Precursor B-acute lymphoblastic leukemia occurring in patients with a history of prior malignancies: is it therapy-related?

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ABSTRACT

Background
Precursor B-acute lymphoblastic leukemia occurring in patients with a history of malignancies is uncommon, and this condition is not well understood.

Design and Methods
A retrospective review of 457 adults with precursor B-acute lymphoblastic leukemia treated at our hospital identified 44 (9.6%) patients with prior malignancies. The clinical and genetic characteristics of this group of patients was compared with those of their counterparts with *de novo* disease and the relationship with prior chemoradiation therapy was assessed.

Results
Thirty of 44(6.2%) patients received cytotoxic therapies, whereas 14 patients did not. The former group showed a significantly shorter interval from prior malignancy to onset of precursor B-acute lymphoblastic leukemia (36 *versus* 144 months; P=0.002). Compared with 413 *de novo* cases, the frequencies of t(4;11)(q21;q23) (P<0.001) and hypodiploidy (P=0.009) with loss of chromosome 5, 7 or 17 were significantly higher in patients who received topoisomerase II inhibitor and/or alkylating agents. By contrast, Philadelphia-positive and normal karyotype were more frequent in patients who either did not receive chemotherapy or received only local radiation or nucleoside analogs. Patients with precursor B-acute lymphoblastic leukemia following prior malignancies and chemoradiation were older, had a lower complete remission rate and showed an inferior survival in univariate, but not multivariate analysis.

Conclusions
The data support the interpretation that therapy-related precursor B-acute lymphoblastic leukemia does occur. In particular, cases associated with t(4;11)(q21;q23) or hypodiploidy with -5, -7, -17 are likely to be therapy-related and have a poor prognosis. The inferior outcome of these patients may be attributable to the high-risk cytogenetic abnormalities that are found in this group of patients.

Key words: precursor B-acute lymphoblastic leukemia, cytotoxic therapy, cytogenetics, overall survival, therapy-related.

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Introduction

Therapy-related myeloid neoplasms (t-MN), also referred to as therapy-related myelodysplastic syndromes and therapy-related acute myeloid leukemia, are well characterized and accepted in current classification systems.[1] t-MN secondary to alkylating agents or ionizing-radiation is frequently associated with unbalanced loss of genetic material, most often involving chromosomes 5 and/or 7. t-MN after DNA topoisomerase II inhibitor therapy is commonly associated with balanced chromosomal translocations, and more commonly presents as overt therapy-related acute myeloid leukemia.[1] In contrast to the accepted pathogenic role of chemoradiation therapy in t-MN,[1] the possible role of prior therapy in patients who subsequently develop precursor B-acute lymphoblastic leukemia (B-ALL) is much less clear.

Precursor B-ALL is characterized by a proliferation of lymphoblasts arrested at an early stage of B-cell maturation. Precursor B-ALL occurring in patients with prior malignancies, for convenience henceforth referred as secondary precursor B-ALL, is uncommon and the relationship of these neoplasms to prior cytotoxic therapy is largely debatable. The *Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto* (GIMEMA) Archive of Adult Acute Leukemia[2] from 62 Hematologic Divisions reported secondary ALL in 21 of 901 (2.3%) adults. Notably, ten of these patients had not received prior chemoradiotherapy but had multiple cancers or a family history of cancer. The study raised the question: is secondary ALL a direct result of prior cytotoxic therapy, does it simply occur as a random event, or is it related to a familial predisposition to cancer? Subsequent reports, mostly of case studies or small case series with a summary of cases reported in the literature, described various findings that led to different conclusions.[3-5] Notably, the subjects reported in those studies were both adults and children with a mixture of precursor B- and T- acute lymphoblastic leukemia/lymphoma, and the clinicopathological and molecular genetic features of these neoplasms are difficult to delineate. Furthermore, "therapy-related ALL" has been used interchangeably with "secondary ALL" in many of the references, adding to the confusion. Nevertheless, there is a suggestion, particularly in the study by Ishizawa *et al.*,[6] that secondary precursor B-ALL with 11q23 abnormalities is related to topoisomerase II inhibitor therapy.[3,4,6] Secondary precursor B-ALL associated with other cytogenetic abnormalities, however, remains to be clarified.

In this study we reviewed all patients with precursor B-ALL diagnosed in or referred to our hospital in the past 6 years to identify patients with a history of other malignancies. In this large cohort, we examined in detail the treatment history, clinical features, and cytogenetic findings in patients with secondary precursor B-ALL to determine whether these cases are therapy-related, in analogy to t-MN.

Design and Methods

Study group

The diagnosis of precursor B-ALL was made according to criteria established by the World Health Organization (WHO).[7] Patients referred to the University of Texas MD Anderson Cancer Center (MDACC) from May 1, 2004 through December 31, 2010 were reviewed. Only adult patients (≥18 years old) were included. For cases with initial diagnosis made at the referring hospital, the pathologic materials used to establish the initial diagnosis were reviewed in our department. To focus this study on precursor B-ALL, cases of acute leukemia of ambiguous lineage, either biphenotypic or bilineal, were excluded. Cases of B-lymphoblastic crisis of chronic myelogeneous leukemia as well as leukemic presentation of Burkitt's lymphoma were also excluded. The study was approved by the institutional review board of the MDACC.

A detailed chart review was conducted in all patients, with specific attention to prior history of malignancies and related therapy. Treatment was categorized as chemotherapy, chemotherapy plus radiation, and radiation alone. The chemotherapy agents were further categorized as alkylating, topoisomerase II inhibitors, and others (antimetabolites and antitubulin agents).[1] Patients who received brachytherapy or radioisotopes (radioactive iodine) only were not considered as having received prior cytotoxic therapy.[1] For the purpose of simplification, patients with prior malignancy who received chemotherapy and/or radiation were designated as group 1 (secondary precursor B-ALL with prior cytotoxic therapy), patients with prior malignancy but who had not received cytotoxic therapy were designated as group 2 (secondary precursor B-ALL without prior cytotoxic therapy); and patients without prior malignancy were designated as group 3 (*de novo* precursor B-ALL).

Frontline chemotherapy was administered using the hyper-CVAD regimen (hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone alternating with high dose methotrexate and cytarabine) in adults with Philadelphia chromosome (Ph)-negative precursor B-ALL as previously described.[8] Patients with Ph-positive precursor B-ALL were treated with the hyper-CVAD regimen concurrently with either imatinib mesylate or dasatinib as previously reported.[9] Treatment was not different for patients with either secondary or *de novo* precursor B-ALL.

Laboratory data and bone marrow assessment

Peripheral blood smears, bone marrow aspirate smears and trephine biopsy specimens were reviewed. Blast percentage, bone marrow cellularity, and background dysplasia were assessed. Cytochemical staining for myeloperoxidase was performed in all cases. White blood cell and platelet counts and hemoglobin, lactate dehydrogenase, creatinine, and albumin levels were recorded in most patients.

Flow cytometric immunophenotyping

Flow cytometry immunophenotyping was performed using a panel of antibodies designed for acute leukemia and further analyzed by an extended panel designed for precursor B-ALL according to the methods described previously.[9] All precursor B-ALL cases expressed CD19 in conjunction with at least one other B-lineage marker (CD22, CD79a, and/or cIgM), and were negative for cCD3 and myeloproxidase. These results were essential to confirm a diagnosis of precursor B-ALL.

Conventional karyotyping and fluorescence in situ hybridization

Conventional chromosomal analysis was performed on G-banded metaphase cells prepared from unstimulated bone marrow aspirate cultures using standard techniques. Twenty metaphases were analyzed and the results were reported using the International System for Human Cytogenetic Nomenclature.[10]

Fluorescence *in situ* hybridization for *BCR-ABL* (dual-color dual-fusion probe, Abbott Molecular, Des Plaines, IL, USA) and *MLL* (MLL Dual Color Break Apart Probe; Abbott Molecular/Vysis, Des

Plaines, IL, USA) was performed on freshly harvested bone marrow cells (metaphase or interphase). The cutoff to define a positive result for *BCR-ABL* and *MLL* was 1.5%.

Statistical analysis

The Mann-Whitney test was used for numerical comparisons between two groups. Fisher's exact and χ^2 tests were applied for categorical variables. The interval from prior malignancy to diagnosis of precursor B-ALL was calculated from the time of therapeutic intervention of the prior malignancy. Overall survival was estimated by the Kaplan–Meier method from the date of diagnosis of precursor B-ALL until death from any cause (censored at last follow-up).[11] Event-free survival was calculated from the date of treatment for precursor B-ALL until relapse or death. Multivariable analysis was performed by the Cox proportional regression model to examine the relationship between survival and age, leukocyte count, platelet count, creatinine, albumin, lactate dehydrogenase, cytogenetic risk categories, history of prior malignancies and prior therapy, and stem cell transplantation (SCT).[12]

Results

Patients

A total of 457 patients with precursor B-ALL were included in this study. The diagnosis of precursor B-ALL was established based on morphology, immunophenotype, determined by flow cytometry, cytochemical stains, and in some cases immunohistochemistry. In this study, we initially also retrieved 83 cases of precursor T-acute lymphoblastic leukemia/lymphoblastic lymphoma. Only four patients had a history of malignancy: two patients had received chemoradiation therapy and two had not. Due to the small number of cases, these cases were not included in this study.

A detailed chart review identified 44 (9.6%) patients with a history of malignancy. Skin basal cell carcinoma and skin non-metastatic squamous cell carcinoma were not considered as prior malignancies. Thirty of 44 patients had received various cytotoxic therapies (group 1) for breast cancer (n=10), prostate cancer (n=4), colorectal cancer (n=2), other solid tumors (lung, cervix, thyroid, n=4), non-Hodgkin's lymphoma (n=6), myeloma (n=3) and Hodgkin's lymphoma (n=1). Fourteen patients had undergone surgery and/or received non-cytotoxic therapy (immune therapy, such as Bacillus Calmette-Guerin, interleukin-2, interferon; or hormonal therapy such as tamoxifen) for solid tumors (group 2) including breast cancer (n=3), prostate cancer (n=3), kidney tumors (n=2), bladder cancer (n=2), thyroid cancer (n=2), squamous cell carcinoma of unknown origin (n=1), and uterine cervical cancer (n=1). No patients had a diagnosis of myelodysplastic syndrome or acute myeloid leukemia and none had cytopenia(s) prior to the onset of precursor B-ALL. The sex distribution difference among these three groups was similar (Table 1). Patients with secondary precursor B-ALL were significantly older than those with *de novo* diseases [median (ranges): 65 (30-86) *versus* 40 (18-83) years, *P*=0.001], whereas there was no age difference between patients in groups 1 and 2 (Table 1). Median leukocyte count, hemoglobin concentration and platelet count were not significantly different among these three groups (*data not shown*). The intervals from prior malignancy to onset of precursor B-ALL in patients with secondary precursor B-ALL were significantly shorter in group 1 than in group 2 [median, 36 months (range, 6-216) *versus* median, 144 months (range, 7-420), respectively; *P*=0.002].

Bone marrow findings

Among the 413 patients with *de novo* precursor B-ALL, 388 (94%) presented with leukemia and 25 (6%) presented with B-lymphoblastic lymphoma with minimal (<25%) or no bone marrow involvement. In contrast, all patients with secondary precursor B-ALL presented with leukemia. All precursor B-ALL cases, whether *de novo* or secondary, had a hypercellular bone marrow with a high

Table 1. Comparison of precursor B-lymphoblastic leukemia: *de novo* and secondary in patients who had or had not receveid prior cytotoxic therapy.

	Group 1 (n=30)	Group 2 (n=14)	Group 3 (n=413)	P*
Age(years) median (range)	64 (30-86)	68 (39-86)	40 (18-83)	0.001
Sex Male/Female	15/15	6/8	219/194	0.583
Cytogenetics available	29 (97%)	14 (100%)	375 (91%)	
Normal karyotype	5 (17%)	2 (14%)	94 (25%)	0.161
t(4;11)(q21;q23) (*AF4/MLL*)	7 (24%)	1 (7%)	18 (4.8%)	<0.001
t(9;22)(q34;q11.2) (*BCR/ABL1*)	7 (24%)	9 (64%)	129 (34%)	0.102
t(1;19)(q23;p13.3)	0	0	9 (2.4%)	
Hyerpdiploidy	2 (7%)	0	22 (5.9%)	0.125
Hypodiploidy	6 (21%)	1 (7%)	19 (5.1%)	0.009
Other abnormalities	2 (7%)	1(7%)	84 (22.4%)	NA
Abnormalities involving chromosomes of 5, 7, 17 (not including cases with recurrent cytogenetic abnormalities)				
-5, -7	7 (24%)	1 (7%)	27 (7.2%)	0.009
-17, -17p	6 (21%)	0	17 (4.5%)	0.005
Treatment				
Complete remission	18/30(60%)	10/14(71%)	370/398(93%)	Group 1 *versus*3: <0.001
Relapse	13/17(75%)	4/4(100%)	182/370(49%)	Group 1 *versus*3: 0.079
3-year event-free survival	4/30(13%)	0/14(0%)	127/398(32%)	Group 1 *versus*3: <0.001

Group 1: secondary precursor B-ALL in patients who had received prior cytotoxic therapy; Group 2: secondary precursor B-ALL in patients who had not received prior cytotoxic therapy; Group 3: de novo *precursor B-ALL. *P comparison between group 1 and groups 2+3.*

percentage of lymphoblasts. Although dysplasia in hematopoietic cells was observed in some cases, in most patients there were too few hematopoietic cells for an accurate assessment.

Cytogenetic and molecular features

The cytogenetic characteristics of precursor B-ALL are shown in Table 1. *BCR/ABL1* fluorescence *in situ* hybridization and/or reverse transcriptase-polymerase chain reaction analysis were performed essentially in all cases. We observed no significant differences in karyotypic findings between cases of *de novo* and secondary precursor B-ALL without prior cytotoxic chemotherapy, except for a slightly higher frequency of the ph chromosome in the latter group. We then compared the cytogenetic findings in patients who had received prior cytotoxic therapy (group 1) with those in patients who had not received such therapy (groups 2 and 3). In group 1, five patients (17%) had a normal karyotype and seven patients (24%) had the *BCR/ABL1* fusion; the incidence of these findings was similar to that in patients who had not received prior cytotoxic therapy. Notably, among these 12 patients, five had received radiation only and two had received only a purine analog. The frequency of t(4;11)(q21;q23) involving *MLL* was significantly higher in group 1 patients (n=7, 24%; $P<0.001$), and all seven patients had received regimens containing topoisomerase II inhibitors and alkylating agents (Table 2). Hypodiploidy, particularly with loss of chromosomes 5, 7, or 17, was significantly more frequent in group 1 patients (n=6, 21%, $P=0.009$); all six patients in this group had received chemotherapy containing an alkylating agent (Table 2). No patients with secondary precursor B-ALL had t(1;19)(q23;p13.3) and other cytogenetic alterations were also uncommon.

Class I mutations, including *FLT3*, *RAS* (*K-RAS/N-RAS*) and *KIT*, were searched for in a subset of patients and the frequencies were similarly low among the three groups: group 1: *FLT3*, 0/18; *RAS*, 1/9; and *KIT*, 0/7; group 2: *FLT3*, 0/7; *RAS*, 1/4; *KIT*, 0/5; and group 3: *FLT3*, 6/227 (2.5%); *RAS*, 8/84 (9.5%) and *KIT*, 0/62.

Flow cytometry immunophenotypic results

Cases of secondary precursor B-ALL, arising in patients who had or had not received prior cytotoxic therapy, shared similar immunophenotypic characteristics with their *de novo* counterparts within the same cytogenetic group. For example, Ph-positive cases generally exhibited a common-precursor B-cell immunophenotype ($CD10^+$, $cIgM^-$, TDT^+), more frequently expressed CD13, CD25, CD33, and CD66c and less frequently expressed CD15. Normal karyotype cases were more heterogeneous, ranging from precursor-B, pro-B ($CD10^-$), to common-B. The cases with t(4;11)(q21;q23) demonstrated a pro-B immunophenotype: $CD10^-$, $CD20^-$, frequent CD34 negativity or partial expression and frequent CD15 expression. Cases with hypodiploidy, including loss of chromosomes 5, 7 and 17, frequently showed a common B-immunophenotype with frequent CD20 (90%) expression.

Survival

All patients received hyper-CVAD induction chemotherapy. Group 1 and group 2 patients had a significantly lower complete remission rate and a significantly inferior 3-year event-free survival as compared with group 3 patients (Table 1). Three patients in group 1, none in group 2 and 103 patients (25%) in group 3 received a SCT. None of the patients with secondary precursor B-ALL died of prior malignancies. With a median follow-up of 24 months (range, 1-286 months), the median overall survival and event-free survival of patients with secondary precursor B-ALL were significantly inferior to those in patients with *de novo* precursor B-ALL, whether patients who received a SCT were included or excluded (overall survival: 12 *versus* 45 months with SCT, $P=0.001$; 12 *versus* 46 months without SCT, $P=0.002$, respectively) (event-free survival: 11 months *versus* 33 months, $P=0.003$). However, no significant differences in overall survival (12 months versus 13.5 months, $P=0.87$) or event-free survival (11 months *versus* 12 months, $P=0.79$) were identified between patients in group 1 and group 2. A history of malignancy ($P=0.002$), older age (≥50 years; $P=0.001$), high white blood cell count ($\geq 11\times10^9$/dL; $P=0.022$), low platelet count ($<46\times10^9$/dL; $P=0.045$) and high-risk cytogenetics ($P=0.033$) were significant hazards to survival (overall and event-free) in the univariate analysis; however, only cytogenetics remained prognostically significant in the multivariate analysis when age, cytogenetic risks, SCT and laboratory parameters including hemoglobin concentration, white blood cell and platelet counts, albumin, lactate dehydrogenase and creatinine concentrations were co-analyzed for both overall and event-free survival (Table 3).

Discussion

In this study we analyzed a large cohort of patients with precursor B-ALL treated at one institution for whom detailed clinical data and follow-up information were available. We show that secondary precursor B-ALL repre-

Table 2. Secondary precursor B-lymphoblastic leukemia: karyotype in relation to prior cytotoxic therapeutic agents.

Karyotype	Cases	Cytotoxic therapy (patients)
Normal (%)	5 (17%)	Both topo II and alkylating (n=1) Alkylating only (n=1) Purine analog only (n=1) Radiation only (n=2)
t(4;11)(q21;q23) (*MLL* gene)(%)	7 (24%)	Both topo II and alkylating (n=7)
BCR/ABL1 (Ph+) (%)	7 (24%)	Both topo II and alkylating (n=2) Akylating only (n=1) Purine analog only (n=1) Radiation only (n=3)
Hyperdiploidy	2 (7%)	Both topo II and alkylating (n=1) Radiation only (n=1)
Hypodiploidy	6(21%)	Both topo II and alkylating (n=3) Alkylating only (n=3)
Abnormalities involving chromosome 5, 7 or 17 (excluding cases with recurrent cytogenetic abnormalities)		
-5, -7	7 (24%)	Both topo II and alkylating (n=3) Akylating only (n=1) Radiation only (n=1)
-17 or 17p	6 (21%)	Both topo II and alkylating (n=3) Alkylating only (n=2) Radiation only (n=1)

topo II: topoisomerase II inhibitors.

sents approximately 9-10% of all precursor B-ALL in adults. Several cytogenetic alterations, including t(4;11)(q21;q23) and hypodiploidy with loss of chromosomes 5, 7, 17, were likely related to prior cytotoxic therapy. In contrast, cases with a Ph chromosome and a normal karyotype may merely be coincidental or reflect individual genetic susceptibility to cancer.

We first categorized the cytogenetic alterations in this study group according the 2008 WHO algorithm for precursor B-ALL. The cytogenetic alterations commonly observed in t-MN,[1,13] including 11q23 involving *MLL* and loss of chromosomes 5, 7, or 17, were significantly overrepresented in group 1 patients. This was further supported by demonstrating that group 2 patients (with prior malignancy but not treated with cytotoxic agents) had cytogenetic results similar to those of patients with *de novo* precursor B-ALL. All patients with *MLL* gene rearrangement in group 1 had received chemotherapy regimens containing a topoisomerase II inhibitor. In contrast to t-MN, in which the *MLL* gene is known to rearrange with many gene partners, *MLL/AF4*/t(4;11)(q21;q23) was the only fusion form in our series, similar to the cases reported by others.[3,4,6] The translocation breakpoints in both *MLL* and *AF4* are DNA topoisomerase II cleavage sites and DNA topoisomerase II causes chromosomal breakage as well as interchromosomal recombination when the breakage is repaired.[14] *MLL/AF4* fusions in precursor B-ALL have not only been found in $CD19^+$ cells, but also in $CD34^+CD19^-$ cells.[15] It appears that in the therapy-related setting, topoisomerase II inhibitors can cause *MLL* gene rearrangements with various partner genes; whereas most of these rearrangements lead to t-MN, *MLL/AF4* occurs in primitive but lymphoid-restricted progenitor/stem cells, leading to clinical precursor B-ALL. Two of 28 *MLL/AF4* cases identified in our files were biphenotypic, but were not therapy-related. An investigation of acute leukemia with ambiguous lineages in relation to prior therapy may be interesting, but was not addressed in this study. We showed that patients with t(4;11)(q21;q23) in group 1 were older than their counterparts with *de novo* disease and, therefore, less likely qualified for SCT. Otherwise, the clinical laboratory findings, morphology and immunophenotype of the blasts, and responses to hyper-CVAD were very similar and appeared to be denoted by the presence of *MLL/AF4* fusion.

Hypodiploidy occurs in approximately 5% or less of all precursor B-ALL in either children or adults[16,17] and confers a poor prognosis. Loss of chromosome 7 is known to be an adverse prognostic indicator in pediatric precursor B-ALL, and is frequently observed in cases with hypodiploidy.[18] Analysis of deleted regions indicated that loss of 7p rather than 7q was critical prognostically;[18] and precursor B-ALL with -7 and -7p was distinct from myeloid disorders with -7 and -7q. Loss of chromosome of 17 in precursor B-ALL has been rarely reported.[19] A recent study using single nucleotide polymorphism oligonucleotide microarray found microdeletion of 17p (*TP53*) in 11% of all adult precursor B-ALL.[20] We found that hypodiploidy, particularly with complete loss of chromosome 5, 7 and 17(17p), was significantly more frequent among group 1 patients and all patients with this anomaly had received alkylating agents. This finding parallels the common cytogenetic alterations reported in t-MN after alkylating chemotherapy. Intriguingly, t-MN with loss of chromosomes 5 and 7 often have 44-46 chromosomes (*data not shown*) whereas precursor B-ALL with hypodiploidy has a lower number of chromosomes, often fewer than 44.

Table 3. Multivariate Cox proportional hazard regression analysis.

Variables	Hazard ratio (95% CI)	*P
Precursor B-ALL group		
Group 1 *vs.* group 3	1.48 (0.85-2.58)	0.163
Group 2 *vs.* group 3	1.49 (0.71-3.15)	0.294
Cytogenetics		
t(4;11)	2.35 (1.14-4.85)	0.021
t(9;22)	1.67 (1.05-2.65)	0.031
Hypodiploidy	2.31 (1.21-4.42)	0.012
Stem cell transplant		
No *vs.* yes	1.01 (0.68-1.51)	0.95
Age		
≥ 50 *vs.* <50 years	1.20 (0.84-1.71)	0.307
White blood cell count		
≥11 *vs.* $<11\times10^9$/dL	1.35 (0.96-1.89)	0.083
Platelet count		
≥ 46 *vs.* $<46\times10^9$/dL	0.87 (0.63-1.20)	0.390

Precursor B-ALL" B- acute lymphoblastic leukemia; Group 1: secondary precursor B-ALL in patients who had received prior cytotoxic therapy; Group 2: secondary precursor B-ALL in patients who had not received prior cytotoxic therapy; Group 3: de novo *precursor B-ALL. *Hemoglobin, lactate dehydrogenase, creatinine and albumin were co-analyzed, and showed no statistical significance.*

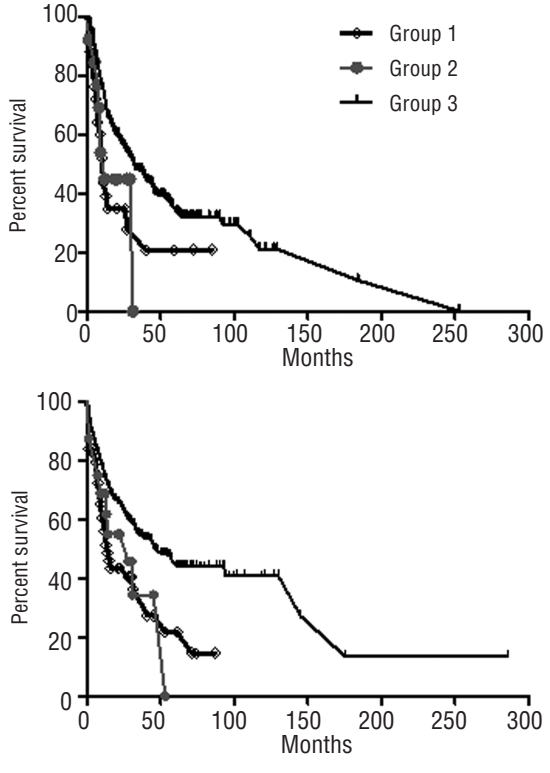

Figure 1. The event-free survival (upper) and overall survival (lower) of patients who did not undergo stem cell transplantation was estimated by the Kaplan–Meier curve from the date of precursor B-lymphoblastic leukemia diagnosis until death. Group 1: secondary precursor B-ALL in patients who had received prior cytotoxic therapy; group 2: secondary precursor B-ALL in patients who had not received prior cytotoxic therapy; group 3: *de novo* precursor B-ALL.

We showed that the frequencies of a normal karyotype and the Ph-chromosome were similar in *de novo* and secondary precursor B-ALL. In fact, the Ph chromosome was slightly more frequent in group 2 patients, which might be explained by a natural increase of Ph-positive precursor B-ALL cases in older patients,[21] and variation due to a small number of cases in group 2. Notably, we showed that of the 12 patients with a normal karyotype (n=5) or Ph-positive (n=7) in group 1, two had received only a purine analog and five had received local radiation. In the past two decades, the field of radiation therapy has moved towards using more conformal treatment techniques that reduce the exposure of hematopoietic bone marrow. We recently reviewed 47 patients who developed myelodysplastic syndrome/acute myeloid leukemia following radiation therapy alone in the past decade suggesting that these diseases may not be a direct consequence of radiation toxicity.[22] On the other hand, single agent purine analog therapy rarely causes t-MN.[23,24] The findings from our series indicate that secondary precursor B-ALL with a normal karyotype or Ph-positive may not be related to prior cytotoxic exposure, and is more likely to be a coincidence or reflect individual genetic susceptibility to cancer.

Patients with secondary precursor B-ALL had an inferior survival; the differences were likely attributed to the poor-risk cytogenetic abnormalities in these patients as a result of cytotoxic exposure, and older age. We observed no difference in outcomes of group 1 and group 2 patients, which could be explained by a high proportion of Ph-positive cases in the group 2 patients. Importantly, some of the "cytotoxic agents" such as local radiation alone and purine analogs might not necessarily be leukemogenic, as discussed above. Unlike therapy-related acute myeloid leukemia,[25,26] a history of prior therapy was not an independent unfavorable factor with respect to survival. The fundamental differences between precursor B-ALL and acute myeloid leukemia with regards to clinical, molecular/genetic and biological characteristics as well as the number of cases studied may be contributing factors. Nevertheless, our data indicate that secondary precursor B-ALL can be stratified by the current precursor B-ALL risk stratification system similar to *de novo* precursor B-ALL, in which cytogenetics appears to be the most important factor in predicting survival.

In summary, we demonstrate that a subset of cases of secondary precursor B-ALL is therapy-related, and linked to previous treatment with alkylating agents or topoisomerase II inhibitors. These cases exhibit characteristic cytogenetic alterations related to different cytotoxic exposures, similar to those described in patients with t-MN. It is biologically interesting and unexplained why similar cytogenetic alterations confer to the development of precursor B-ALL rather than myeloid neoplasms in the post-therapy setting. Class I mutations including *FLT3*, *RAS* and *KIT* were similarly infrequent in *de novo* and therapy-related cases of precursor B-ALL in our study. The prognosis of patients with secondary precursor B-ALL, either therapy-related or not related, is poor, likely attributable to the associated high-risk cytogenetic abnormalities that these cases harbor.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

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