

**Prevalence and prognostic significance of *KIT* mutations in pediatric core binding factor AML patients enrolled on serial pediatric cooperative trials for *de novo* AML**

Jessica A. Pollard<sup>1,2</sup>, Todd A. Alonzo<sup>3,4</sup>, Robert B. Gerbing<sup>4</sup>, Phoenix A. Ho<sup>1,2</sup>, Rong Zeng<sup>1</sup>, Yaddanapudi Ravindranath<sup>5</sup>, Gary Dahl<sup>6</sup>, Norman J. Lacayo<sup>6</sup>, David Becton<sup>7</sup>, Myron Chang<sup>8</sup>, Howard J. Weinstein<sup>9</sup>, Betsy Hirsch<sup>10</sup>, Susana C. Raimondi<sup>11</sup>, Nyla A. Heerema<sup>12</sup>, William G. Woods<sup>13</sup>, Beverly J. Lange<sup>14</sup>, Craig Hurwitz<sup>15</sup>, Robert J. Arceci<sup>16</sup>, Jerald P. Radich<sup>1</sup>, Irwin D. Bernstein<sup>1,2</sup>, Michael C. Heinrich<sup>17</sup> and Soheil Meshinchi<sup>1,2</sup>

<sup>1</sup>Fred Hutchinson Cancer Research Center, Seattle, WA; <sup>2</sup>University of Washington, Seattle, WA; <sup>3</sup>University of Southern California Keck School of Medicine, Los Angeles, CA; <sup>4</sup>Children's Oncology Group, Arcadia, CA; <sup>5</sup>Children's Hospital of Michigan, Detroit, MI; <sup>6</sup>Stanford University Medical Center, Palo Alto, CA; <sup>7</sup>University of Arkansas, Little Rock, AR; <sup>8</sup>Children's Oncology Group, Gainesville, FL; <sup>9</sup>Massachusetts General Hospital, Boston, MA; <sup>10</sup>University of Minnesota Cancer Center, Minneapolis, MN; <sup>11</sup>St. Jude Children's Research Hospital, Memphis, TN; <sup>12</sup>Ohio State University, Columbus, OH; <sup>13</sup>Children's Healthcare of Atlanta/Emory University, Atlanta, GA; <sup>14</sup>Children's Hospital of Philadelphia, Philadelphia, PA; <sup>15</sup>Maine Medical Center, Portland, ME; <sup>16</sup>Johns Hopkins Hospital, Baltimore, <sup>17</sup>Portland VA Medical Center and Oregon Health and Science University Knight Cancer Institute, Portland, OR.

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Address reprint requests to:

Jessica Pollard, MD, Seattle Children's Hospital, 4800 Sandpoint Way NE, Division of Heme/Onc, MS-B-6553, Seattle WA 98105, USA. Phone: 206-987-2106; fax 206-987-3946, email: [jessica.pollard@seattlechildrens.org](mailto:jessica.pollard@seattlechildrens.org)

## ABSTRACT

*KIT* receptor tyrosine kinase mutations are implicated as a prognostic factor in adults with core binding factor (CBF) acute myeloid leukemia (AML). However, their prevalence and prognostic significance in pediatric CBF AML is not well established. We performed *KIT* mutational analysis (exon 8 and exon 17) on diagnostic specimens from 203 pediatric CBF AML patients enrolled on 4 pediatric AML protocols. *KIT* mutations were detected in 38/203 (19%, CI:14-25%) patient samples of which 20/38 (52.5%, CI:36-69%) involved exon 8, 17/38 (45%, CI:29-62%) involved exon 17, and 1 (2.5%, CI:0-14%) involved both locations. Patients with *KIT* mutations had a 5 year event-free survival of  $55 \pm 17\%$  compared with  $59 \pm 9\%$  for those with wild-type *KIT* ( $p=0.86$ ). Rates of complete remission, overall survival, disease free survival, or relapse were not significantly different for patients with or without *KIT* mutations. Location of the *KIT* mutation and analysis by cytogenetic subtype [t(8;21) vs. inv(16)] also lacked prognostic significance. Our study demonstrates that *KIT* mutations lack prognostic significance in a large series of pediatric CBF AML patients. This finding which differs from adult series and a previously published pediatric study, may reflect variations in therapeutic approaches and/or biologic heterogeneity within CBF AML. Two of 4 studies included in this analysis are registered at <http://clinicaltrials.gov> under NCT00002798 (CCG-2961) and NCT00070174 (COG AAML03P1).

## INTRODUCTION

Core binding factor (CBF) acute myeloid leukemia (AML) is characterized by the presence of a t(8;21) (q22;q22) or inv (16) (p13.1q22)/ t(16;16) (p13.1;q22) [hereafter referred to as inv(16)] chromosomal rearrangement and is observed in approximately 20%-30% of pediatric AML cases.<sup>1</sup> Although overall survival (OS) for pediatric CBF AML patients is superior to that of

pediatric AML patients with normal cytogenetics, a subset of these patients do quite poorly, suggesting that disease characteristics of this population are not as homogeneous as their cytogenetic definition and that additional mutational events may impact disease response.<sup>1-3</sup> *KIT* is a proto-oncogene located on chromosome band 4q11-12 and encodes a 145 kD transmembrane glycoprotein that is a member of the type III receptor tyrosine kinase family.<sup>2,4,5</sup> Stem cell factor promotes *KIT* dimerization and transphosphorylation when bound thereby activating downstream signaling pathways integral to proliferation, differentiation and survival of hematopoietic stem cells.<sup>5</sup> Ligand independent activation of *KIT* results from mutations in the extracellular portion of the receptor (exon 8), transmembrane and juxtamembrane domain (exon 10, 11 respectively) and activation loop of the tyrosine kinase domain (exon 17).<sup>5</sup> Mutations of *KIT* are overall infrequent in adult AML (2-8%) and tend to cluster within the activation loop (exon 17) and a region of the extracellular domain integral to receptor dimerization (exon 8).<sup>5</sup> Their prevalence is much higher, however, in adult CBF AML patients (6-48%)<sup>2,6-11</sup> and may be associated with worse clinical outcome in this patient population.<sup>2,6,9-11</sup> The incidence and clinical significance of *KIT* mutations in pediatric CBF AML is less clear as studies have been limited to a small number of patient samples.<sup>12-15</sup>

We screened a total of 203 diagnostic samples obtained from pediatric CBF AML patients enrolled on Pediatric Oncology Group (POG) protocol 9421, Children's Cancer Group (CCG) protocols 2891, 2961 and Children's Oncology Group (COG) protocol AAML03P1 for evidence of mutations in *KIT* exon 8 or 17. Our study, which represents the largest pediatric analysis to date, provides insight into the incidence and prognostic implications of *KIT* mutations in *de novo* pediatric CBF AML patients treated on serial pediatric cooperative clinical trials.

## MATERIALS AND METHODS

Pediatric patients with previously identified CBF AML and enrollment on pediatric cooperative trials POG-9421, CCG-2891, 2961, or COG study AAML03P1 were eligible for this study. This study was approved by the Fred Hutchinson Cancer Research Center Institutional Review Board and the COG Myeloid Disease Biology Committee. Details of treatment protocols POG-9421, CCG-2891, and 2961 have been described previously.<sup>16-18</sup> COG AAML03P1 was a pilot study in which *de novo* AML patients received gemtuzumab ozogamicin, a humanized IgG4 anti-CD33 monoclonal antibody linked to the cytotoxic agent calicheamicin, in combination with a backbone of Medical Research Council (MRC) 12 based conventional chemotherapy.<sup>19</sup>

Cytogenetic data was available for 84%, 53%, 62% and 93% of patients enrolled on POG-9421, CCG-2891, CCG-2961 and COG AAML03P1, respectively. A diagnosis of CBF AML was made if either t(8;21) (q22;22) or inv(16) (p13;q22)/t(16;16)(p13;q22) was detected in pretreatment bone marrow (BM) or peripheral blood (PB) specimens by either conventional cytogenetics and/or fluorescent in situ hybridization techniques. In most cases chromosomal abnormalities were identified by conventional cytogenetics and karyotypes confirmed by central review (SCR: POG 9421, NAH: CCG-2891 and 2961, SCR and BH for COG AAML03P1).

***KIT* mutational analysis:** Genomic DNA was extracted from diagnostic BM or PB specimens using the Puregene Protocol (Gentra Systems, Minneapolis, MN). *KIT* exons 8 and 17 were amplified by genomic PCR in separate reactions.

**PCR amplification of *KIT* exon 17:** PCR amplification of exon 17 was performed using primers 17F, 5'-CCTCCAACCTAATAGTGTATTACAG and 17R, 5-ATGTGTGATATCCCTAGACAGGAT. The PCR mixture contained a maximum of 100 ng

DNA and 10 pmol/μl of 17F and 17R primers. Standard concentrations of reaction mixtures were used as described previously.<sup>20</sup> Negative controls were included with amplification.

Denaturing, annealing and extension steps were performed at 95°C for 30 seconds, 61°C for 25 seconds, and 72°C for 25 seconds respectively for a total of 40 cycles on an MJ Research (Waltham, MA) thermocycler. An initial 5 minute denaturation step at 95°C and a final 8 minute extension step at 72°C were also performed. PCR products were resolved on a 2% agarose gel. After visual confirmation of amplification, 4 μl of the PCR product was purified with 2 μl ExoSAP-IT (USB Corp, Cleveland, OH) and analyzed by bi-directional sequencing on an ABI 377 sequencer, using the BigDye terminator kit (Applied Biosystems Inc, Foster City, CA). Mutational analysis was conducted by Mutation Surveyor (SoftGenetics, State College, PA).

**PCR amplification of *KIT* exon 8:** PCR amplification of exon 8 was performed using primers 8F, 5'-TTCAGATTCTGCCCTTTGAACTTG and 8R, 5-TGAAATTCAAGTGAATTGCAGTCC with reaction conditions identical to those for exon 17 analysis. The 8R primer was labeled with a 5' fluorescent probe to facilitate mutation detection via micro-capillary electrophoresis techniques. PCR products were resolved on a 2% agarose gel and analyzed by the Genescan or Genemapper software (Applied Biosystems, Inc) after appropriate dilution of PCR products to facilitate identification of mutational insertions and/or deletions. Mutations were confirmed by using the aforementioned sequencing techniques when possible.

#### **Denaturing high performance liquid chromatography (DHPLC) analysis of samples from patients enrolled on CCG-2961**

A subset of patient samples obtained from CCG-2961 were also subjected to exon 8 and exon 17 mutational analysis by the Transgenomic WAVE denaturing high performance liquid

chromatography (DHPLC) system (Transgenomic, Inc, Omaha, NE) using previously described methods.<sup>12</sup>

**Statistical methods:** Clinical outcome data from POG-9421, CCG-2891, CCG-2961 and COG AAML03P1 were analyzed through September 5, 2006, January 14, 2004, October 30, 2006 and August 28, 2008, respectively. The median days follow up for all eligible *de novo* AML patients alive at last contact for the clinical trials included in our analysis are: 2003.5 days (range 56 – 3136 days) for POG-9421, 3128 days (range 1 – 5028 days) for CCG-2891, and 1762.5 days (range 0 – 3620 days) for CCG-2961. Median days follow-up for COG AAML03P1 is 783 days (range 0 – 1456 days). For the purpose of this study, patients were defined as being in complete remission (CR) if they had 5% or fewer blasts and trilineage recovery after 2 courses of chemotherapy. OS was defined as the time from study entry until death. Event-free survival (EFS) was defined as the time from study entry until death, induction failure, or relapse. Disease-free survival (DFS) was defined as the time from induction CR until relapse or death and relapse risk (RR) was defined as the time from end of induction to relapse or death due to progressive disease, where deaths from non-progressive disease were considered competing events. Pearson's chi-square test was used to test for differences in the distribution of categorical variables. The Fisher's exact test was used when data were sparse. The Mann-Whitney test was used to analyze differences of medians.<sup>21</sup> The Kaplan-Meier method was used for nonparametric survival curve analyses for OS, EFS and DFS.<sup>22</sup> RR analyses were performed by using methods of cumulative incidence. Patients having a matched family donor were censored at the end of 2 courses of therapy for OS, EFS, DFS and RR. Patients lost to follow-up were censored at their date of last known contact or at a cutoff of 6 months prior to the frozen date of study data to compensate for the tendency of deaths and relapses to be reported sooner than ongoing follow-

up. Differences in OS, EFS, and DFS were tested using Cox proportional hazards models<sup>23</sup> which were stratified by the protocol on which participants were treated in order to account for any differences in treatment protocols. Differences between cumulative incidence curves were tested by using the Gray's test<sup>24</sup> stratified by the study on which participants were enrolled. Confidence intervals for survival estimates were calculated using Greenwood's estimate of the standard error.<sup>25</sup> Binomial confidence intervals (CI) were also provided for prevalence proportions.

## RESULTS

**Study population:** All *de novo* CBF AML patients enrolled on POG-9421, CCG-2891, CCG-2961 and COG-AAML03P1 were eligible for this study. 48 of 97 (49%), 18 of 89 (20%), 98 of 138 (71%) and 39 of 78 (50%) eligible CBF AML patients from POG-9421, CCG-2891, CCG-2961 and COG-AAML03P1 respectively, had diagnostic BM or PB specimens available for *KIT* analysis. In total, 203/402 (50%) CBF AML patient samples were analyzed.

**Patients and treatment:** To determine whether our study population was representative of the overall CBF AML population, we compared laboratory and clinical characteristics of the 203 study patients to those of the remaining 199 CBF AML patients that lacked samples for analysis. Patients included in our study had a significantly higher median WBC (on study 28,800  $\times 10^9/L$  vs. off study 19,600  $\times 10^9/L$ ;  $P=0.036$ ) and median age (on study 11.8 years vs. off study 9.4 years;  $P=0.008$ ) than those for whom samples were unavailable. Percentage of BM blasts at presentation was higher but not significantly different for our study population (52.5%) than for patients off study (46.5%) ( $P=0.117$ ). Moreover, our study population had fewer males (52%) than the population excluded from analysis (61%); this finding approached statistical significance ( $P=0.066$ ). There were no significant differences in 5-year OS (on study  $74 \pm 7\%$

vs. off study  $71 \pm 8\%$ ,  $HR=1.1$ ,  $P=0.685$ ) and EFS (on study  $58 \pm 8\%$  vs. off study  $55 \pm 9\%$ ,  $HR=1.1$ ,  $P=0.628$ ) for the 2 groups. DFS (on study  $59 \pm 9\%$  vs. off study  $59 \pm 10\%$ ,  $HR=0.98$ ,  $P=0.906$ ) and RR (on study  $35 \pm 8\%$  vs. off study  $38 \pm 10\%$ ,  $HR=1.02$ ,  $p=0.830$ ) were also comparable. Clinical outcome of all CBF AML patients (regardless of *KIT* mutational status and/or inclusion in our study) was also compared to that of non-CBF AML patients with known cytogenetic information. Consistent with previous studies, we found that 5 year OS, EFS, DFS and RR was superior for those patients with CBF AML (5yr OS: CBF  $73\% \pm 5\%$  vs. Non-CBF:  $43\% \pm 3\%$ ,  $HR 0.38$ ,  $p<0.001$ ; 5yr EFS: CBF  $57\% \pm 6\%$  vs. Non-CBF  $33\% \pm 3\%$ ,  $HR 0.49$ ,  $p<0.001$ ; 5yr DFS: CBF  $59\% \pm 6\%$  vs. Non-CBF  $40\% \pm 3\%$ ,  $HR 0.58$ ,  $p<0.001$ ; 5yr RR: CBF  $36\% \pm 6\%$  vs. Non-CBF  $55\% \pm 4\%$ ,  $HR 0.55$ ,  $p<0.001$ ).

**Prevalence and type of *KIT* mutations at diagnosis:** *KIT* mutations were detected in 38 (19%, CI:14-25%) of the 203 specimens for which *KIT* mutational analysis (exon 8 and exon 17) was performed. Prevalence of *KIT* mutations ranged from 15% to 28% on individual cooperative studies. Of the 38 mutations detected, 20 (52.5%, CI: 36-69%) involved exon 8, 17 (45%, CI: 29-62%) involved exon 17, and 1 (2.5%, CI: 0-14%) affected both regions (Table 1). Exon 8 mutations were either small deletions or insertions detected in 5% (CI: 2-11%) of t(8;21) and 16% (CI: 9-25%) of inv(16) patient samples (Table 1). All sequenced samples (13 of 20) had deletions or insertions involving codons 416-420. Exon 17 mutations were exclusively point mutations and were found in 12% (CI: 6-19%) of t(8;21) and 4% (CI: 1-11%) of inv(16) patient samples (Table 1). Twelve of 17 exon 17 mutations involved codon 816 (N=9 D816V, N=1 D816H, N=2 D816Y) and 5 involved surrounding codons (N=1 D820G, N=4 N822K). Mutations of both exon 8 (insertion at codon 418) and exon 17 (D816Y) were detected in 1

inv(16) patient sample. Of the 83 samples screened by DHPLC, all previously identified *KIT* mutations were confirmed but no additional mutations were detected.

**Clinical characteristics and CR rates for patients with *KIT* mutations:** Clinical characteristics and CR rates for the 38 patients with *KIT* mutations were compared with those of the 165 CBF AML patients with wild type (WT) *KIT*. There were no significant differences in gender, race, and median age between the 2 groups although more patients with *KIT* mutations were younger than 2 years of age ( $P=0.077$ ; Table 2) Median diagnostic WBC, median BM blast percentage at diagnosis, and CR rates were also similar for both groups (Table 2).

A significant number of our CBF study samples had been previously screened for other AML related molecular abnormalities.<sup>26-30</sup> No additional mutations were detected in the *KIT* mutation positive cohort. Specifically, while FLT3 internal tandem duplications (*FLT3/ITD*) and *WT-1* mutations were observed in 6 and 13 CBF AML patients respectively, these mutations were limited to WT *KIT* CBF AML patients. *CEBPA* and nucleophosmin (*NPM1*) mutations were absent in all CBF samples analyzed (Table 2).

**Prognostic significance of *KIT* mutations in CBF AML:** Five year OS and EFS from study entry (note that 3 year OS/EFS rates were used for COG protocol AAML03P1 due to lack of follow up) was similar for CBF AML patients with and without *KIT* mutations (Table 3, Figure 1). The 5-year cumulative risk of relapse and corresponding DFS from CR were also similar for the 2 groups (Table 3, Figure 2). There were no significant differences in outcome for patients with *KIT* mutations or WT *KIT* within individual studies with exception of POG-9421, wherein 11 patients with *KIT* mutations had poorer 5-year OS but similar 5-year EFS to patients with WT *KIT* (Table 3).

We also evaluated the prognostic significance of *KIT* mutation type (exon 8 vs. exon 17) for this CBF AML cohort. Rates of induction CR for patients with exon 8 and/or exon 17 mutations were comparable to those of patients with WT *KIT* patients (Table 4). Five year EFS and OS from study entry was also similar regardless of *KIT* mutation status as was corresponding 5-year DFS and RR from CR (Table 4).

Patients with t(8;21) AML and *KIT* mutations of exon 17 codon 816 have been found to have a particularly unfavorable prognosis in previous studies.<sup>7,9,10</sup> In our cohort, 12 patients [8 t(8;21) and 4 inv(16) AML] had mutations in codon 816 ( $N=9$  D816V,  $N=1$  D816H,  $N=2$  D816Y). Although small sample size precluded subanalysis by cytogenetic type, patients with CBF AML and *KIT* D816 mutations (*KIT* D816) had similar CR rates compared to those patients with other *KIT* mutations or WT *KIT* (*KIT* D816 100%, other *KIT* mutations 96%, WT *KIT* 94%,  $P=0.649$ ). OS from study entry (*KIT* D816 82%  $\pm$  23%, other *KIT* mutations 74%  $\pm$  18%, WT *KIT* 74%  $\pm$  8%;  $P=0.965$ ) and EFS from study entry (*KIT* D816 61%  $\pm$  33%, other *KIT* mutations 52%  $\pm$  21%, WT *KIT* 59%  $\pm$  9%,  $P=0.894$ ) were also comparable for the 3 groups. Five-year DFS (*KIT* D816 62%  $\pm$  30%, other *KIT* mutations 52%  $\pm$  21%, WT *KIT* 60%  $\pm$  10%;  $P=0.890$ ) and RR from end of induction (*KIT* D816 29%  $\pm$  28%, other *KIT* mutations 38%  $\pm$  21%, WT *KIT* 34%  $\pm$  10%;  $P=0.882$ ) were also similar.

***Prognostic significance of KIT mutations for t(8;21)patients:*** Of the 113 patients with t(8;21) AML, 19 (17%, CI: 10-25%) had a *KIT* mutation [6 involved exon 8 and 13 involved exon 17 (Table 1)]. CR rates for patients with t(8;21) AML were similar regardless of *KIT* mutation status (*KIT* mutation 100% vs. WT *KIT* 92%;  $P=0.599$ ). The 5-year OS (*KIT* mutation 71%  $\pm$  22% vs. WT *KIT* 74%  $\pm$  11%;  $P=0.603$ ) and EFS from study entry (*KIT* mutation 58%  $\pm$  24% vs. WT *KIT* 65%  $\pm$  11%;  $P=0.572$ ) as well as 5-year DFS (*KIT* mutation 64%  $\pm$  25% vs. WT *KIT*

75± 12%;  $P=0.293$ ) and RR from induction CR (*KIT* mutation 31% ± 23% vs. WT *KIT* 23 ± 11%;  $P=0.516$ ) were similar for both groups. Location of the mutation did not impact outcome. CR rates for patients with t(8;21) AML with *KIT* exon 8 or exon 17 mutations were comparable to those with WT *KIT* (Table 4). Five year EFS and OS rates from study entry as well as 5-year DFS and RR rates from end of induction were also similar regardless of *KIT* genotype (Table 4).

**Prognostic significance of *KIT* mutations for inv(16) patients:** Of the 90 patients with inv(16) AML, 19 (21%, CI: 13-31%) had *KIT* mutations (14 involved exon 8, 4 involved exon 17, and 1 involved both exons; Table 1). CR rates for patients with inv(16) AML with or without *KIT* mutations were similar (*KIT* mutation 95% vs. WT *KIT* 97%;  $P=0.547$ ). Rates of 5-year OS (*KIT* mutation 81% ± 19% vs. WT *KIT* 75% ± 12%;  $P=0.607$ ) and EFS from study entry (*KIT* mutation 53% ± 25% vs. WT *KIT* 51% ± 14%;  $P=0.770$ ) were also comparable for both groups. DFS and RR rates from induction CR did not differ for both groups, although outcomes were slightly better for patients with *KIT* mutations than those with WT *KIT*. (DFS: *KIT* mutation 56% ± 25% vs. WT *KIT* 49 ± 16%,  $P=0.528$ ; RR : *KIT* mutation 38% ± 25% vs. WT *KIT* 51 ± 16%;  $P=0.295$ ) Location of mutation did not impact outcome. CR rates for patients with inv(16) AML with *KIT* exon 8 or 17 mutations, or both, were similar to those without mutations (Table 4). 5-year EFS and OS rates from study entry as well as 5-year DFS and RR rates from end of induction were also similar regardless of *KIT* genotype (Table 4).

## **DISCUSSION**

We retrospectively examined the prevalence and prognostic significance of *KIT* exon 8 and 17 mutations in a cohort of 203 children with CBF AML. This study of CBF AML patients enrolled on 4 pediatric cooperative AML trials is the largest *KIT* mutational analysis of this subpopulation to date. The prevalence of *KIT* mutations was approximately 20% in our study population,

which is in the range reported by previous studies of adults (6-48%)<sup>2,6-11</sup> and children (17-41%)<sup>12-15</sup> with CBF AML. We found that *KIT* mutations lacked prognostic significance in pediatric patients with CBF AML. This finding is in contrast to that of previous adult CBF AML studies in which *KIT* mutations predicted a higher rate of relapse,<sup>2,6,10</sup> OS,<sup>2,9-11</sup> EFS,<sup>9,11</sup> and DFS<sup>11</sup> in particular analyses. Specifically, Care et al showed that mutations of exon 8 in adults with inv(16) AML adversely affected relapse rate but not OS<sup>6</sup> whereas Cairoli et al. and Boissel et al. found that *KIT* mutations lacked prognostic significance in adult patients with inv(16) CBF AML.<sup>10,11</sup> A later series by Paschka et al. found that adult patients with inv(16) CBF AML had a higher risk of relapse. Strikingly, RR rates for inv(16) patients with mutations in exon 17 were more than 6 times higher than for those without *KIT* mutations. Mutations in exon 17, exon 8, or both also negatively impacted OS.<sup>2</sup>

The prognostic impact of *KIT* mutations in t(8;21) AML is also debatable. Both Schnittger et al<sup>9</sup> and Cairoli et al<sup>10</sup> found that exon 17 mutations negatively impacted outcome. In the former study, D816 mutations were exclusively evaluated and adversely affected EFS and OS.<sup>9</sup> In the latter, patients samples were screened for mutations in exon 17, 8, and 11 and found, by subset analysis, that only exon 17 mutations had prognostic significance.<sup>10</sup> Boissel et al<sup>11</sup> also showed that *KIT* mutations in t(8;21) AML negatively impacted OS, EFS and relapse free survival, although analysis of outcome by specific genotype was not performed. Paschka et al found that patients with *KIT* mutations in t(8;21) had 5 times higher risk of relapse than, but similar rates of OS to, patients without *KIT* mutations. Nine of 11 mutations detected involved exon 17.<sup>2</sup>

The prognostic significance of *KIT* mutations in pediatric CBF AML has varied in previously published series. Goemans et al identified *KIT* exon 8 and 17 mutations in 10 of 27

(37%) children with CBF AML, with an overall prevalence of 55% for inv(16) (27% exon 8, 27% exon 17) and 31% (12.5% exon 8, 18.8% exon 17) for children with t(8;21) AML. In their study, the presence of a *KIT* mutations was not associated with inferior EFS.<sup>12</sup> Shih et al detected *KIT* mutations in 17 of 41 (41%) patients with CBF AML [5 (29%) involved exon 8, 9 (53%) involved exon 17, and 2 involved mutations at both exon 8 and 17]. No significant difference in OS or EFS was observed for patients with *KIT* mutations versus WT *KIT*. Analysis by cytogenetic subtype also revealed that patients with *KIT* mutations and those with WT *KIT* had similar EFS compared to patients with WT *KIT*.<sup>15</sup> The finding of Goemans et al<sup>12</sup> and Shih et al<sup>15</sup> are in contrast to a study by Shimada et al.<sup>13</sup> In this study, 46 children with t(8;21) AML were screened for *KIT* mutations of exon 8, 9,10, 11, 17 and 18. Eight of 46 patients (17.4%) had *KIT* mutations, all involving the second intracellular kinase domain (exon 17 and 18). Those patients with *KIT* mutations had lower OS and DFS and increased RR than those without *KIT* abnormalities.<sup>13</sup>

The prevalence of *KIT* exon 8 or 17 mutations in our study cohort (19%) is lower than that reported by Goemans et al<sup>12</sup> and Shih et al<sup>12,15</sup> but comparable to that of an analysis by Shimada et al that was limited to patients with t(8;21) AML.<sup>13,14</sup> Exon 8 mutations were observed more frequently in patients with inv(16) AML [74% vs. 32% of t(8;21) samples analyzed], whereas mutations of exon 17 were more prevalent in t(8;21) AML [68% vs. 21% of inv(16) samples analyzed], consistent with published findings.<sup>5,9</sup> Like previous studies, we also found that exon 17 mutations associated with inv(16) disease occurred exclusively at codon D816<sup>2,6,9,10</sup> whereas mutations associated with t(8;21) occurred predominantly at codons D816 and N822.<sup>2,7,8</sup>

Our study of 4 large cooperative pediatric trials highlights that *KIT* mutations lack prognostic significance within pediatric CBF AML. Our findings, which differ from several adult series, may reflect inherent biologic differences between *KIT* mutations in children and adults with CBF AML<sup>2,6,9-11</sup> In vitro studies have shown that exon 8 mutations represent a gain of function mutation that induces KIT receptor hyperactivation in response to stem cell factor stimulation. This results in excessive proliferation and resistance to apoptotic cell death.<sup>31</sup> In contrast, exon 17 mutations promote receptor autophosphorylation without the requirement of receptor-ligand interaction. Such mutations result in constitutive activation of phosphatidylinositol 3 kinase and its downstream targets as well as STAT3.<sup>5,32,33</sup> While the types of exon 8 and 17 mutations observed in adult and pediatric CBF AML are similar, it is possible that the prognostic implications observed in adults may reflect a tendency for the mutation to achieve clonal dominance in a less mature leukemic progenitor that is more difficult to target with conventional chemotherapies. We have previously demonstrated an association between lineage involvement of *FLT3/ITD* mutations in pediatric AML and clinical response to therapy. In that study, pediatric patients with *FLT3/ITD* disease limited to CD34+/CD33+ progenitors had markedly improved outcome compared to patients with CD34+/CD33- *FLT3/ITD* involvement.<sup>34</sup> A similar paradigm may exist for pediatric vs. adult CBF AML patients with *KIT* mutations. Alternatively, *KIT* mutations in adults may evolve from a backbone of greater genetic instability and be associated with additional unidentified mutations that confer prognostic significance/greater resistance to conventional agents. Additional studies aimed at defining the maturational stage of hematopoietic development at which these mutations achieve dominance in adult vs. pediatric CBF AML patients may be illustrative. Alternatively,

with the increased use of whole genome arrays, identification of associated mutations that confer poor outcome in *KIT* mutation positive CBF AML may be detected.

The lack of clinical prognostic significance observed for *KIT* mutations in pediatric compared to adult CBF AML may also reflect the intense nature of pediatric therapeutic regimens. The outcomes of our study cohort are similar to those of 2 smaller pediatric series<sup>12,15</sup> but contrast with that observed by Shimada et al.<sup>13</sup> In that study, *KIT* WT t(8;21) AML outcomes were quite high (EFS 92%)<sup>13</sup> compared to that of similar patients in both our series (WT *KIT* EFS 59%) and that of the Europeans (WT *KIT* EFS 63%<sup>12,13</sup>) which may explain the inferior outcome observed when comparing *KIT* mutation positive vs. WT *KIT* patients within that cohort. We concede that patients included in our analyses were treated in four different clinical trials, which may have introduced an error in analysis of the combined data. However, although clinical outcomes varied from study to study, the prognostic implications of *KIT* mutations remained consistent in each trial.

In childhood AML, the majority of patients with a matched family donor (MFD) undergo allogeneic hematopoietic stem cell transplant (HSCT) in first CR. Children with CBF AML in first CR have been considered a *more* favorable risk group than non-CBF AML given their superior OS and DFS.<sup>35</sup> Moreover, their OS and DFS has not improved with MFD HSCT.<sup>35</sup> These patients are therefore treated solely with chemotherapy even if a MFD is available. As our study demonstrates that pediatric CBF AML patients with *KIT* mutations have similar outcome to CBF AML patients without such mutations, their presence does not provide rationale for alteration of therapy. This finding is in contrast to adult CBF AML, where the inferior outcome observed with *KIT* mutations may justify therapy modification. The utility of receptor tyrosine kinase inhibitors for this patient population remains uncertain. Anecdotal use of

imatinib, both as single agent or in combination with conventional chemotherapy, has been partly effective in patients with exon 8 but not exon 17 D816 mutations, consistent with results from in vitro analysis.<sup>2,8,36-38</sup> The use of second-generation tyrosine kinase inhibitors, such as dasatinib or nilotinib, may broaden the number of mutation types that can be targeted.<sup>39,40</sup> Ultimately, their role in treatment of pediatric CBF AML patients with *KIT* mutations warrants further evaluation in cooperative pediatric clinical trials.

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## **AUTHORSHIP**

JP designed research, performed research, analyzed data, and wrote the manuscript  
TA senior statistician, performed statistical analysis, edited the manuscript  
RG performed statistical analysis, edited the manuscript  
PH analyzed data, edited the manuscript  
RZ performed research, analyzed data, edited the manuscript  
YR analyzed data, edited the manuscript  
GD analyzed data, edited the manuscript  
NL analyzed data, edited the manuscript  
DB analyzed data, edited the manuscript  
MC analyzed data, edited the manuscript  
HW analyzed data, edited the manuscript  
BH analyzed data, edited the manuscript  
SR analyzed data, edited the manuscript  
NH analyzed data, edited the manuscript  
WW clinical study PI, edited the manuscript  
BL clinical study PI, edited the manuscript  
CH clinical study PI, edited the manuscript  
RA analyzed data, edited the manuscript  
JR analyzed data, edited the manuscript  
IB analyzed data, edited the manuscript  
MH designed research, analyzed data, edited the manuscript  
SM designed research, analyzed data, edited the manuscript

**Conflict of interest disclosure:** MCH has an equity interest in MolecularMD and serves as an unpaid consultant for this company. MolecularMD is a molecular diagnostic company that performs molecular testing of leukemia samples. His conflict of interest is managed by Oregon Health and Science University and Portland Veterans Affairs Medical Center Conflict of Interest in Research Committees. The remaining authors declare no competing financial interests.

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**Table 1: Prevalence and type of *KIT* mutations in diagnostic specimens from 203 patients with CBF AML enrolled on 4 pediatric oncology trials**

		# Samples Analyzed	WT <i>KIT</i> N (% samples analyzed)	<i>KIT</i> mutation+ N (% samples analyzed)	exon 8 mutation+ N (% samples analyzed)	exon 17 mutation+ N (% samples analyzed)	Both exon 8/17 mutation+ N (% samples analyzed)
<b>Summation of All Studies</b>							
	All CBF AML	203	165 (81)	38 (19)	20 (10)	17 (8)	1 (<1)
	t(8;21)	113	94 (83)	19 (17)	6 (5)	13 (12)	0 (0)
	inv(16)	90	71 (79)	19 (21)	14 (16)	4 (4)	1 (1)
<b>By Study</b>							
POG-9421	All CBF AML	48	37 (77)	11 (23)	5 (10)	6 (13)	0 (0)
	t(8;21)	25	17 (68)	8 (32)	3 (12)	5 (20)	0 (0)
	inv(16)	23	20 (87)	3 (13)	2 (9)	1 (4)	0 (0)
CCG-2891	All CBF AML	18	13 (72)	5 (28)	4 (22)	1 (6)	0 (0)
	t(8;21)	8	7 (88)	1 (13)	0 (0)	1 (13)	0 (0)
	inv(16)	10	6 (60)	4 (40)	4 (40)	0 (0)	0 (0)
CCG-2961	All CBF AML	98	83 (85)	15 (15)	8 (8)	6 (6)	1 (1)
	t(8;21)	61	53 (87)	8 (13)	3 (5)	5 (8)	0 (0)
	inv(16)	37	30 (81)	7 (19)	5 (14)	1 (3)	1 (3)
COG AAML03P1	All CBF AML	39	32 (82)	7 (18)	3 (8)	4 (10)	0 (0)
	t(8;21)	19	17 (89)	2 (11)	0 (0)	2 (11)	0 (0)
	inv(16)	20	15 (75)	5 (25)	3 (15)	2 (10)	0 (0)

CBF AML indicates core binding factor acute myeloid leukemia; WT, wild-type; +, mutation positive; POG, Pediatric Oncology Group; CCG, Children’s Cancer Group; COG, Children’s Oncology Group.

**Table 2: Clinical characteristics and CR Rates for CBF AML Patients with and without *KIT* mutations**

	<i>KIT</i> mutation +		WT <i>KIT</i>		<i>P</i>
	N	%	N	%	
<b>Total</b>	<b>38</b>		<b>165</b>		
<b>Gender</b>					
Male	22	58%	84	51%	0.437
Female	16	42%	81	49%	
<b>Age (yrs)</b>					
Median (Range)	10.5	(1.1 - 16.6)	12.0	(0.6 - 19.6)	0.127
0 - <2 y	4	11%	6	4%	0.077
2 - <10 y	14	37%	55	33%	0.681
10 - 21 y	20	53%	104	63%	0.236
<b>Race</b>					
White	23	61%	108	66%	0.504
Non White	15	39%	55	34%	
Unknown	0		2		
WBC – median (range)	33.5	(3.9 - 379)	27.1	(1.6 - 373)	0.477
BM Blasts % - median (range)	56	(0 - 92)	52	(0 - 99)	0.915
<b>Additional Mutations</b>					
<b><i>FLT3/ITD</i></b>					
Negative	31	100%	139	96%	0.592
Positive	0	0%	6	4%	
<b><i>NPM1</i></b>					
Negative	17	100%	110	100%	1.000
Positive	0	0%	0	0%	
<b><i>CEBPA</i></b>					
Negative	21	100%	103	100%	1.000
Positive	0	0%	0	0%	
<b><i>WT-1</i></b>					
Negative	22	100%	96	88%	0.124
Positive	0	0%	13	12%	
BM Blasts % - median (range)	56	0-92	52	0-99	0.195
<b>Induction Response</b>					
CR	36	97%	144	94%	0.690
Not in CR	1	3%	9	6%	
Unevaluable or Withdrew	1		12		

CBF AML indicates core binding factor acute myeloid leukemia; WT, wild type; WBC, white blood cell; BM, bone marrow; *FLT3/ITD*= *FLT3* internal tandem duplication; *NPM1*, nucleophosmin; *CEBPA*, CCAAT/enhancer binding protein-alpha; *WT-1*, Wilms tumor gene 1; CR, complete remission

**Table 3: Outcome data for CBF AML patients with and without *KIT* mutations**

	<i>KIT</i> mutation (N=38)		WT <i>KIT</i> (N= 165)		<i>P</i> -value
	N	% ± 2SE%	N	% ± 2SE%	
5 yr OS from Study Entry					
All Studies	38	76 ± 15	165	74 ± 8	0.819
POG-9421	11	64 ± 29	37	89 ± 12	0.014
CCG-2891	5	100 ± 0	13	67 ± 38	0.577
CCG-2961	15	79 ± 22	83	68 ± 12	0.701
COG AAML03P1*	7	67 ± 54	32	77 ± 20	0.589
5 yr EFS from Study Entry					
All Studies	38	55 ± 17	165	59 ± 9	0.857
POG-9421	11	55 ± 30	37	66 ± 18	0.563
CCG-2891	5	80 ± 36	13	53 ± 41	0.925
CCG-2961	15	57 ± 26	83	57 ± 12	0.866
COG AAML03P1*	7	44 ± 44	32	61 ± 21	0.518
5 yr Relapse Risk from End of Induction					
All Studies	36	35 ± 17	144	34 ± 10	0.877
POG-9421	11	36 ± 29	36	35 ± 19	0.961
CCG-2891	4	0 ± 0	13	50 ± 41	0.255
CCG-2961	14	31 ± 26	66	33 ± 14	0.814
COG AAML03P1*	7	56 ± 44	29	30 ± 21	0.512
5 yr DFS from Induction CR					
All Studies	36	56 ± 18	144	60 ± 10	0.786
POG-9421	11	55 ± 30	36	65 ± 19	0.58
CCG-2891	4	100 ± 0	13	50 ± 41	0.541
CCG-2961	14	54 ± 28	66	58 ± 14	0.707
COG AAML03P1*	7	44 ± 44	29	61 ± 22	0.484

CBF AML indicates core binding factor acute myeloid leukemia; WT, wild type; OS, overall survival; POG, Pediatric Oncology Group; CCG, Children’s Cancer Group; COG, Children’s Oncology Group; EFS, event-free survival; RR, relapse risk; DFS, disease-free survival

\* Three-year estimates are used because of lack of follow up.

**Table 4: Clinical outcomes for patients with CBF AML with *KIT* mutations (exon 8, exon 17, or both) compared to patients with WT *KIT*. Data is presented for the entire study population and by cytogenetic subtype.**

Total Study Population													
	KIT exon 8 mutation			KIT exon 17 mutation			KIT exon 8 and exon 17 mutation			No Mutation			exon 8 vs.exon 17 vs. both vs. WT <i>KIT</i>
	N	%	N	%	N	%	N	%	N	%	N	%	P
Total	20		17		1		165						
Outcome from end of induction													
CR	19	95%	16	100%	1	100%	144	94%					0.786
Not in CR	1	5%	0	0%	0	0%	9	6%					
Unevaluable or W/D	0		1		0		12						
	N	HR	% ± 2SE%	N	HR	% ± 2SE%	N	HR	% ± 2SE%	N	HR	% ± 2SE%	P
5-year OS from study entry	20	1.08	71 ± 22	17	0.80	81 ± 20	1	0	100 ± 0	165	1	74 ± 8	0.823
5-year EFS from study entry	20	1.26	48 ± 24	17	0.93	61 ± 25	1	0	100 ± 0	165	1	59 ± 9	0.666
5-year DFS from end of induction	19	1.20	50 ± 25	16	1.07	59 ± 26	1	0	100 ± 0	144	1	60 ± 10	0.722
5-year RR from end of induction	19	1.03	38 ± 24	16	1.03	34 ± 25	1	0	0 ± 0	144	1	34 ± 10	0.829
t(8;21) Samples Only													
	N	%	N	%	N	%	N	%	N	%	N	%	P
Total	6	100%	13	100%	0	0%	94	100%					
Induction CR	6	100%	12	92%	0	0%	82	87%					0.573
	N	HR	% ± 2SE%	N	HR	% ± 2SE%	N	HR	% ± 2SE%	N	HR	% ± 2SE%	P
5-year OS from study entry	6	1.91	60 ± 44	13	1.09	75 ± 25	0	0	0	94	1	74 ± 11	0.695
5-year EFS from study entry	6	1.17	60 ± 44	13	1.33	57 ± 29	0	0	0	94	1	65 ± 11	0.841
5-year DFS from end of induction	6	1.28	60 ± 44	12	1.82	55 ± 30	0	0	0	82	1	68 ± 12	0.510
5-year RR from end of induction	6	0.86	20 ± 36	12	2.25	36 ± 29	0	0	0	82	1	23 ± 11	0.583
inv(16) Samples Only													
	N	%	N	%	N	%	N	%	N	%	N	%	P
Total	14	100%	4	100%	1	100%	71	100%					
Induction CR	13	93%	4	100%	1	100%	62	87%					0.795
	N	HR	% ± 2SE%	N	HR	% ± 2SE%	N	HR	% ± 2SE%	N	HR	% ± 2SE%	P
5-year OS from study entry	14	1.05	75 ± 25	4	0	100 ± 0	1	0	100 ± 0	71	1	75 ± 12	0.662
5-year EFS from study entry	14	1.15	42 ± 29	4	0.48	75 ± 43	1	0	100 ± 0	71	1	51 ± 14	0.710
5-year DFS from end of induction	13	0.99	45 ± 30	4	0.43	75 ± 43	1	0	100 ± 0	62	1	49 ± 16	0.691
5-year RR from end of induction	13	0.84	45 ± 30	4	0.44	25 ± 43	1	0	0 ± 0	62	1	51 ± 16	0.495

CBF AML indicates core binding factor acute myeloid leukemia; WT, wild type; CR, complete remission; W/D, withdrawn; HR, hazard ratio SE, standard error; OS, overall survival; EFS, event-free survival; DFS, disease-free survival; RR, relapse risk

Figure Legends

**Figure 1. Overall survival (OS) for patients with CBF AML with *KIT* mutations or WT *KIT***

**Figure 2: Disease-free survival (DFS) and relapse risk (RR) rates for patients with CBF AML with *KIT* mutations or WT *KIT***

Figure 1

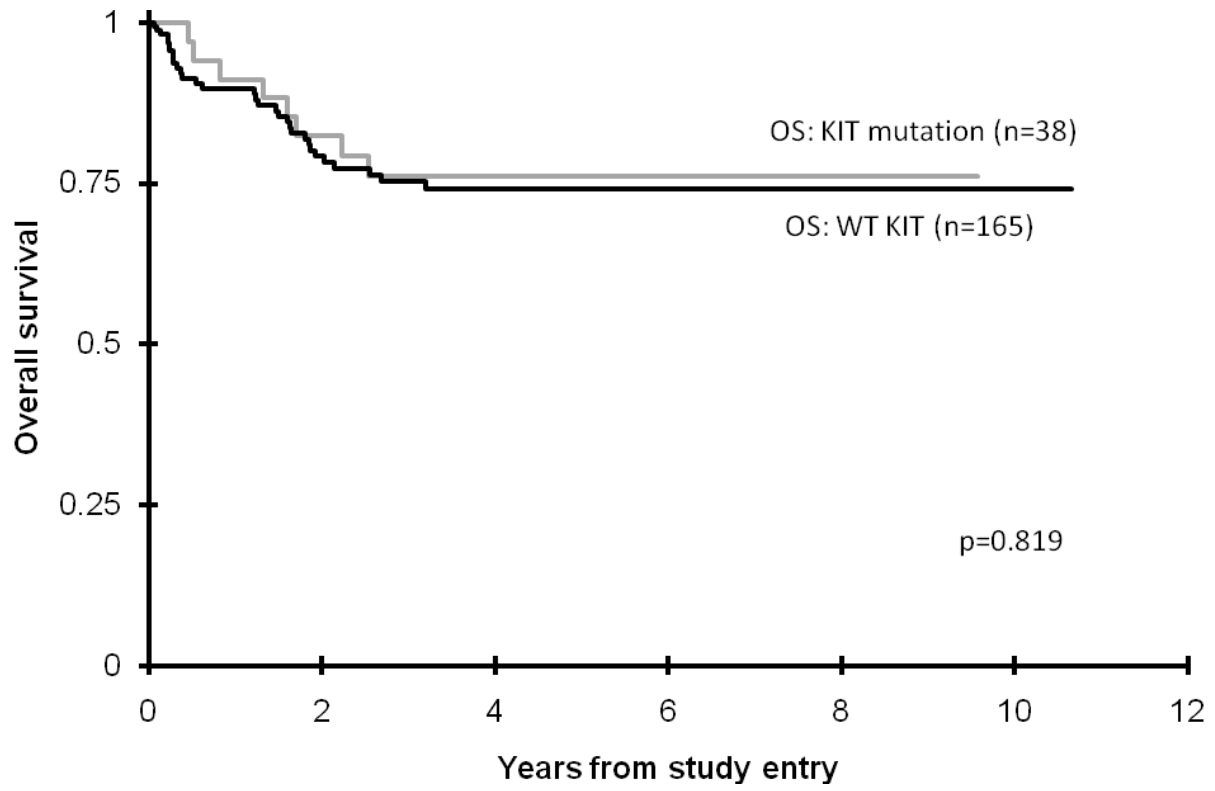


Figure 2

