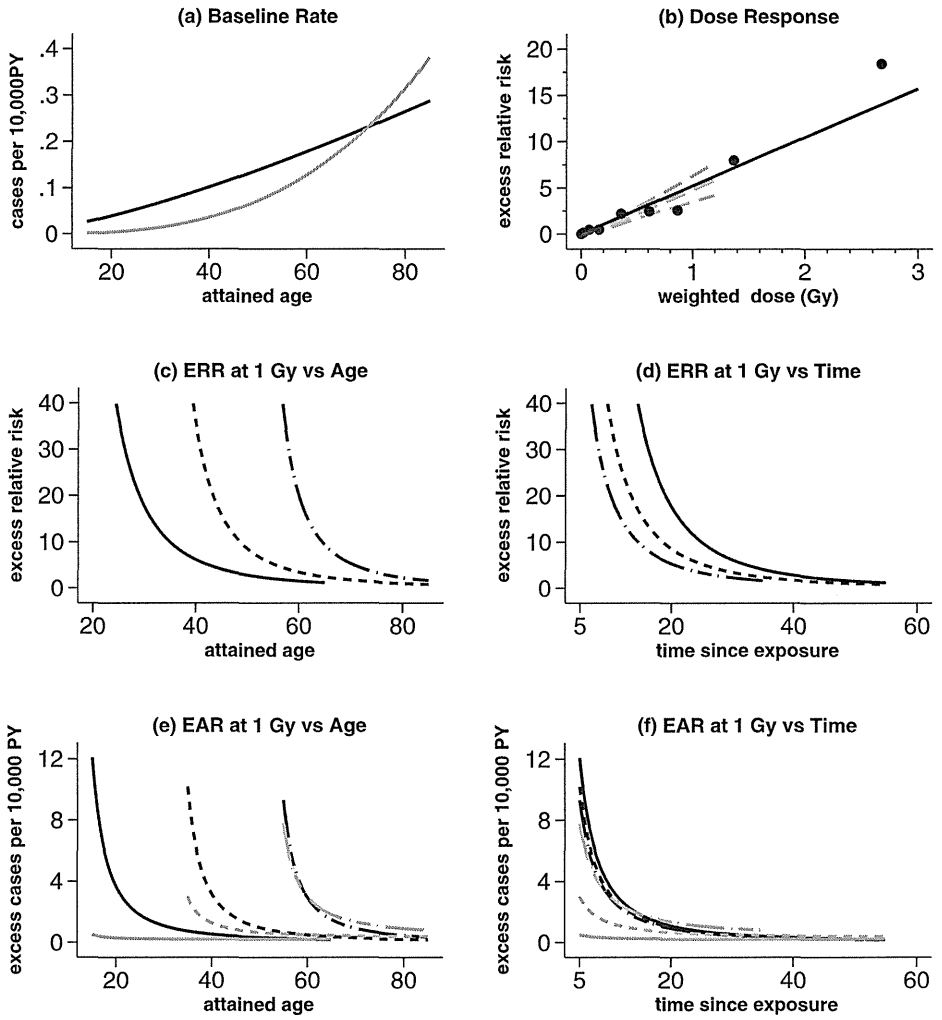

FIG. 4. LSS chronic myeloid leukemia risk summary plots. Panel a: shows age-specific Hiroshima baseline rate for LSS cohort members for men (black line) and women (gray line). Panel b: illustrates the radiation dose response based on the ERR model with gender average risks standardized to time since exposure at 25 and at attained age 55. The solid-black lines illustrates the fitted linear 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 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. Curves are shown for ages at exposure 10 (solid curve), 30 (dash line) and 50 years (dash-dot line) in Hiroshima. The ERR does not depend on gender. Panels e and f: present the temporal pattern and age-at exposure effects by gender based on the preferred EAR model in Hiroshima. Curves are shown for age at exposure 10 (solid line), 30 (dash line) and 50 years (dash-dot line) with black and gray lines for men and for women, respectively.

were identified including 10 cases classified as chronic lymphocytic leukemia and 2 as hairy cell leukemia. The newly identified cases included only one case among the not-in-city cohort members and 7 cases diagnosed after 1987. Using a simple age- and gender-adjusted baseline model, a significant linear dose response was detected, which suggested that CLL risk might be increased at higher doses ($P < 0.05$).

Adult T-Cell Leukemia (ATL), Nagasaki Only

There were a total of 47 eligible ATL cases. Due to the fact that there were only 5 ATL cases in Hiroshima, the analyses were limited to Nagasaki. The background rate exhibited a rapid increase with attained age that was proportional to the power 4.07 (95% CI 2.59–5.74, $P < 0.001$). Age-specific ATL rates in Nagasaki have changed significantly over time ($P = 0.01$), with rates estimated to have increased by about 34% for each decade increase in the year of birth (95% CI 7–71%). There were no statistically significant gender differences in the ATL baseline rates ($P > 0.5$). Figure 5 shows the increasing rates of ATL by age. The ATL baseline rate model and parameter estimates are given in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1).

TABLE 10
Observed and Fitted Background and Excess Cases of Chronic Myeloid Leukemia by Weighted Bone Marrow Dose Category

Dose (Gy)	Person years	Observed cases	Fitted cases†	
			Background	Excess
< 0.005	2,039,093	22	22.5	0.1
0.1	957,889	17	11.6	2.9
0.2	201,935	2	2.5	3.0
0.5	206,749	11	2.6	6.5
1	117,855	6	1.4	7.5
2	64,122	9	0.7	7.5
2+	25,761	8	0.3	5.9
Total	3,613,404	75	41.6	33.4

† Estimates based on the preferred linear ERR model described in the text and Table 11 with more details in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1).

Dose response and effect modification. There was no evidence of a dose response for ATL ($P > 0.5$) and the ERR/Gy in a linear model was estimated as 0.05 (95% CI −0.51–1.54). Because of a lack of cases among women who received high doses, the ERR/Gy for women was estimated to be les than zero. After restricting the excess risk for women to be zero, the fit of the model was improved and the ERR/Gy estimate for men was 0.88 (95% CI −0.60–4.52, $P = 0.28$), which was not statistically significant.

Lymphoma

The 437 eligible lymphoma cases include 402 NHL and 35 HL cases with 143 of these cases diagnosed since 1987 among cohort members who were in the cities at the time of the bombings. Another 102 cases were among cohort members who were not in the cities at the time of bombings. NHL and HL risks were analyzed separately.

Non-Hodgkin lymphoma (NHL). Background rates for NHL increased rapidly with attained age. While this increase was roughly proportional to attained age to the fourth power, the fit was significantly improved when the nature of the increase was allowed to vary with increasing age, with knots at age 40 and 70 ($P = 0.007$, with 3 *df*). Age-specific rates for women were 58% of those for men (95% CI 48–72%, $P < 0.001$) with no significant ($P > 0.5$) gender difference in the nature of the increase with attained age. NHL baseline rates also exhibited a complex nonlinear birth cohort effect ($P < 0.001$) with the highest age-specific rates for cohort members born around 1940 and lower age-specific rates for people born in earlier or later years. As shown in Fig. 6a, the age-specific baseline rates for the 1935 birth cohort were at least twice those for the 1895 birth cohort. This pattern was similar to that seen for the LSS leukemia baseline rates and for Japanese national NHL rates (*33*). There was no indication of a city difference in the baseline rates ($P > 0.5$). The NHL 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 no evidence of a significant dose response in the ERR ($P = 0.23$) in a simple linear dose-response model. However, when the ERR was allowed to differ for men and women, there was a suggestion of an elevated risk in men (ERR/Gy = 0.46; 95% CI −0.08 to 1.29, $P = 0.11$), but no indication of an effect for women (ERR/Gy = 0.02; 95% CI <–0.44 to 0.64, $P > 0.5$). Allowing the ERR for men to vary with attained age led to a significant improvement in the fit relative to a model with no radiation effects ($P = 0.005$, with 2 *df*). Thus, while there was some evidence of a statistically

TABLE 11
Preferred Model, Excess Risk Parameter Estimates for Chronic Myeloid Leukemia

Risk model		Linear dose coefficient (at 1 Gy)	City ratio (N:H)	Gender ratio (F:M)	Attained age (power)	Time since exposure (power)
ERR†	Hiroshima	5.24 (1.92, 11.8)	0.22 (0.03, 0.75)		−1.42 (−3.04, 0.01)	−1.59 (−2.34, −0.95)
	Nagasaki	1.17 (−0.10, 4.71)				
EAR‡	Female Hiroshima	0.57 (0.23, 1.10)	0.23 (0.03, 0.76)	0.84 (0.34, 2.21)	2.10 (0.48, 4.21)	−1.63 (−2.38, −0.97)
	Nagasaki	0.13 (<0, 0.47)				
	Male Hiroshima	0.68 (0.24, 1.49)			−0.20 (−1.03, 0.66)	
	Nagasaki	0.15 (<0, 0.60)				

† The preferred ERR model is linear in dose with log-linear effect modification depending on log (attained age), log (time since exposure) and city. The baseline model parameters and explicit details about the dose effect modification term are given in Table S2 in the supplementary material (http://dx.doi.org/10.1667/RR2892.1.S21). Supplementary Table S9 (http://dx.doi.org/10.1667/RR2892.1.S2) presents information on alternative ERR models. The dose coefficients describe the ERR at 1 Gy at age 55 after exposure at age 30.

‡ The preferred EAR model is linear in dose with log-linear effect modification depending on city, sex, log(time since exposure) and log(attained age) with the attained age effect differing in the two cities. The baseline model parameters and explicit details about the dose effect modification term is given in Table S2 in the supplementary material (http://dx.doi.org/10.1667/RR2892.1.S1). Supplementary Table S9 (http://dx.doi.org/10.1667/RR2892.1.S2) presents information on alternative EAR models. The dose coefficients describe the excess cases per 10,000 person years at 1 Gy at age 55 after exposure at age 30.

TABLE 12
Radiation-Associated Excess Leukemia Cases by Subtype and Period

	Leukemia subtype†						
	AML		ALL		CML		
Period	Excess‡	Percent§	Excess	Percent	Excess	Percent	Total
1950–1955	6.3	20%	8.3	27%	16.3	53%	30.9
1956–1960	4.3	29%	4.1	28%	6.2	43%	14.6
1961–1970	7.6	43%	4.5	25%	5.7	32%	17.8
1971–1980	6.8	57%	2.4	20%	2.7	23%	11.9
1981–1990	6.2	68%	1.4	15%	1.5	17%	9.1
1991–2001	6.1	78%	0.8	10%	0.9	12%	7.8
Total	37.3	41%	21.5	23%	33.3	36%	92.1

† The subtypes considered here are acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), and chronic myeloid leukemia (CML).

‡ Radiation-associated excess case estimates based on the preferred ERR models described in the text and in Tables 6, 9 and 11 with additional details given in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1).

§ Percentage of radiation associated excess cases within the time period.

significant radiation effect in men, there was no indication of a radiation effect in women.

As indicated by the dose-response curves in Fig. 6c, the ERR/Gy for men (dark solid curve) was large at younger ages, but declined dramatically and approached 0 by age 40. It is difficult to interpret the large ERRs for the very young since baseline rates are highly variable and imprecise due to the small number of children and young adults at risk for developing NHL. The EAR model provided a more simple and perhaps more useful description for small excess risks when baseline rates are low. There was a suggestion of a dose response ($P = 0.10$) in a simple constant EAR model. Letting the EAR depend on gender led to a significant improvement in the fit ($P = 0.048$). The estimated EAR for men was 0.54 (95% CI 0.09–1.32, $P = 0.003$) while that for women was essentially 0 (95% CI −0.02–0.31, $P > 0.5$). There was no indication that the EAR varied with attained age ($P = 0.3$), time since exposure ($P = 0.46$), or age at exposure ($P = 0.15$). Supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S21) contains information on the form of the final model than the parameter estimates for this model.

Figure 6d presents the fitted constant EAR for men (dark solid line) and the EAR estimate derived from the age-dependent ERR model (lighter dashed line) described above. While the EAR model suggested a persistent increase in risk, the ERR model suggested that the excess rate peaked around age 20 and that there was little excess after age 30, which also implies that the radiation effects were seen primarily among those exposed as children or young adults. Despite striking differences in the pattern of the excess risk, the goodness of fit of the ERR (AIC = 2,374.8) and EAR (AIC = 2,378.8) models was comparable. Based on the time-constant EAR model, the estimated number of radiation-associated cases over the follow-up period was estimated to be 7.8 for men and 0 for women (Table 13), with about 10% of the cases among men with doses in excess of 5 mGy attributable to the radiation exposure. The time-dependent ERR model predicts about half the number of excess cases as does the EAR model.

Hodgkin Lymphoma (HL)

There were only 35 eligible HL cases in the cohort. Age- and birth-cohort adjusted baseline rates in women were about 40% of those in men (95% CI 21–82%, $P = 0.01$). Baseline rates tended to increase with increasing attained age, but the increase was not statistically significant ($P = 0.35$). However, age-specific rates in later birth cohorts were significantly lower than those for earlier birth cohorts ($P = 0.003$). There was no indication of a city difference in the baseline rates ($P = 0.16$). HL baseline rates are plotted in Fig. 7. The HL baseline rate model and parameter estimates are described in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1).

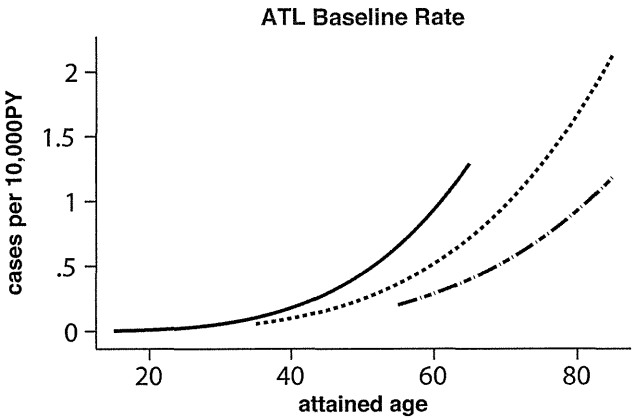

FIG. 5. LSS adult T-cell leukemia risk summary plots. The plot shows age-specific Nagasaki baseline rates (zero dose) for LSS cohort members born in 1895 (dash-dot line), 1915 (dash line) and 1935 (solid line). There is no significant dose response.

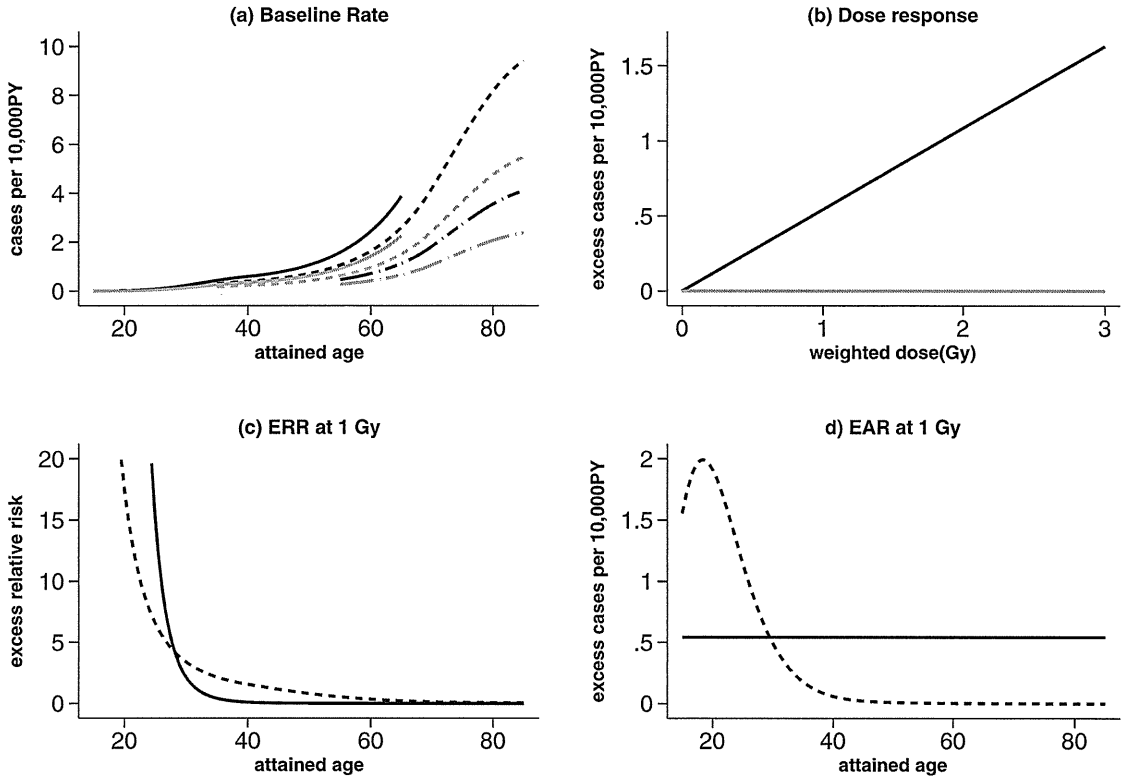

FIG. 6. LSS non-Hodgkin lymphoma risk summary plots. Panel a: shows age-specific Hiroshima baseline rates for LSS cohort members. Curves are shown for birth cohorts 1895 (dash-dot line), 1915 (dash line) and 1935 (solid line) model with black and gray lines for men and for women, respectively. Panel b: illustrates the radiation dose response based on the EAR model with black and gray lines for men and for women, respectively. Panel c illustrates the ERR temporal pattern for men. The solid line shows the predicted ERR based on our preferred ERR model, and the dotted line is the ERR derived from our preferred EAR model. Panel d: presents the EAR temporal pattern for men. The dotted line shows the predicted EAR based on our preferred EAR model and the solid curve is the EAR derived from our preferred ERR model.

Dose response and effect modification. No significant dose response was found for HL (ERR/Gy = 0.20; 95% CI −1.03–2.63, $P > 0.5$). Allowing the dose response to depend on gender did not improve the fit of the model ($P > 0.5$), nor was there any indication of statistically significant variation in the ERR with age ($P > 0.5$). The estimated time constant EAR was essentially 0 ($P > 0.5$).

Multiple Myeloma (MM)

Among the 181 cases of MM identified in this study, 136 were eligible for use in the risk analyses including 36 cases diagnosed in survivors after 1987 and 31 cases diagnosed among cohort members who were not in the cities at the time of the bombings.

Baseline rates varied significantly with both attained age ($P < 0.001$) and birth cohort ($P < 0.001$). The birth cohort effect appeared to be non-monotonic, with the largest age-specific rates seen for people born around 1920 and decreasing by about 35% per decade for earlier and later birth cohorts. This pattern was generally similar to that seen in leukemia other than CLL or ATL in this cohort. The increase in rates was roughly proportional to attained age to the power 5.45 (95% CI 4.41–6.55, $P < 0.001$). However, the model was significantly improved ($P = 0.01$) when the baseline rate was allowed to increase to about age 80 and level out, or even decrease later in life (Fig. 8).

TABLE 13
Observed and Fitted Background and Excess Cases of Non-Hodgkin Lymphoma Cases by Weighted Bone Marrow Dose Category

Dose (Gy)	Person years	Observed cases	Fitted cases†	
			Background	Excess
<0.005	2,039,093	226	221.6	0.02
–0.1	957,889	99	104.2	0.6
–0.2	201,935	21	22.7	0.6
–0.5	206,749	28	22.9	1.3
–1	117,855	13	13.2	1.7
–2	64,122	14	7.1	2.0
2+	25,761	1	2.5	1.6
Total	3,613,404	402	394.2	7.8

† Estimates based on the preferred linear EAR model described in the text with additional details in supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1). This is gender-dependent, time-constant linear EAR model with an EAR of 0.54 cases per 10,000 PY at 1 Gy and no excess risk for women.

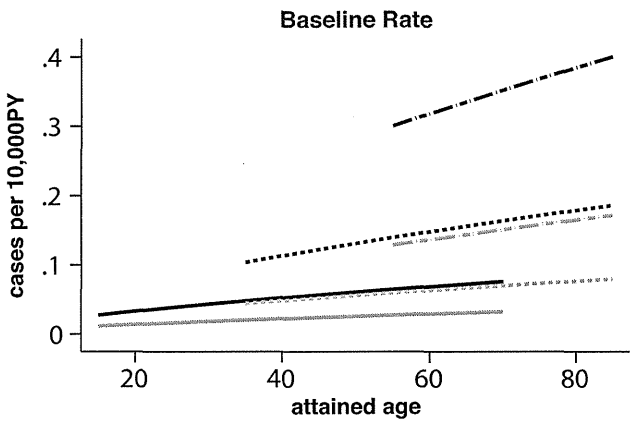

FIG. 7. LSS Hodgkin lymphoma. The plot shows age-specific baseline rates (zero dose) for LSS cohort members born in 1895 (dash-dot line), 1915 (dot line) and 1935 (solid line). The black lines are for men and the gray lines are for women. There is no significant dose response.

The background risk did not vary significantly with either gender ($P = 0.16$) or city ($P = 0.12$). Supplementary Table S2 (http://dx.doi.org/10.1667/RR2892.1.S1) contains information on the form of the final baseline rate model with the associated parameter estimates.

Dose response and effect modification. The ERR/Gy estimate of 0.38 from a linear dose-response model was not statistically significant (95% CI −0.23–1.36, $P = 0.21$) The fit was not improved by the addition of a quadratic term ($P = 0.44$). There was no evidence of statistically significant variation in the ERR with gender ($P > 0.5$), attained age ($P = 0.31$) or age at exposure ($P > 0.5$).

When the data were described using a constant linear EAR model, the estimated EAR was 0.07 cases per 10,000 PY per Gy (95% CI <–0.05–0.29, $P = 0.25$), with no indication of variation with gender ($P = 0.5$), attained age ($P = 0.33$) or time since exposure ($P > 0.5$). This point estimate of the EAR is almost identical to that reported 15 years ago. As in the previous report, we conclude that there is no evidence of a statistically significant radiation-associated excess risk of incident MM in the atomic bomb survivors.

DISCUSSION

Our study extends the follow-up 14 years beyond that used in the last major report on hematological malignancies in the LSS cohort (*4*), providing incidence follow-up for 55 years. This has been made possible because of the active ascertainment and review of hematopoietic malignancies already in place when the cohort was established in late 1950, together with the subsequently established tumor registries in Hiroshima and Nagasaki. Due to incomplete ascertainment of incident cases among cohort members who moved away from Hiroshima or Nagasaki, a probabilistic adjustment for migration was included in the analyses as with all other major LSS incidence reports. Diagnostic criteria and type definitions for hematopoietic malignancies have evolved and been refined over time, but the reclassification of leukemia types for cases diagnosed before the mid-1980s using the recent classifications has enabled us to use diagnostic categories that are consistent with and generally as detailed as those used in other studies of radiation effects on these malignancies.

In contrast to the earlier report (*4*) that focused exclusively on time-since-exposure dependent EAR models with, in most cases, categorical age-at-exposure effects, we assessed excess risk in this report using ERR and EAR models with simple continuous functions of age at exposure and either attained age or time since exposure. We found that those smooth models describe the data as well as the partially categorical models used in ref. (*4*). We also found that while both ERRs and EARs for leukemia other than CLL or ATL as a group have declined over the follow-up period for all ages at exposure (Fig. 1d and f), the excess risks do not appear to have fallen to zero by the end of the follow-up—55 years after exposure. The temporal variation of either the ERR or EAR as described in terms of (post-

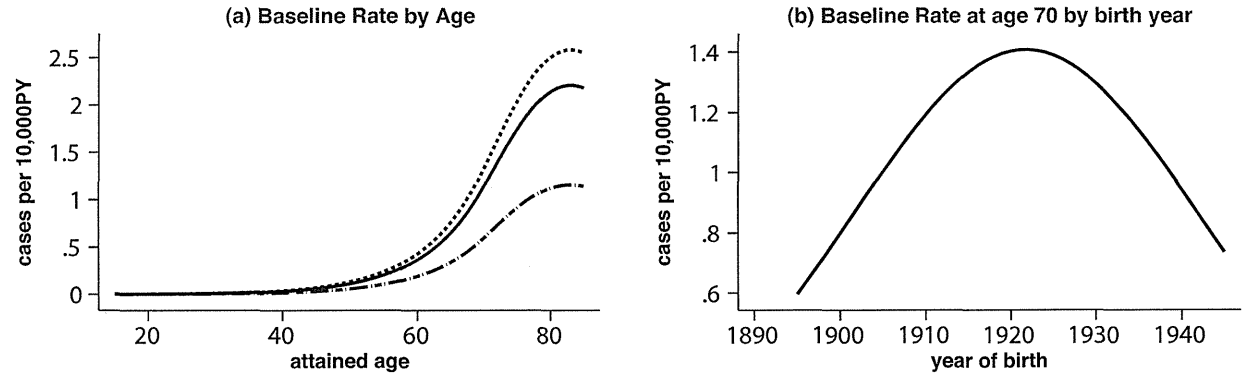

FIG. 8. LSS multiple myeloma summary plots. Panel a: shows age-specific baseline rate (zero dose) for LSS cohort members born in 1895 (dash-dot line), 1915 (dash line) and 1935 (solid line). Panel b: shows the baseline rate by birth year at attained age 70.

TABLE 14
Comparison of Risk Estimates to Selected Cohort Studies

Study cohort	Cancer end point	Follow-up	Cases	Average dose (range)	Dose response model	ERR: Leukemia excluding CLL (95% CI)
3rd NRRW	incidence	1955–2001	234	0.025 Sv (>0, 0.1+)	Linear	1.78 (0.17, 4.36)
A-bomb LSS cohort† (males, 20<=age at exposure <60)	incidence	1950–2001	93	0.50 Gy (0, 3.16)	Linear	2.04 (0.33, 6.85)
Techa River cohort	incidence	1953–2005	70	0.3 Gy (0, 2.0)	Linear	4.9 (1.6, 14.3)
A-bomb LSS cohort‡	incidence	1950–2001	312	0.64 Gy (0, 4.54)	Linear	2.78 (1.84, 4.01)

† The analysis is restricted to men who were exposed to A-bomb radiation at age 20–60 with weighted bone marrow dose ≤4 Gy. The ERR coefficient describes the excess RR at 1 Gy at age 50 after exposure at age 25.

‡ This analysis is for the full LSS cohort excluding unknown dose. The ERR coefficient describes the excess RR at 1 Gy at age 60 after exposure at age 25.

exposure) attained age highlights that, while highest excess risks are seen shortly after exposure for those exposed as children, at any given attained age the excess risks are generally higher for those exposed later in life (Fig. 1c and e). In addition, the NIC group was used in this study to augment the information on the variation in baseline rates. When all leukemias (or subtypes) other than CLL/ATL were examined, the inclusion or exclusion of the NIC group did not substantially change the risk estimates.

Richardson *et al.* (*7*) recently considered an ERR model for the LSS leukemia mortality in which the temporal variation was described in terms of an age-at-exposure dependent log cubic spline in time since exposure. That model (AIC = 2,445.6) did not describe the leukemia incidence data as well as did our preferred ERR model (AIC = 2,431.9).

In the BEIR VII study (*36*) and some other recent work on the LSS leukemia mortality data (*7*), the age-at-exposure effect on the excess risks was not allowed to vary among people who were more than 30 years old at the time of exposure. Imposing this constraint on our models resulted in a markedly poorer fit (AIC = 2,438.4 for the constrained model versus 2,431.89 for our preferred leukemia other than CLL or ATL model).

A major focus of this report was on the leukemia other than CLL or ATL as a group, which includes data on several different types of leukemia. To the extent that the cell-type distribution of the cases in this group in the LSS resembles that of many non-Japanese populations, the LSS risk data for this group can be useful for prediction or comparison with the radiation-related risk of leukemia other than CLL in other irradiated populations. Carrying out such additional analyses, we found that the predicted ERRs in the LSS cohort are consistent and comparable to those estimated in the studies of the National Registry of Radiation Workers (*37*) and the Techa River Cohort (*38*). Table 14 gives the comparison of risk estimate of the LSS cohort based on a relevant subset to the aforementioned cohorts.

Another focus was to characterize the radiation-related risk for specific types of leukemia. Leukemias other than CLL or ATL as a group, as well as ERRs for AML, ALL and CML considered separately, declined over time for all age groups. The temporal patterns in the type-specific EAR estimates are more complicated. Our analyses suggested that AML excess rates may have increased slightly over time, especially among people exposed after about age 30, while the EARs for ALL and CML have decreased over time. Those declines have often been interpreted as suggesting that the leukemia excess rates among the exposed returned to baseline levels 10–20 years after exposure. However, the analyses in this paper indicated that this is not the case for AML and ALL, as there was evidence of persistent risks 30 to 50 years after exposure for each of these types.

Cell-type specific leukemia risks and especially how they are modified by age and time, may be more useful in considering possible biological mechanisms for radiation-associated leukemogenesis than from analyses of a heterogeneous group of several types of leukemia combined. Some striking contrasts were noted between the type-specific excess rates. First, while AML exhibited a nonlinear upward-curving dose response, there was no evidence against linearity for either ALL or CML. Also, as noted above, AML excess rates tended to increase with increasing attained age for those exposed as adults, while ALL and CML excess rates appeared to decrease over time. While the radiation-related risk of CML seemed to decline with both age at exposure and time since exposure, the ALL excess rates decreased with attained age, but did not vary with either age at exposure or time since exposure.

The data suggest that the radiation associated excess rates for AML follow a U-shaped pattern in age, with excess rates falling for the youngest cohort members (who had to have been exposed early in life) and then increasing with attained age regardless of age at exposure. Although the current preferred EAR model for AML is much more simple than the 1994 model that involved complex interactions between age at exposure and time since exposure, the variation in the fitted excess rates with age are quite similar as shown in Fig. 2e and f. The temporal patterns for the ALL and CML excess rates differ significantly from those seen for AML. In

particular, excess rates for both ALL and CML, which were considerably greater than those for AML in the period shortly after the bombings, have decreased markedly over time. Thus, as indicated in Table 12, ALL and CML cases were the most common radiation-associated types and account for about 75% of the excess cases during the first 5 years of follow-up, i.e., years 5–10 after radiation exposure. If radiation markedly increased leukemia risks prior to 1950, as seems especially likely for ALL and CML, then the proportion of the radiation-associated excess ALL and CML cases in the first decade after exposure is likely to be even more striking than that which was suggested by the available data.

As the cohort members have aged, AML has become the predominant radiation-associated leukemia, accounting for over 80% of excess leukemia cases during the last 15 years of follow-up. It has been suspected that leukemia may be induced by specific translocations caused by radiation. However, spontaneous translocations specific to ALL are much more frequent than AML cases bearing the translocations. In addition, radiation-induced DNA damage is essentially random in the genome. Based on these observations, Nakamura (*39*) recently speculated that the radiation-related ALL risk in a population is almost entirely attributable to a small number of predisposed individuals in whom translocation-carrying pre-ALL cells have accumulated. He suggested that the short latency period for radiation-related ALL risk at young ages may be due to the small number of events needed for the conversion of pre-ALL cells present in newborns to full malignancy. Although CML occurs primarily in the elderly, the temporal risk pattern for CML is similar to that of ALL. BCR/ABL fusions have been linked to the majority of CML cases, but are also common in the general population (*40*). It may be that a very few additional mutations are required to convert the translocation-bearing pre-leukemic cells to tumor cells. Nakamura suspected the possibility of two different mechanisms: one for young-onset AML, which is clonally expanded similar to ALL, and the other for non-translocation-type AML, which like solid cancers, predominates in middle- and older-aged individuals, requiring a multi-step leukemogenesis process. The temporal patterns that we find for these three leukemia subtypes are generally consistent with Nakamura's hypothesis.

A recent study of myelodysplastic syndrome (MDS) risks among Nagasaki survivors for the period from 1985–2004 (*41*) showed a significant excess risk of MDS with an ERR/Gy estimate of 4.3 (95% CI 1.6–9.5), which is about twice the AML ERR estimate for the post-1986 period. People with MDS are known to have a higher risk of developing AML. However, it is of interest to note that the ERR for MDS after 1985 is larger than that for AML and that the shape of the dose response is quadratic for AML, but approximately linear for MDS. Further data on MDS incidence among LSS Nagasaki survivors and among Hiroshima survivors would help to better understand the relationship between MDS and AML excess risks.

It is likely that some cases identified as AML in the early years would have been classified as MDS if they were diagnosed with modern criteria. However, we feel that such misclassification is unlikely to have much impact on the inferences about AML risk estimates in the LSS, since (1) AML cases prior to the mid-1980s identified as MDS in the FAB review (*18*) were not used in the current analyses, (2) misclassification is less likely in the more recent years since MDS was recognized as a distinct condition, and (3) the AML baseline risk model includes a birth cohort effect that reduces the impact of period-specific misclassification. Any misdiagnosis of MDS as AML cases is likely to be independent of dose in which case it would not affect the ERR estimate of AML risk, though it would tend to increase the EAR estimate.

Chronic lymphocytic leukemia is very rare in Japan. Due to the small number of CLL cases in the LSS, radiation effects on CLL have not been considered in previous LSS reports. Although there were only 12 eligible cases, four of which occurred among survivors with doses in excess of 0.2 Gy, a simple trend test suggested a statistically significant dose response. Our results are consistent with findings of some, though not all, studies in the literature. A recent case-control study of leukemia in Chernobyl clean-up workers in Ukraine (*29*) and another such study in Belarus, Russia and Baltic countries (*42*) have shown increased risk of both CLL and leukemia other than CLL associated with radiation exposure. Similarly, a significantly increased risk of CLL as well as non-CLL leukemia was associated with radon and/or gamma radiation exposures in uranium miners (*28*). Conversely, no evidence of radiation-associated increases in the risk of CLL were seen in various other studies including the 15-country nuclear worker study (*43*), the Techa River cohort (*38*), the Mayak worker cohort (*44*), or UK radiation workers (*37*). The suggestion of a radiation effect on CLL risks in the LSS should be interpreted with caution and generalization to other populations may be unwarranted. In most Western populations, CLL accounts for 20% or more of all leukemia cases and an even higher proportion of leukemias seen late in life, but accounts for only about 3% of the LSS cases. Clinical data suggest that Japanese CLLs are largely dormant and genetically and biologically different from nonsmoldering CLLs seen in Western populations.

The large proportion of ATL in Nagasaki is not surprising. As reported in a nationwide study on ATL in Japan, ATL is endemic in Nagasaki (*45*). In the Nagasaki LSS, 42 out of 66 leukemia incident cases (63.6%) were diagnosed with ATL. There is no evidence of a radiation effect for ATL among Nagasaki LSS subjects, which may be due to the unusually high HTLV-I infections in the region.

The evidence for radiation effects on the risks of the other types of hematopoietic malignancies considered was less

clear cut. While there is no evidence of a radiation effect or dose response for HL or MM, the finding of a significant radiation-associated increase in NHL risks for men with no evidence of a radiation effect for women is similar to what has been reported in earlier analyses of NHL incidence (*4*) and mortality in the LSS (*46*). In the recent mortality analysis, Richardson *et al.* (*46*) considered ERR models for NHL mortality among LSS men who were 15–64 years old at the time of exposure and found a significant association that was most prominent 35 or more years after exposure. This is quite different than what is seen for the incidence data in which the ERR declines markedly with age (and hence time since exposure) whether or not the analysis is restricted to working age males. In our view, at best the LSS incidence data provide rather weak support for an NHL dose response among men with no evidence to support the idea that the risks are increasing with attained age or time since exposure.

Some early reports on myeloma mortality and incidence in the LSS have shown a statistically significant dose response for MM. The reasons for the differences between the findings of the incidence analyses and these earlier analyses were discussed in (*4*). To understand the difference, we also carried out an analysis of MM mortality in the cohort used for the current study. There were 111 MM deaths during the follow-up period including 86 MM cases used in the incidence analyses, and another 19 cases that were not used in the incidence analyses because they were either second primaries (10 cases) or not resident in the catchment area at the time of diagnosis (9 cases). Four of the remaining 6 cases had been rejected by the registries, while the other 2 cases had reports of solid cancers (both reported as renal cell carcinoma) with no indication of MM or any other hematopoietic malignancies. There was no evidence of statistically significant increases in either the ERR ($P = 0.12$) or the EAR ($P = 0.3$) with dose. The ERR and EAR point estimates were similar to those given above for the incidence data. This difference is largely due to uncertainties about the diagnosis of a small number of high-dose cases for which MM reported as the cause of death was not confirmed by the in-depth hematological review conducted for the incidence study. Taken together, the data provide little, if any, evidence of a radiation effect on MM. Although some worker studies including the most recent analysis of UK National Registry for Radiation Workers (*37*) and a study based on records from the National Dose Registry of Canada (*47*) provide some suggestion of a dose response for MM, the dose-response trends seen in those studies were not statistically significant. Results for MM mortality using extended follow-up are discussed in the latest LSS mortality report (*48*).

Increases in leukemia risks were one of the earliest significant long-term health effects detected among the atomic bomb survivors. However, in view of the declining radiation-related risk over the years, it has been thought that the radiation-associated excess risks would disappear over time. The present analyses that included information on cases diagnosed 43–56 years after exposure have provided important new insights into the persistent nature of the leukemia risks. The data suggest that, while radiation-associated excess risks for ALL and CML among the exposed have essentially returned to baseline levels by the end of follow-up, AML risks have persisted with excess rates that appear to be increasing with attained age (albeit not as rapidly as the baseline rates). It seems likely that the excess AML risks will persist throughout lifetime for people exposed at any age. This is similar to the temporal pattern for solid cancers. Since 40% of the cohort including most of those exposed as children, were still alive at the end of follow-up in this study, with continued follow-up of the LSS and evolving analytical methods, we expect that further insights will be gained into radiation effects on the leukemia and other hematopoietic malignancies.

APPENDIX

The online appendix tables include a listing of the morphological codes for the subtype groups and detailed descriptions of the parameterizations and parameter estimates for the preferred baseline and excess rate (ERR and EAR) models along with tables that describe (1) the impact of migration adjustment on person years (by city, gender, birth cohort and time period), (2) crude rates (by birth cohort, time period and dose category) and (3) alternative models for leukemia other than CLL or ATL, AML, ALL and CML (http://dx.doi.org/10.1667/RR2892.1.S3).

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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 ($Pao_2 \geq 60$ mm Hg or $SpO_2 \geq 93\%$), 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

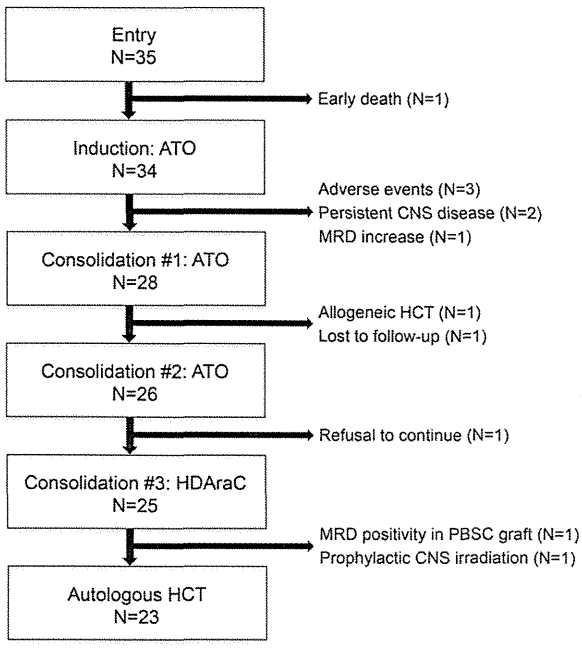

Figure 1. Patient flow diagram. HDAraC, high-dose cytarabine; MRD, minimal residual.

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

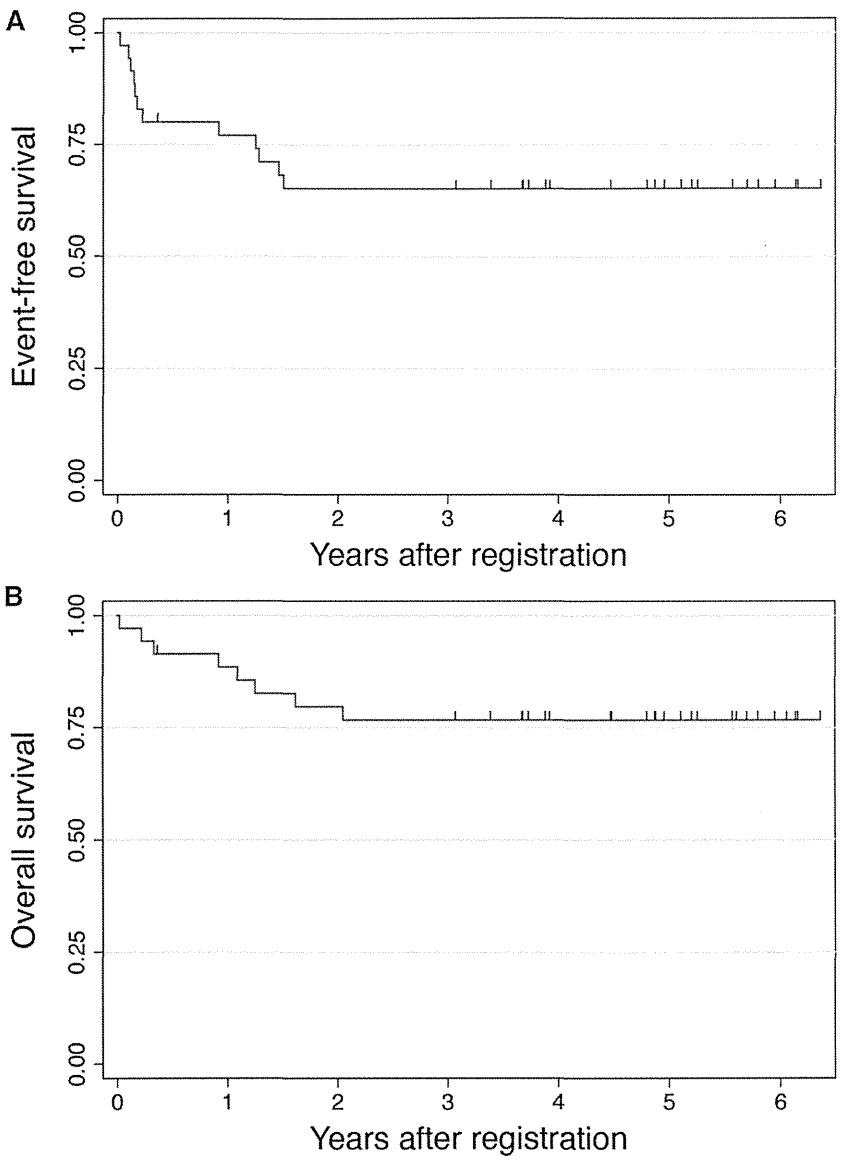

Figure 2. Kaplan-Meier curves for EFS (A) and OS (B). The probabilities of EFS and OS for the entire cohort (N = 35) were 65% and 77% at 5 years, respectively.

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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ORIGINAL ARTICLE: CLINICAL

Clinical evaluation of WT1 mRNA expression levels in peripheral blood and bone marrow in patients with myelodysplastic syndromes

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Abstract

A study to evaluate WT1 mRNA expression levels in peripheral blood (PB) and bone marrow aspirate (BM) was conducted in 172 patients, including 115 with myelodysplastic syndromes (MDS), in Japan. The level of WT1 mRNA expression was evaluated according to the French–American–British (FAB) and World Health Organization (WHO) classifications (2001, 2008) and using the International Prognostic Scoring System and the WHO Prognostic Scoring System scales. WT1 mRNA expression levels in PB and BM were well correlated ($r = 0.85$), and they tended to increase with disease stage progression and in those at higher risk of leukemic transformation. WT1 mRNA expression can be a useful marker for the diagnosis and risk evaluation of MDS.

Keywords: Myelodysplastic syndromes, WT1 mRNA expression, classification system, peripheral blood, bone marrow

Introduction

Myelodysplastic syndrome (MDS), a clonal disorder of pluripotent hematopoietic stem cells, is a blood disease characterized by dysplasia and ineffective hemopoiesis. Approximately 20–30% of cases of MDS undergo transformation to acute myeloid leukemia (AML) [1].

The expression of Wilms' tumor gene (WT1) has been found to be a new prognostic factor and marker for the detection of minimal residual disease (MRD) in acute leukemia, including AML and acute lymphocytic leukemia (ALL) [2]. A recent study has revealed the clinical relevance of measuring WT1 mRNA for monitoring MRD in AML, primarily due to its high rate of expression (93.9%) in the peripheral blood (PB) of incipient untreated patients with AML, secondarily due to its ability to predict relapse after complete remission (CR), and finally because its levels after consolidation therapy

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show a significant correlation between disease-free survival, overall survival and early relapse [3]. WT1 mRNA expression occurs not only in AML but also in the PB and bone marrow (BM) of patients with MDS [4-9].

Tamaki *et al.* [4] examined the level of WT1 mRNA expression in PB and BM from 57 patients with MDS grouped by the French-American-British (FAB) classification, and 12 patients experienced AML-MDS progression. The results revealed that WT1 mRNA expression in both PB and BM progressively increased with disease stage progression, from refractory anemia (RA), refractory anemia with excess of blasts (RAEB), refractory anemia with excess of blasts in transformation (RAEB-t), and to AML, suggesting the possibility that the WT1 mRNA expression level reflects the disease stage progression of MDS. Particularly, the patient group who developed leukemia from RAEB or RAEB-t within 6 months showed significantly higher WT1 mRNA expression in PB compared with the group who did not [4].

In accordance with that study, Cilloni *et al.* [6] measured WT1 mRNA expression levels in PB and BM from 131 patients with MDS, and found that: (1) WT1 mRNA expression in PB and BM was confirmed in 78% and 65% of patients with RA, respectively; (2) WT1 mRNA expression in PB and BM was confirmed in all patients with RAEB and secondary AML; (3) the level of WT1 mRNA expression increased with disease stage progression; and (4) the WT1 mRNA expression level was well correlated with the International Prognostic Scoring System (IPSS) scores established by Greenberg *et al.* [10].

In addition to the IPSS, the World Health Organization (WHO) Classification-Based Prognostic Scoring System (WPSS) has been proposed as a prognostic scoring system for MDS [11]. The WPSS consists of three characteristics: WHO subtype classification, considered to be important as a prognostic factor; IPSS-based karyotype abnormalities; and transfusion dependency.

Both the IPSS and WPSS require a chromosomal test as a primary parameter. However, because there are cases in which chromosomal abnormalities cannot be determined [12-14], it is necessary to establish molecular- and genetic-based methods to diagnose and determine the prognosis of MDS. The relatively rapid quantitation of WT1 mRNA is considered to be a useful test to determine the prognosis of MDS and has potential for clinical application, to become a novel marker to complement the current IPSS and WPSS criteria. We performed a clinical study in patients with MDS to demonstrate the usefulness of measuring the WT1 mRNA expression level in PB and BM in the diagnosis and treatment of MDS.

Patients and methods

This study was conducted in accordance with the Declaration of Helsinki, and preliminary approval was obtained from the Institutional Review Board or equivalent organization of each participating institution. Explanations of the study protocol were provided to all patients, and written informed consent was obtained from them before study enrollment.

Patients

From December 2008 to September 2009, 175 patients with MDS, suspected MDS and AML-MDS examined at 17 Japanese medical institutions were enrolled in the study. The subjects were 20 years of age or older and entered in the study regardless of gender, inpatient/outpatient status, or presence or absence of treatment. The 175 patients comprised 106 men (age range 27-88 years, average 65.5 years) and 69 women (age range 22-85 years, average 64.5 years). PB and BM samples from each patient were collected on the same day and used for WT1 mRNA measurement. Three of the 175 enrolled patients were excluded because BM could not be collected due to a dry tap or because the subtype could not be diagnosed. A total of 172 patients were therefore included in the final analysis set.

Diagnosis

Diagnosis of MDS was carried out using a central review format based on the FAB classification [15], the 2001 WHO classification [16] and the 2008 WHO classification [17]. Central review of the bone marrow smear-stained specimens, blood smear-stained specimens, iron-stained specimens, and clot hematoxylin and eosin-stained specimens was carried out by two individuals, one each in the Department of Hemato-Oncology, Saitama International Medical Center, Saitama Medical University, and the Department of Laboratory Medicine, Kawasaki Medical School.

WT1 mRNA measurement method

mRNA was extracted from PB leukocytes and BM nucleated cells at SRL, Inc., Tokyo, Japan using the RNeasy Mini-Kit (Qiagen, Valencia, CA), and the amount containing WT1 mRNA was measured at the Research Laboratory, Diagnostic Division, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan using a WT1 mRNA Assay Kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). cDNA was synthesized from 1 μg of extracted RNA in a reverse-transcription reaction using random hexamer primers. The amounts of WT1 and GAPDH (glyceraldehyde 3-phosphate dehydrogenase) mRNA were quantitated using real-time polymerase chain reaction (PCR) with a COBAS TaqMan48 analyzer (Roche Diagnostics, Pleasanton, CA), and the respective amounts of WT1 and GAPDH RNA in the sample were calculated by simultaneous reaction with standards of known concentrations.

Method for calculating WT1 mRNA expression

mRNA of the universally expressed housekeeping gene GAPDH was used for correction of variations in the efficiencies of RNA extraction and reverse transcription. As shown in the following formula, the level of WT1 mRNA expression was calculated by dividing the measured amount of WT1 mRNA by the measured amount of GAPDH mRNA and multiplying that value by the average number of copies of GAPDH mRNA found in 1 μg of RNA from PB leukocytes of healthy adults (GAPDH mRNA expression). The average GAPDH mRNA expression in PB leukocytes of healthy adults was reported to be 2.7×10^7 copies/μg RNA based on independent tests in healthy adults [3].