Acute myeloid leukemia: negative prognostic impact of early blast persistence can be in part overcome by a later remission prior to post-induction therapy

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Abstract

In acute myeloid leukemia (AML), an early bone marrow (BM) assessment is widely performed during the induction therapy to guide further decisions regarding therapy. However, a clear consensus concerning its prognostic impact on long-term survival and the optimal time point to perform an early BM assessment does not exist so far. While several studies negate the prognostic value of an early BM assessment, many others discuss early blast clearance as a favorable prognostic parameter with regard to both remission rates and long-term survival. Conversely, early blast persistence has been linked to an unfavorable outcome in patients treated intensively for AML. However, it is still unclear whether a potential negative prognostic impact of early blast persistence can be overcome during subsequent therapy of AML.

The present study was conducted in a large cohort of intensively treated AML patients (n=1,008) with the aims of (i) analyzing the prognostic impact of early blast clearance on overall survival (OS), event-free survival (EFS) and relapse-free survival (RFS), and (ii) of evaluating the long-term prognosis in patients with early blast persistence.

Methods

Clinical characteristics, treatment and endpoints
We have treated 1,340 patients aged ≥18 years with newly diagnosed AML at our clinic within the past two decades (January 1st, 2000 - December 31st, 2018). After application
of the exclusion criteria (Figure 1), 1,008 patients were eligible for this retrospective and non-interventional study, which is in line with local ethical guidelines and the Declaration of Helsinki and was approved by the local ethics committee (EA1/038/21).

Standard first-line induction therapy consisted of cytarabine-/daunorubicin-based chemotherapy according to the “7+3” regimen. Some patients received comparable induction therapy with Idarubicin, cytarabine and etoposide (ICE), thioguanine, cytarabine and daunorubicin (TAD9) or high-dose cytarabine and mitoxantrone (HAM) within particular clinical trial protocols. Targeted therapies such as midostaurin or gemtuzumab-ozogamicin were applied in some patients in addition to “7+3”-based regimens within clinical trials. Consolidation chemotherapy was performed with intermediate- to high-dose cytarabine-based therapy with or without mitoxantrone or TAD9 in particular clinical trials (more details concerning chemotherapy are provided in the Online Supplementary Methods). Allogeneic hematopoietic stem cell transplantation (HSCT) following either myeloablative (MAC) or reduced-intensity conditioning (RIC) regimens was used as consolidation therapy in first remission or in relapsed/refractory patients (further details are given in the Online Supplementary Methods).

BM assessment was performed at baseline, on day 14-21 of the first induction cycle, and prior to post-induction therapy. BM assessment was performed by both morphology (cytology and/or histopathology) and multiparametric flow cytometry (see Online Supplementary Methods). The 2010 European LeukemiaNet (ELN) classification was applied for the assessment of the remission status (see Online Supplementary Methods). For this analysis, combined remission was defined as a combination of complete remission plus complete remission with incomplete hematologic recovery plus morphological leukemia-free state (MLFS). Early partial remission (PR) was defined by a decrease of bone marrow blasts by at least 50% to a blast percentage in the range of 5%-25%. Cytogenetic and molecular risk was defined using the ELN risk stratification of 2010 (due to a lack of some molecular data that are mandatory for the 2017 ELN risk classification). The patients' general condition was measured by the Eastern Cooperative Oncology Group (ECOG) performance score. Comorbidity was assessed using the Charlson Comorbidity Index. OS, EFS, RFS, risk of relapse and non-relapse mortality were defined as clinical endpoints by applying the Cheson criteria and the response criteria of the European Society for Blood and Bone Marrow Transplantation.

**Statistical analysis**

Data were curated and retrospectively analyzed using SPSS 23.0 software (IBM®, 2015, Armonk, NY, USA).

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**Figure 1. Study design and clinical endpoints.** AML: acute myeloid leukemia; APL: acute promyelocytic leukemia; OS: overall survival; EFS: event-free survival; RFS: relapse-free survival; CS-HR: cause-specific hazard ratio; RR: risk of relapse; NRM: non-relapse mortality.
Baseline characteristics were analyzed using the Kruskal-Wallis-H test and the \( \chi^2 \) test followed by post-hoc testing and Bonferroni adjustment. The median follow-up was estimated by the reverse Kaplan-Meier method. Survival was analyzed by the Kaplan-Meier method. The log-rank test was used to detect survival differences between groups. Subsequently, univariate and multivariate Cox regression models, which included factors with a significance level of \( P \leq 0.1 \), were applied. In order to define a hazard ratio (HR) and a cause-specific hazard ratio (CS-HR), the variables were transformed into categorical dichotomous data. To estimate the relapse risk and non-relapse mortality in patients with blast clearance, a multivariate cause-specific Cox proportional hazards model that included confounding factors with a significant impact on relapse and survival was used based on an etiological approach. Within this model, death and relapse were defined as competing events and hence treated as censored observations. Post-hoc survival analysis was conducted using the Benjamini-Hochberg procedure. A \( P < 0.05 \) was considered statistically significant. For graphical presentation, Graph Pad Prism 8 (GraphPad Software, Inc) was applied.

**Table 1.** Baseline characteristics and therapeutic approach in 1,008 acute myeloid leukemia patients with regard to remission status at interim bone marrow assessment.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Early blast clearance</th>
<th>Early PR</th>
<th>Early resistant AML</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (% entire cohort)</td>
<td>572 (57)</td>
<td>196 (19)</td>
<td>240 (24)</td>
<td></td>
</tr>
<tr>
<td>ECOG, median (IQR)</td>
<td>1 (0-1)</td>
<td>0 (0-1)</td>
<td>0 (0-1)</td>
<td>0.144</td>
</tr>
<tr>
<td>CCI, median (IQR)</td>
<td>0 (0-1)</td>
<td>0 (0-1)</td>
<td>0 (0-1)</td>
<td>0.237</td>
</tr>
<tr>
<td>Age (years), median (IQR)</td>
<td>56 (46 - 64)</td>
<td>54 (43 - 61)</td>
<td>57 (45 - 65)</td>
<td>0.060</td>
</tr>
<tr>
<td>Subtype of AML</td>
<td></td>
<td></td>
<td></td>
<td>0.019</td>
</tr>
<tr>
<td>de novo AML, N (% remission subgroup)</td>
<td>387 (68)</td>
<td>136 (69)</td>
<td>137 (57)</td>
<td></td>
</tr>
<tr>
<td>sAML, N (% of remission subgroup)</td>
<td>115 (20)</td>
<td>42 (22)</td>
<td>74 (31)</td>
<td></td>
</tr>
<tr>
<td>tAML, N (% of remission subgroup)</td>
<td>67 (11)</td>
<td>16 (8)</td>
<td>26 (11)</td>
<td></td>
</tr>
<tr>
<td>unknown, N (% of remission subgroup)</td>
<td>3 (1)</td>
<td>2 (1)</td>
<td>3 (1)</td>
<td></td>
</tr>
<tr>
<td>ELN 2010 risk group</td>
<td></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>favorable, N (% of remission subgroup)</td>
<td>97 (17)</td>
<td>19 (10)</td>
<td>8 (3)</td>
<td></td>
</tr>
<tr>
<td>intermediate I/II, N (% of remission subgroup)</td>
<td>280 (49)</td>
<td>96 (49)</td>
<td>123 (51)</td>
<td></td>
</tr>
<tr>
<td>adverse, N (% of remission subgroup)</td>
<td>121 (21)</td>
<td>67 (34)</td>
<td>90 (38)</td>
<td></td>
</tr>
<tr>
<td>unknown, N (% of remission subgroup)</td>
<td>74 (13)</td>
<td>14 (7)</td>
<td>19 (8)</td>
<td></td>
</tr>
<tr>
<td>Allo-HSCT in 1(^{st}) remission, N (% of remission subgroup)</td>
<td>225 (39)</td>
<td>86 (44)</td>
<td>90 (38)</td>
<td>0.423*</td>
</tr>
<tr>
<td>double induction prior to allo-HSCT, N (% allo-HSCT 1(^{st}) CR/CRi/MLFS within remission subgroup)</td>
<td>120 (53)</td>
<td>79 (92)</td>
<td>85 (94)</td>
<td>0.002*</td>
</tr>
<tr>
<td>consolidation chemotherapy prior to allo-HSCT, N (% allo-HSCT 1(^{st}) CR/CRi/MLFS within remission subgroup)</td>
<td>158 (70)</td>
<td>53 (62)</td>
<td>49 (54)</td>
<td>0.230*</td>
</tr>
<tr>
<td>Allo-HSCT in 2(^{nd}) remission or as salvage therapy, N (% of remission subgroup)</td>
<td>156 (27)</td>
<td>63 (32)</td>
<td>67 (28)</td>
<td>0.317*</td>
</tr>
<tr>
<td>Consolidation chemotherapy without allo-HSCT, N (% of remission subgroup)</td>
<td>191 (33)</td>
<td>47 (24)</td>
<td>83 (35)</td>
<td>0.070*</td>
</tr>
<tr>
<td>double induction prior to scheduled consolidation chemotherapy, N (% of non-allo-HSCT within remission subgroup)</td>
<td>87 (46)</td>
<td>38 (81)</td>
<td>59 (71)</td>
<td>0.021*</td>
</tr>
<tr>
<td>CR/CRi/MLFS after double induction, N (% of all non-allo-HSCT patients with double induction)</td>
<td>69 (79)</td>
<td>29 (76)</td>
<td>29 (49)</td>
<td>0.045*</td>
</tr>
</tbody>
</table>

*Significance level adjusted with the Bonferroni correction \( P \leq 0.008 \). PR: partial remission; AML: acute myeloid leukemia; n: number of patients; IQR: interquartile range; ECOG: Eastern Cooperative Oncology Group performance status; CCI: Charlson Comorbidity Index; sAML: secondary acute myeloid leukemia; tAML: therapy-related acute myeloid leukemia; ELN: European LeukemiaNet; allo-HSCT: allogeneic hematopoietic stem cell transplantation; CR: complete remission; CRi: complete remission with incomplete hematologic recovery; MLFS: morphological leukemia-free state.
Results

Clinical characteristics
A total of 1,008 patients, who had undergone intensive therapy, were eligible for this analysis. The median follow-up was 63.1 months (95% confidence interval [95% CI]: 55.3-71.0 months). Fifty-seven percent of the entire cohort showed early blast clearance (n=572), whereas 43% had blast persistence (n=436). Within the latter group, 45% (196/436) had an early PR and 55% (240/436) showed early resistant disease without any response. The distribution of baseline characteristics within the entire cohort and the three “remission groups” (early blast clearance, early PR and early resistant AML) are shown in Table 1. As expected, ELN risk stratification (P<0.001) and subtype of AML differed significantly between the three groups (P=0.019). However, there were no further significant differences with regard to baseline characteristics. The further treatment of patients with early blast persistence beyond induction 1 is outlined in Table 1 and Figure 2.

Early blast clearance and early blast persistence are prognostic in the entire cohort
The entire cohort had a 5-year OS of 35% with a median OS of 28.5 months (95% CI: 24.4 - 32.6 months). The evaluation of early BM results revealed a significant decrease in OS in patients with early blast persistence as compared to those with blast clearance (P<0.001) (Table 2). The 5-year OS of patients with early blast clearance was 41% as compared to 30% for those with early blast persistence (P<0.001). This observation maintained its significance within a multivariate model (HR=1.4, P<0.001) (Table 2) that included all factors with a significant impact on survival within the univariate analysis (ECOG status >1: P=0.004; Charlson Comorbidity Index ≥2: P=0.003; ELN risk group intermediate/adverse: P<0.001 (including FLT3-ITD mutational status); age ≥60 years: P<0.001; and subtype of AML: P<0.001).

In the entire cohort, 5-year EFS and RFS were 24% and 25%. The median EFS and RFS were 13.9 months (95% CI: 12.4-15.5 months) and 13.9 months (95% CI: 11.9-15.9 months), respectively. The negative prognostic impact of early blast persistence also translated into an effect on EFS. Early blast clearance was associated with a 5-year EFS of 26% as compared to 18% in patients with early blast persistence (P=0.001) (Table 2). This significant difference was also maintained within the multivariate analysis (HR=1.3, P=0.001). Comparable results were observed for RFS with a hazard ratio of 1.2 in the multivariate analysis in the presence of the other biologically relevant risk factors that are mentioned above (P=0.031) (Table 2).

The negative prognostic impact of early blast persistence can be overcome if a response is achieved prior to post-induction therapy
In the entire cohort, the combined remission rate was 81%

Figure 2. Further treatment in 436 acute myeloid leukemia patients with early blast persistence. For further details regarding chemotherapy, see the Online Supplementary Methods. AML: acute myeloid leukemia; PR: partial remission; n: number of patients; “7+3”: cytarabine-/daunorubicin-based chemotherapy according to the “7+3” regimen; HAM: high-dose cytarabine and mitoxantrone; IdaFLAG: idarubicin, fludarabine, cytarabine; granulocyte colony-stimulating factor; MitoFLAG: mitoxantrone, fludarabine, cytarabine; granulocyte colony-stimulating factor; allo-HSCT: allogeneic hematopoietic stem cell transplantation.
prior to scheduled post-induction therapy. The combined remission rate was 89% (508/572) in patients with early blast clearance and 70% (305/436) in patients with early blast persistence after additional therapy (P<0.001). The negative prognostic impact of early blast persistence was maintained in patients who achieved blast clearance during further induction therapy. The 5-year OS and RFS were 43% and 26% in patients with an early blast clearance as compared to 31% and 23% in patients with early blast persistence who achieved a remission prior to post-induction therapy (P=0.016 and P=0.013) (Table 2). The negative prognostic impact of early blast persistence was also maintained in the multivariate model that included relevant risk factors for OS (HR=1.3, P=0.024) and RFS (HR=1.4, P=0.002) (Table 2). Furthermore, patients with early blast persistence showed an increased risk of relapse in the cause-specific hazard model which included the same covariates (CS-HR=1.3, P=0.039) (Table 2). Moreover, in patients with early blast persistence, there was a strong trend towards a higher risk of non-relapse mortality, even in the presence of other risk factors (CS-HR=1.4, P=0.069) (Table 2). Interestingly, in the group with early blast persistence, survival was very heterogeneous depending on whether the patients had at least an early PR or showed early resistant disease (Figure 3). The survival of patients with early PR and subsequent combined remission prior to consolidation therapy was comparable to the survival of patients with early blast clearance (5-year OS: 45% vs 44%, P=0.618), whereas early resistant AML maintained its negative prognostic impact throughout the analysis (5-year OS: 28%, P<0.001). Comparable results were observed for RFS (Figure 3). Similarly, early resistant AML (but not early PR) remained prognostically unfavorable in the multivariate analysis for both OS (HR=1.5, 95% CI: 1.2-2.0; P=0.001) and RFS (HR=1.4, 95% CI: 1.1-1.7; P=0.012).

Table 2. Impact of early blast persistence on survival in the entire cohort and in patients with combined remission prior to consolidation therapy.

<table>
<thead>
<tr>
<th>Survival</th>
<th>Early blast clearance</th>
<th>Early blast persistence (PR &amp; resistant AML)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Entire cohort</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (% entire cohort)</td>
<td>572 (57)</td>
<td>436 (43)</td>
<td></td>
</tr>
<tr>
<td>OS (months), median (95% CI)</td>
<td>35.8 (29.4-42.3)</td>
<td>18.0 (14.2-21.8)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RFS (months), median (95% CI)</td>
<td>15.7 (13.2-18.2)</td>
<td>11.5 (9.1-13.9)</td>
<td>0.100</td>
</tr>
<tr>
<td>EFS (months), median (95% CI)</td>
<td>16.2 (13.8-18.6)</td>
<td>11.3 (10.0-12.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>MV-HR* for OS with early blast persistence</td>
<td>1.42 (1.18-1.71)</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>MV HR* for RFS with early blast persistence</td>
<td>1.22 (1.02-1.50)</td>
<td>0.031</td>
<td></td>
</tr>
<tr>
<td>MV-HR* for EFS with early blast persistence</td>
<td>1.34 (1.13-1.59)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Combined remission prior to post-induction therapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (% of group with post-induction combined remission)</td>
<td>508 (62)</td>
<td>305 (38)</td>
<td></td>
</tr>
<tr>
<td>OS (months), median (95% CI)</td>
<td>42.2 (31.8-52.6)</td>
<td>29.3 (20.7-37.8)</td>
<td>0.016</td>
</tr>
<tr>
<td>RFS (months), median (95% CI)</td>
<td>18.8 (15.8-21.9-37.8)</td>
<td>13.0 (10.5-15.6)</td>
<td>0.013</td>
</tr>
<tr>
<td>MV-HR* for OS with early blast persistence</td>
<td>1.29 (1.03-1.60)</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>MV-HR* for RFS with early blast persistence</td>
<td>1.35 (1.11-1.65)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>MV CS-HR* for NRM with early blast persistence</td>
<td>1.42 (0.97-2.07)</td>
<td>0.069</td>
<td></td>
</tr>
<tr>
<td>CS-HR* for relapse with early blast persistence</td>
<td>1.30 (1.01-1.62)</td>
<td>0.039</td>
<td></td>
</tr>
</tbody>
</table>

*The multivariate analysis included the following dichotomized parameters: Eastern Cooperative Oncology Group score ≤ 1 vs. >1, Charlson Comorbidity Index <2 vs. ≥ 2, European LeukemiaNet risk group favorable vs. intermediate/adverse, age <60 years vs. ≥ 60 years, subtype of acute myeloid leukemia de novo vs. secondary/therapy-related. CR: complete remission; CRi: complete remission with incomplete hematologic recovery; MLFS: morphological leukemia-free state; n: number of patients; PR: partial remission; AML: acute myeloid leukemia; 95% CI: 95% confidence interval; OS: overall survival; EFS: event-free survival; RFS: relapse-free survival; MV: multivariate; HR: hazard ratio; NRM: non-relapse mortality; CS-HR: cause-specific hazard ratio.
Figure 3. Entire cohort. Survival outcomes after achievement of combined remission prior to post-induction therapy (allogeneic transplantation or consolidation chemotherapy) with regard to early remission status at the interim bone marrow assessment. (A) Overall survival (OS) and relapse-free survival (RFS) with early blast clearance versus early partial remission. (B) OS and RFS with early blast clearance versus early resistant acute myeloid leukemia. (C) OS and RFS with early partial remission versus early resistant acute myeloid leukemia. AML: acute myeloid leukemia; PR: partial remission; OS: overall survival; RFS: relapse-free survival; n: number of patients; 95% CI: 95% confidence interval.
**Prognostic impact of early blast clearance in patients who underwent allogeneic hematopoietic stem cell transplantation as post-remission therapy**

Of all patients, 68% (687/1,008) underwent allogeneic HSCT. Among these transplanted patients, 5-year OS and RFS were 44% and 28%, and median OS and RFS were 41.8 months (95% CI: 33.1-50.3 months) and 16.5 months (95% CI: 14.1-19.0 months), respectively. Of these patients, 58% (401/687) underwent allogeneic HSCT as consolidation therapy in first remission, whereas the remaining 42% (286/687) received their transplant beyond first remission (Table 1).

Patients who underwent allogeneic HSCT in first remission (n=401) had a 5-year OS of 51% and a 5-year RFS of 47% with a median OS of 62.1 months (95% CI: 33.1-91.0 months) and a median RFS of 38.6 months (95% CI: 17.3-59.9 months). Regarding OS and RFS, there was no significant difference between patients with early blast clearance and early PR in this particular subset of patients (Figure 4A). In contrast, patients with early resistant AML showed both inferior RFS and OS, even after having achieved combined remission prior to allogeneic HSCT (Figure 4B). Precisely, 5-year OS was 57% with early blast clearance, and 46% with early PR (P=0.267) as compared to 37% with early resistant AML (Figure 4B, C, P=0.002). The 5-year RFS was 51% with early blast clearance, 42% with early PR (P=0.333) and 32% with early resistant AML (P=0.001).

Considering the multivariate analysis, early resistant AML was an unfavorable prognostic factor in patients who underwent allogeneic HSCT as consolidation therapy (OS: P=0.011, HR=1.6, 95% CI: 1.1-2.4; RFS: P=0.006, HR=1.7, 95% CI: 1.2-2.4). In patients who had been transplanted in first remission, we further analyzed the influence of both the type of transplant conditioning (MAC vs. RIC) and the type of donor (matched sibling donor [MSD] vs. matched unrelated donor [MUD]) on the clinical outcome. In fact, 136/401 patients received MAC, whereas 261/401 patients were treated with RIC (information on the conditioning regimen was not available in 4 cases). Comparing MAC and RIC, we found a significant difference in median OS (61.2 months vs. 46.8 months, P=0.012) and RFS (58.4 months vs. 33.5 months, P=0.013). However, this finding was mainly attributable to major differences in median age (39 years vs. 58 years, P<0.001) and Charlson Comorbidity Index (upper quartile 0 points vs. 1 point, P<0.001) between both groups. In contrast, ELN subgroups (P=0.182) and median ECOG status (P=0.866) did not differ significantly between patients given MAC or RIC. More importantly, there was no significant difference in early remission status between patients given MAC or RIC since early blast clearance and blast persistence were similarly distributed between these subgroups (early blast clearance 51% with MAC vs. 58% with RIC, early blast persistence 49% with MAC vs. 42 % with RIC, P=0.153) and, vice versa, MAC and RIC application were equally distributed within the remission subgroups. Regarding the type of donor (MSD vs. MUD), there was a trend towards better OS and RFS in patients with MSD (OS 59.4 months vs. 47.0 months, P=0.058; RFS: 51.3 months vs. 34.3 months, P=0.091) However, this seemed to be caused again by differences in median age (50 vs. 53 years, interquartile range 37-58 vs. 43-62 years, P=0.003) or HLA-mismatch (full match vs. mismatch, P<0.001). However, early blast clearance and blast persistence were equally distributed within the subgroups with MSD and MUD (early blast clearance: 56% with MSD and MUD, early blast persistence: 44% with MSD and MUD, P=0.968) and vice versa.

In conclusion, early resistant AML remained an independent unfavorable prognostic factor in the multivariate analysis of patients with allogeneic HSCT as consolidation therapy (OS: P=0.011, HR=1.6, 95% CI: 1.1-2.4; RFS: P=0.006, HR=1.7, 95% CI: 1.2-2.4).

**Prognostic impact of early blast persistence in patients who received chemotherapy as post-remission therapy**

In patients who did not undergo allogeneic HSCT (321/1,008), 5-year OS and RFS were 20% and 17% with a median OS of 12.0 months (95% CI: 9.9-14.1 months) and a median RFS of 8.4 months (95% CI: 6.8-9.9 months). Fifty-one percent (165/321) of the non-transplanted patients had achieved blast clearance prior to consolidation chemotherapy. Of these latter patients, 24% (39/165) had been treated with one cycle of induction chemotherapy and 76% (126/165) had received two cycles of induction therapy. Within the latter subgroup of non-transplanted patients who had received two cycles of induction therapy, 5-year OS and RFS were 32% and 23% with a median OS and RFS of 17.8 months (95% CI: 9.5-26.1 months) and 9.7 months (95% CI: 6.9-12.5 months), respectively. In these patients, early blast clearance was comparable to early PR with regard to OS (5-year OS 40% vs. 32%, P=0.401) (Figure 5A). In contrast, RFS with early PR was significantly worse than with early blast clearance in the univariate analysis (5-year RFS 32% vs. 15%, P=0.037) (Figure 5A), and there was a clear trend towards inferior survival in the multivariate model (HR=1.6, 95% CI: 1.0-2.7, P=0.058), suggesting an adverse prognostic impact of early PR on RFS which was most likely compensated by subsequent salvage therapy with regard to OS.

**Discussion**

Whether an early remission during AML induction therapy is of any prognostic value has remained a matter of debate over the past decade. Even in the era of minimal/ measurable residual disease (MRD)-guided therapeutic decision-
Figure 4. Allogeneic hematopoietic stem cell transplant cohort in first remission. Survival outcomes after achievement of a combined remission prior to allogeneic hematopoietic stem cell transplantation with regard to early remission status at the interim bone marrow assessment. (A) Overall survival (OS) and relapse-free survival (RFS) with early blast clearance versus early partial remission. (B) OS and RFS with early blast clearance versus early resistant acute myeloid leukemia. (C) OS and RFS with early partial remission versus early resistant acute myeloid leukemia. AML: acute myeloid leukemia; PR: partial remission; RD: refractory disease; OS: overall survival; RFS: relapse-free survival; n: number of patients; 95% CI: 95% confidence interval; allo-HSCT: allogeneic hematopoietic stem cell transplantation.
Figure 5. Non-transplanted cohort. Survival after achievement of combined remission after double induction and prior to scheduled consolidation chemotherapy with regard to early remission status at the interim bone marrow assessment. (A) Overall survival (OS) and relapse-free survival (RFS) with early blast clearance versus early partial remission. (B) OS and RFS with early blast clearance versus early resistant acute myeloid leukemia. (C) OS and RFS with early partial remission versus early resistant acute myeloid leukemia. AML: acute myeloid leukemia; PR: partial remission; OS: overall survival; RFS: relapse-free survival; n: number of patients; 95% CI: 95% confidence interval; allo-HSCT: allogeneic hematopoietic stem cell transplantation.
making, this controversy has not been resolved, since early blast clearance can indicate a therapy response at a very early time point when MRD assessment is not yet part of the routine management. Furthermore, there are also patients in whom an adequate MRD marker cannot be established. In these cases, early BM assessment may inform therapeutic decision-making, particularly for those in whom the choice of consolidation therapy (i.e., conventional chemotherapy vs. allogeneic HSCT) is challenging.

Over the past decade, there has been an extensive discussion not only on the general value, but also on the most appropriate time point, of the early BM assessment. Recommendations vary from omitting early BM assessment completely (due to a lack of prognostic information) to its implementation during induction therapy between day 6 and day 21.3,5-9 At our institution, the early BM assessment was generally performed between day 14 and 21, as previous studies had shown that there is no substantial difference between BM evaluation on day 14 and 21 and, thus, results obtained within this interval were merged.37 Certainly, there is some heterogeneity with regard to induction and consolidation therapy within our cohort of AML patients. However, the different treatment protocols were prospectively compared within the German Intergroup trial and no relevant outcome differences were detected and thus they should be comparable with regard to long-term survival.

Our large cohort of 1,008 intensively treated patients with newly diagnosed AML does now confirm a negative prognostic impact of early blast persistence on both OS and RFS. While in our cohort survival was slightly above the upper range of international studies, which might be explained by the large number of patients who underwent allogeneic HSCT, the survival in the transplanted cohort was in the range of other studies in AML.

Thus, the favorable impact of early blast clearance observed in our cohort is in line with previously published data and it seems conceivable that this effect is due to chemosensitivity of AML cells in vivo. Vice versa, the negative impact of early persistent AML most likely reflects resistance to conventional chemotherapy. This assumption is emphasized by the comparison of cause-

**Figure 6. Potential treatment algorithm in European LeukemiaNet intermediate-risk acute myeloid leukemia after implementation of the early response as an additional prognostic parameter (in a notional scenario).** Clinical responses and therapy decisions in 499 patients with intermediate-risk acute myeloid leukemia (according to the European LeukemiaNet classification) with and without implementation of the early bone marrow assessment into further therapeutic decision making. In our cohort, 153/343 patients with at least an early partial remission underwent allogeneic hematopoietic stem cell transplantation (HSCT) and 132/343 were treated with consolidation chemotherapy. In these groups, the implementation of the early response as a prognostic parameter (in addition to minimal residual disease, which was not available in our cohort) would have possibly led to consolidation chemotherapy instead of allogeneic transplantation in 153/343 patients, if they had achieved minimal residual disease negativity. Seventy-seven of 420 patients had early resistant disease. Within this subgroup, 45/77 underwent allogeneic HSCT and 14/77 consolidation chemotherapy. The implementation of the early response as a prognostic parameter (in addition to minimal residual disease) would have possibly led to allogeneic HSCT as consolidation therapy in an additional 14/77 patients. Thus, in our cohort, the implementation of early response would have possibly changed treatment decision in 33% of ELN intermediate-risk patients and in 17% of all patients. ELN: European LeukemiaNet; n: number of patients; PR: partial remission; allo-HSCT: allogeneic hematopoietic stem cell transplantation.
specific hazards for relapse and non-relapse mortality in our cohort. When comparing patients with early blast persistence to patients with early blast clearance, we observed a significant increase in the risk of relapse, but not in the risk of non-relapse mortality. This finding strongly suggests that the unfavorable prognostic impact of early blast persistence is mainly driven by disease relapse and less by toxicity caused by additional therapy.

Interestingly, the achievement of a combined remission prior to post-induction therapy outperforms the negative impact of early blast persistence on OS, if the early BM assessment shows at least a PR. In contrast, the negative prognostic impact of early resistant AML cannot be completely overcome, even if a combined remission is obtained prior to consolidation therapy. Notably, in patients with a later combined remission during induction, the poor prognostic impact of early blast persistence can be in part compensated by consolidation with allogeneic HSCT. This is possibly due to an additional immunological graft-versus-leukemia-effect that may compensate for a lower extent of chemosensitivity in these cases.29-32 This hypothesis is underlined by the observation that the adverse prognostic impact of early blast persistence cannot be overcome in AML patients who do not proceed to allogeneic HSCT in first remission after induction therapy. Notably, early blast persistence only translates into inferior RFS in this subgroup, whereas this is not observed in patients undergoing allogeneic HSCT. Therefore, the adverse impact of early blast persistence might be overcome in the subgroup with at least an early response as assessed by additional induction therapy resulting in subsequent remission prior to post-induction therapy that should comprise allogeneic HSCT consolidation. Unfortunately, MRD assessment by molecular methods and/or highly sensitive multicolor flow cytometry, particularly within the ELN intermediate-risk group, could not be included in our analysis, since these data were not available for all patients throughout the study period. In the future, additional evidence provided by large datasets from molecularly characterized AML cohorts consolidated with either intensive chemotherapy or allogeneic HSCT might pave the way for such a strategy which would then also need further prospective evaluation.

Disclosures
No conflicts of interest to disclose.

Contributions
JI and JW designed the study. AF, MS, NRN, AB, IA, ST, IWB, TB, DH, LAB and JW performed the clinical and diagnostic workup required for this study. JI, SG, LEB and JW collected, analyzed, and interpreted the data. JI and JW wrote the manuscript draft. All authors critically revised the manuscript and approved the final version.

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Data-sharing statement
Data for this study are not publicly available due to ethical restrictions.
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