To the editor:

Presence of high-*ERG* expression is an independent unfavorable prognostic marker in *MLL*-rearranged childhood myeloid leukemia

Childhood acute myeloid leukemia (AML) is a heterogeneous disease, in terms of genetic/molecular abnormalities resulting into marked differences in outcome.[1] A myriad of proteins have been suggested aberrantly regulated in AML, and Ets-related gene (*ERG*, 21q22) expression in normal and aberrant hematopoiesis is currently under evaluation.[2] High *ERG* expression has been associated with poor prognosis in cytogenetically normal adult AMLs.[3,4] Only recently, Staffas et al reported that *ERG* expression was associated with an inferior probability of event-free survival (EFS) in northern European children with AML, although not as independent prognostic factor.[5] We measured *ERG* expression in a cohort of 268 Italian pediatric patients (141 males and 127 females, median age at diagnosis 6 years, range 7 days-22 years) with newly diagnosed de novo AML enrolled in AIEOP LAM-2002 protocol.[6] *ERG* expression, relative to *ABL*, was measured by real-time quantitative–PCR and calculated the relative quantity (RQ) by the comparative ΔΔCt method. ERG expression turned out to be higher (high-*ERG*, RQ > 1) or lower (low-*ERG*, RQ < 1) in AML patients, dividing them into 2 groups significantly different ($N_{high\text{-}ERG}$ = 162, RQ = 4927, $N_{low\text{-}ERG}$ = 106, RQ = 0371, P = .007). Patients were then divided into cytogenetic groups. Of these, 157 carried well-known molecular markers, such as Core Binding Factor (*CBF*) anomalies (n = 52), *FLT*3-ITD (n = 34) and the mixed-lineage leukemia gene (*MLL*) rearrangements (n = 71), whereas the remaining 111 patients were cytogenetically normal (CN). *ERG* was found to be different expressed between each AML subgroups, with statistical significance for all the subgroups analyzed (Figure 1A, Student t test, statistical significance $P < .05$). Overall Survival (OS), defined as the time from diagnosis to last follow-up or death, was measured. Results showed no statistical difference in OS for CN (Kaplan-Meier log-rank test, P = .58), *CBF* (P = .82), nor *FLT*3ITD (P = .54) patients according to *ERG* expression. By contrast, OS and EFS for patients with *MLL*-rearrangements and high-*ERG* expression were found to be significantly worse than those of patients with low-*ERG* ($P < .001$, Figure 1B) expression. Multivariate analysis by Cox regression model confirmed that high-*ERG* expression is an independent prognostic factor for EFS in *MLL*-rearranged patients (hazard risk = 4.22, 95% CI = 1.10-16.18, P = .036). In multivariate analysis complex karyotype remained the only other independent adverse factor in this group (P = .028, HR = 3.51, 95%CI = 1.15-10.74). These data indicate that *ERG* expression strongly influences the probability of OS and EFS of *MLL*-rearranged patients. Since childhood *MLL*-rearranged AML includes patients with marked differences in biology and outcome,[7,8] we suggest the use of *ERG* expression to stratify patient's prognostic risk and to tailor therapeutic approaches.

A

	N (%)	RQ	P value
CBF $_{high\text{-}ERG}$	46 (87 %)	9.143	$p = 0{,}048$
CBF $_{low\text{-}ERG}$	7 (13 %)	0.665	
*FLT3*ITD $_{high\text{-}ERG}$	25 (73.5 %)	3.608	$p = 0.0004$
*FLT3*ITD $_{low\text{-}ERG}$	9 (26.5 %)	0.523	
MLL-Rearranged $_{high\text{-}ERG}$	28 (39.4 %)	2.933	$p < 0.0001$
MLL-Rearranged $_{low\text{-}ERG}$	43 (60.6 %)	0.254	
CN $_{high\text{-}ERG}$	64 (58 %)	3.285	$p < 0.0001$
CN $_{low\text{-}ERG}$	46 (42 %)	0.405	

B

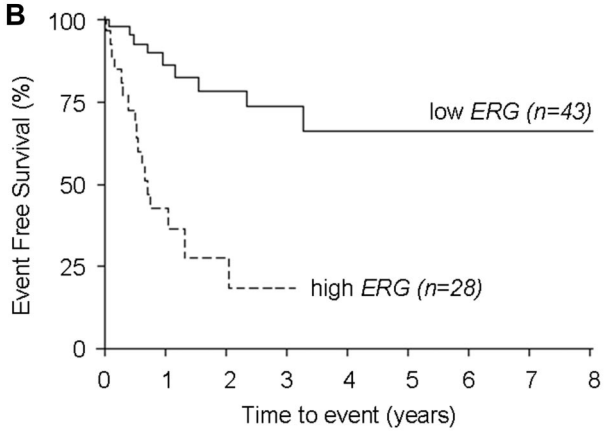

Figure 1. *ERG* expression in AML influences EFS of *MLL*-rearranged patients. (A) *ERG* expression in AML subgroups. *ERG* expression relative to *ABL* housekeeping gene was measured by real-quantitative PCR. RQ is the relative quantity of *ERG* expression with respect to the average of its expression in healthy bone marrows (N = 17) calculated by comparative ΔΔCt method. *CBF* indicates core binding factor rearrangements; and CN, cytogenetically normal. (B) Probability of event-free survival in children with *MLL*-rearranged AML according to *ERG* expression. Event-free survival (EFS) for patients *MLL*-rearranged with high (73.3%) vs low (18.3%) *ERG* expression.

Martina Pigazzi
Department of Pediatrics, Laboratory of Hematology-Oncology, Città Della Speranza, University of Padova, Padova, Italy

Riccardo Masetti
Department of Pediatrics, "Lalla Seràgnoli," Hematology-Oncology Unit, University of Bologna, Bologna, Italy

Francesco Martinolli
Department of Pediatrics, Laboratory of Hematology-Oncology, University of Padova, Padova, Italy

Elena Manara
Department of Pediatrics, Laboratory of Hematology-Oncology, University of Padova, Padova, Italy

Alessandra Beghin
Department of Pediatrics, Laboratory of Hematology-Oncology, University of Padova, Padova, Italy

Roberto Rondelli
Department of Pediatrics, "Lalla Seràgnoli," Hematology-Oncology Unit, University of Bologna, Bologna, Italy

Franco Locatelli
Department of Pediatric Hematology-Oncology, IRCCS Ospedale Bambino Gesù, Rome, Italy

Franca Fagioli
Pediatric Onco-Hemathology, Stem Cell Transplantation and Cellular Therapy Division, Regina Margherita Children's Hospital, Turin, Italy

Andrea Pession
Department of Pediatrics, "Lalla Seràgnoli," Hematology-Oncology Unit, University of Bologna, Bologna, Italy

Giuseppe Basso
Department of Pediatrics, Laboratory of Hematology-Oncology, University of Padova, Padova, Italy

Acknowledgments: The authors thank all Italian AIEOP centers. They also thank Sabrina Gelain, Claudia Tregnago, Samuela Francescato, Maria Grazia Giacometti, Silvia Disarò and Katia Polato for their collaboration.

This study was supported by grants from Fondazione Città della Speranza-Padova, University of Padova, Istituto Superiore di Sanitá, Fondazione Veneto Banca and AIL.

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

Correspondence: Martina Pigazzi, Pediatrics Department, University of Padova-Città della, Speranza, Hematology-Oncology Laboratory via Giustiniani 3, 35 128 Padova-Italy; e-mail: martina.pigazzi@unipd.it.

References

1. Balgobind BV, Hollink IH, Arentsen-Peters ST, et al. Integrative analysis of type-I and type-II aberrations underscores the genetic heterogeneity of pediatric acute myeloid leukemia. *Haematologica.* 2011;96(10):1478-1487.
2. Martens JH. Acute myeloid leukemia: a central role for the ETS factor ERG. *Int J Biochem Cell Biol.* 2011;43(10):1413-1416.
3. Marcucci G, Baldus CD, Ruppert AS, et al. Overexpression of the ETS-related gene, ERG, predicts a worse outcome in acute myeloid leukemia with normal karyotype: a Cancer and Leukemia Group B study. *J Clin Oncol.* 2005;23(36): 9234-9242.
4. Baldus CD, Burmeister T, Martus P, et al. High expression of the ETS transcription factor ERG predicts adverse outcome in acute T-lymphoblastic leukemia in adults. *J Clin Oncol.* 2006;24(29):4714-4720.
5. Staffas A, Kanduri M, Hovland R, et al. Presence of FLT3-ITD and high BAALC expression are independent prognostic markers in childhood acute myeloid leukemia. *Blood.* 2011;118(22):5905-5913.
6. Pession A, C. R., MC. P, et al. Results of the AIEOP AML 2002/01 Study for treatment of children with acute myeloid leukemia. 51st ASH annual meeting and exposition. Orlando, FL. *Blood.* 2009;114: Abstract 17.
7. Balgobind BV, Raimondi SC, Harbott J, et al. Novel prognostic subgroups in childhood 11q23/MLL-rearranged acute myeloid leukemia: results of an international retrospective study. *Blood.* 2009;114(12):2489-2496.
8. Pigazzi M, Masetti R, Bresolin S, et al. MLL partner genes drive distinct gene expression profiles and genomic alterations in pediatric acute myeloid leukemia: an AIEOP study. *Leukemia.* 2011;25(3):560-563.

Response:

High *ERG* gene expression is an unfavorable prognostic marker in pediatric acute myeloid leukemia

It is with great interest we read the scientific letter by Pigazzi et al and their finding that high *ERG* expression is an independent unfavorable prognostic marker for both event-free and overall survival in pediatric acute myeloid leukemia (AML) with *MLL* (11q23) rearrangement treated within the AIEOP LAM-2002 protocol.[1] As the authors also point out, our recent study on pediatric AML patients enrolled in the NOPHO-1993 or NOPHO-2004 protocols supports this finding.[2] We could show that high *ERG* expression is an unfavorable prognostic marker for event-free survival in this cohort of pediatric AML, where patients with *MLL* rearrangement were included.[2] And indeed, this observation was even more pronounced if we analyzed the group of patients with *MLL* rearrangement separately for event free survival (Figure 1A, $P = .03$). However, there was no significant difference between low and high *ERG* expression for overall survival in this group ($P = .16$). High *ERG* expression was not identified as an independent marker in our study but this was mainly because *BAALC* and *ERG* were often found coexpressed at high levels, where high *BAALC* expression came out as a stronger predictor for prognosis in the multivariate analysis when the whole pediatric AML cohort was included. But, importantly, high *BAALC* was not statistically significant for event-free survival within the subgroup with MLL rearrangement (Figure 1B, $P = .43$) in contrast to *ERG*. There were too few patients in the MLL group (n = 40) in our study to perform a meaningful multivariate analysis, so we cannot comment on the independence of the *ERG* expression. However, we think that these 2 separate studies now have strengthened the evidence that also high expression level of *ERG* is an unfavorable prognostic marker in pediatric AML, in particular for the important group of patients with *MLL* rearrangements. The remaining challenge before gene expression levels can be used for prognostic stratification for clinical use is to establish standardized methods for quantitative

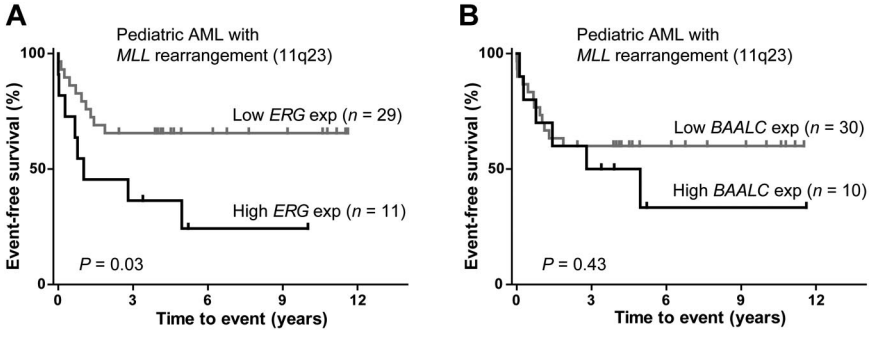

Figure 1. Survival according to *ERG* and *BAALC* expression. Event-free survival for AML patients under the age of 19 with *MLL* (11q23) rearrangement and high or low *ERG* (A) and *BAALC* (B) expression, respectively. Patients were diagnosed 1997-2007 in Denmark, Finland, Iceland, Norway, or Sweden. High and low *ERG* and *BAALC* expression were defined as above and below the median value of all 149 pediatric non-APL AML patients included in the study.