




Quantifying *NPM1* MRD in AML patients prior to allogeneic stem cell transplantation: Where to draw the line?

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Today, assessment of measurable residual disease (MRD) increasingly allows a dynamic risk stratification and informs treatment decisions in acute myeloid leukemia (AML).¹ For AML patients with *NPM1* gene mutations quantitative PCR methods have proven to be reliable tools for MRD measurement.¹ Detection of mutated *NPM1* transcript levels is highly informative at various time points during the disease course,^{2–4} including before allogeneic hematopoietic stem cell transplantation (HSCT).^{3,5–8} Published studies consistently indicate overall survival (OS) differences of approximately 40%–50% at 2 years after HSCT comparing *NPM1* MRD-positive and *NPM1* MRD-negative patients.^{3,6–9} However, the MRD thresholds identified and adapted differ substantially between studies, ranging from the limit of detection of the respective test, often 0.001%,^{6,7} to 0.1%³ or even 1%⁸ *NPM1/ABL1* copies. Reasons for these differences are based on characteristics of the respective MRD assays, varying statistical approaches, or different priorities, for example, avoiding “overtreatment” versus identifying every patient at risk. This has resulted in distinct proportions of patients identified to be at high risk of adverse outcomes in each study.^{2,6–8} Additionally, due to still insufficient interlaboratory harmonization, *NPM1* MRD result comparability remains limited. Here we systematically analyzed different *NPM1* MRD thresholds to identify *NPM1*-mutated AML patients at high risk of relapse following HSCT.

We retrospectively analyzed 81 *NPM1*-mutated AML patients who received an allogeneic HSCT at our institution. All had available material for MRD analysis obtained up to 28 days before HSCT in complete remission (CR) or CR with incomplete recovery (CRI) (bone marrow, $n = 30$, or peripheral blood, $n = 51$). Median age at HSCT was 61.4 (range: 33.1–76.4) years. Most patients received reduced-intensity or non-myeloablative conditioning (81%), and 19% of patients were transplanted following myeloablative conditioning. Further patients' characteristics are given in Supporting Information S1: Table S1. Written informed consent was obtained in accordance with the Declaration of Helsinki. Median follow-up after HSCT was 4.4 years. *NPM1* MRD was measured by

digital droplet PCR using a competitive probe approach and normalized to *ABL1* as previously described.⁷ For patients with a positive *NPM1* MRD test result, distinct cut-offs were analyzed using receiver operator characteristics (ROC) curves to define the clinically most relevant threshold, starting from the limit of detection (0.001% *NPM1/ABL1*) in log steps up to 10% *NPM1/ABL1*. As potentially different detection levels in blood and bone marrow have been reported in some studies,^{2,6,10} ROC curves were analyzed separately for both tissues. However, the largest area under the curve was detected for a 0.01% *NPM1/ABL1* cut-off to define MRD-positive or MRD-negative patients in both bone marrow and blood, respectively (Figure 1A,B and Supplementary Table S2), which is why for further analyses, both were analyzed together. Twenty-five patients (31%) relapsed after HSCT. Adapting the 0.01% *NPM1/ABL1* cut-off, we observed the clearest separation of the cumulative incidence of relapse (CIR, $p < 0.001$, after 2 years 4% vs. 66%, Figure 1C, resulting in a 93.8% relative risk reduction for relapse after 2 years for MRD-negative [$n = 50$] compared to MRD-positive [$n = 31$] patients), as well as OS curves ($p < 0.001$, after 2 years 76% vs. 38%; Supporting Information S1: Figure S1A). Higher cut-offs resulted in steeply declining sensitivities and negative predictive values, while “relapse prediction” in MRD-positive patients did not improve much: Compared to a 0.01% *NPM1/ABL1* cut-off, a stepwise higher proportion of MRD-negative patients experienced relapse for 0.1% and 1% *NPM1/ABL1* cut-offs (at 2 years 11% vs. 67% and 21% vs. 73%, respectively; Figure 1D,E). Only at the highest cut-off of 10% *NPM1/ABL1* copy numbers, all MRD-positive patients relapsed, but at this cut-off “MRD-negative” patients had a CIR as high as 25% after 2 years (Figure 1F), with a negative predictive value of only 0.72 (Supporting Information S1: Table S3). Subsequently, we identified the 0.01% *NPM1/ABL1* cut-off in remission before HSCT as the cut with the highest discriminative power for relapse after HSCT in our cohort. Patients in MRD-positive remission (at least 0.01% *NPM1/ABL1*) had a significantly higher white blood cell count ($p = 0.04$) and higher LDH levels ($p = 0.02$) at diagnosis. Even though the co-mutational profile at diagnosis did not differ between both groups

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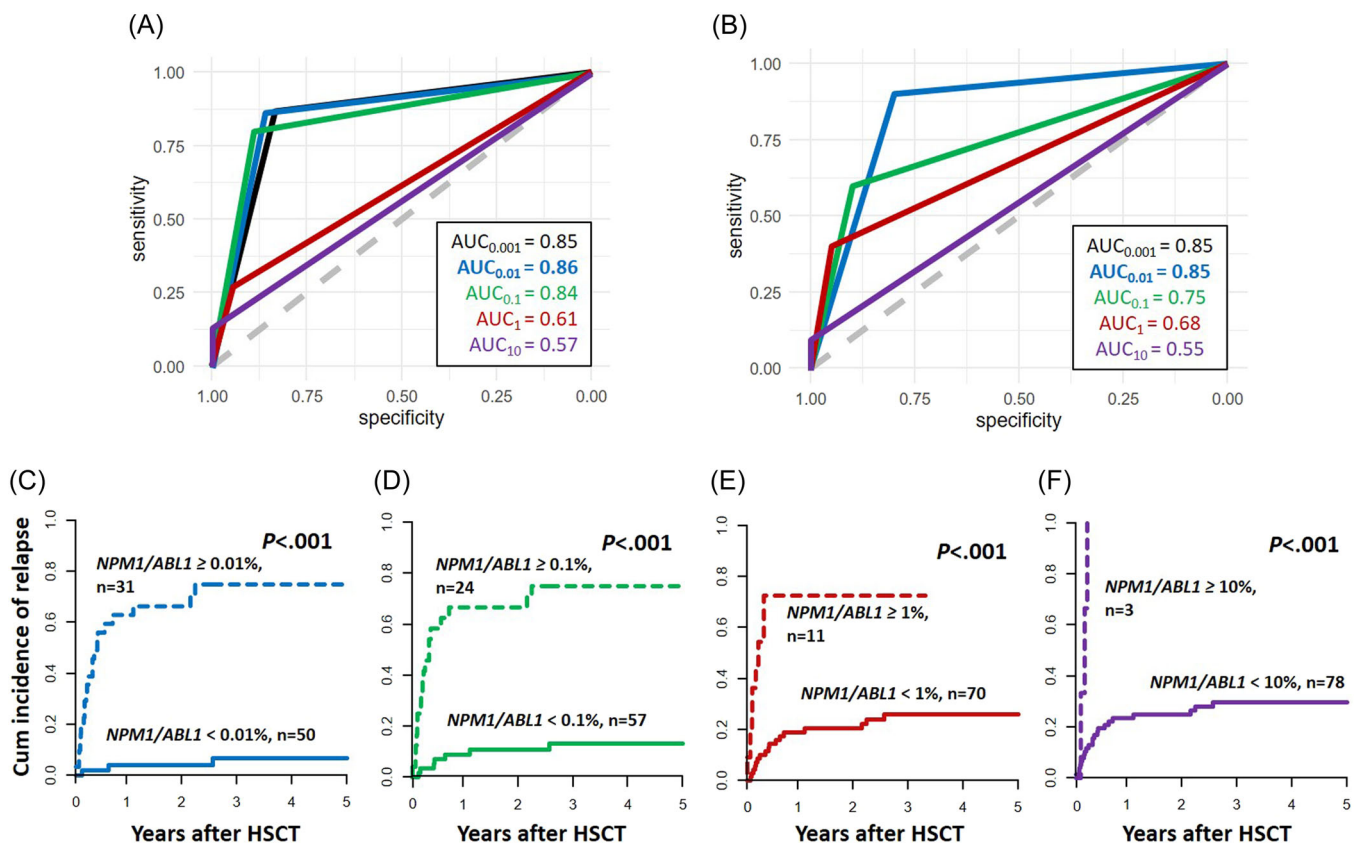


FIGURE 1 Impact of distinct prehematopoietic stem cell transplantation (HSCT) *NPM1/ABL1* measurable residual disease (MRD) thresholds on relapse probability after HSCT. (A, B) Receiver operator characteristics curve for MRD values analyzed in (A) peripheral blood ($n = 50$), and (B) bone marrow ($n = 31$). (C–F) Cumulative incidence of relapse according to pre-HSCT *NPM1/ABL1* MRD levels adapting the thresholds (C) 0.01% (cumulative incidence of relapse [CIR] after 2 years 4% vs. 66%), (D) 0.1% (CIR after 2 years 11% vs. 67%), (E) 1% (CIR after 2 years 21% vs. 73%), and (F) 10% (CIR after 2 years 25% vs. 100%). AUC, area under the curve.

(Supporting Information S1: Table S1 and Figure S2), patients remaining *NPM1* MRD-positive at HSCT by trend more often had a pretreatment *FLT3*-ITD mutant-to-wild-type ratio > 0.5 ($p = 0.08$). Notably, the number of chemotherapy cycles prior to HSCT did not differ between MRD-positive and MRD-negative patients ($p = 0.20$), indicating that MRD-positive patients may not benefit from additional conventional chemotherapy cycles. Whether shifting treatment strategies to, for example, venetoclax plus hypomethylating agents before HSCT may lead to MRD-conversion and outcome improvements in these patients should be evaluated in clinical studies. *NPM1* MRD-positive patients more often were transplanted in a second remission of their AML ($p = 0.03$) and patients transplanted in a second remission had higher *NPM1/ABL1* copy numbers at HSCT compared to those transplanted in first remission ($p = 0.02$), a finding that may contribute to the previously described adverse outcomes of patients transplanted in second remission.¹¹

While AML patients with an MRD burden above 0.01% *NPM1/ABL1* cut-off had significantly higher relapse rates, we observed a negative correlation between *NPM1/ABL1* MRD levels before HSCT and time to relapse; the higher the *NPM1* MRD burden at HSCT, the earlier the relapse occurred ($r = -0.5$, $p = 0.03$; Supporting Information S1: Figure S3). This correlation was strongest in patients with *FLT3*-ITD present at diagnosis ($r = -0.82$, $p = 0.002$). When introducing two cuts for patients with low ($\geq 0.01\%$ but $< 1\%$) or high ($\geq 1\%$) *NPM1/ABL1* MRD levels, there was a significantly shorter time to relapse in AML patients with high *NPM1* MRD levels prior to HSCT

compared to patients with low MRD burden (Figure 2A,B). In addition, compared to patients with low *NPM1* MRD levels, patients with high *NPM1* MRD levels also showed a trend for shorter OS ($p = 0.10$; Figure 2C), indicating that patients transplanted with higher *NPM1* MRD may be harder to salvage after relapse following HSCT.

Recently, Dillon et al.⁶ presented data of 107 *NPM1*-mutated AML patients prior to HSCT, implicating that patients with low *NPM1* MRD burden (less than 1% [blood] or 0.2% [bone marrow]) only had shorter survival when they harbored a *FLT3*-ITD ($n = 22$ vs. $n = 8$).⁶ However, in our cohort the negative impact of a low or high *NPM1* MRD at HSCT was not modified by the presence or absence of an *FLT3*-ITD (Supporting Information S1: Figure S4), which might be a result of the restricted sample size. Of note, in our cohort, none of the patients without a diagnostic *FLT3*-ITD that were MRD-negative at HSCT suffered a relapse during the follow-up time.

Previous studies tried to identify the most informative *NPM1* MRD thresholds at other time points than prior to HSCT. After chemotherapy consolidation, Krönke et al.² and Shayegi et al.¹² suggested 2% and 1% *NPM1/ABL1* MRD, respectively, as the values most informative for relapse. This led to the implementation of a 1% cut in some studies evaluating preemptive treatment strategies in MRD-positive patients.¹³ However, after an allogeneic HSCT, also 10% *NPM1/ABL1* was described as clinically relevant¹²—likely as a result of graft-versus-leukemia (GvL) effects providing continuous therapeutic effects.

One could speculate that the much lower *NPM1* MRD levels observed as most discriminative in our cohort may result from the lower

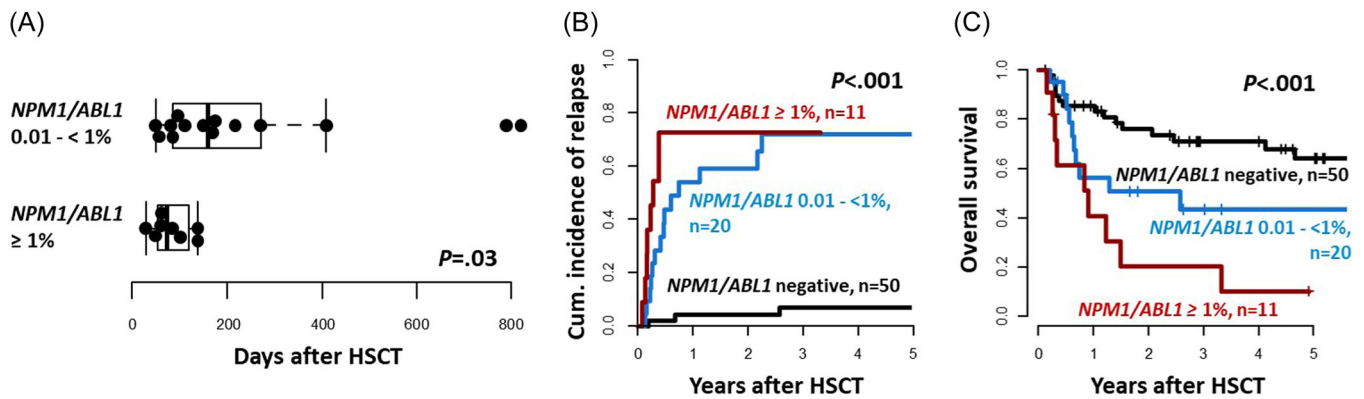


FIGURE 2 Outcomes according to high ($\geq 1\%$) or low ($< 1\%$) *NPM1/ABL1* measurable residual disease (MRD) levels. (A) Time to relapse in relapsing patients (median time to relapse in patients with 0.01% - $< 1\%$ *NPM1/ABL1* burden at hematopoietic stem cell transplantation (HSCT): 159 days; median time to relapse in patients with $\geq 1\%$ *NPM1/ABL1* burden at HSCT: 75 days), (B) cumulative incidence of relapse and (C) overall survival.

intensity conditioning regimens providing less disease control than myeloablative HSCT. However, in separate analyses for reduced-intensity/nonmyeloablative and myeloablative HSCT, despite limited patient numbers, the cut-off had prognostic power irrespective of the conditioning intensity (Supporting Information S1: Figure S5). Also in a recent study adapting NGS, adverse outcomes after HSCT were predicted by a very low MRD burden (0.01% variant allele fraction).⁹ Remarkably, outcomes of MRD-positive patients after reduced-intensity HSCT improved when the conditioning regimen was melphalan-based, showing potential for clinical intervention.⁹

Since mutated *NPM1* has been described as immunogenic, *NPM1*-mutated patients may benefit from HSCT consolidation.^{14,15} We evaluated the prognostic impact of a chronic graft-versus-host disease (GvHD) as a surrogate marker for GvL effects. In a landmark analysis for patients surviving longer than 100 days, CIR was reduced in *NPM1* MRD-positive patients with chronic GvHD (25% after 2 years), compared to *NPM1* MRD-positive patients who did not develop a chronic GvHD (92% after 2 years; Supporting Information S1: Figure S6). However, OS did not differ ($p > 0.99$) as MRD-positive patients with a chronic GvHD tended to have higher nonrelapse mortality (NRM; $p = 0.06$). Thus, patients transplanted with positive *NPM1* MRD may benefit from immunologic interventions such as donor lymphocyte infusions to augment GvL but should be selected very carefully to avoid increasing NRM.

Our study has some limitations, including the small sample size and use of blood and bone marrow for MRD assessment. Additional studies—or meta-analyses of existing ones—will be needed to confirm our findings. In conclusion, in our cohort, *NPM1/ABL1* MRD levels prior to allogeneic HSCT were most informative when applying a 0.01% cut-off. Higher *NPM1/ABL1* thresholds resulted in lower sensitivity and negative predictive values without a meaningful gain of specificity. These results were independent of the analyzed tissue (blood vs. bone marrow), conditioning intensity, or patients' age (Supporting Information S1: Figure S7). Higher levels of *NPM1* MRD burden at HSCT are associated with a shorter time to relapse after HSCT and may be harder to salvage following relapse after HSCT.

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AUTHOR CONTRIBUTIONS

Madlen Jentzsch and Sebastian Schwind contributed to the design and analysis of this study and the writing of the manuscript. Madlen Jentzsch, Lara Bischof, Marius Bill, Juliane Grimm, Jule Ussmann, Donata Backhaus, Tung Pham Thanh, and Dominic Brauer carried out the laboratory-based research. Madlen Jentzsch and Sebastian Schwind performed statistical analyses. Maximilian Merz, Marco Herling, Klaus H. Metzeler, Vladan Vucinic, Georg-Nikolaus Franke, Uwe Platzbecker, and Sebastian Schwind provided administrative support. Madlen Jentzsch, Lara Bischof, Marius Bill, Juliane Grimm, Jule Ussmann, Donata Backhaus, Dominic Brauer, Tung Pham Thanh, Maximilian Merz, Georg-Nikolaus Franke, Klaus H. Metzeler, Vladan Vucinic, Marco Herling, Uwe Platzbecker agreed on the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

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