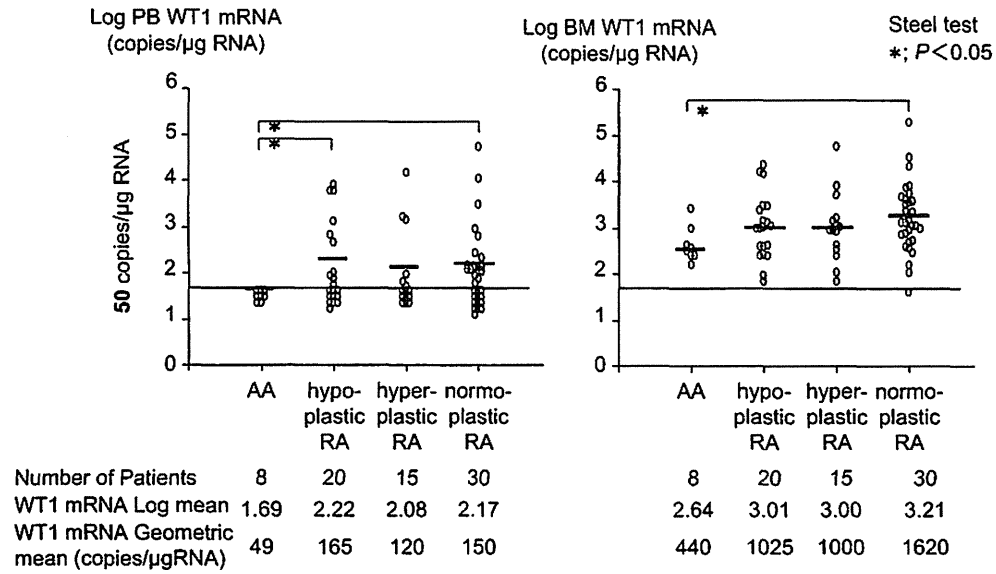

Figure 4. Comparison of WT1 mRNA expression between AA and RA groups (hypoplastic, hyperplastic and normoplastic RA). In intergroup comparison of WT1 mRNA expression, Steel test was performed using log-transformed values of WT1 mRNA expression with a level of significance of $p < 0.05$. Bold lines represent mean WT1 mRNA expression after log transformation. Fine lines represent lower limit of detection of WT1 mRNA (50 copies/μg RNA).

from the correlation between WT1 mRNA expression in PB and BM (Figure 2), BM WT1 mRNA expression became 480 copies/μg RNA. When 500 copies/μg was evaluated as the cut-off value for BM WT1 mRNA expression, the sensitivity was 68.1% (47/69) and the specificity was 75.0% (6/8). Based on these results, 500 copies/μg RNA was considered to be an appropriate cut-off value for the differential diagnosis between RA and AA using WT1 mRNA expression in BM.

Comprehensive analysis using cut-off values

The PB and BM samples in each disease and MDS subtype were further evaluated for their WT1-positive rates, using the WT1 mRNA expression cut-off values determined above (PB: 50 copies/μg RNA; BM: 500 copies/μg RNA) (Table II). For AML-MDS (11 patients), the WT1 mRNA-positive rates were a high 100% (11/11) for PB and 90.9% (10/11) for BM, and in MDS (115 patients), the WT1 mRNA-positive rates were 61.7% (71/115) for PB and 73.0% (84/115) for BM, which were the second highest after AML-MDS. In contrast, all patients with AA, ICUS, ITP, PNH, PRCA and erythroid hypoplasia had low positive rates of 0% for PB and 18.8% (3/16) for BM. The WT1 mRNA-positive rates for PB and BM increased with MDS disease stage progression (Table II).

Table II. WT1 mRNA-positive rate in PB and BM from patients with different MDS subtypes and AML-MDS according to FAB classification.

Subtype	No. of patients	WT1 mRNA-positive rate (%)	
		Peripheral blood	Bone marrow
RA	69	50.7 (35/69)	68.1 (47/69)
RARS	9	44.4 (4/9)	44.4 (4/9)
RAEB	24	83.3 (20/24)	87.5 (21/24)
RAEB-t	13	92.3 (12/13)	92.3 (12/13)
AML-MDS	11	100.0 (11/11)	90.9 (10/11)
Total	126	65.1 (82/126)	74.6 (94/126)

PB, peripheral blood; BM, bone marrow; MDS, myelodysplastic syndromes; AML-MDS, acute myeloid leukemia-evolved MDS; FAB, French-American-British; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess of blasts; RAEB-t, refractory anemia with excess of blasts in transformation.

Discussion

In this study, the clinical usefulness of the measurement of WT1 mRNA expression in risk assessment of MDS was evaluated using a WT1 assay kit. Recently, a steady stream of reports has indicated the usefulness of WT1 mRNA measurement. The group of Cilloni [6] confirmed that WT1 mRNA expression potentially fulfills all the requirements for an additional marker for risk assessment in MDS, compared with the conventional methods. The measurement of WT1 can be effective, particularly in cases in which BM aspiration and/or cytogenetic analysis fail or are not informative [6].

Furthermore, in their findings in a long-term prospective study, Tamura *et al.* [19] reported that a significant correlation ($p = 0.0186$) was seen between WT1 mRNA expression and survival time when WT1 mRNA expression in PB was categorized into three groups of less than 10^2, 10^2–10^4, and greater than 10^4 copies/μg RNA, that the median survival time for each group was 62.7 months, 29.9 months and 11.6 months, respectively; and that the time until transformation to leukemia was the shortest in the group with the highest WT1 mRNA expression. In addition, they reported that in univariate analysis, WT1 mRNA expression was a predictive parameter for transformation to leukemia, and in multivariate analysis, it was a significant predictive parameter along with the IPSS score [19]. As described above, Tamaki *et al.* reported similar findings [4].

This study was conducted using not only the FAB classification system but also the 2001 and 2008 WHO classification systems. It was confirmed that in all three classification systems, WT1 mRNA expression in both PB and BM increases significantly in MDS subtypes with disease stage

progression. In addition, both PB and BM WT1 mRNA expression increased significantly as the risk of transformation to AML rose in the IPSS and WPSS risk groups. Furthermore, a correlation of $r = 0.57$ between the IPSS score and WT1 mRNA expression was seen in both PB and BM. The correlations between the WPSS score and WT1 mRNA expression were $r = 0.61$ in PB and $r = 0.55$ in BM. In comparison with the IPSS, the WPSS allows the assessment of survival time and progression of leukemic transformation at all time periods during the clinical course, leading to continued prognostic evaluation while reviewing the risk. WT1 mRNA expression correlates with the WPSS prognosis, and despite the single-point quantitation, the results in this study indicate that WT1 mRNA is useful as a time-course prognostic marker in the same manner as the WPSS.

At present, allogeneic hematopoietic stem cell transplant is the only curative treatment for MDS. However, determination of the timing of allogeneic transplant is very difficult because many patients are older, treatment-related deaths frequently occur, and there are large individual differences in the rate of disease progression. Allogeneic transplant is selected as the therapeutic regimen for MDS when no increase in blast cells is confirmed, taking into consideration the development of transfusion dependency and frequency of infections [20]. In addition, allogeneic transplant is selected when a future increase in blast cells is predicted by karyotypic analysis even though no increase is currently observed. It is recommended that transplant be performed before the progression to cytopenia caused by an increase in blast cell clones and before the progression to acute leukemia, although induction chemotherapy may be required when an increase in blast cells is observed [21]. On the other hand, another study suggested that delaying transplant until the advanced stage of disease results in a longer survival time for low and intermediate-1 IPSS risk groups, while early transplant was recommended for the intermediate-2 and high groups [22]. The period after CR is achieved is considered to be the standard timing to perform transplant for acute leukemia, but determining CR is extremely challenging. Our results revealed that periodic monitoring of WT1 mRNA expression in patients with MDS provided useful information for predicting the timing of transplant.

RA, a subtype in the early MDS disease stage, is often difficult to differentiate from AA [23]. In a previous study by Iwasaki *et al.*, no difference in WT1 mRNA expression was observed between RA and AA [9]. However, our data revealed the possibility of WT1 expression level to differentiate AA and RA groups using both peripheral blood and bone marrow samples (Figure 4). In the present statistical analysis, significant differences were observed between AA and hypoplastic RA ($p = 0.04$) in PB. The number of subjects was limited, and further trial is required for more detailed analysis. Moreover, tentative cut-off values for WT1 mRNA expression were set at 50 copies/μg RNA in PB and 500 copies/μg RNA in BM. Although the number of patients was small, the results showed that the level of WT1 mRNA expression could differentiate between RA and AA, with specificity in PB and BM of 100% (8/8) and 75.0% (6/8), respectively. This provides evidence that the measurement of WT1 mRNA expression can play a role in the differential diagnosis of RA and AA.

The WT1 assay kit is used clinically in Japan as a marker to monitor MRD in patients wth AML. In MDS, a clonal disorder of pluripotent hematopoietic stem cells, WT1 mRNA expression increases depending on the MDS subtype and disease stage. In contrast, the mechanism by which WT1 mRNA expression increases in MDS is not considered to correlate simply with the fluctuation in leukemic clones, as seen in AML. In normal hematopoiesis, WT1 mRNA is expressed mainly in CD34-positive cells. In contrast, in patients with MDS, WT1 mRNA is also expressed in CD34-negative cells, particularly in lineages exhibiting abnormalities [24]. In our study, the level of WT1 mRNA expression within the RA group was shown to increase with the increase in IPSS risk [Figure 3(c)]. Moreover, a similar trend of increasing WT1 expression was found in the RCUD and RCMD groups according to the 2008 WHO classification, although no significant increase in blast cells in BM was observed in these groups. Taken together, these findings indicate that the increase in WT1 mRNA expression in patients with MDS may reflect the divergence of MDS clones from normal clones and preleukemic changes.

In patients with MDS, evaluating the changes in WT1 mRNA levels simultaneously in PB and BM samples provides useful information on disease stage progression or risk assessment in individual patients. In addition, the WT1 mRNA-positive rate in each subtype of MDS was high (50–90%) in both PB and BM in this study, suggesting that a single measurement of WT1 mRNA is sufficient for MDS diagnosis, particularly for differentiating RA from AA.

Overall, this study provides evidence that the measurement of the level of WT1 mRNA expression in PB and BM serves as a supplemental marker for MDS diagnosis and prognostic assessment. This assay has great potential to contribute to more appropriate diagnoses and therapeutic decisions in patients with MDS and to evaluate the timing of allogeneic transplant.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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Supplementary material available online

Supplementary figure showing ROC analysis of WT1 mRNA expression in BM in RA and AA groups

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Phase 2 study of arsenic trioxide followed by autologous hematopoietic cell transplantation for relapsed acute promyelocytic leukemia

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Regular Article

CLINICAL TRIALS AND OBSERVATIONS

Phase 2 study of arsenic trioxide followed by autologous hematopoietic cell transplantation for relapsed acute promyelocytic leukemia

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Key Points

- We conducted a phase 2 study of ATO followed by autologous HCT for relapsed APL.
- This sequential treatment is effective and feasible.

The optimal treatments for relapsed acute promyelocytic leukemia (APL) remain equivocal. We conducted a phase 2 study to evaluate the efficacy and feasibility of a sequential treatment consisting of induction and consolidation with arsenic trioxide (ATO), peripheral blood stem cell (PBSC) harvest after high-dose cytarabine chemotherapy, and autologous hematopoietic cell transplantation (HCT). Between 2005 and 2009, 35 patients (26 with hematologic and 9 with molecular relapse) were enrolled. Induction therapy resulted in complete remission in 81% of those with hematologic relapse, and most patients became negative for *PML-RARα* after the first ATO consolidation course, but 4 remained positive. Administration of the second ATO consolidation course further decreased the transcript levels in 3 patients. In total, 25 patients proceeded to PBSC harvest, all of whom successfully achieved the target CD34+ cell doses, and 23 underwent autologous HCT with *PML-RARα*–negative PBSC graft. Posttransplant relapse occurred in 3 patients, and there was no transplant-related mortality. With a median follow-up of 4.9 years, the 5-year event-free and overall survival rates were 65% and 77%, respectively. These findings demonstrate the outstanding efficacy and feasibility of the sequential treatment featuring ATO and autologous HCT for relapsed APL. This study was registered at http://www.umin.ac.jp/ctr/ as #C000000302. (*Blood*. 2013;121(16):3095-3102)

Introduction

Outcomes for acute promyelocytic leukemia (APL) have improved significantly since the advent of all-*trans* retinoic acid (ATRA), and the recently introduced frontline therapy that combines ATRA and chemotherapy can provide long-term complete remission (CR) for a majority of patients with newly diagnosed APL.[1-6] Nevertheless, relapse still occurs in ~20% of cases, for which arsenic trioxide (ATO) has been shown to provide high CR rates exceeding 80%,[7-9] thus making it a current recommendation for reinduction therapy.[10,11] After returning to CR, autologous or allogeneic hematopoietic cell transplantation (HCT) for consolidating the CR status is generally considered if the patient is eligible for the procedure.[10-12] However, because there have been few prospective studies for this very small patient population, the therapeutic approach after achievement of second or subsequent CR is mostly based on findings from retrospective studies.

In 2005, the Japan Adult Leukemia Study Group (JALSG) initiated a phase 2 study entitled APL205R for patients with relapsed APL. The main purpose of this study was to evaluate the efficacy and feasibility of a sequential treatment consisting of induction and consolidation with ATO, peripheral blood stem cell (PBSC) harvest after chemotherapy using high-dose cytarabine (AraC), and autologous HCT. This report presents and discusses the results of this study.

Methods

Patients

This study enrolled patients with relapsed APL between December 2005 and June 2009. At least a single documentation of cytogenetic and/or molecular evidence of t(15;17)/*PML-RARα* was required at the time of entry. Eligibility criteria consisted of age between 18 and 65 years; an Eastern Cooperative Oncology Group performance status between 0 and 3; and adequate functioning of the liver (serum bilirubin level <2.0 mg/L), kidneys (serum creatinine level <2.0 mg/dL), lungs (Pao_2 ≥60 mm Hg or SpO_2 ≥93%), and heart (no severe abnormalities detected on electrocardiograms). Patients who

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Table 1. Treatment schedule

Drug	Dose	Route	Days
Induction			
ATO	0.15 mg/kg	IV (2 h)	1-*
IDA	12 mg/m^2	IV (30 min)	†
MTX, AraC, PSL	15 mg, 40 mg, 10 mg	IT	‡
Consolidation #1			
ATO	0.15 mg/kg	IV (2 h)	1-25
MTX, AraC, PSL	15 mg, 40 mg, 10 mg	IT	‡
Consolidation #2			
ATO	0.15 mg/kg	IV (2 h)	1-25
MTX, AraC, PSL	15 mg, 40 mg, 10 mg	IT	‡
Consolidation #3			
AraC	2 g/m^2, every 12 h	IV (3 h)	1-4
PBSCH			§
Autologous HCT			
Busulfan	1 mg/kg, every 6 h	po	−6, −5, −4
Melphalan	70 mg/m^2	IV (bolus)	−3, −2
PBSCT			0

IT, intrathecally; IV, intravenously; MTX, methotrexate; PBSCH, peripheral blood stem cell harvest; PBSCT, peripheral blood stem cell transplantation; po, by mouth; PSL, prednisolone.

*For induction, ATO was administered until complete remission or for 60 d, whichever was shorter.

†IDA was added for 2 d if the WBC count exceeded 20.0 × 10^9/L before or during the induction therapy, if the combined total count of myeloblasts and promyelocytes in the peripheral blood exceeded 5.0 × 10^9/L before or during the induction therapy, or if an extramedullary myeloid tumor was detected before the induction therapy.

‡Intrathecal injection was given when the platelet count recovered after the end of the courses. PSL could be replaced with 4 mg of dexamethasone.

§PBSCH was performed when the WBC count had recovered.

had previously undergone autologous or allogeneic HCT were not eligible for inclusion. Written informed consent was obtained from all patients prior to registration. The protocol was reviewed and approved by the institutional review board of each of the participating centers and was conducted in accordance with the Declaration of Helsinki. This study is registered at http://www.umin.ac.jp/ctr/ as #C000000302.

Treatments

The treatments used during the study are summarized in Table 1. For remission induction, ATO was administered by a 2-hour infusion at a daily dose of 0.15 mg/kg until CR or a maximum of 60 days. In addition, patients received 12 mg/m^2 of idarubicin (IDA) on days 1 and 2 if 1 or more of the following criteria were met when the treatment was started: (1) the white blood cell (WBC) count exceeded 20.0 × 10^9/L; (2) the combined total count of myeloblasts and promyelocytes in the peripheral blood exceeded 5.0 × 10^9/L; and (3) there was the presence of an extramedullary myeloid tumor. Patients who showed evidence of criteria 1 and/or 2 after the start of induction therapy were given 2 extra doses of 12 mg/m^2 of IDA at that point. Those who achieved CR were scheduled to receive an additional 2 courses of ATO (0.15 mg/kg for 25 days) for consolidation. During ATO administration, a 12-lead electrocardiogram, complete blood cell counts, and chemistry parameters including the electrolytes were monitored at least twice a week, and the serum potassium and magnesium levels were maintained above the lower limits of normal. After the end of each ATO course, central nervous system (CNS) prophylaxis was attained by means of intrathecal injection of methotrexate, AraC, and corticosteroids (3 times in total). Patients with cytological evidence of CNS leukemia received intrathecal injections twice a week simultaneously with ATO, until complete clearance of leukemic cells in the cerebrospinal fluid (CSF) had been achieved. Following the third course of ATO, patients proceeded to PBSC harvest. For this purpose, high-dose AraC was administered at 2 g/m^2 for 3 hours twice daily for 4 days, and granulocyte–colony-stimulating factor was initiated from day 6. Upon recovery, autologous PBSCs were harvested by means of apheresis. Patients who attained a target CD34+ cell dose of 2.0 × 10^6/kg or higher were allocated to undergo autologous HCT unless *PML-RARα* transcripts were detected in PBSCs. The conditioning regimen consisted of busulfan (1 mg/kg orally every 6 hours on days −6 to −4) and melphalan (70 mg/m^2 intravenously on days −3 to −2),[13] whereas unpurged autologous PBSCs were infused on day 0. The study flow is shown in Figure 1.

Assessments and definitions

Hematologic CR was defined as the presence of all of the following: <5% of blasts in the bone marrow, no leukemic blasts in the peripheral blood or extramedullary sites, and recovery of peripheral blood counts. Hematologic relapse was defined as the presence of at least 1 of the following: recurrence of >10% leukemic cells in the bone marrow, recurrence of any leukemic cells in the peripheral blood, or development of extramedullary disease.[3] Molecular relapse was defined as the reappearance of polymerase chain reaction (PCR) positivity for *PML-RARα* in a single bone marrow or peripheral blood sample for this study. Prospective molecular monitoring was performed with the real-time quantitative reverse-transcription PCR (qRT-PCR) assay in a single independent laboratory. The *PML-RARα* levels in bone marrow samples were assessed at enrollment and after each course of therapy. Harvested PBSCs were also subjected to the qRT-PCR assay. The number of transcript copies was normalized by means of glyceraldehyde-3-phosphate dehydrogenase, and then converted into molecules per μg RNA. The threshold for quantification was 50 copies per μg RNA, which corresponds to a sensitivity of 10^{-4}, whereas levels below the threshold were differentiated into "not detected" and "detected but not quantifiable," and PCR negativity was categorized as "not detected."

For posttransplant engraftment, neutrophil engraftment was defined as achievement of a neutrophil count of at least 0.5 × 10^9/L for 2 consecutive days, and platelet engraftment as achievement of a platelet count of at least 30 × 10^9/L independent of transfusions for 2 consecutive days.

Statistical analysis

The primary end point was event-free survival (EFS) at 1 year after registration, which was defined as the time from registration to failure to achieve CR, relapse, death, or last visit, whichever came first. The expected and threshold EFS rates at 1 year were estimated to be 50% and 20%, respectively. The threshold EFS rate of 20% was determined based on historical control data of Japanese patients with relapsed APL who were

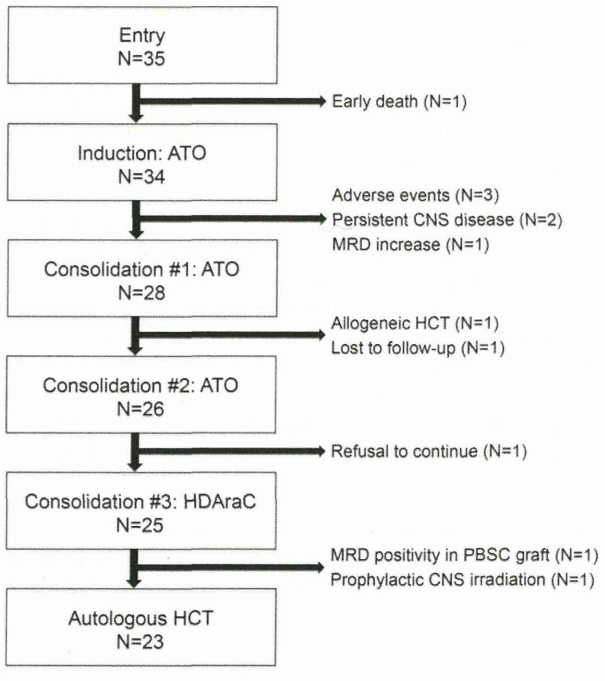

Figure 1. Patient flow diagram. HDAraC, high-dose cytarabine; MRD, minimal residual.

Table 2. Patient characteristics at enrollment

Characteristics	Values
Age in years, median (range)	46 (20-64)
Gender, male/female	23/12
WBC count, x 10^9/L	
Median (range)	2.6 (0.5-18.1)
≤10/>10	34/1
Platelet count, x 10^9/L	
Median (range)	79 (8-260)
≤40/>40	9/26
Performance status, 0/1/2/3	27/6/0/2
Number of prior relapses, 1/2	32/3
Type of relapse, hematologic/molecular	26/9
Interval between primary diagnosis and enrollment in years, median (range)	2.5 (0.8-11.0)

treated with ATRA-based therapy.[14] With a statistical power of 80% and a 1-sided, type I error of 5%, the minimum number of 17 eligible patients required for this study was calculated by means of binomial analysis. Allowing for a premature dropout rate of 15%, we aimed for inclusion of at least 20 patients. Primary end point analysis was performed with the Kaplan-Meier method for the calculation of probability of EFS. The treatment was considered to be effective if the lower limit of the 90% confidence interval (CI) exceeded the threshold EFS (ie, 20%). Overall survival (OS) was defined as the time from registration to death or last visit, and failure-free survival as the time from registration to failure to achieve CR, withdrawal from study, relapse, death, or last visit. Survival estimates and CIs were calculated with the Kaplan-Meier method and Greenwood's formula. The log-rank test was used for group comparison.

Results

Patient characteristics

A total of 35 patients with relapsed APL were enrolled in this study. Patient enrollment was allowed to exceed the originally planned minimum requirement after having ensured it ethical to expand the number of patients. Table 2 summarizes baseline characteristics of the patients. There were 23 males and 12 females, with a median age at enrollment of 46 years (range, 20-64 years). The median interval between primary diagnosis and enrollment was 2.5 years (range, 0.8-11.0 years).

All of the patients had been initially treated with ATRA-based therapy, and most of them in accordance with the protocols of JALSG or modifications thereof.[3,15] Thirty-two patients were in first relapse, and 3 in second relapse, with hematologic relapse accounting for 26, and molecular for 9. None of the patients had received ATO before.

Induction with ATO

ATO was administered to all patients except for 1 who developed intracranial hemorrhage immediately after enrollment and succumbed to early death (unique patient number [UPN] 26). Of the remaining 34 patients who underwent induction therapy, IDA was added for 2 patients on days 1 and 2, and during the induction course for 8 patients as per protocol. None of the patients developed differentiation syndrome. Three patients discontinued the study due to adverse events (grade 3 skin rash [UPN 10], grade 3 QT prolongation [UPN 19], and grade 4 QT prolongation accompanied by frequent ventricular premature contraction [UPN 23]). CSF examination performed at the end of the induction therapy revealed cytological evidence of CNS involvement in 4 patients, 2 of whom discontinued due to persistent CNS disease despite repeated intrathecal injections (UPN 13 and UPN 33). Of the 26 patients with hematologic relapse, 5 were taken off the study as mentioned previously, whereas the other 21 (81%) achieved CR. Of the 9 patients presenting with molecular relapse, 7 proceeded to consolidation therapy, and 2 were withdrawn from the study because of persistent CNS disease (UPN 13) or at the physician's discretion because the *PML-RARα* levels increased significantly after induction therapy (UPN 29).

Consolidation with ATO

During the 2 consolidation courses with ATO, 3 patients were taken off the study: 1 discontinued the protocol after the first consolidation course to receive umbilical cord blood transplantation (UPN 1), 1 was lost to follow-up after completing the first consolidation course (UPN 14), and the other refused to continue for unknown reasons after the second consolidation course (UPN 30). None of the patients discontinued the study because of relapse or adverse events during this phase of the treatment.

High-dose AraC and PBSC harvest

For PBSC harvest, 25 patients were given high-dose AraC as the third consolidation therapy, and all of them attained the target CD34+ cell doses of 2.0×10^6/kg. The median value of the CD34+ cell doses was 6.5×10^6/kg (range, 2.0-42.2 $\times$ 10^6/kg). One patient (UPN 18) whose PBSC sample was positive for *PML-RARα* was taken off the study because of ineligibility for autologous HCT as per protocol. One other patient (UPN 3), who had documented CNS leukemia at the end of induction therapy, but whose leukemic cells in the CSF were completely cleared with intrathecal injections, was withdrawn from the protocol at the physician's discretion to undergo prophylactic CNS irradiation. This patient received autologous HCT, but not as part of this study, and subsequently suffered posttransplant relapse in the CNS with fatal outcome. All of the other patients proceeded to autologous HCT. No dropouts due to relapse or adverse events were reported during this phase of the treatment.

Autologous HCT

The remaining 23 patients underwent autologous HCT as per protocol. The median time until engraftment was 12 days (range, 11-39 days) for neutrophils and 15 days (range, 12-136 days) for platelets. Posttransplant relapse occurred in 3 patients after a median duration of 5 months (range, 3-6 months). There was no transplant-related mortality.

Kinetics of the *PML-RARα* transcript levels

The results of the serial qRT-PCR tests during the treatment are summarized in Table 3. Most patients achieved PCR negativity after the first consolidation, but 4 were still positive for *PML-RARα* at this time. The PCR results turned negative after the second and third consolidation in 1 patient each (UPN 25 and 17, respectively). Of the 2 patients who remained positive for *PML-RARα* after the third consolidation, 1 (UPN 18) showed positive and the other (UPN 5) negative PCR test results for PBSCs. The latter underwent autologous HCT with a *PML-RARα*–negative graft but relapsed 5 months after transplantation.

Overall outcome

The probability of EFS was 77% at 1 year, with the 90% CIs ranging from 63% to 86%, thus demonstrating that this study has met its

Table 3. Kinetics of *PML-RARα* transcript levels

UPN	At entry	After induction	After consolidation #1	After consolidation #2	After consolidation #3
1	3000	N	N	Off study	Off study
2	460	N	N	N	N
3	60 000	<50	N	N	N
4	4200	<50	N	N	NA
5	69 000	28 000	760	140	<50
6	32 000	6000	N	N	N
7	15 000	290	N	N	N
8	360 000	<50	N	N	N
9	NA	1000	N	NA	N
10	NA	Off study	Off study	Off study	Off study
11	950	N	NA	N	N
12	64 000	50	N	NA	N
13	10 000	7100	Off study	Off study	Off study
14	120 000	400	NA	Off study	Off study
15	510 000	150	N	N	NA
16	190 000	<50	N	N	NA
17	95 000	1800	110	110	N
18	67 000	1500	480	390	280
19	130 000	Off study	Off study	Off study	Off study
20	450 000	280 000	N	N	N
21	140 000	170	N	N	N
22	26 000	61	N	N	N
23	24 000	Off study	Off study	Off study	Off study
24	730 000	<50	N	N	N
25	1900	2500	<50	N	N
26	440 000	Off study	Off study	Off study	Off study
27	NA	7800	N	N	N
28	NA	2600	N	N	N
29	510	6300	Off study	Off study	Off study
30	45 000	65	N	N	Off study
31	NA	300 000	NA	N	N
32	NA	50	N	N	N
33	180 000	NA	Off study	Off study	Off study
34	20 000	N	N	N	N
35	150 000	10 000	N	N	N

"Off study" indicates that the patient discontinued the study for reasons detailed in the text.

The threshold for quantification was 50 copies per μg RNA, which corresponds to a sensitivity of 10^{-4}. The levels below the threshold were differentiated into "not detected (N)" and "detected but not quantifiable (<50)."

N, not detected; NA, not assessed.

primary end point. Figure 2 shows Kaplan-Meier estimates for EFS and OS. With a median follow-up for surviving patients of 4.9 years (range, 0.3-6.3 years), the 5-year EFS and OS rates were 65% and 77%, respectively. The probability of failure-free survival was estimated to be 59% at 5 years.

Discussion

Current comprehensive practice guidelines have provided recommendations on the management of APL,[10,11] but what the optimal treatments for relapsed APL are remains equivocal. This is primarily because of the lack of prospective studies due to the rarity of relapses in APL, so that we initiated a phase 2 study for patients with relapsed APL in 2005 to evaluate the efficacy and feasibility of a sequential treatment featuring ATO and autologous HCT and enrolled 35 patients from 25 institutions nationwide. The treatment immediately induced molecular remission in a majority of patients, and only 3 patients were taken off the protocol because of adverse events throughout the entire study period, so that 23 patients could receive autologous HCT with a *PML-RARα*–negative PBSC graft. The 5-year probabilities of EFS and OS for the entire cohort were 65% and 75%, respectively. Of note, the EFS curve reached a stable plateau after 2 years from registration. These results have led us to conclude that this sequential treatment is effective and feasible.

ATO is currently the most active agent available for APL. Accumulated evidence has shown that >80% of patients with relapsed APL can achieve CR with ATO monotherapy.[7-9] In addition to high CR rates, the capability of this agent to induce molecular remission is another significant advantage because molecular remission is a prerequisite for long-term disease control in APL and is thus considered an important therapeutic milestone.[10,16] By contrast, ATRA alone is less likely to induce molecular remission, which results in this agent being used generally in combination with intensive chemotherapy rather than as monotherapy.[17] Although high CR rates can be expected for such combined use, this approach is limited by unsustained CR, especially for patients with hematologic relapse, and, more importantly, by quite high toxicity.[18,19] A retrospective study by Thomas et al[18] reported better survival for relapsed APL patients treated with ATO-based therapy than for historical control patients treated with ATRA-based therapy. The favorable safety profile of ATO is also an important advantage, as was seen in our study, where only 3 patients (8%) had to discontinue the protocol because of adverse events during induction therapy. This ratio seems to be only slightly higher than that observed in a US Intergroup study (5%).[8] It is further worth noting that none of our patients developed differentiation syndrome. This contrasts with a high incidence of this complication (25%) in the American study.[8] It can be assumed that the additional use of IDA for cases with high WBC counts may have contributed to reducing the risk of differentiation syndrome in our cohort.

Although the beneficial effect of ATO for induction has been well documented in relapsed APL, it is far less clear what the best consolidation strategy is after achieving CR. Previous studies showed that patients who achieved second or subsequent CR with ATO but did not receive transplantation thereafter had poor outcome; the proportion of those remaining alive and relapse-free ranged from 22% to 37%.[7,17,20] Although some patients may remain in CR without transplantation, overall prognosis is far from satisfactory, and the outcome seems much better for those who receive autologous or allogeneic HCT.[17,20] Owing to its posttransplant graft-versus-leukemia effect, allogeneic HCT is generally considered the most effective treatment of preventing relapse in acute myeloid leukemia.[21] In APL, however, the relapse rate after autologous HCT may be quite low provided the patient is in molecular remission at the time of transplantation.[22-25] Given the lower risk of transplant-related mortality with autologous HCT, the balance of benefits and risks may well favor autologous HCT over allogeneic HCT. For autologous HCT to be successful, it is imperative to reduce the tumor burden substantially at the molecular level before transplantation. For this reason, what constitutes an adequate number of cycles of ATO therapy is a subject of clinical interest. Similar to the observation by the US Intergroup,[8] our study found that 2 courses of ATO therapy induced most patients into molecular remission, although 4 patients remained positive for *PML-RARα* after the second course (ie, consolidation #1). Administration of the third ATO course reduced the transcript levels in 3 of the patients, whereas the level stayed unchanged in the remaining patient. It was possible to administer the third course of ATO because none of the 26 patients who had received this course had to withdraw from the study due to relapse or adverse events. These findings lead us to consider that

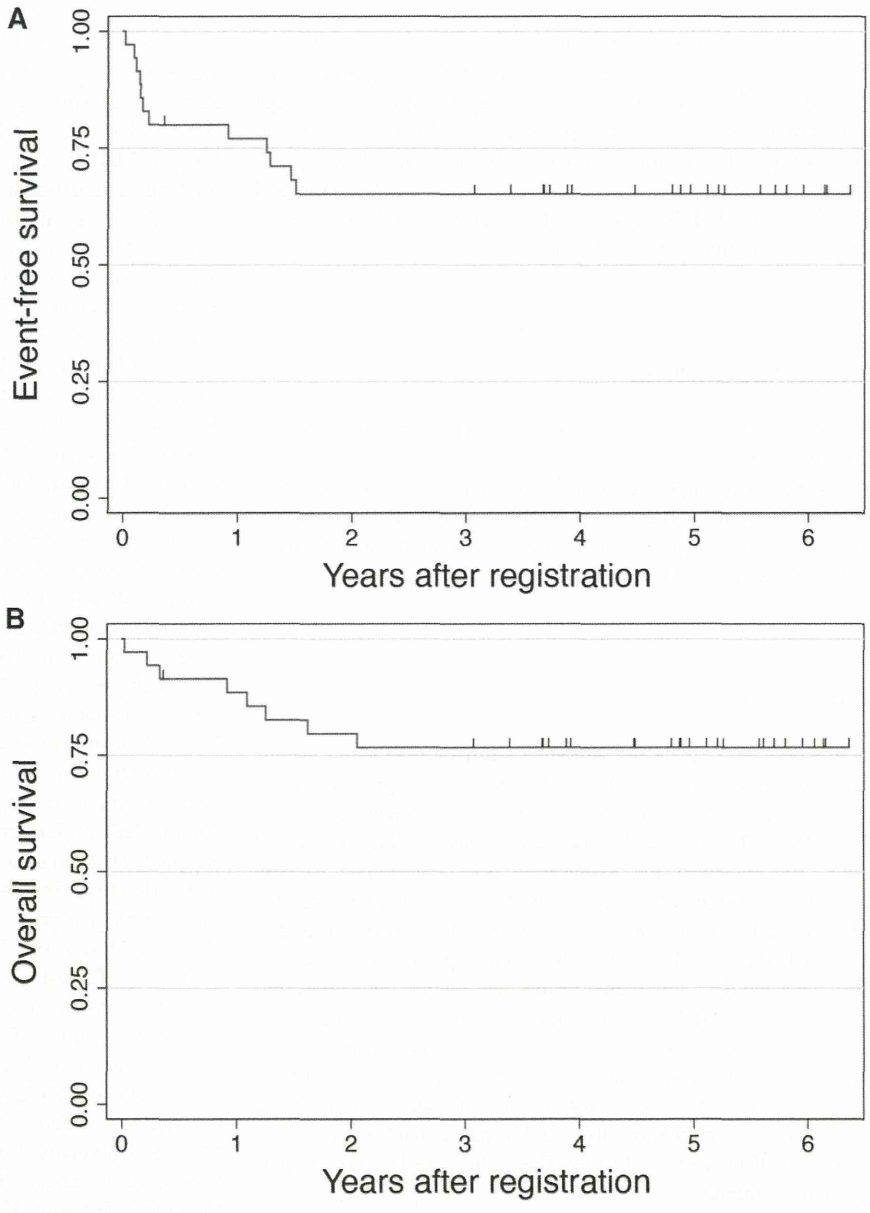

Figure 2. Kaplan-Meier curves for EFS (A) and OS (B). The probabilities of EFS and OS for the entire cohort (N = 35) were 65% and 77% at 5 years, respectively.

administration of a total of 3 courses of ATO before PBSC collection is feasible.

For the PBSC-mobilizing regimen, we chose high-dose AraC, hoping it would produce highly efficient mobilization as well as exert a systemic antileukemic effect. The fact that all the 25 patients undergoing this procedure successfully achieved the target CD34+ cell doses has convinced us of the usefulness of this regimen. In addition, high-dose AraC is known to provide good coverage of the CNS, the most common site of extramedullary involvement in APL.[26,27] Above and beyond our expectations, routine CSF examination at the end of the induction therapy identified 4 patients with cytological evidence of CNS involvement, although they did not show any CNS-related symptoms. This suggests that high-dose AraC may also play a part in protecting against the potential risk of subsequent CNS relapse for these patients.

Except for 1 patient whose PBSC sample was positive for *PML-RARα* and another who was withdrawn from the study to receive off-protocol prophylactic CNS irradiation, all the remaining patients who had undergone PBSC harvest proceeded to autologous HCT without any subsequent transplant-related mortality. This contrasts with a previous prospective study conducted before the advent of ATO, in which a combination of ATRA and intensive chemotherapy was used.[28] In that study, severe toxicity of induction therapy precluded the subsequent conduct of PBSC harvest or autologous HCT for some patients, and nearly 10% of the autografted patients suffered transplant-related mortality. These results highlight the need for active and less toxic therapies that give patients a better chance to proceed to and receive autologous HCT safely. For this reason, ATO can be considered to be an ideal treatment because of its strong antileukemic effect and favorable safety profile.

Although relatively few patients were analyzed in our study, to our knowledge this is the first prospective study to evaluate the use of ATO in conjunction with autologous HCT for relapsed APL. The results presented here provide evidence of the outstanding efficacy and feasibility of the sequential treatment consisting of induction and consolidation with ATO, PBSC harvest after high-dose AraC chemotherapy, and autologous HCT. For patients who are not eligible for this strategy, such as those for whom autologous HCT is

not suitable or whose *PML-RARα* levels do not decrease sufficiently during treatment, other treatment approaches need to be investigated that incorporate, for example, allogeneic HCT,[24,29] gemtuzumab ozogamicin,[30,31] tamibarotene,[32] or novel agents. It is desirable that such studies can be conducted prospectively. Finally, we should remember that the incorporation of ATO into initial therapy is expected to further improve the outcome for newly diagnosed APL,[33,34] which will hopefully lead to reduction in the number of patients who require salvage therapy.

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Authorship

Contribution: M.Y. collected and analyzed data, interpreted results, and drafted the manuscript; M.T., H.F., and A.T. designed the study, collected data, interpreted results, and reviewed the manuscript; K.F., S.F., K.S., M.T., A.O., K.T., and A.M. collected data, interpreted results, and reviewed the manuscript; S.O. contributed to data management, designed the study, collected data, interpreted results, and reviewed the manuscript; Y.M. contributed to data management, interpreted results, and reviewed the manuscript; Y.A. designed the study, analyzed data, interpreted results, and drafted the manuscript; Y.K. designed the study, provided administrative support, interpreted results, and reviewed the manuscript; T.N. provided administrative support, interpreted results, and reviewed the manuscript; and N.E. served as the principal investigator, designed the study, collected and analyzed data, interpreted results, and drafted the manuscript.

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A complete list of the members of the JALSG appears in "Appendix: study group members."

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Appendix: study group members

The members of the JALSG are Nihon University School of Medicine, Kasukabe Municipal Hospital, Tokyo Metropolitan Komagome Hospital, Tokyo Metropolitan Ohtsuka Hospital, Nagoya University Graduate School of Medicine, Nagoya Ekisaikai Hospital, JA Aichi Showa Hospital, Okazaki City Hospital, Daido Hospital, Yokkaichi Municipal Hospital, Ichinomiya Municipal Hospital, Komaki City Hospital, Toyohashi Municipal Hospital, Ogaki Municipal Hospital, Tosei General Hospital, National Center for Geriatrics and Gerontology, Aichi Cancer Center, Toyota Kosei Hospital, Japanese Red Cross Nagoya First Hospital, Fujita Health University School of Medicine, Mie University Graduate School of Medicine, Suzuka Kaisei Hospital, Takeuchi Hospital, Yamada Red Cross Hospital, JA Suzuka General Hospital, Matsusaka Chuo General Hospital, Kinki University School of Medicine, Osaka Minami Medical Center, Sakai Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, Hiroshima Red Cross Hospital & Atomic-Bomb Survivors Hospital, Shikoku Cancer Center, Nagasaki University Graduate School of Biomedical Sciences, Sasebo City General Hospital, Nagasaki Medical Center, Kumamoto University School of Medicine, Kumamoto City Hospital, Kumamoto Shinto General Hospital, Jichi Medical School, Okayama University Hospital, Minami-Okayama Medical Center, Okayama City Hospital, Chugoku Central Hospital, Okayama Medical Center, Okayama Rosai Hospital, Kagawa Rosai Hospital, Gunma University Graduate School of Medicine, Nishi-Gunma National Hospital, Fujioka General Hospital, Fukaya Red Cross Hospital, University of Fukui, Kurashiki Central Hospital, Kanazawa Medical Center, Fukui Red Cross Hospital, Fukui Prefectural Hospital, National Cancer Center Hospital, Saitama Medical School, Hyogo College of Medicine, Osaka National Hospital, Takarazuka Municipal Hospital, Uegahara Hospital, Amagasaki Central Hospital, Kawasaki Medical School, Kochi Health Sciences Center, Chiba University Hospital, Chiba Aoba Municipal Hospital, Funabashi Central Hospital, Saiseikai Narashino Hospital, Oami Hospital, Nara Medical University, Jikei University School of Medicine, Dokkyo University School of Medicine, Nagoya Medical Center, Ohta Nishinouchi Hospital, Kochi Medical School, Shiga University of Medical Science, National Cancer Center East, Anjo Kosei Hospital, St. Marianna University School of Medicine, Yokohama Seibu Hospital, Shinshu University School of Medicine, Nagano Red Cross Hospital, Matsumoto Medical Center Matsumoto Hospital, Showa Inan General Hospital, Tokyo Women's Medical University, Tama-Hokubu Medical Center, Hamamatsu University School of Medicine, Hamamatsu Medical Center, Kagoshima University Hospital, Tochigi Cancer Center, Kanazawa University Graduate School of Medical Science, Keijyu Medical Center, NTT West Kanazawa Hospital, Toyama City Hospital, Ishikawa Central Hospital, JA Takaoka Hospital, Tokyo Medical University, Tokyo Medical University Hachioji Medical Center, Kyorin University School of Medicine, Hokkaido University Graduate School of Medicine, Sapporo Kousei Hospital, Sapporo Aiiku Hospital, Asahikawa City Hospital, Hakodate City Hospital, Hokkaido Cancer Center Hospital, Saiseikai Maebashi Hospital, Nagoya City University Graduate School of Medical Sciences, Enshu General Hospital, Shizuoka Saiseikai General Hospital, Tokai University School of Medicine, Ebina General Hospital, Yamaguchi University School of Medicine, Yamaguchi Prefecture Central Hospital, The University of Tokyo, Osaka City University, Saiseikai Nakatsu Hospital, Osaka University Graduate School of Medicine, University of Tokyo, Niigata University Medical and Dental Hospital, Oita University Faculty of Medicine, Oita Prefectural Hospital, Almeida Memorial Hospital, Kouseiren Tsurumi Hospital, National Kyushu Cancer Center, Kyushu Medical Center, Fukuoka Postal Services Agency Hospital, Aso Iizuka Hospital, Teikyo University School of Medicine, Teikyo University Mizonokuchi Hospital, Sapporo Hokuyu Hospital, Aichi Medical University, Kitasato University Hospital, Yamagata University Faculty of Medicine, Keio University, Aomori Prefectural Central Hospital, Hyogo Cancer Center, Kyoto Prefectural University of Medicine, Kyoto Hospital, Kobe Central Hospital, Matsushita Memorial Hospital, Osaka City General Hospital, National Defense Medical College, Akita University School

of Medicine, NTT Kanto Medical Center, Yokohama City University Hospital, Yokohama City University Medical Center, Kanagawa Cancer Center, Yokosuka City Hospital, Fujisawa City Hospital, Shizuoka Red Cross Hospital, Yamato Municipal Hospital, Saiseikai Yokohama Nanbu Hospital, Tohoku University School of Medicine, Osaki Citizen Hospital, Hiroshima University, Hiroshima-Nishi Medical Center, Kagawa University, Kagawa Prefectural Central Hospital, Sakaide City Hospital, Juntendo University School of Medicine, Kanazawa Medical University, Kobe University Graduate School of Medicine, Imamura Bun-In Hospital, Ehime University School of Medicine, Bokutoh Hospital, Ohtsu Red Cross Hospital, Matsue Red Cross Hospital, Tokyo Medical and Dental University, Yokohama City Minato Red Cross Hospital, Jichi Medical School Omiya Medical Center, Shizuoka Cancer Center Hospital, Ehime Prefectural Central Hospital, International Medical Center of Japan, Kure Medical Center, Nagoya Daini Red Cross Hospital, University of Yamanashi, Heart Life Hospital, Musashino Red Cross Hospital, Saitama Medical School, PL General Hospital, Toyama Prefectural Central Hospital, Shimane Prefectural Central Hospital, Tottori Prefectural Central Hospital, National Disaster Medical Center, Shimane University Hospital, Otemae Hospital, Nakagami Hospital, Tsukuba University Hospital, Mito Medical Center, National Hospital Organization, Tsuchiura Kyodo General Hospital, Hitachi General Hospital, JA Toride Medical Center, Fuchu Hospital, Ibaraki Prefectural Central Hospital, Saga University, Yamanashi Prefectural Central Hospital, Hiroshima City Asa Hospital, Sendai Medical Center, Kurume University of Medicine, Kitakyushu Municipal Medical Center, Kyushu Kosei-Nenkin Hospital, University of Miyazaki Hospital, Hamanomachi Hospital, Kyushu University Graduate School of Medical Sciences, Harasanshin Hospital, Toranomon Hospital, Matsuyama Red Cross Hospital, Fukushima Medical University, Fukuoka University Hospital, Yokohama Municipal Citizen's Hospital, Nagoya City West Medical Center, Takanohara Central Hospital, The University of the Ryukyus, Osaka Red Cross Hospital, and Sapporo Medical University School of Medicine.

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The Incidence of Leukemia, Lymphoma and Multiple Myeloma among Atomic Bomb Survivors: 1950–2001

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A marked increase in leukemia risks was the first and most striking late effect of radiation exposure seen among the Hiroshima and Nagasaki atomic bomb survivors. This article presents analyses of radiation effects on leukemia, lymphoma and multiple myeloma incidence in the Life Span Study cohort of atomic bomb survivors updated 14 years since the last comprehensive report on these malignancies. These analyses make use of tumor- and leukemia-registry based incidence data on 113,011 cohort members with 3.6 million person-years of follow-up from late 1950 through the end of 2001. In addition to a detailed analysis of the excess risk for all leukemias other than chronic lymphocytic leukemia or adult T-cell leukemia (neither of which appear to be radiation-related), we present results for the major hematopoietic malignancy types: acute lymphoblastic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, adult T-cell leukemia, Hodgkin and non-Hodgkin lymphoma and multiple myeloma. Poisson regression methods were used to characterize the shape of the radiation dose-response relationship and, to the extent the data allowed, to investigate variation in the excess risks with gender, attained age, exposure age and time since exposure. In contrast to the previous report that focused on describing excess absolute rates, we considered both excess absolute rate (EAR) and excess relative risk (ERR) models and found that ERR models can often provide equivalent and sometimes more parsimonious descriptions of the excess risk than EAR models. The leukemia results indicated that there was a nonlinear dose response for leukemias other than chronic lymphocytic leukemia or adult T-cell leukemia, which varied markedly with time and age at exposure, with much of the evidence for this nonlinearity arising from the acute myeloid leukemia risks. Although the leukemia excess risks generally declined with attained age or time since exposure, there was evidence that the radiation-associated excess leukemia risks, especially for acute myeloid leukemia, had persisted throughout the follow-up period out to 55 years after the bombings. As in earlier analyses, there was a weak suggestion of a radiation dose response for non-Hodgkin lymphoma among men, with no indication of such an effect among women. There was no evidence of radiation-associated excess risks for either Hodgkin lymphoma or multiple myeloma.

INTRODUCTION

A radiation-related excess of leukemia in radiologists and physicians was recognized in the early 1940s (*1*, *2*). By the late 1940s, physicians in Hiroshima and Nagasaki had noticed an apparent increase in leukemia incidence among survivors (particularly children) who were near the hypocenters at the time of the atomic bombs. The first published report of an increased risk of leukemia among the atomic bomb survivors appeared in 1952 (*3*). Since then, risks of leukemia and other hematological malignancies have been the subject of special and continuing interest in studies of the survivors conducted at the Radiation Effects Research Foundation (RERF), formerly the Atomic Bomb Casualty Commission (ABCC).

The latest comprehensive analysis of the incidence of hematological malignancies in the RERF Life Span Study (LSS) cohort of the atomic bomb survivors (*4*) considered radiation effects on all leukemias as a group and on selected leukemia subtypes for the period from 1950–1987. Radiation effects on leukemia mortality have been considered in most of the periodic LSS mortality reports and

Note. The online version of this article (DOI: 10.1667/RR2892.1) contains supplementary information that is available to all authorized users.

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reports on dosimetry changes and temporal patterns of risk (*5–7*).

Incident cases used in the analyses presented here were identified by the Leukemia and Tumor/Tissue Registries in Hiroshima and Nagasaki with follow-up through the end of 2001, fifty-five years after the bombings and 14 years beyond that used in the previous comprehensive report. Analyses are presented for all leukemias other than chronic lymphocytic leukemia (CLL) or adult T-cell leukemia (ATL) as a group (leukemia other than CLL or ATL) as well as for major leukemia subtypes [acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML)] and CLL, ATL, non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL) and multiple myeloma (MM). In the leukemia analyses herein, we focus on how the excess risk varies with gender, age at exposure and attained age or time since exposure, as well as the characterization of curvature in the leukemia dose response. In contrast to the previous incidence analyses that focused solely on excess absolute rates (*4*), the present analyses focused on both excess relative risks and excess rates. A question of particular interest with regard to leukemia was whether or not there were any indications of a radiation-associated increase in risks 30 or more years after exposure. For lymphomas and MM, where the excess risks, if any, appear to be considerably lower than those for leukemia, our analyses primarily focused on the evidence for a statistically significant dose response.

MATERIAL AND METHODS

Study Population and Cohort Follow-up

In the late 1950s, records from the 1950 special national census of atomic bomb survivors were used by ABCC researchers to establish a fixed cohort of atomic bomb survivors: the LSS cohort. The LSS cohort includes 93,741 survivors who were residents of Hiroshima and Nagasaki, were present within 10 km of the hypocenters at the time of the bombs and were alive on October 1, 1950, and 26,580 Hiroshima and Nagasaki residents who were not in the cities at the time of the bombings. The latter group, which is referred to as the not-in-city (NIC) group, is similar in size and frequency-matched on gender and age at the time of the bombings to survivors in the cohort who were within 2.5 km of the hypocenters. The present analyses were based on the 113,011 cohort members for whom dose estimates are available. Dose estimates are not available for 7,044 cohort members because of uncertain locations or shielding configurations. Almost 60% of the cohort members are women and 41% were less than 20 years old at the time of the bombings. As of the end of follow-up for the present analyses (December 31, 2001), 43% of the cohort members were still alive. Additional information on the characteristics and history of the LSS cohort can be found in previously published articles (*4–6, 8*).

Until recently, it has been customary to exclude the NIC group from risk analyses because concerns about possible differences in socioeconomic status or other factors that might affect risk estimates. However, as in the most recent LSS solid cancer incidence analyses (*9*), the NIC group was used in this work to augment the information on variation in baseline rates by gender, attained age and birth cohort, but not on the overall level of the rates. This was accomplished by the inclusion of fitted increments associated with a city-specific NIC indicator in the baseline risk model.

Vital status for individual LSS cohort members is ascertained by linkage to the national family registration (*koseki*) system on a 3-year cycle. Given the comprehensive nature of the *koseki* system, only 175 (<1%) cohort members have been lost to follow-up. Information from *koseki* records is used to trace death certificates, which provide information on causes of death for those known to have died. Incidence follow-up began on October 1, 1950. The end of follow-up is the earliest date of diagnosis of the first primary malignancy (of any type), the date of death, the date of loss to follow-up or December 31, 2001.

Ascertainment of Hematological Malignancies

Leukemia registry. Two to three years after the atomic bombings of Hiroshima and Nagasaki, a number of physicians in Hiroshima and Nagasaki noted a markedly increased rate of leukemia in children living near the hypocenters (*3*). Therefore, in the early 1950s, ABCC researchers together with hematologists in Hiroshima and Nagasaki launched the Leukemia Registry program to ascertain all potential cases of leukemia and other hematological malignancies in the two areas, including cases that occurred in the late 1940s. The Leukemia Registry remained active until the late 1980s when it was supplanted by the city and prefecture cancer registries. Leukemia Registry data were the basis for a number of reports on radiation-related risk of leukemia and related diseases in the survivors (*10–15*).

The Leukemia Registry also gathered blood smears or other biological specimens used for diagnosis, clinical information, laboratory records and other material relevant to the diagnosis. These materials and information stored at ABCC and later at RERF were reviewed by at least two Leukemia Registry hematologists to develop a consensus diagnosis. Additional information on the Leukemia Registry procedures is available elsewhere (*4, 16*). Accepted cases were assigned a diagnosis date and the type of malignancy was coded. In the mid-1980s, the materials collected by the Leukemia Registry were re-reviewed and 60% of the leukemia diagnoses were classified using the French-American-British (FAB) classification system (*17–20*).

Tumor registries. As the Leukemia Registry activities declined in the mid-1980s, the population-based Tumor Registries, independent of the Leukemia Registry, became the primary source for ascertaining leukemias and other hematological malignancies in Hiroshima and Nagasaki. The Tumor Registries were established in 1957 in Hiroshima and 1958 in Nagasaki (*21*). The Hiroshima and Nagasaki Tumor Registries are operated by RERF entrusted by Hiroshima and Nagasaki prefectures and cities. Active ascertainment from hospital records in the two cities and their outlying areas is the primary method of case identification employed by the registries. Furthermore, this is supplemented by linkage to the cause of death information and to records from the ABCC surgical pathology program, which was superseded in the early 1970s by the regional tissue registries. Records are reviewed by RERF personnel trained in nosology and coded using the International Classification of Diseases for Oncology (ICD-O) codes that were current at the time of coding (*22*).

Assembling the present case series. Incident cases considered for these analyses were ascertained and assembled from the Leukemia Registry and the Hiroshima and Nagasaki Tumor Registries using a series of rules to give precedence to the better information when there were discrepancies. Since the Leukemia Registry involved detailed hematology review, precedence was given to the Leukemia Registry diagnosis if it was at least as detailed as the Tumor Registry diagnosis. Additional review was carried out for a small number of cases in which the Leukemia Registry and Tumor Registry diagnoses appeared to be inconsistent. More detailed information can be found in (*4*).

Classification of hematologic malignancies has been modified and refined over time, particularly for myeloid leukemias. Since most of the cases in these analyses cannot be classified according to the more detailed modern classifications and the number of cases of specific

subtypes tends to be small, we used a broad classification of types for these analyses that parallels the classification used in earlier reports on risks for these cancers in the LSS. In particular, cases identified as aleukemic/subleukemic myeloid leukemias and myeloid leukemia not otherwise specified are included in the AML group. The aleukemic/subleukemic lymphoid leukemias are combined with ALL. The CLL group includes CLL and hairy cell leukemia. ICD-O morphology codes included in the various analysis groups are given in supplementary Table S1 (http://dx.doi.org/10.1667/RR2892.1.S1)

Organization of the data for analysis. The primary Poisson regression analyses for this report were based on a highly stratified tabulation of person-years and case counts. The stratifying factors were: city, gender, age at exposure (5-year categories to age 69 and 70 and over), attained age (5-year categories from age 5–84 and 85 and over), calendar time period (from October 1, 1950, with subsequent cut points on January 1 of 1953, 1956 and 1958, and every 5 years from 1961–2001 except for an additional cut-point at 1988 to facilitate comparison with the previous report), exposure status (<3 km from the hypocenter, 3–10 km from the hypocenter and not in a city), adjusted and truncated weighted (gamma plus 10 times the neutron) bone marrow dose (22 dose categories for survivors), and whether or not an individual's shielded kerma estimate was greater than 4 Gy based on the latest dosimetry system (DS02). The lowest dose category included people whose DS02 weighted bone marrow dose estimates were less than 5 mGy. The lower dose bounds (in Gy) for the subsequent categories were: 0.005, 0.02, 0.04, 0.06, 0.08, 0.10, 0.125, 0.150, 0.175, 0.20, 0.25, 0.30, 0.50, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, and 3.0. As in other LSS incidence reports (*4*, *9*), person-years were adjusted by birth cohort, time period, gender and city-specific residence probabilities to correct for migration from the Tumor Registries' catchment areas (*23*). A table with information on the proportion of person-years lost due to migration is given in supplementary Table S3 (http://dx.doi.org/10.1667/RR2892.1.S2). The data for each stratum included migration-adjusted person-years, counts of the number of eligible cases by outcome type, and person-year-weighted mean values of weighted bone marrow dose, attained age, age at exposure and time since exposure.

Risk Models and Statistical Methods

The previous analyses (*4*) focused on age at exposure dependent excess absolute rate (EAR) models in which the radiation dose effect could vary with time since exposure within age at exposure groups. As we examined the current data, it became apparent that simpler models similar to those used for solid cancers (*9*), in which the excess risk varies smoothly with age at exposure and time can often describe the data at least as well as the models used in the previous analyses. Therefore, we considered both EAR and excess relative risk (ERR) models in which the excess risk varies with age at exposure and either attained age or time since exposure. In an EAR model, the disease rate can be written as:

$$\lambda_0(c,s,a,b) + \rho(d)\varepsilon_a(c,s,a,b).$$

While in the ERR model it is

$$\lambda_0(c,s,a,b)[1 + \rho(d)\varepsilon_r(c,s,t,e)].$$

The term $\lambda_0(c,s,a,b)$ is a parametric model for the baseline (zero dose) rates that depends on attained age (a), gender (s), and factors such as birth cohort (b) and city (c). In the primary dose-response model, $\rho(d)\varepsilon(c,s,t,e)$, $\rho(d)$ describes the shape of the dose response and $\varepsilon(c,s,t,e)$ describes effect modification associated with the effect of dose d, i.e., how the level of the radiation-related excess risk varies with city (c), gender (s), age at exposure (e) and time (t), where time can be functions of either time since exposure or attained age. For this report (as in most analyses of the LSS data) effect modification was described using log-linear functions of the variables of interest. In descriptions of these models, unless explicitly noted, they are based on log attained age and log time since exposure. In general, age at exposure and time since exposure were centered or scaled so that the dose-effect parameters correspond to the risk for a person who was 30 years old at the time of the bombings for incidence 25 (attained age 55) or 40 (attained age 70) years after exposure. For some outcomes, we considered extensions of the effect modification model, including gender-dependent age at exposure and time effects, interactions between age at exposure and time, or categorical age at exposure and time effects.

The dose response functions considered in this report included:

(a) linear $\rho(d) = \beta_1 d;$

(b) linear – quadratic $\rho(d) = \beta_1 d + \beta_2 d^2;$

(c) pure – quadratic $\rho(d) = \beta_2 d^2;$

(d) single knot linear spline/threshold models

$$\rho(d) = \theta_1 d + \theta_2 (d - c)(d>c);$$

(e) nonparametric $\rho(d) = \theta_{dcat}.$

β_1 and β_2 are the linear and quadratic dose-response parameters, respectively. In a linear-quadratic model, the curvature of the dose response is defined as the ratio of the quadratic and linear dose effects: i.e., β_2/β_1. In the single-knot linear-spline/threshold models, c is a dose join point and when θ_1 equals 0 this is a threshold model. In the nonparametric dose-response model the dose response varies by dose category without any smoothing ($\rho(d) = \theta_{dcat}$). Although the bone marrow dose estimates used in all of these analyses were adjusted to allow for the effects of dose uncertainty (*24*), dose-response models also included a multiplicative dichotomous factor for those with shielded kerma estimates in excess of 4 Gy to allow for dose uncertainties not captured by the standard adjustment methods or for high-dose effects such as cell killing.

Analyses were limited to first primary malignancies diagnosed during the follow-up period among cohort members with DS02 dose estimates. Cases that were diagnosed outside of Hiroshima or Nagasaki prefectures were excluded. Maximum likelihood estimates of the parameters in these models were computed using the data in the person-year (PY) table described above. *P* values and confidence intervals (CI) for model parameters were based on the profile likelihood function. Uncertainty in the various risk estimates were summarized using 95% confidence intervals. The models were fit using the Epicure risk regression software (*25*). Akaike information criteria (AIC) (*26*) values were used to aid in the comparison of nonnested models. The models used are described and estimates of some of the key parameters are given in the Results section. However, details of the parameterizations used and the parameter estimates for our preferred models are presented in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1).

RESULTS

A total of 1,215 hematological malignancies were identified among 113,011 LSS cohort members and 944 of these cases were eligible for inclusion in the analyses between 1950 and the end of 2001. Almost 40% of the eligible hematopoietic malignancies were diagnosed after the end of the follow-up (1987) used in the last comprehensive analyses of the LSS data (*4*). Table 1 provides a summary of the numbers of cases eligible for

TABLE 1
Eligible and Ineligible Cases by Exclusion Reason

Malignancy	Eligible	Ineligible				Total
		Not first primary	Nonresident[†]	Unknown dose	Before 10/1/1950	
Leukemia						
Leukemia other than CLL or ATL	312	28	31	36	9	416
Acute myeloid (AML)[‡]	176	20	13	18	2	229
Chronic myeloid (CML)	75	3	5	11	5	99
Acute lymphoblastic (ALL)[§]	43	4	9	4	2	62
Other	18	1	4	3	0	26
Chronic lymphocytic (CLL)[††]	12	4	0	0	0	16
Adult T-cell (ATL)	47	3	7	2	0	59
Any leukemia	371	35	38	38	9	491
Lymphoma and Myeloma						
Non-Hodgkin lymphoma (NHL)	402	34	33	27	5	501
Hodgkin lymphoma (HL)	35	1	4	1	1	42
Multiple myeloma (MM)	136	26	10	9	0	181
Total	944	96	85	75	15	1,215

[†] Residing outside of Hiroshima or Nagasaki prefectures at the time of diagnosis.
[‡] Includes acute myeloid leukemia (146 eligible cases) as well as acute monocytic leukemia (10 eligible cases), a-/sub-leukemic myeloid leukemia (16 eligible cases) and myeloid leukemia NOS (4 eligible cases).
[§] Includes 41 cases classified as acute lymphoblastic leukemia (41 cases) and 2 cases classified as aleukemia/subleukemic lymphoid leukemia.
[††] Includes 10 cases classified as chronic lymphocytic leukemia and 2 as hairy cell leukemia.

dose-response analyses by type of malignancy together with information on the reasons why cases were deemed ineligible. About 40% of the cases were leukemias, another 40% were identified as NHL and almost 15% were MM. Hodgkin lymphoma was uncommon. Almost half of the leukemia cases were classified as AML, 20% were CML and about 12% were ALL. All but five of 47 ATL cases were diagnosed in Nagasaki and constitute almost 40% of all of the Nagasaki leukemia cases. As with other populations in Japan, the incidence of CLL is remarkably low. All except 3 of 18 cases in the *other leukemia* group were diagnosed in Hiroshima. Eleven of the cases in this group were classified as acute leukemia not otherwise specified (NOS) and other specific types of leukemia while 7 were classified as aleukemia, subleukemia or leukemia NOS. The cases in this group were included in the leukemia other than the CLL or ATL analyses discussed below but were not analyzed separately. The crude rates for leukemia, lymphoma and multiple myeloma by age at exposure, period and dose category are given in supplementary Tables S4 and S5 (http://dx.doi.org/10.1667/RR2892.1.S2).

Leukemia Other Than CLL or ATL

While a few studies suggest that CLL risk may be affected by radiation exposure (*27–30*), a number of others do not (*31, 32*). It is generally believed that radiation has little effect on CLL rates and it is common practice in studies of radiation effects to focus on the risk of leukemia other than CLL. In view of the unusual nature of ATL incidence, we also excluded ATL from the pooled leukemia analyses. A total of 312 cases of leukemia other than CLL or ATL were used in these analyses (Table 1).

Baseline rates for this outcome were described reasonably well by a model in which the rates increased in proportion to age. This simple pattern was significantly improved ($P < 0.001$) by allowing the power to increase with increasing attained age (i.e., by adding a quadratic term in log attained age). The nature of the increase with attained age did not differ significantly by gender ($P > 0.5$), nor did it appear to vary significantly with birth cohort ($P = 0.30$). However, at any given age the risk for women was about half that for men (female:male ratio 0.50 95% CI 0.40–0.63). Baseline rates in Nagasaki were 35% lower than in Hiroshima ($P = 0.004$). There was a significant ($P < 0.001$) nonlinear birth cohort effect with the highest age-specific rates for those born around 1920, which decreased by about 30% for people born 20 years earlier or later than this—a pattern similar to that seen in the Japanese national leukemia mortality rates (*33*). The fitted age-specific baseline rate estimates for three birth cohorts are shown in Fig. 1a. The baseline rate model and parameter estimates for leukemias other than CLL or ATL are given in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1).

Dose response and effect modification. Using a simple time-constant linear ERR model with no effect modification, there was a statistically significant ($P < 0.001$) dose-response relationship. Allowing for attained age and time since exposure effects (discussed below), a concave upward linear-quadratic (LQ) model described the data significantly better than either a linear dose response ($P = 0.001$) or pure-quadratic ($P = 0.04$) dose-response model. The estimated linear dose effect in the LQ ERR model at attained age 70 after exposure at age 30 was 0.79 per Gy and the estimated curvature was 1.20, as given in Table 3. Figure 1b illustrates the fitted dose response together with dose-category-

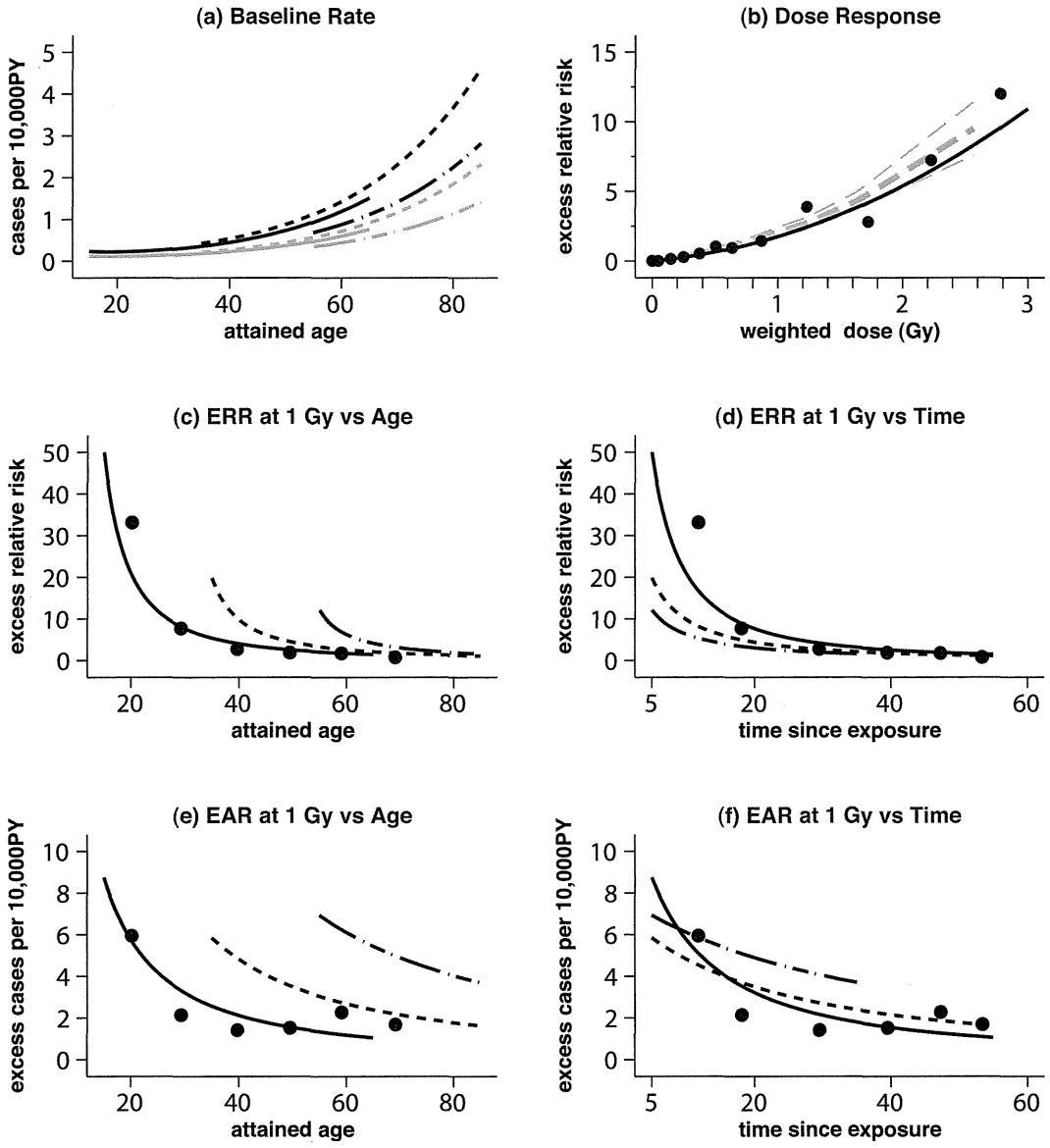

FIG. 1. Summaries of the risk of leukemia other than CLL or ATL in the LSS. Plot (panel a) shows age-specific baseline (zero dose) rates in Hiroshima for men (black lines) and women (gray lines) for LSS cohort members born in 1895 (dash-dot line; age at exposure 50), 1915 (dash line; age at exposure 30) and 1935 (solid line; age at exposure 10). Panel b: illustrates the radiation dose response based on the ERR model with risks standardized to attained age 70 for a person exposed at age 30 (born in 1915). The solid-black line illustrates the fitted linear-quadratic dose response. The points are based on a nonparametric dose-response model, while the middle-dashed-gray line is a smoothed version of the dose category-specific estimates from the nonparametric fit. The upper- and lower-dashed-gray line are plus and minus one standard error from the smoothed fit. Panels c and d: illustrate the temporal pattern and age-at-exposure effects for our preferred ERR model. The fitted ERR did not depend on either gender or city. Panels e and f: present the temporal pattern and age-at-exposure effects for Hiroshima males based on the preferred EAR model. The points in panels c–f are nonparametric estimates for exposure at age 10.

specific standardized ERR estimates and a dose-response function defined by smoothing the category-specific standardized estimates.

Based on our preferred ERR model (described below), it was estimated that about 94.1 of the 312 cases of leukemia other than CLL or ATL used in these analyses were associated with the radiation exposure (Table 2). The radiation-associated excess cases account for about 49% of the 192 cases among cohort members with doses in excess of 5 mGy.

Parameter estimates and confidence intervals for the preferred model for leukemia other than CLL or ATL are given in Table 3. The ERR was found to depend jointly on log attained age ($P < 0.001$) and either age at exposure ($P = 0.01$) or time since exposure ($P = 0.003$). These two models (age and age at exposure or age and time since exposure) led to similar patterns of the excess risk. However, since the fit of the age and time since exposure model (AIC = 2431.89) was somewhat better than that for the age and age at exposure model (AIC = 2433.97), our preferred model

TABLE 2
Observed and Fitted Cases of Leukemia Other than CLL or ATL by Weighted Bone Marrow Dose Categories

Dose (Gy)	Person years	Mean dose (Gy)	Observed cases	Fitted cases†	
				Background	Excess
<0.005	2,039,093	0.0006	120	116.9	0.1
–0.1	957,889	0.03	63	60.7	3.6
–0.2	201,935	0.14	16	13.7	4.1
–0.5	206,749	0.32	25	13.6	11.1
–1	117,855	0.71	24	7.5	18.2
–2	64,122	1.37	35	4.0	28.4
2+	25,761	2.68	29	1.5	28.6
Total	3,613,404	0.10	312	217.9	94.1

† Estimates based on the preferred ERR linear-quadratic model described in the text and Table 3 with additional details in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1).

includes age and time since exposure as effect modifiers. As indicated in the upper portion of Table 3, with both of these temporal factors in the model, the decrease in the ERR with increasing attained age was proportional to attained age to the power −1.09 and simultaneously proportional to time since exposure to the power −0.81. The model predicted that the highest ERRs were seen shortly after exposure among those exposed early in life (Fig. 1d). However, due to the rapid decline in the ERR with time, at any given attained age the ERR was greater for those who were exposed at older ages (Fig. 1c). There was no indication that the ERR varied significantly with gender ($P = 0.29$) or city ($P = 0.42$), nor did it appear that the dose-response curvature varied with city ($P > 0.5$). The precise form of this model is indicated in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1) and information on the fit of alternative models is given in supplementary Table S6 (http://dx.doi.org/10.1667/RR2892.1.S2).

The excess absolute rates for leukemia other than CLL or ATL could also be described by a linear-quadratic EAR model in which the radiation-associated excess rate depended on attained age ($P < 0.001$), age at exposure ($P < 0.001$) and city ($P = 0.03$), with a suggestion of a statistically significant gender difference ($P = 0.08$). This EAR model (AIC = 2433.2) describes the data slightly worse than the preferred ERR model discussed above. An EAR model with log-time since exposure and age-at-exposure effects (AIC = 2,438.8) fit worse than the preferred attained age and age-at-exposure model. Adding attained age to this time-since-exposure model led to a statistically significant improvement in fit ($P < 0.001$). The resulting model was virtually identical to the attained-age model. Thus, attained age with an age-at-exposure effect provided a better description of the temporal variation of EAR than did attained age with a time-since-exposure effect.

In our preferred EAR model (Table 3 and supplementary Table S6: http://dx.doi.org/10.1667/RR2892.1.S2), the linear dose coefficient estimates (standardized to age 70 after exposure at age 30) in Hiroshima were 1.06 excess cases per 10,000 PY per Gy for men and 0.7 for women with estimated curvature similar to the ERR model. The decrease

TABLE 3
Preferred Model, Excess Risk Parameter Estimates for Leukemia Other than CLL or ATL

Risk model	Dose coefficients (at 1 Gy)		Curvature	Gender ratio (F:M)	City ratio (N:H)	Attained age (power)	Time since exposure (power)	Age at exposure
	Linear	Quadratic						
ERR†	0.79	0.95	1.20			−1.09	−0.81	
	(0.03, 1.93)	(0.34, 1.80)	(0.23, 49.35)			(−2.01, −0.27)	(−1.31, −0.28)	
EAR‡								
Women	0.70	0.71	1.03	0.66	0.52	−1.45		0.41
	(0.13, 1.53)	(0.24, 1.41)	(0.20, 8.52)	(0.41, 1.04)	(0.26, 0.93)	(−2.13, −0.80)		(0.2, 0.64)
Men	1.06	1.09						
	(0.16, 2.42)	(0.37, 2.13)						

† The preferred ERR model is linear quadratic in dose with log-linear effect modification depending on log (attained age) and log (time since exposure). The baseline model parameters and explicit details about the dose effect modification term are given in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1). Supplementary Table S6 (http://dx.doi.org/10.1667/RR2892.1.S2) presents information on other ERR models. The dose coefficients describe the ERR at 1 Gy at age 70 after exposure at age 30.

‡ The preferred EAR model is linear-quadratic in dose with log-linear effect modification depending on log (attained age) and age at exposure. The baseline model parameters and explicit details about the dose effect modification term is given in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1). Supplementary Table S6 (http://dx.doi.org/10.1667/RR2892.1.S2) presents information on other EAR models. The dose coefficients describe the excess cases per 10,000 person years at 1 Gy at age 70 after exposure at age 30.

TABLE 4
Observed and Fitted Excess Cases of Leukemia Other than CLL or ATL by Time Period and Age at Exposure with Category-Specific ERR Estimates

	Age at exposure									
	0–19		20–39		40+		Total			
Period	Obs	Exc[†]	Obs	Exc	Obs	Exc	Obs	Exc	ERR[‡] (95%CI)	
1950–1955	18	16.2	11	6.5	13	7.6	42	30.3	15.2	(8.8 to 25)
1956–1959	17	5.4	4	4.1	18	4.7	39	14.2	13.3	(7.2 to 23)
1960–1969	14	6.3	17	6.5	19	6.0	50	18.8	4.8	(2.3 to 8.4)
1970–1979	11	4.7	33	5.3	18	3.2	62	13.2	3.4	(1.5 to 6.3)
1980–1989	17	4.4	25	4.3	12	1.3	54	9.9	1.8	(0.5 to 3.8)
1990–2001	29	4.3	32	3.0	4	0.3	65	7.6	2.1	(0.8 to 4.3)
Total	106	41.3	122	29.7	84	23.1	312	94.0		
ERR[‡] (95% CI)	6.5 (4.0 to 10.3)		3.9 (2.3 to 6.1)		4.0 (2.1 to 6.9)		4.7 (3.3 to 6.5)			

[†] Excess cases based on preferred ERR model described in the text and Table 3 with additional details in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1).
[‡] ERR at 1 Gy for a linear dose response model with categorical period or age at exposure effects.

in the EAR with attained age was proportional to age to the power −1.45, while the EARs for a given attained age were estimated to increase by about 51% per decade increase in age at exposure (95% CI 23–89%). Excess rates for Nagasaki survivors were estimated to be about 52% of those for Hiroshima survivors of the same gender and exposure age, and excess rate estimates for women were about 66% of those for men. The variation in the fitted EAR estimates with attained age and time since exposure for various ages at exposure are shown in Fig. 1e and f, respectively.

We used a simple ERR model with categorical main effects for six time periods and three age-at-exposure groups to examine whether or not the risks had persisted throughout the follow-up period. As indicated by the results summarized in Table 4, there was evidence of statistically significant increased risks in each of the six time periods considered. The largest ERRs were seen for the two earlier periods. However, even for the last 12 years of follow-up (1990–2001 or 45–55 years after exposure), the radiation-associated leukemia risk at 1 Gy was estimated to be twice the baseline risk.

Acute Myeloid Leukemia (AML)

There were 176 eligible AML cases, including 42 cases diagnosed after 1987 among LSS cohort members who were in the cities at the time of the bombings and 15 cases among cohort members who were NIC at the time of the bombings.

As indicated in Fig. 2a, AML baseline rates increased with attained age, but the level of risk and the nature of the increase with age differed for men and for women ($P < 0.001$). Baseline rates for women were about 40% (95% CI 29–56%) of those for men, and the rate of increase with attained age was more rapid for men than for women ($P = 0.04$). The baseline rates also exhibited a complex birth cohort effect. Age-specific rates were larger for people born between 1915 and 1925 than for people born before or after this period ($P < 0.001$). This pattern is similar to that seen in the Japanese national leukemia mortality rates, but somewhat more pronounced in the LSS cohort. The baseline AML rates in Nagasaki were 25% lower, though not significantly lower ($P = 0.14$), than those in Hiroshima. The AML baseline rate model and parameter estimates are given in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1).

Dose response and effect modification. There was strong evidence for a radiation dose-response relationship ($P < 0.001$). As shown in Fig. 2B, the dose-response curve was concave upward ($P = 0.01$). A pure-quadratic model with an estimated ERR at 1 Gy of 1.11 (95% CI 0.53–2.08, standardized to age 70 after exposure at age 30) as shown in Table 6 described the data as well as a linear-quadratic model ($P > 0.5$). Inference about effect modification in the ERR and EAR was based on a pure-quadratic model. In our preferred AML models (described below), the number of radiation-associated cases was estimated to be 37.4 (Table 5). The fraction attributable to radiation was 38% among cohort members with doses in excess of 5 mGy.

ERRs for AML exhibited a statistically significant [$P = 0.004$, with 2 degrees of freedom (*df*)] non-monotone dependence on age at exposure. As suggested in Fig. 2c, for any attained age (after exposure), the ERR for the people exposed around age 30 tended to be lower than for those exposed later or younger in life. The decrease in the ERR with attained age (AIC = 1,552.14) was well described as proportional to age to the power −0.89 (Table 6). More complex patterns for the age/time dependence were also considered. Neither the addition of a quadratic term in log age nor the use of splines in log age significantly improved the fit, nor did the use of functions of time since exposure result in better fits ($P > 0.5$ in every case). There was no indication of an attained age by age-at-exposure interaction ($P > 0.5$), nor did the attained age or age-at-exposure effects appear to vary with gender ($P > 0.5$). Parameter estimates with confidence intervals for the preferred AML,

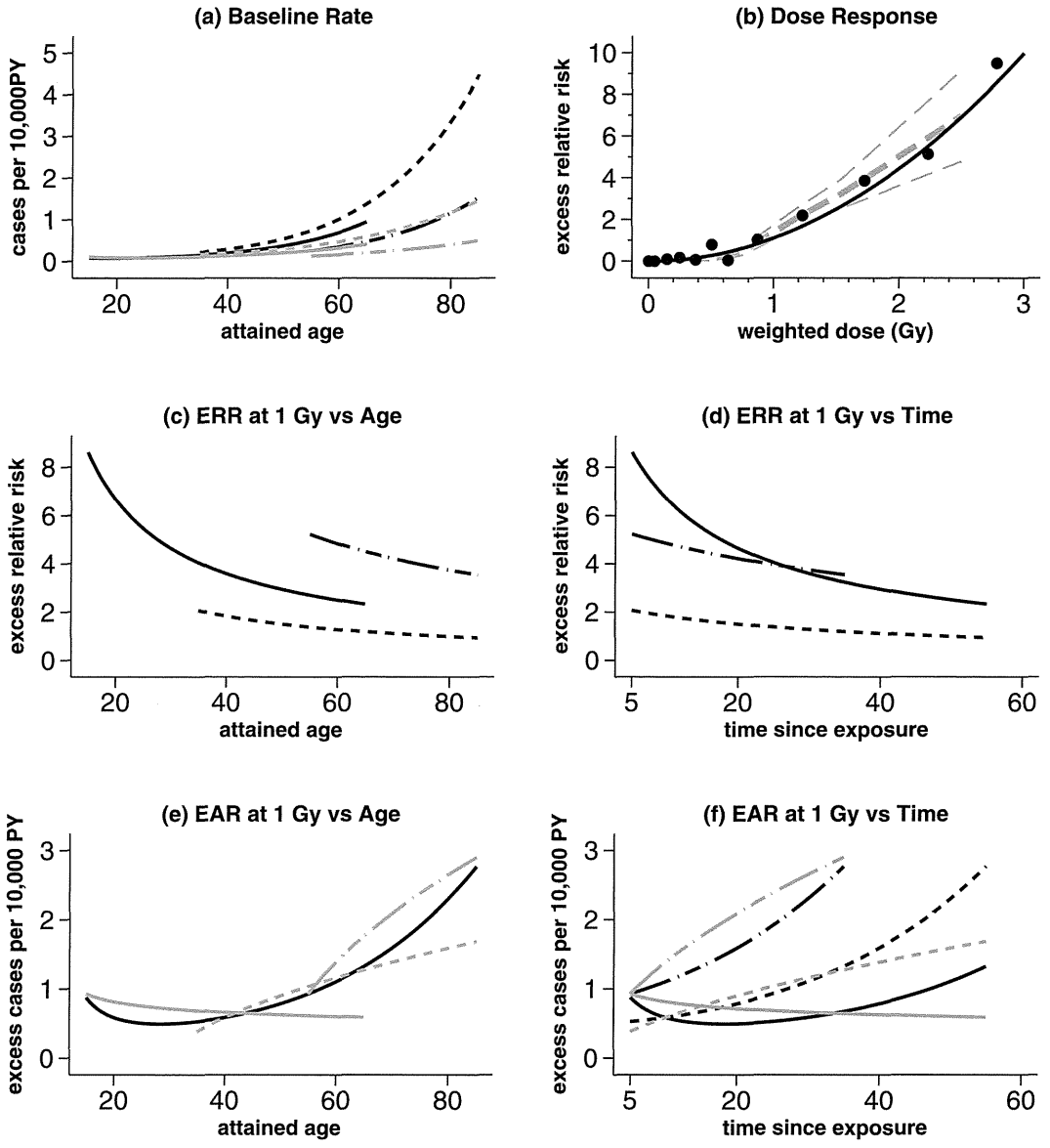

FIG. 2. LSS acute myeloid leukemia risk summary plots. Panel a: shows age-specific rates in the Hiroshima baseline (zero dose) for men (black lines) and women (gray lines) for LSS cohort members born in 1895 (dash-dot line; age at exposure 50), 1915 (dash line; age at exposure 30) and 1935 (solid line; age at exposure 10). Panel b: illustrates the radiation dose response based on the preferred ERR model with risks standardized to attained age 70 for a person exposed at age 30 (born in 1915). The solid-black line illustrates the fitted pure-quadratic dose response. The points are based on a nonparametric dose-response model, while the middle-dashed-gray line is a smoothed version of the dose category-specific estimates from the non-parametric fit. The upper- and lower-dashed-gray lines are plus and minus one standard error from the smoothed fit. Panels c and d: illustrate the temporal pattern and age-at-exposure effects for our preferred ERR model. Panels e and d: present the temporal pattern and age-at-exposure effects for Hiroshima males based on the preferred EAR model. Black lines are shown for ages at exposure of 10 (solid line), 30 (dash line) and 50 years (dash-dot line). The gray lines are the EAR temporal patterns using the model specified in a previous report (*4*). For the ERR and EAR models shown here, the excess risks did not depend on either gender or city.

ERR and EAR models are given in Table 6. Additionally, the precise form of this model is given in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1) and information on the fit of alternative models is given in supplementary Table S7 (http://dx.doi.org/10.1667/RR2892.1.S2).

The AML EAR was also described equally well (AIC = 1,550.0) using a model with a linear-quadratic effect in log attained age. This description of the temporal pattern of the AML, which is illustrated in Fig. 2e, is considerably simpler than the model used in the previous LSS leukemia incidence report (*4*). In that model, there were separate temporal patterns for each of three age-at-exposure groups, while all of the temporal variation excess rates in the current model are expressed in terms of age without the need for dependence on either age at exposure or time since exposure. Figure 2e and f shows the variation of the AML EAR with attained age and time since exposure based on the