REGULAR ARTICLE

blood advances

Calcitonin receptor-like (CALCRL) is a marker of stemness and an independent predictor of outcome in pediatric AML

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Key Points

- Expression of CALCRL is an independent prognostic factor and a potential therapeutic target in pediatric AML.
- CALCRL is linked with relapse risk in p ediatric AML, which supports its role as a master regulator of relapse-initiating drug-tolerant cells.

We have recently identified the G protein-coupled neuropeptide receptor calcitonin receptor-like (CALCRL) as an independent prognostic biomarker and a therapeutic target in more than 1500 adult patients with acute myeloid leukemia (AML). Here, we confirmed *CALCRL* expression as a prognostic factor in a cohort of 284 pediatric patients with AML. High *CALCRL* expression was independently associated with event-free survival (hazard ratio [HR], 1.87; 95% confidence interval [CI], 1.36-2.57; $P = .0001$), overall survival (HR, 1.55; 95% CI, 1.06-2.27; $P = .025$), and cumulative incidence of relapse (HR, 2.10; 95% CI, 1.49-1.96; $P < .0001$) when adjusting for age, white blood cell count, and genetic risk. Despite its association with leukemia stem cell signatures, *CALCRL* expression remained associated with all end points when compared with the 17-gene leukemic stem cell score. The strong association of *CALCRL* expression with the risk of relapse also in the pediatric population supports its role as novel age-independent master regulator of relapse-initiating, drug-tolerant AML cells in humans.

Introduction

Despite improvements in outcome over the past few decades, acute myeloid leukemia (AML) remains a major cause of childhood cancer mortality.[1] Treatment relies on intensive chemotherapy, followed by allogeneic hematopoietic stem cell transplantation (HSCT) in high-risk genetic subgroups and in poor responders to induction therapy and is associated with significant morbidity.[2] Careful risk stratification is key for the reliable identification of patients not in need of an allogeneic transplant to avoid significant late effects. Furthermore, to prevent relapses currently occurring in 30% of patients despite intensive frontline therapy, novel targets for pharmacological interventions with higher efficacy and a better therapeutic index are urgently needed.[3]

Calcitonin receptor-like (CALCRL) is a G-protein coupled neuropeptide receptor involved in various biological processes such as proliferation, apoptosis, and inflammation.[4,5] It can be activated by 2 ligands, adrenomedullin (ADM) and calcitonin gene-related peptide. Inhibition of CALCRL signaling has demonstrated therapeutic activity in preclinical models of solid malignancies[6-10] and has emerged as a novel therapeutic principle in migraine.[11] We recently demonstrated that higher messenger RNA (mRNA) and

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protein levels of CALCRL in leukemic blasts are consistently associated with adverse outcomes across various genetic backgrounds in 5 independent international cohorts collectively comprising more than 1500 adult AML patients.[12] *CALCRL* knockout reduces colony formation of human AML cell lines, confirming a functional role of the receptor in AML.[12] Together, these finding suggest CALCRL as an attractive novel therapeutic target in adult AML.

The molecular landscape of pediatric AML has been characterized in detail in recent years.[13] Significant differences in molecular profiles have been found in comparison with adult AML.[13] Thus, the clinical role of *CALCRL* expression demands validation in pediatric patients with AML. Here, we investigated the prognostic impact of *CALCRL* expression in a cohort of 284 pediatric AML patients treated within the AAML03P1, AAML0531, and CCG-2961 trials.[13-16]

Methods

Patients, samples, and treatment

The expression of *CALCRL* was analyzed in the publicly available mRNA-sequencing cohort from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET)-AML project (284 patients) comprising children, including infants and adolescents, with de novo AML who were treated within the CCG-2961, AAML03P1, and AAML0531 trials of the Children's Oncology Group.[13-16] The institutional review board of the Children's Oncology Group approved these trials. CCG-2961 incorporated idarubicin, fludarabine, and interleukin-2 on a standard treatment backbone. AAML03P1 and AAML0531 introduced the monoclonal anti-CD33 antibody-drug conjugate gemtuzumab ozogamicin into standard first-line AML therapy. The TARGET-AML mRNA-sequencing and clinical data were downloaded from the TARGET Data Matrix on 26 March 2021.[13]

The 17-gene leukemic stem cell score (LSC17) was calculated as previously described.[17] Gene set enrichment analysis was done with the curated "C2" collection of the Molecular Signatures Database version 7.4 (http://software.broadinstitute.org/gsea/msigdb/) that consists of 6290 gene sets from various sources.

Statistical analyses

Maximally selected rank statistics on event-free survival (EFS) as the primary end point was used to dichotomize *CALCRL* expression.[18] Baseline characteristics were compared between *CALCRL* expression groups using χ^2 or Fisher's exact test for categorical and the Mann-Whitney test for continuous variables. Time-to-event variables were defined as described.[12,13] Survival probabilities were estimated using the Kaplan-Meier method and compared using log-rank test. Cumulative incidence of relapse (CIR) was evaluated with the Aalen-Johansen estimator and compared using Gray's test. All survival probabilities are given at 5 years. The follow-up time was calculated by the reverse Kaplan-Meier method. We used multivariable Cox proportional hazards models to assess statistical significance of prognostic factors with respect to overall survival (OS) and EFS, multivariable logistic regression models for achievement of complete remission (CR), and multivariable Fine-Gray proportional hazards regression models for CIR. Age, white blood cell count (WBC), and genetic risk[19] were included in the multivariable models in addition to *CALCRL* expression. Cox proportional hazards models and Wald test for interaction was used to examine the potential heterogeneity of the prognostic impact of *CALCRL* expression across subgroups. The proportional hazards assumption was verified for each variable individually by inspection of scaled Schoenfeld residuals. Missing data were not imputed. Two-sided P values $< .05$ were considered to indicate significant differences. All analyses were performed using the R software package, version 4.0.3.

Results

CALCRL expression was dichotomized using maximally selected rank statistics on EFS that was adjusted for multiple testing (supplemental Data).[18] Baseline characteristics of patients from the TARGET-AML cohort according to *CALCRL* expression levels are listed in Table 1. High *CALCRL* expression levels were significantly associated with inv(16)/t(16;16) ($P = .0009$) and internal tandem duplication of the *FLT3* gene (*FLT3*-ITD; $P = .0001$), whereas low *CALCRL* expression was associated with t(8;21) ($P < .0001$), *CEBPA* mutations ($P = .0076$), and with a higher bone marrow blast count ($P = .023$). Overall, pediatric patients with high leukemic *CALCRL* expression trended to be allocated to adverse cytogenetic and molecular risk groups ($P = .068$). We found no association of *CALCRL* expression with age, sex, French-American-British classification type, involvement of the central nervous system, myeloid sarcoma, WBC, or peripheral blast count. The frequency of allogeneic HSCT in first CR was numerically higher in patients with high *CALCRL* expression, although not statistically significant (19.4% and 12.2% for high and low *CALCRL* expression, respectively; $P = .21$).

The median follow-up time was 6.1 years (interquartile range, 5.2, 7.6 years). In patients with pediatric AML, high *CALCRL* expression was associated with an inferior probability of EFS (12.2% and 40.3% at 5 years for high vs low *CALCRL* expression; $P < .0001$; Figure 1A). High *CALCRL* expression also predicted an inferior probability of OS (37.0% and 59.9% at 5 years; $P = .0023$; Figure 1B). High *CALCRL* expression trended to be associated with lower CR rates after induction therapy (82.7% and 90.6% for high and low *CALCRL* expression, respectively; $P = .060$). Of note, in patients who achieved a CR to induction therapy, high *CALCRL* expression predicted a significantly higher CIR at 5 years (85.2% and 51.7%; $P < .0001$; Figure 1C). We found no consistent heterogeneity of the associations between *CALCRL* expression levels by age, sex, WBC, genetic risk factors, type of trial, or allogeneic HSCT vs no HSCT in CR1 (Figure 2; supplemental Data).

Next, we performed multivariable analyses to investigate whether the adverse prognostic impact of *CALCRL* was independent from established risk factors in pediatric AML (Table 2). Besides *CALCRL* expression, established risk factors such as age, WBC, and genetic risk factors were included in the models. Indeed, *CALCRL* expression levels remained independently associated with EFS (hazard ratio [HR], 1.87; 95% confidence interval [CI], 1.36-2.57; $P = .0001$) and OS (HR, 1.55; 95% CI, 1.06-2.27; $P = .025$) after multivariable adjustment. High *CALCRL* expression tended to be associated with a lower probability of CR to induction therapy, though not statistically significant (odds ratio, 0.57; 95% CI, 0.25-1.29; $P = .17$). Of note, in patients who achieved a CR after induction therapy, high *CALCRL* expression was associated with a twofold increased hazard risk of relapse (HR, 2.10; 95% CI, 1.49-2.96; $P < .0001$).

Table 1. Patient characteristics according to *CALCRL* expression

Variables	*CALCRL* expression		*P* value
	Low	High	
N	203	81	
Age, y			.38*
Median (range)	10 (0-23)	10 (0-20)	
Sex, no. (%)			.15†
Male	102 (50.2)	49 (60.5)	
Female	101 (49.8)	32 (39.5)	
FAB, no. (%)			.059†
M0	6 (3.0)	1 (1.2)	
M1	28 (13.8)	8 (9.9)	
M2	47 (23.2)	23 (28.4)	
M4	41 (20.2)	24 (29.6)	
M5	47 (23.2)	7 (8.6)	
M6	3 (1.5)	1 (1.2)	
M7	8 (3.9)	1 (1.2)	
NOS	10 (4.9)	7 (8.6)	
Missing	13 (6.4)	9 (11.1)	
WBC, $\times 10^9$/L			.27*
Median (range)	40.5 (0.9-519.0)	51.1 (2.0-446.0)	
PB blasts, %			.67*
Median (range)	61 (0-97)	61 (0-97)	
BM blasts, %			**.023***
Median (range)	77 (20-99)	70 (14-100)	
CNS involvement, no. (%)			.81*
Present	16 (7.9)	5 (6.2)	
Absent	187 (92.1)	76 (93.8)	
Extramedullary AML, no. (%)			.64*
Present	23 (11.3)	7 (8.6)	
Absent	179 (88.2)	74 (91.4)	
Missing	1 (0.5)	0 (0.0)	
Trial, no. (%)			.68†
AAML03P1	44 (21.7)	16 (19.8)	
AAML0531	132 (65.0)	51 (63.0)	
CCG-2961	27 (13.3)	14 (17.3)	
HSCT in 1 CR, no. (%)			.21†
Yes	22 (10.8)	13 (16.0)	
No	159 (10.8)	54 (66.7)	
Missing	22 (10.8)	14 (17.3)	
Cytogenetics, no. (%)‡			
t(8;21)	44 (22.8)	1 (1.4)	**<.0001§**
inv(16)/t(16;16)	21 (10.9)	21 (28.4)	**.0009†**
Normal	47 (24.4)	23 (31.1)	.34†
t(9;11)	15 (7.8)	1 (1.4)	.080§
t(6;9)	1 (0.5)	2 (2.7)	.19§
t(9;22)	–	–	
t(*v*;11q23)	20 (10.4)	3 (4.1)	.14§
inv(3)/t(3;3)	0 (0.0)	1 (1.4)	.27§
del(5q)/-5	1 (0.5)	0 (0.0)	1.00§
−7	1 (0.5)	1 (1.4)	.48§
−17/abn(17p)	2 (1.1)	3 (5.3)	.092§
Complex	15 (7.8)	4 (5.4)	.60§
Monosomal	3 (1.6)	3 (4.1)	.35§
Other	30 (15.5)	16 (21.6)	.32†
Missing	10 (4.9)	7 (8.6)	
***FLT3*-ITD, no. (%)**			**.0001†**
Present	22 (10.8)	25 (30.9)	.43†
High allelic ratio	14 (63.6)	12 (48.0)	
Low allelic ratio	8 (36.4)	13 (52.0)	
Absent	181 (89.2)	56 (69.1)	
***NPM1*, no. (%)**			.54§
Mutated	12 (5.9)	7 (8.6)	
Wild type	185 (91.1)	70 (86.4)	
Missing	6 (3.0)	4 (4.9)	
***CEBPA*, no. (%)**			**.0076§**
Mutated	16 (7.9)	0 (0.0)	
Wild type	185 (91.1)	79 (97.5)	
Missing	2 (1.0)	2 (2.5)	
Risk group, no. (%)‖			.068†
Favorable	89 (43.8)	27 (33.3)	
Intermediate	86 (42.4)	34 (42.0)	
Adverse	16 (7.9)	13 (16.0)	
Missing	12 (5.9)	7 (8.6)	

Significant *P* values are marked in bold.BM, bone marrow; CEPBA, CCAAT/enhancer binding protein α; CNS, central nervous system; FAB, French-American-British classification; FLT3-ITD, internal tandem duplication of the FLT3 gene; HSCT, allogeneic hematopoietic stem cell transplantation; NPM1, nucleophosmin-1; PB, peripheral blood.

*Mann-Whitney test.

†χ^2 test.

‡Patients may be counted more than once in cases with 2 or more coexisting cytogenetic abnormalities.

§Fisher's exact test.

‖Risk groups were defined as described[19] as favorable [t(8;21), inv(16)/t(16;16), *NPM1* or *CEBPA* mutations in the absence of *FLT3*-ITD], adverse [-5/del(5q), -7, or high *FLT3*-ITD allelic ratio], and intermediate (all other patients with available genetic data).

Overall, *CALCRL* expression levels were associated with the differential expression of 2262 genes. Among those genes, 516 were up- and 1746 were downregulated. A heatmap of the 200 most significantly regulated genes is shown in Figure 3. High expression of *CALCRL* was strongly associated with high expression of *TNFRSF18,* which has been described to contribute to immune evasion of AML.[20] It was also positively

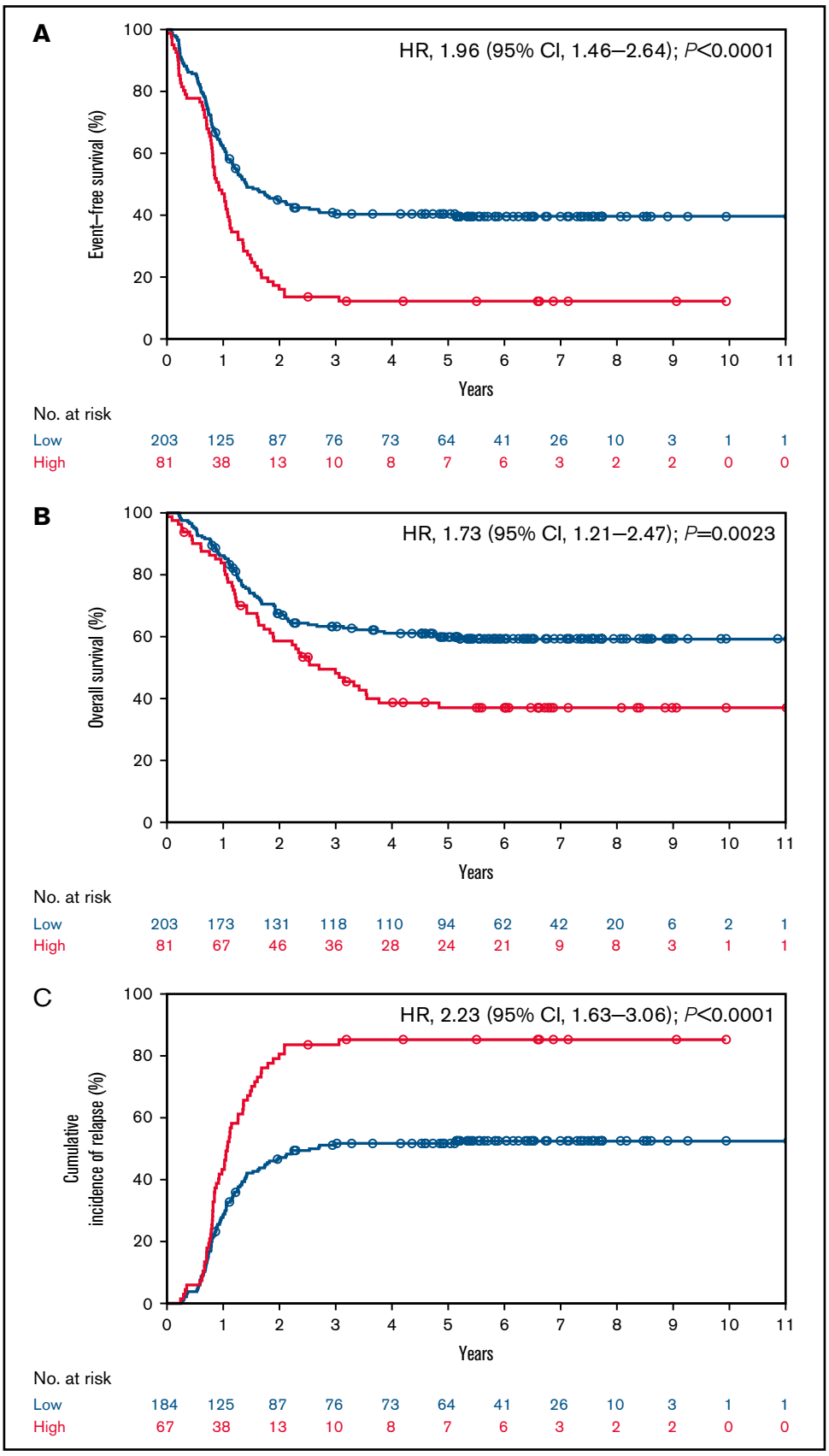

Figure 1. *CALCRL* expression and survival in the pediatric TARGET cohort.[13] (A) Event-free survival, (B) overall survival, and (C) cumulative incidence of relapse according to dichotomized *CALCRL* expression levels.

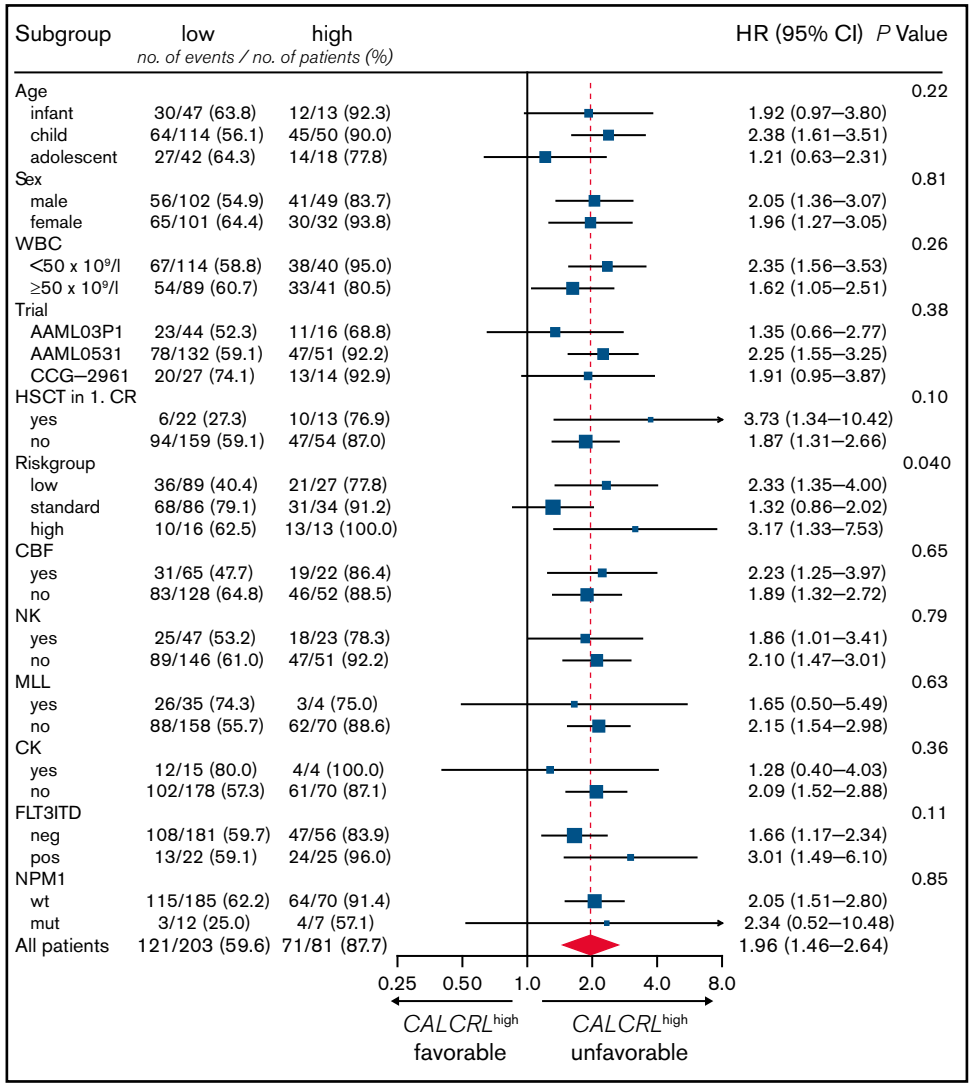

Figure 2. Forest plot of the association of *CALCRL* expression on event-free survival in selected subgroups of the pediatric TARGET cohort. Unadjusted hazard ratios (HRs) for high vs low *CALCRL* expression in selected subgroups are shown. Position of the squares represents the point estimate and horizontal lines represent the 95% confidence intervals (CI). The size of the squares is proportional to the precision of the estimate. The position of the diamond and the dotted vertical line represent the overall HR from the entire cohort. The lateral points of the diamond represent the 95% CI. The *P* values are for interaction of unadjusted hazard ratios by subgroups and represent heterogeneity. CBF, core-binding factor; CK, complex karyotype; MLL, mixed-lineage leukemia; NK, normal karyotype; *NPM1*, nucleophosmin-1.

correlated with the upregulation of markers of stemness such as *CDCP1*.[21,22] In turn, it was correlated with the downregulation of *MSX2*, which has been described as a negative regulator of stem cell functions.[23] In addition, *CALCRL* expression inversely correlated with genes that sensitize toward chemotherapy *MSX2* and *CADM1*.[23,24] Overall, samples with high *CALCRL* expression were significantly enriched for genes defining signatures of LSCs on gene set enrichment analyses (supplemental Data). Because LSC signatures are strong predictors of outcome, we tested the prognostic performance of *CALCRL* expression compared with the LSC17 score.[17] The LSC17 score has been described to be superior to other LSC scores and does not include *CALCRL* as a component. Here, we found that *CALCRL* expression remained prognostic when the LSC17 score was included in the multivariable analyses (supplemental Data) and *CALCRL* expression further stratified survival in pediatric AML patients with low and high LSC17 scores (Figure 3).

Discussion

The neuropeptide receptor *CALCRL* has been described as an independent prognostic factor and novel therapeutic target in adult patients with AML.[12] In this study, we investigated the clinical and prognostic role of *CALCRL* in pediatric patients with AML. The expression of *CALCRL* and its associations with outcome were retrospectively analyzed in a publicly available cohort of 284 patients with childhood AML.[13]

Relapse is still a major cause of death in children and adults with AML.[2,25] On a biological level, quiescent LSCs that survive chemotherapy have been proposed to cause relapse.[26]

Table 2. Multivariable regression analyses

Variables in the model	OR/HR	95% CI	*P* value
Complete remission			
Age: per 10-y increase	0.91	0.46-1.81	.79
WBC: per 50 × 10^9/L increase	0.91	0.75-1.11	.36
Risk group*			.0064
Favorable vs intermediate	7.67	2.19-26.80	.0014
Adverse vs intermediate	0.66	0.23-1.85	.43
CALCRL expression: high vs low	0.57	0.25-1.29	.17
Overall survival			
Age: per 10-y increase	1.29	0.93-1.79	.13
WBC: per 50 × 10^9/L increase	0.98	0.88-1.09	.71
Risk group*			<.0001
Favorable vs intermediate	0.35	0.22-0.53	<.0001
Adverse vs intermediate	1.16	0.66-2.04	.61
CALCRL expression: high vs low	1.55	1.06-2.27	.025
Event-free survival			
Age: per 10-y increase	1.11	0.85-1.45	.44
WBC: per 50 × 10^9/L increase	1.03	0.94-1.12	.55
Risk group*			<.0001
Favorable vs intermediate	0.40	0.28-0.55	<.0001
Adverse vs intermediate	1.04	0.63-1.70	.89
CALCRL expression: high vs low	1.87	1.36-2.57	.0001
Cumulative incidence of relapse			
Age: per 10-y increase	0.98	0.72-1.32	.87
WBC: per 50 × 10^9/L increase	1.01	0.91-1.12	.83
Risk group*			<.0001
Favorable vs intermediate	0.46	0.32-0.64	<.0001
Adverse vs intermediate	0.79	0.44-1.41	.42
CALCRL expression: high vs low	2.10	1.49-2.96	<.0001

ORs greater or less than 1.0 indicate higher or lower CR rates, respectively, for the first category listed. HRs greater or less than 1.0 indicate an increased or decreased risk, respectively, of an event for the first category listed.

OR, odds ratio.

*Risk groups were defined as described[19] as favorable [t(8;21), inv(16)/t(16;16), *NPM1* or *CEBPA* mutations in the absence of *FLT3*-ITD], adverse [-5/del(5q), -7, or high *FLT3*-ITD allelic ratio], and intermediate (all other patients with available genetic data).

On the clinical level, this is supported by the fact that LSC signatures are strong predictors of outcome.[17] Here, we show that *CALCRL* expression is associated with stemness signatures in pediatric AML. It remains significantly associated with outcome even when the LSC17 score is included in the multivariable models, suggesting an added value over this existing stemness score. In recent years, evidence has been increasing that relapse of AML is caused by relapse-initiating drug-tolerant cells (RICs) that are not enriched for LSCs or quiescent cells in vivo.[27,28] Interestingly, *CALCRL* has just been proposed as a master regulator of RICs in preclinical models of AML.[29] Although the association of *CALCRL* with OS and EFS has previously been shown for adult patients with AML, a direct association with the risk of relapse has not been described before, to the best of our knowledge. The clinical association of *CALCRL* expression with the risk of relapse in the pediatric AML population supports the biological role of CALCRL as a marker of stemness and a master regulator of RICs in human AML. Overall, our findings support CALCRL as an attractive target for novel pharmaceutical interventions that are directed against mechanisms causing relapse of AML, now including children and adolescents with their high medical need.

Antibody-based inhibition of CALCRL has been proposed as a novel therapeutic principle in preclinical models of solid tumors.[6-10] Here, inhibition of CALCRL results in a reduction of tumor growth by the disruption of angiogenesis and an antiproliferative impact on cancer cells. Interestingly, CALCRL-targeting drugs are approved for patients with migraine, based on the critical role of a neuropeptide acting on CALCRL signaling in this disease.[11] These agents are well-tolerated in humans and therefore attractive candidates for repurposing as antileukemic agents. Recently, knockdown of *CALCRL* in human AML cell lines has been shown to prolong the survival of mice in AML xenotransplantation models and increase the sensitivity toward chemotherapy.[29] Further studies are necessary to determine the therapeutic efficacy of antibody- or small

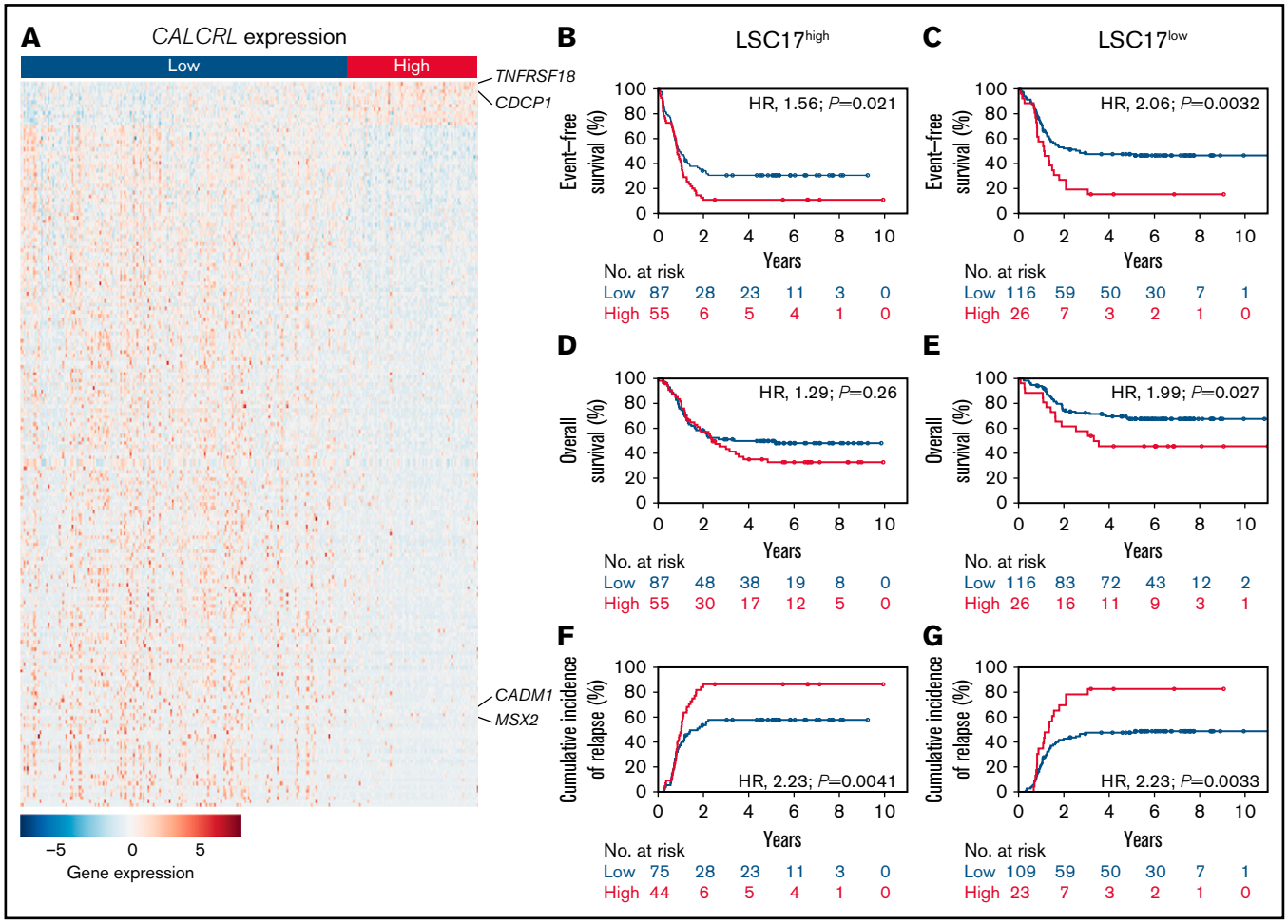

Figure 3. Biological insights. (A) Heat map of the 200 differentially expressed genes with the lowest adjusted *P* values ordered by log fold change between pediatric patients with AML with high and low *CALCRL* expression levels. Rows represent the color-coded genes with blue indicating low, white intermediate, and red high expression values for the given gene. Columns represent patients, ordered from left to right by *CALCRL* expression. Genes that are mentioned in the text are indicated. The complete list of the 200 most differentially regulated genes can be found in the supplemental Data. (B,C) Event-free survival, (D,E) overall survival, and (F,G) cumulative incidence of relapse according to *CALCRL* expression in patients with (B,D,F) high and (C,E,G) low LSC17 scores.[17]

molecule-based inhibition of CALCRL and/or its ligands ADM and calcitonin gene-related peptide alone and in combination with chemotherapy in pediatric and adult AML.

Our study has some limitations. First, our analysis is limited by its retrospective nature and needs validation in a prospective manner. Second, information on the time of transplantation was not available in the TARGET data matrix; thus, we were not able to investigate the impact of allogeneic HSCT in *CALCRL* high expressors in a time-dependent manner. In addition, we were not able to look for a potential bias caused by events unrelated to leukemia biology by censoring at allogeneic HSCT. However, we did not find any significant heterogeneity of the prognostic impact of *CALCRL* expression according to transplant status. Thus, the prognostic impact of *CALCRL* exists in both transplanted and nontransplanted patients and is not determined by the type of consolidation.

In summary, we identified *CALCRL* as a marker of stemness and an independent prognostic factor in pediatric AML, reinforcing its suitability as a novel therapeutic target in these patients. We demonstrated that the expression of *CALCRL* is strongly connected with the risk of relapse in pediatric AML, which supports its role as a master regulator of RICs. Results of this study will stimulate prospective analysis of the prognostic value of *CALRCL* expression in future trials and clinical investigation of repurposed CALCRL-targeting drugs in children and adolescents with AML.

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Authorship

Contribution: L.A. designed the study; L.A. performed statistical studies and analyzed the data; M.W. performed the GSE and DGE analyses; S.M. coordinated the studies providing the material and the public database used for this analysis; L.A., A.A., W.E.B., S.M., C.S., and C.R. wrote the manuscript; L.A., M.W., J.-H.M., M.F.A., A.A., S.S., W.E.B., G.L., M.D., S.M., C.S., and C.R. interpreted the data and made the decision to submit the manuscript for publication.

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References

1. Steliarova-Foucher E, Colombet M, Ries LAG, et al; IICC-3 contributors. International incidence of childhood cancer, 2001-10: a population-based registry study. *Lancet Oncol.* 2017;18(6):719-731.
2. Rasche M, Zimmermann M, Borschel L, et al. Successes and challenges in the treatment of pediatric acute myeloid leukemia: a retrospective analysis of the AML-BFM trials from 1987 to 2012. *Leukemia.* 2018;32(10):2167-2177.
3. Conneely SE, Stevens AM. Acute myeloid leukemia in children: emerging paradigms in genetics and new approaches to therapy. *Curr Oncol Rep.* 2021;23(2):16.
4. Russell FA, King R, Smillie SJ, Kodji X, Brain SD. Calcitonin gene-related peptide: physiology and pathophysiology. *Physiol Rev.* 2014;94(4):1099-1142.
5. Larráyoz IM, Martínez-Herrero S, García-Sanmartín J, Ochoa-Callejero L, Martínez A. Adrenomedullin and tumour microenvironment. *J Transl Med.* 2014;12(1):339.
6. Chen P, Huang Y, Bong R, et al. Tumor-associated macrophages promote angiogenesis and melanoma growth via adrenomedullin in a paracrine and autocrine manner. *Clin Cancer Res.* 2011;17(23):7230-7239.
7. Kaafarani I, Fernandez-Sauze S, Berenguer C, et al. Targeting adrenomedullin receptors with systemic delivery of neutralizing antibodies inhibits tumor angiogenesis and suppresses growth of human tumor xenografts in mice. *FASEB J.* 2009;23(10):3424-3435.
8. Ouafik L, Sauze S, Boudouresque F, et al. Neutralization of adrenomedullin inhibits the growth of human glioblastoma cell lines in vitro and suppresses tumor xenograft growth in vivo. *Am J Pathol.* 2002;160(4):1279-1292.
9. Toda M, Suzuki T, Hosono K, et al. Neuronal system-dependent facilitation of tumor angiogenesis and tumor growth by calcitonin gene-related peptide. *Proc Natl Acad Sci USA.* 2008;105(36):13550-13555.
10. Berenguer-Daizé C, Boudouresque F, Bastide C, et al. Adrenomedullin blockade suppresses growth of human hormone-independent prostate tumor xenograft in mice. *Clin Cancer Res.* 2013;19(22):6138-6150.
11. Dodick DW. Migraine. *Lancet.* 2018;391(10127):1315-1330.
12. Angenendt L, Bormann E, Pabst C, et al. The neuropeptide receptor calcitonin receptor-like (CALCRL) is a potential therapeutic target in acute myeloid leukemia. *Leukemia.* 2019;33(12):2830-2841.
13. Bolouri H, Farrar JE, Triche T Jr, et al. The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions [published corrections appear in *Nat Med.* 2018;24(4):526 and *Nat Med.* 2019;25(3):530]. *Nat Med.* 2018;24(1):103-112.
14. Cooper TM, Franklin J, Gerbing RB, et al. 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(3):761-769.
15. Gamis AS, Alonzo TA, Meshinchi S, et al. Gemtuzumab ozogamicin in children and adolescents with de novo acute myeloid leukemia improves event-free survival by reducing relapse risk: results from the randomized phase III Children's Oncology Group trial AAML0531. *J Clin Oncol.* 2014;32(27):3021-3032.
16. Lange BJ, Smith FO, Feusner J, et al. Outcomes in CCG-2961, a Children's Oncology Group phase 3 trial for untreated pediatric acute myeloid leukemia: a report from the children's oncology group. *Blood.* 2008;111(3):1044-1053.
17. Ng SW, Mitchell A, Kennedy JA, et al. A 17-gene stemness score for rapid determination of risk in acute leukaemia. *Nature.* 2016;540(7633):433-437.
18. Hothorn T, Lausen B. On the exact distribution of maximally selected rank statistics. *Comput Stat Data Anal.* 2003;43(2):121-137.
19. Lim EL, Trinh DL, Ries RE, et al. MicroRNA expression-based model indicates event-free survival in pediatric acute myeloid leukemia [published correction appears in *J Clin Oncol.* 2019;37(3):261]. *J Clin Oncol.* 2017;35(35):3964-3977.
20. Baessler T, Krusch M, Schmiedel BJ, et al. Glucocorticoid-induced tumor necrosis factor receptor-related protein ligand subverts immunosurveillance of acute myeloid leukemia in humans. *Cancer Res.* 2009;69(3):1037-1045.

21. Conze T, Lammers R, Kuci S, et al. CDCP1 is a novel marker for hematopoietic stem cells. *Ann N Y Acad Sci.* 2003;996(1):222-226.

22. Kimura H, Morii E, Ikeda JI, et al. Role of DNA methylation for expression of novel stem cell marker CDCP1 in hematopoietic cells. *Leukemia.* 2006; 20(9):1551-1556.

23. Yin Y, Xie CM, Li H, et al. The FBXW2-MSX2-SOX2 axis regulates stem cell property and drug resistance of cancer cells. *Proc Natl Acad Sci USA.* 2019;116(41):20528-20538.

24. Fisser MC, Rommer A, Steinleitner K, et al. Induction of the proapoptotic tumor suppressor gene Cell Adhesion Molecule 1 by chemotherapeutic agents is repressed in therapy resistant acute myeloid leukemia. *Mol Carcinog.* 2015;54(12):1815-1819.

25. Short NJ, Rytting ME, Cortes JE. Acute myeloid leukaemia. *Lancet.* 2018;392(10147):593-606.

26. Pollyea DA, Jordan CT. Therapeutic targeting of acute myeloid leukemia stem cells. *Blood.* 2017;129(12):1627-1635.

27. Boyd AL, Aslostovar L, Reid J, et al. Identification of chemotherapy-induced leukemic-regenerating cells reveals a transient vulnerability of human AML recurrence. *Cancer Cell.* 2018;34(3):483-498.e5.

28. Farge T, Saland E, de Toni F, et al. Chemotherapy-resistant human acute myeloid leukemia cells are not enriched for leukemic stem cells but require oxidative metabolism. *Cancer Discov.* 2017;7(7):716-735.

29. Larrue C, Guiraud N, Mouchel PL, et al. Adrenomedullin-CALCRL axis controls relapse-initiating drug tolerant acute myeloid leukemia cells. *Nat Commun.* 2021;12(1):422.