Molecular minimal residual disease negativity and decreased stem cell mobilization potential predict excellent outcome after autologous transplant in *NPM1* mutant acute myeloid leukemia

Although most solid tumors are genetically more complex than acute myeloid leukemia (AML), the disease is heterogeneous at the cytogenetic and molecular level.[1] Despite comprehensive clarification of the mutational landscape, treatment concepts remain based on cytogenetics, a limited collection of molecular markers, and cytomorphologic and immunophenotypic remission assessment. Accordingly, allogeneic stem cell transplantation (SCT) is proposed to patients with an adverse-risk disease, but not to those with a favorable risk. Ambiguity on the preferred consolidation option exists in cytogenetically normal AML, which affects ~50% of younger adult patients. Autologous SCT may be performed in younger adults with good- or intermediate-risk AML.[2-4] The advantages are the high anti-leukemic efficacy, while avoiding the morbidity of graft-*versus*-host disease associated with allogeneic SCT.

The most common mutations, observed in a third of AML patients and more than half of normal karyotype AML, occur in exon 12 of the *NPM1* gene.[5] The implementation of mutated *NPM1* as a target for minimal residual disease (MRD) has greatly consolidated the armamentarium how best to advise cytogenetically normal AML patients.[6] It has been suggested that AML patients <50 years in first complete remission (CR1) with MRD-negativity may have no benefit from allogeneic SCT, whereas patients with MRD-positivity after induction may benefit from myeloablative conditioning.[7]

At our center, we consistently consolidate *NPM1*mut/*FLT3*-wild-type patients with autologous SCT, but also *NPM1*mut/*FLT3*-ITD-positive patients in the absence of an available donor or according to patient preference.[4,8-10]

In previous studies including various AML subtypes, we observed a wide variation in mobilized circulating CD34^{+} cells at the day of peripheral stem cell collection after two induction cycles.[11-12] We identified a poor stem cell mobilization potential (CD34^{+low}) to predict favorable long-term outcome after autologous SCT, whereas high levels of mobilized CD34^{+} cells (CD34^{+high}) were associated with adverse outcome. We therefore hypothesized that the combined use of molecular *NPM1* MRD information and stem cell mobilization potential may provide more refined prognostic information, thereby identifying those *NPM1*mut AML patients, which may benefit most from autologous SCT in CR1.

In this retrospective single-center analysis, we investigated 42 consecutive adult AML patients with mutated *NPM1* at first diagnosis. All patients received intensive induction at Bern University Hospital (01/2008-06/2018). The study was approved by a decision of the local ethics committee of Bern (#223/15). A median value of 45 CD34^{+} cells/μL peripheral blood at the day of autologous stem cell collection dichotomized patients between low (<45 CD34^{+} cells/μL; CD34^{+low}) and high mobilizers (≥45 CD34^{+} cells/μL; CD34^{+high}). Patients' clinical characteristics are presented in the *Online Supplementary Tables S1-S2*. There were no significant differences between CD34^{+low} and CD34^{+high} mobilizing patients, and the proportion of patients with concomitant *FLT3*-ITD mutations was comparable. *NPM1* muta-

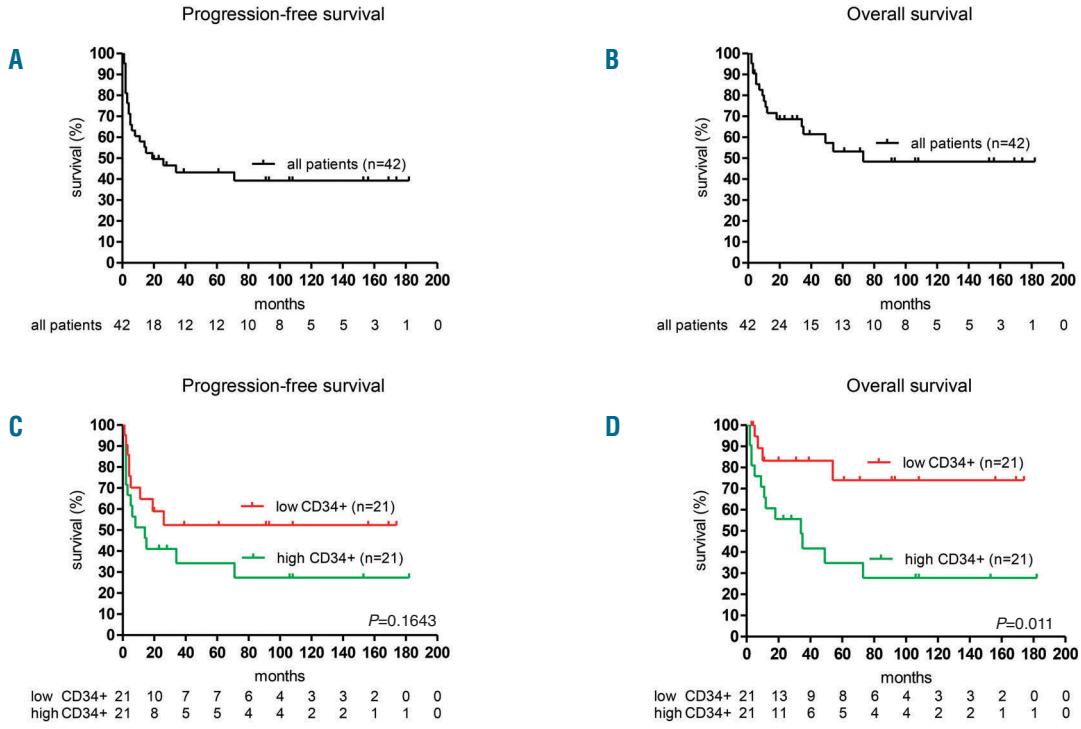

Figure 1. Survival estimates comparing CD34^{+} low *versus* high mobilizing AML patients with *NPM1* mutations. Kaplan-Meier curves are presented for progression-free survival (PFS) in panels (A) and (C), and for overall survival (OS) in panels (B) and (D). The entire study cohort (42 patients) is studied in panels (A) and (B) and dichotomized for patients with a mobilization of autologous peripheral circulating CD34^{+} cells below the median value of 45 CD34^{+} cells/μl blood *versus* above this value in panels (C) and (D) at the day of peripheral stem cell collection.

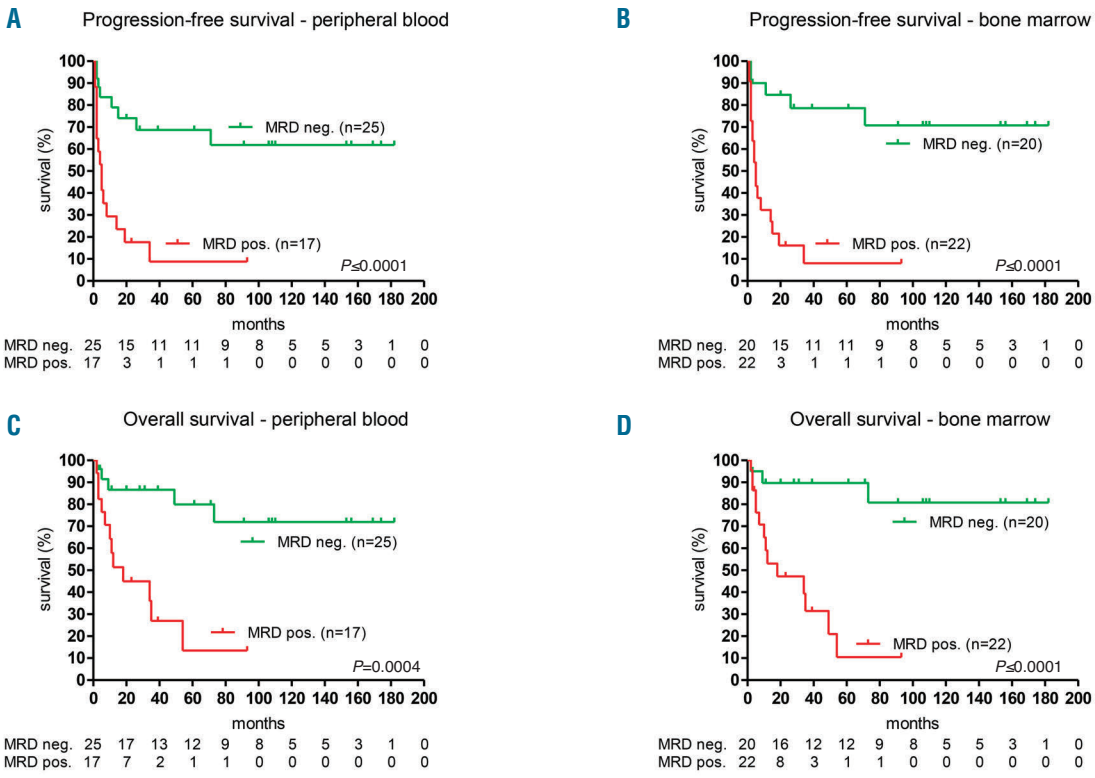

Figure 2. Survival estimates comparing MRD-positive *versus* MRD-negative AML patients with *NPM1* mutations. Kaplan-Meier curves are depicted for progression-free survival (PFS) in panels (A) and (B), and for overall survival (OS) in panels (C) and (D). The threshold separating MRD-positive from MRD-negative *NPM1*mut AML is detectable *NPM1*mut transcripts at a level of 10^{-5} after two cycles of induction treatment. Panels (A) and (C) depict PFS and OS using peripheral blood as a source for MRD assessment, whereas panels (B) and (D) show PFS and OS using bone marrow as a source for MRD assessment.

tion subtypes were distributed as follows: type A: n=31, B: n=7, D: n=3. One patient had a rare subtype (Trp290Serfs*10 c869_875delins CCCTCTCCCAG). According to European Leukemia Net (ELN), 69% of the patients had a favorable-risk, and 31% had an intermediate-risk disease.

The collection of peripheral stem cells was triggered by identifying the first day of circulating $CD34^+$ cells exceeding 20/µL peripheral blood following neutrophil recovery after the second induction cycle under continued G-CSF stimulation (which had been started at the first day of the neutrophil count exceeding 0.5 G/L). Stem cell collection was performed after a median of 24 days following the start of the second induction cycle. The collection of peripheral stem cells occurred later in $CD34^{+low}$ mobilizers than in $CD34^{+high}$ mobilizers (median 28 days *versus* 23 days; P=0.01), whereas the number of apheresis days needed was similar in the two groups (*Online Supplementary Tables S3-S4*). The median number of circulating peripheral $CD34^+$ cells at the day of stem cell collection was higher in $CD34^{+high}$ mobilizers (106/µL *versus* 21/µL peripheral blood; P<0.0001). Additionally, the median number of collected $CD34^+$ cells was higher in $CD34^{+high}$ mobilizers (12 *versus* $5x10^6$/kg; P<0.0001). Finally, the median number of transfused $CD34^+$ cells was higher in $CD34^{+high}$ mobilizers (5.5 *versus* $3.8x10^6$/kg; P=0.0072).

We used a threshold of 10^{-5} to identify MRD^{pos} patients for *NPM1*mut. After two induction cycles, 60% of all patients were MRDneg in the peripheral blood, and 48% in bone marrow. Among the 42, 11 patients were $MRD^{neg}/CD34^{+low}$, 12 patients were $MRD^{pos}/CD34^{+high}$, 9 patients were $MRD^{neg}/CD34^{+high}$, and 10 patients were $MRD^{pos}/CD34^{+low}$. The median PFS of the entire cohort was 13 months and the median OS was 26 months (Figure 1). The median PFS for $CD34^{+low}$ was not reached, while it was reached at only 14 months in $CD34^{+high}$ patients. Similarly, the OS was better for $CD34^{+low}$ compared to $CD34^{+high}$ (not reached at 34 months; P=0.011). We observed that 48% (20/42) of all patients relapsed after autologous SCT, and 40% died due to disease progression. More patients in the $CD34^{+high}$ group died due to disease progression compared to $CD34^{+low}$ patients (62% *versus* 19%; with P=0.01; *Online Supplementary Table S5*).

Survival rates were better in *NPM1*mut MRD^{neg} patients. Patients who were MRD^{neg} in the peripheral blood and bone marrow had better PFS (P<0.0001 for both) and better OS (P=0.0004 and P<0.0001, respectively) when compared to MRDpos patients as presented in Figure 2. When both *NPM1* MRD status and $CD34^+$ mobilization potential were combined (*Online Supplementary Figure S1* and *Table S6*), we observed an excellent outcome in $CD34^{+low}/MRD^{neg}$ patients, with no deaths so far. In contrast, $CD34^{+high}/MRD^{pos}$ patients had a dismal outcome, with a median PFS of only 5.5 months and an OS of 15 months. Finally, we assessed the MRD-

status and CD34+ mobilization potential in a multivariate analysis (*Online Supplementary Table S7*). The analysis further included the pre-treatment parameters age, lactate dehydrogenase (LDH), leukocytes, and ELN risk (favorable *versus* intermediate). The analysis identified an independent prognostic significance for the MRD status, CD34+ mobilization potential and ELN risk, both for PFS and OS.

Even in molecularly defined subgroups of patients with AML receiving identical treatment, outcomes remain diverse. Some patients may achieve cytomorphologic complete remission (CR) but relapse, while others never have recurrence and appear cured. A variety of patient- and disease-related pre-treatment variables predict the prognosis of subgroups of AML patients.[1] We previously reported in 78 patients with good- and intermediate-risk AML undergoing autologous SCT consolidation of CR1 that the levels of circulating CD34+ cells at the day of peripheral stem cell collection confer important prognostic information.[11] In this study, we investigated the potential of two post-treatment assessments, including MRD negativity and decreased stem cell mobilization potential, to define a subgroup of patients with *NPM1*mut AML with excellent outcome after autologous SCT consolidation. We found that the PFS and OS were better in *NPM1*mut patients with a low stem cell mobilization potential compared to high mobilizers. Moreover, our results are in line with previous studies reporting a higher relapse incidence and an inferior leukemia-free survival in AML patients in CR1 who received autografts with higher CD34+ cell numbers.[13] In particular, high CD34+ cell numbers in the autografts were associated with a higher relapse rate and shorter disease-free survival (DFS).[14-15]

The importance of MRD detection of *NPM1*mut levels in the follow-up of AML patients after consolidation treatment in CR1 is well established.[1,6] Ivey *et al*. reported that persistence of *NPM1* mutated transcripts in the peripheral blood was associated with a greater risk of relapse after three years and a lower survival rate.[6] However, these data are based on chemotherapy consolidation, whereas such information is lacking for *NPM1*mut AML patients consolidated with autologous SCT. We found that 40% of *NPM1*mut patients remain MRD-positive in the peripheral blood and 52% in the bone marrow after two induction cycles. These proportions are higher than those reported by Ivey *et al*., most likely due to the fact that we used 10^{-5} as a cut-off for MRD (and not 10^{-4}). Importantly, however, the outcome of *NPM1*mut AML patients with MRD-positivity in the bone marrow after two cycles of induction treatment remained poor despite subsequent autologous SCT consolidation, and both PFS and OS were decisively worse when compared with MRD-negative patients ($P<0.001$ for both).

Finally, we combined the prognostic information obtained from the *NPM1*mut MRD status and stem cell mobilization potential after two induction cycles. The results suggest that the subgroup of $MRD^{neg}/CD34^{+low}$ *NPM1*mut patients enjoys an excellent long-term outcome following autologous SCT, with no deaths observed so far during the study period. In contrast, the subgroup of $MRD^{pos}/CD34^{+high}$ *NPM1*mut patients has a dismal outcome. Noteworthy, the prognostic information of the MRD status and stem cell mobilization seem to be independent as demonstrated in our multivariate analysis above.

Our data propose a rationale for a prospective study investigating whether embedding additional consolidation cycles before autologous SCT in MRD-positive patients in CR1 might improve outcome. Patients with *NPM1* mutations may be a candidate subgroup for such an approach given the option of highly sensitive real-time PCR for MRD measurement. In conclusion, our study of intensively treated *NPM1*mut AML patients suggests that the outcome varies widely if such patients receive consolidation treatment with autologous SCT in CR1 depending on the *NPM1*mut MRD status and on the stem cell mobilization potential. These results may contribute to improve the selection of appropriate candidates for autologous SCT within the subgroup of *NPM1* mutated AML patients and to identify those that possibly should rather undergo allogeneic SCT if possible.

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