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by Dimitrios Kotsos, Patrycja Gradowska, Sjoerd J.F. Hermans, Thomas Pabst, Johan Maertens, Markus Manz, Dimitri A. Breems, Bjorn T. Gjertsen, Soren Lehmann, Bart J. Biemond, H. Berna Beverloo, Peter J.M. Valk, Jeroen J.W.M. Janssen, Gert Ossenkoppele, Bob Löwenberg, Gerwin Huls, Marc H.G.P. Raaijmakers and Jurjen Versluis

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Treatment of therapy-related acute myeloid leukemia and acute myeloid leukemia with myelodysplasia-related changes: a comparative analysis of higher-dose intensive 7+3 induction chemotherapy *versus* liposomal cytarabine and daunorubicin

Dimitrios Kotsos,[1] Patrycja Gradowska,[1,2] Sjoerd J.F. Hermans,[1] Thomas Pabst,[3,4] Johan Maertens,[5] Markus Manz,[4,6] Dimitri A. Breems,[7] Bjorn T. Gjertsen,[8] Soren Lehmann,[9] Bart J. Biemond,[10] H. Berna Beverloo,[1] Peter J.M. Valk,[1] Jeroen J.W.M. Janssen,[11] Gert Ossenkoppele,[10] Bob Löwenberg,[1] Gerwin Huls,[12] Marc H.G.P. Raaijmakers,[1] Jurjen Versluis.[1]

1 *Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands*

2 *HOVON Foundation, Rotterdam, The Netherlands*

3 *Department of Medical Oncology, University Hospital, Inselspital, Bern, Switzerland*

4 *Swiss Group for Clinical Cancer Research (SAKK), Bern, Switzerland*

5 *University Hospital Gasthuisberg, Leuven, Belgium*

6 *University Hospital Zurich, Zurich, Switzerland*

7 *ZAS, Cadix Hospital, Antwerp, Belgium*

8 *Haukeland University Hospital, University of Bergen, Norway*

9 *Karolinska University Hospital, Stockholm, Sweden*

10 *Amsterdam UMC, Cancer Center Amsterdam, University of Amsterdam , Amsterdam, The Netherlands*

11 *Radboud University Medical Center, Nijmegen, The Netherlands*

12 *University Medical Center, University Groningen, Groningen, The Netherlands*

RUNNING HEAD: Higher-intensity 7+3 vs CPX-351 in t-AML and AML-MRC

CORRESPONDING AUTHOR: Jurjen Versluis, Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands; j.versluis.1@erasmusmc.nl

AUTHOR CONTRIBUTIONS: DK performed the formal analysis, data curation, visualization, wrote the first draft of the manuscript and contributed to manuscript review and editing. PG contributed to data curation and manuscript review and editing. SJFH, TP, JM, MM, DAB, BTG, SL, BJB, HBB, PJMV, JJWMJ, GO, and BL contributed to manuscript review and editing. GH contributed to writing the original draft and manuscript review and editing. MHGPR contributed to conceptualization, writing the original draft, and manuscript review and editing. JV supervised the study, contributed to conceptualization, data curation, visualization, writing the original draft, and manuscript review and editing. All authors approved the final version of the manuscript.

KEYWORDS: therapy-related acute myeloid leukemia, acute myeloid leukemia with MDS-related changes, intensive 7+3 induction, CPX-351

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ABSTRACT

Therapy-related acute myeloid leukemia (t-AML) and AML with myelodysplasia-related changes (AML-MRC) are associated with poor outcomes. The liposomal formulation of cytarabine and daunorubicin (CPX-351) improved complete remission (CR) and CR with incomplete hematologic recovery (CRi) rates and overall survival (OS) compared with 'standard' induction (7+3) chemotherapy in a phase-III trial for patients aged 60-75 years. However, 7+3 dosing varies among trials and in clinical practice and it remains unknown whether CPX-351 is superior to 7+3 double-induction regimens including intermediate-dose cytarabine, as the one employed in the HOVON-SAKK-Nordic clinical trials. To address this question, we conducted a post-hoc analysis on t-AML/AML-MRC patients aged ≥60 years enrolled in three HOVON-SAKK-Nordic trials and defined a subset of patients that met the eligibility criteria of the CPX-351 trial and compared their outcomes with those of the CPX-351 arm using reconstructed survival data. CR/CRi rates were higher in the higher-intensity 7+3 cohort (67.8%) compared with CPX-351 (47.7%) with similar median OS between the two cohorts (10.1 months versus 8.9 months respectively, HR = 0.99; 95% CI 0.78-1.26, p=0.95). Thirty-day mortality (4.4% for higher-intensity 7+3 versus 5.9% for CPX-351) and adverse events, including febrile neutropenia (61% for higher-intensity 7+3 versus 68% for CPX-351), were comparable. The data suggest that obligatory double-induction may achieve outcomes similar to CPX-351 in these patients and provide a strong rationale for ongoing clinical trials comparing these regimens.

INTRODUCTION

Acute myeloid leukemia (AML) is a biologically and clinically heterogeneous neoplasm characterized by proliferation of hematopoietic stem cells with a median age of 68 years at diagnosis.[1] AML disease ontogeny is clinically defined as de novo, secondary (s-AML) from antecedent hematologic disorders, such as myelodysplastic syndrome (MDS), or myeloproliferative neoplasm (MPN), or therapy-related (t-AML) after previous exposure to chemotherapy or radiotherapy.[2,3] Recent classification systems have refined AML ontogeny by adding a genetic definition termed AML with MDS-related gene mutations. [2,3] The incidence of AML with MDS-related gene mutations or t-AML increases with age, and is associated with lower response rates to treatment and shorter overall survival (OS).[4–10]

For five decades, the conventional intensive remission induction chemotherapy regimen for AML has consisted of a combination of cytarabine and an anthracycline (7+3). Although targeted treatments such as FLT3 inhibitors have been efficacious when added to 7+3,[11,12] these mutations are less frequent in the subset of AML with myelodysplasia-related changes (AML-MRC) or t-AML. CPX-351, a liposomal formulation of cytarabine and daunorubicin, has been approved for patients with AML-MRC or t-AML.[13,14] In the pivotal randomized trial, CPX-351 prolonged median OS by 3.4 months compared to 7+3 induction chemotherapy and was associated with higher complete remission (CR) and CR with incomplete hematologic recovery (CRi) rates after induction (48% versus 33%, respectively). [15]

Nevertheless, the 7+3 regimen is not uniform across clinical practice, with differences in the cumulative doses and scheduling of cytarabine and anthracyclines between cooperative groups and geographical regions. For example, the Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON) and the Swiss Group for Clinical Cancer Research (SAKK) clinical trial protocols use double-induction, including intermediate-dose cytarabine (IDAC) in the second cycle, in contrast to the control arm of the CPX-351 trial. These intensity differences may partly explain the lower post-induction CR/CRi rates reported in the CPX-351 trial

compared with those observed in the HOVON-SAKK-Nordic trials, where CR/CRi rates for elderly patients were ~66%.[16]

The objective of our study was to evaluate key clinical features and treatment outcomes in older and intensively-treated patients with t-AML and AML-MRC. Moreover, given the lower dose of cytarabine in the control arm of the pivotal CPX-351 trial compared with the standard induction regimen of the HOVON-SAKK-Nordic trials, we retrospectively analyzed the response rates and OS of patients with AML-MRC and t-AML treated in HOVON-SAKK-Nordic protocols and compared them with patients treated with CPX-351 in the pivotal phase III trial.

METHODS

Study Population

Patients with newly diagnosed and previously untreated t-AML or AML-MRC aged 60 years and older who were treated between 2010 and 2017 with intensive chemotherapy in the HOVON-SAKK 102/30-09, 103/30-10 and 132/30-13 clinical trials were included.[16–18] Patients with high-risk MDS and blast count between 10–19% were also included in these trials. s-AML was defined as having a documented diagnosis of prior MDS, chronic myelomonocytic leukemia (CMML) or MPN >3 months prior to AML diagnosis. For t-AML, prior exposure to chemotherapy or radiotherapy for an unrelated disease was considered. De novo AML with MDS-related cytogenetics was defined according to the 2008 World Health Organization (WHO) criteria.[19] MDS-related gene mutations were defined as the presence of at least one mutation in *SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR,* or *STAG2* genes.[20]

A total of 2,399 patients were enrolled in HOVON-SAKK 102/30-09, 103/30-10, and 132/30-13 clinical trials, of which 1,223 patients were excluded because of age <60 years. After excluding patients with de novo AML without MDS-related cytogenetics, 428 patients were included in the final analysis and constituted the t-AML/AML-MRC cohort (Figure 1). This broader cohort was used to identify factors impacting OS in these older patients with t-AML

and AML-MRC. Risk stratification in this cohort was performed according to the 2022 European LeukemiaNet (ELN2022) classification.[3]

To enable a comparison with the CPX-351 phase III trial, the inclusion and exclusion criteria of the CPX-351 phase III trial[14] were applied to patients in the three HOVON-SAKK-Nordic trials to obtain a similar 7+3 cohort (HOVON 7+3 cohort) (Figure 1). Patients with high-risk MDS with increased blast counts were included per eligibility criteria. Patients were excluded if they were aged >75 years, had prior diagnosis of MPN, or if central nervous system (CNS) AML was present at diagnosis. Other clinical inclusion and exclusion criteria were similar across HOVON-SAKK-Nordic trials and the CPX-351 trial. Additionally, only patients assigned to the control arms of the three HOVON-SAKK-Nordic clinical trials were considered for comparison. FLT3 inhibitors were not used in the HOVON-SAKK-Nordic clinical trials as part of protocol treatment. Similar to the CPX-351 trial, responses in the HOVON-SAKK-Nordic trials were defined using the Revised International Working Group Criteria[21] and cytogenetic risk was stratified according to the National Comprehensive Cancer Network (NCCN) classification.[22]

The original HOVON-SAKK-Nordic trials were approved by the institutional review boards and ethics committees of all participating centers and conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

Induction protocols

Patients aged ≥60 years enrolled in the HOVON-SAKK 102/30-09, 103/30-10, and 132/30-13 trials received double-induction chemotherapy per protocol, consisting of cytarabine at 200 mg/m^2/day for 7 days and an anthracycline for 3 days (either daunorubicin 60 mg/m^2/day or idarubicin at 12 mg/m^2/day) in the first cycle. The second cycle in the HO102/30-09 consisted of IDAC with 1,000 mg/m^2 twice daily for 6 days and amsacrine 120 mg/m^2 for 3 days, whereas HO103/30-10 and HO132/30-13 used IDAC only in the control arm (1,000 mg/m^2 cytarabine

twice daily for 6 days).[16–18]Patients in the experimental arm of the pivotal trial received CPX-351 with 100 mg/m^2 of cytarabine and 44 mg/m^2 of daunorubicin for 3 days and refractory patients received a second induction cycle with the same dose for 2 days. Patients assigned to the control arm received one induction cycle with cytarabine at 100 mg/m^2/day for 7 days, combined with daunorubicin at 60 mg/m^2 for 3 days. A second cycle consisting of cytarabine at 100 mg/m^2/day for 5 days with daunorubicin at 60 mg/m^2 for 2 days was given to patients with refractory disease.[14]

Statistical analysis

The primary endpoint was OS, and secondary endpoints included CR/CRi rates within two induction cycles, adverse events (AEs) frequency, early mortality rates, time to hematologic recovery, and duration of hospital stay. These endpoints were compared between the selected HOVON 7+3 cohort and the CPX-351 trial patients in the experimental arm. OS was defined as the time from randomization to death by any cause. Continuous variables were described using the mean and standard deviation (SD) or the median and the interquartile range (IQR), in case of not normally distributed data. Continuous data were compared using the Student's t-test or Mann-Whitney U test, in case of not normally distributed data. Categorical variables were summarized using counts and percentages and were compared using the chi-square test, or the Fisher's exact test, when required. Survival curves were estimated using the Kaplan-Meier method and the log-rank test was applied for comparison. The reverse Kaplan-Meier method was used to calculate the median follow-up. Survival estimates of the CPX-351 arm were regenerated using the number of patients at risk and the number of censored patients at each time point from the original publication.[15]

A multivariable Cox proportional hazards regression model was developed for the t-AML/AML-MRC cohort using a backward stepwise approach involving variables with a Cox p-value<0.1 in univariable analysis. To account for the inherent heterogeneity of the three different HOVON-SAKK-Nordic trials, trial and treatment arm ("standard" or "experimental") were added in the

multivariable model as stratifying factors. Allogeneic hematopoietic cell transplantation (allo-HCT) was considered as a time-varying variable.

Results were considered statistically significant when the 2-sided p-value was <0.05 and confidence intervals (CI) were computed with 95% coverage. Statistical analyses were performed using R version 4.2.2 or higher.

RESULTS

Characteristics and outcomes of all t-AML and AML-MRC patients

In the t-AML/AML-MRC cohort, the median age of patients was 66 years (IQR: 63-70) and 272 (63.6%) were male. The most frequent disease subtype was de novo AML with MDS-related cytogenetics (n=232, 54.2%), followed by s-AML with antecedent hematologic disorders (n=136, 31.8%), and t-AML (n=60, 14.0%) (Table S1). Next-generation sequencing (NGS) was available for 327 patients, where *TP53* was the most frequently mutated gene (n=128, 39.1%, Figure 2). MDS-related gene mutations were found in 45.0% (n=147), of which *RUNX1* (n=72, 22.0%), *ASXL1* (n=63, 19.3%), and *SRSF2* (n=55, 16.8%) were the most frequent. Consequently, the majority of patients were assigned to the ELN2022 adverse risk subgroup (87.8%). A total of 223 (52%) patients obtained CR/CRi after the first induction cycle, which increased to 276 (64%) after two induction cycles. The median follow-up was 68.0 months (IQR: 54.8-88.4). OS was poor in the entire t-AML/AML-MRC cohort, with a median of 8.9 months (95% CI: 8.0-10.1). *TP53* mutation, adverse risk cytogenetics, higher baseline WHO score and white blood cell (WBC) count were associated with worse OS after adjustment for covariates and stratification for trial and treatment arm (Table S2 and S3). Focusing on patients who obtained CR/CRi, allo-HCT recipients had significantly better OS compared with patients who did not receive allo-HCT as consolidation treatment (hazard ratio [HR] = 0.64; 95% CI 0.46-0.89, p=0.008) (Table S4).

Higher intensity 7+3 induction versus CPX-351

Patient characteristics

A total of 180 t-AML/AML-MRC patients were identified fulfilling the key eligibility criteria of the CPX-351 trial,[14] excluding patients aged >75 years, patients with prior MPN or CNS leukemia, and patients assigned to the experimental treatment arms (HOVON 7+3 cohort) (Figure 1). These HOVON 7+3 patients were compared with patients enrolled in the CPX-351 arm of the phase III trial (Table 1). HOVON 7+3 patients were significantly younger as compared with CPX-351 trial patients (mean age 66.1 versus 67.8 years, p<0.001) and more frequently classified as de novo AML with MDS cytogenetics (57.7% vs 26.8% for the CPX-351 arm), whereas s-AML was mostly found in the CPX-351 cohort (53.6% vs 25.6% for the HOVON 7+3 cohort). Adverse cytogenetic risk according to NCCN criteria was more frequent in the HOVON 7+3 cohort compared with CPX-351 (67.6% versus 50.3%, respectively).

Induction treatment efficacy

A total of 130/180 (72.2%) patients in the HOVON 7+3 cohort received two cycles of 7+3 induction chemotherapy, which was higher compared with the CPX-351 arm (48/153, 31.4%) and the control 7+3 arm (51/156, 32.7%). First, we compared CR/CRi rates after one induction cycle between the two cohorts. After the first induction cycle, the CR/CRi rate was 54.4% (98/180) in the HOVON 7+3 cohort, compared with 37.9% (58/153) in the CPX-351 cohort and 21.7% (34/156) in the 7+3 arm of the CPX-351 study. After the second cycle with IDAC, remission rates further increased, with 122/180 patients (67.8%) in the HOVON 7+3 cohort achieving CR/CRi and 111/180 (61.7%) achieving CR. In contrast, the CPX-351 arm and the 7+3 arm of the CPX-351 study showed lower rates (CR/CRi 73/153, 47.7% and CR 57/153, 37.3% for the CPX-351 arm, CR/CRi 52/156, 33.3% and CR 40/156, 25.6% for the 7+3 arm) (Table 2). Higher remission rates were consistently observed in the HOVON 7+3 cohort across the cytogenetic groups per NCCN and age groups, whereas remission rates were similar in treatment-naive s-AML patients (Table 2).

The median follow-up was 67.3 months (IQR: 54.8-90.3) in the HOVON 7+3 cohort and 60.9 months (IQR: 60.1-63.0) in the CPX-351 cohort. After regenerating and superimposing the

CPX-351 survival curve onto the HOVON 7+3 survival curve, OS was not significantly different with a median OS of 8.9 months (95% CI 7.1-11.9) in the CPX-351 cohort versus 10.1 months (95% CI 9.1-13.4) in the HOVON 7+3 cohort (HR = 0.99; 95% CI 0.78-1.26, p=0.95) (Figure 3). Since the CPX-351 trial included patients with prior exposure to hypomethylating agents (HMAs), we then focused on the subset of patients that had not received prior HMAs. CPX-351 and HOVON 7+3 were again associated with similar OS (HR = 0.84; 95% CI 0.63-1.11, p=0.22) (Figure 4A). Similarly, no significant difference in OS was observed across the two age groups, 60-69 and 70-75 years (Figure 4B and 4C), as well as within t-AML, s-AML, and de novo AML with MDS cytogenetics subtypes (Figures S1-3).

Allo-HCT in CR/CRi was applied comparably in HOVON 7+3 and CPX-351 cohorts (66/122, 54.1% versus 41/73, 56.2%, p=0.88, Figure S4A). Within this selected subgroup of CR/CRi patients who proceeded to allo-HCT, OS was significantly longer for patients who received CPX-351 as induction therapy compared with HOVON 7+3 (median OS: not reached vs 14.4 months, p = 0.007, respectively) (Figure S4B).

Since *TP53* was the dominant mutation in the HOVON 7+3 cohort (Figure S5), we specifically analyzed outcomes of this subset of patients. This revealed that CR/CRi rate was 56.9% in the HOVON 7+3 cohort, with a median OS of 8.0 months (95% CI: 5.8-9.6). In a separate analysis of *TP53* mutated patients of the CPX-351 trial, a CR/CRi rate of 33.3% was observed with a median OS of 5.0 months (95% CI: 2.7-7.6).[23] In patients with MDS-related gene mutations without mutated *TP53*, HOVON 7+3 patients were associated with a median OS of 15.8 months (95% CI, 10.6–30.8). In comparison, the CPX-351 pivotal trial reported a median OS of 9.7 months (95% CI, 6.2–13.7) for the same subgroup.[24]

Induction treatment toxicity

Early mortality rates between the CPX-351 and HOVON 7+3 cohorts were comparable, with a 30-day mortality of 5.9% for the CPX-351 cohort versus 4.4% for the HOVON 7+3 cohort, and

a 60-day mortality of 13.7% for the CPX-351 cohort versus 11.7% for the HOVON 7+3 cohort. Duration of hospital stay was comparable in both initial and second cycle of induction (cycle 1: 28 days for the CPX-351 cohort versus 29 days for the HOVON 7+3, cycle 2: 28 days for the CPX-351 cohort versus 28 days for the HOVON 7+3 cohort).[25] For patients that reached CR/CRi, median time until neutrophil recovery (≥500/μL) or platelet recovery (≥50,000/μL) was longer for CPX-351 compared with HOVON 7+3 (neutrophil recovery cycle 1: 35 versus 26 days, cycle 2: 35 versus 25, respectively, platelet recovery cycle 1: 37 versus 25 days and cycle 2: 35 versus 33 days, respectively). The most common grade 3 to 5 adverse events were similarly observed in CPX-351 and HOVON 7+3 cohorts (febrile neutropenia: 68% versus 61%, respectively; pneumonia: 20% versus 23%, respectively), while hypoxia was more frequent in the CPX-351 cohort (13% versus 3%, respectively).

DISCUSSION

Outcomes of patients with t-AML and AML-MRC remain poor despite recent advancements in the induction treatment paradigm of AML. CPX-351 has demonstrated improved OS compared with 7+3 in this patient population aged 60-75 years, but CR/CRi rates were relatively low, particularly for patients enrolled in the control 7+3 arm of the pivotal trial.[14] The obligatory double-induction 7+3 regimen used within the HOVON-SAKK-Nordic trials included IDAC in the second cycle, urging a comparison between patients with t-AML or AML-MRC aged 60–75 years treated in these trials and patients enrolled in the CPX-351 pivotal trial. These trials had similar key eligibility criteria and used similar response definitions. We observed higher CR/CRi rates for HOVON-SAKK-Nordic patients, while OS was similar between HOVON 7+3 and CPX-351 cohorts. Additionally, the rate of allo-HCT, early mortality and toxicity were similar between the two cohorts. These data indicate that induction regimens incorporating IDAC, as used within HOVON-SAKK-Nordic trials might benefit these patients and highlight the need for further investigation into the comparative efficacy of CPX-351 and an obligatory double-induction regimen, which randomized clinical trial is currently underway (NCT03897127).

Optimal dosing of 7+3 has been extensively studied in the last decades.[26–28] The obligatory double-induction regimen used in recent HOVON-SAKK-Nordic trials differs from the 7+3 regimen used in the control arm of the CPX-351 trial with double doses of cytarabine (200 mg/m^2/day versus 100mg/m^2/day, respectively) in the first cycle. However, these doses are generally considered clinically equivalent.[29] More importantly, all eligible HOVON-SAKK-Nordic patients were scheduled for a second IDAC induction cycle with 2,000mg/m^2/day of cytarabine, whereas the CPX-351 trial protocol only scheduled a second cycle of 5+2, including 100mg/m^2/day cytarabine, for refractory patients. In these selected trial populations, the CR/CRi rates after the first cycle and within two cycles were consistently higher in the HOVON 7+3 cohort compared with the 7+3 arm used in the CPX-351 trial. Cytarabine dosing for patients with AML has been previously studied in randomized clinical trials. A previous HOVON-SAKK trial showed that intensification of cytarabine dosing beyond intermediate levels did not provide any therapeutic benefit and instead led to increased toxicity.[30] Similarly, Röllig et al. reported higher CR rates after induction with intermediate dose cytarabine for 4 days combined with mitoxantrone compared with 100 mg/m^2/day cytarabine for 7 days and daunorubicin, while early mortality was similar.[31] In addition, double-induction with 7+3-like regimens in elderly patients has been associated with CR/CRi rates estimating 65-70% confirming the results observed in this selected t-AML and AML-MRC population.[16,32,33] Building on these observations and our findings, the control arm of the pivotal CPX-351 trial has been compared with real-world IDAC in a propensity score analysis, where significantly higher CR rates and longer OS were observed with IDAC, highlighting the benefit of higher doses of cytarabine during induction and consolidation for these high-risk AML patients.[34]

There is heterogeneity of response rates reported by real-world CPX-351 studies. Some of these studies show CR/CRi rates of 60-70%,[35,36] whereas others report CR/CRi rates of 47-53% in these high-risk t-AML and AML-MRC patients.[37,38] Additionally, therapy-related morbidity and mortality is a major concern in the elderly AML subgroup. The HOVON 7+3 and CPX-351 cohorts were comparable regarding 30-day and 60-day mortality, duration of

hospitalization per induction cycle, and most frequent adverse events (febrile neutropenia and pneumonia), while days to hematologic recovery were shorter with the higher intensity 7+3. Real-world studies identified similar toxicity rates,[35,37–39] although gastrointestinal events are reported with a lower incidence and are of lower grade in the CPX-351 setting.[40] Although OS was similar between the two cohorts in our study, the potential for lower gastrointestinal toxicity with CPX-351 observed in real-world reports may be considered when selecting therapy, particularly in patients at higher risk of treatment-related morbidity. These observations from real-world use of CPX-351 again warrant a follow-up trial comparing CPX-351 with higher doses of cytarabine (NCT03897127).

Overall, survival of elderly t-AML/AML-MRC patients remains poor with a median OS of less than 12 months, emphasizing the significant unmet medical need in the management of those patients. In our t-AML/AML-MRC cohort, the adverse cytogenetic risk group and the presence of *TP53* mutations were observed with high incidence and associated with particularly worse OS in accordance with previous studies.[9,14,41] This confirms the known challenges of treating t-AML and AML-MRC in patients with *TP53* mutations, a subgroup with a dismal prognosis and resistance to chemotherapy-based therapies.[42–44] A post-hoc analysis of the phase III trial showed that CPX-351 also did not substantially improve the short OS associated with *TP53* mutated AML.[23] Recent studies indicate that the benefit of CPX-351 over standard chemotherapy is driven by the improved OS of patients with MDS-related gene mutations, while those harboring high-risk cytogenetics or *TP53* alterations derive limited benefit.[24,45] The HOVON 7+3 cohort of patients with MDS-related gene mutations appeared to have relatively favorable survival, although a formal comparison to the CPX-351 cohort with MDS-related gene mutations was not possible.[24]

Allo-HCT remains the ultimate path to cure for these high-risk patients. In our total cohort, allo-HCT was associated with a 36% reduction in risk of death compared with non-transplanted patients with CR/CRi after induction. In the subset of patients with a CR/CRi who received allo-

HCT, patients who received CPX-351 induction were associated with higher post-transplant OS compared with those treated with HOVON 7+3. Favorable OS rates have also been previously observed in real-world CPX-351 studies that reported a high rate of allo-HCT.[35,36,38,46] Ultimately, allo-HCT remains the cornerstone of curative treatment for t-AML and AML-MRC. Thus, the primary objective of intensive induction therapy is to achieve a (deep) CR with minimal toxicity, especially in older patients, allowing patients to proceed to transplantation.

Our study has some limitations. The HOVON 7+3 cohort was treatment-naïve, while the CPX-351 trial enrolled also patients with prior exposure to HMAs. However, a subgroup analysis did not show a significant survival difference when the subset of CPX-351 trial patients that had no prior exposure to HMAs was compared with the HOVON 7+3 cohort. Secondly, the actual data of the CPX-351 trial were not available in order to perform a propensity score matched analysis or multivariable analysis given the inherent differences in baseline characteristics. Although we cannot fully adjust for potential confounders and this is a comparison across trials, we were able to stratify by clinically relevant subgroups (age groups, AML subtype). Moreover, both cohorts consisted of patients enrolled in clinical trials and were selected using similar strict inclusion and exclusion criteria. Therefore, the cohorts were likely comparable with regards to frailty and comorbidities, as shown by the similar WHO performance scores and age. Additionally, the trials were conducted during the same time period (HOVON-SAKK-Nordic trials 2010-2017 and CPX-351 trial 2012-2014), minimizing potential confounding by different decade treatment trends. Despite harmonizing key predictors, these results should be interpreted with caution since residual differences in baseline disease biology and ontogeny, and variations in response evaluation timing may have lowered the CR/CRi rates in the CPX-351 arm. Additionally, details of salvage treatment in the CPX-351 trial are not available which might explain why OS is similar, despite different first CR/CRi rates between the CPX-351 arm and the HOVON 7+3 cohort.

In conclusion, in older patients with t-AML or AML-MRC, OS with CPX-351 induction treatment was not significantly different compared with the obligatory double-induction treatment that included a second cycle with IDAC. These results were similarly observed in different subgroups, whereas CR/CRi rates were higher with the HOVON 7+3 regimen. Toxicity analysis also indicated comparable outcomes, with fewer days to hematologic recovery with the HOVON 7+3. Further randomized studies, including the ongoing trial (NCT03897127), are needed to clarify whether CPX-351 achieves better outcomes than more intensive 7+3, and to identify possible molecular features or patient populations that might benefit from CPX-351. Importantly, allo-HCT remains the most effective curative strategy for these patients, and should be offered to as many t-AML/AML-MRC patients as possible.

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TABLES

Variable	HOVON 7+3 N (%)	CPX-351 N (%)	p-value
No. of patients	**180**	**153**	
Age (years)			
Mean (SD)	66.1 (4.1)	67.8 (4.2)	<0.001
Age group	**180**	**153**	0.005
60-69	139 (77.2)	96 (62.7)	
≥70	41 (22.8)	57 (37.3)	
Sex	**180**	**153**	0.82
Male	108 (60.0)	94 (61.4)	
WHO PS	**177**	**153**	0.21
0-1	167 (94.4)	138 (90.2)	
2	10 (5.6)	15 (9.8)	
AML subtype	**180**	**153**	<0.001
t-AML	30 (16.7)	30 (19.6)	
s-AML	46 (25.6)	82 (53.6)	
s-AML with antecedent MDS	45 (25.0)	71 (46.4)	
Without prior HMAs	45 (25.0)	21 (13.7)	
With prior HMAs	0 (0.0)	50 (32.7)	
s-AML with antecedent CMML	1 (0.6)	11 (7.2)	
dnAML with MDS cytogenetics	104 (57.7)	41 (26.8)	
WBC count	**179**	**153**	0.24
<20 × 10^9/L	161 (89.9)	131 (85.6)	
≥20 × 10^9/L	18 (10.1)	22 (14.4)	
NCCN risk group	**176**	**143**	0.002
Favorable/Intermediate	57 (32.4)	71 (49.7)	
Adverse	119 (67.6)	72 (50.3)	
***FLT3* mutation**	**140**	**138**	0.04
Absent	129 (92.1)	116 (84.1)	
Present	11 (7.9)	22 (15.9)	
Prior anthracycline exposure	6 (3.3)	6 (3.9)	0.78
Allo-HCT	**180**	**153**	
Yes	67 (37.2)	53 (34.6)	0.65

Table 1. Demographic and baseline characteristics of the HOVON 7+3 cohort and the CPX-351 arm of the phase III clinical trial of the liposomal drug.

Abbreviations: 7+3, 7 days of cytarabine and 3 days of an anthracycline; allo-HCT, allogeneic hematopoietic cell transplantation; AML, acute myeloid leukemia; CPX-351, liposomal formulation of cytarabine and daunorubicin; CMML, chronic myelomonocytic leukemia; dnAML, de novo acute myeloid leukemia, HMAs, hypomethylating agents; HOVON, Dutch-Belgian Hemato-Oncology Cooperative Group; MDS, myelodysplastic syndrome; NCCN, national comprehensive cancer network; s-AML, secondary acute myeloid leukemia; SD, standard deviation; t-AML, therapy-related acute myeloid leukemia; WBC count, white blood cell count; WHO PS, World Health Organization performance status.

Response	HOVON 7+3 N (%)	CPX-351 N (%)	p-value
No. of patients	**180**	**153**	
CR/CRi	122 (67.8)	73 (47.7)	<0.001
CR	111 (61.7)	57 (37.3)	<0.001
Age group			
No. in 60-69-year age-group	**139**	**96**	
CR/CRi	98 (70.5)	48 (50.0)	0.002
CR	90 (64.7)	38 (39.6)	<0.001
No. in ≥70-year age-group	**41**	**57**	
CR/CRi	24 (58.5)	25 (43.9)	0.22
CR	21 (51.2)	19 (33.3)	0.10
AML subtype			
No. with t-AML	**30**	**30**	
CR/CRi	20 (66.7)	14 (46.7)	0.19
CR	19 (63.3)	11 (36.7)	0.07
No. with s-AML	**46**	**82**	
s-AML with antecedent MDS (no prior HMAs)	**45**	**21**	
CR/CRi	31 (68.9)	14 (66.7)	1.00
CR	25 (55.6)	12 (57.1)	1.00
s-AML with antecedent MDS (with prior HMAs)	**0**	**50**	
CR/CRi	0 (0.00)	18 (36.0)	
CR	0 (0.00)	13 (26.0)	
s-AML with antecedent CMML	**1**	**11**	
CR/CRi	1 (100.0)	4 (36.4)	0.42
CR	1 (100.0)	2 (18.2)	0.25
No. with dnAML with MDS cytogenetics	**104**	**41**	
CR/CRi	70 (67.3)	23 (56.1)	0.25
CR	66 (63.5)	19 (46.3)	0.06
Cytogenetic risk at screening by NCCN			
No. with Favorable/Intermediate	**57**	**71**	
CR/CRi	47 (82.5)	39 (54.9)	0.001
CR	42 (73.7)	30 (42.3)	<0.001
No. with Adverse	**119**	**72**	
CR/CRi	72 (60.5)	31 (43.1)	0.02
CR	66 (55.5)	25 (34.7)	0.007
***FLT3* mutation status**			
No. with *FLT3* mutation	**11**	**22**	
CR/CRi	7 (63.6)	15 (68.2)	1.00
CR	6 (54.5)	12 (54.5)	1.00

Table 2. Best response rates after induction of the HOVON 7+3 cohort and the CPX-351 arm of the phase III clinical trial of the liposomal drug.

Abbreviations: 7+3, 7 days of cytarabine and 3 days of an anthracycline; AML, acute myeloid leukemia; dnAML, de novo acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; CPX-351, liposomal formulation of cytarabine and daunorubicin; CR, complete remission; CRi,

complete remission with incomplete hematologic recovery; HOVON, Dutch-Belgian Hemato-Oncology Cooperative Group; MDS, myelodysplastic syndrome; HMAs, hypomethylating agents; NCCN, national comprehensive cancer network; s-AML, secondary acute myeloid leukemia; t-AML, therapy-related acute myeloid leukemia

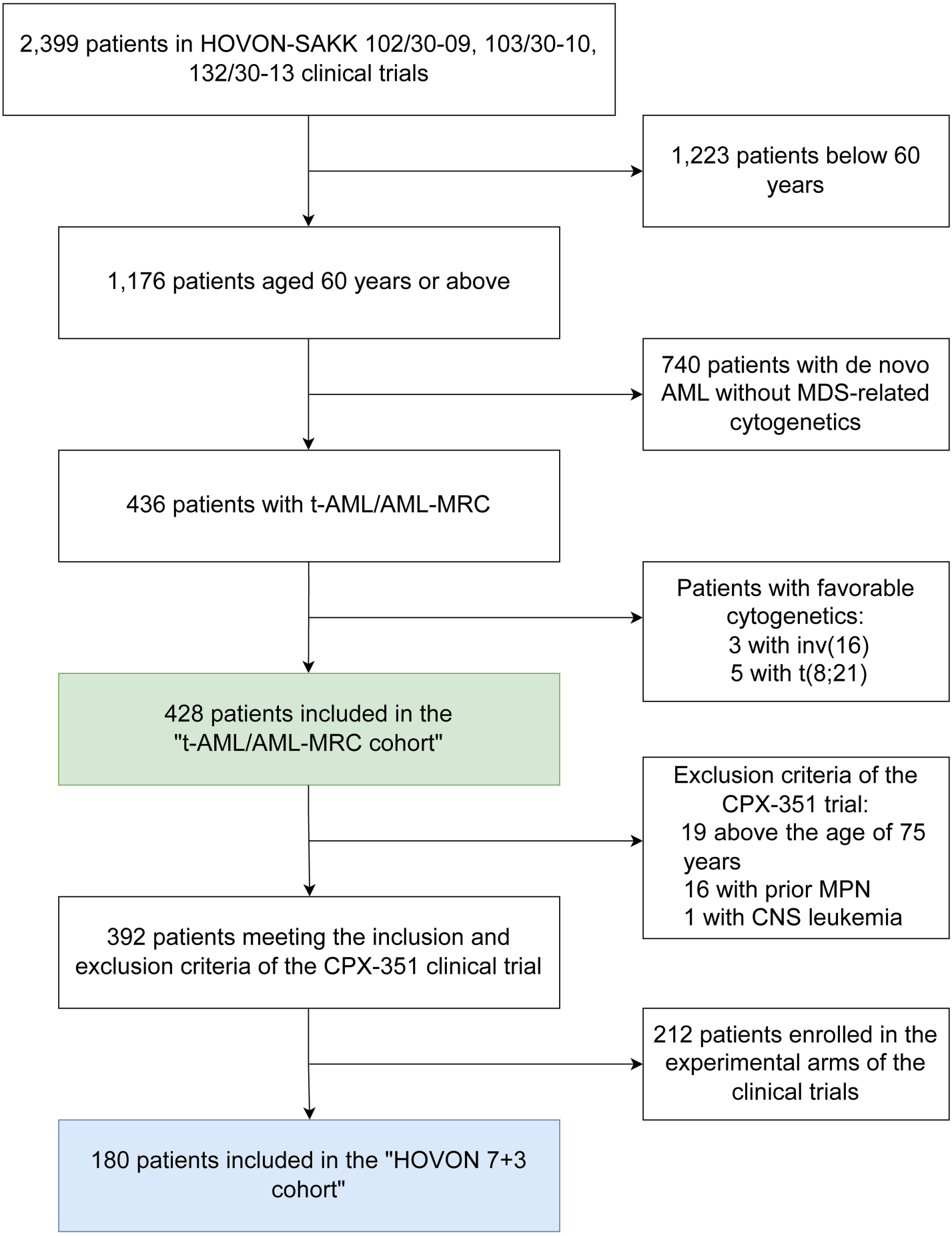
FIGURE LEGENDS

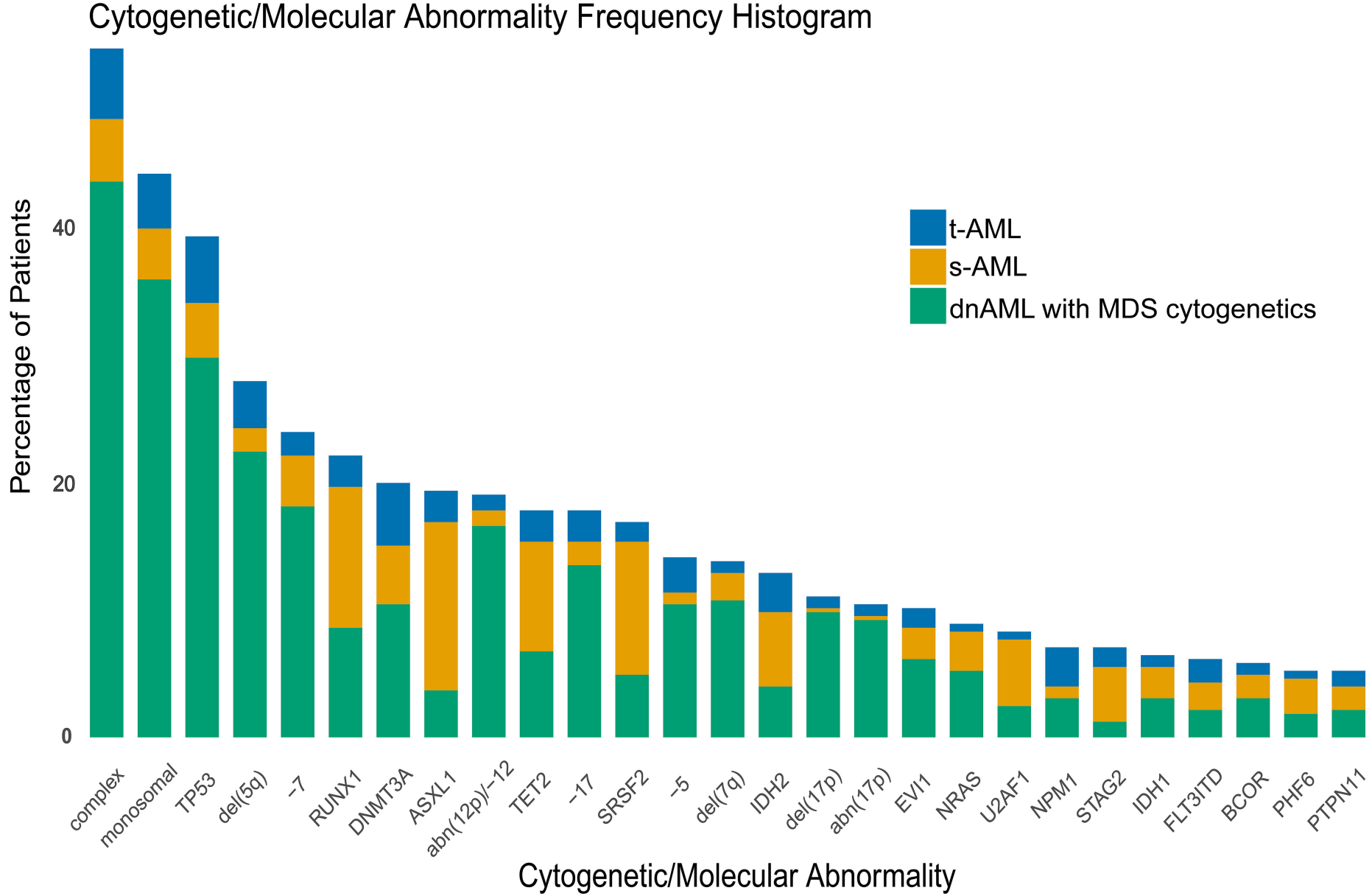
Figure 1. Flow diagram of patient selection for the t-AML/AML-MRC and HOVON 7+3 cohorts.
Abbreviations: 7+3, 7 days of cytarabine and 3 days of an anthracycline; AML, acute myeloid leukemia; AML-MRC, AML with myelodysplasia-related cytogenetics; CNS, central nervous system; CPX-351, liposomal formulation of cytarabine and daunorubicin; HOVON-SAKK, Dutch-Belgian Hemato-Oncology Cooperative Group and the Swiss Group for Clinical Cancer Research Collaborative Group; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; t-AML, therapy-related AML.

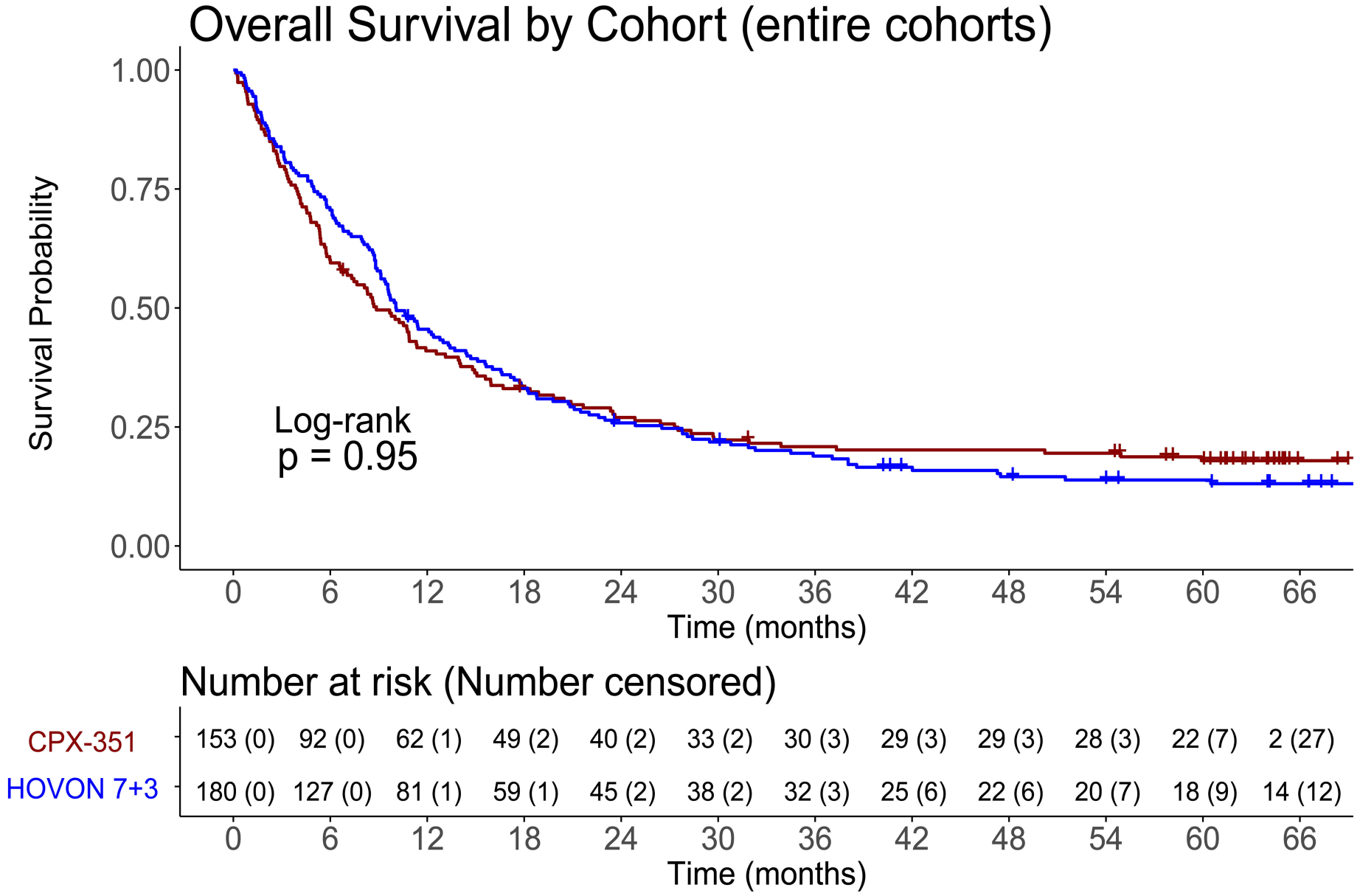
Figure 2. Driver events found in 327 out of 428 t-AML/AML-MRC patients with newly diagnosed AML. Each bar represents a driver lesion, including gene mutations and chromosomal abnormalities.
Abbreviations: dnAML, de novo acute myeloid leukemia; MDS, myelodysplastic syndrome; s-AML, secondary acute myeloid leukemia; t-AML, therapy-related acute myeloid leukemia.

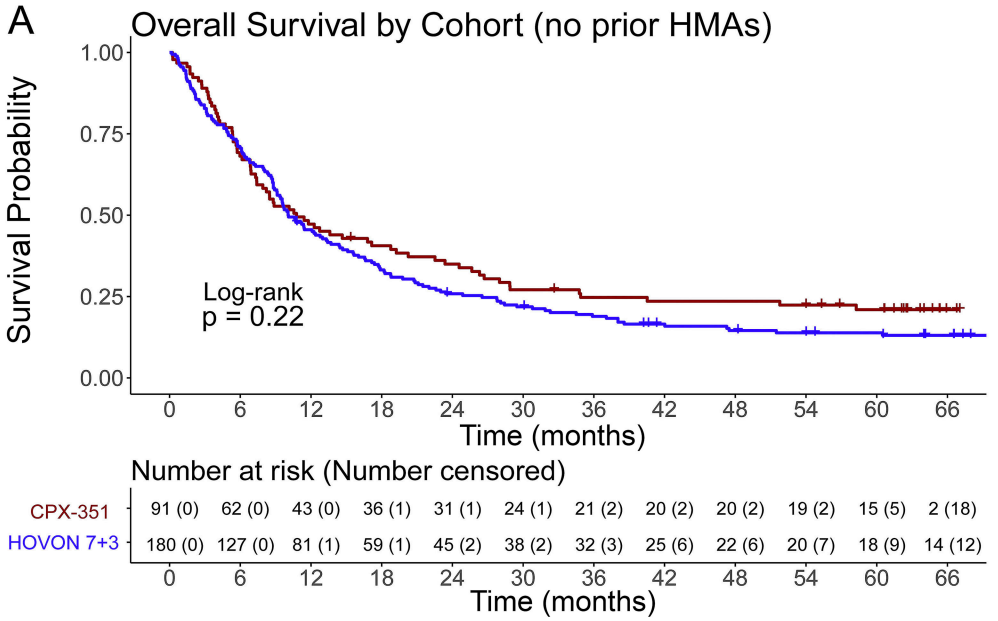
Figure 3. Overall survival of the entire CPX-351 arm of the phase III clinical trial versus the selected HOVON 7+3 cohort
Abbreviations: 7+3, 7 days of cytarabine and 3 days of an anthracycline; CPX-351, liposomal formulation of cytarabine and daunorubicin.

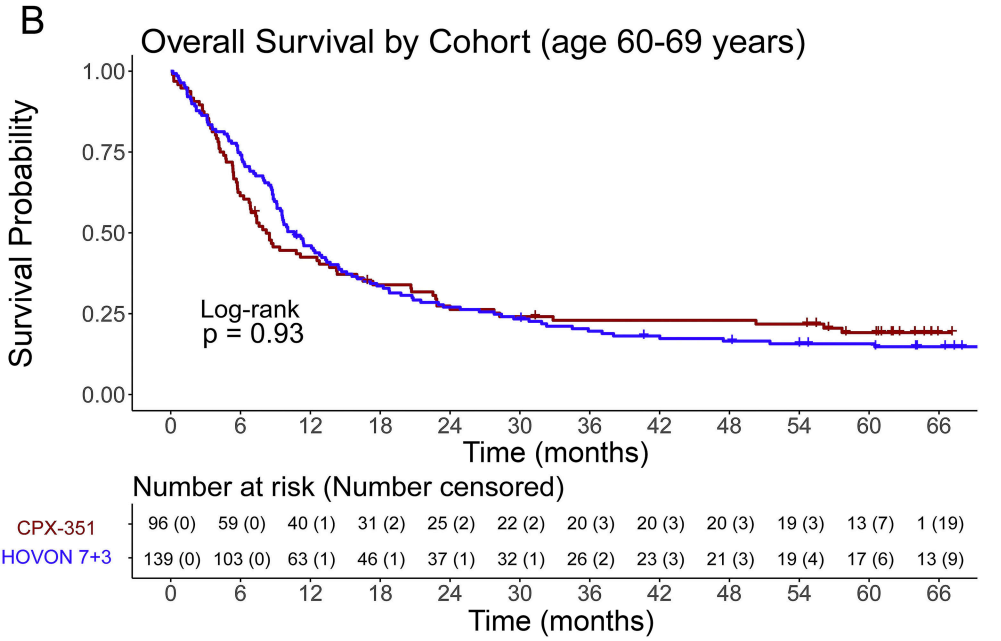
Figure 4. Overall survival in subgroups of the CPX-351 arm of the phase III clinical trial versus the selected HOVON 7+3 cohort. **(A)** Overall survival of the patients without prior exposure to hypomethylating agents of the CPX-351 arm of the phase III clinical trial versus the selected HOVON 7+3 cohort (all patients were treatment-naïve) **(B)** Overall survival of the 60-69-year age group of the CPX-351 arm of the phase III clinical trial versus the 60-69-year age group of the selected HOVON 7+3 cohort **(C)** Overall survival of the 70-75-year age group of the CPX-351 arm of the phase III clinical trial versus the 70-75-year age group of the selected HOVON 7+3 cohort.
Abbreviations: 7+3, 7 days of cytarabine and 3 days of an anthracycline; CPX-351, liposomal formulation of cytarabine and daunorubicin; HMAs, hypomethylating agents.

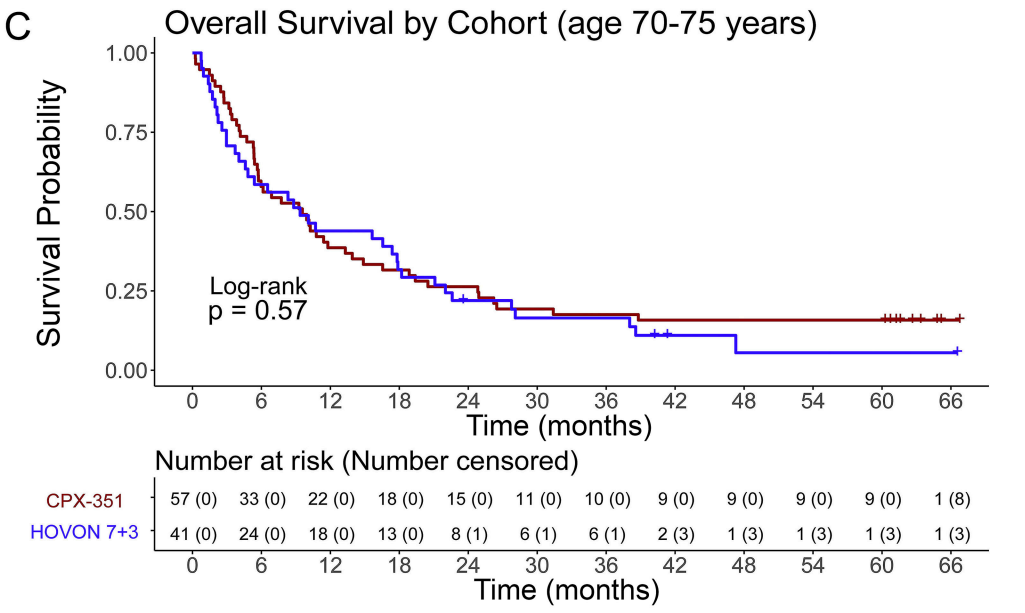

2,399 patients in HOVON-SAKK 102/30-09, 103/30-10, 132/30-13 clinical trials
1,223 patients below 60 years
1,176 patients aged 60 years or above
740 patients with de novo AML without MDS-related cytogenetics
436 patients with t-AML/AML-MRC
Patients with favorable cytogenetics:
3 with inv(16)
5 with t(8;21)
428 patients included in the "t-AML/AML-MRC cohort"
Exclusion criteria of the CPX-351 trial:
19 above the age of 75 years
16 with prior MPN
1 with CNS leukemia
392 patients meeting the inclusion and exclusion criteria of the CPX-351 clinical trial
212 patients enrolled in the experimental arms of the clinical trials
180 patients included in the "HOVON 7+3 cohort"


Cytogenetic/Molecular Abnormality Frequency Histogram
Percentage of Patients
0
20
40
t-AML
s-AML
dnAML with MDS cytogenetics
complex
monosomal
TP53
del(5q)
−7
RUNX1
DNMT3A
ASXL1
abn(12p)/−12
TET2
−17
SRSF2
−5
del(7q)
IDH2
del(17p)
abn(17p)
EVI1
NRAS
U2AF1
NPM1
STAG2
IDH1
FLT3ITD
BCOR
PHF6
PTPN11
Cytogenetic/Molecular Abnormality


Overall Survival by Cohort (entire cohorts)
Survival Probability
1.00
0.75
0.50
0.25
0.00
Log-rank
p = 0.95
0
6
12
18
24
30
36
42
48
54
60
66
Time (months)
Number at risk (Number censored)
CPX-351
153 (0)
92 (0)
62 (1)
49 (2)
40 (2)
33 (2)
30 (3)
29 (3)
29 (3)
28 (3)
22 (7)
2 (27)
HOVON 7+3
180 (0)
127 (0)
81 (1)
59 (1)
45 (2)
38 (2)
32 (3)
25 (6)
22 (6)
20 (7)
18 (9)
14 (12)
0
6
12
18
24
30
36
42
48
54
60
66
Time (months)


A
Overall Survival by Cohort (no prior HMAs)
Survival Probability
1.00
0.75
0.50
0.25
0.00
Log-rank
p = 0.22
0 6 12 18 24 30 36 42 48 54 60 66
Time (months)
Number at risk (Number censored)
CPX-351 91 (0) 62 (0) 43 (0) 36 (1) 31 (1) 24 (1) 21 (2) 20 (2) 20 (2) 19 (2) 15 (5) 2 (18)
HOVON 7+3 180 (0) 127 (0) 81 (1) 59 (1) 45 (2) 38 (2) 32 (3) 25 (6) 22 (6) 20 (7) 18 (9) 14 (12)
0 6 12 18 24 30 36 42 48 54 60 66
Time (months)


B
Overall Survival by Cohort (age 60-69 years)
Survival Probability
1.00
0.75
0.50
0.25
0.00
Log-rank
p = 0.93
0 6 12 18 24 30 36 42 48 54 60 66
Time (months)
Number at risk (Number censored)
CPX-351 96 (0) 59 (0) 40 (1) 31 (2) 25 (2) 22 (2) 20 (3) 20 (3) 20 (3) 19 (3) 13 (7) 1 (19)
HOVON 7+3 139 (0) 103 (0) 63 (1) 46 (1) 37 (1) 32 (1) 26 (2) 23 (3) 21 (3) 19 (4) 17 (6) 13 (9)
0 6 12 18 24 30 36 42 48 54 60 66
Time (months)

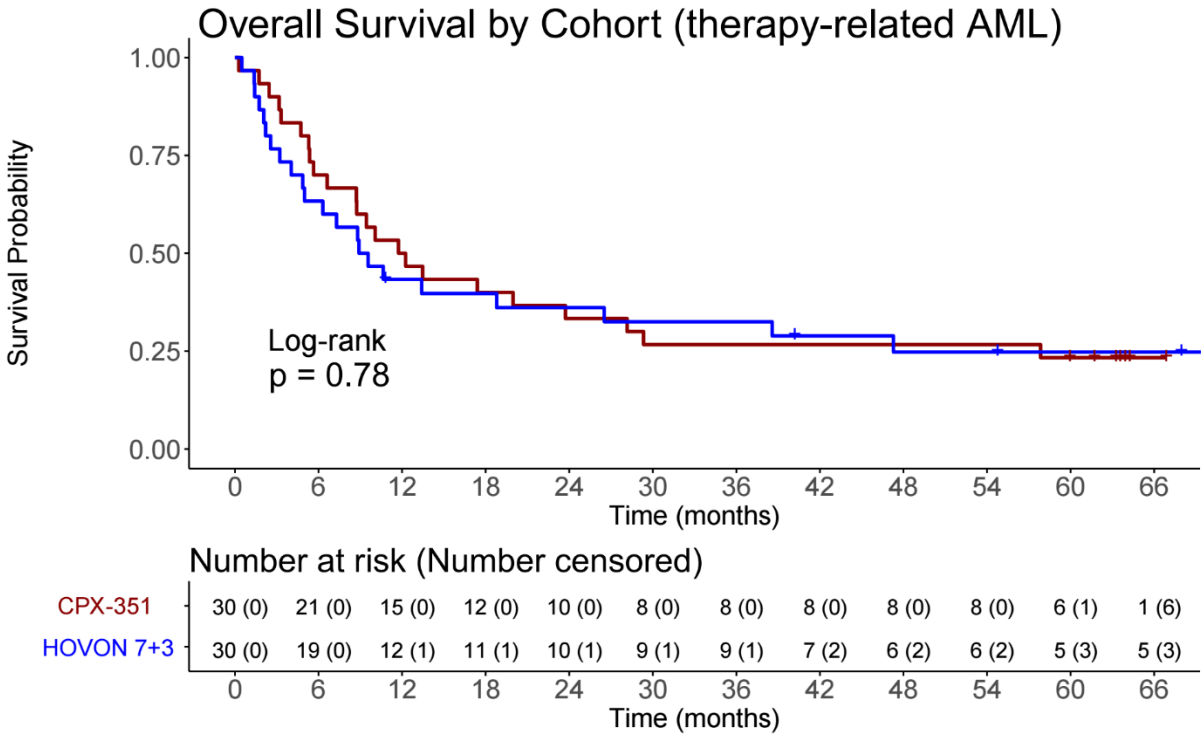

C
Overall Survival by Cohort (age 70-75 years)
Survival Probability
1.00
0.75
0.50
0.25
0.00
Log-rank
p = 0.57
0 6 12 18 24 30 36 42 48 54 60 66
Time (months)
Number at risk (Number censored)
CPX-351 57 (0) 33 (0) 22 (0) 18 (0) 15 (0) 11 (0) 10 (0) 9 (0) 9 (0) 9 (0) 9 (0) 1 (8)
HOVON 7+3 41 (0) 24 (0) 18 (0) 13 (0) 8 (1) 6 (1) 6 (1) 2 (3) 1 (3) 1 (3) 1 (3) 1 (3)
0 6 12 18 24 30 36 42 48 54 60 66
Time (months)

SUPPLEMENTARY APPENDIX

to

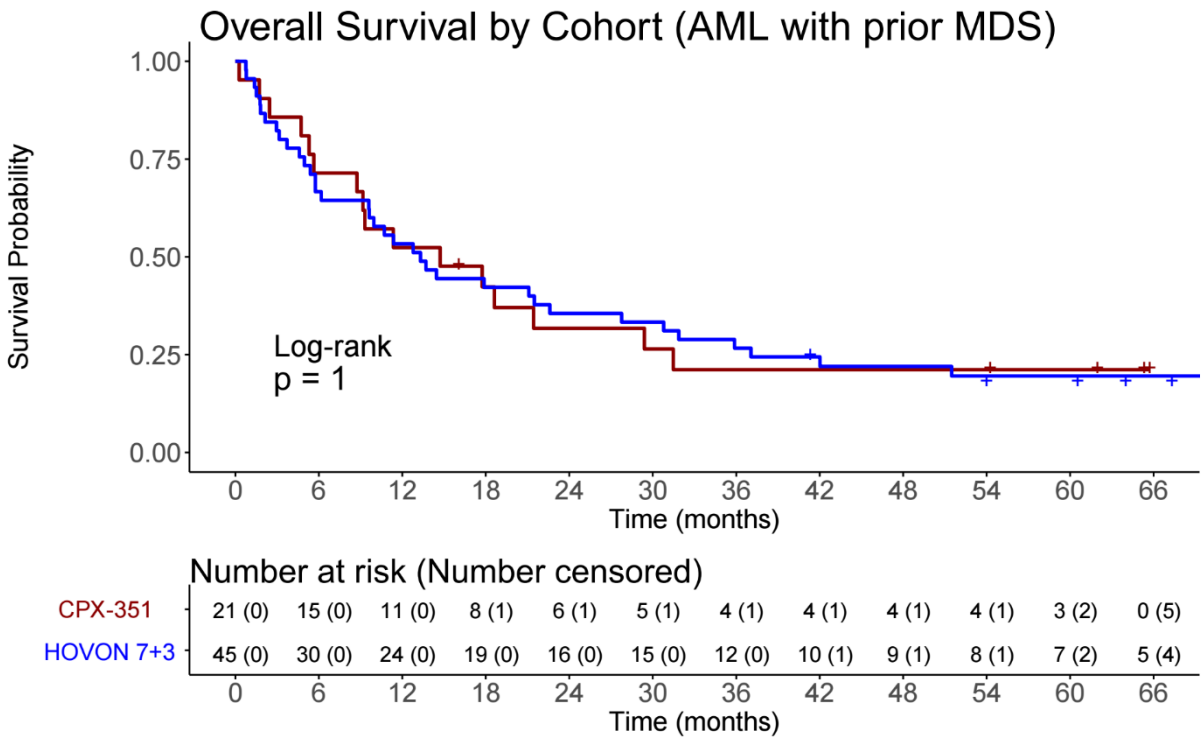
Treatment of therapy-related acute myeloid leukemia and acute myeloid leukemia with myelodysplasia-related changes: A comparative analysis of higher-dose intensive 7+3 induction chemotherapy versus liposomal cytarabine and daunorubicin

by Kotsos et al.



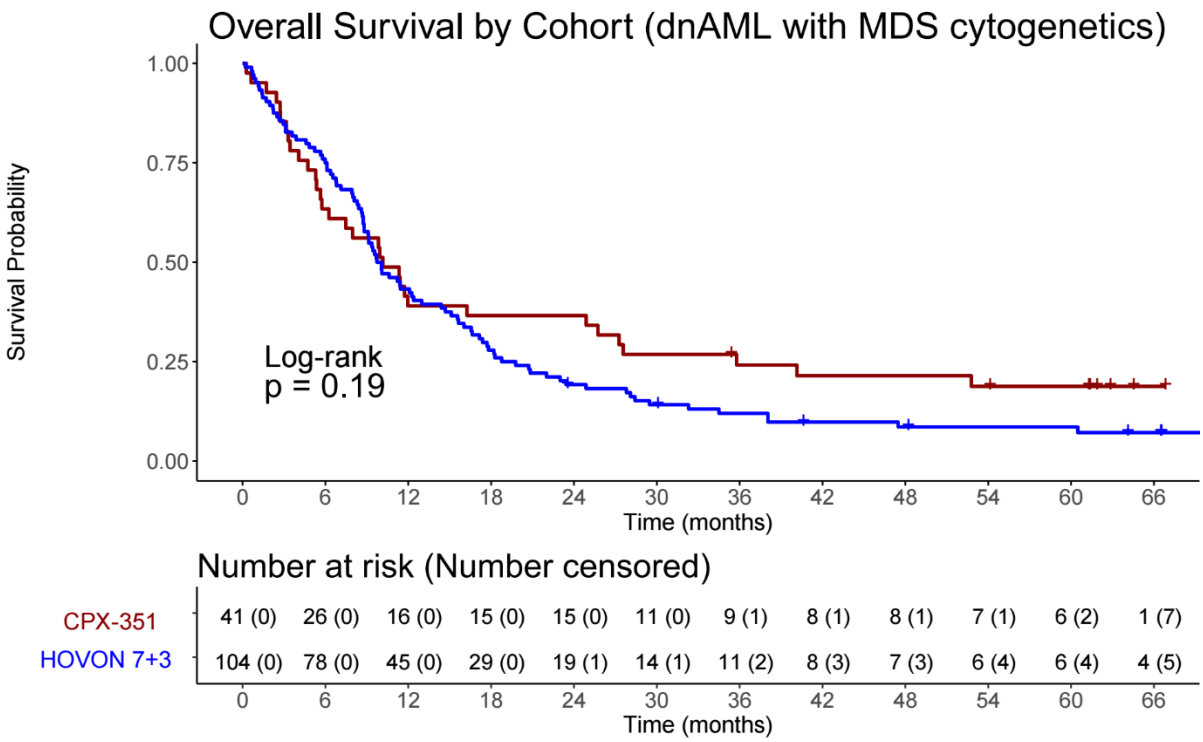
Supplementary Figure 1. Overall survival of the therapy-related AML subgroup of the CPX-351 arm of the phase III clinical trial versus the therapy-related AML subgroup of the HOVON 7+3 cohort.

Abbreviations: 7+3, 7 days of cytarabine and 3 days of an anthracycline; AML, acute myeloid leukemia; CPX-351, liposomal formulation of cytarabine and daunorubicin.



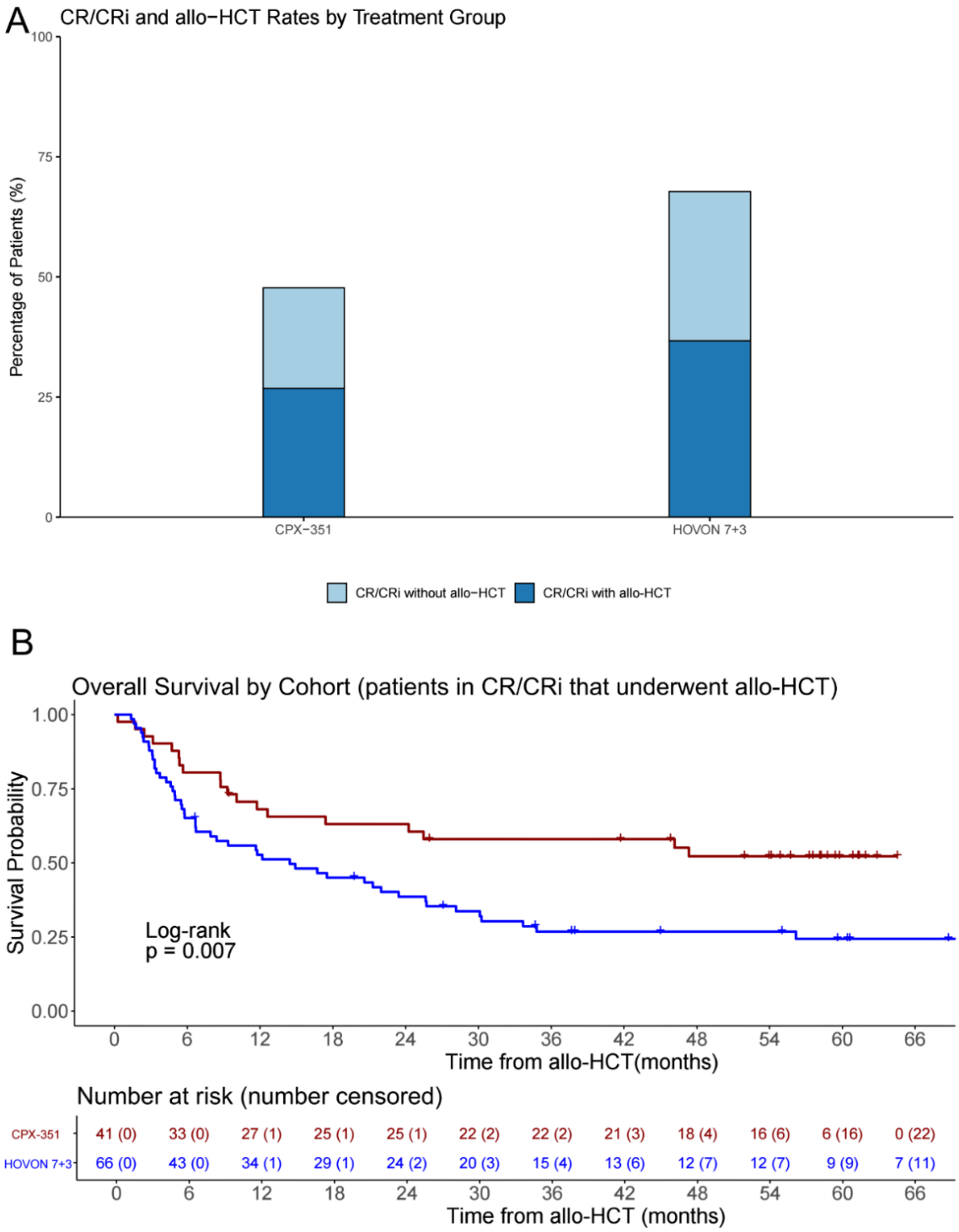
Supplementary Figure 2. Overall survival of the secondary AML with prior MDS subgroup of the CPX-351 arm of the phase III clinical trial versus the secondary AML with prior MDS subgroup of the HOVON 7+3 cohort.

Abbreviations: 7+3, 7 days of cytarabine and 3 days of an anthracycline; AML, acute myeloid leukemia; CPX-351, liposomal formulation of cytarabine and daunorubicin; MDS, myelodysplastic syndrome.



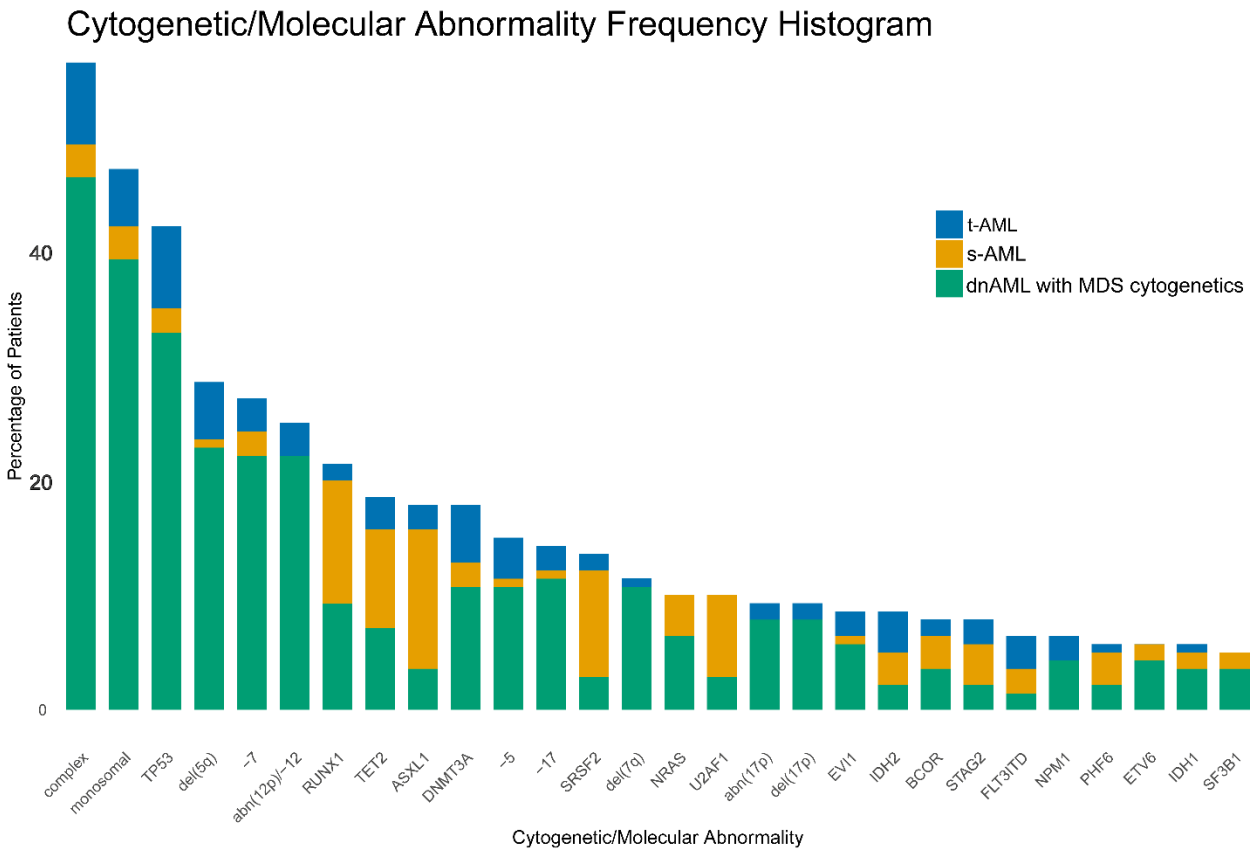
Supplementary Figure 3. Overall survival of the de novo AML with MDS cytogenetics subgroup of the CPX-351 arm of the phase III clinical trial versus the de novo AML with MDS cytogenetics subgroup of the HOVON 7+3 cohort.

Abbreviations: 7+3, 7 days of cytarabine and 3 days of an anthracycline; dnAML, de novo acute myeloid leukemia; MDS, myelodysplastic syndrome; CPX-351, liposomal formulation of cytarabine and daunorubicin.



Supplementary Figure 4. Allo-HCT rates and overall survival after allo-HCT in patients achieving CR/CRi. **(A)** Each bar represents the percentage of patients that proceeded to allo-HCT (dark blue) out of all patients in CR/CRi **(B)** Overall survival of the patients in CR/CRi that proceeded to allo-HCT of the CPX-351 arm of the phase III clinical trial versus the selected HOVON 7+3 cohort.

Abbreviations: 7+3, 7 days of cytarabine and 3 days of an anthracycline; allo-HCT, allogeneic hematopoietic cell transplantation; CPX-351, liposomal formulation of cytarabine and daunorubicin; CR, complete remission; CRi, complete remission with incomplete hematologic recovery.



Supplementary Figure 5. Driver events found in 140 out of 180 HOVON 7+3 patients with newly diagnosed AML. Each bar represents a driver lesion, including gene mutations and chromosomal abnormalities.

Abbreviations: 7+3, 7 days of cytarabine and 3 days of an anthracycline; dnAML, de novo acute myeloid leukemia; MDS, myelodysplastic syndrome; s-AML, secondary acute myeloid leukemia; t-AML, therapy-related acute myeloid leukemia.

Supplementary Table 1. Demographic and baseline characteristics of the entire t-AML/AML-MRC cohort and characteristics stratified by AML subtype.

Variable	Overall	t-AML	s-AML	dnAML with MDS cytogenetics	p-value
	N (%)	N (%)	N (%)	N (%)	
No. of patients	**428**	**60**	**136**	**232**	
Age (years)					
Median (IQR)	66 (63-70)	67 (64-70)	67 (64-71)	66 (63-70)	0.33
Age group	**428**	**60**	**136**	**232**	0.33
60-69	301 (70.3)	41 (68.3)	90 (66.2)	170 (73.3)	
≥70	127 (29.7)	19 (31.7)	46 (33.8)	62 (26.7)	
Sex	**428**	**60**	**136**	**232**	<0.001
Female	156 (36.4)	35 (58.3)	49 (36.0)	72 (31.0)	
Male	272 (63.6)	25 (41.7)	87 (64.0)	160 (69.0)	
WHO PS	**418**	**58**	**133**	**227**	0.83
0	209 (50.0)	29 (50.0)	72 (54.1)	108 (47.6)	
1	182 (43.5)	25 (43.1)	53 (39.8)	104 (45.8)	
2	27 (6.5)	4 (6.9)	8 (6.0)	15 (6.6)	
WBC count	**426**	**60**	**135**	**231**	0.07
<20 × 10^9/L	368 (86.4)	54 (90.0)	109 (80.7)	205 (88.7)	
≥20 × 10^9/L	58 (13.6)	6 (10.0)	26 (19.3)	26 (11.3)	
NCCN Risk Group	**420**	**59**	**129**	**232**	<0.001
Favorable/Intermediate	150 (35.7)	25 (42.4)	89 (69.0)	36 (15.5)	
Adverse	270 (64.3)	34 (57.6)	40 (31.0)	196 (84.5)	
ELN 2022 Risk Group	**327**	**51**	**95**	**181**	<0.001
Favorable	17 (5.2)	6 (11.8)	4 (4.2)	7 (3.9)	
Intermediate	23 (7.0)	11 (21.6)	6 (6.3)	6 (3.3)	
Adverse	287 (87.8)	34 (66.6)	85 (89.5)	168 (92.8)	
Best Response Rates	**428**	**60**	**136**	**232**	
CR/CRi	276 (64.5)	44 (73.3)	83 (61.0)	149 (64.2)	0.25
CR	237 (55.4)	42 (70.0)	66 (48.5)	129 (55.6)	0.02
Allo-HCT in CR/CRi	**276**	**44**	**83**	**149**	
Yes	125 (45.3)	18 (40.9)	38 (45.8)	69 (46.3)	0.81

Abbreviations: allo-HCT, allogeneic hematopoietic cell transplantation; AML; acute myeloid leukemia; CR, complete remission; CRi, complete remission with incomplete hematologic recovery; dnAML, de novo acute myeloid leukemia; ELN, European LeukemiaNet; IQR, interquartile range; MDS, myelodysplastic syndrome; NCCN, national comprehensive cancer network; s-AML, secondary acute myeloid leukemia; t-AML, therapy-related acute myeloid leukemia; WBC count, white blood cell count; WHO PS, World Health Organization performance status.

Supplementary Table 2. Univariable analysis for OS in the t-AML/AML-MRC cohort.

Variable	Levels	n/(N=428) (%)	Hazard Ratio* (95% CI)	p-value
Age (years)	(continuous)		1.02 (0.98-1.05)	0.42
Age group (years)	60-69	301 (70.3)	(reference)	
	≥70	127 (29.7)	1.29 (0.99-1.69)	0.06
Sex	Female	156 (36.4)	(reference)	
	Male	272 (63.6)	0.95 (0.77-1.18)	0.65
WHO PS	0	209 (48.8)	(reference)	
	1	182 (42.5)	1.11 (0.89-1.37)	0.35
	2	27 (6.3)	2.07 (1.37-3.15)	<0.001
AML subtype	dnAML with MDS cytogenetics	232 (54.2)	(reference)	
	s-AML	136 (31.8)	0.76 (0.61-0.96)	0.02
	t-AML	60 (14.0)	0.55 (0.39-0.77)	<0.001
WBC count	< 20 × 10^9/L	368 (86.0)	(reference)	
	≥ 20 × 10^9/L	58 (13.6)	1.27 (0.94-1.70)	0.11
Platelets	≤ 50 × 10^9/L	187 (43.7)	(reference)	
	> 50 × 10^9/L	239 (55.8)	0.83 (0.68-1.02)	0.08
BM blast (%)	0-20	128 (29.9)	(reference)	
	20-40	117 (27.3)	0.74 (0.57-0.98)	0.03
	40-60	56 (13.1)	1.01 (0.73-1.39)	0.97
	≥60	84 (19.6)	0.89 (0.66-1.20)	0.43
Hemoglobin (g/dL)	≤9	341 (79.7)	(reference)	
	>9	3 (0.7)	0.48 (0.12-1.95)	0.31
Cytogenetic risk category (per NCCN)	Non-adverse	150 (35.0)	(reference)	
	Adverse	270 (63.1)	1.81 (1.45-2.27)	<0.001
MDS-related gene mutations	Absent	180 (42.1)	(reference)	
	Present	147 (34.3)	0.80 (0.63-1.02)	0.07
TP53 mutation	Absent	199 (46.5)	(reference)	
	Present	128 (29.9)	2.51 (1.95-3.21)	<0.001
RUNX1 mutation	Absent	255 (59.6)	(reference)	
	Present	72 (16.8)	0.91 (0.69-1.22)	0.54
DNMT3A mutation	Absent	262 (61.2)	(reference)	
	Present	65 (15.2)	0.66 (0.48-0.90)	0.008
ASXL1 mutation	Absent	264 (61.7)	(reference)	
	Present	63 (14.7)	1.09 (0.81-1.46)	0.59
TET2 mutation	Absent	269 (62.9)	(reference)	
	Present	58 (13.6)	1.06 (0.78-1.45)	0.56
SRSF2 mutation	Absent	272 (63.6)	(reference)	
	Present	55 (12.9)	0.89 (0.65-1.24)	0.49
IDH2 mutation	Absent	285 (66.6)	(reference)	
	Present	42 (9.8)	0.79 (0.55-1.12)	0.19
IDH1 mutation	Absent	306 (71.5)	(reference)	
	Present	21 (4.9)	0.62 (0.37-1.04)	0.07
EVI1 mutation	Absent	175 (40.9)	(reference)	
	Present	33 (7.7)	1.48 (1.01-2.19)	0.048
NRAS mutation	Absent	298 (69.6)	(reference)	

	Present	29 (6.8)	0.89 (0.58-1.36)	0.58
U2AF1 mutation	Absent	300 (70.1)	(reference)	
	Present	27 (6.3)	1.11 (0.73-1.69)	0.63
NPM1 mutation	Absent	304 (71.0)	(reference)	
	Present	23 (5.4)	0.63 (0.38-1.02)	0.06
STAG2 mutation	Absent	304 (71.0)	(reference)	
	Present	23 (5.4)	0.81 (0.50-1.32)	0.42
FLT3ITD mutation	Absent	307 (71.7)	(reference)	
	Present	20 (4.7)	0.98 (0.60-1.62)	0.95
BCOR mutation	Absent	308 (72.0)	(reference)	
	Present	19 (4.4)	0.81 (0.48-1.34)	0.43
PHF6 mutation	Absent	310 (72.4)	(reference)	
	Present	17 (4.0)	0.65 (0.37-1.14)	0.13
PTPN11 mutation	Absent	310 (72.4)	(reference)	
	Present	17 (4.0)	0.92 (0.55-1.55)	0.76
EZH2 mutation	Absent	312 (72.9)	(reference)	
	Present	15 (3.5)	1.11 (0.64-1.90)	0.72
ETV6 mutation	Absent	314 (73.4)	(reference)	
	Present	13 (3.0)	0.66 (0.34-1.28)	0.22
JAK2 mutation	Absent	314 (73.4)	(reference)	
	Present	13 (3.0)	1.33 (0.74-2.39)	0.34
KRAS mutation	Absent	314 (73.4)	(reference)	
	Present	13 (3.0)	1.50 (0.81-2.75)	0.22
SF3B1 mutation	Absent	315 (73.6)	(reference)	
	Present	12 (2.8)	0.60 (0.30-1.22)	0.16
SETBP1 mutation	Absent	315 (73.6)	(reference)	
	Present	12 (2.8)	1.77 (0.96-3.26)	0.07

*****Cox model stratified by treatment arm and trial number.

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; CI, confidence interval; dnAML, de novo acute myeloid leukemia; MDS, myelodysplastic syndrome; MDS-related gene mutations, myelodysplasia-related gene mutations; NCCN, national comprehensive cancer network; s-AML, secondary acute myeloid leukemia; t-AML, therapy-related acute myeloid leukemia; WBC count, white blood cell count; WHO PS, World Health Organization performance status.

Supplementary Table 3. Multivariable analysis for OS in the t-AML/AML-MRC cohort.

Variable	Levels	n/(N=428) (%)	Hazard Ratio* (95% CI)	p-value
Age group (years)	60-69	301 (70.3)	(reference)	
	≥70	127 (29.7)	1.13 (0.86-1.49)	0.38
WHO PS	0	209 (48.8)	(reference)	
	1	182 (42.5)	1.14 (0.91-1.42)	0.24
	2	27 (6.3)	2.08 (1.32-3.26)	0.002
AML subtype	dnAML with MDS cytogenetics	232 (54.2)	(reference)	
	s-AML	136 (31.8)	1.06 (0.81-1.39)	0.66
	t-AML	60 (14.0)	0.64 (0.45-0.91)	0.01
WBC count	<20 × 10^9/L	368 (86.0)	(reference)	
	≥20 × 10^9/L	58 (13.6)	1.52 (1.11-2.07)	0.008
Cytogenetic risk category (per NCCN)	Non-adverse	150 (35.0)	(reference)	
	Adverse	270 (63.1)	1.47 (1.12-1.95)	0.006
TP53 mutation	Absent	199 (46.5)	(reference)	
	Present	128 (29.9)	2.17 (1.62-2.91)	<0.001
DNMT3A mutation	Absent	262 (61.2)	(reference)	
	Present	65 (15.2)	0.68 (0.49-0.93)	0.02
SETBP1 mutation	Absent	315 (73.6)	(reference)	
	Present	12 (2.8)	2.63 (1.39-4.99)	0.003

*Cox model stratified by treatment arm and trial number.

Abbreviations: AML, acute myeloid leukemia; CI, confidence interval; dnAML, de novo acute myeloid leukemia; MDS, myelodysplastic syndrome; NCCN, national comprehensive cancer network; s-AML, secondary acute myeloid leukemia; t-AML, therapy-related acute myeloid leukemia; WBC count, white blood cell count; WHO PS, World Health Organization performance status.

Supplementary Table 4. Multivariable analysis for OS in the t-AML/AML-MRC cohort with allo-HCT as a time-dependent variable.

Variable	Levels	n/(N=276) (%)	Hazard Ratio* (95% CI)	p-value
Age group (years)	60-69	203 (73.6)	(reference)	
	≥70	73 (26.4)	1.04 (0.71-1.52)	0.85
WHO PS	0	148 (53.6)	(reference)	
	1	112 (40.6)	0.94 (0.70-1.25)	0.65
	2	10 (3.6)	0.78 (0.38-1.62)	0.51
AML subtype	dnAML with MDS cytogenetics	149 (54.0)	(reference)	
	s-AML	83 (30.1)	1.03 (0.72-1.47)	0.88
	t-AML	44 (15.9)	0.66 (0.42-1.02)	0.06
WBC count	<20 × 10^9/L	252 (91.3)	(reference)	
	≥20 × 10^9/L	24 (8.7)	1.52 (0.92-2.50)	0.10
Cytogenetic risk category (per NCCN)	Non-adverse	110 (39.9)	(reference)	
	Adverse	162 (58.7)	1.48 (1.03-2.13)	0.03
TP53 mutation	Absent	141 (51.1)	(reference)	
	Present	79 (28.6)	2.54 (1.76-3.66)	<0.001
DNMT3A mutation	Absent	174 (63.0)	(reference)	
	Present	46 (16.7)	0.70 (0.46-1.04)	0.08
SETBP1 mutation	Absent	216 (78.3)	(reference)	
	Present	4 (1.4)	1.21 (0.37-4.00)	0.75
Allo-HCT	No	151 (54.7)	(reference)	
	Yes	125 (45.3)	0.64 (0.46-0.89)	0.008

* Cox model stratified by treatment arm and trial number.

Abbreviations: allo-HCT, allogeneic hematopoietic cell transplantation; AML, acute myeloid leukemia; CI, confidence interval; dnAML, de novo AML; MDS, myelodysplastic syndrome; NCCN, national comprehensive cancer network; s-AML, secondary acute myeloid leukemia; t-AML, therapy-related acute myeloid leukemia; WBC count, white blood cell count; WHO PS, World Health Organization performance status.