REGULAR ARTICLE

blood advances

The prognostic impact of *FLT3*-ITD and *NPM1* mutation in adult AML is age-dependent in the population-based setting

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Key Points

- *FLT3*^{ITD} marks poor survival in younger (<60 years) but not in older (60-74 years) patients with AML.
- *NPM1*^{mut} marks good survival in older, but not younger patients, with AML.

In acute myeloid leukemia (AML) *FLT3* internal tandem duplication (ITD) and nucleophosmin 1 (*NPM1*) mutations provide prognostic information with clinical relevance through choice of treatment, but the effect of age and sex on these molecular markers has not been evaluated. The Swedish AML Registry contains data on *FLT3*-ITD and *NPM1* mutations dating to 2007, and 1570 adult patients younger than 75 years, excluding acute promyelocytic leukemia, had molecular results reported. Females more often had *FLT3*^{ITD} and/or *NPM1*^{mut} (*FLT3*^{ITD}: female, 29%; male, 22% [$P = .0015$]; *NPM1*^{mut}: female, 36%; male, 27% [$P = .0001$]), and more males were double negative (female, 53%; male, 64%; $P < .0001$). Patients with *FLT3*^{ITD} were younger than those without (59 vs 62 years; $P = .023$), in contrast to patients with *NPM1*^{mut} (62 vs 60 years; $P = .059$). Interestingly, their prognostic effect had a strong dependence on age: *FLT3*^{ITD} indicated poor survival in younger patients (<60 years; $P = .00003$), but had no effect in older patients (60-74 years; $P = .5$), whereas *NPM1*^{mut} indicated better survival in older patients ($P = .00002$), but not in younger patients ($P = .95$). In *FLT3*^{ITD}/*NPM1*^{mut} patients, the survival was less dependent on age than in the other molecular subsets. These findings are likely to have clinical relevance for risk grouping, study design, and choice of therapy.

Introduction

Genetic markers are increasingly important in acute myeloid leukemia (AML) for knowledge on pathophysiology,[1,2] risk classification, and choice of specific treatment.[3] Cytogenetic results have been essential in the clinical work-up for more than 20 years.[4] More recently, the prognostic effect of *FLT3* internal tandem duplication (ITD) and nucleophosmin 1 (*NPM1*) mutations in AML have become well established.[5-11] *FLT3*-ITD is associated with higher white blood cell (WBC) counts and blast counts at diagnosis, and a poorer prognosis,[5-9] whereas *NPM1* mutation associates with a better outcome if *FLT3*^{ITD} is absent.[8,9] Both *FLT3* and *NPM1* status have influence on European Leukemia Net risk stratification,[12] and if *FLT3*-mutation is present, the recommendation is to include the multikinase inhibitor midostaurin into primary treatment[13] and to perform allogeneic stem cell transplantation (alloSCT) in first complete remission, if feasible.

Age is also a strong prognostic marker,[14] but the prognostic effect of genetic markers may differ by age,[15] and the age-dependence for molecular aberrations has not been fully evaluated. We have therefore used the high-coverage nationwide population-based Swedish AML Registry[14,16] to study the effect of *FLT3*^{ITD} and *NPM1*^{mut} in different age groups of adult AML.

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The full-text version of this article contains a data supplement.

Table 1. Clinical and laboratory data at diagnosis in patients with AML (APL excluded) according to *FLT3*-ITD, *NPM1*, and age

	No FLT3-ITD				FLT3-ITD			
	NPM1-mutated		NPM1 wildtype		NPM1-mutated		NPM1 wildtype	
Number/median values	<60 y	60-74 y	<60 y	60-74 y	<60 y	60-74 y	<60 y	60-74 y
Number of patients	100	144	386	475	95	109	83	69
Sex, male/female, n	48/52	68/76	223/163	290/185	41/54	52/57	46/37	32/37
Median age, y	51	67	47	67	51	67	44	67
de novo AML, %	92	85	89	69	91	87	96	75
Intensive treatment, %	100	94	97	89	95	97	100	96
ECOG/WHO PS, % 0-II	96	93	95	95	88	90	96	91
Hemoglobin, g/L	93	94	95	95	94	96	92	94
Platelet count, $\times 10^9$/L	91	81	62	73	54	62	45	72
WBC, $\times 10^9$/L	14.8	21.1	6.3	4.0	49.7	59.2	44.9	39.7
Blood blast count, $\times 10^9$/L	3.7	5.9	2.6	0.8	23.4	22.0	17.2	13.7
Bone marrow blasts, %	50	59	50	42	70	72	70	54
LDH, ukat/L (normal <4.2)	7.0	6.4	5.9	5.2	10.1	10.1	10.1	9.7
Death <28 days, %	6.0	4.2	1.3	5.7	5.3	11.0	0	8.7
Allogeneic SCT, %	32	12.5	55	21.7	57	18.3	71	26.1
Median OS, y	>12	3.3	10.1	1.3	1.9	2.0	1.9	1.0

ECOG/WHO, Eastern Cooperative Oncology Group/World Health Organization; LDH, lactic dehydrogenase; PS, performance status.

Patients and methods

The Swedish AML Registry collects data on all adult (18 years or older) patients with AML,[14] including secondary AML, diagnosed since 1997, and the registry now contains more than 8000 patients.[16] The coverage has consistently been higher than 98% as compared with the Swedish National Cancer Registry, established in 1958, with compulsory dual reporting from both pathology and clinical departments. Missing data are actively requested and data quality maintained through monitoring by the Regional Cancer Center, funded by national grants, as previously reported.[14] Data collection is facilitated by the Swedish personal ID code system, established in 1947. Survival is automatically updated daily through the population registry. The AML Registry became web-based in 2007, when more clinical data at diagnosis, including genetics, and details on primary treatment also were introduced. *FLT3*^{ITD} and *NPM1*^{mut} analyses have been performed in the university hospital laboratories, using established standard techniques, as part of the routine diagnostic workup in all Swedish AML centers. The molecular results (aberration present/absent/not evaluated) have been recorded in the AML registry since 2007. Molecular analysis was recommended for patients who had normal or inconclusive karyotype and were scheduled for intensive therapy, but in practice, most patients considered for active AML therapy had molecular analyses performed. *FLT3*^{ITD} allelic ratio and other *FLT3*-mutations than ITD have not been recorded.

Patients have been uniformly treated according to the Swedish National Guidelines,[17] established in 2005 and revised biannually; however, with minimal changes in primary treatment and indication for transplantation.[16] Treatment consists of daunorubicin 60 mg/m^2 per 8-hour infusion days 1 to 3 and cytarabine 1 g/m^2 per 2-hour infusion twice daily on days 1 to 5, with a second identical course as consolidation #1, daunorubicin reduced to 2 days in consolidation #2, and optional cytarabine alone as consolidation #3. Few patients have been treated according to other clinical study protocols, and few patients up to 75 years have had dose reductions or modifications, primary treatment with hypomethylating agents, or palliation only (see details in Juliusson et al[16]). Patients diagnosed before 2019 did not receive *FLT3*-inhibitor as part of primary therapy. Patients were considered for alloSCT in first complete remission if medically fit, except for patients with favorable risk genetics (ie, core-binding factor AML or *NPM1*^{mut} without *FLT3*^{ITD}). During recent years, assessment of measurable residual disease by multicolor flow cytometry has been used to guide the indication for alloSCT. Current data were extracted from the registry in August 2019. Standard robust statistical analyses (parametric and non-parametric analyses, χ^2 analysis, log rank analysis of overall survival [OS]) were performed using Statistica software version 12 (Tulsa, OK). In addition, parallel statistics on survival including hazard ratios with 95% confidence intervals were performed with R software (version 3.6.1). Studies on data from the AML Registry are approved by ethics review.

Results

At data extraction, the database contained 4716 patients overall (median age, 71 years; mean, 68 years) diagnosed since 2007, of whom 200 had acute promyelocytic leukemia (APL). For the purpose of this study, we selected the 2665 patients without APL who were younger than 75 years (median age, 63 years; mean age, 59 years) at diagnosis. Of them, 1827 (69%) had de novo AML, 1482 were males (56%) and 1183 females, and 2225 (83%) received intensive treatment, 119 (4%) primary hypomethylation, and the remaining palliation only.

Representativity of the study population

We identified 1461 patients with molecular results on both *FLT3*^{ITD} and *NPM1*, 14 patients with only *NPM1* results, and 95 patients with only *FLT3*^{ITD} results. These molecularly characterized patients

Table 2. OS (median in years, and 3-year percentage) by age, sex, genetic, and clinical subgroup

	<60 y					60-74 y					<60 vs 60-74 y	
Patients and comparison	**n**	**Median OS, y**	**3-year OS, %**	***P***	**HR (95% CI)**	**n**	**Median OS, y**	**3-year OS, %**	***P***	**HR (95% CI)**	***P***	**HR (95% CI)**
All												
FLT3-ITD	194	1.87	45.1	.00008	1.60 (1.26-2.02)	193	1.33	34.8	.50226	1.07 (0.88-1.30)	.00083	1.54 (1.10-1.99)
No *FLT3*-ITD	515	>8	63.4		1	654	1.49	35.5		1	0	2.32 (1.96-2.74)
Males												
FLT3-ITD	95	2.55	50.0	.0194	1.49 (1.06-2.09)	90	1.4	35.4	.91331	0.99 (0.75-1.29)	.0099	1.63 (1.12-2.37)
No *FLT3*-ITD	287	>8	65.2		1	380	1.45	33.5		1	0	2.48 (1.99-3.10)
Females												
FLT3-ITD	99	1.72	40.8	.0012	1.71 (1.23-2.38)	103	1.13	34.3	.25597	1.17 (0.89-1.55)	.0344	1.46 (1.03-2.06)
No *FLT3*-ITD	228	>5	61.1		1	274	1.64	38.4		1	0	2.10 (1.63-2.70)
De novo/intensive Tx												
FLT3-ITD	176	2.23	48.2	.00005	1.70 (1.31-2.19)	155	1.6	39.5	.19051	1.16 (0.93-1.46)	.0033	1.52 (1.15-2.02)
No *FLT3*-ITD	454	>12	66.9		1	455	2.16	42.8		1	0	2.24 (1.84-2.71)
All												
NPM1 mut	198	11.83	59.4	.5428	0.92 (0.72-1.19)	258	2.92	49.8	0	0.64 (0.53-0.78)	.00038	1.63 (1.24-2.14)
NPM1 wt	473	7.53	58.3		1	546	1.24	28.9		1	0	2.33 (1.97-2.76)
Males												
NPM1 mut	92	>12	68.2	.1126	0.73 (0.49-1.08)	124	3.01	50.6	.00086	0.64 (0.49-0.83)	.00061	2.07 (1.35-3.17)
NPM1 wt	270	7.53	58.4		1	322	1.28	28.4		1	0	2.29 (1.83-2.85)
Females												
NPM1 mut	106	5.02	52.3	.5158	1.12 (0.80-1.58)	134	2.92	49.0	.00233	0.65 (0.50-0.86)	.095	1.35 (0.95-1.94)
NPM1 wt	203	7.47	58.1		1	224	1.23	29.8		1	0	2.36 (1.82-3.07)
De novo/intensive Tx												
NPM1 mut	175	11.83	62.2	.5643	0.92 (0.69-1.22)	210	3.01	50.5	.01514	0.76 (0.61-0.95)	.00012	1.80 (1.33-2.43)
NPM1 wt	420	>8	61.7		1	367	1.7	36.8		1	0	2.16 (1.78-2.63)
All												
FLT3-ITD/*NPM1* mut	95	1.93	46.1	.00035	1.45 (1.05-1.99)	109	1.99	46.7	.00002	0.76 (0.59-0.99)	.1401	1.32 (0.91-1.91)
FLT3-ITD/*NPM1* wt	83	2.06	44.3		1.56 (1.13-2.17)	69	0.96	18.9		1.25 (0.94-1.66)	.00029	2.03 (1.37-3.00)
No *FLT3*-ITD/*NPM1* mut	100	>12	71.5		0.69 (0.48-1.01)	144	3.3	51.5		0.59 (0.46-0.76)	.00025	2.13 (1.41-3.23)
Double negative	386	10.06	61.5		1	475	1.28	30.6		1	0	2.45 (2.03-2.97)
De novo/intensive Tx												
FLT3-ITD/*NPM1* mut	81	11.83	52.0	.0002	1.44 (0.99-2.07)	93	2.38	48.8	0.01237	0.90 (0.67-1.20)	.0515	1.51 (0.99-2.30)
FLT3-ITD/*NPM1* wt	80	2.06	44.7		1.85 (1.32-2.61)	51	1.21	24.2		1.40 (0.99-1.97)	.0059	1.82 (1.18-2.79)
no *FLT3*-ITD/*NPM1* mut	92	>12	71.3		0.77 (0.51-1.15)	114	3.3	52.0		0.73 (0.54-0.97)	.00027	2.26 (1.44-3.54)
Double negative	337	>10	66.0		1	314	1.85	39.1		1	0	2.36 (1.88-2.95)

P values and hazard ratios calculated with R statistics comparing OS by genetic subset within each patient selection, and in the far-right column by age group within a genetic subset.

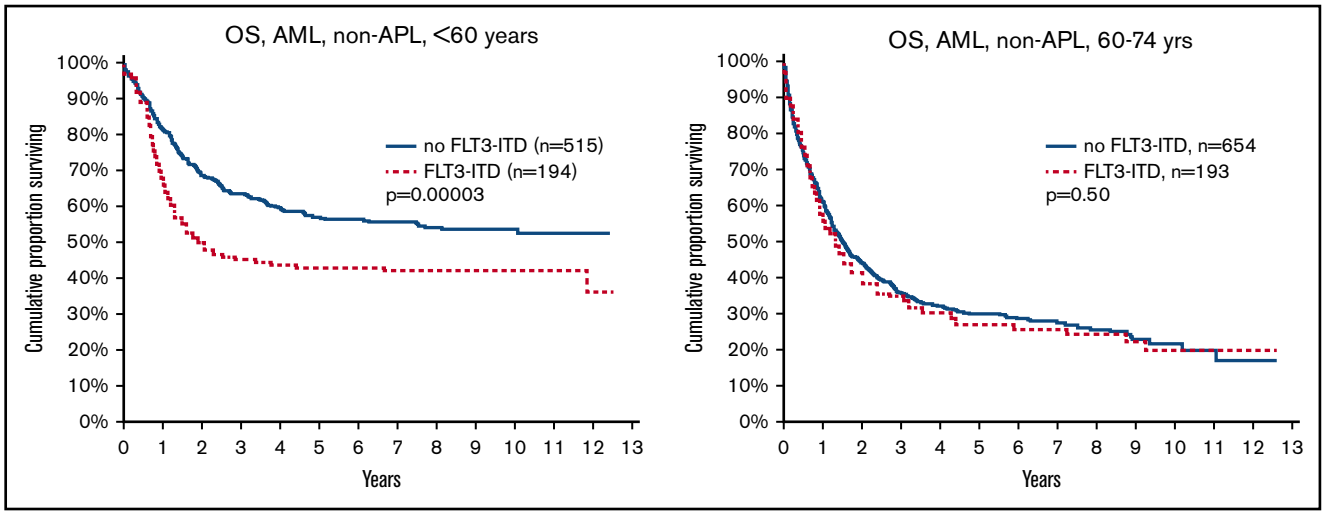

Figure 1. OS by *FLT3*-ITD and age. (Left) Aged younger than 60 years ($P = .00003$). (Right) Aged 60 to 74 years ($P = .5$).

constitute 59% of the overall population, 73% of those younger than 60 years, 57% of patients aged 60 to 69 years, and 41% of those aged 70 to 74 years, as well as 67% of all receiving intensive treatment (76% of those <60 years, 60% of those 60-74 years) and 20% of those receiving nonintensive treatment (32% <60 years, 18% of those 60-74 years). Patients with de novo AML had molecular reports in 70% overall (80% if <60 years, 71% if 60-69 years, 51% if 70-74 years), and 33% of patients with secondary AML. There was no difference in molecular testing by sex. The median observation time of 640 surviving patients with reported data on *FLT3* and *NPM1* status was 4.6 years (quartile range, 2.0-7.6 years).

Sex differences

AML is overall more common in males,[16] but more female than male patients with AML had $FLT3^{ITD}$ and/or $NPM1^{mut}$. $FLT3^{ITD}$ was found in 202 (29%) of 704 females vs 185 (22%) of 852 males (χ^2 analysis $P = .0015$), and $NPM1^{mut}$ in 240 (36%) of 667 females vs 216 (27%) of 808 males ($P = .0001$). $FLT3^{ITD}$ was more common in de novo AML (339/1278, 27%) than in secondary AML (48/278, 17%), but the sex difference remained in secAML (female, 32/133, 24%; male, 16/145, 11%; $P = .004$). $FLT3^{ITD}/NPM1^{mut}$ double mutation was found in 111 (17%) of 661 females and 93 (12%) of 800 males ($P = .005$), and correspondingly more males than females were double negative (male, 513/800, 64%; female, 348/661, 53%; $P < .0001$).

$FLT3^{ITD}$ was more common in younger (<60 years) than in older (60-74 years) males (25% vs 19%; $P = .044$), whereas there was no such age difference in females (30% vs 27%; $P = .4$). Thus, $FLT3^{ITD}$ was more common in older females than in older males (female, 27%; male, 19%; $P = .0048$).

$NPM1^{mut}$ was more common in females than in males among both younger (female, 34%; male, 25%; $P = .012$) and older (female, 37%; male, 28%; $P = .004$) patients. $NPM1^{mut}$ was also more common in de novo (400/1210, 33%) than in secAML (56/265,

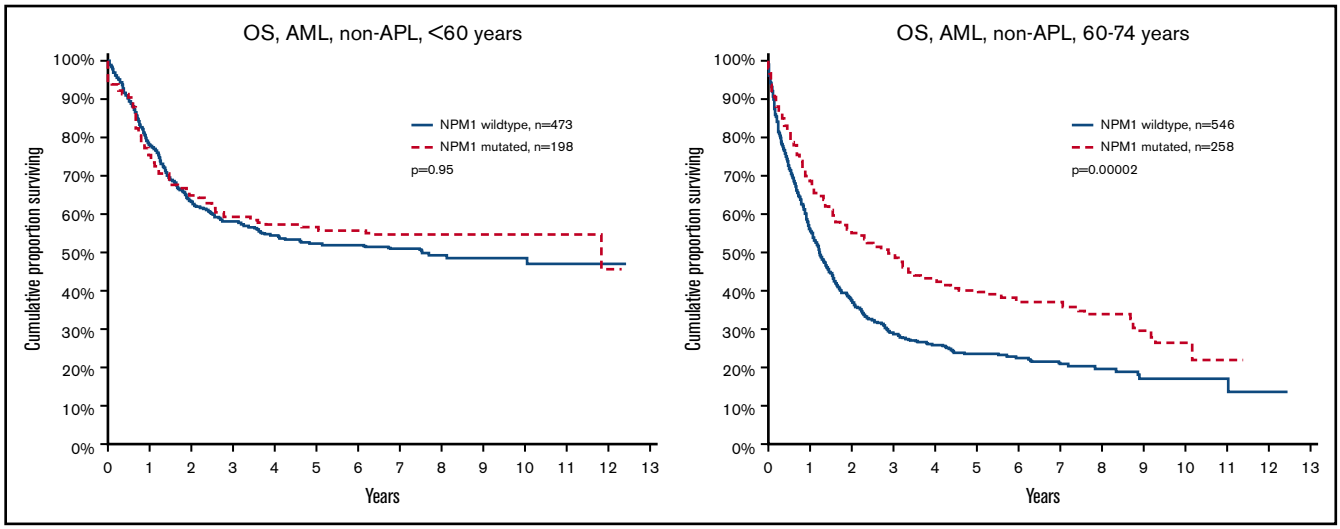

Figure 2. OS by *NPM1* mutation and age. (Left) Aged younger than 60 years ($P = .95$). (Right) Aged 60 to 74 years ($P = .00002$).

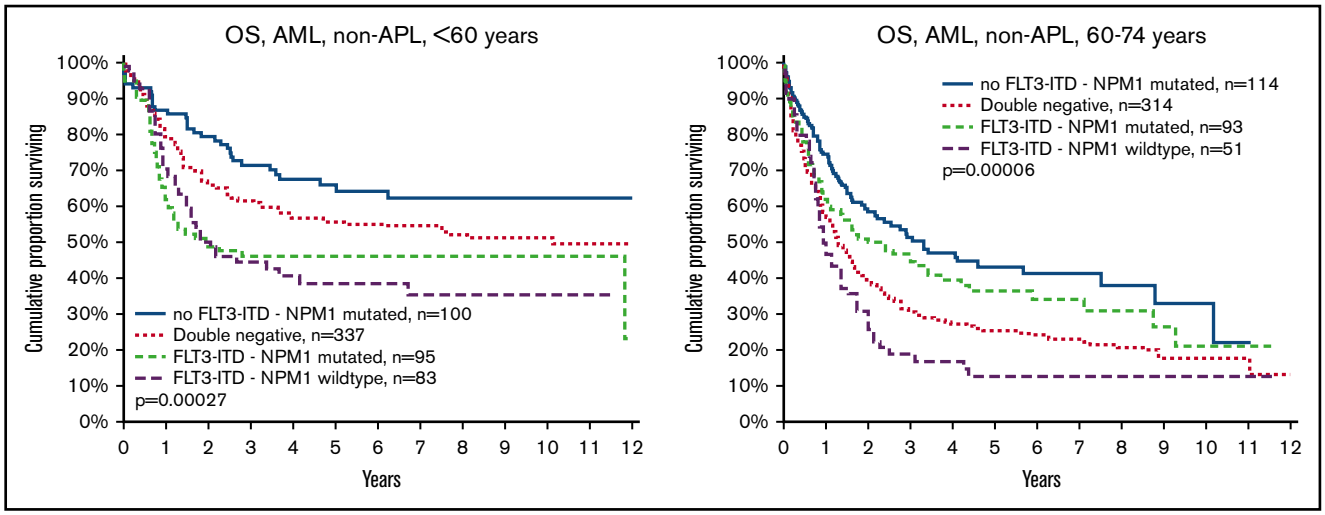

Figure 3. OS by *FLT3*-ITD, *NPM1* mutation, and age. (Left) Aged younger than 60 years. (Right) Aged 60 to 74 years. Log rank analyses of survival in patients younger than 60 years (left): comparison of all 4 subsets ($P = .00027$); no *FLT3*-ITD/*NPM1* mutated (group A) vs double-negative (group B; $P = .049$); *FLT3*-ITD/*NPM1* mutated (group C) vs *FLT3*-ITD/*NPM1* wild type (group D; $P = .62$); group A vs C ($P = .0005$); group A vs D ($P = .0004$); group B vs C ($P = .007$); group B vs D ($P = .018$). Patients aged 60 to 74 years (right): all 4 subsets ($P < .0001$, group A vs B ($P < .0001$), group C vs D ($P = .049$), group A vs C ($P = .073$), group A vs D ($P < .0001$), group B vs C ($P = .10$), and group B vs D ($P = .37$).

21%; $P = .0001$), and the sex difference of $NPM1^{mut}$ was similar in de novo and secAML (de novo: female, 38%; male, 29% [$P = .002$]; and secAML: female, 28%; male, 15% [$P = .008$]). Clinical data by mutation status and age are shown in Table 1.

Patients with $FLT3^{ITD}$ were slightly younger than those without $FLT3^{ITD}$ (mean, 59.0 vs 61.6 years, also including evaluated patients aged 75 years and older; $P = .023$). The mean age of patients with and without $NPM1^{mut}$ was 61.9 and 60.4 years, respectively ($P = .059$) compared with 72.9 years for patients who did not have mutation analysis performed.

Survival by mutation status, age, and sex

The main purpose of this study was to evaluate the effect of $FLT3^{ITD}$ and $NPM1^{mut}$ on OS in different patient subsets (Table 2). We could validate the established knowledge that $FLT3^{ITD}$ marks poor prognosis, and $NPM1^{mut}$ good prognosis in the absence of $FLT3^{ITD}$. However, unexpectedly, we found a strong age dependence: $FLT3^{ITD}$ was a strong marker for poor survival in young patients (<60 years), but not in older patients (60-74 years; Figure 1). In contrast, $NPM1^{mut}$ was a strong marker for good survival in older patients, but not in young patients (Figure 2). To avoid bias from patient selection and treatment intensity, we performed corresponding studies in patients with de novo AML given intensive treatment, with very similar results (Table 2; supplemental Data).

It is also well established that $NPM1^{mut}$ is associated with good survival in the absence of $FLT3^{ITD}$. This was confirmed and was valid in both young and old patients (Figure 3). We also show that $FLT3^{ITD}$ in the absence of $NPM1^{mut}$ constitutes the worst survival in both younger and older patients. The beneficial effect of $NPM1^{mut}$ on survival was greater in old compared with young patients, both in patients with $FLT3^{ITD}$ (group C vs D; old, $P = .049$ vs young, $P = .6$) and in those without (group A vs B; $P < .0001$ vs $P < 0.049$, respectively), and the adverse effect of $FLT3^{ITD}$ was greater in young as compared with old patients both in patients with $NPM1^{mut}$ (group C vs A; young, $P = .0005$ vs old, $P = .073$) and $NPM1^{wt}$ (group D vs B; $P = .018$ vs $P = .37$, respectively; Figure 3).

Patients with the double mutation $FLT3^{ITD}/NPM1^{mut}$ had similar survival in the younger and the older age groups (Table 2), whereas age had a strong negative effect in double-negative patients (Figure 3). Thus, the prognostic rank between double mutated vs double negative was reversed when comparing young and old patients.

$FLT3^{ITD}$ had stronger negative effect on survival in females (all aged <75 years; $P = .0057$) than in males ($P = .28$). In contrast, $NPM1^{mut}$ had a stronger positive effect on survival in males (aged <75 years; $P = .0024$) than in females ($P = .21$; supplemental Data). No other significant differences in survival by sex were found.

Blood counts by genetics and age

$FLT3^{ITD}$ is, as previously well documented, strongly associated with increased leukemia cell counts, such as WBC counts at diagnosis, blood blast counts, and bone marrow blast percentages. We here also found higher lactate dehydrogenase and lower platelet counts in patients with $FLT3^{ITD}$ (Table 3).

Patients with $NPM1^{mut}$ also had higher WBC as compared with $NPM1^{wt}$ patients, but this was not seen in patients with concomitant $FLT3^{ITD}$ (Table 3).

There were few differences by age in the effect of mutation status on blood counts (ie, groups A vs D and groups A vs B in Table 3). Nonparametric analyses (Kolmogorov-Smirnov 2-test analysis) of laboratory values comparing younger and older patients did not show significant differences in any of the 4 molecular subgroups (A-D).

Discussion

$FLT3^{ITD}$ and $NPM1^{mut}$ are late-occurring events in the development of AML, not found in preleukemia[1,2] and rarely in myelodysplastic

Table 3. Laboratory data at diagnosis by *FLT3*-ITD, *NPM1*, and age (mean with standard deviation), with Kolmogorov-Smirnov 2-sample test for pairwise comparison of genetic subgroups A, B, C, and D

	No FLT3-ITD				FLT3-ITD				<60 y						60-74 y					
	NPM1-mut		NPM1 wt		NPM1-mut		NPM1 wt													
Group	A		B		C		D		A vs B	A vs C	A vs D	B vs C	B vs D	C vs D	A vs B	A vs C	A vs D	B vs C	B vs D	C vs D
Age group, y	<60	60-74	<60	60-74	<60	60-74	<60	60-74												
Age, y	48 (10)	67 (4)	44 (11)	67 (4)	48 (9)	67 (4)	42 (13)	67 (4)			*	*		**						
Hemoglobin, g/L	95 (20)	95 (20)	95 (23)	94 (19)	96 (22)	98 (22)	91 (22)	92 (18)												
Platelet count, $\times 10^9$/L	109 (86)	106 (113)	98 (104)	100 (103)	86 (87)	87 (82)	71 (73)	99 (86)	*	***	***									
WBC, $\times 10^9$/L	33 (47)	49 (70)	26 (53)	19 (39)	80 (78)	74 (67)	71 (79)	68 (77)	**	****	***	****	****		****	****		***	****	
Blood blast count, $\times 10^9$/L	14 (27)	28 (54)	17 (45)	9 (28)	53 (71)	48 (57)	49 (65)	32 (47)		****	****	****	****		****	****		***	****	
Bone marrow blasts, %	51 (24)	56 (25)	53 (25)	47 (23)	66 (23)	66 (24)	65 (22)	57 (22)		***	***	****	****		***	*		***	***	*
LDH, ukat/L (normal <4.2)	8 (5)	9 (8)	10 (17)	8 (12)	14 (12)	8 (13)	13 (9)	11 (11)	*	****	***	****	****		***	****	*	***	****	

Values are mean (standard deviation).
*$P < .05$.
**$P < .01$.
***$P < .005$.
****$P < .001$.

syndromes, and thus mostly regarded as markers for de novo AML. The established knowledge that *FLT3*^{ITD} with wild-type *NPM1* confers poor prognosis, whereas the reverse, *NPM1*^{mut} without *FLT3*^{ITD}, is favorable was true in all ages, and is currently included in the European Leukemia Net risk classification.[12] However, the main finding in our study was that *FLT3*^{ITD} is a strong marker for poor survival only in the young, whereas *NPM1*^{mut} strongly predicts good survival only in the older (60-74 years) patients. These are new findings that likely have clinical impact. Our data are strong, with large patient numbers and highly significant differences using robust standard statistics, and importantly, this study is the only population-based study so far with focus on the dominant age group (ie, patients older than 60 years).

FLT3^{ITD} was, in 2010, reported to have a negative effect on survival also in older patients[18]; however, this was based on 41 study patients aged 60-69 years with *FLT3*^{ITD}, who had an exceptionally poor survival (median OS, 0.6 years; 3-year OS, 10%) as compared with 137 such patients in our present study (median OS, 1.4 years; 3-year OS, 38%). Furthermore, 131 patients with *FLT3*^{ITD}, aged 61-70 years, who received intensive therapy according to Swedish guidelines without a kinase inhibitor seem to have a similar 2-year survival as 86 such patients treated with intensive therapy and midostaurin in a recent German-Austrian study[19] (46.0% vs 45.6%). One possible explanation might be that older Swedish AML patients mostly receive intensive treatment similar to younger patients,[16] which is more intensive than most published protocols. As a consequence, clinical studies with targeted and other therapies should be performed in all relevant age groups, and extrapolation of data from studies in young patients will not suffice for older patients.

Previous studies have identified the *NPM1*^{mut}/*FLT3*^{wt} group as a good prognosis subset, with no specific rank between the other molecular subsets,[6,7,9,20] whereas Gale et al also identified a worse prognosis in *FLT3*^{ITD}/*NPM1*^{wt},[11] as seen in our study. The ranking between double mutated vs double negative was not consistent in previous studies, and we here show that this could be a result of the age distribution of the study population (Figure 3). Together, our results indicate biological differences of these genetic markers in young as compared with older patients.

Both *FLT3*^{ITD} and *NPM1*^{mut} seem to increase AML cell proliferation, leading to higher leukemia cell counts (Table 1), despite their different prognostic effect. Outcome is clearly more dependent on the biology of the AML cells than the resulting blood counts. The mix between the molecular subsets in unselected AML populations may result in a low effect of WBC and blast counts in prognostic models. There were few differences in the clinical and diagnostic data between the age groups, and these differences can hardly explain any of the outcome differences by age.

Both *FLT3*^{ITD} and *NPM1*^{mut} are more common in females than in males. We could here find a modest effect of sex on outcome (supplemental Data), where *FLT3*^{ITD} had more prognostic effect in females, and *NPM1*^{mut} a stronger effect in males. It should be noted that the beneficial effect of midostaurin in the RATIFY study on *FLT3*-mutated patients was restricted to males.[13] Whether this finding is relevant to ours needs further evaluation. Our present study included patients diagnosed since 2007, and we have recently shown an equal outcome by sex during the most recent decade[16]; no molecular data from previous decades have been available.

Our results are not the consequence of patient selection or different management. The Swedish AML Registry is population-based with high coverage (>98%) and low drop-out rates, with full survival update. Similar data on survival by age and molecular subgroups was found in separate analysis of patients with de novo AML and intensive treatment (supplemental Data). However, not all patients have been sampled for gene mutations, as data emerge from the patient population clinically selected for the recommended opinion that molecular analyses would be helpful in deciding on initial therapy, indication for alloSCT, and deep monitoring of remission status. Most evaluated patients have been treated with a high-intensity treatment, with high response and survival rates,[16] as compared with published multicenter trials. Our results are clearly also influenced by the high transplantation rates, where presence of *FLT3*^{ITD} has been a strong indication for alloSCT (Table 1). Among patients younger than 70 years, 47% of the patients with *FLT3*^{ITD} had alloSCT reported as compared with 39% in those without *FLT3*^{ITD} ($P = .013$). In contrast, patients with *NPM1*^{mut} had alloSCT in 33% as compared with 46% in patients with *NPM1*^{wt} ($P < .0001$). However, the high alloSCT rate still failed to neutralize the adverse prognostic effect of *FLT3*^{ITD} in young patients.

Age is a strong prognostic marker in AML.[14-16] Our current data, however, indicate that age has a limited effect on survival in AML with *FLT3*^{ITD}/*NPM1*^{mut}, and consequently, even greater effect in AML lacking both these molecular drivers. Broad molecular analyses on large clinical study samples have recently evaluated the incidence of further combinations of gene mutations,[21] mainly of epigenetic modifiers that may develop early in preleukemic states.[2] Most patients with *NPM1*^{mut} were thus found to have mutations also involving *DNMT3A*, *IDH1/2*, and/or *TET2*. In patients with the subset *NPM1*/*DNMT3A* double mutation, *FLT3*^{ITD} had a strong prognostic effect, but not so in other combinations of *NPM1* and *DNMT3A*.[21] In the study by Papaemmanuil et al,[21] only 11% of the patients were older than 60 years, and thus not were representative for the overall AML population. We do not yet have access to other molecular results, but this will be an important task for the future. Our data could fit into the concept that younger double-negative (*FLT3*^{wt}/*NPM1*^{wt}) patients have a higher incidence of favorable molecular and/or cytogenetic changes (eg, core-binding factor leukemias), whereas older double-negative patients more often have other driver mutations and dysplastic changes leading to poor outcome, such as del(5q), monosomy 7, *TP53* mutations, and complex and monosomal karyotypes. We have recently participated in a large international cooperative study to assess the influence of cytogenetic abnormalities on the prognostic effect of *NPM1*^{mut}.[22] A similar effort is likely required and warranted to analyze the additional effect of cytogenetic risk on outcome by *FLT3* status, including the effect of *FLT3*^{ITD} allelic ratio.

In conclusion, about 30% of the non-APL adult patients with AML who are younger than 75 years had *NPM1*^{mut}, and 25% had *FLT3*^{ITD},[14] with a female dominance, in contrast to the greater proportion of males in the 60% of patients with double-negative AML. *FLT3*^{ITD} was slightly more common in younger patients, whereas *NPM1* had a similar prevalence in all ages. *FLT3*^{ITD} indicated poor prognosis in younger (<60 years) and female patients, but less so in older (60-74 years) and male patients, whereas the favorable effect of *NPM1* was greater in older and male patients than in younger and female patients. In patients with strong driver mutations (ie, *FLT3*^{ITD}/*NPM1*^{mut} double mutated), age did not affect survival. This implies a biological age-dependent interaction between these genetic markers that is likely to be of clinical relevance.

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Authorship

Contribution: G.J. designed study, performed analyses, and wrote the manuscript; and all authors contributed to the Swedish AML registry and critically reviewed and approved manuscript.

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