Prognostic value of *FLT3* mutations in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline monochemotherapy

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ABSTRACT

Background

Fms-like tyrosine kinase-3 (*FLT3*) gene mutations are frequent in acute promyelocytic leukemia but their prognostic value is not well established.

Design and Methods

We evaluated *FLT3*-internal tandem duplication and *FLT3*-D835 mutations in patients treated with all-trans retinoic acid and anthracycline-based chemotherapy enrolled in two subsequent trials of the *Programa de Estudio y Tratamiento de las Hemopatías Malignas* (PETHEMA) and *Hemato-Oncologie voor Volwassenen Nederland* (HOVON) groups between 1996 and 2005.

Results

FLT3-internal tandem duplication and *FLT3*-D835 mutation status was available for 306 (41%) and 213 (29%) patients, respectively. Sixty-eight (22%) and 20 (9%) patients had internal tandem duplication and D835 mutations, respectively. Internal tandem duplication was correlated with higher white blood cell and blast counts, lactate dehydrogenase, relapse-risk score, fever, hemorrhage, coagulopathy, BCR3 isoform, M3 variant subtype, and expression of CD2, CD34, human leukocyte antigen-DR, and CD11b surface antigens. The *FLT3*-D835 mutation was not significantly associated with any clinical or biological characteristic. Univariate analysis showed higher relapse and lower survival rates in patients with a *FLT3*-internal tandem duplication, while no impact was observed in relation to *FLT3*-D835. The prognostic value of the *FLT3*-internal tandem duplication was not retained in the multivariate analysis.

Conclusions

FLT3-internal tandem duplication mutations are associated with several hematologic features in acute promyelocytic leukemia, in particular with high white blood cell counts, but we were unable to demonstrate an independent prognostic value in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline-based regimens.

Key words: acute promyelocytic leukemia, *FLT3* mutations, prognostic factors, all-trans retinoic acid, anthracyclines.

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Introduction

Constitutive activation of the fms-like tyrosine kinase 3 (*FLT3*) receptor due to internal tandem duplication (*FLT3*-ITD) and a point mutation in the activation loop (*FLT3*-D835) may confer proliferative and survival advantages to acute myeloid leukemia blasts.[1,2] The presence of *FLT3* mutations, in particular of the ITD, has been consistently associated with a worse outcome in patients with acute myeloid leukemia with normal cytogenetics or intermediate-risk cytogenetics.[3,4] However, the prognostic value of *FLT3* mutations in patients with acute promyelocytic leukemia (APL), a form of acute myeloid leukemia characterized by the specific t(15;17), is still a matter of controversy. Although several studies in APL have reported an association between *FLT3*-ITD and various characteristics, including elevated white blood cell (WBC) count, BCR3 isoform, and microgranular morphology (M3v),[5-15] the prognostic value of these mutations remains to be established. Two previous studies used multivariate analysis including both WBC count, a well-known poor risk factor in APL,[16] and ITD in order to assess the independent value of each one. The study by Gale *et al.*[12] showed that the WBC count was the sole independent prognostic factor for relapse and survival. In contrast, Chillón *et al.*[17] analyzed the prognostic value of the ratio and size of ITD and found that both the length of the duplications and WBC count were independent prognostic factors in APL. With regards to the *FLT3*-D835 mutation, most authors were unable to demonstrate that this mutation has an impact on prognosis,[7,9-11] although in some studies patients with the D835 mutation were found to have worse outcomes.[12,14]

To provide new insights into the characterization of *FLT3* mutations in APL, we set out to evaluate the frequencies of ITD and D835 mutations, as well as the size and ratio of ITD, and their relationships with a broad variety of clinical and biological features. We also assessed the prognostic value of these mutations in a large cohort of APL patients enrolled in two successive trials of the *Programa de Estudio y Tratamiento de las Hemopatías Malignas* (PETHEMA) and *Hemato-Oncologie voor Volwassenen Nederland* (HOVON) groups (LPA96 and LPA99).

Design and Methods

Patients and eligibility

Between November 1996 and June 2005, 739 patients enrolled in the consecutive multicenter PETHEMA LPA96 and PETHEMA/HOVON LPA99 trials were required to have a diagnosis of *de novo* APL with demonstration of the t(15;17) or *PML/RARA* rearrangement. More details about the general exclusion and inclusion criteria for these trials are reported elsewhere.[18-20] According to the Declaration of Helsinki, informed consent was obtained from all patients, and the protocol was approved by the Research Ethics Board of each participating hospital.

Therapy for acute promyelocytic leukemia

The induction regimen consisted of oral all-trans retinoic acid (ATRA, 45 mg/m^2/day) divided into two daily doses, which was maintained until morphological complete remission was achieved, and intravenous idarubicin (12 mg/m^2/day) on days 2, 4, 6, and 8. For patients 20 years of age or younger, the dose of ATRA was adjusted to 25 mg/m^2/day. From November 1999, the idarubicin on day 8 was omitted for patients older than 70 years. Patients in complete remission received three monthly consolidation courses. The detailed consolidation schedule has been reported elsewhere. Briefly, the first and the third courses consisted of idarubicin and the second of mitoxantrone. From November 1999 (LPA99 study), intermediate- and high-risk patients, as previously defined,[21] received ATRA (45 mg/m^2/day for 15 days) combined with the three chemotherapy courses,[19,20] and the idarubicin dose was slightly increased. Patients who tested negative for *PML/RARA* at the end of consolidation were started on maintenance therapy with oral mercaptopurine, intramuscular methotrexate, and intermittent oral ATRA over 2 years. Details of the supportive therapy have been described elsewhere.[18,22]

Analysis of FLT3-*ITD* and FLT3-*D835* mutations

Analyses for *FLT3*-ITD and *FLT3*-D835 mutations were performed on bone marrow samples collected at the time the APL had been diagnosed. Samples were sent to reference laboratories, in sodium citrate or EDTA vials, and processed within 24 h. DNA was obtained using QIAmp DNA Blood Mini Kit extraction kits (QIAGEN GmbH Hilden, Germany) and UltraClean™ extraction kits (MO BIO Carlsbad, CA, USA). RNA was obtained using Rneasy Mini kits (QIAGEN GmbH Hilden, Germany) or Trizol reagent (Invitrogen Carlsbad, CA, USA). Then 0.5 µg of RNA were reverse transcribed into cDNA in a 25 µL reaction volume with the TaqMan Gold RT-PCR Kit (PE Applied Biosystems Branchburg, NJ, USA).

FLT3-ITD and *FLT3*-D835 mutations were studied qualitatively in DNA or cDNA samples using the methods described by Nakao *et al.*[23] and Moreno *et al.*[24] In selected cases, the presence of *FLT3*-ITD and *FLT3*-D835 mutations was confirmed by sequencing the amplified products.

Quantitative assessment of *FLT3*-ITD mutations was performed only in patients with available genomic DNA, by Genescan analysis using a fluorescently labeled primer with 6-FAM to determine the allelic *FLT3*-ITD ratio and size, following the method described by Thiede *et al.*[25] If several mutant alleles were detected by Genescan, the mutant allele with the highest allelic ratio was selected for the size analyses.

Definitions and study endpoints

Induction of remission was assessed according to the recently revised criteria described by Cheson *et al.*[26] For morphological assessment of leukemia resistance, sufficient time had to have passed to allow for full terminal differentiation of the malignant promyelocytes (up to 40-50 days). Molecular remission was defined as the disappearance on an ethidium bromide gel of the *PML/RARA*-specific band visualized at diagnosis, using reverse transcriptase polymerase chain reaction (RT-PCR) assays with a sensitivity level of one cell in 10^{-4}, or as the disappearance of the *PML/RARA* rearrangement detected by real-time quantitative PCR (RQ-PCR).[27] Molecular persistence was defined as PCR positivity in two consecutive bone marrow samples collected at the end of consolidation therapy. The positive cases with a very low number of copies (<5 copies) by RQ-PCR were confirmed to be positive using a low sensitivity method (nested RT-PCR assay). Molecular relapse was defined as the reappearance of PCR positivity in two consecutive bone marrow samples at any time after consolidation therapy.[28] A genetic diagnosis of APL using PCR, anti-PML staining or cytogenetic tests was required for the diagnosis of overt hematologic relapse.

Differentiation syndrome was diagnosed and graded according to previously defined criteria.[29] Coagulopathy was defined as a prolonged prothrombin time and/or activated partial thromboplastin time in addition to hypofibrinogenemia and/or increased levels of fibrin degradation products or D-dimers. Patients were

classified as having t(15;17) with or without additional chromosomal abnormalities in accordance with previously defined criteria.[30] The patients' performance status at diagnosis was measured using the Eastern Cooperative Oncology Group (ECOG) scale. The risk of relapse was estimated at diagnosis using a predictive model based on the patients' leukocyte and platelet counts at diagnosis, as reported elsewhere.[21] Relapse-risk groups were defined as reported elsewhere[19] and summarized here: low-risk patients had a WBC count less than 10×10^9/L and a platelet count more than 40×10^9/L; intermediate-risk patients had a WBC count less than 10×10^9/L and a platelet count less than 40×10^9/L; and high-risk patients had a WBC count of 10×10^9/L of more.

Statistical methods

The χ^2 test, with Yates' correction if necessary, was used to analyze differences in the distribution of categorical variables between subsets of patients. The Student's t-test was used to analyze continuous variables with a normal distribution and Mann-Whitney's U test for data that failed the normality test. Thirty-four characteristics of the patients and the disease were examined to establish their relationship to *FLT3*-ITD and *FLT3*-D835 mutations. These characteristics are listed in Tables 1 and 2. In addition we analyzed the following variables: total body surface, levels of serum creatinine, uric acid, albumin, total bilirubin, and fibrinogen, peripheral blood platelet count and hemoglobin levels, peroxidase reactivity of bone marrow blasts, and CD9, CD13, CD33, CD56 and CD117 surface antigen markers. For comparison, unadjusted time-to-event analyses were performed using the Kaplan-Meier estimate,[31] log-rank tests and their generalizations.[32] For all estimates in which the event "relapse" was considered as an endpoint, hematologic and molecular relapse, as well as molecular persistence (*PML/RARA* positive by RT-PCR at the end of consolidation), were each considered as uncensored events. Overall survival was calculated from the time of diagnosis to the time of death from any cause, and relapse-free survival from the date of achieving complete remission to the time of relapse. All *P* values reported are two-sided. The patients' follow-up was updated on March 15, 2010. The characteristics selected for inclusion in the multivariate analysis, using the Cox proportional hazards model, were those for which there was some indication of a significant association in univariate analysis ($P<0.1$) and, if available, those for which prior studies had suggested a possible relationship. Computations were performed using the 4F, 3D, 1L and 2L programs from the BMDP statistical library (BMDP Statistical Software Inc, Los Angeles, CA, USA).

Results

Incidence of FLT3 mutations

We were able to perform an analysis of *FLT3*-ITD mutations at diagnosis in 306 (41%) patients (57 patients in the LPA96 and 249 in the LPA99 trial), and *FLT3*-D835 in 213 (29%) of 739 patients with APL. The following characteristics were observed more frequently in the patients tested for *FLT3* mutations than in those who were not tested (n=433): LPA99 trial ($P=0.01$), fibrinogen concentration less than 170 mg/dL ($P= 0.04$), and hemoglobin concentration less than 10 g/dL ($P=0.06$). The patients came from 53 institutions in Spain and The Netherlands (see *Appendix*), and *FLT3* analyses were routinely performed in eight reference laboratories (see *Appendix*). The median follow-up of the series was 97 months (range, 15-158 months) from diagnosis.

Overall, 68/306 patients (22%) had a *FLT3*-ITD mutation and 20/213 patients (9%) had a *FLT3*-D835. One patient presented with both mutations concomitantly.

Genescan analysis and sequencing of FLT3 mutations

Genescan analysis to determine size and ratio was conducted in 39 patients with *FLT3*-ITD. The ratio of the *FLT3*-ITD mutations ranged from 0.06 to 1.3 with a median of 0.66, and the length varied from 6 to 180 bp with a median of 40 bp. Thirty-five patients showed only one mutant allele, and four showed two mutant alleles of different sizes.

Sequence analysis performed in 20 *FLT3*-ITD positive and 10 *FLT3*-D835 positive APL samples showed that in all cases ITD mutations consisted of in-frame duplications involving exon 11, while D835 mutations consisted of replacement of aspartic residue by tyrosine (8 cases) or histidine (2 cases).

Patients' characteristics according to FLT3 mutation status

The main clinical and biological characteristics of the patients according to *FLT3* mutation status are summarized in Tables 1 and 2. *FLT3*-ITD was significantly associated with higher WBC count, higher relapse-risk score, more than 70% blasts in peripheral blood, BCR3 isoform, M3v subtype ($P<0.001$ for all), lactate dehydrogenase levels greater than 600 UI/L ($P=0.005$), presence of fever or coagulopathy at diagnosis (both $P=0.02$), more than 70% blasts in the bone marrow ($P=0.03$), and hemorrhage ($P=0.04$). *FLT3*-ITD was also associated with expression of the following surface markers: CD2, CD34 (both $P<0.001$), HLA-DR ($P=0.03$), and CD11b ($P=0.04$)

By contrast, *FLT3*-D835 was not significantly associated with higher WBC count or any other hematologic characteristic (Table 2).

Induction outcome

The overall induction death rate in the series was 8.8%. As shown in Table 3, there was a significantly higher induction death rate in patients with *FLT3*-ITD than in patients without *FLT3*-ITD mutations (16% *versus* 7%, $P=0.03$). The incidence of severe differentiation syndrome was 22% among patients with *FLT3*-ITD mutations and 13% among those without *FLT3*-ITD ($P=0.05$). Multivariate analysis identified a WBC count greater than 10×10^9/L ($P<0.001$), age greater than 60 years ($P=0.001$), and creatinine levels greater than 1.4 mg/dL ($P=0.02$) as independent prognostic factors for induction death.

No significant associations were found with induction outcomes according to the *FLT3*-D835 mutation status (Table 4).

Post-remission outcomes

Relapse-free survival

Fourteen relapses, 11 overt and 3 molecular, occurred among the *FLT3*-ITD positive cohort at a median time of 16 months (range, 4-75 months) after achievement of complete remission; and 27 relapses, 17 overt and 10 molecular, occurred among the *FLT3*-ITD negative cohort at a median time of 23 months (range, 4-85 months) after attainment of complete remission.

Univariate analysis showed a lower 5-year relapse-free survival rate in *FLT3*-ITD-positive patients than in *FLT3*-ITD-negative ones (77% *versus* 88%; $P=0.02$) and no impact of *FLT3*-D835 mutation status (93% *versus* 84%; $P=0.29$) (Figure 1 and Tables 3 and 4). We did not find sig-

Table 1. Disease and patients' characteristics according to *FLT3*-ITD mutations.

Characteristic	*FLT3*-ITD positive Median (range)	*FLT3*-ITD positive N. (%)	*FLT3*-ITD negative Median (range)	*FLT3*-ITD negative N. (%)	P
Overall		68 (100)		238 (100)	
PETHEMA trial					
LPA96		15 (22)		42 (18)	0.41
LPA99		53 (78)		196 (82)	
Age, years	40 (6-72)		41 (2-81)		
≤ 18		5 (7)		23 (10)	0.81
19-40		31 (46)		89 (37)	
41-60		21 (31)		81 (34)	
> 60		11 (16)		45 (19)	
Gender					
Male		34 (50)		121 (51)	0.91
Female		34 (50)		117 (49)	
ECOG score (n=284)	1 (0-3)		1 (0-3)		
0-1		45 (78)		173 (71)	0.26
2-3		18 (22)		48 (29)	
Fever (n=305)					
No		33 (49)		155 (65)	0.02
Yes		34 (51)		83 (35)	
Hemorrhage					
No		5 (7)		45 (19)	0.04
Yes		63 (93)		193 (81)	
Hepatosplenomegaly (n=299)					
No		58 (88)		218 (94)	0.13
Yes		8 (12)		15 (6)	
WBC count,×10⁹/L	16.9 (0.6-148)		1.2 (0.2-133)		<0.001*
≤ 5		16 (23)		176 (74)	<0.001
5-10		8 (12)		20 (8)	
10-50		32 (47)		35 (15)	
> 50		12 (18)		7 (3)	
Relapse-risk group					
Low		5 (7)		52 (22)	<0.001
Intermediate		19 (28)		144 (60)	
High		44 (65)		52 (18)	
Blasts in PB, % (n = 276)	82 (0-98)		32 (0-100)		
≤ 70		22 (34)		160 (76)	<0.001
> 70		42 (66)		52 (24)	
Blasts in BM, % (n = 287)	90 (30-100)		89 (6-100)		
≤ 70		6 (10)		48 (21)	0.03
> 70		57 (90)		176 (79)	
Coagulopathy					
No		9 (13)		66 (28)	0.02
Yes		59 (87)		172 (72)	
LDH, IU/L (n = 297)	778 (225-2904)		505 (206-3950)		
≤ 600		21 (32)		121 (48)	0.005
> 600		44 (68)		111 (52)	
Morphological subtype					
Hypergranular		39 (57)		210 (88)	<0.001
Microgranular		29 (43)		28 (12)	
Cytogenetics (n = 223)					
t (15;17)		32 (73)		124 (69)	0.65
t (15;17) plus other**		12 (27)		55 (31)	
PML/RARα isoform (n = 298)					
BCR1/BCR2		13 (19)		158 (68)	<0.001
BCR3		54 (81)		73 (32)	
CD2 (n = 201)					
Positive		20 (44)		26 (17)	< 0.001
Negative		25 (56)		130 (83)	
CD11b (n = 164)					
Positive		0 (0)		19 (15)	0.04
Negative		33 (100)		112 (85)	
CD34 (n = 233)					
Positive		29 (45)		26 (86)	<0.001
Negative		24 (55)		154 (14)	
HLA-DR (n = 215)					
Positive		6 (3)		5 (12)	0.03
Negative		43 (97)		161 (88)	

continued in the next column

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**P compares continuous variables (mean WBC count 28.4 versus 7.4); **plus other additional chromosomal abnormalities; PB: peripheral blood; BM: bone marrow; LDH: lactate dehydrogenase.*

nificant differences in relapse-free survival according to several levels of *FLT3*-ITD ratio (0.5, 0.66, and 0.8) or size (40, 50, and 60 bp) (Table 3 and Figure 2).

Multivariate analysis was performed including the following variables: age, gender, WBC count, relapse-risk score, protocol, M3 morphology, BCR isoform, *FLT3*-ITD positive (*versus* negative), and *FLT3*-ITD size greater than 60 bp (*versus* <60 bp and negative together). A WBC count greater than 10×10^9/L was the only independent prognostic factor for relapse ($P<0.001$).

Overall survival

Univariate analysis showed a lower 5-year overall survival rate in *FLT3*-ITD-positive patients than in *FLT3*-ITD-negative ones (71% *versus* 81%; $P=0.05$). In contrast, similar 5-year overall survival rates were observed when comparing *FLT3*-D835-positive with FLT3-D835-negative patients (80% *versus* 80%; $P=0.80$) (Tables 3 and 4).

Multivariate analyses showed that a WBC count greater than 10×10^9/L and age over 60 years were independent prognostic factors for survival (both, $P<0.001$).

Discussion

This study shows that *FLT3*-ITD mutations, but not *FLT3*-D835 ones, are associated with notable hematologic and clinical features, such as high WBC counts, high percentages of peripheral blood and bone marrow blasts, coagulopathy, M3v subtype, BCR3 isoform, and expression of CD2, CD34, HLA-DR, and CD11b surface antigens. Although univariate analyses showed higher induction death and lower relapse-free survival rates among *FLT3*-ITD-positive patients, multivariate analyses revealed that the *FLT3*-ITD had no independent prognostic value in patients with APL treated with ATRA and anthracycline-based chemotherapy.

As far as we know, our study represents the largest single series addressing the clinical significance of *FLT3* mutations in APL. It should be noted that, although patients came from 53 institutions, the *FLT3* analyses were performed in a limited number of reference laboratories. Since not all centers performed the *FLT3* analyses, which were not compulsory in the initial diagnostic set, we cannot definitely exclude a selection bias. Activating mutations of *FLT3* have been reported in APL at frequencies within the range of 12% to 39% for *FLT3*-ITD (median 21%),[5-15,17,33,34] and 6% to 22% for *FLT3*-D835 (median 9%).[6-12,14,15,17] The incidence of these mutations in our

Table 2. Disease and patients' characteristics according to *FLT3*-D835 mutations.

Characteristic	*FLT3*-D835 positive Median (range)	N. (%)	*FLT3*-D835 negative Median (range)	N. (%)	*P*
Overall		20 (100)		193 (100)	
PETHEMA trial					
LPA96		3 (15)		39 (20)	0.79
LPA99		17 (85)		154 (80)	
Age, years	34 (8-71)		41 (2-81)		
≤ 18		4 (20)		15 (8)	0.35
19-40		9 (45)		84 (44)	
41-60		5 (25)		55 (28)	
> 60		2 (10)		39 (20)	
Gender					
Male		9 (45)		99 (51)	0.59
Female		11 (55)		94 (49)	
ECOG score (n=194)	1 (0-2)		1 (0-3)		
0-1		12 (80)		140 (78)	0.99
2-3		3 (20)		39 (22)	
WBC count, $\times 10^9$/L	4.7 (0.6-40)		2.6 (0.3-118)		0.25*
≤ 5		10 (50)		120 (62)	0.29
5-10		3 (15)		19 (10)	
10-50		7 (35)		41 (21)	
> 50		0 (0)		13 (7)	
Relapse-risk group					
Low		3 (15)		36 (19)	0.79
Intermediate		10 (50)		103 (53)	
High		7 (35)		54 (28)	
Blasts in PB, % (n = 192)	73 (0-100)		46 (0-100)		
≤ 70		8 (44)		120 (69)	0.07
> 70		10 (56)		54 (31)	
Blasts in BM, % (n = 201)	89 (59-100)		89 (7-100)		
≤ 70		3 (16)		32 (18)	0.85
> 70		16 (84)		150 (82)	
Morphological subtype					
Hypergranular		14 (70)		154 (80)	0.46
Microgranular		6 (30)		39 (20)	
PML/RARα isoform (n = 205)					
BCR1/BCR2		12 (60)		104 (56)	0.75
BCR3		8 (40)		81 (44)	

**P compares continuous variables (mean WBC count 10.2 versus 11.8); PB: peripheral blood; BM: bone marrow.*

Table 3. Treatment results according to *FLT3*-ITD mutations in the study population.

	FLT3-ITD negative N. of patients	%	*FLT3*-ITD positive N. of patients	%	*P* value
Overall	238	100	68	100	
Induction outcome					
Complete remission	220*	92	57	84	0.06
Induction death	16	7	11	16	0.03
Positive PCR after induction** (n=249)	111	56	28	56	0.97
Differentiation syndrome					
Severe	30	13	15	22	0.05
Moderate/absent	208	87	53	78	
Post-remission outcomes at 5 years					
Overall survival		81		71	0.05
Relapse-free survival		88		77	0.02
ITD size > 60 bp. (n=7)		NA		57	0.61
ITD size < 60 bp. (n=24)		NA		71	
ITD ratio > 0.66 (n=16)		NA		63	0.74
ITD ratio ≤ 0.66 (n=15)		NA		73	

**Two patients among the FLT3-ITD negative cohort were considered as resistant; **Positive PCR of PML/RARA in a bone marrow sample after induction; NA: Not applicable.*

Table 4. Treatment results according to *FLT3*-D835 mutations in the study population.

	FLT3-D835 negative N. of patients	%	*FLT3*-D835 positive N. of patients	%	*P* value
Overall	193	100	20	100	
Induction outcome					
Complete remission	176*	91	15*	75	0.06
Induction death	16	8	4	20	0.19
Differentiation syndrome					
Severe	33	17	2	10	0.62
Moderate/absent	160	83	18	90	
Post-remission outcomes at 5 years					
Relapse-free survival		84		93	0.29
Overall survival		80		80	0.80

**Two patients were considered as having resistant disease (1 among the FLT3-D835 negative cohort and 1 among the positive cohort).*

study, 22% and 9%, respectively, are consistent with the reported data. As in previous studies,[9,11,12,14] in our series the simultaneous presence of both *FLT3*-ITD and *FLT3*-D835 mutations was extremely rare.

The mutant allelic ratio of *FLT3*-ITD in APL has been analyzed in two studies in which median ratios of 1.0 (range, 0.11 to 6.55),[11] and 0.66 (range, 0.3 to 1.0),[17] were found in 30 and 19 patients, respectively. In our series of 39 patients for whom the mutant allelic ratio was known, the median was 0.66 (range, 0.06 to 1.3) which was the same as that reported by Chillón *et al.*[17] Unlike in the study by Xue *et al.*,[11] patients with a ratio greater than 2, indicating loss of wild-type allele, were not observed.

In line with previous studies,[5-7,9,11-15] we found a strong association between ITD and high WBC counts. We also found a clear correlation between ITD and M3v and BCR3, as has previously been reported by others.[6-10,12,15] However, the relationship found between ITD and CD34, CD2, and HLA-DR expression had not been previously noted. This relationship could be explained by the association of the ITD with M3v and BCR3 APL which, in turn, have been shown to express the CD34, CD2, and HLA-DR surface markers more frequently.[35,36] We confirm that high WBC counts, like elevated lactate dehydrogenase levels and coagulopathy, are more frequent in *FLT3*-ITD-positive APL.[5] The increased frequency of hemorrhage and high percentages of peripheral blood and bone marrow blasts that we report here have not been described previously. In contrast to Xue *et al.*,[11] and Gale *et al.*,[12] we were unable to show an association between *FLT3*-D835 and elevated WBC counts or any other clinical or biological characteristic.

As previously reported,[11,13,14,33] patients with ITD had an increased risk of induction death also in our study. However, the multivariate analysis made clear that this was due to the association with hyperleukocytosis, a well-established risk factor for induction death.[22] One study suggested that there is an inverse relationship between molecular remission rate and ITD in patients treated with arsenic trioxide.[15] An association between *FLT3*-ITD and molecu-

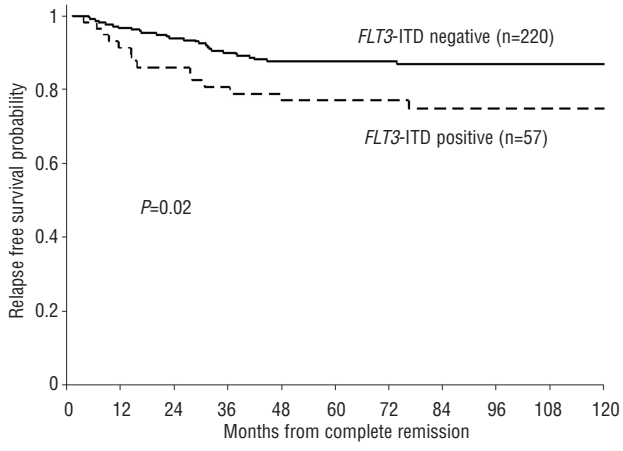

Figure 1. Relapse-free survival in APL patients according to *FLT3*-ITD mutation status.

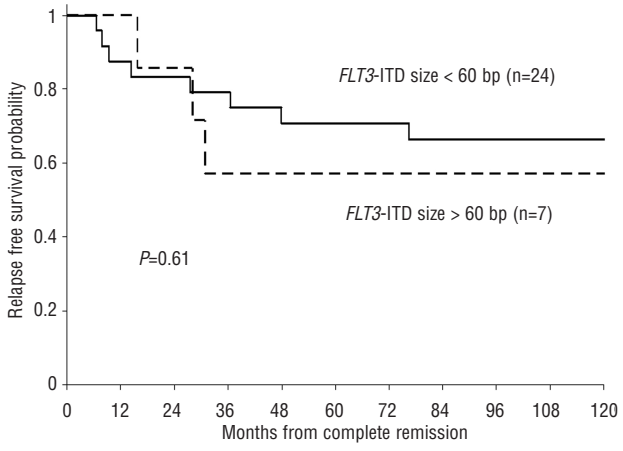

Figure 2. Relapse-free survival in *FLT3*-ITD positive APL patients according to ITD size.

lar remissions was not apparent in the present study.

The associations reported between ITD and overall survival and relapse-free survival rates have previously been established by univariate but not by multivariate analysis.[9,17,37] In our study, *FLT3*-ITD status was removed from the regression equation when WBC count was included in the multivariate analysis, showing that the adverse long-term outcome of *FLT3*-ITD mutations is attributable to its relationship with elevated WBC count. Furthermore, in a much larger series, we were unable to demonstrate the adverse prognostic impact of the ratio and length of *FLT3*-ITD mutations reported by Chillón *et al.*[17] We should, however, be very cautious when interpreting the potential prognostic impact of the ratio and size of *FLT3*-ITD mutations because of the low number of datasets available. Regarding the *FLT3*-D835, unlike Gale *et al.*,[12] we found no prognostic significance of this mutation in our series.

In conclusion, *FLT3*-ITD mutations, but not *FLT3*-D835 ones, are associated with characteristic diagnostic hematologic features of APL, in particular with high WBC counts. However, in a large series of APL patients treated with ATRA and anthracycline-based chemotherapy, we were unable to demonstrate an independent prognostic value of *FLT3* mutations. Given the relationship between *FLT3*-ITD and high WBC count, targeted therapy with *FLT3* inhibitors could be an attractive strategy to improve long-term outcome in high-risk patients.[12]

Appendix

The following institutions and clinicians participated in the study: Spain *(Programa Español de Tratamiento de las Hemopatías Malignas, PETHEMA)—Basurtuko Ospitalea, Bilbao: J. M. Beltrán de Heredia; Complejo Hospitalario de Segovia: J.M. Hernández; Complexo Hospitalario Xeral-Calde, Lugo; J. Arias; Complejo Hospitalario, León: F. Ramos; Fundación Jiménez Díaz, Madrid: A. Román; Hospital 12 de Octubre, Madrid: J. de la Serna; Hospital Carlos Haya, Málaga: S. Negri; Hospital Central de Asturias, Oviedo: C. Rayón; Hospital Clinic, Barcelona: J. Esteve; Hospital Clínico de Valladolid: F.J. Fernández-Calvo; Hospital Clínico San Carlos, Madrid: J. Díaz-Mediavilla; Hospital Clínico San Carlos (H. Infantil), Madrid: C. Gil; Hospital Clínico Universitario, Santiago de Compostela: M. Pérez-encinas; Hospital Clínico Universitario, Valencia: M. Tormo; Hospital Clínico Universitario Lozano Blesa, Zaragoza: M. Olave; Hospital de Cruces, Barakaldo: E. Amutio; Hospital del Mar, Barcelona: C. Pedro; Hospital de Navarra, Pamplona: A. Gorosquieta; M. Viguria; M. Zudaire ; Hospital Dr Negrín, Las Palmas: T. Molero; Hospital Dr Peset, Valencia: M. J. Sayas; Hospital Dr Trueta, Girona: R. Guardia; Hospital General de Albacete: F. Manso; Hospital General de Alicante: C. Rivas; Hospital General de Alicante (Oncología Pediátrica): C. Esquembre; Hospital General de Castellón: R. García; Hospital General de Especialidades Ciudad de Jaén: A. Alcalá; J.A. López; Hospital General de Jerez de la Frontera: V. Rubio; Hospital General de Murcia: M.L. Amigo; Hospital General de Valencia: M. Linares; Hospital Germans Trias i Pujol, Badalona: J. M. Ribera; Hospital Insular de Las Palmas: J. D. González San Miguel; Hospital Juan Canalejo, A Coruña: G. Debén; Hospital Joan XXIII, Tarragona: L. Escoda; Hospital La Princesa, Madrid: R. de la Cámara; Hospital Materno-Infantil de Las Palmas: A. Molines; Hospital do Meixoeiro, Vigo: C. Loureiro; Hospital Montecelo, Pontevedra: M.J. Allegue; L. Amador; Hospital Mutua de Terrasa: J.M. Martí; Hospital Niño Jesús, Madrid: L. Madero; A. Lassaletta; Hospital Ntra. Sra. de Sonsoles, Ávila: M. Cabezudo; Hospital Ramón y Cajal, Madrid: J. García-Laraña; Hospital Reina Sofía, Córdoba: R. Rojas; Hospital Río Carrión, Palencia: F. Ortega; Hospital Río Hortega, Valladolid: M. J. Peñarrubia; Hospital San Jorge, Huesca: F. Puente; Hospital San Rafael, Madrid: B. López-Ibor; Hospital Sant Pau, Barcelona: S. Brunet; Hospital San Pedro de Alcántara, Cáceres: J. M. Bergua; Hospital Santa María del Rosell, Cartagena: J. Ibáñez; Hospital Severo Ochoa, Leganés: P. Sánchez; Hospital Son Dureta, Palma de Mallorca: A. Novo; Hospital de Tortosa: LL. Font; Hospital Txagorritxu, Vitoria: J. M. Guinea; Hospital Universitario del Aire, Madrid: A. Montero; Hospital Universitario de Salamanca: M. González; Hospital Universitario La Fe, Valencia: M. A. Sanz, G. Martín, J. Martínez, P. Montesinos; Hospital Universitario La Fe (Hospital Infantil), Valencia: A. Verdeguer; Hospital Universitario La Paz (Hospital Infantil), Madrid: P. García; Hospital Universitario Marqués de Valdecilla, Santander: E. Conde; Hospital Universitario Príncipe de Asturias, Alcalá de Henares: J. García; Hospital Universitario Puerta del Mar, Cádiz: F.J. Capote;*

Hospital Universitario Puerta de Hierro, Madrid: I. Krsnik; Hospital Universitario Vall D'Hebron, Barcelona: J. Bueno; Hospital Universitario Materno-Infantil Vall D'Hebron, Barcelona: P. Bastida; Hospital Universitario Virgen de la Arrixaca, Murcia: A. Rubio; Hospital Universitario Virgen de la Arrixaca (Pediatría), Murcia: J.L. Fuster; Hospital Universitario Virgen del Rocío, Sevilla: J. González; Hospital Universitario Virgen de la Victoria, Málaga: I. Pérez; Hospital Virgen del Camino (Infantil), Pamplona: J. Molina; Hospital Virgen del Camino (Adultos), Pamplona: M.C. Mateos; M.A. Ardaiz; Clínica San Miguel, Pamplona: M. Rodríguez-calvillo; Hospital Xeral Cíes, Vigo; C. Poderós; Institut Català d'Oncologia, Hospitalet de Llobregat; M. Arnán, R. Duarte; Hospital de Fuenlabrada, Fuenlabrada: J.A. Hernández; Hospital General de Guadalajara, Guadalajara: M. Díaz-Morfa; Hospital Juan Ramón Jimenez, Huelva: E. Martín-Chacón; Hospital Doctor José Molina Orosa, Lanzarote: J.M. Calvo-Villas; Hospital Madrid Norte Sanchinarro, Madrid: D. García-Belmonte; Hospital U. La Paz, Madrid: D. Hernández-Maraver; The Netherlands *(The Dutch-Belgian Hemato-Oncology Cooperative Group, HOVON) —VU Medical Center Amsterdam: G. J. Ossenkoppele; Academic Medical Center, University of Amsterdam: J. van der Lelie; Erasmus University Medical Center, Rotterdam: B. Lowenberg, P. Sonneveld, M. Zijlmans, GC de Greef, M. Jongen-Lavrencic; University Medical Center, Groningen: E. Vellenga; Gasthuisberg Hospital, Leuven: J. Maertens; OLVG Hospital, Amsterdam: B. de Valk; Den Haag Hospital, Leyenburg: P.W. Wijermans; Medical Spectrum Twente Hospital, Enschede: M.R. de Groot; Academic Hospital Maastricht: H.C. Schouten; St. Antonius Hospital, Nieuwegein: D.H. Biesma; Sophia Hospital, Zwolle: M. van Marwijk Kooy*

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Authorship and Disclosures

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