To the editor:

Cladribine added to daunorubicin-cytarabine induction prolongs survival of *FLT3*-ITD$^+$ normal karyotype AML patients

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Internal tandem duplication in the *FLT3* gene (*FLT3*-ITD) has been recognized as a marker conferring poor outcome in patients with normal karyotype acute myeloid leukemia (NK-AML).[1] Because of the inferior outcome of *FLT3*-ITD$^+$ NK-AML patients when treated with standard daunorubicin-cytarabine (AraC) 3+7 induction (DA), this group prompts the use of investigational therapy. So far, clinical experiences of targeting the *FLT3* receptor signaling failed to demonstrate efficacy of such an approach.[2]

Cladribine is a purine analog that acts by increasing cellular uptake of AraC in leukemic blasts as well as by directly inhibiting DNA synthesis.[3] As demonstrated in 2 subsequent studies by the Polish Adult Leukemia Group (PALG), the addition of cladribine to DA (DAC) was associated with an increased complete remission (CR) rate and prolonged overall survival (OS) in the general AML population, with the most prominent effect in patients with unfavorable cytogenetics.[4,5] Because the previous studies did not include molecular results, we have performed a retrospective analysis of *FLT3*-ITD and nucleophosmin 1 (*NPM1*) mutations in the DNA of AML patients treated either in randomized trials or according to the same protocols outside prospective studies. The aim of this study was to compare efficacy of DAC and DA regimens in different subgroups according to the presence or absence of *FLT3*-ITD, with OS as the primary end point of the study.

A total of 227 samples from newly diagnosed NK-AML patients were analyzed retrospectively for *FLT3*-ITD and *NPM1* mutations.[6,7] The main characteristics of patients treated using DA vs DAC are summarized in Table 1 (part A). Patients were treated in 9 PALG centers in the years 1999 through 2014. Details of the treatment, response end points, and statistical methods are provided in the supplemental Material, available on the *Blood* Web site. All samples were obtained after written informed consent was obtained in accordance with the Declaration of Helsinki. The study was approved by the local bioethical committees.

In the entire group of patients, CR was achieved in 77.1% (175 patients), which was consistent with the observations of others.[1,8-10] In the DA-treated group, the CR rate was 73.4% (91/124), whereas in DAC-treated patients, the CR rate was 81.6% (84/103), with a trend toward a statistically significant difference between both arms ($P = .14$). Interestingly, among the *FLT3*-ITD$^+$ patients, the CR rate after DAC was 86% (19/22), whereas it was 61% (20/33) after the DA regimen, with the statistically significant difference between both induction groups ($P = .04$; see Table 1, part B). We could not demonstrate the same difference for *FLT3*-ITD$^-$ patients. In multivariate analysis adjusted for age and *NPM1* mutations, there was a tendency toward reduced risk of induction failure in the group of *FLT3*-ITD$^+$ patients treated in the DAC arm (odds ratio [OR] = 0.25; 95% confidence interval [CI], 0.06-1.14; $P = .07$; see Table 1, part C).

With a median follow-up for survivors of 3.69 years, the 4-year probability of OS for the whole group was 34% (standard error ± 3), and the median survival was 18 months. For the whole study group, OS was improved in the DAC (41%) when compared with the DA arm (30.4%), but only a trend toward statistical significance was observed ($P = .15$). However, when the group was stratified according to *FLT3*-ITD, an OS advantage could be demonstrated for *FLT3*-ITD$^+$ patients treated with DAC (37%) when compared with the DA arm (14%; $P = .05$), with the most prominent OS improvement after censoring the observation at the time of allogeneic hematopoietic stem cell transplantation (alloHSCT; $P = .007$; see Table 1, part B and Figure 1). Interestingly, cladribine seems to present OS advantage for patients with high as well as low *FLT3*-mutant–to–wild-type allelic ratio, although the most prominent effect was observed in the poor-risk patients with high mutant–to–wild-type ratio (60% vs 18% for DAC- and DA-treated patients, respectively; $P = .04$; see supplemental Figure 1). In regard to co-occurring *NPM1*$^+$ mutation, we observed statistically different OS between the DAC and DA arms in the *NPM1*$^+$/*FLT3*-ITD$^+$ population (38% vs 8%, respectively; $P = .026$). Cladribine seems to also be effective in poor-risk *NPM1*$^-$ patients; however, the results need to be confirmed in a larger group of patients (supplemental Figure 2A-B). In multivariate analysis, DAC induction was independently associated with a reduced risk of mortality in *FLT3*-ITD$^+$ patients and this effect was particularly prominent when observations were censored at the time of alloHSCT (hazard ratio [HR] = 0.36; 95% CI, 0.167-0.775; $P = .009$).

In the entire group of patients, independently from the type of induction, we did not see a negative impact of *FLT3*-ITD on the CR rate (70.1% vs 79% for *FLT3*-ITD$^+$ vs *FLT3*-ITD$^-$ AML; NS). Different studies showed the ambiguous impact of *FLT3*-ITD on the CR rate: in some groups, *FLT3*-ITD$^+$ patients were associated with a decreased CR,[8] whereas others reported a lack of significant difference between 2 genetic subgroups.[9,10] Thus,

Table 1. Biological, hematologic, and clinical data of NK-AML patients

A. Demographic data of the patients

Characteristics	DAC (n = 103)	DA (n = 124)	*P*	Total no. (N = 227)
Median age (y)	49	50	.49*	50 y‡
***NPM1*(+/−)/*FLT3*-ITD(+/−)§**				
1st N^-/F^-	54 (54%)	60 (49%)	.54†	114
2nd N^+/F^-	25 (25%)	29 (24%)	.88†	54
3rd N^-/F^+	3 (3%)	11 (9%)	.09†	14
4th N^+/F^+	19 (19%)	22 (18%)	.89†	41
Median initial WBC ($\times 10^6$)	33	21.2	.88*	28‡
Sex	55 F, 48 M	72 F, 52 M	.48†	127 F, 100 M
De novo AML	101 (98%)	113 (91%)	.03†	214
Sec MDS AML	2 (2%)	10 (8%)	.07†	12
Sec Th AML	0	1 (0.8%)	.9†	1
FAB				
M0	8 (8%)	8 (6%)	.8†	16
M1	28 (27%)	35 (29%)	.86†	63
M2	25 (24%)	34 (28%)	.59†	59
M4	36 (35%)	27 (21%)	.03†	63
M5	5 (5%)	20 (16%)	.07†	25
M6	1 (1%)	0 (0%)	.45†	1

B. Results of univariate analysis for all patients (N = 227)

End point and variables	DAC (n = 103)	DA (n = 124)	*P*
CR rate, number of patients (%)			
***FLT3*-ITD**			
NEG	65/81 (80%)	71/91 (78%)	.72†
POS	19/22 (86%)	20/33 (61%)	.04†
OS, number of patients, 4-y rate, % (SD)			
***FLT3*-ITD**			
NEG	n = 81; 43% (6)	n = 91; 36% (5)	.6¶
POS	n = 22; 37% (11)	n = 33; 14% (7)	.051¶
OS censored at allograft, number of patients, 4-y rate, % (SD)			
***FLT3*-ITD**			
NEG	n = 81; 37% (8)	n = 91; 28% (6)	.61¶
POS	n = 22; 37% (13)	n = 33; 5% (5)	.007¶

C. Multivariate analysis restricted to *FLT3*-ITD$^+$ patients (N = 55)

End point and variables	Hazard ratio (95% CI)	*P*
CR		
DAC vs DA	0.25 (0.06-1.14)	.07‖
Age (continuous)	1.08 (1-1.16)	.04‖
NPM1$^+$	0.61 (0.14-2.62)	.5‖
OS		
DAC vs DA	0.507 (0.257-1.001)	.05‖
NPM1$^+$	1.084 (0.52-2.262)	.83‖
Age (continuous)	1.018 (0.984-1.053)	.3‖
OS censored at allograft		
DAC vs DA	0.36 (0.167-0.775)	.009‖
NPM1$^+$	1.074 (0.468-2.464)	.87‖
Age (continuous)	1.022 (0.988-1.058)	.21‖

CRR, complete remission rate; FAB, French American British classification; MDS, myelodysplastic syndrome; *NPM1*$^+$), mutations in *nucleophosmin* gene; SD, standard deviation; sec MDS AML, secondary to MDS AML; sec Th AML, secondary to chemotherapy AML; WBC, white cells blood count.

A, Patients' characteristics; B-C, early and long-term outcome by treatment arms in subgroups according to *FLT3*-ITD status.

*Computed by the Mann-Whitney *U* test.

†Computed by the Fisher exact test or χ^2.

‡Median value for total patient group.

§For 4 *FLT3*-ITD$^-$ patients, *NPM1* status was not established.

¶Computed by log-rank test.

‖Computed by Cox regression analysis.

it is possible that this effect might depend on the type of induction because it was already documented for *DNMT3A* mutation.[11] In our group, the *FLT3*-ITD$^+$ patients treated with the standard DA protocol presented a lower CR rate when compared with *FLT3*-ITD$^-$ NK-AML patients (64.5% vs 82.5%, respectively; $P = .038$). In contrast, in the DAC arm the CR rate was not statistically different between *FLT3*-ITD$^+$ and *FLT3*-ITD$^-$ subgroups of patients (86% vs 80%, respectively; $P = .3$). Thus, we might conclude that the addition of cladribine abolishes the negative effect of *FLT3*-ITD on the CR of NK-AML patients.

Recent data suggest that the negative effect of *FLT3*-ITD could be potentially overcome by escalation of the drug doses used in induction. Indeed, Boissel et al[9] documented that reinforced double induction regimens could overcome the negative prognostic impact of *FLT3*-ITD in patients enrolled prospectively in the ALFA-9000 trial. The study by Luskin et al[12] showed that escalated doses of daunorubicin might improve the outcome of *FLT3*-ITD$^+$ AMLs, suggesting again that successful treatment of AML depends on the optimal drug doses used at the very beginning of the therapy.[10-13]

However, further drug dose intensification during induction might be a false approach in *FLT3*-ITD$^+$ patients. This group presented particularly frequent variants of single-nucleotide polymorphisms in genes encoding the AraC-metabolizing enzymes, which correlated with higher toxicity of high-dose Ara-C and thus shorter survival of *FLT3*-ITD$^+$ patients.[14,15] In our setting, the standard dose of AraC accompanied by cladribine allowed *FLT3*-ITD$^+$ patients to achieve CR rates comparable with the *FLT3*-ITD$^-$ subgroup. Thus, our data suggest that cladribine presents an effective and relatively safe solution for *FLT3*-ITD$^+$ AMLs.

Among current postremission therapy protocols, alloHSCT remains until now the standard procedure, increasing long-term survival of *FLT3*-ITD$^+$ patients.[10] Interestingly, the effect of cladribine on OS was the strongest when observations were censored at the time of alloHSCT. Moreover, we found that OS in the DA arm differed significantly between *FLT3*-ITD$^+$ and *FLT3*-ITD$^-$ patients in the group receiving chemotherapy alone as postremission therapy (7% vs 20%, respectively; $P = .018$). In contrast, in the DAC arm receiving chemotherapy alone, we could not document the same difference (33% vs 29% for *FLT3*-ITD$^+$ vs *FLT3*-ITD$^-$ patients, respectively; $P = .57$). Therefore, the addition of cladribine might be of particular importance when alloHSCT for various reasons cannot be done, because of advanced age of patient or lack of an HLA-matched donor that cannot be foreseen at the time of diagnosis. Thus,

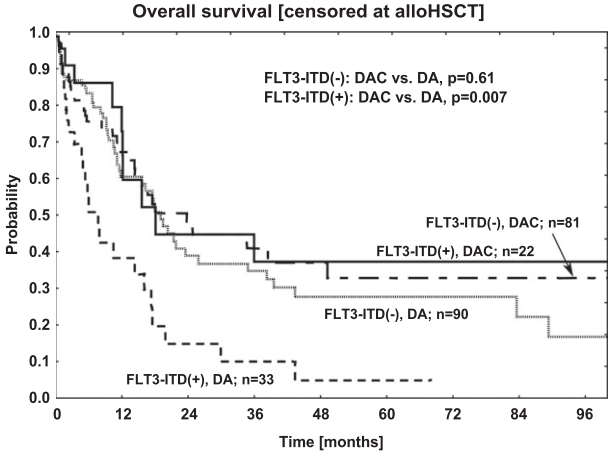

Figure 1. Kaplan-Meier estimates for the probability of OS.

the addition of cladribine seems a reasonable option to be considered for all *FLT3*-ITD$^+$ patients.

In recent years, targeted therapy has been proposed as the most promising treatment option for *FLT3*-ITD$^+$ patients. Recent data, however, does not show convincing results regarding their clinical application.[2] In summary, our data demonstrate that cladribine could abolish the negative effect of *FLT3*-ITD on survival of NK-AML patients and thus might present an interesting and safe solution for *FLT3*-ITD$^+$ AML patients.

The retrospective nature of our study, as well as the relatively small number of *FLT3*-ITD$^+$ AML patients included in the analysis, is the most serious limitation of the obtained data. Our results, however, provide a rationale to carry out prospective studies on the role of cladribine in the treatment of different genetic subgroups of NK-AML patients.

The online version of this article contains a data supplement.

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To the editor:

Germline *RBBP6* mutations in familial myeloproliferative neoplasms

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