Table 5
Transplantation Outcome

	AML (n = 91)	ALL/LBL (n = 51)	ATL (n = 68)
Alive (dead)	48 (43)	33 (18)	25 (43)
Cause of death			
Bacterial infection	3	-	6
Fungal infection	3	-	2
CMV disease	2	-	2
Viral infection other than CMV	-	1	2
Disease progression	24	12	21
GVHD with or without any infection	5	2	4
Bleeding	1	-	2
Organ failure without any infection	5	3	3
Secondary malignancy	-	-	1

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; LBL, lymphoblastic lymphoma; ATL, adult T cell leukemia/lymphoma; CMV, cytomegalovirus; GVHD, grant-versus-host disease.

showed a better OS than without cGVHD. Episode of fungal infection provided a negative impact on OS for the AML (P = .0015) and ALL/LBL groups (P = .0459). Episodes of bacterial infection (P = .0102) and CMV infection (P = .0184) had a significant negative impact in the ATL group (Figure 2B, C). In CMV-seropositive patients of each of the 3 groups, episode of CMV infection negatively affected survival with a borderline difference in the ATL groups (P = .0615), but there was no relationship in the AML and ALL/LBL groups (P = .4680 and P = .4620, respectively).

There was no significant relationship between the difference of institution and OS in each of the 3 groups. For the ATL group, neither the use of anti-thymocyte globulin in the conditioning regimen nor the HTLV-1 serostatus of donor was associated with survival.

Multivariate Analysis for Survival

Multivariate analysis in all 210 patients revealed 6 factors that adversely affected OS: ATL (HR 1.944; 95% CI: 1.204 to 3.141, P = .0066), older age (HR 2.204; 95% CI 1.364 to 3.562, P = .0012), nonremission (HR 3.153; 95% CI 2.041 to 4.868, P < .0001), bacterial infection (HR 2.121; 95% CI 1.267 to 3.550, P = .0042), fungal infection (HR 2.718; 95% CI 1.507 to 4.901, P = .0009), and myeloabrative conditioning (HR 2.064; 95% CI 1.149 to 3.707, P = .0154).

To clarify the distinct unfavorable features in ATL groups, we also performed multivariate analysis for survival respectively in each of the 3 groups (Table 6). There were 4 factors that adversely affected OS in the AML group: patient age (≥ 42 years; HR 2.283; 95% CI: 1.164 to 4.476, P = .0163), lack of CR at transplantation (HR 2.975; 95% CI: 1.285 to 6.888, P = .0109), the existence of aGVHD (grade II-IV) (HR 1.731; 95% CI: 1.327 to 2.258, P < .0001), and episodes of fungal infection (HR 3.934; 95% CI: 1.357 to 11.406, P = .0117). In the ALL/LBL group, 2 factors were associated with worse

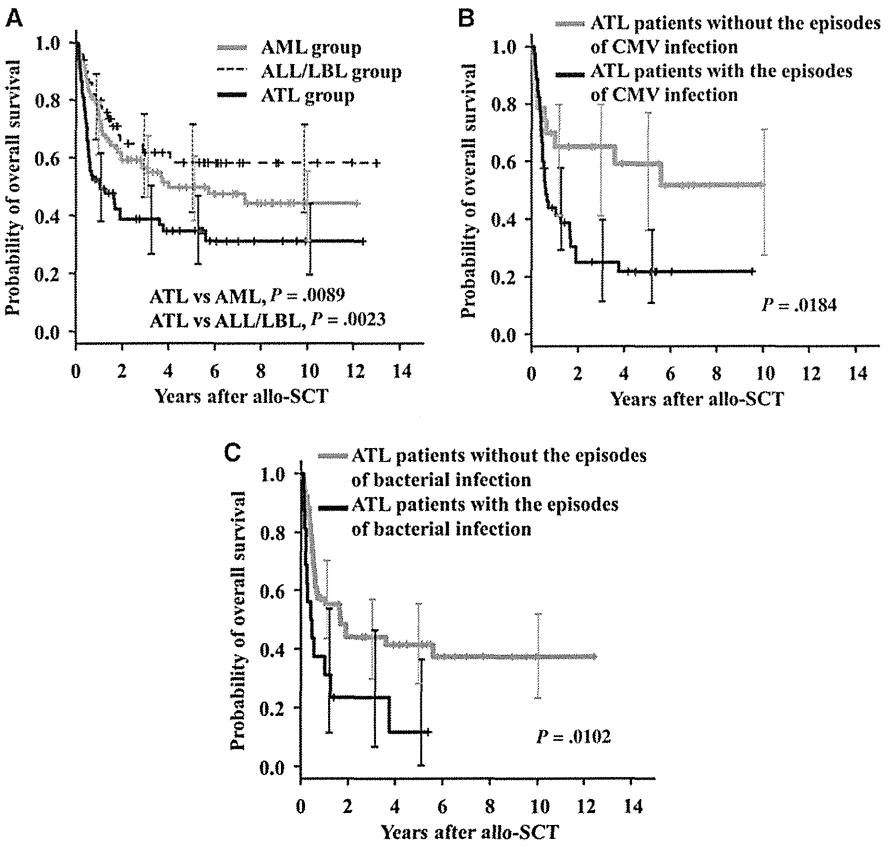

Figure 2. Kaplan-Meier estimate of overall survival after transplantation. The 95% confidence intervals at 1, 3, 5, and 10 years are shown in each overall survival (OS). (A) OS of the adult T cell leukemia/lymphoma (ATL) group was significantly worse than that of either the acute myeloid leukemia (AML) or acute lymphoblastic leukemia/lymphoblastic lymphoma (ALL/LBL) group. (B) In ATL group, the OS of patients with episodes of cytomegalovirus (CMV) infection was significantly worse than those without. (C) In the ATL group, the OS of patients with episodes of bacterial infection was significantly worse than those without.

Table 6
Multivariate Analysis of Risk Factors for Overall Survival

	AML		ALL/LBL		ATL	
	P Value	Hazard Ratio	*P* Value	Hazard Ratio	*P* Value	Hazard Ratio
Age ≥ median age	$P = .0163$	2.283	Not selected		$P = .3487$	1.363
Without CR or PR at allo-SCT	$P = .0109$	2.975	$P = .0058$	3.874	Not selected	
aGVHD (grade II -IV)	$P < .0001$	1.731	Not selected		Not selected	
Unrelated BM	Not selected		Not selected		$P = .0248$	2.568
Cord blood	Not selected		Not selected		$P = .8645$	0.936
CMV infection	Not selected		Not selected		$P = .0171$	2.514
Invasive fungal infection	$P = .0117$	3.934	$P = .0448$	3.430	Not selected	

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; LBL, lymphoblastic lymphoma; ATL, adult T cell leukemia/lymphoma; CR, complete remission; PR, partial remission; GVHD, grant-versus-host disease; BM, bone marrow; CMV, cytomegalovirus.
In the ATL group, episodes of CMV infection significantly correlated with worse overall survival. However, there was no such association in either the AML or ALL/LBL group.

survival: lack of CR at transplantation (HR 3.874; 95% CI: 1.481 to 10.134, $P = .0058$) and episodes of fungal infection (HR 3.430; 95% CI: 1.029 to 11.435, $P = .0448$). In the ATL group, 2 factors that differed from those in the AML or ALL/LBL group had a negative impact on OS with significance: the use of unrelated bone marrow (HR 2.568; 95% CI: 1.127 to 5.854, $P = .0248$) and episodes of CMV infection (HR 2.514; 95% CI: 1.178 to 5.366, $P = .0171$). Although the difference of institution was analyzed in multivariate analysis as a factor, this difference was not selected as a factor in each of 3 groups.

DISCUSSION

The success or failure of allo-SCT is mostly determined in the first 6 months after allo-SCT. Outcome closely correlates with the reconstitution of donor cell derived immunity, which affects the survival of recipients through GVHD and the graft-versus-leukemia effect, and the degree of immune competence achieved against infectious agents. Recently, some reports have indicated that the incidence of fungal infection and CMV infection has increased after engraftment, particularly among patients with severe immunodeficiency. For example, after allo-SCT using in vitro or in vivo T cell depletion, fungal infection, and reactivation of the Epstein-Barr virus and CMV are closely related to serious complications [42-49]. Also, the risk of infectious complications, including HHV-6 encephalitis and CMV diseases, is higher in patients who received cord blood transplantation [42,50-54]. These results suggest that the incidence of infectious complications depends not only on infectious disease-causing pathogens but also on the background of the patient or the cause of immunosuppression.

In our study, although the cumulative incidence of either bacterial or fungal infection was similar among the 3 groups, the ATL group showed the highest cumulative incidence of infection-related death, mainly caused by these infections. Importantly, bacteria resistant to many antibiotic agents emerged as a cause of death after 100 days in patients with ATL. It is suggested that after allo-SCT, these patients would be more susceptible to life-threatening bacterial infections even on late phase, compared to those with acute leukemia, and that the current strategy for infection would not be sufficient for allo-SCT to ATL. Hence, it seems that the development of an adoptive strategy in post-transplant patients with ATL is required.

The appearance of a CMV infection showed a negative impact for OS as an independent variable in the ATL group. Interestingly, while there were only 2 patients with ATL having CMV disease at the time of death, the risk of infectious death in patients with ATL who experienced CMV infection (including CMV antigenemia) was likely to be higher. Namely, episodes of CMV infection could predict a higher risk of death caused by not only CMV disease, but also other infections, which may help to identify the ATL patients who should receive more intensive management for infection.

It is not clear why episodes of CMV infection correlated with the outcome of patients with ATL, although multivariate analysis identified episodes of CMV infection as an independent variable only in the ATL group. It has been shown that episodes of CMV infection were associated with a worse outcome in post-transplant patients with defective cellular immunity [43,44,55]. Therefore, it is speculated that persistent compromised cellular immunity after transplantation led to the higher susceptibility of CMV and other infections among ATL patients, resulting in worse outcomes than leukemia patients. Considering that the reactivation of CMV itself and the prolonged administration of GCV could induce greater immune suppression [56,57], we hypothesize that the direct and indirect influence of CMV infection adversely promoted immunosuppression attendant on ATL patients. It remains to be elucidated how immunologic recovery was delayed after transplantation in ATL patients. Since the immune system recovery following allo-SCT was not sufficiently evaluated in our study, the monitoring of immune function after transplant, such as analysis of lymphocyte subset and quantitative estimation of immunoglobulin, should be considered in a future study.

It is possible that CMV-serostatus affected the result of statistical analysis in our study, because CMV-serostatus, which was unexamined in 37.6% patients, was not included in the statistical analysis. Therefore, to remove the bias of this point, the analysis for ATL patients with CMV-seropositive revealed that CMV infection was also identified as a risk factor in infection-related mortality and OS. Considering that it has been reported that about 90% of the Japanese population tests CMV-seropositive, the difference of CMV-serostatus would not have a big impact in our study. However, a larger analysis for matched patients' background regarding with CMV-serostatus would help to confirm our findings.

The ATL group showed that the highest cumulative incidence of infection-related mortality and the various pathogens causing death, indicating that it was difficult to establish the uniform management to reduce the fetal infectious complications for post-transplant patients with ATL. However, it is speculated that more intensive management for bacterial infection might provide the reduction of

infection-related death in some post-transplant patients with ATL, since ATL group would be more likely to show the higher risk of fatal antibiotic-resistant bacterial infection, even on late phase in our study. Therefore, appropriate antibiotic treatment using prolonged bacterial surveillance culture should be considered, particularly in ATL patients with persistent compromised cellular immunity. Moreover, because of a limitation of treatment active on multi-drug resistant gram negative rods, particularly *Pseudomonas aeruginosa*, at the present situation, the introduction of new treatment options, including antibiotic combination therapy using a "break-point checker board plate" and developing antibiotic agents such as colistin [58-62], are expected in patients who developed such infection after allo-SCT.

Our results showed the higher risk of fatal infectious complications in post-transplant patients with ATL. However, the number of patients is limited and the detailed treatment protocols were not completely uniform. Thus, it is possible that these factors exerted a bias and affected results. For instance, the small number of patients in our study resulted in wide and overlapping confidence intervals despite P values $<.05$. Our finding should be interpreted carefully, and they should be confirmed in larger prospective studies.

In conclusion, we found that the clinical features of infectious complications after allo-SCT in ATL patients are different from those in AML and ALL/LBL patients. Because allo-SCT offers the best chance of prolonged survival by inducing graft-versus-ATL effect, developing supportive care to minimize fatal infectious complications would be important, in particular, for post-transplant patients with ATL. Our data suggested that ATL patients require more intensive management for infections according to individualized risk such as the appearance of CMV infection. Such a strategy may be beneficial in reducing transplantation-related mortality in post-transplant patients with ATL.

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ORIGINAL ARTICLE

The role of HLA-matched unrelated transplantation in adult patients with Ph chromosome-negative ALL in first remission. A decision analysis

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The efficacy of unrelated transplantation for patients with ALL who lack an HLA-matched sibling remains unclear. We performed a decision analysis to determine the efficacy of myeloablative transplantation from a genetically HLA-A, -B, -DRB1 allele-matched unrelated donor for patients with Ph chromosome-negative ALL aged 21–54 years. The transition probabilities were estimated from the Japan Adult Leukemia Study Group studies (ALL93; $n=80$, ALL97; $n=82$), and the Japan Marrow Donor Program database (transplantation in first CR (CR1): $n=177$). The primary outcome measure was the 10-year survival probability with or without quality of life (QOL) adjustment. Subgroup analyses were performed according to risk stratification based on the WBC count and cytogenetics, and according to age stratification. In all patients, unrelated transplantation in CR1 was shown to be superior in analyses both with and without QOL adjustment (40.8 vs 28.4% and 43.9 vs 29.0%, respectively). A similar tendency was observed in all subgroups. The decision model was sensitive to the probability of leukemia-free survival following chemotherapy and the probability of survival after transplantation in standard-risk and higher-aged patients. Unrelated transplantation in CR1 improves the long-term survival probability in patients who lack an HLA-matched sibling. However, recent improvements in treatment strategies may change this result.

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Keywords: ALL; decision analysis; first remission; unrelated SCT

INTRODUCTION

The outcome of chemotherapy for Ph chromosome (Ph)-negative ALL in adult patients is inferior to that in children. Although about 90% of patients achieve CR, most of them eventually relapse, and leukemia-free survival is only 30–40%.[1] Therefore, allogeneic hematopoietic SCT (HSCT) in first CR (CR1) has been investigated to decrease the relapse rate. The efficacy of this approach has been evaluated through clinical studies using genetic randomization, in which patients with a HLA-matched sibling donor are allocated to the allogeneic HSCT arm, and those without a donor are placed in the chemotherapy or autologous HSCT arm.[2–10] These studies, as well as a meta-analysis of seven similar studies, confirmed that the donor group had a superior outcome compared with the no-donor group, and that autologous HSCT was not superior to chemotherapy in patients with adult ALL in CR1.[11] However, the efficacy of unrelated HSCT in patients with ALL in CR1, who lack an HLA-matched sibling, is still unclear. Although retrospective studies have reported a similar outcome for related and unrelated HSCT for ALL, a major problem was that the duration between the achievement of remission and HSCT was considered to be longer in unrelated HSCT due to the coordination process.[12,13] Therefore, patients who relapsed early after achieving remission might have been excluded in the unrelated HSCT group. On the other hand, it is practically difficult to perform a prospective clinical trial, in which patients with ALL in CR1, who lack an HLA-matched sibling but who have an HLA-matched unrelated donor, are randomly assigned to receive unrelated HSCT or chemotherapy alone.

A decision analysis is a statistical technique that aids the clinical decision making process under conditions of uncertainty. We previously demonstrated through a decision analysis that allogeneic HSCT is superior to chemotherapy alone in CR1 for adult patients with Ph-negative ALL who have an HLA-matched sibling, even after adjusting for quality of life (QOL).[14] In the

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present study, we performed a decision analysis to evaluate the efficacy of unrelated myeloablative HSCT for adult patients with Ph-negative ALL in CR1 who lack an HLA-matched sibling. We used a decision tree based on the results of prospective studies by the Japan Adult Leukemia Study Group (JALSG) (ALL93[3] and ALL97[15]), in which conventional-intensity regimens were used, the database of the Japan Marrow Donor Program (JMDP),[16] and the literature. Patients with Ph-positive ALL were not included in our analysis, because the outcome of treatment in these patients has improved dramatically as tyrosine kinase inhibitors became available.[17] In addition, patients aged less than 21 years were excluded from this analysis because the outcome of treatment in these patients has also improved greatly using intensified chemotherapy based on a pediatric regimen.[18]

MATERIALS AND METHODS

Model structure

We constructed a decision tree (Figure 1) to identify the optimal treatment strategy for adult patients with Ph-negative ALL in CR1, who lack an HLA-matched sibling, but who have an HLA-matched unrelated donor. At a decision node, we can decide to either proceed to unrelated HSCT or continue chemotherapy in CR1. Each decision is followed by chance nodes, which have possible outcomes with a transition probability (TP), and every branch finally ends with terminal nodes, which have utilities according to different health states. The sum of the products of the transition probabilities and utilities of all branches following each chance node become the expected value of each chance node, and the expected value of each decision is calculated as the sum of the expected values in all of the chance nodes following each decision. The following analyses were performed using TreeAge Pro 2009 software (Williamstown, MA, USA). This study was approved by the Institutional Review Boards of JMDP and Jichi Medical University.

Data sources

Outcomes after continuing chemotherapy in CR1 were estimated from JASLG studies (ALL93[3] and ALL 97[15]). Patients with Ph-negative ALL aged 21–54 years were included, and those who never achieved remission with chemotherapy were excluded. The data from 80 patients in ALL93 and 82 patients from ALL97 were analyzed separately and then combined by weighting the number of patients. Outcomes after unrelated HSCT in various disease statuses were estimated from the database of JMDP. Patients with Ph-negative ALL aged 21–54 years who underwent a first myeloablative allogeneic HSCT from a genetically HLA-A, -B, -DRB1 allele-matched unrelated donor between 1993 and 2008 were included. Of these, 177, 45 and 62 patients were in first remission, second remission and non-remission, respectively, at unrelated HSCT. All patients received BM graft.

The characteristics of the patients included in this study are summarized in Table 1. There was no significant difference in baseline characteristics among the JALSG studies and the JMDP data. To determine the following transition probabilities, OS and leukemia-free survival with a 95% confidence interval (CI) were calculated using the Kaplan–Meier method, whereas the cumulative incidences of non-relapse mortality and relapse with 95% CI were calculated using Gray's method,[19] where the other event was considered a competing risk. Probabilities that we could not estimate from these data were estimated from the literature.

Transition probabilities and utilities

Transition probabilities of the entire population were determined as summarized in Table 2. Each TP has a baseline value and a plausible range. Baseline decision analyses were performed based on the baseline value.

Patients may have been precluded from the undergoing unrelated HSCT due to early relapse or comorbidities even if they decided to undergo HSCT, and therefore the TP of actually undergoing unrelated HSCT in CR1 after the decision branch to undergo HSCT was determined as follows. First, the median duration between the achievement of CR1 and HSCT without relapse was calculated as 270 days based on the JMDP data. Next, leukemia-free survival rates at 270 days after achieving CR1 were calculated using the data for all patients who achieved remission in the JALSG studies, and the combined leukemia-free survival was 0.70 (95% CI: 0.64–0.77). We considered this to be the TP for actually receiving HSCT in CR1, and assigned a baseline value of 0.70 and 95% CI to the plausible range. The TP of undergoing unrelated HSCT in second remission (CR2) after the patient had a relapse following a decision to continue chemotherapy could not be calculated from our data. We assigned a plausible range of 0.5–0.70; the former value was the only available rate in a large study,[20] and the latter was the TP calculated above. The median of this range was taken as the baseline value. Probabilities regarding the actual rate of receiving HSCT in other disease statuses could not be obtained, even in the literature. Therefore, a baseline value of 0.5 was assigned with a wide plausible range of 0.3–0.7. The TP values for 'Alive at 10 years' following HSCT in various disease statuses were determined based on the JMDP data. We assigned 95% CI to the plausible ranges. Recently, results of HSCT in more specific disease statuses, such as HSCT following an early or late relapse after chemotherapy[21] and HSCT following

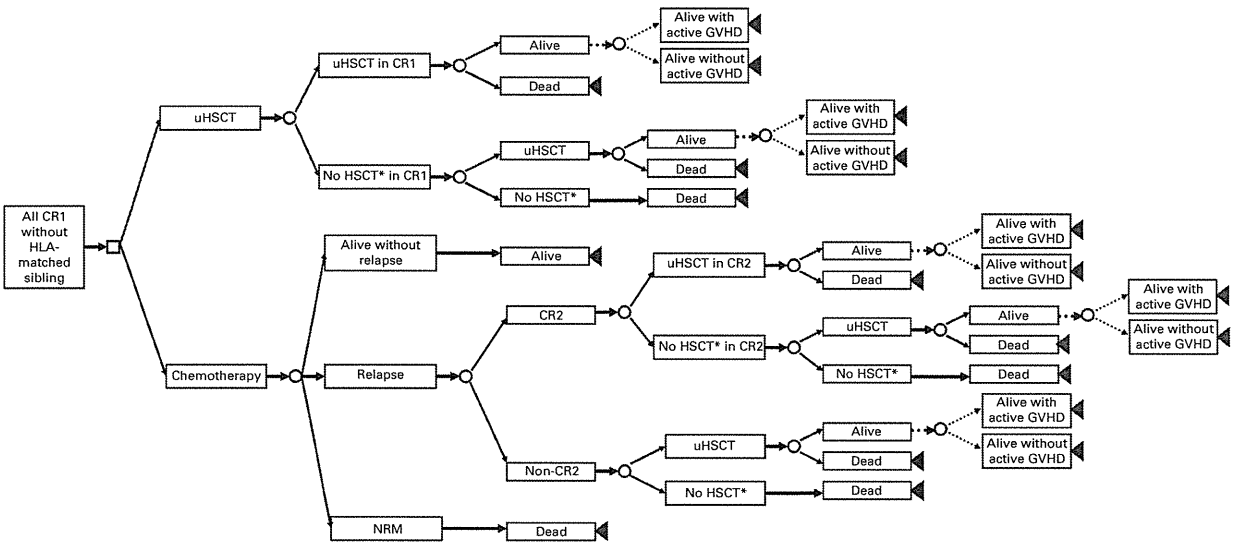

Figure 1. Decision tree used in this study. Decision analysis was performed based on this decision tree. A square indicates a decision node and open circles indicate chance nodes. In analyses with a QOL adjustment, 'Alive' after transplantation was followed by two branches with or without active chronic GVHD (dotted arrow). *Unrelated hematopoietic SCT (uHSCT) was not performed due to early relapse, death and so on. ALL, acute lymphoblastic leukemia; CR, complete remission; NRM = non-relapse mortality.

Table 1. Patient characteristics in the three data sources

	Chemotherapy in CR1		*HSCT in CR1*	*P-value*[a]
	JALSG ALL 93	*JALSG ALL97*	*JMDP*	
No. of patients	80	82	177	
Median age (range) (years)	38 (21–54)	37 (21–54)	35 (21–54)	0.58
No. of males/females	44/36	33/49	97/80	0.07
No. of each phenotype T/B/other	8/49/11	13/66/3	32/104/26	0.19
Median WBC count at diagnosis (range) ($\times 10^9$/L)	8.8 (0.7–301.1)	10.4 (1.3–398.0)	9.0 (0.6–480.0)	0.49
Karyotype standard: high[b], ratio	12:1	8:1	12: 1	0.66

Abbreviations: CR1 = first CR; HSCT = hematopoietic SCT; JALSG = Japan Adult Leukemia Study Group; JMDP = Japan Marrow Donor Program. [a]Statistical analyses were performed using the Kruskal-Wallis test for continuous variables and the Fisher's exact test for categoric variables. [b]t(4;11) and complex karyotype (five or more chromosomal abnormalities) were classified as high-risk karyotypes, and other karyotypes were classified as standard-risk.

Table 2. Transition probabilities of the overall population and all subgroups

	baseline value (plausible range)				
	All patients	*Standard-risk*	*High-risk*	*Lower age*	*Higher age*
HSCT in CR1	0.70 (0.64–0.77)	0.74 (0.65–0.83)	0.58 (0.44–0.72)	0.72 (0.63–0.81)	0.69 (0.60–0.78)
Alive at 10 years following HSCT in CR1	0.58 (0.49–0.66)	0.55 (0.41–0.66)	0.71 (0.53–0.83)	0.61 (0.49–0.72)	0.54 (0.39–0.67)
HSCT after failure of HSCT in CR1	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)
Alive at 10 years following HSCT after failure of HSCT in CR1[a]	0.22 (0.15–0.29)	0.27 (0.25–0.28)	0.16 (0.08–0.23)	0.23 (0.19–0.26)	0.23 (0.11–0.35)
Alive at 10 years without relapse following CTx,	0.20 (0.13–0.28)	0.24 (0.11–0.37)	0.13 (0.01–0.16)	0.14 (0.03–0.25)	0.25 (0.16–0.35)
NRM at 10 years following CTx	0.09 (0.04–0.14)	0.08 (0.01–0.16)	0.12 (0.01–0.24)	0.06 (0–0.12)	0.11 (0.05–0.18)
Achievement of CR2 after relapse following CTx	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.4 (0.3–0.5)	0.4 (0.3–0.5)
HSCT in CR2	0.6 (0.5–0.7)	0.62 (0.5–0.74)	0.54(0.5–0.58)	0.61 (0.5–0.72)	0.60 (0.5–0.69)
Alive at 10 years following HSCT in CR2	0.29 (0.16–0.44)	0.28 (0.09–0.51)	0.23 (0.04–0.51)	0.26 (0.10–0.45)	0.35 (0.13–0.59)
HSCT after failure of HSCT in CR2	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)
Alive at 10 years following HSCT after failure of HSCT in CR2[b]	0.15 (0.07–0.26)	0.25 (0.08–0.45)	0.08 (0.01–0.24)	0.19 (0.07–0.35)	0.11 (0.03–0.26)
HSCT in non-CR after relapse following CTx	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.3–0.7)
Alive at 10 years following HSCT in non-CR after relapse	0.15 (0.07–0.26)	0.25 (0.08–0.45)	0.08 (0.01–0.24)	0.19 (0.07–0.35)	0.11 (0.03–0.26)
Rate of active GVHD at 10 years[c]	0.18 (0.1–0.25)	0.18 (0.1–0.25)	0.18 (0.1–0.25)	0.18 (0.1–0.25)	0.18 (0.1–0.25)

Abbreviations: HSCT = hematopoietic SCT; CTx = chemotherapy; NRM = non-relapse mortality. [a]This rate was estimated from the survival rate following HSCT in CR2 and HSCT in non-CR. [b]The same rate of survival following HSCT in non-CR was used. [c]The same baseline value and plausible range were used as the rate of active GVHD at 10 years following HSCT in various disease statuses, but one-way sensitivity analyses were performed separately for each status.

a relapse after first HSCT,[22] have been reported, but sufficient data for this decision analysis were not provided in these reports.

The transition probabilities for 'Alive without relapse at 10 years' and non-relapse mortality following chemotherapy in CR1 were determined based on the JALSG studies, and the TP of relapse following chemotherapy was determined by subtracting the sum of these TPs from one. The TP of achieving CR2 after relapse in patients who decided to continue chemotherapy in CR1 was estimated to have a baseline value of 0.4 with a plausible range of 0.3–0.5 based on the literature.[10,20,23]

Utilities were calculated based on a 10-year survival probability, which was the primary outcome measure, with or without adjusting for QOL. The survival curve nearly reaches a plateau after 5 years, and therefore 'Alive at 10 years' reflects 'Cure of leukemia', which is the primary goal of HSCT. In an analysis without an adjustment for QOL, we considered only two kinds of health states, 'Alive at 10 years' and 'Dead', and assigned utility values of 100 to the former and 0 to the latter. On the other hand, in an analysis with an adjustment for QOL, 'Alive after chemotherapy without relapse at 10 years', 'Alive with active GVHD at 10 years' and 'Alive without active GVHD at 10 years' were considered as different health states. The proportion of patients with active GVHD among those who were alive at 10 years was determined based on the literature.[24–26] We assigned a value of 100 to the utility for being alive without relapse at 10 years after chemotherapy alone, and a value of 0 to the utility for being dead in all situations. We assigned a fixed value of 98 to the utility for being alive without active GVHD at 10 years following HSCT because a part of patients had suffered from complications other than active GVHD, such as cataract.[27] Moreover, we assigned a value of 70 with a wide plausible range of 0–98 to the utility for being alive with active GVHD at 10 years. These utilities were determined based on the opinions of 10 doctors who were familiar with HSCT and the literature.[28,29]

Subgroup analyses were also performed according to risk stratification based on the WBC count and cytogenetics, and according to age stratification with a cutoff of 35 years. This cutoff value is based on the age used in the Medical Research Council/Eastern Cooperative Oncology Group trial for risk stratification.[9] Patients with a high WBC count (more than 30×10^9/L for B lineage and more than 100×10^9/L for T lineage) and/or with t(4;11) or complex karyotype (5 or more chromosomal abnormalities) were classified as high-risk, and all other patients were classified as standard-risk. It was difficult to perform other subgroup analyses regarding the possible prognostic factors like phenotypes, due to the limited number of patients involved. All transition probabilities, based on the JALSG studies and the JMDP data, were recalculated using the data for patients in each subgroup (Table 2).

Sensitivity analyses

To evaluate the robustness of the decision model, we performed one-way sensitivity analyses for all transition probabilities, in which the decision tree was recalculated by varying each TP value in its plausible range, and confirmed whether or not the decision of the baseline analyses changed. In analyses with an adjustment for QOL, the utility for being alive with

active GVHD at 10 years was also subjected to a one-way sensitivity analysis.

We also performed a probabilistic sensitivity analysis using a Monte Carlo simulation[30], in which the uncertainties of all transition probabilities were considered simultaneously. The distribution of the random variables for each TP was determined to follow a normal distribution, with 95% of the random variables included in the plausible range. One thousand simulations were performed based on the decision tree, and the mean and s.d. of the expected value for each decision were calculated.

RESULTS

Baseline analysis

The baseline analysis in the overall population without adjusting for QOL revealed an expected 10-year survival of 43.9% for the decision to perform unrelated HSCT in CR1, which was better than the value (29.0%) for the decision to continue chemotherapy. The decision to perform unrelated HSCT was superior even after adjusting for QOL (40.8% for HSCT vs 28.4% for chemotherapy, Table 3).

Sensitivity analysis

First, we performed one-way sensitivity analyses for all transition probabilities in the decision model without adjusting for QOL. A better expected survival for the decision to perform HSCT was consistently demonstrated in all transition probabilities within the plausible ranges. In the probabilistic sensitivity analysis, the mean value and s.d. of the expected survival probability for HSCT were 44.0 and 3.5% (Figure 2a), and those for chemotherapy were 29.1 and 3.9% (Figure 2b), respectively.

Next, we performed one-way sensitivity analyses for all transition probabilities and for the utility for being alive with active GVHD at 10 years in the decision model adjusted for QOL. Even in these analyses, the results of the baseline analysis were not reversed for any of the transition probabilities. In addition, a higher expected survival probability for HSCT was retained in a sensitivity analysis, in which the utility for being alive with active GVHD was changed between 0 and 98 (Figure 3a). In the probabilistic sensitivity analysis, the mean value and s.d. of the expected survival probability for HSCT were 40.9 and 3.4% (Figure 2c), and those for chemotherapy were 28.4 and 3.9% (Figure 2d), respectively.

Table 3. Expected 10-year survival probabilities with and without adjusting for quality of life (QOL)

	Expected survival probability without a QOL adjustment		*Expected survival probability with a QOL adjustment*	
	HSCT	*Chemotherapy*	*HSCT*	*Chemotherapy*
All patients	43.9%	29.0%	40.8%	28.4%
Standard-risk patients	44.2%	35.1%	41.1%	34.3%
High-risk patients	44.5%	19.1%	41.4%	18.7%
Lower-aged patients	47.1%	24.8%	43.8%	24.1%
Higher-aged patients	40.8%	33.1%	38.0%	32.5%

Abbreviation: HSCT = hematopoietic SCT.

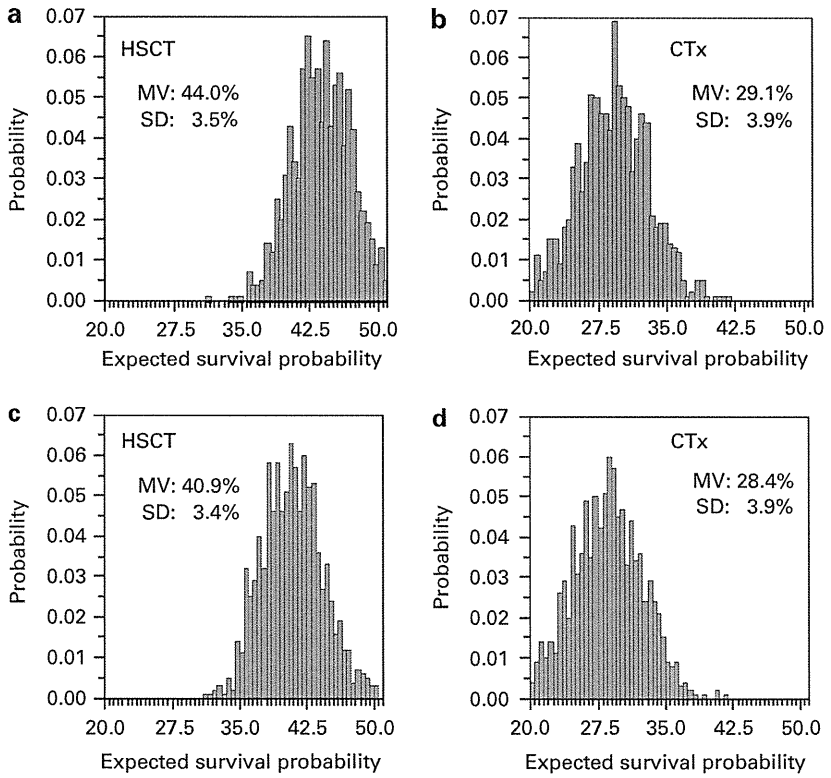

Figure 2. Probabilistic sensitivity analysis (PSA) using a Monte Carlo simulation. We performed a PSA using a Monte Carlo simulation. In the analysis without a QOL adjustment, the mean value (MV) and s.d. of the expected survival probability for unrelated HSCT were 44.0 and 3.5% (**a**), and those for chemotherapy (CTx) were 29.1 and 3.9% (**b**), respectively. In the analysis with a QOL adjustment, the MV and s.d. of the expected survival probability for HSCT were 40.9 and 3.4% (**c**), and those for CTx were 28.4 and 3.9% (**d**), respectively.

Subgroup analyses

In subgroup analyses both with and without adjusting for QOL, a better expected survival probability for HSCT was consistently observed in all of the subgroups (Table 3).

We also performed one-way sensitivity analyses in all of the subgroups. In high-risk and lower-aged patients, the results of baseline analyses were not affected when each TP value was varied within its plausible range in the decision models both with and without adjusting for QOL. In standard-risk patients, the results reversed in favor of chemotherapy if the probability of leukemia-free survival at 10 years without relapse following chemotherapy was higher than 0.35 (Figure 3b) or the probability of OS at 10 years following HSCT in CR1 was lower than 0.42 (Figure 3c) in the decision model without adjusting for QOL. In the decision model with adjusting for QOL, the results reversed in favor of chemotherapy if the probability of leukemia-free survival at 10 years without relapse following chemotherapy was higher than 0.32 (Figure 3f) or the probability of OS at 10 years following HSCT in CR1 was lower than 0.45 (Figure 3g). In older patients, the decision models both with and without adjusting for QOL were also sensitive to both the probability of leukemia-free survival at 10 years without relapse following chemotherapy and the probability of OS at 10 years following HSCT in CR1 (Figures 3d, e, h and i). We also performed one-way sensitivity analyses for a utility for being alive with active GVHD within the range of 0–98. A higher expected survival probability for HSCT was retained in all of the subgroups.

DISCUSSION

About two-thirds of patients with adult ALL lack an HLA-matched sibling, and for these patients, allogeneic HSCT from an HLA-matched unrelated donor might be an alternative treatment. Several studies have suggested that unrelated HSCT may be effective for high-risk adult ALL patients in various disease statuses.[31,32] In addition, two retrospective studies showed no difference between related and unrelated HSCT for adult ALL patients, including those in CR1,[12,13] and the recent evidence-based review from the American Society for Blood and Marrow Transplantation supported this.[33,34] However, patients who undergo unrelated HSCT in CR1 are a select population of patients who have maintained their remission status during the donor-coordination process. We performed a decision analysis to identify the optimal strategy for patients with ALL in CR1, who lack an HLA-matched sibling but who have an HLA-matched unrelated donor. We tried to exclude selection bias in patients who underwent unrelated transplantation by considering patients who did not undergo unrelated HSCT in CR1 due to early relapse or comorbidities even if they decided to undergo unrelated HSCT.

We used data from JALSG prospective studies to estimate outcomes after continuing chemotherapy. On the other hand, we used the database of JMDP to estimate outcomes after unrelated HSCT, due to the limited number of patients who underwent unrelated HSCT in the JALSG prospective studies. The outcomes after unrelated HSCT in CR1 were not significantly different among the JALSG prospective studies and the JMDP database. (OS at 10 years in patients who underwent unrelated HSCT in CR1 was 54.2, 50 and 58.2% in JALSG ALL93 study, JALSG ALL97 study, and the JMDP database, respectively ($P = 0.56$ in log-rank test)).

In our baseline analysis both with and without adjusting for QOL, unrelated HSCT in CR1 was shown to give a superior outcome in both the overall population and in all of the subgroups. In the overall population, probabilistic sensitivity analysis using a Monte Carlo simulation also supported this result (Figure 2). However, in a one-way sensitivity analysis, the decision model was sensitive to the probability of leukemia-free survival following chemotherapy in CR1 in both standard-risk and older patients (Figures 3b, d, f and h). The adaptation of high-intensified chemotherapy, especially the adaptation of chemotherapy according to pediatric regimens up to young adult, has led to improved outcomes in recent trials,[1,9,10] but the JALSG studies in this analysis included less-intensified regimens. Therefore, this improvement in chemotherapy might change our result. In a one-way sensitivity analysis, the decision model was also sensitive to the probability of OS at 10 years following HSCT in CR1 in both standard-risk and older patients (Figures 3c, e, g and i). This study only included data on unrelated HSCT from a genetically HLA-A, -B, -DRB1 allele-matched donor. It has been

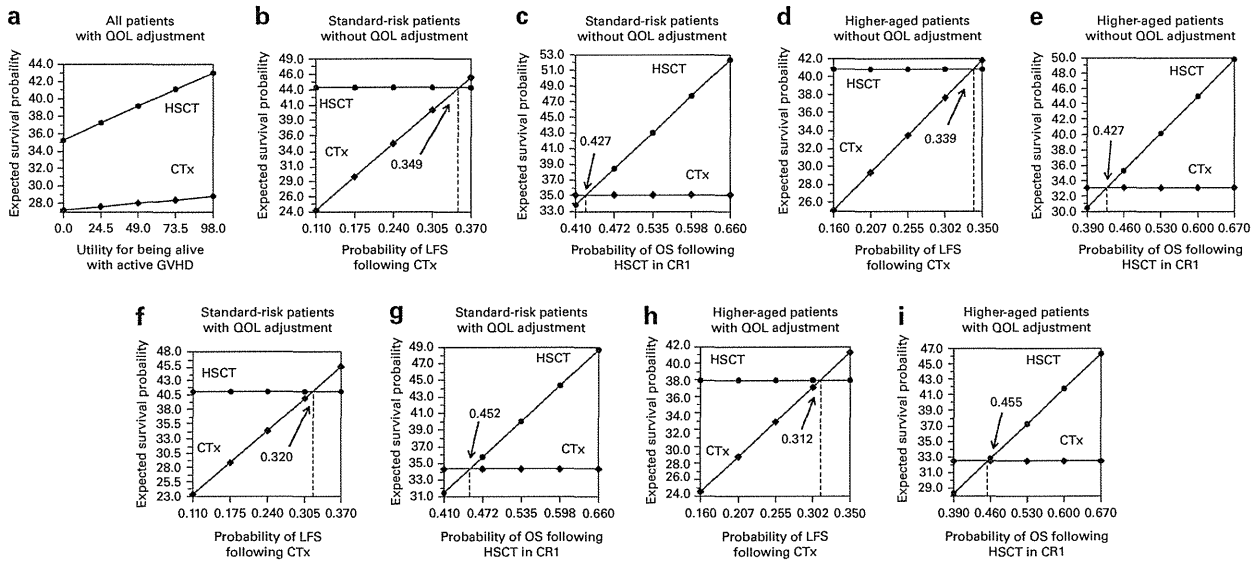

Figure 3. One-way sensitivity analysis. We performed a one-way sensitivity analysis. The superiority of unrelated HSCT compared with CTx was consistently observed with a wide plausible range of the utility for being alive with active GVHD in the overall population (**a**). On the other hand, the models both without adjusting for QOL were sensitive to the probability of leukemia-free survival at 10 years following CTx and the probability of OS at 10 years following HSCT in CR1 in standard-risk (**b**, **c**, **f**, **g**) and older patients (**d**, **e**, **h**, **i**).

reported that the presence of an HLA allele mismatch, especially in some specific combinations, significantly affects the outcome of serologically HLA-matched unrelated HSCT.[35] Therefore, the indications for HSCT from an unrelated donor with an HLA allele mismatch should be considered with great caution, especially in standard-risk and older patients.

Recently, minimal residual disease assays are increasingly involved in the evaluation of treatment response for ALL,[36] and the prevalence of minimal residual disease after the induction therapy or early consolidation therapy has been demonstrated as an important prognostic factor. In the current study, we considered only hematological response, and minimal residual disease status was not included in risk stratification. Minimal residual disease status should be taken into account in the future analysis.

In this study, the median duration from achieving CR1 to unrelated HSCT without relapse was 270 days, which precluded HSCT in CR1 in 30% of patients after a decision to perform HSCT (mainly due to early relapse). This duration was 4 months longer than the duration from achieving CR1 to related HSCT without relapse in our previous study, as the coordination process for an unrelated donor through JMDP requires a longer duration. A meta-regression analysis by Yanada *et al.*[11] showed that the proportion of patients who actually underwent allogeneic HSCT among patients with a donor was positively correlated with survival. The coordination process for a JMDP donor is currently getting shorter, and, as a consequence, the efficacy of unrelated HSCT in CR1 may increase.

The low incidence of severe GVHD has been demonstrated in Japanese patients,[37,38] and this might have influenced the superior outcome of unrelated HSCT in CR1 in our analysis. Therefore, caution should be paid when the current results are applied to patients of other origins.

In conclusion, to improve the probability of long-term survival, myeloablative HSCT from a genetically HLA-A, -B, -DRB1 allele-matched unrelated donor in CR1 is recommended for patients, aged 21–54 years, who lack an HLA-matched sibling donor. Even when we considered QOL, the superiority of unrelated HSCT was confirmed in the overall population and in all of the subgroups. However, recent improvements in treatment strategies, like high-intensified chemotherapy, may change this result.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Mechanisms of action and resistance to all-*trans* retinoic acid (ATRA) and arsenic trioxide (As_2O_3) in acute promyelocytic leukemia

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Abstract Since the introduction of all-*trans* retinoic acid (ATRA) and arsenic trioxide (As_2O_3) for the treatment of acute promyelocytic leukemia (APL), the overall survival rate has improved dramatically. However, relapse/refractory patients showing resistance to ATRA and/or As_2O_3 are recognized as a clinically significant problem. Genetic mutations resulting in amino acid substitution in the retinoic acid receptor alpha (RARα) ligand binding domain (LBD) and the PML-B2 domain of PML-RARα, respectively, have been reported as molecular mechanisms underlying resistance to ATRA and As_2O_3. In the LBD mutation, ATRA binding with LBD is generally impaired, and ligand-dependent co-repressor dissociation and degradation of PML-RARα by the proteasome pathway, leading to cell differentiation, are inhibited. The PML-B2 mutation interferes with the direct binding of As_2O_3 with PML-B2, and PML-RARα SUMOylation with As_2O_3 followed by multimerization and degradation is impaired. To overcome ATRA resistance, utilization of As_2O_3 provides a preferable outcome, and recently, a synthetic retinoid Am80, which has a higher binding affinity with PML-RARα than ATRA, has been tested in the clinical setting. However, no strategy attempted to date has been successful in overcoming As_2O_3 resistance. Detailed genomic analyses using patient samples harvested repeatedly may help in predicting the prognosis, selecting the effective targeting drugs, and designing new sophisticated strategies for the treatment of APL.

Keywords APL · PML-RARα · ATRA · Arsenic trioxide (As_2O_3) · Drug resistance

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Introduction

Almost two decades ago, the prognosis of acute promyelocytic leukemia (APL) was critically poor due to fatal coagulation disorders at diagnosis [1, 2]. Even with conventional chemotherapy using anthracyclines, more than 70 % of APL patients showed poor prognosis [3, 4]. After introduction of all-*trans* retinoic acid (ATRA) in the clinical setting in combination with conventional chemotherapy, the prognosis of APL has improved dramatically, with the result that more than 85 % of patients now achieve complete remission (CR) and nearly 70 % of patients can be cured [5–8]. Since 1994, the marked effectiveness of As_2O_3 in APL patients, even in relapsed patients after combination therapy with ATRA, has been confirmed [9–12]. When As_2O_3 is utilized as a single agent, ~70 % of patients can be cured, whereas nearly 90 % of patients can be cured if As_2O_3 is utilized in combination with ATRA [13, 14]. Although outcomes of APL treatment with ATRA and/or As_2O_3 in combination with conventional chemodrugs have improved, relapsed/refractory patients are still observed in the clinical setting and drug resistance to ATRA and As_2O_3 has been recognized as a critical problem.

More than 98 % of APL patients carry the t(15;17) translocation, which results in fusions of the retinoic acid

receptor alpha (RARα) gene with the promyelocytic leukemia (PML) gene, *PML-RARα* (Fig. 1) [15–17]. A very limited number of patients, showing APL phenotype without t(15;17), exhibit a variety of X-RARα fusions (Fig. 1) [18–25]. Interestingly, some patients expressing X-RARα show clinical resistance to ATRA and/or As_2O_3. Previous reports have indicated that both ATRA [26, 27] and As_2O_3 [28–30] have rigorously defined molecular targets, an improved understanding of their molecular mechanisms of action and resistance may be important to further improving clinical outcomes in APL treatment.

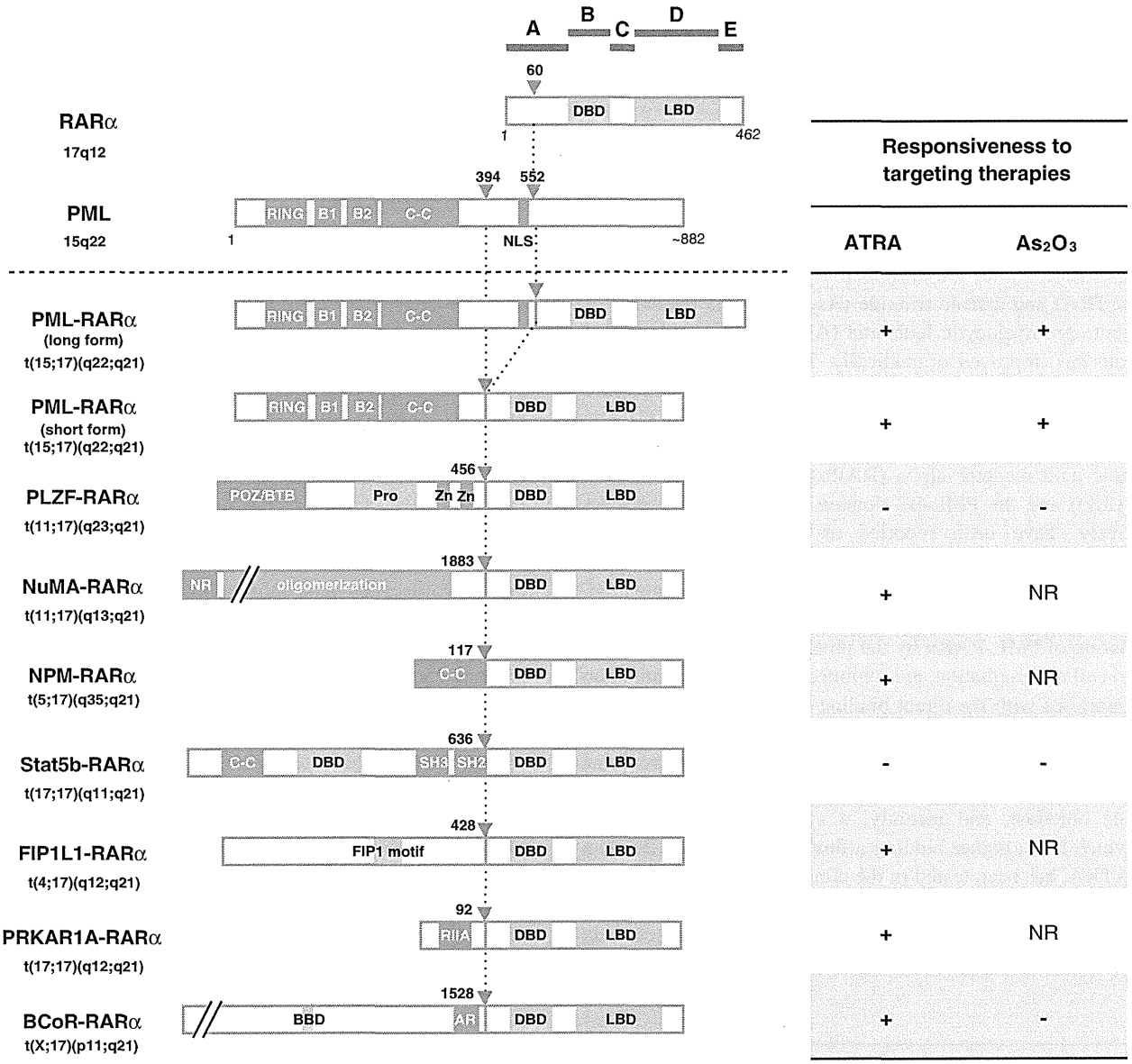

Fig. 1 Schematic representation of PML-RARα and X-RARα fusion protein confirmed in APL. Chromosomal translocations resulting in the fusion protein are also indicated under the name of fusion protein. Long and short forms of PML-RARα with or without nuclear localizing signal (NLS) are reported [86]. ATRA and As_2O_3 responsiveness in the clinical setting and/or in vitro analyses is indicated in the *right panel*. *Gray triangles* indicate break points of chimeric protein. *Numbers* indicate the amino acid positions. *A* to *E* functional domains in RARα, *DBD* DNA binding domain, *LBD* ligand binding domain, *RING* really interesting new gene finger domain, *B1 and B2* B-box motifs, *C–C* coiled-coil domain, *POZ/BTB* pox virus and zinc finger/BR–C, ttk and bab domain, *Pro* proline rich domain, *Zn* zinc finger domain, *NR* nuclear reassembly, *RIIA* dimerization domain, *BBD* BCL6-binding domain, *AR* ankyrin repeats, + sensitive, − resistant, *NR* not reported

Mechanisms of action of molecular targeting drugs to APL cells

ATRA

Wild-type RARα is a nuclear hormone receptor that binds to consensus sequence DR5 (five bases spaced between two AGGTCA motifs) in target gene promoters, normally as heterodimer with retinoid X receptor (RXR) [31–33]. Without ligands, ATRA and 9-*cis* retinoic acid, RAR-RXR heterodimer induces transcription repression throughout chromatin remodeling by recruiting transcription co-repressors, such as N-CoR/SMRT large protein complexes, that contain histone deacetylases (HDACs) [27, 34–37] and histone methyltransferases [38–40]. In the presence of ligand ($\sim 10^{-7}$ M), the co-repressor complexes dissociate from RAR-RXR, and transcriptional de-repression and activation are induced [34–37, 41]. PML-RARα binds to DR5 of target gene promoters primarily as a homodimer, but also as a heterodimer with RXR [42, 43], and induces transcription repression by recruiting N-CoR/SMRT complexes and polycomb group repressive complex 1 and 2 (PRC1/2) [39, 40], which contain histone methyl transferases, in the absence of ligands [27] (Fig. 1). PML-RARα can be SUMOylated at K160 of the PML protein to recruit death domain-associated protein (DAXX), resulting in the transcriptional repression of target genes [44]. Even in the presence of physiological concentration of ligand (10^{-7} M), the co-repressor complex still binds with PML-RARα and the transcriptional repression cannot be dissolved. In the presence of pharmacological concentration of ATRA (10^{-6} M), transcription activation can be induced by dissociation of co-repressor complexes from PML-RARα and proteasome-dependent PML-RARα degradation [45–47].

As_2O_3

The efficacy of As_2O_3 on APL cells was first reported by Chen et al. in 1996 [28], who showed the dual effect of apoptosis at relatively high concentrations (0.5–2 μM/L) and partial differentiation at low concentrations (0.1–0.5 μM/L) in both ATRA-responsive and ATRA-resistant APL cells. As_2O_3 induces the targeting of nucleoplasmic PML-RARα with a micro speckled pattern into nuclear bodies with a normal speckled pattern prior to degradation [30, 48–50]. As_2O_3 induces the formation of reactive oxygen species (ROS) [30], which induce multimerization of PML-RARα through intermolecular disulphide crosslinks at PML B1-domain (Fig. 2) and PML-RARα SUMOylation by ubiquitin-conjugating enzyme 9 (UBC9) [30]. A recent report indicated that As_2O_3 directly binds with PML at the C–C motif in the PML B2-domain, and that PML SUMOylation can be induced by enhancement of UBC9 binding at the PML RING domain [29, 30, 50]. SUMOylated PML recruits RING finger protein 4 (RNF4), which is known as a SUMO-dependent ubiquitin ligase [51], and polyubiquitylated PML-RARα can be degraded by ubiquitin–proteasome pathway [29, 49, 51].

Molecular mechanisms of drug resistance in APL cells

From the molecular mechanisms of ATRA and As_2O_3 effectiveness as indicated above, several mechanisms of drug resistance have been speculated [52]. In this section, we outline the molecular mechanisms of resistance that are thought to be significant from the clinical perspective.

RARα fusion proteins in APL

In very limited cases with APL phenotype, *RARα* translocations with *X*-genes other than *PML* (*PLZF* [18], *NuMA* [19], *NPM* [20], *STAT5b* [21, 53], *FIP1L1* [22], *PRKAR1A* [23, 24], and *BCOR* [25]) resulting in the production of X-RARα fusion protein have been reported (Fig. 1). PML-RARα forms mainly homodimers, and it has been reported that homodimerization of PML-RARα is critical for the pathogenesis of APL [42, 43]. Sternsdorf et al. [54] indicated that forced homodimerization of RARα induces ALP-like leukemia in a mouse model, indicating that the dimerization domain of the fusion protein may be critical to the induction of leukemogenesis by X-RARα. In fact, homodimerization through specific domains (coiled-coil; PML-, NPM-, and STAT5b-, POZ/BTB; PLZF-, RIIA; PRKAR1A-, and so on) has been confirmed in all X-RARα proteins. Interestingly, in PML-, PRKAR1A- [24], and BCOR-RARα [25], heterodimerization with RXR is also important for transformation and/or RARE binding.

Since those chimeric proteins all hold RARα DNA binding domain (DBD) and ligand binding domain (LBD), ATRA responsiveness is speculated in all cases. However, ATRA resistance has been confirmed clinically in cases showing *PLZF-RARα* [18, 34, 41] and *STAT5b-RARα* [21, 53, 55] fusions. One explanation for ATRA resistance is that the N-CoR/SMRT-corepressor complex interacts with PLZF, even in the presence of pharmacological concentration of ATRA, such that transcriptional de-repression cannot occur at RARα target gene promoters [34, 41]. The molecular mechanisms of ATRA resistance in STAT5b-RARα-expressing cells has not been fully explicated. Wild-type Stat5b is localized in cytoplasm, but STAT5b-RARα aberrantly localizes in nucleus [21]. STAT5b is a component of the janus kinase (JAK)-STAT signaling pathway, and phosphorylation of STAT5b by JAK causes homodimerization and translocation into the nucleus, where it acts as a transcription factor [56]. Aberrant transcription

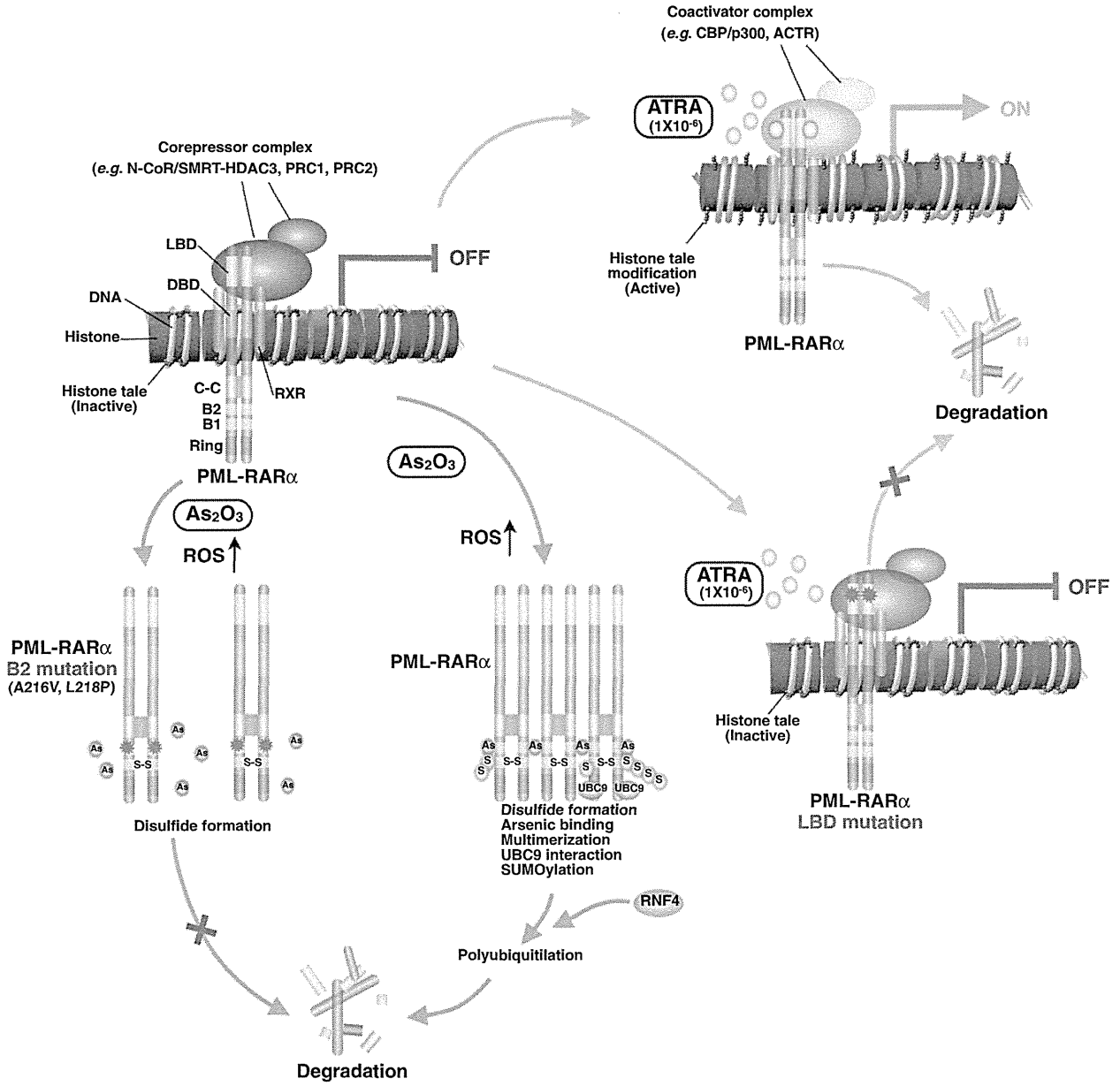

Fig. 2 Molecular mechanisms of action and resistance to ATRA and As_2O_3 in APL cells. PML-RARα are found mainly as homodimers through the C–C domain of PML, and partially as heterodimers with RXR. PML-RARα binds with target gene promoter in the absence of ligand, and recruits co-repressor complexes, such as N-CoR/SMRT complexes containing histone deacetylases (e.g. HDAC3) [34–37, 41] and PRC1/2 complex containing histone methyltransferases (e.g. EZH2) [39] to repress the gene expression. Histone tail deacetylation and/or methylation are related to transcription repression. In the presence of pharmacological concentration (1×10^6 μM) of ligand (ATRA), co-repressor complexes are dissociated from RARα, while co-activator complexes containing histone acetyltransferases (e.g. p300/CBP) are recruited, and transcription activation occurs. In the cases of PML-RARα with LBD mutations, ligand binding with LBD is interfered and co-repressor dissociation does not occur in the presence of pharmacological concentrations of ATRA. In the presence of As_2O_3, the formation of reactive oxygen species (ROS) is induced, and PML intermolecular disulfide crosslinks through B1 domain, that induce multimerization, and SUMOylation of PML by ubiquitin-conjugating enzyme 9 (UBC9) occur. As_2O_3 directly bind with PML-B2 domain and enhancing UBC9 binding and SUMOylation of PML. SUMOylated PML recruits RING finger protein 4 (RNF4), and is polyubiquitylated by RNF4, and proteasome-dependent degradation occurs. If PML-RARα has PML-B2 mutation, direct binding of As_2O_3 with PML is impaired, and polyubiquitylation and degradation are perturbed

regulation of STAT5b target genes in addition to RARα target genes by STAT5b-RARα may be related to ATRA resistance.

On the other hand, As_2O_3 resistance in clinical setting was observed in patients expressing PLZF- [57, 58], STAT5b- [55], and BCoR-RARα [25]. The As_2O_3-binding C–C motif is confirmed in PML-B2 domain, and As_2O_3 binding is critical for the multimerization followed by PML-RARα degradation [29, 30, 42]. Lack of As_2O_3 binding sites in X-RARα protein may be one explanation of loss of As_2O_3 responsiveness. However, no direct effect of As_2O_3 on RARα has been reported.

Mechanisms of resistance to ATRA

A number of mechanisms have been proposed to explain ATRA resistance in APL patients expressing PML-RARα, such as amino acid substitution in RARα LBD domain by genetic mutations, increased catabolism of ATRA, presence of cytoplasmic retinoic acid binding protein (CRABP), and abnormal ATRA delivery to the cell nucleus. Only genetic mutations on the RARα LBD domain in PML-RARα have been confirmed as an ATRA-resistant mechanism, from both clinical observations and in vitro molecular analyses [59–66]. Genetic mutations (missense, nonsense, and deletions) on RARα LBD domain have been confirmed in ATRA-resistant patients and APL cell lines, which grow despite pharmacological concentrations of ATRA, as summarized in Fig. 3. These mutations accumulate in the three subregions (zones I, II, and III in Fig. 3) of the LBD domain [66]. Gallagher et al. [66] reported that *PML-RARα* LBD mutation was confirmed 18 of 45 (40 %) relapse patients treated with ATRA/chemotherapy. In vitro analyses using ATRA-resistant NB4 cells (NB4-R1, -R2 [67], -R4 [60], and -RA [61]) and mutated-*PML-RARα* expressing Cos-1 cells [65] indicated that ATRA binding affinity with mutated PML-RARα was generally lower than that with PML-RARα without mutations, due to conformational changes in LBD. Furthermore, ligand-dependent N-CoR/SMRT co-repressor release and co-activator recruitment (e.g. ACTR histone acetyltransferase), which are critical for the transcriptional activation of genes with RARE sites and morphological cell differentiation, was impaired under the therapeutic dose of ATRA [60, 65, 67].

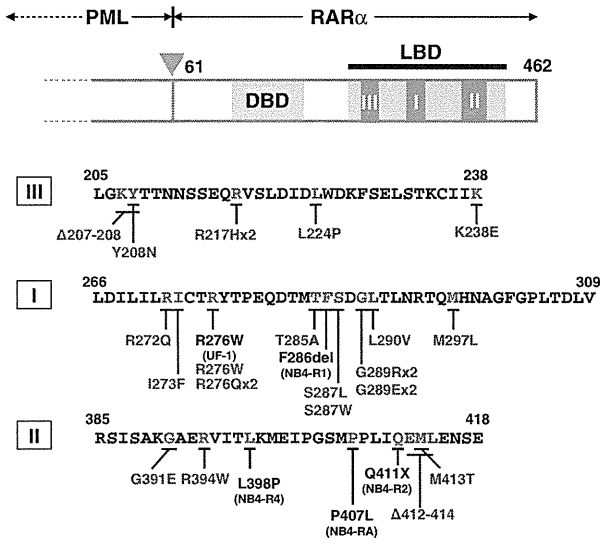

Fig. 3 Genetic mutations resulting in amino acid substitution in PML-RARα LBD confirmed in clinically ATRA-resistant patients and ATRA-resistant cell lines. Mutations are confirmed in 3 cluster regions (zones I to III) in RARα-LBD [66]. *Red letters* indicate amino acids substituted in specific patients and/or cells. Amino acid substitutions and deletions in ATRA-resistant patients are indicated in *blue letters*. Substitution in ATRA-resistant *cell lines* indicated in *black*. Names of *cell lines* are indicated in *brackets*. The position of the mutation is described with reference to normal amino acid sequence of RARα1 [31]

To overcome ATRA resistance, a number of therapeutics has been tested in vitro and in vivo. Several clinical reports indicated that As_2O_3 rescue most of relapsed/refractory patients treated with ATRA/chemotherapy [9–12, 68]. Am80, a synthetic retinoid that shows higher binding affinity with PML-RARα than ATRA, is utilized in the clinical setting [69–71]. Am80 is approximately 10 times more potent than ATRA as an in vitro inducer of differentiation in NB-4 and HL60 cells, and is chemically more stable than ATRA [72, 73]. Histone deacetylase (HDAC) inhibitors [74], such as sodium butyrate (NaF), valproic acid (VPA), and trichostatin A (TSA), have been utilized with ATRA and are expected to transcriptionally activate PML-RARα target genes to inhibit co-repressors complexes that contain HDACs [75–77]. Another approach to overcoming the resistance uses other molecular targeting therapeutics, such as gemtuzumab ozogamicin (GO), an anti-CD33 monoclonal antibody linked with calicheamicins [78, 79].

Molecular mechanisms of resistance to As_2O_3

Even for relapsed/refractory patients following treatment with ATRA/chemotherapy, As_2O_3 therapy is highly effective, with a complete remission rate of more than 80 % [80–82]. Although the CR rate is high even in relapsed patients, resistance to As_2O_3 treatment has been recognized as a clinically critical problem. Information on As_2O_3 resistance remains limited compared with that on ATRA resistance.

Recently, we reported two cases showing clinical As_2O_3 resistance after treatment with ATRA/chemotherapy, which exhibited missense mutations leading to substitution of amino acids in the PML-B2 domain in PML-RARα [50, 68, 83]. One patient with the M3 variant, expressing PML-

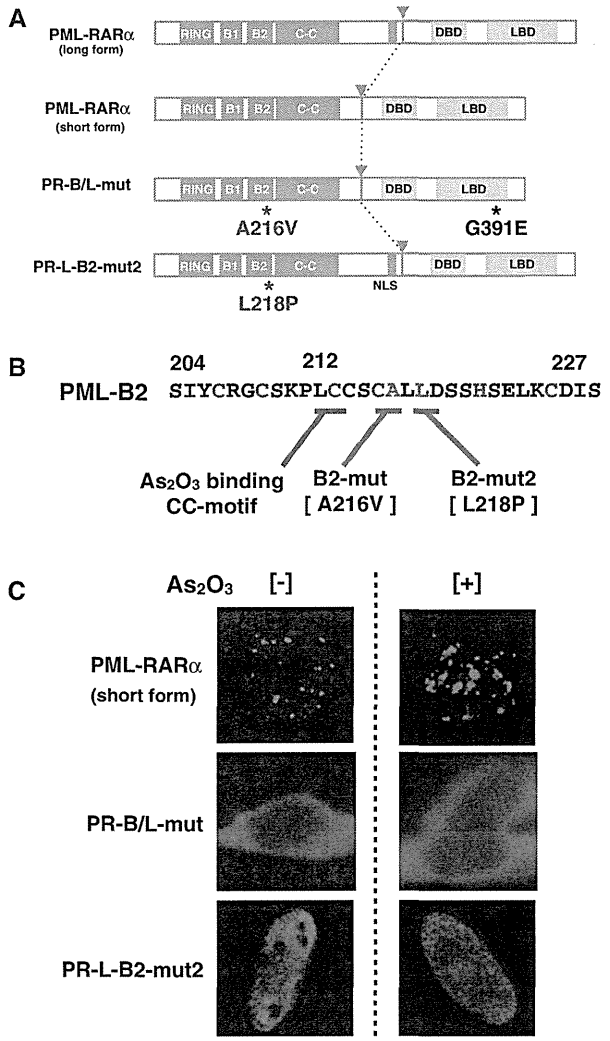

Fig. 4 Genetic mutations resulting in amino acid substitution in PML-B2 domain confirmed in clinically As_2O_3 resistant APL patients. **a** Schematic representation of PML-RARα chimeric protein with B2-domain mutation. One patient held PML-B2 mutation (A216V) and RARα-LBD mutation (G391E) on short form PML-RARα (PR-B/L-mut), and another patient held PML-B2 mutation (L218P) on long form PML-RARα [68]. **b** As_2O_3 direct binding dicysteine motif (C212/C213) [29, 30] and mutated positions in As_2O_3-resistant patients (C216 and L218) occur quite close to each other. **c** Flag-tagged PML-RARα short form, PR-B/L-mut, and PR-B2-mut2 were over expressed in HeLa cells with or without As_2O_3. Over expressed PML were detected by immunofluorescence staining using anti-Flag antibody. When using PML-RARα short form without As_2O_3, PML body was confirmed in the microspeckled pattern in cytoplasm. After incubation with As_2O_3, PML bodies showed macro granular patterns. When using PR-B/L-mut or PR-B2-mut2, the PML body showed diffuse pattern in cytoplasm or nucleus. No difference was seen with/without As_2O_3

RARα short form without nuclear localizing signal (NLS) [84], showed ATRA and As_2O_3 resistance at his terminal stage. Significant clonal expansion of *PML-RARα* mutant leading to A216V (PML-B2 domain mutation) and G391E (RARα-LBD mutation) was confirmed in leukemia cells harvested at the terminal stage (Fig. 4a, b). In vitro analysis using wild-type and mutant PML-RARα (PR-B/L-mut)-expressing HeLa and HL60 cells indicated that PML-RARα (short form) localized in cytoplasm as micro speckled pattern without As_2O_3, and as a macro granular pattern after adding As_2O_3 (Fig. 4c; PML-RARα). In contrast, PR-B/L-mut localized in cytoplasm with diffuse pattern without As_2O_3, and no change was confirmed in the presence of As_2O_3(Fig. 4c; PR-B/L-mut). Another case carried an L218P mutation, also in the PML-B2 domain (PR-B2-mut2), in PML-RARα long form with NLS. PML-RARα long form localized in nucleus, while PR-B2-mut2 was diffusely localized in the nucleus. No change was confirmed with or without As_2O_3 (Fig. 4c; PR-B2-mut2). Further in vitro analysis using PML-RARα overexpressed HeLa cells indicated that SUMOylation of PR-B/L-mut and PR-B2-mut2 after As_2O_3 treatment was strictly impaired. Recent reports have indicated that direct As_2O_3 binding to PML-B2 domain is critical for the serial reaction including SUMOylation, multimerization, and degradation [29, 30]. Jeanne et al. conclude that dicysteine C212/C213 in PML-B2 domain may be the direct As_2O_3 binding motif. From these results, genetic mutations identified in As_2O_3-resistant patients resulting in A216V and L218P may contribute to As_2O_3 resistance through impairment of direct As_2O_3 binding to PML-RARα due to conformational changes in As_2O_3 binding sites. Further accumulation of patients for genetic analyses is required for confirming the clinical significance of PML-B2 domain mutations in As_2O_3 resistance.

Conclusion

Although the overall survival of APL has been significantly prolonged since the introduction of ATRA and As_2O_3, relapse/refractory disease due to ATRA and/or As_2O_3 resistance remains a serious clinical problem. Additional genetic mutations in PML-RARα and another gene, such as FLT3-ITD or TP53 [66, 85], may contribute to disease progression and drug resistance in APL. Detailed genomic analyses using clinical samples harvested repeatedly from patients may help for predicting prognosis, selecting effective targeting drugs, understanding molecular backgrounds, and designing sophisticated new therapeutic strategies.

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