monosomy of chromosome 7, deletion of the long arm of chromosomes 5 and 7, and a translocation involving the long arm of chromosome 7. Our study did not identify any RIT-related cytogenetic marker, thus failing to identify any distinct contribution of ^{90}Y-ibritumomab tiuxetan to the development of sMDS/AML.

Pretransplantation and post-transplantation levels of multipotent, myeloid, and fibroblast progenitors were reduced significantly compared with the levels in normal controls.[42] The decreased number of hematopoietic progenitors coexisting with normal blood cell counts indicates that the hematopoietic system is capable of compensating for the deficiency of early hematopoietic progenitor cells, as reported previously in patients who received highly aggressive chemotherapy or received allogeneic BM transplantation.[26,43] This suggest that, even after high doses of ^{90}Y-ibritumomab tiuxetan, an increased demand on stem cells for differentiation takes precedence over the demand for self-renewal, thus compensating and maintaining the supply of end cells to the peripheral blood.

Likely because of the direct effect of HD BM radiation, post-transplantation serial analysis of BM CFC growth revealed a transient and early impairment of all classes of BM progenitors, and only CFU-GM was reduced significantly compared with baseline. However, this early impairment of CFC growth usually recovered to baseline levels within 1 year after transplantation. This short-lasting decrease in BM hematopoietic progenitor cell growth is in striking contrast to data reported in patients who received chemotherapy-based conditioning, and, indeed, experienced a marked and long-lasting decrease in BM progenitors after transplantation.[26,32] Taken together, it can be concluded that HD-RIT has only limited toxicity on BM hematopoietic and stromal progenitors.[28,32]

In contrast to the transient damage of CFC growth, a significant and progressively worsening reduction of post-transplantation BM TL usually was detected 1 year after myeloablative doses of ^{90}Y-ibritumomab tiuxetan. Such a progressive telomere shortening represents an unusual finding that has not been documented equally in patients who were conditioned with chemotherapy-based regimens. Indeed, a possible telomere gain within the first year after ASCT has been reported by Bhatia et al.[32] In patients who received HDS-CT and underwent ASCT, we previously reported a significant shortening of TL compared with that in an age-matched normal control.

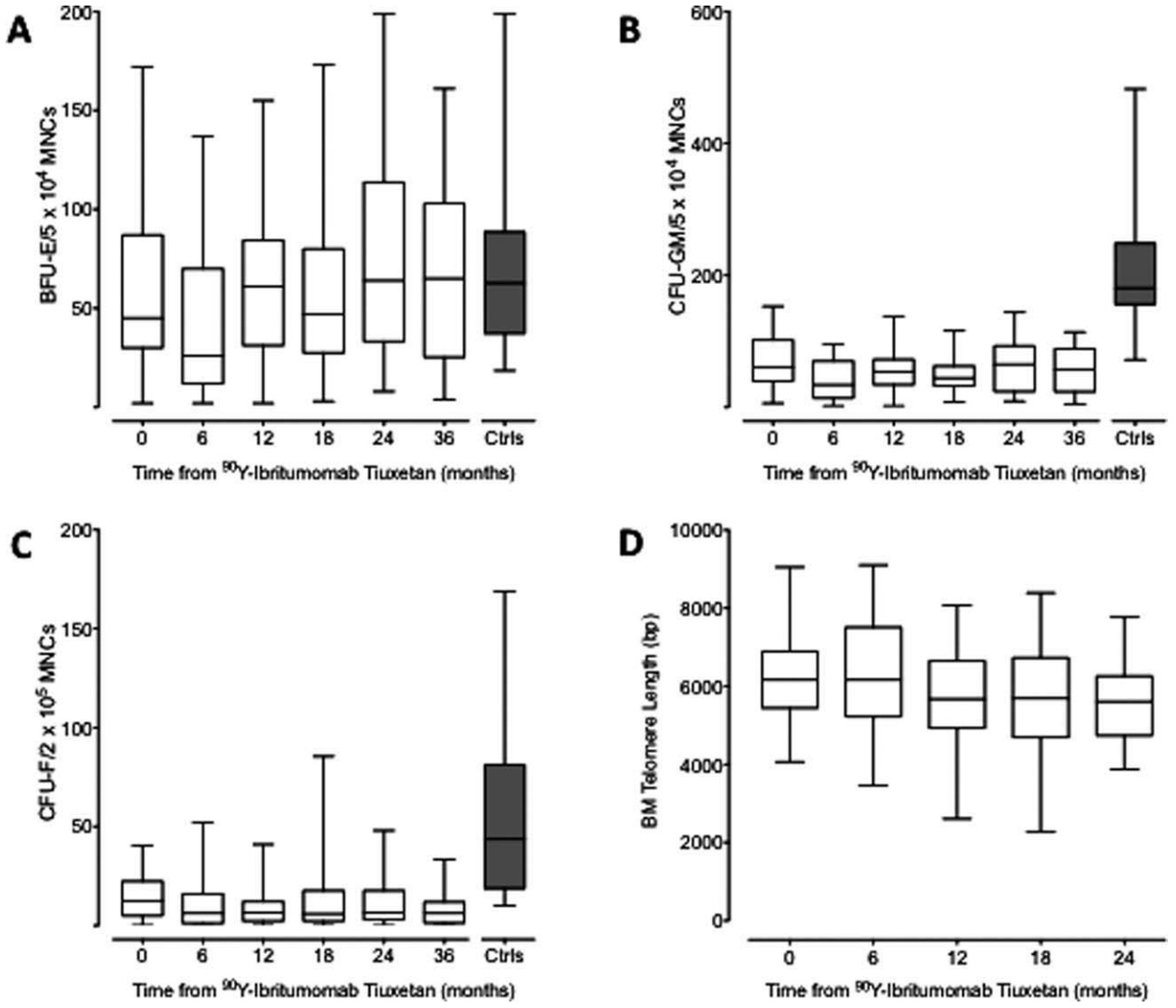

Figure 2. Bone marrow (BM) committed progenitor frequency and telomere lengths of BM mononuclear cells (MNCs) are illustrated. Box plots illustrate (A) erythroid burst-forming units (BFU-E), (B) granulocyte-macrophage colony-forming units (CFU-GM), (C) fibroblast colony-forming units (CFU-F), and (D) and telomere length at baseline and during follow-up after high-dose radioimmunotherapy. Ctrls indicates controls.

group. Such a telomere loss appears to be permanent but remains unaltered for several years after ASCT.[28] Conversely, more severe effects of chemotherapy on the replicative capacity of blood cells might be expected in older patients. In fact, a previous study documented that elderly patients who received chemotherapy had more pronounced telomere loss during follow-up compared with younger patients.[44] Thus, the progressive telomere loss detected in the current series appears to be a distinctive feature, and the advanced age of the treated patients seems the most reasonable explanation. However the continuous post-transplantation telomere loss might be ascribed to other factors, including the use of ^{90}Y-ibritumomab tiuxetan at high doses, or the exposure of reinfused PBSCs to residual ^{90}Y-ibritumomab tiuxetan radiation, or the combinations of all these features. Whatever the reason, the observation of marked and continuous TL shortening after autograft, along with the increased incidence of sMDS/AML, are in keeping with previously published reports describing a correlation between accelerated telomere loss and the development of sMDS/AML.[32,45]

In addition to biologic insights, the current study allowed us to prospectively define the real risk of developing sMDS/AML after HD-RIT. The cumulative

Table 3. Laboratory Data of Patients Who Developed sMDS/AML Secondary Myelodysplastic Syndrome and Acute Myelogenous Leukemia

		Peripheral Blood				Bone Marrow		
UPN	Time After ^{90}Y-Ibritumomab Tiuxetan, mo	ANC, 10^9/L	Platelets, 10^9/L	Hemoglobin, g/dL	Blasts, %	CFU-Mix, 5×10^4 MNCs	BFU-E, 5×10^4 MNCs	CFU-GM, 5×10^4 MNCs
5	0	2.8	169	9.6	NE	NE	NE	NE
	6	0.7	82	9.2	NE	NE	NE	NE
	12	3.5	232	11.5	NE	0	6	2
	24	1.28	116	13.1	NE	0.3	47	24
	30	1.13	115	12.2	NE	NE	NE	NE
	36[a]	1.33	122	12.8	2	0.8	65	22
34	0	4.3	428	10.9	NE	0	30	45
	6[a]	1.1	159	15	2	2.3	92	73
	12	1.6	135	13.5	2	1.8	68	77
	18	4.5	182	11.1	5	0.3	12	28
50	0	3.5	237	11.3	NE	7.5	121	129
	6	1.2	142	15.3	NE	0.5	45	38
	12[a]	2.1	72	14.5	3	3.0	81	87
	14	2.4	42	14.7	4	0	17	33
48	0	2.7	178	9.2	NE	2	11	38
	6	4.5	116	13.4	NE	0.3	12	14
	12	6.0	115	13.5	NE	0.5	63	65
	18	5.4	110	13.8	NE	NE	NE	NE
	27[a]	3.8	65	12.3	10	0.3	12	21

Abbreviations: ANC, absolute neutrophil count; BFU-E, erythroid burst-forming units; CFU, colony-forming units; CFU-GM, granulocyte-macrophage colony-forming units; CFU-Mix, multilineage colony-forming units; MNCs, mononuclear cells; NE, not evaluated; UPN, unique patient number; ^{90}Y, yttrium-90.
[a] The first occurrence of cytogenetic abnormalities.

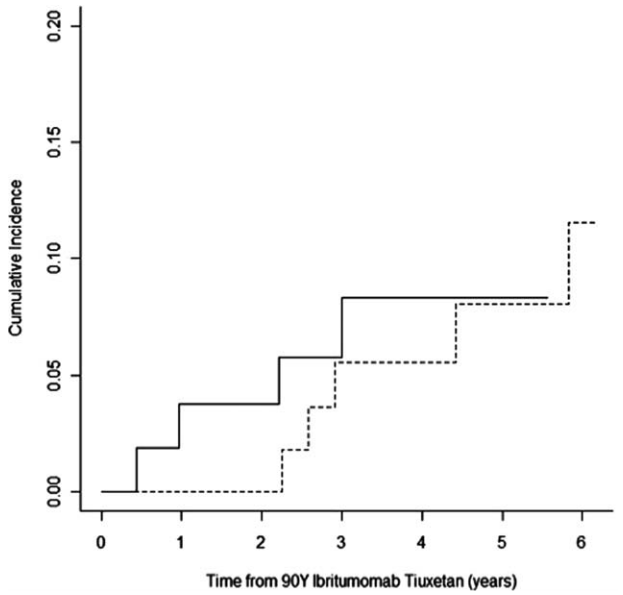

Figure 3. The cumulative incidence of secondary myelodysplastic syndrome and acute myelogenous leukemia is illustrated in 53 patients who received a high-dose radioimmunotherapy myeloablative conditioning regimen (solid line) and 55 patients who received a chemotherapy myeloablative conditioning regimen (dashed line). ^{90}Y indicates yttrium-90.

incidence indicates an sMDS/AML occurrence of 8.29% at 5 years, as discussed above, which is definitely higher compared with the most recently reported surveys on this severe, late complication. Furthermore, we had the opportunity of comparing the results from HD-RIT-treated patients with the behavior of patients who received a quite similar treatment schedule but without HD-RIT. Indeed, a long-term survey recently was concluded on a large series of 1347 patients with NHL who received the HDS-CT schedule without HD-RIT.[38] The cumulative incidence of sMDS/AML in that series was of 3.09%, 4.52%, at 5 years and 10 years, respectively, and, thus, definitely was lower compared with the incidence observed in the HD-RIT series. However, our matched-pair analysis allowed us to identify among the 1347 HDS-CT-treated patients a group of 55 patients who had the same clinical features as patients in the HD-RIT-treated series. Surprisingly, the 2 groups of pair-matched patients had a virtually identical risk of developing sMDS/AML, suggesting that other factors, including advanced age, extensive pretreatment, and type of PBSCs harvested and reinfused during the autograft procedure, are likely to play a critical causative role.

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REFERENCES

1. Wrench D, Gribben JG. Stem cell transplantation for non-Hodgkin's lymphoma. *Hematol Oncol Clin North Am*. 2008;22:1051-1079, xi.
2. Tarella C, Zanni M, Magni M, et al. Rituximab improves the efficacy of high-dose chemotherapy with autograft for high-risk follicular and diffuse large B-cell lymphoma: a multicenter Gruppo Italiano Terapie Innnovative nei Linfomi survey. *J Clin Oncol*. 2008;26:3166-3175.
3. Gianni AM, Bregni M, Siena S, et al. High-dose chemotherapy and autologous bone marrow transplantation compared with MACOP-B in aggressive B-cell lymphoma. *N Engl J Med*. 1997;336:1290-1297.
4. Gordon LI, Molina A, Witzig T, et al. Durable responses after ibritumomab tiuxetan radioimmunotherapy for CD20+ B-cell lymphoma: long-term follow-up of a phase 1/2 study. *Blood*. 2004;103:4429-4431.
5. Witzig TE, Flinn IW, Gordon LI, et al. Treatment with ibritumomab tiuxetan radioimmunotherapy in patients with rituximab-refractory follicular non-Hodgkin's lymphoma. *J Clin Oncol*. 2002;20:3262-3269.
6. Witzig TE, White CA, Gordon LI, et al. Safety of yttrium-90 ibritumomab tiuxetan radioimmunotherapy for relapsed low-grade, follicular, or transformed non-Hodgkin's lymphoma. *J Clin Oncol*. 2003;21:1263-1270.
7. Gisselbrecht C, Bethge W, Duarte RF, et al. Current status and future perspectives for yttrium-90 ([90]Y)-ibritumomab tiuxetan in stem cell transplantation for non-Hodgkin's lymphoma. *Bone Marrow Transplant*. 2007;40:1007-1017.
8. Krishnan A, Nademanee A, Fung HC, et al. Phase II trial of a transplantation regimen of yttrium-90 ibritumomab tiuxetan and high-dose chemotherapy in patients with non-Hodgkin's lymphoma. *J Clin Oncol*. 2008;26:90-95.
9. Palanca-Wessels MC, Press OW. Improving the efficacy of radioimmunotherapy for non-Hodgkin lymphomas. *Cancer*. 2010;116:1126-1133.
10. Park SI, Press OW. Radioimmunotherapy for treatment of B-cell lymphomas and other hematologic malignancies. *Curr Opin Hematol*. 2007;14:632-638.
11. Nademanee A, Forman S, Molina A, et al. A phase 1/2 trial of high-dose yttrium-90-ibritumomab tiuxetan in combination with high-dose etoposide and cyclophosphamide followed by autologous stem cell transplantation in patients with poor-risk or relapsed non-Hodgkin lymphoma. *Blood*. 2005;106:2896-2902.
12. Gopal AK, Gooley TA, Maloney DG, et al. High-dose radioimmunotherapy versus conventional high-dose therapy and autologous hematopoietic stem cell transplantation for relapsed follicular non-Hodgkin lymphoma: a multivariable cohort analysis. *Blood*. 2003;102:2351-2357.
13. Ferrucci PF, Vanazzi A, Grana CM, et al. High activity 90Y-ibritumomab tiuxetan (Zevalin) with peripheral blood progenitor cells support in patients with refractory/resistant B-cell non-Hodgkin lymphomas. *Br J Haematol*. 2007; 139:590-599.
14. Devizzi L, Guidetti A, Tarella C, et al. High-dose yttrium-90-ibritumomab tiuxetan with tandem stem-cell reinfusion: an outpatient preparative regimen for autologous hematopoietic cell transplantation. *J Clin Oncol*. 2008;26:5175-5182.
15. Armitage JO, Carbone PP, Connors JM, Levine A, Bennett JM, Kroll S. Treatment-related myelodysplasia and acute leukemia in non-Hodgkin's lymphoma patients. *J Clin Oncol*. 2003;21:897-906.
16. Stone RM, Neuberg D, Soiffer R, et al. Myelodysplastic syndrome as a late complication following autologous bone marrow transplantation for non-Hodgkin's lymphoma. *J Clin Oncol*. 1994;12:2535-2542.
17. Krishnan A, Bhatia S, Slovak ML, et al. Predictors of therapy-related leukemia and myelodysplasia following autologous transplantation for lymphoma: an assessment of risk factors. *Blood*. 2000;95:1588-1593.
18. Pedersen-Bjergaard J, Andersen MK, Christiansen DH. Therapy-related acute myeloid leukemia and myelodysplasia after high-dose chemotherapy and autologous stem cell transplantation. *Blood*. 2000;95:3273-3279.
19. Lenz G, Dreyling M, Schiegnitz E, et al. Moderate increase of secondary hematologic malignancies after myeloablative radiochemotherapy and autologous stem-cell transplantation in patients with indolent lymphoma: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group. *J Clin Oncol*. 2004;22:4926-4933.
20. Kalaycio M, Rybicki L, Pohlman B, et al. Risk factors before autologous stem-cell transplantation for lymphoma predict for secondary myelodysplasia and acute myelogenous leukemia. *J Clin Oncol*. 2006;24:3604-3610.
21. Miller JS, Arthur DC, Litz CE, Neglia JP, Miller WJ, Weisdorf DJ. Myelodysplastic syndrome after autologous bone marrow transplantation: an additional late complication of curative cancer therapy. *Blood*. 1994;83:3780-3786.
22. Tarella C, Passera R, Magni M, et al. Risk factors for the development of secondary malignancy after high-dose chemotherapy and autograft, with or without rituximab: a 20-year retrospective follow-up study in patients with lymphoma. *J Clin Oncol*. 2011;29:814-824.
23. Czuczman MS, Emmanouilides C, Darif M, et al. Treatment-related myelodysplastic syndrome and acute myelogenous leukemia in patients treated with ibritumomab tiuxetan radioimmunotherapy. *J Clin Oncol*. 2007;25:4285-4292.
24. Bennett JM, Kaminski MS, Leonard JP, et al. Assessment of treatment-related myelodysplastic syndromes and acute myeloid leukemia in patients with non-Hodgkin lymphoma treated with tositumomab and iodine I131 tositumomab. *Blood*. 2005;105:4576-4582.
25. Greenberger JS. Toxic effects on the hematopoietic microenvironment. *Exp Hematol*. 1991;19:1101-1109.
26. Soligo DA, Lambertenghi Deliliers G, Servida F, et al. Haematopoietic abnormalities after autologous stem cell transplantation in lymphoma patients. *Bone Marrow Transplant*. 1998;21:15-22.
27. Domenech J, Linassier C, Gihana E, et al. Prolonged impairment of hematopoiesis after high-dose therapy

followed by autologous bone marrow transplantation. *Blood*. 1995;85:3320-3327.
28. Rocci A, Ricca I, Dellacasa C, et al. Long-term lymphoma survivors following high-dose chemotherapy and autograft: evidence of permanent telomere shortening in myeloid cells, associated with marked reduction of bone marrow hematopoietic stem cell reservoir. *Exp Hematol*. 2007;35:673-681.
29. Akiyama M, Asai O, Kuraishi Y, et al. Shortening of telomeres in recipients of both autologous and allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2000;25:441-447.
30. Ricca I, Compagno M, Ladetto M, et al. Marked telomere shortening in mobilized peripheral blood progenitor cells (PBPC) following 2 tightly spaced high-dose chemotherapy courses with G-CSF. *Leukemia*. 2005;19:644-651.
31. Ruella M, Rocci A, Ricca I, et al. Comparative assessment of telomere length before and after hematopoietic SCT: role of grafted cells in determining post-transplant telomere status. *Bone Marrow Transplant*. 2010;45:505-512.
32. Bhatia R, Van Heijzen K, Palmer A, et al. Longitudinal assessment of hematopoietic abnormalities after autologous hematopoietic cell transplantation for lymphoma. *J Clin Oncol*. 2005;23:6699-6711.
33. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. *N Engl J Med*. 1993;329:987-994.
34. Magni M, Di Nicola M, Testi A, et al. Radioimmunotherapy and secondary leukemia: a case report [serial online]. *Leuk Res*. 2010;34:e1-e4.
35. Gianni AM, Magni M, Martelli M, et al. Long-term remission in mantle cell lymphoma following high-dose sequential chemotherapy and in vivo rituximab-purged stem cell autografting (R-HDS regimen). *Blood*. 2003;102:749-755.
36. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009;114:937-951.
37. Cilloni D, Carlo-Stella C, Falzetti F, et al. Limited engraftment capacity of bone marrow-derived mesenchymal cells following T-cell-depleted hematopoietic stem cell transplantation. *Blood*. 2000;96:3637-3643.
38. Tarella C, Passera R, Magni M, et al. Male gender, quality of grafted cells, advanced age, rituximab and radiotherapy are the main factors that variously influence the occurrence of secondary malignancies following high-dose therapy and autograft: a GITIL (Gruppo Italiano Terapie Innovative nei Linfomi) survey in 1347 lymphoma patients [abstract]. *Blood (ASH Annual Meeting Abstracts)*. 2009;114. Abstract 519.
39. Gray RJ. A class of K-sample tests for comparing the cumulative incidence of a competing risk. *Anal Stat*. 1988;16:1141-1154.
40. Forrest DL, Hogge DE, Nevill TJ, et al. High-dose therapy and autologous hematopoietic stem-cell transplantation does not increase the risk of second neoplasms for patients with Hodgkin's lymphoma: a comparison of conventional therapy alone versus conventional therapy followed by autologous hematopoietic stem-cell transplantation. *J Clin Oncol*. 2005;23:7994-8002.
41. Smith SM, Le Beau MM, Huo D, et al. Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. *Blood*. 2003;102:43-52.
42. Betticher DC, Huxol H, Muller R, Speck B, Nissen C. Colony growth in cultures from bone marrow and peripheral blood after curative treatment for leukemia and severe aplastic anemia. *Exp Hematol*. 1993;21:1517-1521.
43. Hong SS, Karayan L, Tournier J, Curiel DT, Boulanger PA. Adenovirus type 5 fiber knob binds to MHC class I alpha2 domain at the surface of human epithelial and B lymphoblastoid cells. *EMBO J*. 1997;16:2294-2306.
44. Unryn BM, Hao D, Gluck S, Riabowol KT. Acceleration of telomere loss by chemotherapy is greater in older patients with locally advanced head and neck cancer. *Clin Cancer Res*. 2006;12:6345-6350.
45. Chakraborty S, Sun CL, Francisco L, et al. Accelerated telomere shortening precedes development of therapy-related myelodysplasia or acute myelogenous leukemia after autologous transplantation for lymphoma. *J Clin Oncol*. 2009;27:791-798.