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Multimerin-1 (*MMRN1*) as Novel Adverse Marker in Pediatric Acute Myeloid Leukemia: A Report from the Children's Oncology Group

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Abstract

PURPOSE—Exploratory gene expression array analyses suggested multimerin-1 (*MMRN1*) to be a predictive biomarker in acute myeloid leukemia (AML). Following-up on these studies, we evaluated the role of *MMRN1* expression as outcome predictor in 2 recent Children's Oncology Group trials.

EXPERIMENTAL DESIGN—We retrospectively quantified *MMRN1* expression in 183 participants of AAML03P1 and 750 participants of AAML0531 by reverse-transcriptase polymerase chain reaction and correlated expression levels with disease characteristics and clinical outcome.

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RESULTS—In AAML03P1, the highest quartile of *MMRN1* expression (expression ≥0.5 relative to β-*glucuronidase*; n=45) was associated with inferior event-free survival (EFS; *P*<0.002) and higher relapse risk (*P*<0.004). In AAML0531, in which we quantified *MMRN1* mRNA for validation, patients with relative *MMRN1* expression ≥0.5 (n=160) less likely achieved remission (67% vs. 77%, *P*=0.006), and more frequently had minimal residual disease (43% vs. 24%, *P*=0.001) after one induction course. They had inferior overall survival (44±9% vs. 69±4% at 5 years; *P*<0.001) and EFS (32±8% vs. 54±4% at 5 years; *P*<0.001) and higher relapse risk (57±10% vs. 35±5% at 5 years; *P*<0.001). These differences were partly attributable to the fact that patients with high *MMRN1* expression less likely had cytogenetic/molecular *low-risk* disease (*P*<0.001) than those with low *MMRN1* expression. Nevertheless, after multivariable adjustment, high *MMRN1* expression remained statistically significantly associated with shorter OS (hazard ratio [HR]=1.57 [95% confidence interval: 1.17–2.12] p=0.003) and EFS (HR=1.34 [1.04–1.73] p=0.025), and higher relapse risk (HR=1.40 [1.01–1.94] p=0.044).

CONCLUSIONS—Together, our studies identify *MMRN1* expression as a novel biomarker that may refine AML risk-stratification.

INTRODUCTION

Acute myeloid leukemia (AML) is a challenging disease with outcomes that vary widely between individual patients (1). Numerous disease-related risk factors have so far been recognized; among those, cytogenetic abnormalities and somatic mutations are the most important ones and provide the framework for diagnostic classification and risk-stratification schemes (1). While such schemes are increasingly used for risk-tailored treatment assignment, there are only a small number of informative predictive markers, and it is a recurrent clinical observation that this limited battery fails to accurately predict outcome for many patients. Thus, there remains a need for refined tools to characterize disease risk in AML. Our recent studies indicate that multimerin-1 (*MMRN1*) may be such a biomarker.

MMRN1, a member of the elastin microfibrillar interface protein (*EMILIN*)/multimerin family, has so far primarily been described as a component of secretory granules found in platelets and endothelial cells that may mediate cellular adhesion via integrin receptors (2). During recent discovery studies using gene expression array data from diagnostic specimens of 211 recently treated pediatric AML patients, we identified *MMRN1* as a SOCS2 (3) co-segregating gene whose expression varied widely (Supplemental Figure 1) and appeared to be related to patient outcomes (Supplemental Figure 2). To follow-up on these studies, we retrospectively quantified *MMRN1* expression in pre-treatment bone marrow specimens from participants of the Children's Oncology Group (COG) AML protocol, AAML03P1, and then validated findings in participants of AAML0531 to investigate the potential role of *MMRN1* as a predictive biomarker in pediatric AML.

PATIENTS AND METHODS

Patient samples

Cryopreserved pretreatment ("diagnostic") specimens from patients enrolled on AAML03P1 or AAML0531 who consented to the biology studies and had bone marrow samples were available were included in this study. AAML03P1 (registered at ClinicalTrials.gov as NCT00070174) was a multicenter phase 3 pilot study that determined the safety and feasibility of adding gemtuzumab ozogamicin (GO) to intensive chemotherapy among 339 eligible children and adolescents (aged 1 month to 21 years) with newly diagnosed *de novo* AML, excluding those with acute promyelocytic leukemia (APL), bone marrow failure syndromes, juvenile myelomonocytic leukemia, or Down syndrome between 2003 and 2005 (4). AAML0531 (NCT00372593) was the subsequent multicenter phase 3 study that determined the addition of GO to intensive chemotherapy among 1,070 eligible patients aged <30 years with newly diagnosed *de novo* non-APL AML, excluding those with bone marrow failure syndromes, juvenile myelomonocytic leukemia, or Down syndrome (if ≤3 years of age) between 2006 and 2010 (5). The patient and disease (cytogenetic/molecular) characteristics of the subsets of AAML03P1 and AAML0531 patients studied in this analysis were relatively comparable to patients not studied in this analysis. Specifically, for AAML03P1, there were differences with regard to some disease characteristics (i.e., higher WBC counts [$P<0.001$] and higher proportion of patients with *NPM1* mutation [$P=0.011$] and *low-risk* disease [$P<0.001$]), but short-term outcomes were similar (i.e. complete remission [CR] rate after 1 course of therapy [$P=0.08$] and rates of minimal residual disease [MRD; $P=0.55$]), as were overall survival (OS; $P=0.22$) and event-free survival ($P=0.93$). For AAML0531, there were also some differences in disease characteristics (i.e., higher proportion of patients with inv(16)/t(16;16) [$P=0.011$] and *low-risk* disease [$P<0.001$]) as well as better short-term outcomes (i.e. CR rate after 1 course of therapy [$P=0.001$] albeit not rate of MRD [$P=0.95$]), but OS was similar ($P=0.52$) and EFS was only slightly better ($P=0.04$). Informed consent was obtained from all study subjects in accordance with the Declaration of Helsinki, and the institutional review boards (IRBs) of all participating institutions approved the clinical protocol. IRB approval was obtained from Fred Hutchinson Cancer Research Center before conduct of this biological study, which was also approved by the COG Myeloid Disease Biology Committee and the National Cancer Institute Cancer Therapy Evaluation Program.

Risk stratification

A combination of cytogenetic and molecular abnormalities was used to stratify participants into risk groups. A patient was considered *low-risk* if a chromosomal abnormality/mutation was present in core-binding factors [CBF, t(8;21) or inv(16)/t(16;16)], *nucleophosmin [NPM1]* (unless a *FLT3*-ITD mutation with high allelic ratio [≥0.4] was also present), or CCAAT/enhancer binding protein (C/EBP), alpha [*CEBPA*]; for CEBPA, both single and double mutations were considered favorable (6). Patients were classified as *high-risk* if they had monosomy 5 or deletion of 5q (-5/5q-), monosomy 7 (-7), or *FLT3*-ITD with high allelic ratio (0.4 or higher). All other patients with data sufficient for classification were considered *standard-risk*.

Detection and quantification of MRD

Residual AML was quantified in bone marrow aspirates collected at the end of the first induction course by multiparameter flow cytometry using a “different-from-normal” approach as previously described.(7)

Quantification of MMRN1 expression in unsorted AML specimens and fluorescence-activated cell sorting (FACS)-isolated $CD34^+/CD33^-$ and $CD34^+/CD33^+$ cells

Total RNA from unsorted diagnostic AML specimens was extracted with the AllPrep DNA/RNA Mini Kit using the QIAcube automated system (Qiagen, Valencia, CA). After quantification with a microvolume spectrophotometer (NanoDrop; Thermo Scientific, Wilmington, DE), 10 ng of total RNA was subjected to quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR; 7900 Real-Time PCR System; Applied Biosystems; Foster City, CA) using taqman primers per manufacturer's instructions to determine expression of *MMRN1* and, for normalization, the housekeeping gene, β-glucuronidase (*GUSB*). Primer probe sets were as follows: *MMRN1* was designed to amplify sequence at the junction of exon 3 and 4 and *GUSB* was designed to amplify sequence at the junction of exon 8 and 9 (Hs00201182_m1 and Hs00939627_m1, respectively; Applied Biosystems). Patient samples were run in duplicate, and the ΔΔCT method quantified as $2^{(-\Delta\Delta CT)}$ (8, 9) was used to determine the expression levels of *MMRN1* relative to *GUSB*.

In a set of 10 AML specimens, $CD34^+/CD33^-$ and $CD34^+/CD33^+$ cells were separated after thawing and staining with directly labeled antibodies (CD34-fluorescein isothiocyanate [FITC; clone 8G12] and CD33-phycoerythrin [PE; clone P67]; both from BD Biosciences, San Jose, CA) using a FACSAria flow cytometer (BD Biosciences). $CD34^+$ cells were defined as the cells that fell outside the bottom 99.5% of the FITC-isotype control staining. $CD34^+/CD33^-$ cells were defined as $CD34^+$ cells that fell within the bottom 85% of the PE-isotype control staining; the collection window was narrowed in samples with dim CD33 expression so that no more than 25% of $CD34^+$ cells would be collected. $CD34^+/CD33^+$ cells were defined as the $CD34^+$ cells that fell outside the bottom 99.5% of the PE-isotype control staining. Total RNA was then extracted from isolated cell populations with the AllPrep DNA/RNA Mini Kit. After quantification, 5–10 ng of total RNA (equal amounts for cell population pairs) was subjected to qRT-PCR for *MMRN1* and *GUSB* as described above.

Statistical analysis

Data from AAML03P1 and AAML0531 were current as of December 31, 2013. The median (range) of follow-up for patients alive at last contact was 7.8 (0–9.3) years for AAML03P1 and 4.3 (0.02–7.1) years for AAML0531. The Kaplan-Meier method (10) was used to estimate OS (defined as time from study entry to death) and EFS (time from study entry until failure to achieve CR during induction, relapse, or death). Relapse risk (RR) was calculated by cumulative incidence methods defined as time from the end of induction I for patients in CR to relapse or death where deaths without a relapse were considered competing events (11). Patients who withdrew from therapy due to relapse, persistent central nervous system (CNS) disease, or refractory disease with >20% bone marrow blasts by the end of induction I were defined as induction I failures. The significance of predictor variables was

tested with the log-rank statistic for OS, EFS and with Gray's statistic for RR. All estimates are reported with two times the Greenwood standard errors. Children lost to follow-up were censored at their date of last known contact. Cox proportional hazards models (12) were used to estimate the hazard ratio (HR) for defined groups of patients in univariate and multivariate analyses of OS and EFS. Analyses of OS for AAML0531 patients across all risk groups and for standard risk patients violated the proportional hazards assumption, and therefore a parametric Weibull regression model was used to estimate the HR. Competing risk regression models were used to estimate HRs for univariate and multivariate analyses of RR. The chi-squared test was used to test the significance of observed differences in proportions, and Fisher's exact test was used when data were sparse. Differences in medians were compared by the Mann-Whitney or Wilcoxon signed-rank test, as appropriate. A *P*-value <0.05 was considered statistically significant.

RESULTS

Identification of MMRN1 as predictive biomarker in participants of AAML03P1

Among 339 eligible patients enrolled on AAML03P1, 309 (91%) consented to contribute to the biologic aims of the study and provided diagnostic bone marrow specimens. At the time this study was conducted, RNA from 188 of these 309 patients (61%) was available for quantitation of *MMRN1* expression levels by qRT-PCR. Five samples were excluded because of inadequate RNA as determined by low GUSB expression (97.5th percentile cut-off for low GUSB in AAML03P1: Ct >33.09). Within the remaining 183 specimens, *MMRN1* mRNA was detected in all samples and its abundance varied >80,000-fold relative to *GUSB* mRNA (0.0002–15.14 [median: 0.1793]; Figure 1A). Of note, the median expression of *MMRN1* in the AML specimens was similar to the median expression found in a small subset of normal whole bone marrows obtained from volunteers aged 22, 26, 31, and 44 years (median relative expression 0.170 [range: 0.094–0.232], *n*=4).

Studying the relationship between *MMRN1* expression and clinical outcome, we initially analyzed patient outcomes per quartile of *MMRN1* expression and noticed that the 45 patients with the highest relative *MMRN1* expression (4th quartile, corresponding to an expression of ≥0.5 relative to GUSB) fared worse than patients in the first, second, or third quartile of *MMRN1* expression, respectively, with little difference between the first three quartiles. We therefore subsequently compared patients with the highest relative *MMRN1* expression (i.e. relative expression ≥0.5) to patients with lower expression (i.e. relative expression <0.5); their baseline characteristics are summarized in Supplemental Table 1. We found that patients with the highest *MMRN1* expression had an inferior EFS (*P*=0.002; at 5 years: 33±15% vs. 58±9%) and higher RR (*P*=0.004; at 5 years: 52±20% vs. 24±8%) than the 138 patients within the lower 3 quartiles of *MMRN1* expression, while OS was not statistically significantly different (*P*=0.135; at 5 years: 57±15% vs. 71±8%; Figure 2A–C). Of note, exploratory multiple cutpoint analyses for EFS indicated that the most statistically significant results were centered around the Q4 cutpoint region, supporting our approach of comparing patients with the highest quartile of relative *MMRN1* expression with those having lower relative *MMRN1* expression (data not shown).

Validation of MMRN1 as predictive biomarker in participants of AAML0531

To further validate the role of *MMRN1* expression as predictive biomarker in pediatric AML, we quantified *MMRN1* expression among 1,070 eligible patients enrolled on AAML0531, and correlated expression levels with clinical outcome and disease characteristics. Among these patients enrolled on AAML0531, 980 (92%) consented to the use of biospecimens for correlative research, with RNA available from 765 patients, 15 of which had inadequate GUSB levels (Ct >33.09). The remaining 750 patients (77%) were used for quantitation of *MMRN1* expression levels. In 740 of the 750 patient specimens, *MMRN1* mRNA was detectable, varied >130,000-fold relative to *GUSB* mRNA (0.0001–18.21 [median: 0.1263]; Figure 1B), and was distributed across quartiles similarly to *MMRN1* expression in AAML03P1.

Association between MMRN1 expression and characteristics of study population—To investigate associations between relative *MMRN1* expression and demographics, baseline laboratory findings, and pretreatment characteristics of the study cohort, we used the same cut-off as identified in the AAML03P1 training cohort and compared patients with high *MMRN1* expression (i.e., relative expression of ≥0.5; n=160) with those having low *MMRN1* expression (i.e., relative expression of <0.5; n=590). As summarized in Table 1, patients with high *MMRN1* expression were younger (P<0.001), whereas there was no statistically significant difference in gender distribution, white blood cell (WBC) count, hemoglobin, platelet count, and proportion of patients with hepatomegaly, splenomegaly, or extramedullary disease (chloroma and/or CNS involvement). There was also no significant correlation between *MMRN1* mRNA levels and the percentage of bone marrow blasts (Supplemental Figure 3). Importantly, patients with high *MMRN1* expression less likely had CBF translocations (t(8;21): 0% *vs.* 19%, P<0.001; inv(16): 2% *vs.* 15%, P<0.001) and *NPM1* mutations (3% *vs*. 9%, P=0.016); conversely, they were more likely to have leukemia with monosomy 7 (7% *vs*. 1%, P<0.001) and abnormalities involving 11q23 (33% *vs*. 17%, P<0.001). Consistently, patients with high *MMRN1* expression less likely had *low-risk* disease (5% *vs.* 48%, P<0.001) and more likely had *standard-risk* disease (73% *vs*. 41%, P<0.001) and *high-risk* disease (23% *vs*. 11%, P<0.001) than those with lower *MMRN1* expression.

Association between MMRN1 expression and clinical outcome—To investigate the relationship between *MMRN1* expression and clinical outcome in the AAML0531 cohort, we first studied responses to initial chemotherapy. We found that the 160 patients with high relative *MMRN1* expression were statistically significantly less likely to have achieved CR after one course of chemotherapy than the 590 patients with lower *MMRN1* expression (67% *vs.* 77%, P=0.006) and more likely had MRD at the end of the first induction course (43% vs. 24%, P=0.001). Some patients with high *MMRN1* expression were able to achieve remission with re-induction therapy, and the proportion of patients with high *MMRN1* expression in CR after 2 courses of induction chemotherapy approached that of patients with low *MMRN1* expression (83% vs. 88%, P=0.153). We subsequently evaluated how *MMRN1* expression related to parameters of long-term outcome and found that high *MMRN1* expression was associated with inferior OS (P<0.001; at 5 years: 44±9% vs. 69±4%) and EFS (P<0.001; at 5 years: 32±8% vs. 54±4%), and higher RR (P<0.001; at

5 years: 57±10% vs. 35±5%) (Figure 3A–C). The 5-year survival and relapse estimates, stratified by disease-risk and relative *MMRN1* expression, are summarized in Table 2.

MMRN1 as independent predictive factor—We next evaluated the potential role of *MMRN1* expression as independent predictor of OS, EFS, and RR in regression models (Table 3). Given the association between disease risk and *MMRN1* expression, one might attribute the worse outcome for patients with high *MMRN1* expression to the lower prevalence of leukemias with more favorable prognoses in this subgroup. However, after adjustment for disease risk, age, bone marrow blast percentage, and treatment arm, high *MMRN1* expression remained statistically significantly associated with inferior OS (HR=1.57 [1.17–2.12], *P*=0.003), inferior EFS (HR: 1.34 [1.04–1.73], *P*=0.025), and higher RR (HR: 1.40 [1.01–1.94], *P*=0.044; Table 3).

Correlation of MMRN1 expression with outcome in individual risk groups—Finally, we performed subgroup analyses to investigate the potential role of *MMRN1* expression as a predictor for outcome in specific risk groups; these studies were of exploratory nature since our ability to perform these analyses was relatively limited because of the sample size of the individual risk groups. As summarized in Table 2, patients with high *MMRN1* expression had generally worse outcomes than those with low MMRN1 expression across all 3 disease risk categories, although these differences approached statistical significance only in the subset of *standard-risk* patients (e.g. RR: *P*=0.045). Of note, fewer patients with high *MMRN1* expression underwent HCT as consolidation therapy relative to those with lower *MMRN1* expression (*low-risk*: 0/7 [0%] vs. 17/276 [6%]; *standard-risk*: 20/111 [18%] vs. 42/238 [18%]; and *high-risk*: 12/35 [34%] vs. 27/63 [43%]). This difference was at least partly explained by primary failures to achieve remission on study, or early relapse after short remission duration. Nevertheless, analyses in which patients were censored at the time of HCT indicated that the predictive significance of *MMRN1* expression was retained (Supplemental Table 2).

Relationship between MMRN1 expression and differentiation stage of AML cell

In the analyses presented thus far, we used unsorted bone marrow specimens to quantify MMRN1 expression. To investigate whether levels of MMRN1 mRNA were related to the differentiation stage of the AML cell, we used bone marrow specimens from 10 patients and isolated less mature $CD34^+/CD33^-$ and more mature $CD34^+/CD33^+$ cell subsets by FACS. In these 10 specimens, the relative MMRN1 expression was slightly higher in $CD34^+/CD33^-$ cells (median: 0.33 [range: 0.01–4.82]) than corresponding $CD34^+/CD33^+$ cells (median: 0.14 [range: 0.02–1.44]; $p<0.05$; Supplemental Table 3).

DISCUSSION

Multimerins encompass an elusive family of secreted glycoproteins that are characterized by an N-terminal cysteine-rich EMI domain thought to be involved in multimerization, a long central region predicted to form coiled-coil structures, and a C-terminal globular C1q domain that mediates binding to integrins (2). The biological function of *MMRN1* is poorly understood. Originally identified as a multimeric glycoprotein released by, and associated

with, the surface of platelets following platelet activation (13), MMRN1 was later found in α-granules in both platelets and endothelial cells and implicated in factor V sequestration as well as platelet adhesion to collagens via binding to integrins following its cellular release. Forming a variety of di-sulfide linked multimers, ranging from a trimeric complex to multimers of many megadaltons in size (14), it is conceivable that MMRN1 could play a dynamic role in the cytoarchitectural and adhesive changes that accompany platelet aggregation and clot formation. Together, MMRN1 may therefore serve as an extracellular matrix (ECM) or adhesive protein mediating cellular attachment through the binding of ECM proteins and integrin receptors (15, 16).

So far, *MMRN1* has neither been implicated in myeloid biology nor described as individual predictive marker for any human cancer. In AML, global gene expression profiling studies indicated that *MMRN1* expression was higher in leukemic stem cell (LSC)-enriched populations than in leukemic progenitor cells. Data on *MMRN1* and 30 other genes that were more highly expressed in LSC populations was then combined to derive a LSC signature, which was found to be associated with clinical outcome, most notably worse OS, EFS, and relapse-free survival, in subsequent correlative studies.(17) More recently, our analyses of gene expression profiling data obtained from diagnostic specimens from pediatric and adolescent AML patients suggested a possible role for *MMRN1* as an individual outcome biomarker in AML. Through qRT-PCR analyses of diagnostic specimens from a large number of pediatric AML patients treated homogeneously on 2 recent cooperative group trials, our findings presented in this study indeed showed that *MMRN1* expression, which is highly variable in AML, was associated with certain disease characteristics and outcome. Specifically, patients with high *MMRN1* expression had a significantly lower likelihood of early remission achievement (i.e. achievement of CR with one course of induction therapy) and a higher risk of relapse and, consequently, inferior survival expectations.

Our analyses demonstrate significant heterogeneity of *MMRN1* expression in AML, with relative levels that varied widely in bone marrows of patients with active disease. Patients with high *MMRN1* expression were less likely to have cytogenetic/molecular abnormalities that are considered low risk, most notably leukemias with translocations affecting CBFs and, perhaps, *NPM1* mutations. In turn, high *MMRN1* expression is associated with adverse features such as monosomy 7 or abnormalities involving the 11q23 locus. As a result, patients with high *MMRN1* expression less likely had *low-risk* disease and more likely had *standard-risk* and *high-risk* disease than those with lower *MMRN1* expression. Thus, it may not be surprising that univariate analyses showed high *MMRN1* expression to be statistically significantly associated with lower CR rates, shorter survival estimates, and higher risk of relapse. However, even after adjustment for cytogenetic/molecular disease risk, high *MMRN1* remained statistically significantly associated with inferior OS, EFS, and RR, indicating that *MMRN1* expression is an independent biomarker of poor outcome in pediatric AML.

Previous studies have established that proteins involved in cellular adhesion such as L-selectin, β-integrin, and VLA-4 constitute biological features that can serve as predictive markers in AML (18–21). Ultimately, mechanistic studies will be necessary to fully understand this association between high *MMRN1* expression and adverse outcome in AML

and to investigate whether secreted levels of MMRN1 also bear predictive information. Still, given the emerging recognition of the importance of cellular adhesion for resistance to chemotherapy in hematologic malignancies including AML (22), and the ability of multimerins to bind to ECM and integrins, it is interesting to speculate that elevated MMRN1 levels could alter AML cell function and adhesion-dependent resistance to chemotherapy. Future studies will be required to test whether elevated expression of *MMRN1* in AML cells provides further support for the concept of cellular adhesion being a pivotal biological factor in AML. Additionally, MMRN1 has been identified as a TGF-β1-interacting protein (23), and the multimerin family member, EMILIN-3, acts as a TGF-β1 antagonist *in vitro* (24), raising the possibility that MMRN1 could exert an effect on AML cells via modulation of cytokine signaling (e.g. attenuation of TGF-β1 signaling).

Our study has several strengths, most notably the inclusion of a large number of the diagnostic specimens, thereby increasing the precision of outcome estimates, the use of data from patients treated homogeneously on recent cooperative group trials, and the use of data from 2 independent trials that allow validation of our findings across independent patient cohorts. On the other hand, several limitations have to be acknowledged. First, despite the use of a large number of specimens, our ability to perform subset analyses was relatively limited because of the sample size of the individual risk groups, for example for risk-stratified analyses. Likewise, our study was not sufficiently powered to allow for extensive multivariate adjustments. Second, since unsorted bone marrow specimens were used for our studies, differences in *MMRN1* abundance between specimens may not necessarily reflect differences in AML blasts but, rather, other cell types such as megakaryocytes or vascular cells. Gene expression studies in humans and mice indicate that higher *MMRN1* mRNA levels are found in less mature hematopoietic cells, including LSC populations.(17, 25) Consistent with the latter, we found in a small set of AML specimens that *MMRN1* levels were higher in less mature less mature $CD34^+/CD33^-$ cells than the more mature $CD34^+/CD33^+$ cell counterparts. Identifying the exact cellular origins of the greatly variable amounts of *MMRN1*, and more detailed analyses of relative expression levels along the cellular differentiation path of AML cells, may be a subject for future work. And third, only cryopreserved specimens were available for our analyses, and additional studies will be required to determine to what degree, if any, *MMRN1* expression changes in the cryopreservation process. Nevertheless, our data indicate that *MMRN1* expression is a novel independent adverse predictive marker in pediatric and adolescent AML. For outcome prediction, high *MMRN1* expression characterizes patients at significantly increased risk for primary treatment failure, relapse, and poor leukemia-free survival. Thus, *MMRN1* may be of use for the refinement of risk-stratification, e.g. via assignment of intensified chemotherapy or use of allogeneic hematopoietic cell transplantation in future cooperative study group trials or routine off-protocol care, and improve our abilities to individualize treatment decisions in AML.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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STATEMENT OF TRANSLATIONAL RELEVANCE

Although several predictive biomarkers have been described in acute myeloid leukemia (AML), current models are unable to accurately forecast therapeutic response and survival. Exploratory gene expression array analyses suggested that multimerin-1 (*MMRN1*), a hitherto very poorly described gene that may be involved in cellular adhesion via integrin receptors, could be a novel predictive biomarker in AML. Following-up on these studies, we investigated the *MMRN1* expression in 2 recent Children's Oncology Group trials, AAML03P1 and AAML0531. While associated with some adverse disease-risk features, we found that high *MMRN1* expression was independently associated with shorter overall- and event-free survival as well as a higher relapse risk in a large set of homogenously treated pediatric patients with AML. Together, these studies identify *MMRN1* expression as a novel biomarker that may refine AML risk-stratification.

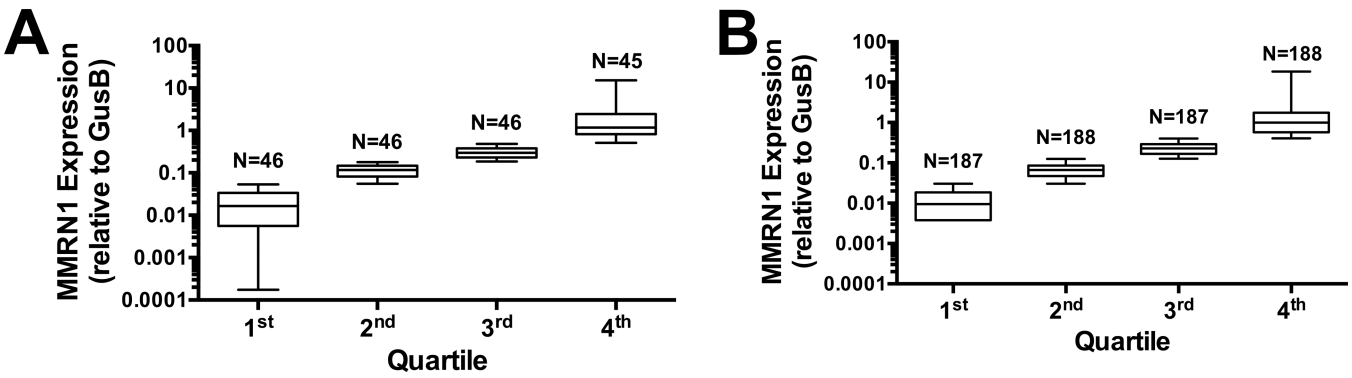
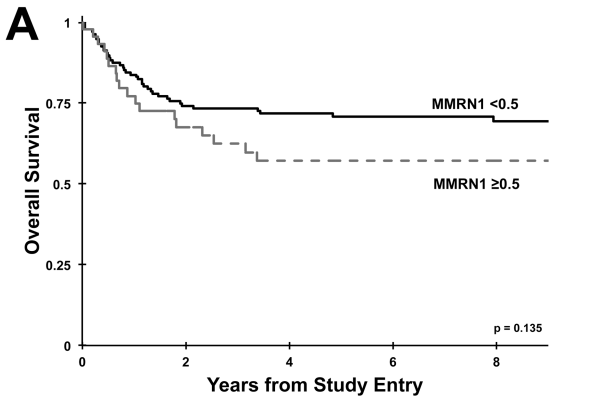
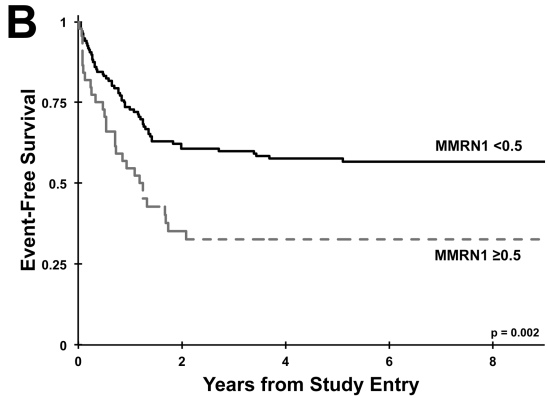


Figure 1. Relative *MMRN1* expression in AAML03P1 and AAML0531

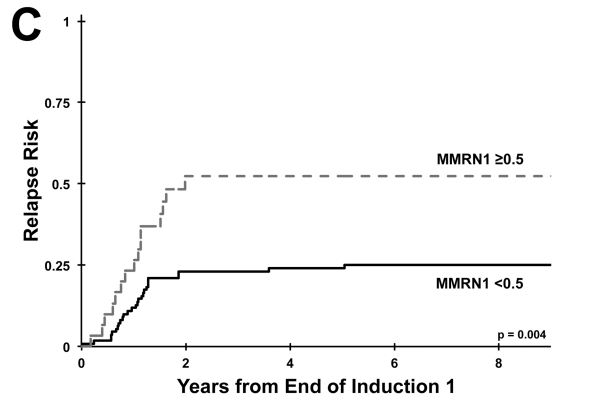
Distribution of relative *MMRN1* expression in diagnostic specimens of **(A)** 183 patients enrolled on AAML03P1 and **(B)** 750 patients enrolled on AAML0531 who were included in this study.



Number at Risk										
At Year	0	1	2	3	4	5	6	7	8	9
Low MMRN1	138	112	99	94	92	81	73	71	43	6
High MMRN1	45	33	26	24	21	19	19	17	12	1



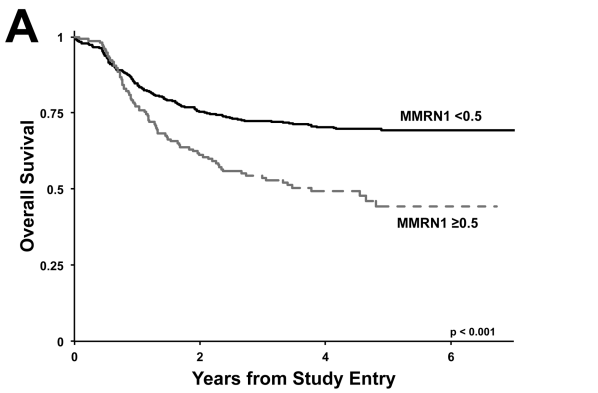
Number at Risk										
At Year	0	1	2	3	4	5	6	7	8	9
Low MMRN1	138	97	81	76	73	63	55	53	33	5
High MMRN1	45	24	13	12	11	10	10	8	4	1



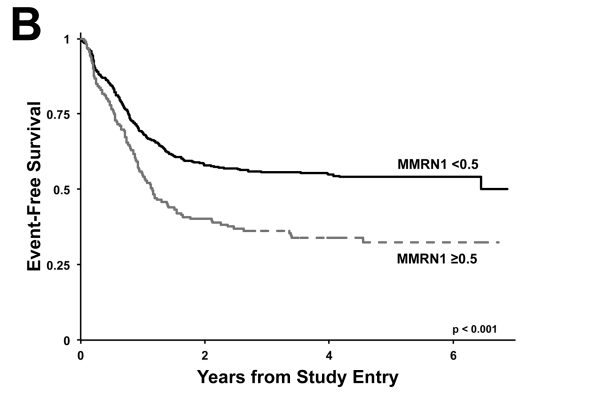
Number at Risk										
At Year	0	1	2	3	4	5	6	7	8	9
Low MMRN1	110	84	71	68	65	57	51	49	27	2
High MMRN1	30	21	10	10	10	9	9	8	4	1

Figure 2. Clinical outcome in patients with high and low *MMRN1* expression in AAML03P1

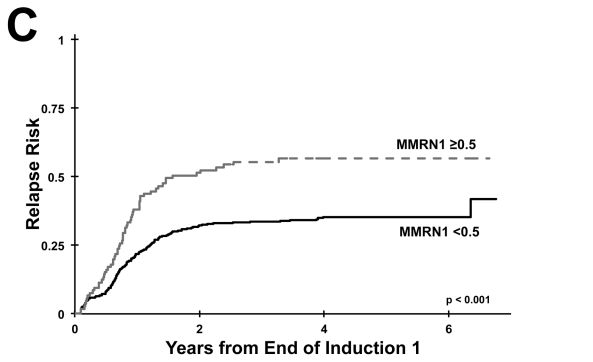
Estimates of the probability of OS **(A)**, EFS **(B)**, and RR **(C)** in patients with high (relative mRNA expression ≥0.5) vs. low (relative mRNA expression <0.5) *MMRN1* expression.



Number at Risk								
At Year	0	1	2	3	4	5	6	7
Low MMRN1	590	490	428	369	256	128	40	1
High MMRN1	160	121	94	73	44	22	5	0



Number at Risk								
At Year	0	1	2	3	4	5	6	7
Low MMRN1	590	399	330	283	204	100	31	0
High MMRN1	160	87	64	50	32	19	4	0



Number at Risk								
At Year	0	1	2	3	4	5	6	7
Low MMRN1	449	320	268	230	158	72	24	0
High MMRN1	106	63	48	38	25	13	3	0

Figure 3. Clinical outcome in patients with high and low *MMRN1* expression in AAML0531

Estimates of the probability of OS **(A)**, EFS **(B)**, and RR **(C)** in patients with high (relative mRNA expression ≥0.5) vs. low (relative mRNA expression <0.5) *MMRN1* expression.

TABLE 1

Comparison of Baseline Characteristics of Patients with Low (<0.5) vs. High (≥0.5) *MMRN1* Expression in AAML0531

Patient Characteristics	Relative MMRN1 Expression		P-value
	Low (<0.5) n = 590	High (≥0.5) n = 160	
Median Age, years (range)	10.5 (0.01–29.8)	6.2 (0.05–19.8)	<0.001
Male Sex, n (%)	298 (51%)	76 (48%)	0.500
WBC ($\times 10^3/\mu L$) - median (range)	27.8 (0.2 – 827)	27.9 (0.9 – 385)	0.467
Median bone marrow blasts, %	68 (0.4–100)	71 (0–100)	0.112
Platelet count ($\times 10^3/\mu L$) - median (range)	48 (1 – 571)	47 (2 – 1,177)	0.984
Hemoglobin (g/dL) - median (range)	8.1 (1.8 – 17)	8.1 (2.4 – 17.0)	0.502
Cytogenetics, n (%)			
Normal	137 (24%)	26 (17%)	0.064
t(8;21)(q22;q22)	111 (19%)	0 (0%)	<0.001
inv(16)/t(16;16)(p13.1;q22)	86 (15%)	3 (2%)	<0.001
t(9;11)(p22;q23) or other abn 11q23	99 (17%)	50 (33%)	<0.001
t(6;9)(p23;q34)	11 (2%)	0 (0%)	0.133
Monosomy 7	3 (1%)	11 (7%)	<0.001
Del7q	5 (1%)	1 (1%)	1.000
-5/5q-	6 (1%)	4 (3%)	0.232
Trisomy 8	28 (5%)	19 (12%)	0.001
Other	84 (15%)	39 (25%)	0.003
Unknown	20	7	
Risk Group, n (%)			
Standard	238 (41%)	111 (73%)	<0.001
Low	276 (48%)	7 (5%)	<0.001
High	63 (11%)	35 (23%)	<0.001
Unknown	13	7	
Molecular alterations, %			
***FLT3*/ITD**	16%	15%	0.708
***NPM1* mutation**	9%	3%	0.016
***CEBPA* mutation**	7%	1%	0.006
***WT1* mutation**	7%	4%	0.175
Hepatomegaly, n (%)	24%	31%	0.087
Splenomegaly, n (%)	24%	31%	0.067
Extramedullary disease, n (%)	15%	13%	0.567
CNS disease, n (%)	6%	9%	0.234
Chloroma, n (%)	15%	13%	0.564
Treatment Arm, n (%)			0.909

Patient Characteristics	Relative MMRN1 Expression		P-value
	Low (<0.5) n = 590	High (≥0.5) n = 160	
Arm A – no GO	298 (15%)	80 (50%)	
Arm B – with GO	292 (49%)	80 (50%)	

TABLE 2

Comparison of Treatment Responses of Patients with Low (<0.5) vs. High (≥0.5) *MMRN1* Expression in AAML0531

Outcome at 5-years	Relative MMRN1 Expression				P-value
	Low (<0.5)	High (≥0.5)	Hazard Ratio	95% Confidence Interval	
All Patients	*n=590*	*n=160*			
OS	69 ± 4%	44 ± 9%	2.01	1.54 – 2.63	<0.001
EFS	54 ± 4%	32 ± 8%	1.72	1.37 – 2.15	<0.001
RR	35 ± 5%	57 ± 10%	1.91	1.42 – 2.57	<0.001
***Low-Risk* Patients**	*n=276*	*n=7*			
OS	82 ± 5%	71 ± 34%	1.93	0.47 – 7.95	0.357
EFS	68 ± 6%	43 ± 37%	2.25	0.82 – 6.13	0.105
RR	26 ± 6%	40 ± 50%	1.89	0.46 – 7.84	0.392
***Standard-Risk* Patients**	*n=238*	*n=111*			
OS	60 ± 7%	47 ± 11%	1.33	0.95 – 1.87	0.092
EFS	44 ± 7%	35 ± 10%	1.24	0.93 – 1.65	0.144
RR	46 ± 8%	61 ± 11%	1.45	1.02 – 2.08	0.045
***High-Risk* Patients**	*n=63*	*n=35*			
OS	52 ± 13%	31 ± 23%	1.40	0.79 – 2.48	0.243
EFS	34 ± 12%	20 ± 17%	1.17	0.72 – 1.92	0.527
RR	44 ± 16%	48 ± 26%	1.07	0.47 – 2.45	0.841

TABLE 3

Univariate and Multivariate Regression Models of OS, EFS, and RR for the AAML0531 Study

	OS				EFS				RR			
	n	**HR**	**95% CI**	***P*-value**	**n**	**HR**	**95% CI**	***P*-value**	**n**	**HR**	**95% CI**	***P*-value**
Univariate Model												
MMRN1 Expression												
<0.5	590	1 (Ref)			590	1 (Ref)			449	1 (Ref)		
≥0.5	160	2.014	1.54–2.63	<0.001	160	1.716	1.37–2.15	<0.001	106	1.908	1.42–2.57	<0.001
Disease Risk*												
Standard-risk	349	1 (Ref)			349	1 (Ref)			251	1 (Ref)		
Low-risk	283	0.346	0.25–0.48	<0.001	283	0.439	0.34–0.56	<0.001	233	0.412	0.30–0.56	<0.001
High-Risk	98	1.263	0.91–1.75	0.157	98	1.386	1.05–1.83	0.020	56	0.878	0.58–1.34	0.543
BM Blast % (per 20%)	697	1.111	0.99–1.24	0.076	697	1.215	1.11–1.33	<0.001	519	1.251	1.10–1.42	<0.001
Platelet Count (per $50\times10^3/\mu L$)	748	1.048	0.99–1.11	0.092	748	1.038	0.99–1.09	0.102	553	1.033	0.97–1.10	0.309
Multivariate Model**												
***MMRN1* Expression**												
<0.5	538	1 (Ref)			538	1 (Ref)			415	1 (Ref)		
≥0.5	144	1.572	1.17–2.12	0.003	144	1.339	1.04–1.73	0.025	93	1.399	1.01–1.94	0.044
Disease Risk*												
Standard-risk	323	1 (Ref)			323	1 (Ref)			232	1 (Ref)		
Low-risk	267	0.316	0.22–0.46	<0.001	267	0.420	0.32–0.56	<0.001	223	0.438	0.31–0.63	<0.001
High-Risk	92	1.031	0.73–1.47	0.865	92	1.177	0.87–1.58	0.283	53	0.838	0.54–1.30	0.434
BM Blast % (per 20%)	682	1.007	0.90–1.13	0.909	682	1.141	1.04–1.26	0.006	508	1.221	1.07–1.39	0.002

* See methods section for definition of cytogenetic/molecular disease risk

** Models were also adjusted for treatment arm and age.