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Letter to *Blood*

TO THE EDITOR:

Prognostic impact of CSF3R mutations in favorable risk childhood acute myeloid leukemia

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Colony-stimulating factor 3 receptor (CSF3R) is a driver of neutrophil differentiation, proliferation, and activation following granulocyte colony-stimulating factor binding, resulting in downstream signaling of tyrosine kinases, including JAK/STAT and SRC.[1-3] Activating mutations in *CSF3R* are highly prevalent in chronic neutrophilic leukemia, but rarely identified in acute myeloid leukemia (AML).[4,5] We previously reported on the identification of somatic *CSF3R* mutations in pediatric AML with a high degree of overlap with *CEBPA* mutations,[6] which are associated with favorable prognosis.[7] Small series have also identified *CSF3R* mutations among patients with the favorable risk lesions of core binding factor (CBF) AML (*RUNX1-ETO* fusion [t(8;21)] or *CBFB-MYH11* [inv(16)/t(16;16)]).[8]

In this letter, we report on the prevalence and prognostic significance of *CSF3R* mutations and the impact of cooperating *CEBPA* mutations and t(8;21) on outcome. A total of 2150 pediatric patients treated on the Children's Oncology Group phase 3 trials AAML0531 (n = 917) and AAML1031 (n = 1233) with comprehensive clinical and karyotype information were eligible for this study (NCT00372593, NCT01371981). *CSF3R* mutational status was determined by next-generation sequencing, and *CEBPA* mutation status was determined by fragment length analysis and Sanger sequencing, both described previously.[7,9] Research was approved by the appropriate review boards and conducted in accordance with the Declaration of Helsinki. The Kaplan-Meier method was used to determine 5-year overall survival (OS), event-free survival (EFS), and relapse rate (RR).[10] The significance of predictor variables was tested using log-rank statistics for OS and EFS, and Gray statistic for RR. The significance of observed difference in proportions was analyzed by Fisher's exact test. Complete remission was defined as <5% blasts (AAML0531) or measurable residual disease of <0.1% blasts (AAML1031) at the end of induction 1 (EOI1).

We identified 35 patients (1.6%) with *CSF3R* mutations (*CSF3R*$^+$). Mutations occurred in the proximal region of the transmembrane domain (T618I, T640N) or in the cytoplasmic domain as truncating events (Q754X, Y767fs, P7872fs, and S783fs; Figure 1A). T618I (n = 17, 49%) was the most commonly detected mutation. Proximal transmembrane domain mutations result in ligand-independent activation, whereas those in the cytoplasmic domain result in cell surface overexpression with increased activation of downstream signaling pathways.[4,11] Among *CSF3R*$^+$ patients, the overwhelming majority (89%) had a cooccurring t(8;21) fusion (n = 18, 51%) or *CEBPA* mutation (*CEBPA*$^+$; n = 12, 34%), whereas 1 patient harbored both a t(8;21) and *CEBPA*$^+$ (Figure 1B). Among the 13 *CEBPA* mutations detected, 11 were biallelic and 2 harbored single bZip mutations. Of the 4 patients lacking t(8;21) or *CEBPA*$^+$, 1 harbored an inv(16). Among non-CBF *CSF3R*$^+$ patients, n = 6 (50%) had normal cytogenetics (Figure 1B). Additional genomic mutations frequently detected to cooccur with *CSF3R* included *KIT* (23%), *NRAS* (7%), and *FLT3*/ITD (14%) (Figure 1B). Variant allele frequencies (VAF) were available in 15 patients (43%) with a range of 0.07 to 0.74 (median, 0.29). Among patients with available VAF data, 9 harbored cooccurring signaling mutations (supplemental Figure 1, available on the *Blood* Web site). Analysis of outcome across patients who relapsed (n = 4; median, 0.28; range, 0.27-0.75) vs no relapse (n = 8; median, 0.325; range, 0.07-0.59) vs induction failure (n = 3; median, 0.29; range, 0.07-0.48) demonstrated similar median VAFs (P = .965).

Based on the strong cooccurrence patterns, we analyzed the outcomes for *CSF3R*$^+$ patients with *CEBPA* or t(8;21) variants. The EFS for the 31 patients with either of these mutations was 61% ± 18%. However, when stratified based on the cooccurring *CEBPA* or t(8;21) (n = 30), patients with dual *CSF3R*$^+$/*CEBPA*$^+$ had an EFS of 25% ± 25% vs 83% ± 18% for *CSF3R*$^+$/t(8;21) patients (P < .001; Figure 2A). This was driven by a high RR of 75% ± 28% among *CSF3R*$^+$/*CEBPA*$^+$ patients vs 15% ± 21% among *CSF3R*$^+$/t(8;21) (P = .003; Figure 2B). Despite significantly higher RR and inferior EFS, *CSF3R*$^+$/*CEBPA*$^+$ patients experienced an OS of 73% ± 26%, although differences persisted because the *CSF3R*$^+$/t(8;21) patients experienced an OS of 100% ± 0% (P = .026; Figure 2C). Patients with t(8;21) had a comparable EFS, with a trend toward more favorable outcomes, according to CSF3R$^+$ vs wild-type (WT) of 83% ± 18% vs 67% ± 16% (P = .151); however, a converse relationship was observed in *CEBPA*$^+$ patients according to *CSF3R*$^+$ vs WT of 25% ± 15% vs 69% ± 19% (P < .001). Comparison of initial response to therapy of *CSF3R*$^+$/*CEBPA*$^+$ vs *CSF3R*$^+$/t(8;21) patients were similar as measured by achievement of CR (100% vs 76.5%; P = .121) and undetectable measurable residual disease (90% vs 88.2%; P = 1.00).

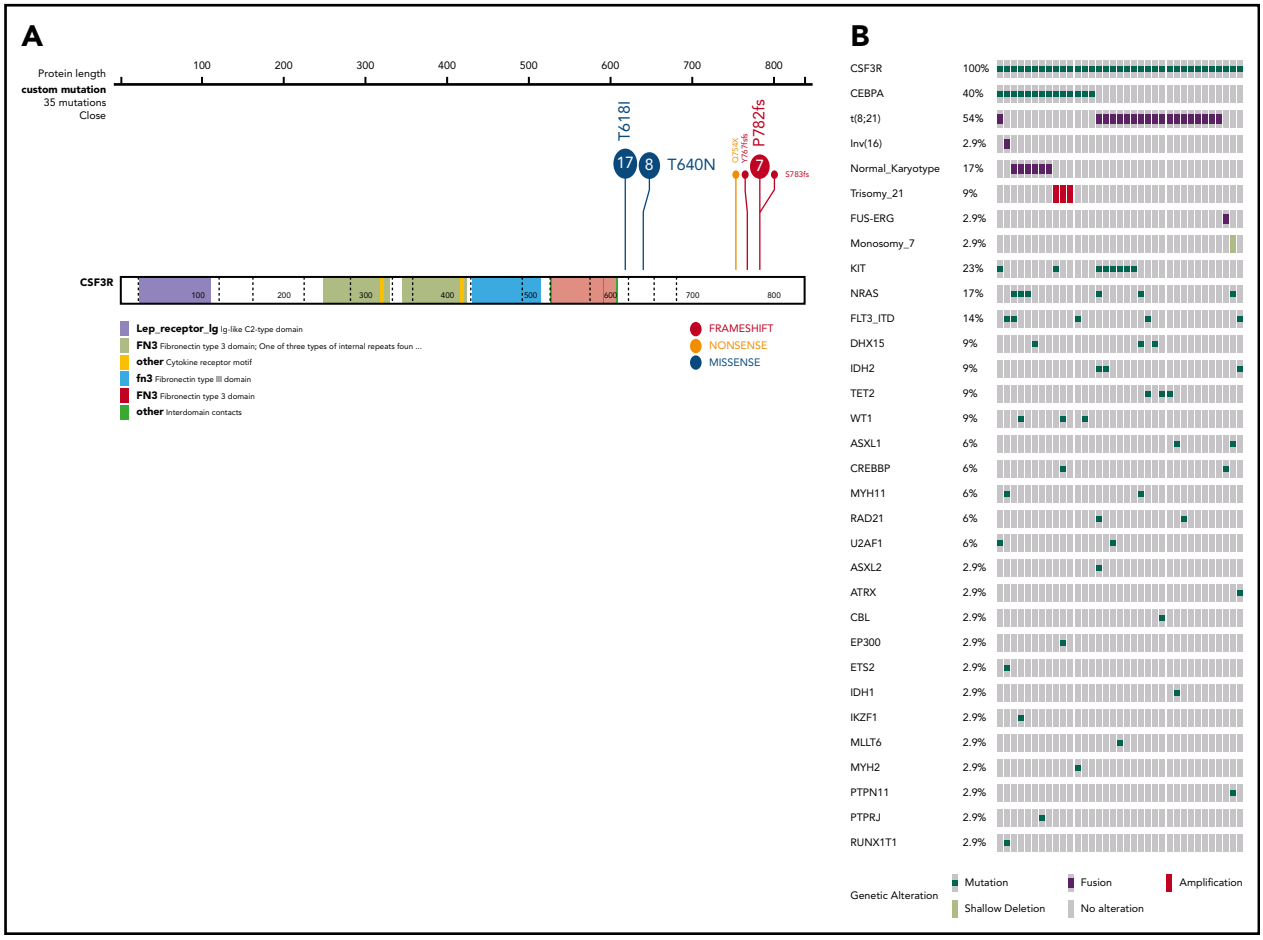

Figure 1. Location and cooccurring genomic profile of CSF3R mutations in pediatric AML. (A) Protein structure of CSF3R showing location of mutations detected. (B) Mutational profile of *CSF3R*-mutant samples with cooccurring mutation and cytogenetic changes.

We report the incidence of *CSF3R* mutations in large cohort of pediatric AML and found that these mutations almost always cooccurred in a mutually exclusive manner with either *CEBPA*$^+$ or t(8;21). Our findings on the prevalence of *CSF3R* mutations is in line with prior smaller studies in pediatric AML.[6,12] Although t(8;21) and *CEBPA*$^+$ are associated with favorable outcomes in AML, our findings demonstrate that *CSF3R* mutations had significant and variable prognostic effects in these groups. This was particularly notable among *CEBPA*$^+$ patients because dual *CSF3R*$^+$/*CEBPA*$^+$ patients experienced poor outcomes with chemotherapy and very high relapse rates. Our studies are in line with recent reports by Su et al describing inferior outcomes with decreased relapse-free survival among *CSF3R*$^+$/*CEBPA*$^+$ patients.[13,14] In the larger of these 2 studies, *CSF3R*$^+$ was associated with inferior relapse-free survival and OS in a cohort of adult and pediatric *CEBPA*$^+$ patients.[14] Our findings in a more homogeneously treated pediatric cohort differ as we demonstrate *CSF3R*$^+$/*CEBPA*$^+$ patients were highly salvageable. Given that hematopoietic stem cell transplantation (HSCT) is the standard treatment of relapsed AML, our findings implicate efficacy of HSCT in dual *CSF3R*$^+$/*CEBPA*$^+$ patients. The use of HSCT has been shown to be an effective therapeutic strategy in *CEBPA*$^+$ patients, and thus may especially efficacious for a subset with higher risk disease.[15]

CSF3R mutations occur in patients with severe congenital neutropenia who develop AML, with cooperativity implicated in leukemogenesis, but not solely capable of malignant transformation.[16,17] In this setting, strong cooperation between *CSF3R* and *RUNX1*, a lineage determining transcription factor, is well recognized, with some studies finding 80% of patients are dual positive for *RUNX1* mutations (*RUNX1*$^+$) and *CSF3R*$^+$.[18] Further, *RUNX1*$^+$/*CSF3R*$^+$ CD34$^+$ cells demonstrate enhanced proliferation and diminished proliferation compared with WT or single-mutant cells.[18] The t(8;21) fusion similarly perturbs normal RUNX1 function, leading to altered expression of many downstream genes, including repressed *CEBPA* expression.[19,20] Like *RUNX1* alterations, *CEBPA* mutations inhibit myeloid differentiation. Signaling through the *CSF3R* T618I mutation promotes proliferation and differentiation of neutrophil precursors.[21] *CEBPA* mediates the expression of differentiation-associated genes downstream of *CSF3R*.[22] *CEBPA* mutations block the pro-differentiation program downstream of *CSF3R*, but not the proliferative program, leading to an expansion of myeloid cells, with arrested differentiation resulting in a lethal AML phenotype.[21] This robust cooperation is consistent with the high rate of relapse observed in our cohort.

Mutations in *CSF3R* may be amenable to tyrosine kinase inhibitors directed against JAK as robust inhibitory activity has

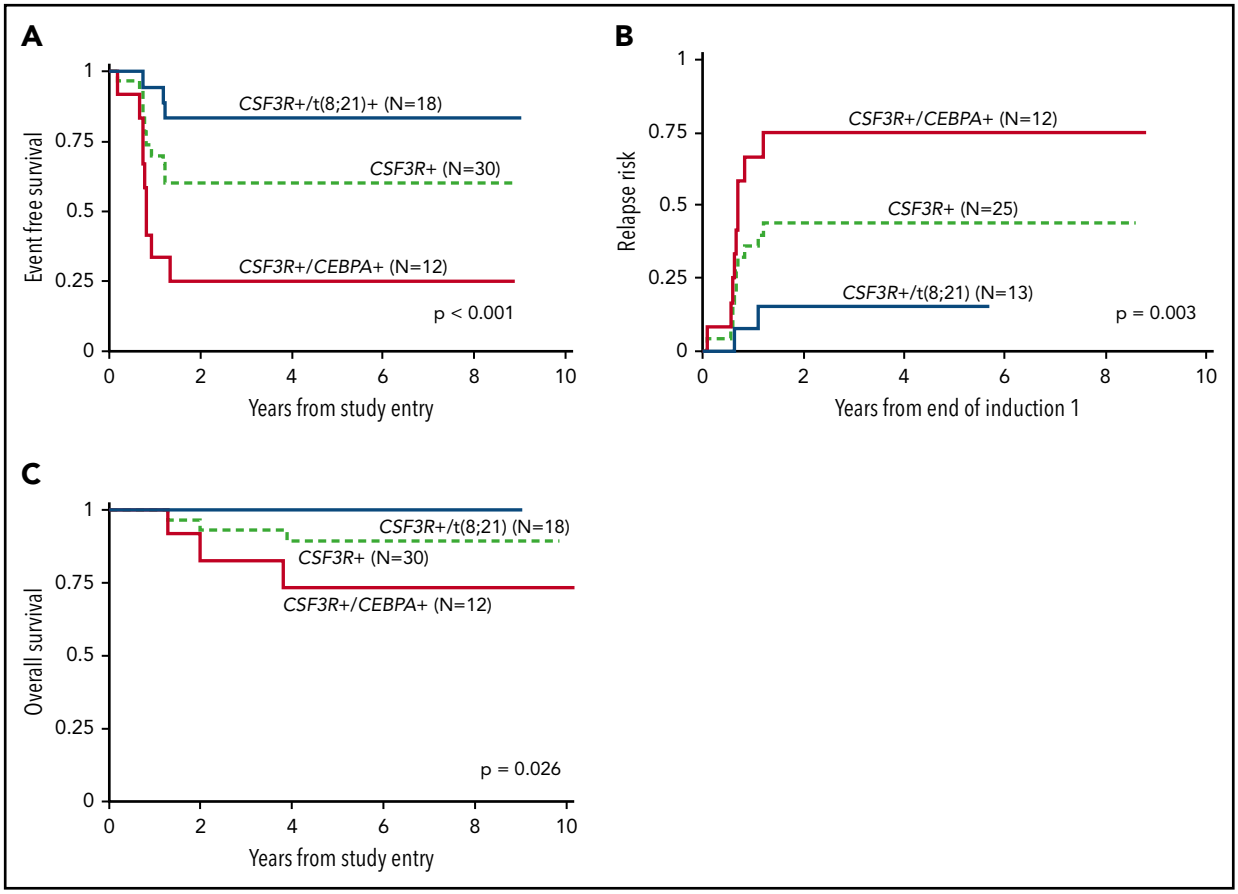

Figure 2. Outcomes according to *CSF3R* mutation and cooccurring *CEBPA* or t(8;21). Outcomes for *CSF3R* mutant patients according to cooccurring t(8;21) or *CEBPA* mutation, with overall outcomes of all *CSF3R*$^+$ patients, including either of the 2 mutations, shown as reference for (A) EFS; (B) RR; (C) OS. *P* values indicate differences between the t(8;21) vs *CEBPA*$^+$ subgroups.

been demonstrated in vitro; however, variable clinical efficacy has been observed in *CSF3R*$^+$ chronic neutrophilic leukemia.[4,23-25] Targeted therapy as well as the conserved and divergent biology between *CSF3R*$^+$ AML with *CEBPA* and *RUNX1* alterations warrants further investigation. Additional functional studies will also elucidate the clinical differences between *CSF3R*$^+$/*CEBPA*$^+$ vs *CSF3R*$^+$/t(8;21) AML. Our findings highlight that *CSF3R* mutations can further risk stratify pediatric patients with favorable risk AML. Patients with cooccurring t(8;21) experience excellent outcomes, while those with *CEBPA* mutations should not be considered favorable risk based solely on presence of a *CEBPA* mutation or an initial good response to therapy, as they experience very poor outcomes with high relapse rates with chemotherapy alone.

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Authorship

Contribution: K.T. and S.M. designed the research; K.T., R.E.R., and T.H. performed the research; T.A., Y.-C.W., R.B.G., and J.L.S. analyzed the data; J.E.M. provided general scientific guidance; and K.T., J.E.M., and S.M. wrote the manuscript.

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Footnotes

For original data, please e-mail the corresponding author.

The online version of this article contains a data supplement.

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