

# The clinical spectrum of adult acute myeloid leukaemia associated with core binding factor translocations

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## Summary

To better understand the spectrum of adult acute myeloid leukaemia (AML) associated with core binding factor (CBF) translocations, 370 patients with newly diagnosed CBF-associated AML were analysed. Patients' age ranged from 16–83 years (median 39 years) with a slight male predominance (55%); 53% had *inv*(16); 47% had *t*(8;21). Patients with *t*(8;21) tended to be younger ( $P = 0.056$ ), have lower peripheral blood white cell counts ( $P < 0.0001$ ) and were more likely to have additional cytogenetic abnormalities ( $P < 0.0001$ ). Loss of sex chromosome, *del*(9q) and complex abnormalities were more common among patients with *t*(8;21), while +22 and +21 were more common with *inv*(16). Overall, 87% [95% confidence interval (CI) 83–90%] of patients achieved complete response (CR) with no difference between *t*(8;21) and *inv*(16); however, the CR rate was lower in older patients due to increased resistant disease and early deaths. Ten-year overall survival (OS) was 44% (95% CI 39–50%) and, in multivariate analysis, was shorter with increasing age ( $P < 0.0001$ ), increased peripheral blast percentage ( $P = 0.0006$ ), in patients with complex cytogenetic abnormalities in addition to the CBF translocation ( $P = 0.021$ ), and in patients with *t*(8;21) ( $P = 0.025$ ). OS was superior in patients who received regimens with high-dose cytarabine, a combination of fludarabine and intermediate-dose cytarabine, or haematopoietic cell transplantation.

**Keywords:** acute myeloid leukaemia, core binding factor leukaemia, cytogenetics of leukaemia, leukaemia treatment, adult leukaemia.

The core binding factors (CBFs) are a group of heterodimeric transcriptional regulators containing a common beta (CBFB) and one of three alpha components. One of the three alpha components, *RUNX1* (formerly known as CBFA2 or AML1), is restricted to myeloid and lymphoid tissues, and murine knockouts of either *CBFB* or *RUNX1* die in utero without developing haematopoiesis (Downing, 2003).

The CBF acute myeloid leukaemias (AMLs) result from translocations involving either *RUNX1* or *CBFB*. In *t*(8;21) AML, *RUNX1T1* (formerly known as CBFA2T3 or ETO) on chromosome 8 is fused with *RUNX1* on chromosome 21 (Licht, 2001). In *inv*(16) AMLs, CBFB located at 16q22 is fused to the *MYH11* gene located at 16p13 (Liu *et al*, 1993). Both translocations are thought to lead to leukaemia by creating fusion products that are dominant negative inhibitors of normal myeloid differentiation (Speck & Gilliland, 2002; Downing, 2003). In addition to sharing a similar pathogenetic

mechanism, the CBF leukaemias share the characteristics of sensitivity to high-dose cytarabine treatment and having a relatively favourable prognosis compared with most other forms of adult AML (Grimwade *et al*, 1998; Slovak *et al*, 2000; Byrd *et al*, 2002).

While the CBF AMLs share a number of features, there is also considerable heterogeneity within this group of diseases. For example, AML associated with *t*(8;21) is more often of French–American–British (FAB) M2 morphology and has secondary cytogenetic changes, including loss of a sex chromosome (LOS) or loss of part or all of 9q. AML associated with *inv*(16) more often is of FAB M4Eo morphology and is less likely to have secondary cytogenetic changes (Byrd *et al*, 1999, 2004; Nguyen *et al*, 2002; Delaunay *et al*, 2003; Schlenk *et al*, 2004; Marcucci *et al*, 2005). When CBF leukaemias are considered in total, patient age has been reported to alter the behaviour of the disease. Understanding the heterogeneity of

CBF AML may be instructive for clinical decision-making and, perhaps as importantly, may provide insights into how various factors interact with a known pathogenetic mechanism in the evolution of AML.

Core binding factor AMLs are relatively uncommon, comprising perhaps 15% of AML cases at most (Grimwade *et al*, 1998; Slovak *et al*, 2000). Thus, to identify biologically interesting or clinically significant heterogeneity within this category of disease, retrospective analyses combining studies are required. Accordingly, we have collected the records of 370 adults with CBF AML treated on Southwest Oncology Group (SWOG) ( $n = 96$ ), Eastern Cooperative Oncology Group (ECOG) ( $n = 67$ ) and MD Anderson Cancer Center (MDA) ( $n = 207$ ) clinical trials and have analysed them for heterogeneity within the entire group and for comparisons between patients with t(8;21) and inv(16) AMLs.

## Methods

This analysis included all patients with CBF abnormalities who were enrolled on completed SWOG, ECOG and MDA protocols for treatment of adults with previously untreated AML that required cytogenetics as part of the patient evaluation. SWOG trials included S8600, S9031, S9034, S9126, S9333 and S9500 (Weick *et al*, 1996; Cassileth *et al*, 1998; Godwin *et al*, 1998; List *et al*, 2001; Anderson *et al*, 2002). MDA trials included DM79-95, DM82-86, DM86-00, DM87-080, DM91-004, DM93-048, DM95-020, DM98-208, DM00-097 and ID02-266 (Estey *et al*, 2001). ECOG trials included E1490, E3489, E3993, E3997, E4995 and PC486 (Cassileth *et al*, 1993, 2005; Rowe *et al*, 1995, 2004; Cassileth *et al*, 1998; Cripe *et al*, 2000). Treatments varied greatly over these trials. For purposes of this retrospective analysis, induction regimens were classified as fludarabine plus intermediate-dose cytarabine (FA), high-dose cytarabine containing (HDAC), or other. Patients who achieved CR and received postremission therapy on protocol were also classified according to the postremission therapy they received: FA, HDAC, haematopoietic cell transplantation (HCT), or other.

Data sets from ECOG and MDA were submitted to the SWOG statistical center and combined with SWOG data for statistical analyses. Demographic and clinical variables available for analysis included age at start of treatment, sex, race, FAB classification, AML onset (secondary versus *de novo*, available for certain studies), white blood cell (WBC) count, marrow and peripheral blast percentages, haemoglobin and platelet count. Cytogenetic data included the specific CBF abnormality [inv(16), which included t(16;16), or t(8;21)], as well as indicators of the following chromosome loss or gain: loss of either sex chromosome, -X, -Y, +8, +22, +21, +4, del(9q)(q23) and -7 or del(7q). Karyotypes were also scored for the presence of any clonal abnormality other than those just listed (called 'other abnormalities'), of complex abnormality defined as three or more unrelated abnormalities, and for tetraploidy. Comparisons between groups of patients were

based on logistic and proportional hazards regression analyses for dichotomous and time-to-event data respectively. Outcomes analysed included complete response (CR) and resistant disease (RD). Overall survival (OS) was measured from date of study entry until death from any cause with observation censored at the date of last contact for patients last known to be alive. For remitting patients, relapse-free survival (RFS) was measured from the date CR was achieved until AML relapse or death from any cause, with observation censored at the date of last contact for patients last known alive without report of relapse. For remitting patients who received protocol-directed postremission therapy, disease-free survival (DFS) was measured from the start of postremission therapy until the same endpoints as RFS. Statistical significance was characterised by two-sided *P*-values. Significance tests and confidence intervals were not adjusted for multiple testing due to the exploratory nature of these analyses.

## Results

### Demographics

A total of 370 patients with inv(16)/t(16;16) ( $n = 196$ ) or t(8;21) ( $n = 174$ ) were identified. Clinical characteristics of these patients are shown in Tables I–III. The median age of patients was relatively young (39 years), although the age

**Table I.** Characteristics of 370 Adult patients with core binding factor leukaemia.

	Total ( $n = 370$ )	inv (16) ( $n = 196, 53\%$ )	t(8;21) ( $n = 174, 47\%$ )	<i>P</i> -value
Sex (%)				
Female	165 (45)	88 (53)	77 (47)	NS
Male	205 (55)	108 (52)	97 (48)	
Race (%)				
Black	33 (9)	12 (36)	21 (64)	0.095
Hispanic	56 (15)	29 (52)	27 (48)	
White	269 (73)	151 (56)	118 (44)	
Other	12 (3)	4 (33)	8 (67)	
Age (years) (%)				
16–30	101 (27)	43 (43)	58 (57)	0.056
31–40	96 (26)	53 (55)	43 (45)	
41–55	102 (27)	60 (59)	42 (41)	
56–65	34 (9)	17 (50)	17 (50)	
66–83	37 (10)	23 (62)	14 (38)	
FAB classification (%)				
M1	27 (7)	4 (15)	23 (85)	<0.0001
M2	154 (42)	29 (19)	125 (81)	
M4	150 (40)	140 (93)	10 (7)	
Other	39 (10)	23 (59)	16 (41)	
AML onset (%)				
<i>De novo</i>	184 (79)	105 (57)	79 (43)	0.11
Secondary	50 (21)	22 (44)	28 (56)	
Unknown	136	69	67	

NS, not significant.

**Table II.** Characteristics of 370 adult patients with core binding factor leukaemia.

	inv(16) ( <i>n</i> = 196)		t(8;21) ( <i>n</i> = 174)		Total ( <i>n</i> = 370)	
	Med.	Min–Max	Med.	Min–Max	Med.	Min–Max
Age (years)	41	16–83	36	16–77	39	16–83
Marrow blasts (%)	56	8–99	56	9–95	56	8–99
WBC ( $\times 10^9/l$ )	30.2	0.6–387	10.6	0.3–107	17	0.3–387
Peripheral blasts (%)	40	0–96	43	0–94	40	0–96
Haemoglobin (g/dl)	8.7	0.6–14.4	8.7	0.8–15.6	8.7	0.6–15.6
Platelets ( $\times 10^9/l$ )	38	4–247	37	3–658	37	3–658

**Table III.** Additional cytogenetic abnormalities in core binding factor acute myeloid leukaemia.

Category	Overall (%)	t(8;21) (%)	inv (16) (%)	<i>P</i>
None	167 (46)	50 (29)	117 (60)	<0.0001
–Y	56 (27)	53 (55)	3 (3)	<0.0001
–X	29 (8)	28 (16)	1 (1)	<0.0001
del (9q)	25 (7)	25 (14)	0 (0)	<0.0001
+22	36 (10)	1 (1)	35 (18)	<0.0001
+21	13 (4)	2 (1)	11 (6)	0.023
+8	28 (8)	11 (6)	17 (9)	0.43
Complex*	57 (15)	33 (19)	24 (12)	0.085

\*Defined as three or more independent cytogenetic abnormalities.

distribution was influenced to a certain degree by the availability of age-specific trials. As shown in Table I, the proportion with t(8;21) compared with inv(16) decreased with increasing age ( $P = 0.056$ ). t(8;21) was somewhat more frequent than inv(16) among Black patients (64%) compared with Hispanic (48%) or White patients (44%) ( $P = 0.095$ ). In addition to being younger, patients with t(8;21) presented with lower peripheral blood WBC counts ( $P < 0.0001$ ), were much more likely to have an FAB morphology of M1 or M2, and less likely to have an M4 morphology ( $P < 0.0001$ ) compared to patients with inv(16).

### Cytogenetics

Additional clonal chromosomal abnormalities were seen in 54% of patients overall. As shown in Table III, patients with t(8;21) AML were far more likely to have additional chromosomal abnormalities ( $P < 0.0001$ ) and, in particular, were more likely to have del(9q) ( $P < 0.0001$ ) or the LOS ( $P < 0.0001$ ), including both –Y ( $P < 0.0001$ ) and –X ( $P < 0.0001$ ). In contrast, patients with inv(16) were more likely to also have +22 ( $P < 0.0001$ ) or +21 ( $P = 0.023$ ). Trisomy 8 and –7/7q– were seen in 8% and 5% of cases overall, and did not differ significantly between the inv(16) and t(8;21) groups ( $P = 0.43$  and  $0.80$  respectively). Abnormalities other than the specific chromosomal losses or gains shown in Table III were also somewhat more frequent in patients with t(8;21) ( $P = 0.088$ ), as were complex abnormalities involving three or more aberrations ( $P = 0.085$ ).

Univariate logistic regression analyses revealed that the presence of additional abnormalities varied significantly among FAB classes ( $P < 0.0001$  for heterogeneity among M1, M2, M4 and other), and decreased significantly with increasing WBC count ( $P < 0.0001$ ). However, the apparent effect of FAB classification was due to its association with specific CBF abnormality: in multivariate analysis t(8;21) ( $P < 0.0001$ ) and lower WBC count ( $P = 0.018$ ) retained their significant associations with additional abnormalities, while FAB did not ( $P = 0.21$ ).

As LOS occurred almost entirely in the patients with t(8;21), further analysis of LOS was limited to that group. All 28 cases with –X were women while the 53 –Y cases were, of course, all men. In multivariate analysis the prevalence of LOS varied significantly between the sexes ( $P = 0.0076$ ) and among FAB classes (M1 vs. M2 vs. M4,  $P < 0.0001$ ). For both sexes, loss of sex chromosome occurred almost exclusively in patients with M1 or M2 morphology.

Trisomy 22 occurred almost exclusively in patients with inv(16), and among them the prevalence of +22 decreased significantly with increasing age ( $P = 0.019$ ). Only two (9%) of 23 inv(16) patients aged 66 years or older had +22. In multivariate analysis, the frequency of –7/7q– was found to increase with increasing age ( $P = 0.013$ ) and to be higher among males ( $P = 0.018$ ). None of the factors listed in Tables I and II was significantly associated with del(9q) in patients with t(8;21) or with complex abnormalities in all 370 patients.

### Achievement of complete response

A total of 321 [87%, 95% confidence interval (CI) 83–90%] of the 370 patients achieved CR. There was no significant difference in CR rates between t(8;21) (155/174 = 89%) and inv(16) (166/196 = 85%,  $P = 0.21$ ), although 30% of patients with t(8;21) required two or more cycles of induction to achieve CR, compared to 20% for patients with inv(16) ( $P = 0.040$ ). Among those who achieved a CR, the need for a second cycle of induction did not affect RFS ( $P = 0.56$ ). In univariate analyses, the CR rate decreased with increasing patient age ( $P = 0.021$  based on logistic regression treating age as a continuous variable), and was lower in patients with secondary AML due in most cases to a history of myelodysplasia

( $P = 0.035$ ). Overall the presence of additional cytogenetic abnormalities did not significantly affect the CR rate ( $P = 0.69$ ). However, the CR rate was somewhat higher in patients with LOS (79/85 = 93% vs. 241/284 = 85%,  $P = 0.041$ ). This trend was found both for  $-Y$  in men (52/56 = 93% vs. 126/149 = 85%,  $P = 0.099$ ), and  $-X$  in women (27/29 = 93% vs. 115/135 = 85%,  $P = 0.22$ ) (the presence/absence of X was unknown in one woman). The CR rate also tended to be lower in patients with +8 ( $P = 0.081$ ),  $-7/7q-$  ( $P = 0.075$ ) and complex abnormalities ( $P = 0.075$ ), and was significantly lower in patients with abnormalities other than the specific losses or gains listed in Table III (42/59 = 71% vs. 278/309 = 90%,  $P = 0.0003$ ). The impact of complex abnormalities on CR rate was seen predominantly in patients with complex abnormalities but not  $del(9q)$  ( $P = 0.015$  compared to complex abnormalities with  $del(9q)$ ). In multivariate analysis, after adjusting for the effects of age ( $P = 0.065$ ) and the presence of other abnormalities ( $P = 0.006$ ), the CR rate was marginally higher in the  $t(8;21)$  patients compared with  $inv(16)$  ( $P = 0.096$ ). The effects of age and other abnormalities did not differ significantly between the  $t(8;21)$  and  $inv(16)$  groups ( $P = 0.12$ ).

**Resistant disease**

Twenty-eight patients (8%, 95% CI 5–11%) had RD following remission induction chemotherapy, including 15 (8%) with  $inv(16)$  and 13 (7%) with  $t(8;21)$  ( $P = 0.95$ ). In univariate analyses the RD rate increased with increasing platelet count ( $P = 0.038$ ) and was significantly higher for patients with  $-7/7q-$  (5/17 = 29% vs. 23/350 = 7%;  $P = 0.0058$ ) or with the miscellaneous other abnormalities (12/59 = 20% vs. 16/309 = 5%;  $P = 0.0004$ ) (the presence/absence of  $-7/7q-$  was unknown in three patients and the presence/absence of other abnormalities was unknown in two). In multivariate analysis adjusting for the effects of these factors, the RD rate did not differ significantly between the  $inv(16)$  and  $t(8;21)$  groups ( $P = 0.46$ ).

**Overall survival**

Of the 370 patients, 197 have died, and the remaining 173 were last known to be alive between 8 months and 21.6 years after starting treatment (median follow-up time 9.0 years). The median survival for all patients was 3.8 years (95% CI 2.4–8.6 years), and the estimated probability of survival was 48% (CI 42–53%) at 5 years and 44% (CI 39–50%) at 10 years. OS was somewhat poorer for patients with  $t(8;21)$  compared to  $inv(16)$ , with an estimated hazard ratio (HR) of 1.30 (95% CI 0.99–1.73); however, the difference was not statistically significant ( $P = 0.063$ , Fig 1). In univariate analysis, increasing age ( $P = 0.0001$ ), increasing peripheral blast percentage ( $P = 0.0076$ ), and increasing marrow blast percentage ( $P = 0.042$ ) were significantly associated with shorter survival. OS was also shorter in patients with +8 ( $P = 0.035$ ), and

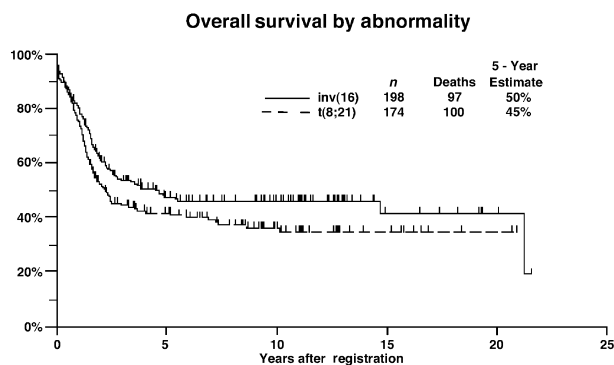


Fig 1. Kaplan–Meier estimates of overall survival of 196 patients with  $inv(16)$  acute myeloid leukaemia (AML) and 174 with  $t(8;21)$  AML.

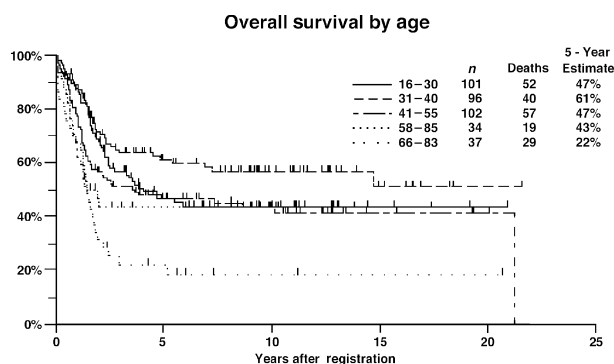


Fig 2. Kaplan–Meier estimates of overall survival of 370 patients with core binding factor-related acute myeloid leukaemia according to age (years).

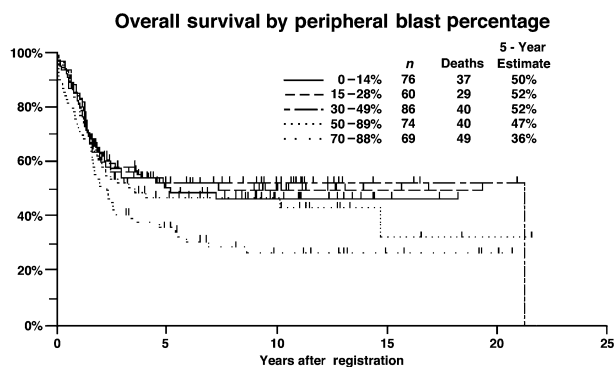


Fig 3. Kaplan–Meier estimates of overall survival of patients with core binding factor-related acute myeloid leukaemia in 365 patients with known peripheral blast percentage.

tended to be shorter in patients with complex abnormalities ( $P = 0.057$ ) and longer in patients with +22 ( $P = 0.053$ ), but did not vary significantly with loss of sex chromosome ( $P = 0.86$ ), or  $del(9q)$  ( $P = 0.97$ ). In multivariate analysis, age ( $P < 0.0001$ ), peripheral blast percentage ( $P = 0.0006$ ) and complex abnormality ( $P = 0.021$ ) retained independent prognostic significance (See Fig 2–4). After accounting for these

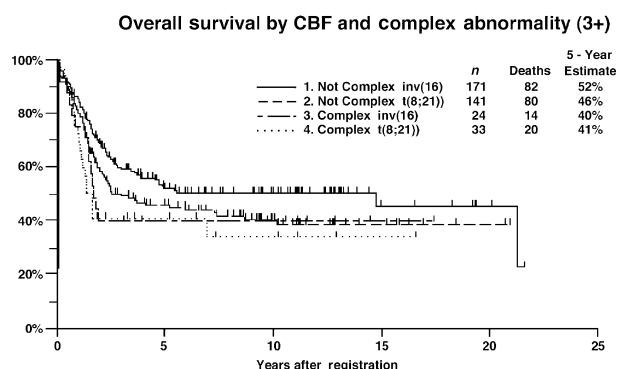


Fig 4. Kaplan–Meier estimates of overall survival by specific core binding factor abnormality and the presence or absence of complex abnormalities involving 3 or more chromosomes.

effects, the difference in OS between t(8;21) and inv(16) was essentially unchanged, with HR 1.38 (CI 1.04–1.84,  $P = 0.025$ ). The effects of age, peripheral blast percentage, and complex abnormality did not differ significantly between patients with t(8;21) and inv(16) AML ( $P = 0.97$ ). Similar to what was seen for CR rates, the influence of complex cytogenetics were largely restricted to those with complex abnormalities without del(9q).

#### Relapse-free survival

Of the 321 patients who achieved CR, 134 have relapsed and another 45 have died without report of relapse. The overall estimated probability of RFS was 44% (CI 39–50%) at 5 years and 42% (CI 36–48%) at 10 years. RFS did not differ significantly between the t(8;21) and inv(16) patients (HR 1.16 with 95% CI 0.86–1.56,  $P = 0.33$ , Fig 5). The analysis looking for factors predictive of RFS gave results similar to those found for OS. In univariate as well as multivariate analysis, RFS decreased with increasing age ( $P = 0.013$ ) and increasing peripheral blast percentage ( $P = 0.0053$ ), and tended to be longer in patients with +22 ( $P = 0.061$ ).

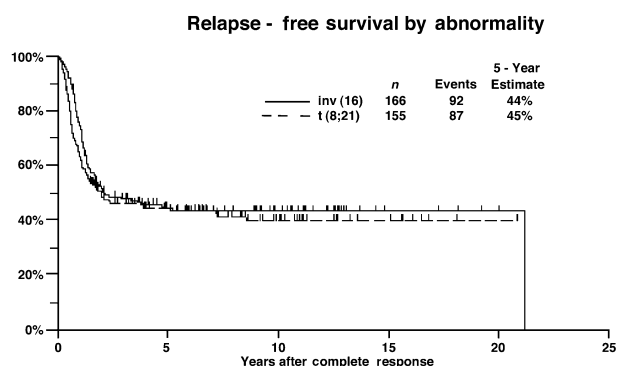


Fig 5. Kaplan–Meier estimates of relapse-free survival of 166 patients with inv(16) acute myeloid leukaemia (AML) and 155 with t(8;21) AML.

Adjusting for the effects of age and blast percentage left the difference between t(8;21) and inv(16) groups almost unchanged (HR 1.20,  $P = 0.23$ ). The effects of age and peripheral blast percentage on RFS were similar in t(8;21) and inv(16) cases ( $P = 0.86$ ).

Among the 134 patients who relapsed from CR, the 60 with t(8;21) had significantly poorer survival after relapse than the 74 with inv(16), with an estimated HR of 1.84 (95% CI 1.26–2.67;  $P = 0.0015$ ).

#### Impact of del(9q)

Given previous reports suggesting that the presence of del(9q) in t(8;21) AML cases is associated with a poor prognosis (Schoch *et al*, 1996), additional analyses were conducted looking at this specific association. Among the 174 patients with t(8;21) the CR rates were 92% and 89% with and without del(9q) respectively, and did not differ significantly in either univariate analysis ( $P = 0.62$ ) or after adjusting for the effects of age and the presence of other cytogenetic abnormalities ( $P = 0.48$ ). OS was not significantly influenced by the presence of del(9q) in either univariate analysis (HR = 0.85, CI 0.47–1.52,  $P = 0.57$ ) or after adjusting for the effects of age, peripheral blast percentage, and complex abnormalities (HR = 0.99, CI 0.53–1.85,  $P = 0.98$ ). Similarly, RFS did not differ significantly according to the presence of del(9q) in univariate (HR = 0.92, CI 0.51–1.66,  $P = 0.78$ ) or multivariate (HR = 1.26, CI 0.68–2.36,  $P = 0.46$ ) analysis.

#### Treatment effects

An analysis examining possible treatment effects showed no significant difference in CR rates among patients treated with 'FA', 'HDAC' or other induction regimens. However, both OS ( $P = 0.0063$ ) and RFS ( $P = 0.019$ ) varied significantly among the three treatment groups, due primarily to superior outcomes in both the FA and HDAC groups. This association persisted after adjusting for the effects of age and peripheral blast percentage.

Of the 321 patients who achieved CR, 264 received protocol-directed postremission therapy (53 FA, 44 HDAC, 31 HCT, 136 other). Of these 264, 121 have relapsed and another 30 have died without relapse. DFS did not differ significantly between the 141 patients with inv(16) and the 123 with t(8;21); the estimated HR for t(8;21) relative to inv(16) was 1.08 (95% CI 0.78–1.49,  $P = 0.64$ ). There was significant heterogeneity of DFS among the four treatment groups ( $P = 0.0071$ ). This was largely due to superior DFS in patients receiving FA, HDAC or HCT; there was no significant heterogeneity of DFS among these three intensively treated groups ( $P = 0.51$ ; Fig 6). After adjusting for the effects of age and peripheral blast percentage, the superiority of the three intensively treated groups persisted.

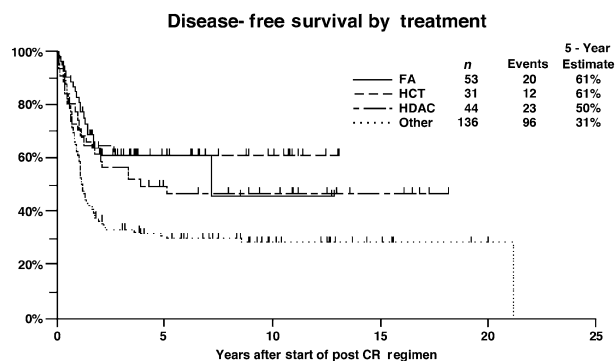


Fig 6. Kaplan–Meier estimates of disease-free survival of 264 patients with core binding factor acute myeloid leukaemia given postremission therapy with FA, HDAC, HCT or other.

## Discussion

The results presented here confirm previous reports showing significant differences between patients with t(8;21) and those with inv(16) AMLs. Specifically, in this study we found that patients with t(8;21) AML tended to be younger, had a lower WBC count at diagnosis, and more often had an FAB morphology of M1 or M2 as opposed to M4eo, which was commonly seen with inv(16). In addition, patients with t(8;21) AML had disease that was more often associated with additional cytogenetic abnormalities, particularly LOS or del(9q), more often required two cycles of induction therapy, and had a poorer survival following relapse from CR. In contrast, patients with inv(16) more often also exhibited +22 or +21, more often responded to a single cycle of induction chemotherapy, and had longer survival after relapse from CR, suggesting that they were easier to reinduce.

When all patients with CBF leukaemias were considered together, although high remission rates were seen in virtually every subset of patients examined, in multivariate analysis, older patients, those with inv(16) and those with uncommon cytogenetic abnormalities in addition to the CBF alteration, had lower complete response rates. Multivariate analysis of OS identified age, peripheral blast percentage and the presence of complex cytogenetic abnormalities as significant prognostic factors and, after adjusting for these effects, OS was somewhat poorer for t(8;21) AML ( $P = 0.025$ ). In an analysis of treatment effects, the best OS was seen when more intensive regimens employing intermediate or high dose cytarabine or HCT were applied.

Two other large retrospective analyses of CBF AMLs have recently been reported (Schlenk *et al*, 2004; Marcucci *et al*, 2005). While many of the findings of the three retrospective analyses were similar, a few differences are noteworthy. First, concerning age, the German Acute Myeloid Leukemia Intergroup report (Schlenk *et al*, 2004) and the Cancer and Leukemia Group B (CALGB) study (Marcucci *et al*, 2005) both noted similar median ages for patients with t(8;21) and inv(16) AML. However, neither study examined overall age

distribution by cytogenetic group. The current study also found a similar median age for patients with t(8;21) and inv(16) AML, but the proportions changed among the young and old, with t(8;21) more common in young patients (age 16–30 years) and inv(16) more common in the older populations (age 66–83 years) (see Table I). Like the report from CALGB (Marcucci *et al*, 2005), we found that the ratio of t(8;21) cases compared with inv(16) cases was higher in blacks than in Hispanics or whites. The German study (Schlenk *et al*, 2004) did not report on race. All three reports noted lower WBC counts, percentage of peripheral blasts and percentage of marrow blasts with t(8;21) than with inv(16), suggesting that t(8;21) is a somewhat less rapidly proliferating disease. Similarly, all three reports noted the more common presence of secondary cytogenetic abnormalities with t(8;21), particularly the loss of X or Y or del(9q), the higher incidence of +22 and +21 with inv(16) and the presence of +8 with similar frequencies in both groups. Neither of the other two reports commented specifically on the incidence of complex cytogenetic abnormalities, which were found in 15% of patients in the current report, with no significant distinction between t(8;21) and inv(16). The remarkable association of t(8;21) AMLs with LOS or del(9q) and the contrasting association of inv(16) AML with +22 and +21 are entirely unexplained.

The 87% CR rate seen in the present study is similar to that reported by the German group (87%; Schlenk *et al*, 2004) and the CALGB (88%; Marcucci *et al*, 2005). CALGB reported that higher BM blasts, older age, lower platelets and non-white race were each in multivariable analysis associated with lower CR rates when CBF leukaemias were considered together. In the German study, higher WBC count and older age were associated with an increased incidence of early death and lower CR rates in patients with inv(16) but not in patients with t(8;21). The French AML study group has published separate analyses of patients with t(8;21) and those with inv(16) (Nguyen *et al*, 2002; Delaunay *et al*, 2003). The report on t(8;21) provided no details about induction outcome, but the inv(16) analysis reported that higher WBC counts and lower platelet counts were associated with lower CR rates. Data from the current study support the association of older age with lower CR rates and are at least consistent with the idea that increased peripheral blasts are associated with decreased CR rates ( $P = 0.053$ ), but do not support an effect of race ( $P = 0.52$ ) or platelet count ( $P = 0.13$ ) on remission induction outcome. Neither the CALGB, German, nor French studies specifically discussed factors predictive for having RD following initial induction, which we saw in 8% of patients overall and which was associated with -7/7q- and with the presence of uncommon ‘miscellaneous’ cytogenetic abnormalities.

In the current study, the only factors, apart from form of treatment, that in multivariable analysis predicted for lower OS, were increasing age and peripheral blast percentage, the presence of complex abnormalities, and t(8;21). The effects of the first three factors were similar in t(8;21) and inv(16) AML

cases. The CALGB study (Marcucci *et al*, 2005) similarly found shorter survival in older patients, and in those with t(8;21), but also in patients with lower platelet counts at diagnosis. The German report (Schlenk *et al*, 2004) did not include an analysis of OS for all CBF AMLs together, but did note that, for patients with t(8;21), increasing WBC count, lower platelet count, and LOS were each associated with poorer outcome, while in patients with inv(16), they found no single factor predictive of OS. When RFS was considered, the current study also found that, apart from treatment, only age and peripheral blast percentage independently influenced outcome. CALGB did not analyse RFS, while the German study reported that RFS appeared shorter in t(8;21) patients with higher WBC counts and lower platelet counts, while for patients with inv(16), also having +22 appeared to be associated with better DFS, but had no impact on OS. In the French AML study of patients with t(8;21), peripheral WBC count at diagnosis was the only identified prognostic factor for both DFS and OS. For patients with inv(16), higher WBC counts and lower platelet counts were associated with lower CR rates, but older age was the only factor associated with shorter DFS, while older age and lower platelet counts both were associated with poorer OS. Thus, while there are some differences among these various studies, in general, older age and higher white counts or blast percentage (the two are linked) appeared to be associated with poorer survival and DFS as might a lower platelet count at diagnosis.

Whether among patients with t(8;21) the presence of del(9q) confers an unfavourable prognosis or not has been the subject of some uncertainty. This study found no significant decreases in CR rates, OS or RFS when del(9q) was present with t(8;21). These findings are in contrast to one previous report (Schoch *et al*, 1996), but are consistent with other recent reports (Rege *et al*, 2000; Peniket *et al*, 2005).

The negative impact of patient age on CR rates, DFS and OS can be explained, at least in part, by an inability of older patients to tolerate chemotherapy as easily as younger patients and thus the use of less aggressive therapy in older patients. However, even after accounting for induction regimen, older patients more often failed to clear leukaemic blasts following induction, implying that increasing patient age is inherently linked to the development of chemoresistant leukaemia. Certainly this has been noted previously for AML in general, and has been suggested for CBF leukemias specifically (Schoch *et al*, 2004). Why age *per se* should contribute to chemoresistance is not understood, but various arguments have been made. Some suggest that AML in older patients is more often the result of a string of mutational events, leading to multiple leukaemic subclones with the opportunity to develop multiple mechanisms of chemoresistance. We and others have suggested that perhaps the development of leukaemia in an older stem cell in and of itself is sufficient to lead to greater drug resistance (Appelbaum *et al*, 2006). The reasons that higher WBC counts or blast counts lead to poorer RFS and OS are likewise not entirely clear, but could be related to an

association between higher blast counts and mutations in *KIT*. In several recent studies, *KIT* mutations were commonly found in patients with CBF AML, and among patients with t(8;21), the presence of a c-*KIT* tyrosine kinase domain mutation at codon 816 was associated with a high WBC count at diagnosis and a significantly higher incidence of relapse and poorer OS (Care *et al*, 2003; Cairoli *et al*, 2006; Schnittger *et al*, 2006). Unfortunately, the *KIT* mutational status of most of the 370 patients included in the current study is unknown. Confirmation of the link between *KIT* mutations, elevated WBC counts, and poorer outcomes in a subset of patients with CBF AML would be useful, particularly since pharmacologic inhibition of c-*KIT* is a real possibility.

In the current study, when analysing the impact of treatment regimens on outcome, although CR rates tended to be higher with FA than with other regimens, the difference was not statistically significant. However, those patients treated with FA or with high-dose cytarabine during induction had significantly improved DFS and OS. An analysis of consolidation therapy showed that patients receiving FA, HDAC, or HCT had superior survival compared with those receiving 'other' (usually conventional or low dose) therapy. Because those patients who received FA or HDAC during induction usually received the same treatment during consolidation, independent effects during induction and consolidation could not be determined. These results are consistent with those published by the CALGB who found, in an analysis limited to patients aged <60 years, that receiving multiple cycles of HDAC regimens significantly reduced the risk of relapse in CBF AML patients, both those with t(8;21) and those with inv(16) (Byrd *et al*, 1999; Byrd *et al*, 2004). This resulted in improved RFS, but did not translate into improved OS. In contrast, the German group reported no prognostic impact of the cumulative dose of cytarabine on RFS or OS (Schlenk *et al*, 2004). The French group reported inferior survival in patients with t(8;21) treated with a single cycle of intermediate dose cytarabine compared with patients receiving more intensive therapy, but did not find a similar effect in patients with inv(16) AML (Nguyen *et al*, 2002; Delaunay *et al*, 2003).

In summary, although patients with CBF AMLs have a better prognosis than most patients with AML, considerable room for improvement exists, particularly among older patients, those presenting with a high WBC count or blast percentage and those with complex cytogenetic changes in addition to the CBF translocation. When comparing and contrasting patients with t(8;21) AML and inv(16) AML, a number of marked differences were noted, including the younger age of t(8;21) patients, the marked associations of each subtype of CBF AML with other specific cytogenetic abnormalities, and the greater difficulty of salvaging t(8;21) patients who relapse following initial chemotherapy. The fact that t(8;21) and inv(16) AMLs have a similar pathogenetic mechanism yet these substantial differences exist suggests that this may be a fruitful setting to investigate the molecular basis leading to these differences.

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