Published in final edited form as:
Leuk Lymphoma. 2014 July ; 55(7): 1523–1532. doi:10.3109/10428194.2013.842985.

Arsenic trioxide in front-line therapy of acute promyelocytic leukemia (C9710): prognostic significance of *FLT3* mutations and complex karyotype

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Abstract

The addition of arsenic trioxide (ATO) to frontline therapy of acute promyelocytic leukemia (APL) has been shown to result in significant improvements in disease-free survival (DFS). *FLT3* mutations are frequently observed in APL, but its prognostic significance remains unclear. We analyzed 245 newly diagnosed adult patients with APL treated on intergroup trial C9710 and evaluated previously defined biological and prognostic factors and their relationship to *FLT3* mutations and to additional karyotypic abnormalities. *FLT3* mutations were found in 48% of patients, including 31% with an internal tandem duplication (*FLT3*-ITD), 14% with a point mutation (*FLT3*-D835) and 2% with both mutations. The *FLT3*-ITD mutant level was uniformly low, <0.5. Neither *FLT3* mutation had an impact on remission rate, induction death rate, DFS or

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

overall survival (OS). The addition of ATO consolidation improved outcomes regardless of *FLT3* mutation type or level, initial white blood cell count, *PML–RARA* isoform type or transcript level. The presence of a complex karyotype was strongly associated with an inferior OS independently of post-remission treatment. In conclusion, the addition of ATO to frontline therapy overcomes the impact of previously described adverse prognostic factors including *FLT3* mutations. However, complex karyotype is strongly associated with an inferior OS despite ATO therapy.

Keywords

Acute promyelocytic leukemia; arsenic trioxide; *FLT3* mutations; mutant level; complex karyotype; prognosis

Introduction

Acute promyelocytic leukemia (APL) is defined by the presence of t(15;17)(q22;q21) resulting in the fusion gene *PML–RARA* [1,2]. This genetic abnormality is necessary but not sufficient to induce APL [3], and the need for additional genetic changes has been suggested in murine models of the disease [4–6]. *FLT3* mutations have previously been reported to occur frequently in APL, and may cooperate with *PML–RARA* in disease pathogenesis or maintenance [5–8].

FLT3 mutations have been reported in 20–45% of cases of APL [9–11], but studies of their clinical significance have yielded conflicting results. Most studies have found an association between *FLT3* genes harboring an internal tandem duplication in the juxtamembrane domain (*FLT3*-ITD) and adverse prognostic factors, including an elevated initial white blood cell count (iWBC), hypogranular variant morphology (M3 variant) and the short (bcr3) isoform of *PML–RARA* [7,9–15]. However, the prognostic significance of *FLT3* mutations remains unclear, since an independent impact on survival has not been clearly demonstrated [7,10–16]. In these studies, the presence of a recurrent activating point mutation in asparagine-835 of the tyrosine kinase domain (*FLT3*-D835) was not strongly associated with diagnostic features [10,12,13]. Nevertheless, a worse outcome for both *FLT3*-ITD and *FLT3*-D835 mutations has been reported [9,11,14,15,17–19]. Of note, the *FLT3* mutant level, suggested to be a critical determinant of prognosis in cytogenetically normal AML [20,21], has not been thoroughly evaluated in APL, with inconsistent reported results [10,22]. Some publications have also reported on an inverse relationship between the frequency of *FLT3*-ITD and the presence of additional chromosomal abnormalities (ACAs) accompanying t(15;17) [6,15,23]. However, the prognostic significance of ACAs in APL has remained a matter of debate. Some studies have observed a negative impact on outcome [24–26] but others were not able to show any significant independent relationship with survival [27–29].

We report here on the incidence and clinical impact of *FLT3* mutations and on their relationship to previously described biological risk factors in a large subset of adult patients with previously untreated APL who were enrolled on the North American Intergroup phase III randomized trial C9710. The clinical results of this trial demonstrated a significant disease-free survival (DFS) advantage for patients who were randomized to receive arsenic trioxide (ATO) as early post-remission therapy [30]. The frequency, mutant allele level and

insertional length of *FLT3* mutations were determined and correlated with other clinicopathological parameters at disease presentation, including: age, sex, iWBC, platelet count, cytogenetics and *PML–RARA* isoform and transcript level. We also report the novel observation that a complex karyotype (two or more ACAs) is associated with a significantly worse survival in this patient subset.

Patients, materials and methods

Study design

The North American Leukemia Intergroup Protocol C9710 opened in June 1999 and closed to accrual in March 2005. All patients required a clinical diagnosis of APL with confirmation of *PML–RARA* by reverse transcription-polymerase chain reaction (RT-PCR) assay. Informed consent for the treatment and correlative studies was obtained from the 481 adult patients enrolled on this study. Adult patients were randomized to a standard induction and consolidation regimen or to the same induction and consolidation plus two 25-day courses of ATO consolidation given immediately following induction. Details of treatment and clinical outcome have been previously reported [30]. A subset of 245 patients with available tissue from the C9710 protocol was evaluated for the presence of *FLT3* mutations.

Determination of *FLT3* mutation status

Pretreatment marrow and blood samples were obtained at the time of registration and mononuclear cells were isolated by Ficoll separation and cryopreserved in the leukemia cell banks of the three adult cooperative oncology groups (Cancer and Leukemia Group B [CALGB], Eastern Cooperative Oncology Group [ECOG] and Southwestern Oncology Group [SWOG]). All samples contained more than 70% of leukemic cells. Genomic DNA was extracted from those samples using a DNA purification kit (Puregene Gentra Systems, Minneapolis, MN) according to the manufacturer's instructions. When unavailable, cDNA was used instead of DNA. PCR was performed using the *FLT3* Mutation Assay from InVivoScribe (San Diego, CA) for both ITD and D835 mutations using 1 μg of genomic DNA and *Taq* polymerase (*Taq* Gold; Applied Biosystems, Foster City, CA) according to the manufacturer's instructions.

For determination of the *FLT3* mutant level, only genomic DNA was used. Primers for ITD were labeled with 6FAM and HEX, and the forward primer for D835 was labeled with NED [31]. Amplified products were run on an ABI 3100 DNA sequencer after a subsequent 1:10 dilution in formamide (Applied Biosystems) and analyzed using the Peak Scanner software 1.0 (Applied Biosystems). For *FLT3*-ITD, we used 50 ng of genomic DNA with the manufacturer's master mix and *Taq* polymerase. The PCR program consisted of initial preheating at 95°C for 7 min; 25 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min; and a final extension step at 72°C for 1 h. *FLT3*-ITD was detected when a fluorescence peak appeared for a size over 330 bp in both 6FAM and HEX colors. The *FLT3*-ITD mutant level was calculated only with 6FAM using the area under the peak and expressed as a ratio of ITD to the total *FLT3* alleles. For *FLT3*-D835, we used 50 ng of genomic DNA with the manufacturer's master mix and *Taq* polymerase. The PCR program consisted of initial preheating at 95°C for 7 min; 35 cycles of denaturation at

94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 1 min; and a final extension step at 72°C for 1 h. The *FLT3*-D835 mutant level was calculated using the area under the peak and expressed as the ratio of D835 to total *FLT3* alleles. Each reaction was performed in duplicate and each PCR product was run using capillary electrophoresis in duplicate. *FLT3* mutant level was reported as mean ± standard deviation.

PML–RARA real-time RT-PCR

Total RNA was prepared from the purified cells using RNA STAT-60 (Tel-Test, Friendswood, TX), a "single-step method" reagent, or by the RNeasy Total RNA isolation procedure (Qiagen, Valencia, CA). One to five micrograms of total RNA was synthesized into cDNA according to standard methods of the Transcriptor Reverse Transcriptase Protocol (Roche Diagnostics, Indianapolis, IN). Patient specimens, serial log dilutions of plasmid DNA and no template controls were amplified within a LightCycler instrument (Roche Diagnostics) using methods previously described [32]. Transcript level was reported using a dichotomized cut-point as either "high" or "low" (above or below the median normalized quotient value).

Cytogenetic analysis

Bone marrow or blood samples were processed for cytogenetic analysis in institutional laboratories by standard techniques using direct method and short-term (24 and/or 48 h) unstimulated cultures. Chromosomes were G-banded. Karyotypes were interpreted according to the International System for Human Cytogenetic Nomenclature (ISCN 2009) [33]. The presence of t(15;17)(q22;q21) and any additional genetic changes were reported. Patients positive for the presence of a *PML–RARA* transcript by PCR who had normal karyotype were excluded from the analysis because of insufficient evidence of a cryptic insertion. Complex karyotype was defined by the presence of two or more karyotypic abnormalities in addition to the translocation t(15;17).

Statistical analysis

The primary objective of this study was to compute the correlation between different biological markers and outcome and to report the incidence and prognostic significance of *FLT3* mutations and complex karyotype in a subset of patients enrolled on the C9710 trial with available tissue for the mutation analyses. Fisher's exact test using categorical variables was used to compare *FLT3* mutations with other diagnostic and prognostic features of APL, including iWBC (> and < 10 000/μL), initial platelet count (> and < 40 000/μL), *PML–RARA* isoform type (short versus long isoform), *PML–RARA* copy number (> and < median), bone marrow morphology, cytogenetics data (no, one versus two or more additional abnormalities), age (> and < median) and sex. Induction death and remission rate were evaluated in our cohort and associated with *FLT3* mutations and other prognostic features as categorical variables using a Fisher's exact test. DFS was defined as the time from maintenance randomization to relapse or death for patients who achieved a complete remission (CR) during induction. Overall survival (OS) was defined as the time from study entry to death. Five-year disease-free survival (DFS) and OS were estimated using the Kaplan–Meier method and compared across two levels of several factors (such as *FLT3*

status and iWBC count) using a *t* -test. A multivariable Cox proportional hazards model was used to correlate OS and DFS with clinical and biological characteristics after the effect of treatment was removed. The same analysis was used to compare the effect of treatment between both therapeutic arms. *FLT3* mutant level and length were studied as categorical variables (> and < median) and correlated with other prognostic features using Fisher's exact test. *FLT3* mutant level was correlated with OS and DFS in a multivariable Cox proportional hazards model. A logistic regression model was used to associate the iWBC and *FLT3* mutant level as continuous variables with outcomes. Statistical analyses for this report were completed on 20 December 2011. Median, minimum and maximum follow-up times of surviving patients were 52 months, 1.4 months and 98.7 months, respectively.

Results

FLT3 mutations: association with clinicopathological parameters and outcomes

A subset of 245 patients was evaluable for the presence of *FLT3* mutations and for correlation with pretreatment clinicopathological characteristics and treatment outcome. These 245 patients had similar presenting features to the overall cohort of 481 randomized patients. Exceptionally, 31% of these patients had an iWBC > 10 000/μL in our cohort, compared with 17% in the cohort of C9710 patients who were not examined in this analysis ($p < 0.001$), likely resulting from the greater availability of these samples in the cooperative group leukemia tissue banks. One-hundred and seventeen had a *FLT3* mutation (48%), including 77 patients with *FLT3*-ITD (31%), 35 with *FLT3*-D835 (14%) and five with both *FLT3*-ITD and *FLT3*-D835 (2%). As shown in Table I, the different mutational subgroups were well balanced between the two therapeutic arms. The presence or absence of *FLT3* mutations was not associated with age, sex or platelet count at diagnosis. *FLT3* mutations were associated with iWBC over 10 000/μL ($p < 0.001$), for *FLT3*-ITD cases ($p < 0.001$) but not for *FLT3*-D835 ($p = 0.11$). *FLT3*-ITD was further associated with the S (bcr3) isoform ($p < 0.001$), initial high *PML–RARA* transcript level ($p < 0.001$) and hypogranularM3 variant subtype ($p < 0.001$). None of these latter associations appeared in patients with *FLT3*-D835.

Both treatment arms received the same induction therapy and had approximately the same CR rates of 90% in our 245 patient subset (same as entire cohort of 481 C9710 patients). *FLT3* mutation status had no effect on the CR rate: wild-type (91.2%), *FLT3*-ITD (91.5%, $p = 1.0$), *FLT3*-D835 (92.3%; $p = 1.0$) (Table II), nor was there a relationship of mutation status to induction death (ID): *FLT3*-ITD ($p=0.58$), *FLT3*-D835 ($p = 0.48$). The lack of association of CR with *FLT3*-ITD was also observed in our 73-patient subset with a high iWBC (> 10 000/μL; $p = 0.16$). Of note, in our subset, there were no differences in the CR or ID rates related to pretreatment features, except for a weak association of the iWBC with ID ($p = 0.08$; Table III). These observations contrast with the strong association of a high iWBC with decreased CR and increased ID rates in the entire adult C9710 cohort [30]. Together, these observations indicate the incidental selection in this study of a high iWBC subset with a lower ID rate (11%) than in the comparable overall C9710 cohort (20%) [30], despite the increased proportion of subset cases with a high iWBC.

We found that DFS was not influenced by the presence of either *FLT3*-ITD ($p = 0.54$) or *FLT3*-D835 ($p = 0.18$) in a multivariable Cox model adjusting for treatment effect. Further,

there was no association between the presence of either *FLT3*-ITD (p= 0.30) or *FLT3*-D835 (p = 0.54) and OS in a Cox proportional hazards model adjusted for treatment effect. We also demonstrated the lack of association of *FLT3* mutation status on DFS by Kaplan–Meier analysis without adjusting for treatment effect. As illustrated in Figures 1(A) and 1(B), there was a large difference in DFS between treatment arms. However, within treatment type, *FLT3* mutation status had no significant effect, and the addition of ATO improved DFS regardless of *FLT3* mutation status. As in the entire C9710 cohort [30], the primary association accounting for the difference in DFS between treatment arms was the iWBC. In the standard all-*trans* retinoic acid (ATRA) arm, a high iWBC was strongly associated with reduced DFS (p = 0.002), while this parameter was not significantly associated with DFS in the ATO arm (p = 0.06; Table III). In the standard arm, the DFS rate at 5 years was 68% for patients with a iWBC< 10 000/μL and only 31% for patients with a WBC > 10 000/μL. In comparison, in the ATO arm the DFS rate at 5 years was 95% for patients with an iWBC less than 10 000/μL, and 74% for patients with an iWBC above 10 000/μL. In the overall subset, high iWBC remained associated with worse DFS in a Cox proportional hazards model adjusting for treatment effect (p = 0.0006). There was also a weak association of reduced DFS with the S isoform of *PML–RARA* in the standard arm (p = 0.02) but not in the ATO arm (p = 0.52; Table III). No other associations with presenting features were demonstrated in either arm. Overall, we conclude that the addition of ATO improved DFS in our 245-patient subset independently of any previously identified pretreatment risk factor.

FLT3 mutant level and insertion length

The *FLT3* mutant level relative to the total *FLT3* allele level was determined in 80 cases (50 *FLT3*-ITD, 30 *FLT3*-D835) in which sufficient material was available for analysis. The median mutant level was 0.39 (range 0.03–0.48) for *FLT3*-ITD [Figure 2(A)] and 0.33 (range 0.03–0.62) for *FLT3*-D835 [Figure 2(B)]. None of the *FLT3*-ITD levels were above 0.5 and only six cases with *FLT3*-D835 had a mutant level over 0.5. Neither *FLT3*-ITD nor *FLT3*-D835 mutant level influenced DFS or OS. Higher *FLT3*-ITD mutant levels were, however, strongly associated with increasing iWBC (p< 0.001). We also determined the length of insertion into the mutant allele. The median length of the duplicated insertion was 52 bp (range, 18–201 bp). There was no association of insertion length with clinical outcomes.

Cytogenetics: complex karyotype and relationship to *FLT3* mutations and treatment response

One hundred and ninety-four samples of the 245 evaluable patients had cytogenetic analysis available for central review (Table IV). The presence of t(15;17) as a sole abnormality was found in 71% of pretreatment samples. This sole abnormality was not statistically associated with either *FLT3*-ITD (p = 0.10) or *FLT3*-D835 (p = 0.83). One or more ACAs were found in 29% of the patients. Trisomy 8 was the most frequent secondary change reported in 9%, followed by ider(17q), gain of 8q, del(9q) and trisomy 21. Fifteen patients had a complex karyotype, defined as the presence of t(15;17) and at least two additional aberrancies. Complex karyotype was not associated with *FLT3* mutations (p = 1.00), nor was there any association with sex, age, initial WBC, specific isoform, initial *PML–RARA* transcript level or morphologic subset.

There was no association between the presence or absence of a single ACA and clinical outcome. However, the presence of a complex karyotype (two or more ACAs) defined a poor risk group regardless of treatment arm (Table V). The fifteen patients with complex karyotype had a lower CR rate of 73% (11/15) than those with either one ACA or a sole t(15;17), where CR rates were 95% (40/41) and 93% (125/138), respectively. While there was not a significant difference in DFS for patients with a complex karyotype, their OS was significantly worse ($p = 0.001$) [Figures 3(A) and 3(B)]. Deaths among these patients occurred in both treatment arms; in the standard treatment group, three of seven patients with complex karyotypes died and in the ATO arm, four of eight died. Death occurred at a median of 12 months from registration; all DFS events occurred before 24 months. The main cause of death was relapse: three with bone marrow relapse, one with central nervous system relapse. Notably, three of these relapse deaths occurred in patients treated on the ATO arm, in which only seven relapses in the entire ATO cohort were recorded at the time of this analysis. The other three patients died earlier during treatment, two during induction of central nervous system hemorrhage [1], one with differentiation syndrome [1] and one patient of pneumonia approximately 2 months after achieving remission. With a median follow-up of 51 months (1.3–99 months) for the surviving patients, the DFS rate was 60% (95% confidence interval [CI]: 30%, 90%) for those with a complex karyotype in comparison to 69% (95% CI: 59%, 80%) for patients with t(15;17) alone or with one additional abnormality. The OS was only 53% (95% CI: 28%, 79%) for patients with complex karyotypes compared to 81% (95% CI: 75%, 88%) for patients with sole t(15;17) or with one additional abnormality.

Discussion

We report a high incidence of *FLT3* (48%) mutations in a cohort of 245 patients with newly diagnosed APL enrolled on the first randomized trial to examine the impact of ATO consolidation (C9710) [30]. The 31% incidence of *FLT3*-ITD is similar to its incidence in cytogenetically normal (CN)-AML [34]. *FLT3*-D835 was present in 14% of cases, which is twice as high as in CN-AML but within the range of previously reported APL [17]. This high incidence confirms *FLT3* mutation as a frequent event in APL and as a potential key component in leukemogenesis [5]. It is likely that our overall detection rate of *FLT3* mutations is slightly higher than in other series due to its strong association with high iWBC and to the enrichment of high iWBC cases in the cooperative group leukemia cell banks. We also confirm the association of *FLT3*-ITD and -D835 mutations with higher iWBC, and the strong association of *FLT3*-ITD with the M3 variant, short *PML–RARA* isoform and high initial *PML–RARA* transcript levels [7,9,11 – 15,17].

Our study demonstrates that *FLT3* mutations were not prognostic for post-remission clinical outcome as assessed by univariate or multivariate analysis in our subset of the C9710 trial. The lack of an independent prognostic association of *FLT3* mutations with post-remission outcome is consistent with the preponderance of previous studies involving treatment regimens with ATRA and chemotherapy [9,10,12,14,15]. Other smaller studies (non-randomized) that have utilized ATO in frontline therapy have also reported a lack of prognostic significance of *FLT3* mutations [35–37]. Our results extend the findings of previous studies by demonstrating in a single randomized trial that *FLT3* mutations have no

impact on post-remission clinical outcome after receiving treatment with or without ATO consolidation therapy. This lack of impact, notably, was observed despite the very different outcomes in the two treatment arms [Figures 1(A) and 1(B)]. As shown both in our subset [Figures 1(A) and 1(B)] and in the overall C9710 trial [30], the markedly improved outcome with the addition of ATO consolidation was primarily related to the ability of the early ATO consolidation therapy to overcome the adverse effect of a high iWBC. At variance with several previous reports, including the pediatric component of C9710 [7,13,18,37], *FLT3*-ITD mutations were not or were very weakly associated with reduced CR ($p = 0.72$) and increased ID ($p = 0.08$). We suggest that the lack of these associations may be spurious, since the ID rate was considerably lower in the enriched proportion of high iWBC cases in our study set than in the high iWBC subset of the entire C9710 cohort [30]. On the other hand, it seems improbable that this selection bias had an impact on the post-remission results, since *FLT3* mutations consistently lacked an association with DFS despite the great difference in outcome between the two treatment arms, as noted above.

The genetic burden, or the level of mutant *FLT3* relative to wild-type *FLT3*, may be a key determinant of adverse outcomes in patients with CN-AML [20,21,38,39]. In these cases, a high mutant level occurs in approximately 10–20% of cases, as the result of duplication of the mutant allele by uniparental disomy (UPD) [21]. Others have reported that the length of the ITD insertion also has been shown to have independent prognostic value in CN-AML [40,41]. These prognostic features have not previously been thoroughly evaluated in *FLT3* mutant APL. Thus, we examined 80 of the 245 patients in our study group to evaluate the impact of mutant level of both the *FLT3*-ITD and the *FLT3*-D835 as well as the length of the ITD insertion. None of the *FLT3*-ITD cases had a mutant level above 0.5 relative to total *FLT3* alleles, suggesting that UPD is uncommon in APL and may not confer additional proliferative advantage [42]. Even when analyzed as a continuous variable, higher *FLT3*-ITD mutant levels up to 0.5 relative to total *FLT3* alleles were not associated with worse clinical outcome. This contrasts with two recent reports but is in line with another report, in all of which ATO was not included in frontline therapy [22,42]. It also seems noteworthy that in common with the other negative report [43], our analysis of mutant levels was DNA-based, whereas in the two positive studies, the mutant level was determined using cDNA expression [22,42]. Additionally, in contrast to CN-AML in which the incidence of UPD is markedly increased at relapse [44], the *FLT3*-ITD level was < 0.5 in eight C9710 cases of APL studied at relapse [8]. Similarly, there was no impact of the length of *FLT3*-ITD on outcome, which agrees with the results from the larger of two PETHEMA group (Programa Español de Tratamientos en Hematología) studies [22,43]. Overall, only six patients in our series with *FLT3*-D835 had a high mutant burden, and their outcome was not worse than in those with a low mutant level.

Biologically, APL with a *FLT3* mutation appears to be different from *FLT3* wild-type APL [8,45]. Gene expression profiling of APL cases identified two main groups of expression based on the presence or absence of *FLT3*-ITD [46,47]. In APL mouse models, *FLT3*-ITD was associated with a sole chromosomal abnormality involving *PML–RARA*, suggesting that mutation of *FLT3* may substitute for additional cooperating events and emphasizing its potential role in leukemogenesis of APL [6]. Three independent studies reported that *FLT3*

mutations were more common in patients with a sole abnormality of the t(15;17) than in those with additional cytogenetic changes [15,23,27]. However, in our large series, we found no association of the t(15;17) as a sole abnormality with *FLT3*-ITD or *FLT3*-D835, which is similar to data reported by a few other groups [24,26,29]. Nevertheless, a latent association between these genetic aberrations is suggested by our observation of a strong negative relationship of *FLT3*-ITD mutations and additional chromosome abnormalities in a subset of C9710 patients exclusively at relapse [8]. Although further investigation of this variably reported association may provide additional insights into factors that affect the evolution and selection of APL propagating clones under natural or suboptimal treatment conditions, it seems unlikely to be of clinical relevance at diagnosis in the era of high potency ATO-containing therapy in which relapses now rarely occur.

Genetic complexity in APL may, nevertheless, be prognostically significant even with highly effective therapy. The CR rate, DFS and OS were worse for the small group of patients with a complex karyotype (two or more ACAs) [Figures 3(A) and 3(B)] and were independent of ATO treatment. Of the seven relapses reported amongst the 119 patients who received ATO in our cohort, three (42%) had a complex karyotype. There was no association of complex karyotype with any specific clinical or other biological features. Fifty-six (29%) of the 194 evaluable patients had one or more karyotypic abnormalities in addition to t(15;17), but the presence of a single additional chromosomal change did not influence outcome in patients treated on C9710, in contrast to what has been reported by others [27–29]. Our new observation suggests that the presence of two or more additional cytogenetic abnormalities, although relatively rare in APL, renders the leukemic blasts genetically more complex and less sensitive to the therapeutic benefit of the *PML–RARA* targeting agents, ATRA and ATO. This interesting observation requires confirmation in a larger cohort of patients treated with both ATO and ATRA but may define a new clinically relevant subset. This observation is related to the specific schedule of ATO applied in the protocol C9710, and it is possible that earlier and more prolonged exposure to ATO, as recently reported in the Italian/German/Austrian cooperative group study [48], might overcome the negative effect of complex karyotypes. Detailed information on outcome by cytogenetic subset has not been reported for that study.

In conclusion, *FLT3* mutations occur very commonly in APL and, particularly cases with *FLT3*-ITD, are associated with several pretreatment features that have been variably identified in previous studies as adverse prognostic factors [7,9,11–15,17]. Of these features, only high iWBC has consistently been identified as an independent adverse prognostic indicator in ATRA–chemotherapy treatment regimens [49]. In the prospective, randomized trial C9710, the addition of ATO to ATRA–chemotherapy was shown to markedly improve post-remission outcome [30]. Thus, it is remarkable that in this study we found no association of *FLT3* mutations with post-remission outcome whether relatively poor (ATRA–chemotherapy) or excellent (added ATO). This strongly supports the conclusion that *FLT3* mutations at disease presentation are not effective prognostic indicators for postremission outcome in current APL treatment regimens. Our results are inconclusive related to the possible prognostic value of *FLT3* mutations for the remission induction period. In addition, we report for the first time that a complex karyotype was associated with

significantly inferior survival despite ATO frontline therapy. If this observation is confirmed in future studies, a complex karyotype may identify a small subset of patients who are at higher risk of relapse from a disease in which the vast majority of patients are long-term survivors with ATRA–ATO based therapy. Thus, if confirmed, patients with APL and a complex karyotype may benefit from novel therapeutic approaches, such as the addition of gemtuzumab ozogomycin or autologous transplant in first remission.

Acknowledgments

We thank Drs. Loren Joseph and Reddy Poluru from the Molecular Oncology Diagnostic Laboratory at the University of Chicago Medical Center for their valuable assistance.

W.S. and C9710 received support by grants from the National Cancer Institute (CA31946) to the Cancer and Leukemia Group B and to the CALGB Statistical Center. X.P. is supported by a Franqui-De Roover grant (Salus Sanguinis Foundation), Brussels, Belgium. R.E.G. is supported by NCI grant CA56771. E.P. is supported by NCI grants CA021115 and CA114737.

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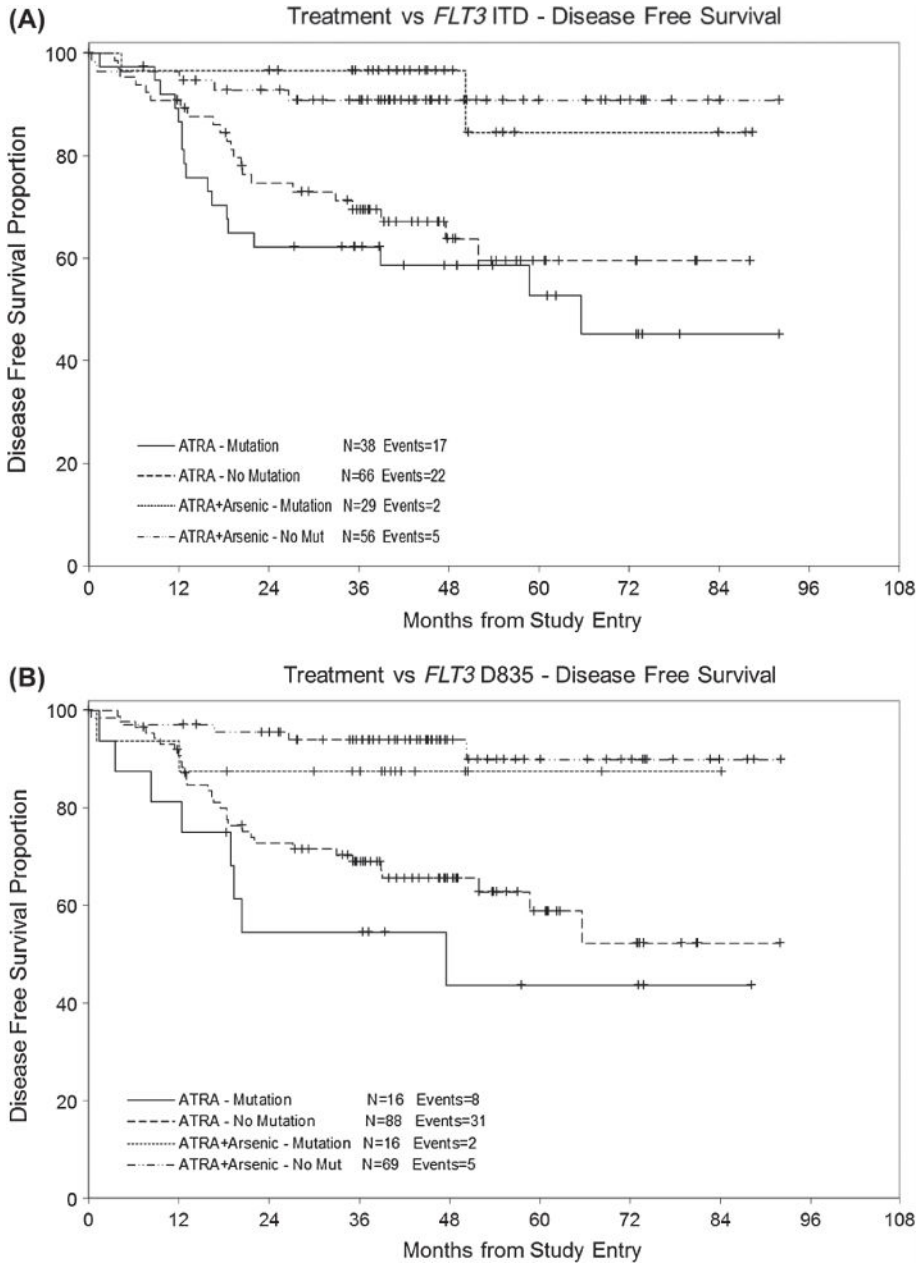

Figure 1.

Disease-free survival by *FLT3* mutational status and treatment arm. (A) DFS in standard arm (— and – –) and in arsenic trioxide arm (— and — – —). DFS is significantly better in arsenic than in standard arm ($p < 0.0001$). Presence of *FLT3*-ITD (— and —) did not influence DFS in either treatment arm. (B) DFS by treatment arm and *FLT3*-D835 mutational status in standard arm (— and – –) and in arsenic arm (— and — – —). *FLT3*-D835 (— and —) did not significantly influence DFS in either treatment arm.

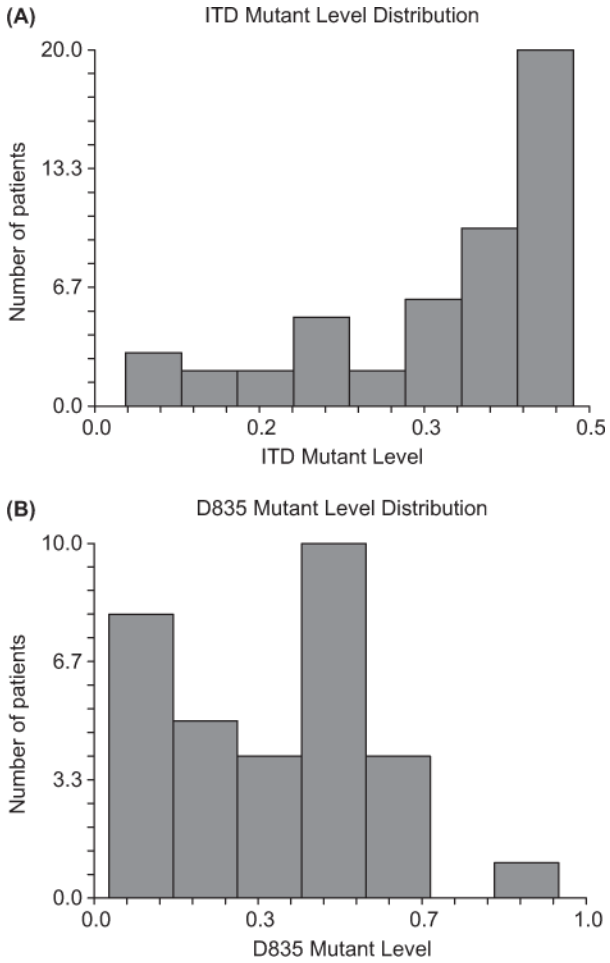

Figure 2.

Distribution of relative *FLT3*-ITD mutant level and relative *FLT3*-D835 mutant level in 80 patients. The *x*-axis shows values of mutant allele level relative to total *FLT3* allele and the *y*-axis indicates number of patients. Shaded columns show absolute number of patients among relative mutant levels. (A) Distribution of relative *FLT3*-ITD mutant level and (B) distribution of relative *FLT3*-D835 mutant level.

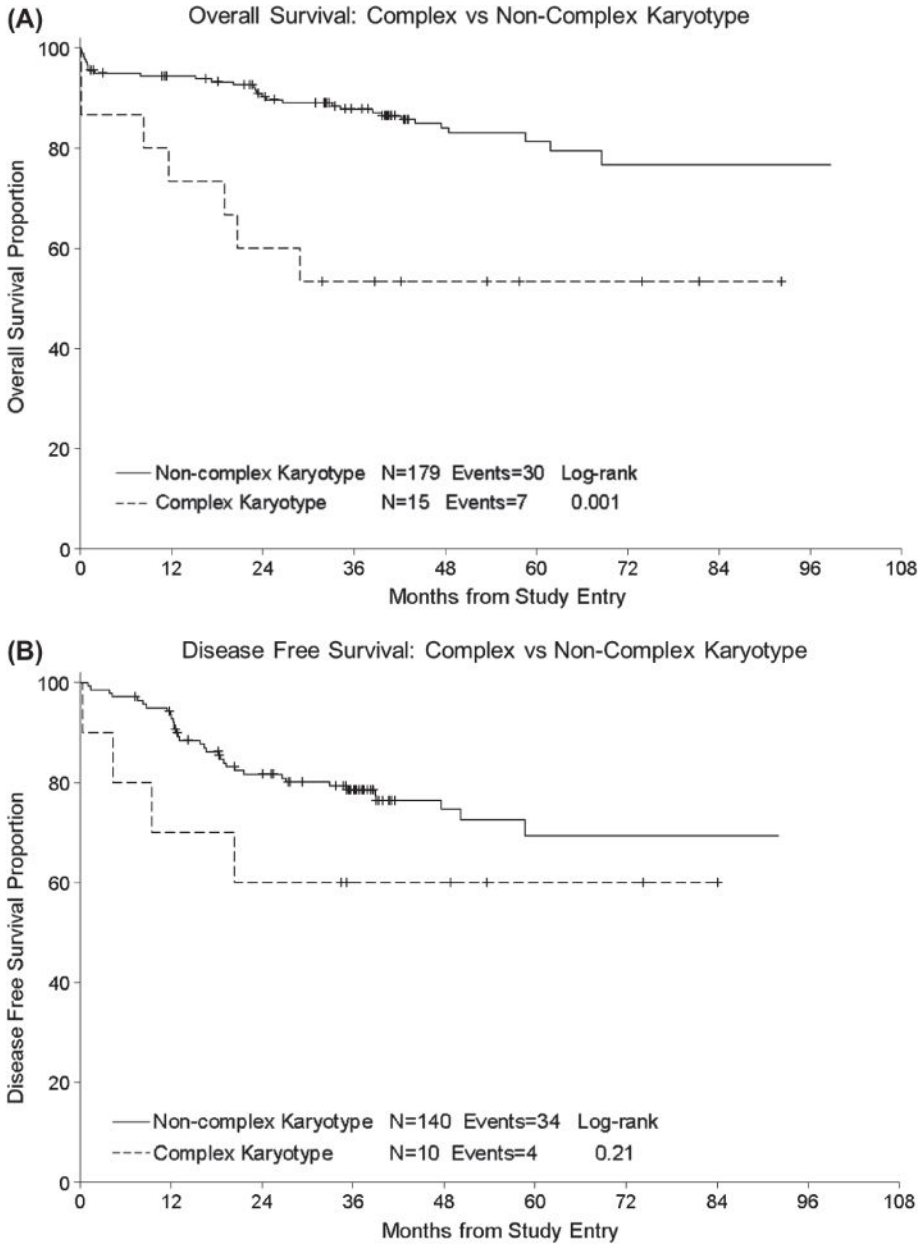

Figure 3.

Overall survival and disease-free survival between complex and non-complex karyotype. (A) OS for non-complex karyotype (—) is significantly better ($p = 0.001$). (B) There is no significant difference in DFS for complex compared to non-complex karyotype (—). Median DFS has not been reached in either group.

Table I

Relationship between *FLT3* mutation status and patient characteristics.

	***FLT3* wild type (*n* = 128)**		***FLT3*-ITD only (*n* = 77)**			***FLT3*-D835 only (*n* = 35)**			***FLT3*-ITD + *FLT3*-D835 (*n* = 5)**	
	ATRA	**ATRA + ATO**	**ATRA**	**ATRA + ATO**	***p***	**ATRA**	**ATRA + ATO**	***p***	**ATRA**	**ATRA + ATO**
Sex	M: 37 F: 33	M: 32 F: 26	M: 18 F: 19	M: 20 F: 20	0.27	M: 10 F: 5	M: 13 F: 7	0.20	M: 3 F: 1	M: 1 F: 0
Age (years), median, range	42.5, 13–79	43.5, 17–80	41, 22–73	41, 17–68	0.96	31, 20–56	39.5, 26–73	0.10	39.5, 32–54	56, —
Initial WBC, median, range	1.8K, 0.4–96.7K	1.5K, 0.2–45.4K	12.2K, 0.6–117.4K	11.8K, 0.2–102.5K	< 0.0001	16.6K, 0.5–87K	4.8K, 0.5–47.7K	0.11	12.9K, 6.1–20.4K	5.3K, —
Initial plts, median, range	29.5K, 7–218K	29K, 4–232K	24K, 1–163K	26K, 4–88K	0.03	37K, 7–111K	28K, 5–102K	0.35	20.5K, 13–95K	57K, —
Isoform	S: 17 L: 33 V: 3	S: 6 L: 30 V: 5	S: 17 L: 9 V: 1	S: 19 L: 11 V: 1	< 0.0001	S: 2 L: 6 V: 2	S: 6 L: 11 V: 0	0.63	S: 2 L: 1 V: 0	S: – L: – V: –
PML – RARA level, median, range	0.0337, 0.0033–0.227 (*n* = 6)	0.0263, 0.01–0.0782 (*n* = 13)	0.0675, 0.00128–0.35 (*n* = 12)	0.124, 0.0446–3.79 (*n* = 8)	0.11	0.0124, 0.0003–0.0795 (*n* = 5)	0.0796, 0.0154–0.273 (*n* = 6)	0.13	0.00321, 0.002–0.0901 (*n* = 3)	–
BM morphology	M3: 56 M3v: 3	M3: 48 M3v: 5	M3: 21 M3v: 12	M3: 24 M3v: 13	< 0.0001	M3: 12 M3v: 3	M3: 17 M3v: 3	1.0	M3: 4 M3v: 0	M3: 1 M3v: 0

ATRA, all-*trans* retinoic acid; ATO, arsenic trioxide; M, male; F, female; WBC, white blood count; plts, platelets; K, 10^9/L; BM, bone marrow; S, short isoform; L, long isoform; V, variable isoform; *p*, *p*-value resulting from Fisher's exact test or rank-sum test between *FLT3* status and patient characteristics.

Leuk Lymphoma. Author manuscript; available in PMC 2014 December 22.

Table II

Association of outcome with *FLT3* mutation status in patients with APL.

	Wild type	*FLT3*-ITD	*p*	*FLT3*-D835	*p*	Either mutant	*p*
No.	128	82		40		117	
CR, %	91.2	91.5	1.000	92.3	1.000	91.3	1.000
ID, %	6.3	7.3	0.58	2.5	0.48	6.0	1.000
5-year DFS, %							
ATRA	63.6	52.7	0.57	43.6	0.33	50.3	0.25
ATRA + ATO	92.3	84.5	0.61	87.5	0.88	84.7	0.44
5-year OS, %							
ATRA	73.6	76.3	0.87	77.9	0.79	76.8	0.71
ATRA + ATO	89.2	68.8	0.10	90.0	0.34	76.3	0.16

CR, complete remission rate; ID, induction death rate; DFS, disease-free survival; OS, overall survival; ATRA, standard arm; ATRA + ATO, arm with early addition of arsenic.

Table III

Fisher's *p*-values of association of non-*FLT3* biological parameters and outcome.

			DFS		OS	
	CR	**ID**	**ATRA**	**ATRA + ATO**	**ATRA**	**ATRA + ATO**
Initial WBC > vs. < 10K	0.21	0.08	0.002	0.06	0.001	0.03
Isoform L vs. S	1.0	0.53	0.02	0.52	0.52	0.18
PML–RARA level > vs. < median	0.61	1.00	0.38	0.83	0.64	0.24

ATRA, all-*trans* retinoic acid; ATO, arsenic trioxide; CR, complete remission rate; ID, induction death rate; DFS, disease-free survival; OS, overall survival; WBC, white blood count; K, 10^9/L; S, short isoform; L, long isoform.

Table IV

Cytogenetics studies.

	Total	*FLT3*-ITD	*FLT3*-D835
Number of patients analyzed	194	69	30
t(15;17) as sole abnormality	138	49	22
Any additional abnormality	56	20	8
One additional abnormality	41	15	6
Two or more additional abnormalities	15	5	2
Trisomy 8	18	6	2
ider(17)(q10)t(15;17)	7	2	1
Gain of 8q	3	2	0
del(9q)	4	0	1
Trisomy 21	3	1	1

Table V

Detailed cytogenetics study on 15 patients with complex karyotype.

Case 1: 48,XX,t(15;17)(q22;q21), + 21, + mar[4]/49,idem, + mar[13]/46,XX[3]
Case 2: 46,XX,der(15)t(15;17)(q22;q21),ider(17)(q10)t(15;17)(q22;q21)[12]/47,idem, + 8[8]
Case 3: 46,XY,del(6)(q23q27),der(7)t(7;8)(q22;q13),t(15;17)(q22;q21)[10]
Case 4: 46,XY,der(15)t(15;17)(q22;q21),ider(17)(q10)t(15;17)(q22;q21)[16]/46,XX,del(1)(q32q42),der(2)t(2;17)(p13;q23),del(13)(q12q14), der(15) t(15;17)(q22;q21)t(2;17)(p13;q23),ider(17)(q10)t(15;17)(q22;q21)[4]
Case 5: 46,XY,del(9)(p22p24),der(15)ins(15;17)(q22;q22.1q2?3),del(16)(q23),der(17)ins(15;17)(q22;q21.1q2?3)del(17)(q21q21)t(17;20) (q2?3;q13.?3),del(19)(p13.2),der(20)t(17;20)(q2?3;q13.?3)[20]
Case 6: 46,XY,t(15;17)(q22;q21)[4]/46,idem,der(12)t(12;17)(p13;q11.2)[3]/46,idem,der(12)t(12;17)(p13;q11.2),der(15)t(9;15)(q13;p13)[3]/49,idem,+ 8,+ 10,+ der(15)t(15;17)(q22;q21)[6]
Case 7: 47,XY,+ 8,t(15;17)(q22;q21)[16]/46,XY,der(15)t(15;17)(q22;q21),ider(17)(q10)t(15;17)(q22;q21)/46,XY[2]
Case 8: 46,XY,add(4)(p11.2),del(6)(q13q23),del(13)(q12q14),t(15;17)(q22;q21),− 16,− 18, + 2mar[7]
Case 9: 46,Y,add(X)(q28),t(4;13)(q27;q22),− 7,− 8,t(15;17)(q22;q21), + 2mar[19]/46,XY[1]
Case 10: 46,XY,t(7;12)(q32;q22),t(15;17)(q22;q21)[5]/46,XY,t(7;12)(q32;q22),der(15)t(15;17)(q22;q21),ider(17)(q10)t(15;17)[4]/46,XY[11]
Case 11: 46,XY,t(15;17)(q22;q21),add(21)(p11.2),add(22)(p11.2)[18]/46,XY,add(13)(p11.2),t(15;17)(q22;q21),add(22)(p11.2)[2]
Case 12: 46,XX,−2,add(6)(p23),+ add(7)(q11.2),del(9)(q13q22),− 10,der(15)t(15;17)(q22;q21),− 17, + 2mar[19]/46,XX[1]
Case 13: 46,XY,add(3)(p12),der(4)t(4;17)(p15.2;q21),add(9)(q12),del(10)(q21),add(12)(p13),t(15;17)(q22;q12),− 17,− 18, + 2mar[13]/46,XY[7]
Case 14: 46,XY,t(15;17)(q22;q21)[14]/46,XY,der(1)t(1;7)(p32;q34),der(7)del(7)(q22q34)t(1;7),del(9)(q13q22),t(15;17)(q22;q21)/46,XY[3]
Case 15: 45−46,XX,add(13)(p11.2),t(15;17)(q22;q21),− 20,− 22,+ 1−2mar[cp20]