Published in final edited form as:
Br J Haematol. 2014 July ; 166(2): 254–259. doi:10.1111/bjh.12852.

Acute Myeloid Leukaemia (AML) with t(6;9)(p23;q34) is Associated with Poor Outcome in Childhood AML Regardless of *FLT3*-ITD Status: A Report from the Children's Oncology Group

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Abstract

Acute myeloid leukaemia (AML) with t(6;9)(p23;q34) is a rare subtype associated with *FLT3*-internal tandem duplication (ITD) and poor outcomes. The clinical outcomes of paediatric patients with t(6;9) with and without *FLT3*-ITD treated on six consecutive cooperative trails were evaluated. In contrast to patients without t(6;9), those with t(6;9) had a significantly lower complete remission rate, higher relapse rate (RR), and poor overall survival (OS). Within t(6;9) patients, those with and without *FLT3*-ITD had an OS of 40% and 27% respectively (p>0.9),

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Authorship Contribution: K.T. and S.M. designed and performed research, analysed data, and wrote the manuscript; T.A.A. analysed the data, performed statistical analyses and edited the manuscript; R.B.G. performed statistical analyses and edited the manuscript; P.M.M, S.C.R, B.A.H., Y.R., B.L., W.G.W., and A.S.G. performed research and edited the manuscript.

Conflict-of-interest disclosure: The authors have no competing financial interests to declare.

demonstrating that t(6;9) is a high-risk cytogenetic feature in paediatric AML and its clinical impact is independent of the presence of *FLT3*-ITD.

Keywords

acute myeloid leukaemia; paediatric; t(6;9)(p23;q34); *FLT3*-ITD; clinical outcome

INTRODUCTION

Translocation t(6;9) is a rare recurring cytogenetic aberration resulting in the formation of a chimeric fusion gene, *DEK-NUP214* that occurs in <2% of adult and paediatric acute myeloid leukaemia (AML) cases (Schwartz *et al*, 1983; Slovak *et al*, 2006). This translocation creates a nucleoporin fusion protein thought to result in altered nuclear transport of key proteins, although its role in leukaemogenesis is not yet defined. This translocation has also been identified in myelodysplastic syndromes and Ph-negative chronic myeloid leukaemia. In AML, patients positive for the translocation have been reported to have a poor response to induction chemotherapy and high rate of post-remission relapse (Harrison *et al*, 2010). Although t(6;9) is known to be highly associated with *FLT3*-internal tandem duplication (ITD), the clinical significance of this association has not been well defined (Meshinchi *et al*, 2006; Theide *et al*, 2002). The lack of clarity of the impact of this association raised the question that the adverse outcome in t(6;9) may be due to the presence of *FLT3*-ITD and not inherent to the *DEK-NUP214* translocation. Currently t(6;9) is considered a high-risk cytogenetic abnormality in many adult trials, although some current paediatric trials, including those of theChildren's Oncology Group (COG), allocate patients with *FLT3*-ITD to high-risk treatment arms but those with t(6;9) without *FLT3*-ITD are treated as standard risk. This study defines the prognostic implications t(6;9) in paediatric AML and the prevalence and contribution to clinical outcome of *FLT3*-ITD in this population.

MATERIALS AND METHODS

Patients and mutation analysis

All paediatric patients with *de novo* AML enrolled on the previous six phase III paediatric AML clinical trials conducted by COG or its predecessors, Pediatric Oncology Group (POG) or Children's Cancer Group (CCG) over a period of 22 years (1988-2010) were eligible for this study. 3790 eligible patients enrolled in AML trials CCG-2861, CCG-2891, POG-9421, CCG-2961, COG-AAML03P1 and COG-AAML0531, of which 2839 with available cytogenetic data were included in this study. Study population and treatment regimens have been previously described (Ravindranath *et al*, 2005; Lange *et al*, 2005; Woods *et al*, 1996, Gamis *et al*, 2010; Cooper *et al*, 2012). Diagnostic specimens from patients with t(6;9) underwent *FLT3* mutational analysis as previously described (Meshinchi *et al*, 2001).

Statistical methods

Data were analysed from POG-9421 and CCG-2861, -2891, -2961, up to 24 April 2006, 21 September 2001, 14 January 2004 and 6 November 2009, respectively. For patients treated on COG-AAML03P1 and - AAML0531, data were analysed up to 30 September 2012. Significance of differences in proportions was tested using Chi-squared test and Fischer's exact test for sparse data. The Mann-Whitney test was used to test differences in medians. The Kaplan-Meier method was used to estimate overall survival (OS), event-free survival (EFS) and disease-free survival (DFS) (Kaplan & Meier, 1958). Estimates of relapse risk (RR) were obtained using methods that account for competing events (Kalbfleish & Prentice, 1980). Definitions of OS, EFS, DFS and RR have been previously described (Cooper *et al*, 2012). The significance of predictor variables was tested with the log-rank statistic for overall OS, EFS and DFS using Gray's statistic for RR. Estimates were reported with corresponding two standard errors calculated by Greenwood's formula. Children without an event were censored at date of last known contact. Statistical significance was defined as a P-value < 0.05.

RESULTS AND DISCUSSION

There were 3790 eligible *de novo* paediatric AML patients, of whom 2839 had known cytogenetic data and 48 cases (1.7%) of t(6;9)(p23;q34) were identified[CCG-2861 (n=2), CCG-2891 (n=5), POG 9421 (n=7), CCG-2961 (n=10), COG-AAML03P1 (n=7) and COG-AAML0531 (n=17)]. We initially evaluated the disease characteristics and clinical impact of t(6;9) in paediatric AML. Comparison of patient demographics and disease characteristics revealed that patients with and without t(6;9) had similar gender distribution, diagnostic white blood cell (WBC) count and diagnostic marrow blast percentage (Table I). However, t(6;9) was highly associated with older age (p<0.001) and French-American-British (FAB) type, with significantly higher association with FAB M2 (p=0.03), and inverse association with FAB M5 (p<0.001). None of the patients with t(6;9) had FAB M0, M5 or M7. Patients with t(6;9) had a worse induction response, with a complete remission (CR) rate of 67% compared to 79% for those without, (p=0.04). Patients with t(6;9) had a worse outcome with an OS from study entry of 39±15% compared to 57±2% for those without (p=0.03, Figure 1A), with a corresponding EFS from study entry of 32±14% and 45±2%, respectively (p=0.2). RR for patients who achieved an initial CR was 64±18% vs. 42±2% (p=0.04) for those with and without t(6;9) with a corresponding DFS from CR of 33±17% and 50±2% (p=0.1, Figure 1B). We found that patients with t(6;9) had similar outcome to patients with the poor prognostic features monosomy 7 or monosomy5/del5q (Figure 1C, D).

Of the 48 patients with t(6;9), diagnostic specimens were available for 36 (75%) patients and were tested for *FLT3* mutations. *FLT3*-ITD was identified in 24 out of 36 evaluable patients (**67%**), and mutations of the activation loop domain of *FLT3* (*FLT3*-ALM) were identified in 2 patients (6%). Patient demographics and disease characteristics were compared in the t(6;9) cohort with and without *FLT3*-ITD. Among patients with t(6;9), those with *FLT3*-ITD had a significantly higher median diagnostic WBC count (51.6×10^9/l vs. 17×10^9/l, **p=0.02**) as well as a higher median marrow blasts percentage (70% vs. 47%, p=0.006). Within the t(6;9) cohort, black race was significantly associated with *FLT3*-ITD negative

status, whereas no association between *FLT3*-ITD status and racial classification has been demonstrated within the entire study population (Meshinchi *et al*, 2006). *FLT3*-ITD status was not associated with FAB type. CR after induction chemotherapy was achieved in 20 of the 36 evaluable patients (56%), and patients with *FLT3*-ITD had a CR rate of 46% vs. 75% in those without *FLT3*-ITD (**p=0.1**). We then examined the impact on clinical outcome for t(6;9) patients based on the presence or absence of *FLT3*-ITD. There was no difference in 5-year actuarial OS from study entry for t(6;9) patients with and without *FLT3*-ITD with an OS of 40±20% vs. 27±30% respectively (**p>0.9**) (Figure 1E). For patients who achieved a remission after induction chemotherapy, RR for those with and without *FLT3*-ITD was 55±32% vs. 87±31% (**p=0.3**) (Figure 1F) with a corresponding DFS from remission of 45±30% vs. 13±23% (**p=0.3**).

As patients in this study were treated over a 22-year period, we examined the impact of treatment era on outcome in t(6;9). Patients with t(6;9) treated on more contemporary studies COG-AAML03P1/0531 (2004-2010) had an EFS of 48% compared to 14% for those treated on CCG-2861/2891 (1989-1992) and 14% for those treated on POG-9421/ COG-2961 (p=0.003) (Figure S1A). Closer analysis of t(6;9) patients treated on AAML03P1/0531 demonstrated those with *FLT3*-ITD had an EFS of 68% compared to 0% in those without *FLT3*-ITD (p=0.02), suggesting the outcome for those with *FLT3*-ITD may have improved with contemporary therapies, whereas the outcome for t(6;9) only patients remains dismal (Figure S1B). We initially described the clinical significance of *FLT3*-ITD in paediatric AML in 2001, and AAML0531 was the first study to allocate patients with high-risk *FLT3*-ITD to receive haematopoietic stem cell transplantation (HSCT) in first CR. This intervention may have contributed to improvement in outcome in patients with t(6;9) and *FLT3*-ITD. Prior to the increased use of HSCT in *FLT3*-ITD, which includes regimens used in the period 1988-2002, EFS for t(6;9) patients was very poor for those with and without *FLT3*-ITD, at 9% and 0%, respectively (p=0.8) (Figure S1C).

We inquired whether HSCT impacted clinical outcome in patients with t(6;9) and *FLT3*-ITD. Of the 24 patients identified, 10 received allogeneic HSCT either in first (N=5) or second CR (N=5). The remaining patients (N=14) received conventional chemotherapy. Six of the 10 (60%) HSCT recipients are long-term survivors compared to only 3/14 (21%) chemotherapy-only recipients.

This study provides conclusive data that paediatric AML patients with t(6;9) are at a high risk of relapse and have a poor outcome independent of the presence of *FLT3*-ITD. Although the presence of *FLT3*-ITD may provide a therapeutic target for directed therapy, it is unclear if FLT3-targeted interventions will provide survival benefit in t(6;9) patients. In addition, further understanding of the *DEK-NUP214* fusion in leukaemogenesis and its potential role in therapeutic resistance may lead to the development of novel and targeted therapeutic strategies in this population. Although the small sample size precludes analysis of statistical significance, it appears that t(6;9) patients have very poor survival when treated with chemotherapy alone compared to HSCT. This supports the data by Ishiyama *et al*. (2012) that patients with t(6;9) who receive HSCT have a more favourable outcome and this benefit was most significant for patients in CR at the time of transplant. We and others have previously shown that HSCT provides survival benefit to patients with *FLT3*-ITD

(Meshinchi *et al*, 2006; Bornhäuser *et al*, 2007) and the previous COG study AAML0531 as well as the current paediatric phase III AML trial COG AALM1031 risk-stratifies patients with high allelic-ratio *FLT3*-ITD to consolidation with HSCT. The data presented here highlights the high risk nature of patients with t(6;9) regardless of *FLT3*-ITD status and suggests that t(6;9) patients should be considered at high risk for treatment failure and may benefit from risk-adapted therapy with consolidation HSCT in first CR. Evaluation of treatment strategies that investigate the role of FLT3-targeted therapies as well as those that could target *DEK-NUP214* and the resultant altered nuclear transport, as well as further investigation of the role of HSCT for all patients with t(6;9,) are needed to improve outcomes in this high-risk population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are thankful to the COG AML Reference Bank for providing diagnostic specimens. This work was supported by the National Cancer Institute of the National Institutes of Health grants T32 CA009351 (K.T.), R01 CA114563 (S.M.), R21 CA102624 (S.M.), Human Banking Specimen U24 CA114766, and the COG Grant's Chair U10 CA98543.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

Bornhäuser M, Illmer T, Schaich M, Soucek S, Ehninger G, Thiede C. Improved outcome after stem-cell transplantation in FLT3/ITD-positive AML. Blood. 2007; 109:2264–5. author reply 2265. [PubMed: 17312001]

Cooper TM, Franklin J, Gerbing RB, Alonzo TA, Hurwitz C, Raimondi SC, Hirsch B, Smith FO, Mathew P, Arceci RJ, Feusner J, Iannone R, Lavey RS, Meshinchi S, Gamis AS. AAML03P1, a pilot study of the safety of gemtuzumab ozogamicin in combination with chemotherapy for newly diagnosed childhood acute myeloid leukemia: a report from the Children's Oncology Group. Cancer. 2012; 118:761–769. [PubMed: 21766293]

Gamis AS, Alonzo TA, Gerbing RB, Aplenc R, Sung L, Meshinchi S, Raimondi SC, Hirsch BA, Kahwash S, Heerema-McKenney A, Winter L, Glick K, Byron P, Burden L, Wallas T, Davies SM, Smith FO. Remission Rates In Childhood Acute Myeloid Leukemia (AML) Utilizing a Dose-Intensive Induction Regimen with or without Gemtuzumab Ozogamicin (GO): Initial Results From the Children's Oncology Group Phase III Trial, AAML0531. Blood (ASH Annual Meeting Abstracts). 2010; 116:182.

Harrison CJ, Hills RK, Moorman AV, Grimwade DJ, Hann I, Webb DK, Wheatley K, deGraaf SS, van den Berg E, Burnett AK, Gibson BE. Cytogenetics of childhood acute myeloid leukemia: United Kingdom Medical Research Council Treatment Trials AML10 and 12. Journal of Clinical Oncology. 2010; 1:2674–2681. [PubMed: 20439644]

Ishiyama K, Takami A, Kanda Y, Nakao S, Maeda T, Naoe T, Tanigcuhi S, Kawa K, Nagamura T, Tabuchi K, Atsuta Y, Sakamaki H. Prognostic factors for acute myeloid leukemia patients with t(6;9)(p23;q34) who underwent an allogeneic hematopoietic stem cell transplant. Leukemia. 2012; 26:1416–1419. [PubMed: 22157737]

Kalbfleish, JD.; Prentice, RL. The Statistical Analysis of Failure Time Data. John Wileve & Sons; New York: 1980.

Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association. 1958; 53:457–481.

Lange BJ, Gerbing RB, Feusner J, Skolnik J, Sacks N, Smith FO, Alonzo TA. Mortality in overweight and underweight children with acute myeloid leukemia. Journal of the American Medical Association. 2005; 293:203–211. [PubMed: 15644547]

Meshinchi S, Woods WG, Stirewalt DL, Sweetser DA, Buckley JD, Tjoa TK, Bernstein ID, Radich JP. Prevalence and prognostic significance of Flt3 internal tandem duplication in pediatric acute myeloid leukemia. Blood. 2001; 97:89–94. [PubMed: 11133746]

Meshinchi S, Alonzo TA, Stirewalt DL, Zwaan M, Zimmerman M, Reinhardt D, Kaspers GJL, Heerema NA, Gerbing R, Lange BJ, Radich JP. Clinical implications of FLT3 mutations in pediatric AML. Blood. 2006; 108:3654–3661. [PubMed: 16912228]

Ravindranath Y, Chang M, Steuber CP, Becton D, Dahl G, Civin C, Camitta B, Carroll A, Raimondi SC, Weinstein HJ. Pediatric Oncology Group (POG) studies of acute myeloid leukemia (AML): a review of four consecutive childhood AML trials conducted between 1981 and 2000. Leukemia. 2005; 19:2101–2116. [PubMed: 16136167]

Schwartz S, Jiji R, Kerman S, Meekins J, Cohen MM. Translocation (6;9)(p23;q34) in acute nonlymphocytic leukemia. Cancer Genetics and Cytogenetics. 1983; 10:133–138. [PubMed: 6616433]

Slovak ML, Gundacker H, Bloomfield CD, Dewald G, Appelbaum FR, Larson RA, Tallman MS, Bennett JM, Stirewalt DL, Meshinchi S, Wilman CL, Ravindranath Y, Alonzo TA, Carroll AJ, Raimondi SC, Heerema NA. A retrospective study of 69 patients with t(6;9)(p23;q34) AML emphasizes the need for a prospective, multicenter initiative for rare ‘poor prognosis’ myeloid malignancies. Leukemia. 2006; 20:1295–1297. [PubMed: 16628187]

Thiede C, Steudel C, Mohr B, Schaich M, Schäkel U, Platzbecker U, Wermke M, Bornhäuser M, Ritter M, Neubauer A, Ehninger G, Ilmer T. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood. 2002; 99:4326–35. [PubMed: 12036858]

Woods WG, Kobrinsky N, Buckley JD, Lee JA, Sanders J, Neudorf S, Gold S, Barnard DR, SeSwarte J, Dusenbery K, Kalousek D, Arthur DC, Lange BJ. Timed-sequential induction therapy improves postremission outcome in acute myeloid leukemia: a report from the Children’s Cancer Group. Blood. 1996; 87:4979–4989. [PubMed: 8652810]

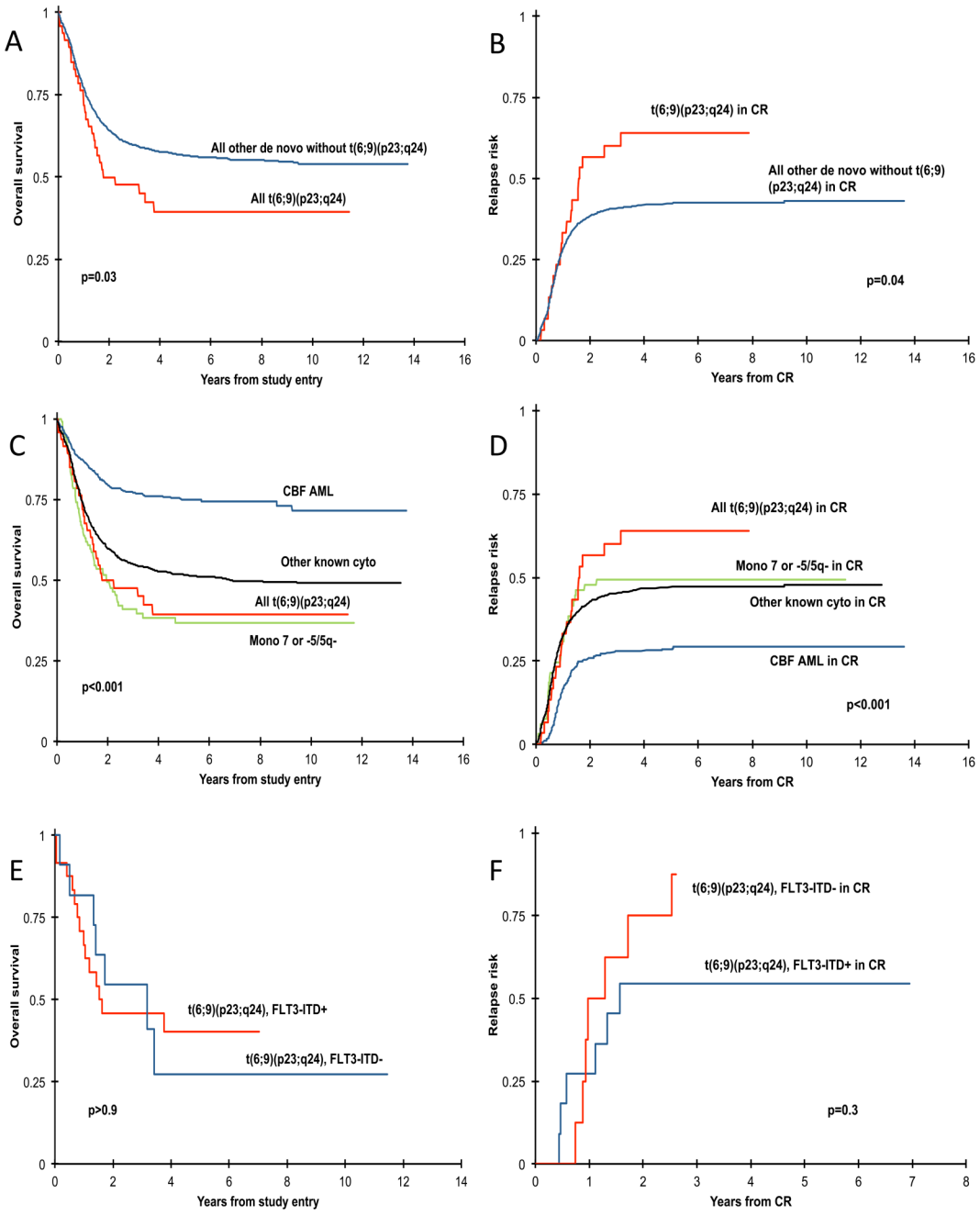

Figure 1.

Clinical outcome of patients with t(6;9) based on the presence or absence of *FLT3*-ITD: Patients with t(6;9) have a worse overall survival (A) and Relapse Risk (B) in contrast to those without t(6;9). Outcome for patients with t(6;9) is similar to those with high-risk cytogenetics (C and D). In patients with t(6;9) those with and without *FLT3*-ITD have similar survival and relapse risk (E and F). *FLT3*-ITD, *FLT3* internal tandem duplication;

CR, complete remission; CBF AML, core-binding factor acute myeloid leukemia; cyto, cytogenetics;

Table I

Patient demographics and disease characteristics

	All t(6;9) patients (n=48)		All non-t(6;9) patients (n=2791)			t(6;9) with *FLT3*-ITD (n=24)		t(6;9) *FLT3*-ITD negative (n=12)		
	N	**%**	**N**	**%**	**P**	**N**	**%**	**N**	**%**	**P**
Gender										
Male	19	40%	1441	52%	0.1	10	42%	4	33%	0.7
Female	29	60%	1350	48%		14	58%	8	67%	
Race										
White	32	73%	1936	79%	0.3	20	87%	4	40%	**0.01**
Black	10	23%	320	13%	0.06	2	9%	6	60%	**0.004**
Asian	2	5%	103	4%	0.7	1	4%	0	0%	1
Other	0	0%	82	3%	0.4	0	0%	0	0%	1
Unknown	4		350			1		2		
Ethnicity										
Hispanic	5	11%	433	16%	0.4	2	8%	2	20%	0.6
Not Hispanic	41	89%	2289	84%		22	92%	8	80%	
Unknown	2		69			0		2		
Age (years): median (range)	12.6	(2.6 - 20.4)	8.9	(0 - 29.8)	**<0.001**	12.4	(2.6 - 17.3)	12.7	(3.6 - 16.9)	0.8
WBC count (x10^9 /l): median (range)	25.5	(1.7 - 245.7)	22.4	(0 - 827.2)	0.9	51.6	(1.8 - 245.7)	17	(7.1 - 29.8)	**0.02**
BM Blasts %	60	(25 - 95)	68	(0 - 100)	0.2	70.2	(42 - 95)	47	(25 - 90)	**0.006**
FAB Classification										
M0	0	0%	90	3%	0.4	0	0%	0	0%	1
M1	7	15%	357	14%	0.7	4	17%	1	8%	0.64
M2	19	41%	705	27%	**0.03**	10	4%	6	50%	0.7
M4	13	28%	598	23%	0.4	8	24%	3	25%	0.7
M5	0	0%	517	20%	**<0.001**	0	0%	0	0%	1
M6	2	4%	52	2%	0.2	0	0%	0	0%	1

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	All t(6;9) patients (n=48)		All non-t(6;9) patients (n=2791)			t(6;9) with *FLT3*-ITD (n=24)		t(6;9) *FLT3*-ITD negative (n=12)		
	N	**%**	**N**	**%**	**P**	**N**	**%**	**N**	**%**	**P**
M7	0	0%	170	6%	0.1	0	0%	0	0%	1
Other	5	11%	149	6%	0.2	1	4%	2	17%	0.3
Unknown	2		152			1		0		
End of course 1 response										
CR	32	67%	2162	79%	**0.04**	11	46%	9	75%	0.1
Not in CR	16	33%	577	21%		13	54%	3	25%	
Unevaluable	0		51			0		0		

WBC, white blood cell; BM, bone marrow; FAB, French-American-British; CR, complete remission

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