Flt3 Internal Tandem Duplication and P-Glycoprotein Functionality in 171 Patients with Acute Myeloid Leukemia

Christophe Marzac,[1,2] I. Teyssandier,[2] Ors'Anton Calendini,[2] Jean-Yves Perrot,[2] Anne-Marie Faussat,[1] Ruoping Tang,[1] Nicole Casadevall,[2] Jean-Pierre Marie,[1,3] and Ollivier Legrand[1,3]

Abstract

Purpose: Patients with adult acute myeloid leukemia (AML) with intermediate cytogenetics remain a heterogeneous group with highly variable individual prognoses. New molecular markers could help to refine cytogenetic stratification.

Experimental Design: We assessed P-glycoprotein (Pgp) activity and Flt3 internal tandem duplication (ITD+) because of their known prognostic value and because they might lead to targeted therapy. We did a multivariate analysis on 171 patients with adult AML treated in the European Organization for Research and Treatment of Cancer protocols.

Results: ITD+ and high Pgp activity (Pgp+) were found in 26 of 171 (15%) and 55 of 171 (32%) of all patients, respectively. ITD and Pgp activities were negative in 94 of 171 (55%, Pgp−ITD− group), mutually exclusive in 73 of 171 (43%, Pgp−ITD+ and Pgp+ITD− groups), and only 4 of 171 (2%, Pgp+ITD+ group) patients were positive for both. In multivariate analyses, Pgp+ITD+ ($P < 0.0001$) and age ($P = 0.0022$) were independent prognostic factors for the achievement of complete remission (CR). Overall survival (OS), CR achievement ($P < 0.0001$), WHO performance status ($P = 0.0007$), and Pgp+ITD+ status ($P = 0.0014$) were also independent prognostic factors. In 95 patients with intermediate cytogenetics, the CR rates of ITD+ patients were 40% versus 62% for ITD− ($P = 0.099$) and 41% versus 67% ($P = 0.014$) for Pgp+ versus Pgp− patients. In the Pgp−ITD− group (41 of 95), CR rates were 70% versus 44% for others ($P = 0.012$), OS achieved 48% versus 16% ($P < 0.0001$) and disease-free survival was 56% versus 27% ($P = 0.024$), respectively. Furthermore, the OS curves of the intermediate cytogenetics-Pgp−ITD− group were not significantly different from the favorable cytogenetic group.

Conclusion: Flt3/ITD and Pgp activity are independent and additive prognostic factors which provide a powerful risk classification that can be routinely used to stratify the treatment of patients with intermediate cytogenetic AML. ITD+ and Pgp+ patients should be considered for targeted therapy.

Despite improvements accomplished during the last three decades with the use of combinations of cytarabine and intercalating agents, the overall prognosis for adult acute myeloid leukemia (AML) remains poor (1). A number of clinical and biological features that reflect the heterogeneity of AML are used to predict the likelihood of response to standard treatment (2). Adverse prognostic factors include age >60 years, a poor performance score before treatment, AML resulting from prior chemotherapy, or an antecedent hematologic disorder such as myelodysplastic syndrome, a white cell count of $>20 \times 10^9$/L and high P-glycoprotein (Pgp) activity (3–6) at presentation. The major effect of cytogenetics (7–11) as an independent prognostic factor has paved the way for risk-adapted treatment (12–14).

However, ~50% to 60% of patients with adult AML present normal karyotypes or cytogenetic aberrations not otherwise classifiable, and are therefore classified in the intermediate risk group. In this vast group, therapeutic results are highly variable, ranging from refractory to chemosensitive diseases, which may reflect the molecular heterogeneity of these AMLs. There has been much interest in identifying new molecular markers of prognostic value in this group (15–20). Of these, the Flt3 internal tandem duplication (Flt3/ITD), which is found in up to 30% of normal karyotypes of AML, has been associated with reduced rates of complete remission (CR), disease-free survival (DFS), and overall survival (OS; refs. 21, 22). The *Flt3* gene encodes a class III receptor tyrosine kinase that has an important role in cell proliferation, differentiation, and survival (23). The Flt3/ITD mutation leads to constitutively activated proteins, confers ligand-independent proliferation, inhibits apoptosis, and impairs granulocytic differentiation (24). Furthermore, the presence of a predominant mutated allele at the DNA level or the

Authors' Affiliations: [1]Université Pierre et Marie Curie-Paris 6, UMRS 736, Les Cordeliers, INSERM, UMR S 736, Paris F-75006, [2]Service d'Hématologie Biologique, and [3]Département d'Hématologie Clinique, AP-HP, Hôpital Hôtel-Dieu, Paris, France

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Requests for reprints: Ollivier Legrand, Département d'Hématologie et d'Oncologie Médicale, AP-HP, Hôpital Hôtel-Dieu, 75181 Paris cedex 04, France. Phone: 33-14234-8585; Fax: 33-14234-8406; E-mail: ollivier.legrand@htd.ap-hop-paris.fr.

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loss of a wild-type allele, thus reflecting a high proportion of blasts bearing the mutation, is associated with a very poor prognosis (25). On the other hand, we have previously shown that high activity of Pgp and other ATP-binding cassette transporters are also a frequent phenomenon in adult AML with a normal karyotype, and may serve as a prognostic marker (26).

In this study, we did a multivariate analysis of all well-known major risk factors in a series of 171 patients with AML, while paying particular attention to Flt3 mutational status and Pgp functionality. We show that the predominant role of the Flt3/ITD clone and high Pgp functionality are nonredundant events in AML blasts, and that the absence of both abnormalities clearly identifies a new good risk AML group in the intermediate risk category.

Materials and Methods

Patients and treatment

Between January 1997 and January 2005, we assessed the Flt3/ITD and Pgp activity status of 171 consecutive samples from *de novo* adult AML treated in the European Organization for Research and Treatment of Cancer protocols. The diagnosis was based on French-American-British (FAB) criteria. Immunophenotyping was done by flow cytometry. Patients with acute promyelocytic leukemia were excluded from the study (due to retinoic acid treatment). Patients with t(9;22) were also excluded. For each patient, several clinical and biological characteristics were analyzed (age, WHO performance status, WBC count at diagnosis, CD34 expression, FAB subtypes, and cytogenetics). Karyotypes were defined as previously reported (7, 9).

None of the patients in this study had a history of prior therapies with anticancer drugs. All patients were treated with uniform chemotherapy and transplantation. They received a combination of cytarabine (100 mg/m^2/d) for 10 days, daunorubicin (45 mg/m^2/d) for 3 days, and etoposide (100 or 50 mg/m^2/d) for 5 days. Those who achieved CR after one or two cycles of therapy received one cycle of consolidation therapy (cytarabine, 500 mg/m^2/12 hours for 6 days, and daunorubicin, 45 mg/m^2/24 hours for 3 days). Patients achieving CR were subsequently scheduled to proceed to allogeneic bone marrow transplantation if a matched sibling donor was available (10 patients); patients >50 years old or lacking a suitable donor received an autograft or chemotherapy. Patients receiving one allograft were censored at the time of transplant.

Flt3/ITD detection

DNA isolation and amplification. DNA was extracted from frozen bone marrow or peripheral blood samples using Nucleon kits (Amersham Biosciences) according to the manufacturer's protocol.

Flt3 exons 14 and 15 (27) were amplified with 35 PCR cycles (94°C for 30 seconds, 60°C for 1 minute, and 72°C for 1 minute) using 500 ng of genomic DNA, 0.3 μmol/L each of primers S (TggTg-TTTgTCTCTTCTTCATTgT) and AS (gTTgCgTTCATCACTTTTCCAA), 750 μmol/L of deoxynucleotide triphosphate, 5 mmol/L of $MgCl_2$, 1.25 units of Taq GOLD polymerase, and 1× buffer GOLD I (Perkin-Elmer, Norwalk, CT) in a total volume of 50 μL.

ITD analysis. Amplicons were migrated in 2% agarose gels, allowing the detection of a 330-bp fragment for wild-type alleles and/or longer fragments for Flt3/ITD alleles. Two categories were distinguished: samples showing an absence or a weak detection of the mutated allele were considered negative (ITD−) for this study, and samples with marked or predominantly mutated bands were considered positive (ITD+).

Pgp functional assay with JC-1 probe

Cells (5×10^5 cells/mL) were incubated with 0.1 μmol/L of JC-1 monomer at 37°C for 15 minutes with or without modulator (cyclosporin A, 2 μmol/L). Cells were washed twice in cold PBS. Cell fluorescence was recorded using a FACSORT flow cytometer (Becton-Dickinson, Meylan, France) equipped with a 488 nm argon laser and three fluorescence detectors FL1 (530 nm bandpass filter), FL2 (585 nm bandpass filter), and FL3 (650 nm bandpass filter). JC-1 fluorescence was analyzed on the FL1 and FL2 channels for the detection of the dye monomer and liquid crystal form, respectively. The function of Pgp was established in the whole blast cell population selected by CD45 antibody weak expression (FL3 channel, $HPCA_2$ clone; Becton-Dickinson, Le Pont de Claix, France). High Pgp functionality (Pgp+) was reached when Pgp inhibition by cyclosporin A, assessed by the D of Kolmogorov-Smirnov statistical test, was at least ≥0.5 (28).

Statistical analyses

The associations between variables were analyzed by Fisher's exact test for categorical variables and by the Mann-Whitney U test or Kruskal-Wallis test for continuous variables. Clinical and biological factors were investigated for their influence on remission rate by Fisher's exact test for binary variables and by the Mann-Whitney U test or Kruskal-Wallis test for continuous variables or by regression logistic model: (*a*) DFS was measured from the establishment of CR until relapse or death from any cause, with observation censored for patients last known to be alive without report of relapse; (*b*) OS was measured from diagnosis until death from any cause, with observation censored for patients last known to be alive. They were estimated by the Kaplan-Meier method and compared by the log-rank test or by Cox model. Analyses of prognostic factors for treatment outcomes were based on proportional hazards regression models for DFS and OS. Significance was defined as a two-tailed $P \leq 0.05$. The Cox proportional model was used for the multivariate analyses. The median follow-up time for censored patients was 721 days. The time point used for the proportion of DFS and OS was May 31, 2005. We used StatView software (version 5.0) for statistical analysis (SAS Institute, Inc., San Diego, CA).

Results

Patient characteristics. Patient characteristics are shown in Table 1. We assessed both Flt3/ITD and Pgp activity in 171 adult patients with a median age of 54 years. Twenty-six out of 171 (15%) were ITD+ and 55 out of 171 (32%) presented high Pgp activity. Ninety-four patients (55%) were considered negative for both (ITD−/Pgp−), 51 patients (30%) were ITD−/Pgp+, 22 patients (13%) were ITD+/Pgp−, and only 4 patients (2%) were both ITD+/Pgp+. Pgp+ patients had significantly less Flt3/ITD mutations than Pgp− patients [7% (4 of 55) versus 19% (22 of 116), $P = 0.049$] and ITD+ patients had a significantly lower activity of Pgp (0.29 ± 0.05) than ITD− patients (0.4 ± 0.02, $P = 0.04$).

Patient characteristics according to Flt3 and Pgp status are shown in Table 2. The frequency of ITD+ patients [22% (21 of 95)] was significantly higher in the intermediate cytogenetic group than in others ($P = 0.03$). In contrast, ITD+ was rarely observed in patients with favorable cytogenetics [5% (1 of 20) with a variant CBFβ/MYH11 type D transcript] or with poor cytogenetics [6% (2 of 32)]. There was also a significant difference in the frequency of ITD+ between patients with CD34+ blasts (13%) and patients with CD34− blasts (26%; $P = 0.026$). The leukocyte count was significantly higher in the ITD+ than in the ITD− group (73 ± 13 versus 44 ± 5, respectively; $P = 0.03$). Significant differences were also found among FAB subtypes concerning ITD+ frequency: 29% for M1, 22% for M5, 9% for M2, 6% for M4, and 0% for both M0 and M6 ($P = 0.01$). Symmetrically, significant differences were found among FAB subtypes with Pgp+ frequency: 55% for M2,

Table 1. Characteristics of 171 patients

Characteristics	
Age	54 ± 17 y (18-80 SD)
WHO performance status	
0 or 1	124 (72%)
≥2	47 (28%)
Leukocyte (10^9/L)	49 ± 61 (0.6-284)
FAB subtypes	
0	7 (4%)
1	43 (25%)
2	46 (27%)
4	24 (14%)
4E	6 (3.5%)
5	34 (20%)
6	5 (3%)
Not done	6 (3.5%)
Karyotype	
Favorable	20 (12%)
Intermediate	95 (55%)
Poor	32 (19%)
Not done or not assessable	24 (14%)
CD34	
Positive	100 (58%)
Negative	56 (33%)
Not done	15 (9%)
Pgp activity	
Mean ± SD (range)	0.36 ± 0.27 (0-0.98)
Positive	55 (32%)
Negative	116 (68%)
Flt3/ITD	
Positive	26 (15%)
Negative	145 (85%)
Flt3/Pgp	
Negative/negative	94 (55%)
Negative/positive	51 (30%)
Positive/negative	22 (13%)
Positive/positive	4 (2%)

29% for M1, 28% for M4, 20% for M6, 14% for M0, and 13% for M5 ($P = 0.01$). A high Pgp activity was also more frequently observed in patients with CD34+ blasts (47%) than in patients with CD34− blasts (12%, $P < 0.0001$).

Subsequently, we divided the 171 patients into two groups, the ITD−/Pgp− and all other patients, as described in Materials and Methods. The only significant differences were found among FAB subtypes ($P = 0.01$) and cytogenetic groups: double-negative patients represented 17 of 20 (85%) of favorable cytogenetics, 41 of 95 (43%) of the intermediate group, and 18 of 32 (56%) of poor prognosis patients ($P = 0.003$).

Clinical outcome and prognostic significance for all patients

Ninety-eight out of the 171 patients achieved CR (57%). The percentage of DFS was 33 ± 7% at 4 years (median DFS, 460 days) and the 4-year OS was 27 ± 4% (median OS, 356 days).

Univariate analysis. The CR rates were 38% for ITD+ patients and 61% for ITD− patients ($P = 0.034$), 47% for Pgp+ patients and 62% for Pgp− patients ($P = 0.06$). In continuous variable analyses, patients in CR had a mean Pgp activity of 0.32 ± 0.02 versus 0.42 ± 0.03 for nonresponders ($P = 0.02$). The 4-year probability of DFS was 37% (SE, 6%) for ITD− patients versus 42% (SE, 20%) for ITD+ patients (P = NS), and 33% (SE, 8%) for Pgp− patients versus 41% (SE, 10%) for Pgp+ patients (P = NS). The estimated 5-year OS of ITD− patients was 28% (SE, 5%) compared with 17% (SE, 9%) for ITD+ patients ($P = 0.03$) and 26% (SE, 6%) in Pgp− patients compared with 21% (SE, 6%) in Pgp+ patients ($P = 0.027$).

We divided patients into four groups according to combined Flt3/ITD and Pgp activity: −/−, −/+, +/−, and +/+. CR was achieved in 66%, 51%, 45%, and 0%, respectively ($P = 0.016$). The curves of DFS and OS of these groups are presented in Fig. 1A-C and show a slight advantage of ITD−/Pgp− OS ($P = 0.0094$). Subsequently, we divided the patients into two groups: ITD−/Pgp− and other patients. The CR rate was 46% for ITD+ and/or Pgp+ patients and 66% for the double-negative patient subgroup ($P = 0.011$). The estimated 4-year probability of DFS by Kaplan-Meier's test was 36% (SE, 8%) for double-negative patients versus 41% (SE, 9%) for others (P = NS; Fig. 1B). The estimated 4-year OS for double-negative patients was 36% (SE, 7%) compared with 22% (SE, 5%) for others ($P = 0.0032$; Fig. 1D).

To further determine which factors were independent prognostic factors for poor outcome (CR, DFS, and OS) we included prognostic factors significant in univariate analysis , in a multivariate analysis. Age ($P = 0.0022$) and ITD+Pgp+ association ($P = 0.023$, four patients) were independent prognostic factors for the achievement of CR. The achievement of CR ($P < 0.0001$), WHO performance status ($P = 0.0007$), and ITD+/Pgp+ association ($P = 0.0014$) were independent prognostic factors for OS (Table 3). Among the youngest patients (<60 years), the prognostic factors found were the same (data not shown).

Clinical outcome and prognostic significance of 95 patients within the subgroup with intermediate cytogenetics

Univariate analyses. The CR rates were 40% for ITD+ patients, 62% for ITD− patients ($P = 0.099$), 41% for Pgp+ patients, and 67% for Pgp− patients ($P = 0.014$). In continuous variable analyses, patients in CR had a mean Pgp activity of 0.32 ± 0.03 versus 0.47 ± 0.05 for nonresponders ($P = 0.018$). The Kaplan-Meier estimate for the 4-year probability of DFS was 38% (SE, 9%) for ITD− patients versus 31% (SE, 24%) for ITD+ patients (P = NS), and was 41% (SE, 11%) in Pgp− patients compared with 25% (SE, 14%) in Pgp+ patients ($P = 0.04$). The estimated 4-year OS of ITD− patients was 32% (SE, 7%) compared with 16% (SE, 13%) for ITD+ patients ($P = 0.05$), and was 39% (SE, 9%) in Pgp− patients compared with 12% (SE, 6%) in Pgp+ patients ($P = 0.0001$).

We again divided patients into four groups, with respect to Flt3/ITD and Pgp activity: −/−, −/+, +/−, and +/+. CR was achieved in 70%, 46%, 54%, and 0%, respectively ($P = 0.018$). The curves of DFS and OS of these groups are shown in Fig. 2A and C. Subsequently, we divided the patients into two groups: the CR rates were 70% for ITD−/Pgp− patients and 44% for others ($P = 0.012$). The Kaplan-Meier estimate for the 4-year probability of DFS was 56% (SE, 12%) for double-negative patients versus 27% (SE, 12%) for others ($P = 0.024$; Fig. 2B). The estimated 4-year OS of double-negative patients was 48% (SE, 9%) compared with 16% (SE, 6%) for others ($P < 0.0001$; Fig. 2D).

In a multivariate analysis, we again found age ($P = 0.0005$) and ITD−/Pgp− association ($P = 0.05$) as independent prognostic factors for CR achievement, but ITD−/Pgp− association ($P = 0.004$) was the sole independent prognostic factor for DFS. Achievement of CR ($P < 0.0001$), age ($P = 0.04$),

Table 2. Comparison of clinical and biological variables of all patients according to Pgp activity and Flt3 mutational status

Characteristics	Pgp+, 55 patients (32%)	Pgp−, 116 patients (68%)	*P* value	Flt3+, 26 patients (15%)	Flt3−, 145 patients (85%)	*P* value	Other patients, 77 patients (45%)	Negative/ negative, 94 patients (55%)	*P* value
Age (y)	57 ± 2.2	52 ± 1.6	NS*	50 ± 3	54 ± 1	NS	54 ± 17	53 ± 17	NS
WHO performance status									
0 or 1	40 (32%)	84	NS†	19 (18%)	105	NS	57	67	NS
≥2	15 (32%)	32		7 (17%)	40		20	27	
Leukocytes ($\times 10^9$/L)	42 ± 8	52 ± 6	NS*	73 ± 13	44 ± 5	*P* = 0.03	49 ± 61	48 ± 62	NS
FAB subtypes (%)									
M0	1 (14%)	6	*P* = 0.01†	0 (0%)	7	*P* = 0.01†	6	1 (14%)	*P* = 0.01
M1	11 (29%)	26		11 (29%)	26		15	22 (59%)	
M2	22 (55%)	18		2 (9%)	38		16	24 (60%)	
M4	7 (28%)	21		2 (6%)	26		20	8 (28%)	
M5	4 (13%)	26		7 (22%)	23		19	11 (36%)	
M6	1 (20%)	4		0 (0%)	5		4	1 (20%)	
Karyotype (G/I/P)‡									
Favorable	2 (27%)	18	*P* = 0.03†	1 (11%)	19	*P* = 0.03§	3	17	*P* = 0.003
Intermediate	35 (35%)	60		21 (21%)	74		54	41	
Poor	14 (37%)	18		2 (5%)	30		14	18	
CD34‖									
Positive	44 (47%)	49	*P* < 0.0001†	11 (12%)	82	*P* = 0.026†	51	42	*P* = 0.06
Negative	6 (12%)	43		13 (26%)	36		19	30	
Flt3									
Positive	4	22	*P* = 0.049	—	—		—	—	
Negative	51	94		—	—		—	—	

NOTE: Using Fisher's exact test for binary variables and a univariate logistic model for continuous variables.
*Using the log-rank test for binary variables and univariate Cox models for continuous variables.
†Using the Cox model.
‡One hundred and forty-seven patients were analyzed, 15 without 20 mitosis.
§Using a multivariate logistic model.
‖One hundred and forty-two patients were analyzed. Favorable karyotypes included t(8;21) and inv(16); poor karyotypes included 11q23 translocations, 3q26 rearrangements, t(6;9), del(7), del(5), and three or more abnormalities. All other cases were classified as intermediate.

WHO performance status (P = 0.006), and ITD−/Pgp− association (P = 0.0002), were independent prognostic factors for OS. Among the youngest patients (<60 years), the prognostic factors found were the same (data not shown).

Therefore, the good outcome reported here for ITD−/Pgp− patients suggests that we define a new group of good risk AML, especially in the intermediate cytogenetic group (28% of 147 patients, and 24% of 171 patients with available cytogenetics). Indeed, although ITD−/Pgp− patients with intermediate cytogenetics were older than patients with favorable cytogenetics (54 ± 16 versus 40 ± 9, respectively; P = 0.0004), these two groups did not have a significantly different prognosis

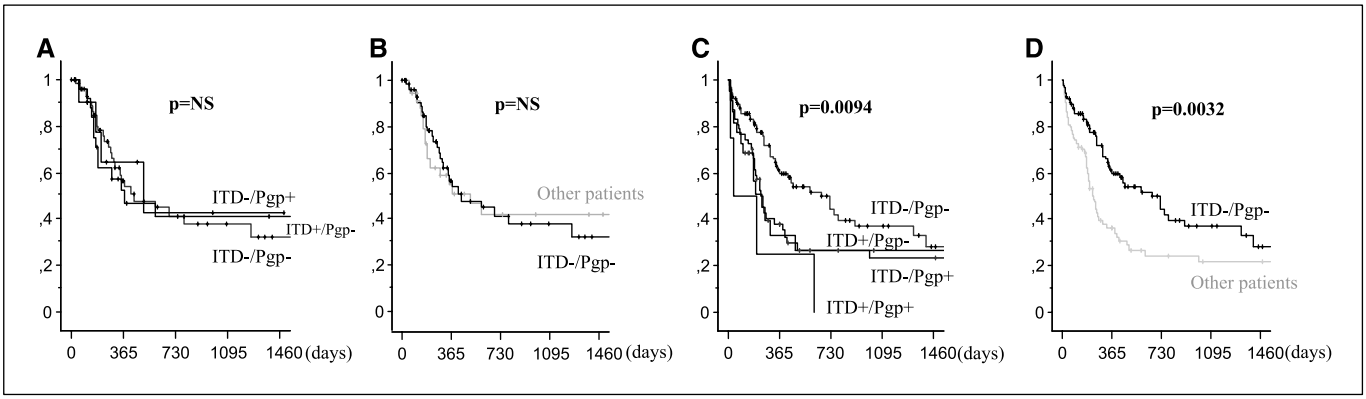

Fig. 1. DFS (*A*/*B*) and OS (*C*/*D*) of all patients according to Flt3/ITD and Pgp status.

Table 3. Prognostic factors for CR, DFS, and OS in all patients and in the intermediate cytogenetic group, in multivariate analysis

Variables	All patients			Intermediate cytogenetics group		
	CR (171 patients)	DFS (98 patients)	OS (171 patients)	CR (95 patients)	DFS	OS (95 patients)
Achievement of CR (yes versus no)	—	—	$P < 0.0001$ ($\chi^2 = 57 \pm 0.31$)	—	—	$P < 0.0001$ ($\chi^2 = 29.77 \pm 0.41$)
Age (continuous variable)	$P = 0.0022$ ($\chi^2 = 9.41 \pm 0.016$)	—	P = NS	$P = 0.0005$ ($\chi^2 = 12.06 \pm 0.021$)	—	$P = 0.04$ ($\chi^2 = 3.95 \pm 0.012$)
WHO performance status (0, 1 versus 2, 3, 4)	—	P = NS	$P = 0.0007$ ($\chi^2 = 11.55 \pm 0.27$)	—	—	$P = 0.006$ ($\chi^2 = 7.44 \pm 0.35$)
Leukocytes, continuous variable (10^9/L)	P = NS	P = NS	—	—	—	—
Cytogenetics (G versus I + P)	$P = 0.03$ ($\chi^2 = 4.12 \pm 0.28$)	$P = 0.023$ ($\chi^2 = 5.33 \pm 0.45$)	$P = 0.01$ ($\chi^2 = 6.22 \pm 0.28$)	—	—	—
CD34 (% positive)	P = NS	—	—	P = NS	—	P = NS
Flt3/Pgp function	$P = 0.025$ ($\chi^2 = 5.12 \pm 0.48$)	—	$P = 0.0014$ ($\chi^2 = 10.18 \pm 0.25$)	$P = 0.05$ ($\chi^2 = 2.79 \pm 0.57$)	$P = 0.004$ ($\chi^2 = 8.18 \pm 0.5$)	$P = 0.0002$ ($\chi^2 = 14.19 \pm 0.37$)

NOTE: NS, not significant in multivariate analyses; —, not evaluated or not significant in univariate analyses. *, Using Fisher exact test for binary variables and a univariate logistic model for continuous variables. **, Using a multivariate logistic model. ***, Using the log-rank test for binary variables and univariate Cox models for continuous variables. ****, Using the Cox model.

(CR, $\chi^2 = 1.34 \pm 0.70$; P = NS by logistic regression model; DFS, $\chi^2 = 0.52 \pm 0.58$; P = NS, by Cox model; and OS, $\chi^2 = 0.74 \pm 0.51$; P = NS by Cox model; Fig. 3).

Discussion

We have analyzed the frequency and prognostic significance of Flt3/ITD and high Pgp activity in 171 adult AML. Consistent with other reports, the overall incidence of ITD+ and Pgp+ were 15% and 32%, respectively, and reached 22% and 37% in the intermediate cytogenetic group. Also, in accordance with previous reports, Flt3/ITD was associated with significantly higher WBC, CD34− marker, AML FAB M5, and with the intermediate cytogenetic group (29, 30). In contrast, high Pgp activity was associated with the CD34+ marker, and Pgp activity was decreased with AML FAB M5. Twenty-two out of 26 (85%) ITD+ patients did not express a functional Pgp, and only 4 out of 51 (8%) Pgp+ patients were ITD+. This mutual exclusion might be explained by the origin of the leukemic cells in which both abnormalities occur: CD34+ cells physiologically express a functional Pgp, usually at low levels, protecting them from xenobiotics (31). On the contrary, FAB M5 monoblasts are typically CD34− cells expressing nonfunctional Pgp. Thus, Flt3/ITD that has been related to environmental factors (32) would occur with a higher frequency in monocytic AML (FAB M4/M5; ref. 33). However, in our series, only 35% (9 of 26) of ITD+ patients were classified in the monocytic group and FAB M1 mostly

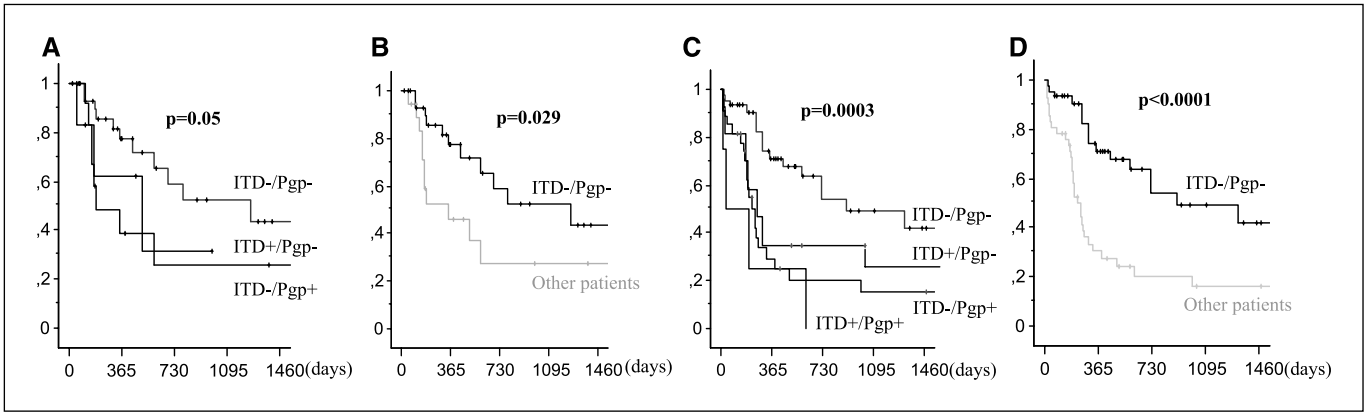

Fig. 2. DFS (*A*/*B*) and OS (*C*/*D*) of intermediate cytogenetic patients according to Flt3/ITD and Pgp status.

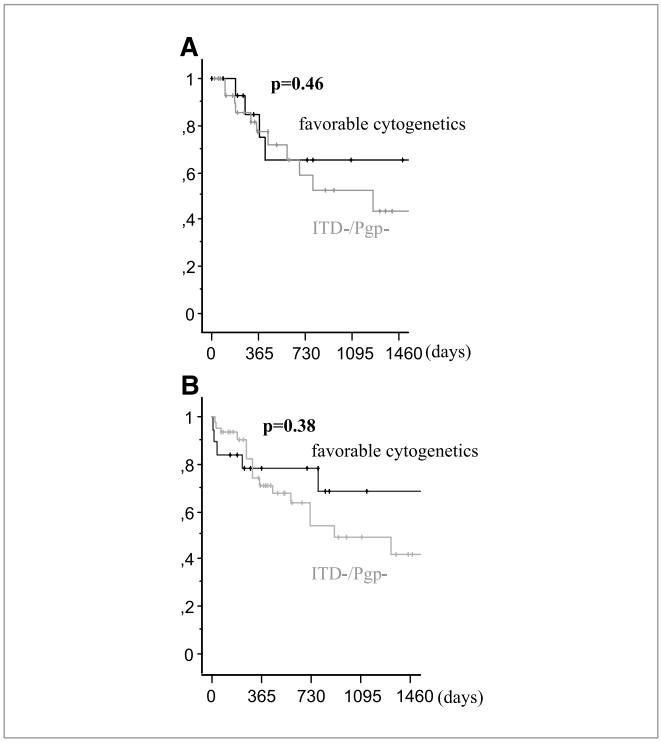

Fig. 3. DFS (*A*) and OS (*B*) of intermediate cytogenetic-ITD−/Pgp− and favorable cytogenetic patients.

represents other ITD+ cases. Whether these cases reflect the differentiation blockade induced by the mutated Flt3 remains speculative (24, 34). Thus, Pgp activity and Flt3/ITD provide additional prognostic information.

Our results on the prognostic relevance are in line with other reports, showing that the presence of Flt3/ITD mutations or a high Pgp activity is associated with worse clinical response (35). The strongest negative effect was found in the intermediate cytogenetic population, with respect to CR achievement, DFS, and OS. On the other hand, the good outcome reported here in ITD−/Pgp− patients suggests that most of these patients represent a group of chemosensitive AML. As this group may account for 29% of all adult AML, further stratification with other molecular markers such as CCAAT enhancer binding protein α mutations could be of definite interest.

In this study, Pgp positivity was retained when the "*D*" for Pgp modulation was at least 0.5 using JC-1/cyclosporin assay (28). In the same way, we considered ITD positivity in patients who showed marked or predominantly mutated *Flt3* alleles in the whole blast population (25, 30). Thus, this study mostly highlights the role of major Flt3/ITD clones and, on the other hand, could underscore the cooperative role of Pgp in the chemoresistance of Flt3/ITD subclones in some patients (36). In spite of variable clinical results, we suggest that ITD+ or Pgp+ patients, as defined in this study, should be considered for clinical studies of specific therapies using selective Pgp modulators such as zosuquidar (37–40) or small Flt3 inhibitors (41–44).

Conclusion

Numerous genetic abnormalities will permit us to better stratify the treatment of patients with AML, whereas new technologies will probably be helpful in order to reach that goal (45). In this study, we have shown that combined Pgp and Flt3 status could provide a powerful risk classification. As they may be routinely assessed with widely used techniques and because targeted therapies are available for both, we suggest they should be used systematically to stratify the treatment of AML patients with intermediate cytogenetics. Our approach might be improved through further assessment of more recently described prognostic factors such as Flt3 tyrosine kinase domain point mutation (Flt3/TKD), overexpression of wild-type Flt3, nucleophosmin mutations, point mutations of the CCAAT enhancer binding protein α gene and overexpression of the brain and acute leukemia, cytoplasmic gene (46, 47). For example, Flt3/ITD and nucleophosmin mutations seem closely related (48) but CCAAT enhancer binding protein α mutations might be independent (49); thus, providing additional information. Nevertheless, the high frequency and the particular distribution of Flt3/ITD and high Pgp activity make them good candidates for a first dichotomy of intermediate cytogenetic AML.

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