REGULAR ARTICLE

blood advances

# Clinical impact of minimal residual disease in blood and bone marrow of children with acute myeloid leukemia

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**Key Points**

- In pediatric patients with AML, early assessment of blood MRD may help refine risk classification.
- The poor sensitivity and specificity of immunophenotypically identified MRD indicate that molecular MRD is urgently needed in pediatric AML.

The prognostic significance of bone marrow minimal residual disease (MRD) in pediatric patients with acute myeloid leukemia (AML) is well characterized, but the impact of blood MRD is not known. We, therefore, used flow cytometric assessment of leukemia-specific immunophenotypes to measure levels of MRD in both the blood and bone marrow of patients treated in the AML08 (NCT00703820) clinical trial. Blood samples were obtained on days 8 and 22 of therapy, whereas bone marrow samples were obtained on day 22. Among patients who tested as having MRD-negative bone marrow on day 22, neither day-8 nor day-22 blood MRD was significantly associated with the outcome. However, day-8 blood MRD was highly predictive of the outcome among patients who tested as having MRD-positive bone marrow on day 22. Although the measurement of blood MRD on day 8 cannot be used to identify patients who have day-22 MRD–negative bone marrow who are likely to relapse, our findings suggest that day-8 blood MRD results can identify patients with MRD-positive bone marrow who have a dismal prognosis and may be candidates for the early use of experimental therapy.

## Introduction

A meta-analysis[1] that included more than 80 publications and >11 000 patients indicated that in adults and children with acute myeloid leukemia (AML), early response to therapy, as assessed via flow cytometric detection of leukemia-specific immunophenotypes[2] or abnormal phenotypes,[3] detection of fusion transcripts, or next-generation sequencing to measure clearance of leukemia-associated variants,[4-6] is associated with event-free and overall survival. Among adults with AML, the prognostic significance of minimal residual disease (MRD) is independent of specimen source (blood or bone marrow).[7-9] In contrast, studies of MRD in children with AML have focused exclusively on bone marrow MRD.[10] Clinical trials for childhood AML that demonstrated the predictive importance of a bone marrow MRD include AML02, the first such trial, to our knowledge, to prospectively use a real-time assessment of MRD to adapt a therapy.[11] Several other pediatric trials, such as the Nordic Society for Paediatric Haematology and Oncology AML 2004 study[12] and the Children's Oncology Group AAML0531 trial[3] confirmed that assessing the bone marrow MRD is superior to the morphologic assessment of response and is significantly associated with outcome. However, to our knowledge, the prognostic

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RNA seq data is deposited to public sites.

Deidentified individual participant data that underlie the reported results will be made available 3 months after publication.

MRD data are available upon request from the authors, Jeffrey Rubnitz (jeffrey.rubnitz@stjude.org) and Stanley Pounds (stanley.pounds@stjude.org).

The full-text version of this article contains a data supplement.

significance of blood MRD in children with AML has not previously been evaluated. We, therefore, sought to determine the clinical impact of blood MRD among pediatric patients with AML treated in a recent clinical trial.

## Methods

### Patients and material

Pediatric patients with newly diagnosed AML who were treated in the AML08[13] (NCT00703820) clinical trial and had immunophenotypes that were suitable for MRD analysis were included in this study. As previously described, patients received either high-dose cytarabine, daunorubicin, and etoposide (HD-ADE) or clofarabine and cytarabine (Clo/AraC) as the initial therapy.[13] Details regarding therapy, risk classification, and outcomes have been reported. Patients underwent bone marrow aspiration for the morphologic assessment of response and MRD measurement on day 22 (±1 day) of induction therapy, the results of which were used for risk classification and treatment assignment. Blood for MRD analysis was obtained on days 8 (±1 day) and 22 (±1 day) of therapy, but these results were not used for risk assignment. MRD was determined via the flow cytometric assessment of leukemia-specific immunophenotypes that were identified in diagnostic blood or bone marrow specimens. Marker combinations that allowed for the detection of 10 leukemia cells per 10 000 mononuclear bone marrow cells were applied to subsequent samples; at least 100 000 viable mononuclear cells were analyzed in each sample. Results were reported as the percentage of mononucleated cells expressing the leukemia-associated immunophenotype, and MRD positivity was defined as ≥0.1%. RNA was extracted from bulk tumor samples obtained at diagnosis and used to identify recurrent fusion transcripts, as previously described.[14] The AML08 protocol was approved by the review boards of all participating institutions, and written informed consent and assent was obtained from patients, their guardians, or parents.

### Statistical design and analysis

Overall survival was defined as the time elapsed from protocol enrollment to death and was censored at the last follow-up for living patients. Event-free survival was defined as the time elapsed from study enrollment to resistant disease, relapse, second malignancy, or study withdrawal, whichever occurred first, and was censored at the last follow-up for patients without any of these events. The Kaplan-Meier method was used to estimate the overall and event-free survival distributions. The log-rank test was used to compare overall and event-free survival across groups. All tests were two-sided. No multiple-testing adjustments were performed. Statistical analyses were performed using R (www.r-project.org).

## Results

Among the 285 patients treated in AML08, 262 had MRD-evaluable, day-22 bone marrow samples, and 223 and 212 had evaluable, day-8 and day-22 blood samples, respectively. Among patients who tested as having MRD-negative bone marrow, 22% (29 of 134) were had MRD-positive blood results on day 8, whereas only 1 had MRD-positive blood results on day 22. In contrast, among patients with MRD–positive bone marrow results, the blood MRD was positive in 71% (66 of 93) on day 8, and in 72% (60 of 83) on day 22. Rates of blood MRD positivity were higher among patients with a bone marrow MRD ≥1% (49 of 56 [88%] on day 8, and 44 of 52 [85%] on day 22) compared with those with a bone marrow MRD from 0.1% to <1% (14 of 34 [41%] on day 8, and 16 of 31 [52%] on day 22; $P < .01$ for both comparisons).

Patients with negative bone marrow MRD results had favorable outcomes regardless of blood MRD results (Table 1). Among patients with MRD-negative bone marrow results, the outcome of those who had day-8 blood MRD–positive results was nearly identical to the outcome to those who had day-8 blood MRD–negative results, with event-free and overall survival rates of 68.1% ± 8.8% and 82.1% ± 7.3%, respectively, compared with 66.2% ± 4.7% and 81.0% ± 3.9%, respectively (Table 1). Although small numbers of patients preclude statistical comparisons, it appears that the favorable outcome of patients who had day-8 MRD–positive/day-22 bone marrow MRD–negative results was restricted to those with core-binding factor (CBF) leukemia (0 relapses among 9 patients with CBF compared with 9 relapses among 20 patients with non-CBF leukemia). Hematopoietic cell transplantation (HCT) in the first remission was performed only among patients who had day-8 MRD–positive/day-22 bone marrow MRD–negative results and high-risk genetic features. Of the 7patients who underwent HCT, 5 relapsed; of the 22 who did not receive HCT, only 3 relapsed.

Among patients with positive bone marrow MRD, day-22 blood MRD positivity was not significantly associated with event-free or overall survival (supplemental Table 1; supplemental Figure 1). In contrast, patients who had day-8 blood MRD–positive/day-22 bone marrow MRD–positive results had significantly worse outcomes than those who had day-8 blood MRD–negative/day-22 bone marrow MRD–positive results (3-year event-free survival, 31.5% ± 5.9% vs 63.0% ± 9.3% [$P < .01$]; 3-year overall survival, 46.5% ± 6.4% vs 77.0% ± 8.3% [$P = .01$]; Table 1; Figure 1). In addition, the impact of day-8 blood MRD differed dramatically per the treatment arm (Table 2). Among patients who were initially treated with Clo/AraC, there were no significant differences in event-free or overall survival based on the day-8 blood MRD. In contrast, patients who had day-8 blood MRD–positive/day-22 bone marrow MRD–positive results after the initial treatment with HD-ADE had event-free and overall survival rates of only 10.7% ± 5.8% and 26.8% ± 8.6%, respectively, compared with 80.0% ± 2.6% and 90.0% ± 9.5%, respectively, for those who had day-8 blood MRD–negative/bone marrow MRD–positive results (Figure 2; $P < .01$ for both comparisons). Overall, 39 of 66 patients who had day-8 blood MRD–positive/day 22 bone marrow MRD–positive results underwent HCT, of whom 16 relapsed.

Multivariable analysis was performed in each treatment arm and included day-8 blood MRD, day-22 bone marrow MRD, genetically-defined risk (excluding MRD response–driven risk changes), and age (supplemental Table 3) as the criteria. In patients treated with HD-ADE, day-22 bone marrow MRD, intermediate-risk (vs low-risk) genetics, and older age were associated with inferior event-free and overall survival. High-risk (vs low-risk) genetics was associated with an inferior overall and marginally inferior event-free survival. Day-8 blood MRD was associated with marginally inferior event-free with no difference in the overall survival. Among patients treated with Clo/AraC, intermediate-risk genetics were

**Table 1. Outcome based on day-22 bone marrow and day-8 blood MRD results**

| MRD | 3-year EFS | *P* value | 3-year OS | *P* value |
|---|---|---|---|---|
| Bone marrow–positive (n = 93) | | | | |
| Blood-positive (n = 66) | 31.5% ± 5.9% | <.01 | 46.5% ± 6.4% | .01 |
| Blood-negative (n = 27) | 63.0% ± 9.3% | | 77.0% ± 8.3% | |
| Bone marrow–negative (n = 133) | | | | |
| Blood-positive (n = 29) | 68.1% ± 8.8% | NS | 82.1% ± 7.3% | NS |
| Blood-negative (n = 104) | 66.2% ± 4.7% | | 81.0% ± 3.9% | |

EFS, event-free survival; NS, not significant; OS, overall survival.

associated with an inferior overall survival and marginally inferior event-free survival, whereas high-risk genetics were associated with a marginally inferior overall survival.

Transcriptome sequencing to identify recurrent fusion transcripts was successfully performed for 249 patients. The most frequently detected fusion transcripts were *RUNX1*::*RUNX1T1* (n = 38; 15%), *CBFB*::*MYH11* (n = 28; 11%), and *KMT2A* fusions (n = 76; 31%). Among the 66 patients with *RUNX1*::*RUNX1T1* or *CBFB*::*MYH11*, 8 relapsed, including 2 who tested positive for MRD in the blood and bone marrow and 6 who tested negative for MRD. Thus, the sensitivities of the day-8 blood MRD and day-22 bone marrow MRD at predicting relapse were only 25%, with specificities of 79% and 86%, respectively. Of the 76 patients with *KMT2A* rearrangements, 28 relapsed, including 9 with positive blood and bone marrow MRD results and 3 with only positive bone

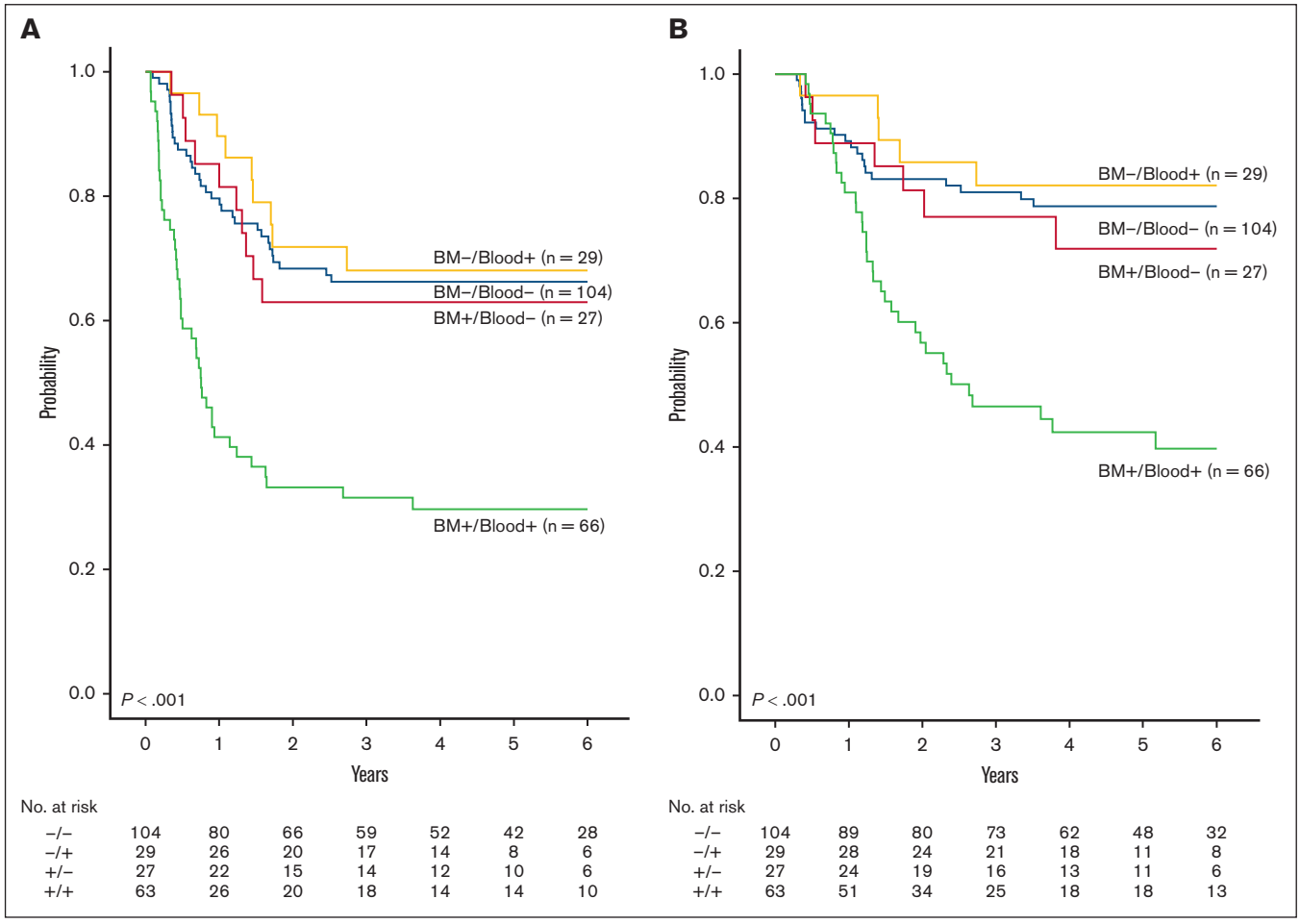


**Figure 1. Outcome based on day-22 bone marrow and day-8 blood MRD results.** (A) Event-free survival. *P* value is shown for overall comparison. *P* < .01 for bone marrow (BM)-positive/blood-negative vs BM-positive/blood-positive MRD result comparison. (B) Overall survival. *P* value is shown for overall comparison. *P* = .01 for BM-positive/blood-negative vs BM-positive/blood-negative MRD result comparison.

**Table 2. Outcome based on day-22 bone marrow and day-8 blood MRD results and the reatment arm**

| | HD-ADE | | | Clo/AraC | | |
|---|---|---|---|---|---|---|
| **MRD** | **N** | **3-year EFS** | **3-year OS** | **N** | **3-year EFS** | **3-year OS** |
| BM-positive | | | | | | |
| Blood-positive | 28 | 10.7% ± 6% | 26.8% ± 9% | 35 | 49.8% ± 9% | 64.0% ± 8% |
| Blood-negative | 10 | 80.0% ± 3% | 90.0% ± 10% | 14 | 53.3% ± 13% | 72.2% ± 12% |
| BM-negative | | | | | | |
| Blood-positive | 18 | 71.8% ± 11% | 83.0% ± 9% | 9 | 55.6% ± 17% | 77.8% ± 14% |
| Blood-negative | 52 | 62.1% ± 7% | 76.1% ± 6% | 44 | 71.2% ± 7% | 88.1% ± 5% |

BM, bone marrow; EFS, event-free survival; OS, overall survival.

marrow MRD results. Like the findings observed among patients with CBF leukemia, the sensitivities of the day-8 blood MRD and day-22 bone marrow MRD results at predicting relapse were only 21% and 46%, respectively, with specificities of 76% and 70%.

Genetic alterations associated with high rates and levels of MRD included *DEK*::*NUP214*,[15] *KMT2A*::*USP2*,[16] *CBFA2T3*::*GLIS2*,[17] *PICALM*::*MLLT10*, *NUP98* fusions,[18] and tandem duplications in *UBTF* (*UBTF*-TD).[19] Among 35 patients with these high-risk alterations, 33 had positive day-8 blood MRD results and 27 had positive bone marrow MRD results. Relapse rates were particularly high among the 23 patients with *CBFA2T3*::*GLIS2*, *NUP98* fusions, or *UBTF*-TD, of whom 15 relapsed, including 13 who had at least 1 sample that was tested MRD positive (sensitivity, 87%; specificity, 17%).

## Discussion

Although the bone marrow MRD results have consistently proven to be prognostically important in pediatric AML, the role of blood MRD is yet to be evaluated. The use of blood MRD is attractive because it is more readily accessible than bone marrow and may allow for earlier, noninvasive, and more frequent evaluation of response to therapy. These issues are particularly saliant in pediatrics because children are routinely sedated for bone marrow evaluations, a practice associated with adverse neurocognitive

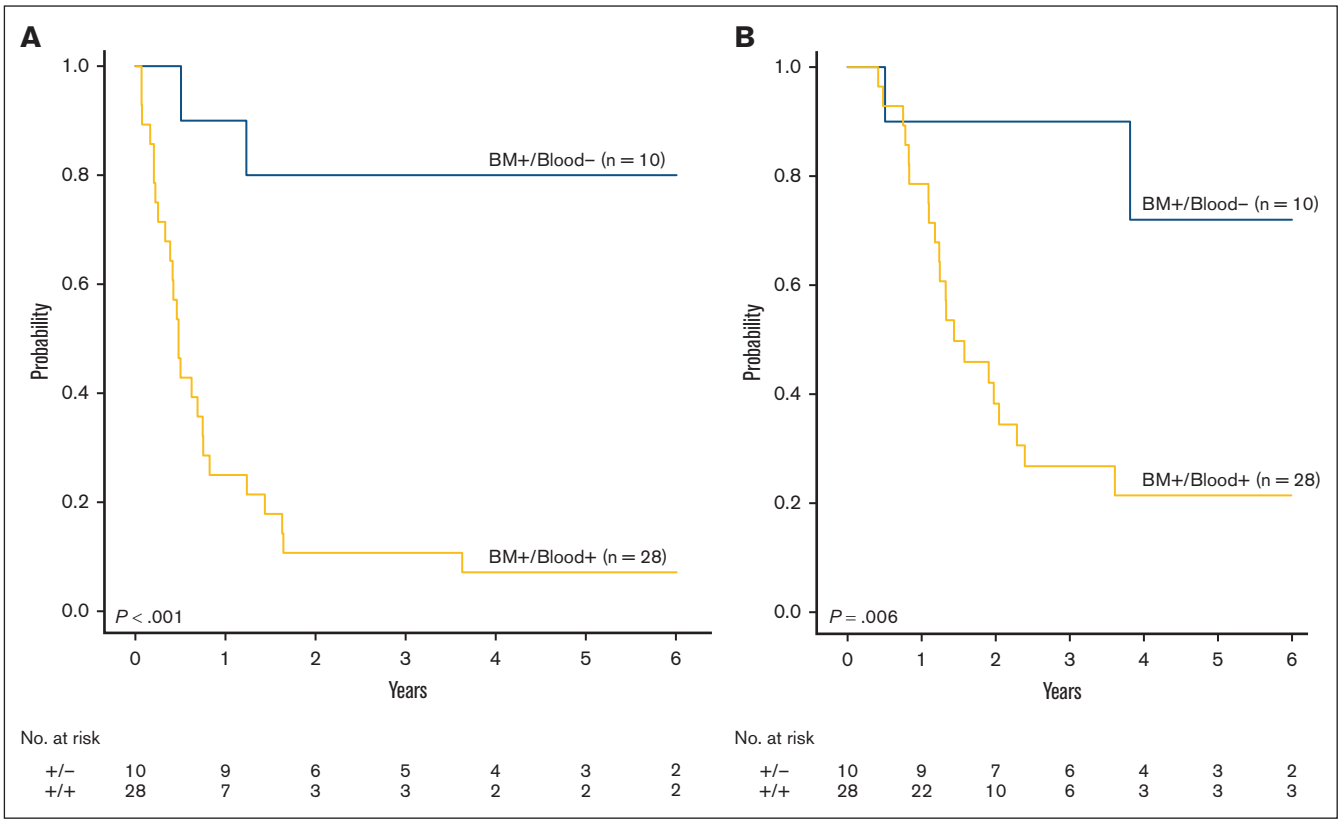


**Figure 2. Outcome of patients with MRD-positive bone marrow results treated in the HD-ADE arm of AML08 based on day-8 blood MRD results.** (A) Event-free survival and (B) overall survival.

outcomes.[20,21] Our data show that among patients who had bone marrow MRD–negative resultson day 22, neither day-8 nor day-22 blood MRD was associated with the outcome. Patients who had positive blood MRD results on day 8 but cleared their MRD by day 22 had outcomes that were similar to those of patients who had MRD-negative outcomes at both points, indicating that the day-8 blood MRD alone cannot be used for risk stratification, nor can it identify patients who will have bone marrow MRD–negative results on day 22 and are likely to relapse. However, it must be noted that the favorable outcome of patients with day-8 MRD–positive blood/day-22 MRD–negative bone marrow results was largely attributable to the excellent outcome of patients with CBF leukemia. Patients without CBF leukemia who had MRD-positive results on day 8 had a poor outcome even if they had MRD-negative results on day 22.

By contrast, day-8 blood MRD was highly predictive of the outcome among patients who had bone marrow MRD–positive results on day 22. In this study, day-8 blood MRD results distinguished patients with MRD-positive bone marrow results who had a dismal prognosis regardless of subsequent treatment from those whose outcome was the same as that of patients with MRD-negative bone marrow results. Of the patients with MRD-positive bone marrow results, ~30% had MRD-negative blood results on day 8 and had a 77% survival rate, suggesting that risk stratification and treatment allocation may be improved by incorporating an early measurement of blood MRD with an end-of-induction assessment of bone marrow MRD. Because the end-of-induction bone marrow MRD is currently used for risk stratification in many clinical trials, such a strategy could potentially reduce the percentage of patients for whom HCT is recommended, thus sparing these patients the toxicities associated with transplant. However, our results must be interpreted cautiously because the favorable outcome of the patients with day-8 MRD–positive bone marrow/day-8 MRD–negative blood results may be partly attributed to the use of HCT in some of these cases.

The dismal outcome of patients with positive MRD results at both time points indicates that conventional therapy is unable to cure most of these patients despite the MRD-guided intensification and suggests that these patients may be candidates for the earlier use of experimental therapy. Currently, enrollment in most early-phase clinical trials is limited to patients who have achieved remission and subsequently relapsed and patients who have refractory leukemia after 2 courses of induction therapy. However, in the standard (3-drug) treatment arm of AML08, the event-free survival of patients who had MRD-positive blood and bone marrow results was only 11%, indicating that they did not benefit from a second induction course of conventional chemotherapy. We believe that the dismal outcome of this subgroup of patients is related to their persistent disease and likely resistance to 3 major classes of chemotherapy (anthracyclines, nucleoside analogues, and topoisomerase inhibitors), whereas patients in the Clo/AraC arm had received only 1 class of agent. Thus, resistant disease on day 8 in the ADE arm implies a greater resistance to all relevant classes of agent. In contrast, persistent disease in the clofarabine arm may still include disease that is sensitive to anthracyclines or topoisomerase inhibitors, thus allowing these patients to be more effectively salvaged via induction-2 and subsequent therapy.

The small numbers of patients in the high-risk genetic subgroups make it difficult to determine the impact of MRD within each group. All 5 patients who tested positive for *CBFA2T3*::*GLIS2* fusion and MRD after 1 course of induction remained with MRD-positive results after their second course, as did 4 of the 8 patients who tested positive for *NUP98* fusion. This indicates that the second cycle of therapy might have increased the toxicity without providing an apparent benefit for most of these patients and suggests that they, too, should be eligible for experimental therapies after 1 course of induction therapy if the promising targeted therapies are available. By contrast, all 6 patients with *DEK*::*NUP214* or *KMT2A*::*USP2* fusions had persistent blood and marrow MRD results, but only 1 relapsed, and all 6 are alive. The good outcome of those with *DEK*::*NUP214* fusions is consistent with the observation that this subgroup of patients has a favorable outcome if they undergo HCT during the first remission.[15] Among the 30 patients with *CBFA2T3*::*GLIS2*, *NUP98*, or *UBTF*-TD fusions, 15 relapsed, of whom, 13 had MRD-positive day-8 blood or day-22 bone marrow (or both) results. The deaths of 19 of 30 patients confirm the dismal prognosis of these subgroups and the urgent need for novel therapies.[17-19] Preclinical data indicate that menin inhibitors may be active in patients with *NUP98* fusions,[22] and at least 5 inhibitors are in clinical development.[23]

Although our data suggest that the combination of day-8 blood MRD and day-22 bone marrow MRD results should be considered in future trials to refine risk classification, our study is limited by small patient numbers and multiple comparisons. In addition, as demonstrated in AML08,[13] the prognostic impact of MRD depends on the treatment given. Thus, another limitation of the present study is that the impact of day-8 blood MRD results may not hold true for patients who are treated differently. However, we do not have a replication data set by which we can confirm these findings, but we will continue to study the clinical significance of early detection of blood MRD in future studies. A minor limitation was that blood MRD sample data were missing for a subset of patients, although this most likely did not affect our conclusions because these patients were similar to patients for whom samples were available.

Our results also confirm the limitations of flow-based MRD, in which detection of leukemia-associated immunophenotypes was used to detect residual leukemia. For example, among patients with *CBFA2T3*::*GLIS2*, *NUP98*, or *UBTF*-TD fusions, the sensitivity of MRD was high, but the specificity was low, because 10 patients with MRD-positive bone marrow results did not relapse. By contrast, the sensitivity of MRD at predicting relapse among patients with CBF or *KMT2A*-rearranged leukemia was low, with 66% of relapses occurring in patients who had no detectable blood or bone marrow MRD. These findings align with our those in a prior report that patients with CBF AML who had MRD-positive results had excellent outcomes, whereas those with high-risk *KMT2A* rearrangements had poor outcomes even in the context of a bone marrow with no detectable MRD at the end of induction.[24] These results strongly suggest that flow-based MRD detection should be supplemented with molecular techniques. For adults with AML, RT-PCR detection of fusion transcripts or *NPM1* mutations[6] as well as next-generation sequencing to measure the clearance of leukemia-associated variants[4,5,25,26] have been successfully used to monitor MRD and have been shown to be highly associated with the outcome. Such methods have recently been incorporated into the consensus guidelines for the assessment of MRD in adults with AML[27] but have not yet been thoroughly evaluated in children. To address this need, we are currently

performing comprehensive genomic testing on all patients enrolled in our ongoing AML clinical trial and using next-generation sequencing techniques to detect the persistence (or lack thereof) of mutant alleles in DNA obtained from bone marrow and blood so that we can prospectively compare flow-based MRD with the molecular detection of somatic mutations.

Although small numbers preclude definitive conclusions, the apparent discrepant results in different genomic subsets relative to bone marrow and blood MRD suggest some possible reasons for the observed results. For example, because CBF AML is sensitive to intensified therapy, the peripheral leukemic burden as measured via a day-8 blood MRD has less prognostic significance. In contrast, several high-risk genetic subgroups have relatively chemoresistant stem cells, resulting in a high risk of treatment failure despite apparent disease clearance as measured via a blood MRD negativity by day 8. Replication of our findings in additional cohorts could further support or refute these possibilities.

We hope to improve the risk classification of children with AML in our next trial by incorporating a combination of molecular and immunophenotypic detection of MRD in the blood on day 8 and the bone marrow at the end of the first course of induction therapy so that we can tailor each patient's therapy based on their genomics and response to therapy.

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## Authorship

Contribution: S.E.K. and J.E.R. provided patient care, analyzed the data, and prepared the manuscript; E.C.-S. and J.M.K. performed MRD analyses; S.P. and L.W. provided statistical support; H.I., R.C.R., and C.-H.P. provided patient care and analyzed data; and all authors read and approved the final manuscript.

Conflict-of-interest disclosure: E.C.-S. is a coinventor of the US patent application 16/757,137 filed on 26 October 2018 and titled, "A new approach for universal monitoring of minimal residual disease in acute myeloid leukemia." The remaining authors declare no competing financial interests.

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