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ORIGINAL ARTICLE

A phase 2, multicenter, clinical trial of CPX-351 in older patients with secondary or high-risk acute myeloid leukemia: PETHEMA-LAMVYX

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Abstract

Background: LAMVYX was a multicenter, single-arm, phase 2 trial designed to validate the safety and efficacy of CPX-351 in patients aged 60–75 years with newly diagnosed, secondary acute myeloid leukemia and to generate evidence on key issues not addressed in the preceding regulatory pivotal trial.

Methods: The primary end point of the study was the complete remission (CR)/CR with incomplete hematologic recovery (CRi) rate after induction. Eligible patients were recommended to undergo allogeneic hematopoietic stem cell transplantation after the first consolidation cycle. Alternatively, patients could undergo up to six maintenance cycles with CPX-351.

Results: Twenty-nine patients (49%; 95% exact confidence interval [CI], 37%–62%) patients achieved a CR/CRi after one or two cycles of induction, with a measurable residual disease negativity rate of 67% as assessed by centralized, multiparameter flow cytometry. Among patients who had serial next-generation sequencing analyses available, clearance of somatic mutations that were present at diagnosis was achieved in 7 (35%). The median follow-up among survivors was 16.8 months (range,

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8.7–24.3 months). The median event-free survival was 3.0 months (95% CI, 1.4–7.3 months), and the median overall survival was 7.4 months (95% CI, 3.7–12.7 months). In landmark analyses at day +100 from diagnosis, the 1-year overall and event-free survival rate among patients who underwent allogeneic hematopoietic stem cell transplantation was 70% (95% CI, 47%–100%) and 70% (95% CI, 47%–100%), respectively. The corresponding values were 89% (95% CI, 71%–100%) and 44% (95% CI, 21%–92%), respectively, for patients who entered the maintenance phase. No significant longitudinal changes were observed in severity index or quality-of-life visual analog scale scores.

Conclusions: The current data provide novel insights that might inform the clinical positioning and optimal use of CPX-351, complementing previous results (ClinicalTrials.gov identifier NCT04230239).

KEYWORDS
acute myeloid leukemia, allogeneic hematopoietic stem cell transplantation, maintenance therapy, measurable residual disease, quality of life

INTRODUCTION

Despite the incorporation of novel agents to the therapeutic armamentarium, outcomes for older patients (aged 60 years and older) with acute myeloid leukemia (AML) remain poor irrespective of frontline therapy choice.[1–4] In this regard, the treatment of secondary AML (sAML)—which is more prevalent in the older patient population—represents a particularly challenging scenario because of convergence of disease and patient-related factors, including adverse cytogenetic and molecular features, prior exposure to hypomethylating agents (HMAs), and comorbidities.[5–9] Notably, one third of older patients with AML have a history of myelodysplastic syndrome (MDS) or chronic myeloproliferative neoplasm and/or have received previous therapies with leukemogenic potential.[10] In addition, cytogenetic and molecular abnormalities associated with MDS are frequently present in older patients with AML arising de novo.[11,12] Overall, this constellation of intersecting biologic and clinical factors translates into low response rates and high treatment-related mortality, with few patients achieving long-term survival. Thus, the development of effective therapies for sAML in older patients represents a major unmet need.

CPX-351, a liposomal formulation of cytarabine and daunorubicin at a fixed 5:1 synergistic molar ratio, obtained the regulatory approval for the treatment of adults with newly diagnosed, therapy-related AML (t-AML) or AML with myelodysplasia-related changes after a report of positive results from a randomized phase 3 trial.[13,14] Compared with standard 7 + 3 frontline therapy, treatment with CPX-351 led to statistically significant improvements in the complete remission (CR)/CR with incomplete hematologic recovery (CRi) rate and overall survival (OS) in patients aged 60–75 years, with especially encouraging results among those who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) in the CPX-351 arm. Although the study established the indication for CPX-351 in sAML, relevant aspects concerning the impact of measurable residual disease (MRD), quality-of-life measures, the role of granulocyte colony–stimulating factor (G-CSF) priming, or the applicability of a CPX-351 maintenance schedule were not addressed in the trial.

Here, we report the results of a phase 2, academic, multicenter clinical trial of CPX-351 in older adults with sAML conducted by the Spanish PETHEMA group (Programa Español para el Tratamiento de las Hemopatias Malignas). This study aimed to validate the results reported in the pivotal trial in a similar patient population while also providing novel data on unexplored questions such as the potential role of CPX-351 maintenance in nontransplanted patients and the impact of G-CSF priming on response rates. In addition, correlative studies were performed to evaluate the depth of response and clonal dynamics—including MRD testing by centralized multiparameter flow cytometry (MFC) and mutational clearance analyses—and the predictive ability of an ex-vivo sensitivity testing platform.

MATERIALS AND METHODS

Study design

This was a prospective, multicenter, open label, single-arm, phase 2 trial designed to evaluate the safety and efficacy of CPX-351 in patients with newly diagnosed sAML or high-risk AML. All patients underwent induction chemotherapy consisting of subcutaneous G-CSF priming (300 mcg/m^2 daily) on days −1, 1, and 2, CPX-351 (100 units/m^2 daily; equivalent to 100 mg/m^2 of cytarabine and 44 mg/m^2 of daunorubicin) on days 1, 3, and 5; and subcutaneous G-CSF (5 mg/kg daily) from day 10 until peripheral count recovery. If a partial response (PR) were obtained, patients could receive an adjusted second induction cycle in which the last CPX-351 dose was omitted. Patients obtaining a CR/CRi after induction 1 or 2 could

proceed to consolidation therapy consisting of up to two cycles of CPX-351 (65 units/m^2 daily) on days 1 and 3 and the same G-CSF regimen administered during induction. Allo-HSCT in first CR/CRi was recommended according to the protocol in fit patients aged 60–65 years who had an available matched-related or unrelated donor and in patients aged 66–70 years, provided that they had a hematopoietic cell transplantation (HCT) comorbidity index[15] value <4 and a matched-related donor available. In contrast, allo-HSCT was advised against for patients older than 70 years, although it could be performed at the investigator's discretion. Eligible patients were recommended to undergo allo-HSCT after the first consolidation cycle. Alternatively, patients not considered candidates for allo-HSCT could undergo six additional maintenance cycles with CPX-351 (50 units/m^2 daily) on day 1, administered every 4 to 8 weeks. The study design flow chart is provided in Figure S1.

Eligibility criteria

We included patients aged 60–75 years with a diagnosis of sAML or high-risk AML (per World Health Organization 2016 criteria, excluding acute promyelocytic leukemia) within any of the following categories: (1) therapy-related AML (t-AML), (2) AML preceded by a diagnosis of MDS (mds-AML), (3) AML with a prior history of chronic myelomonocytic leukemia (CMML; cmml-AML), or (4) de novo AML with MDS-related cytogenetic abnormalities according to World Health Organization 2016 criteria (dn-AML).[16] Patients were required to have an Eastern Cooperative Oncology Group (ECOG) performance status score of 0–2 and to have adequate organ function, as defined by serum creatinine <2.0 mg/mL, serum alanine or aspartate aminotransferase levels or total bilirubin less than three times the upper limit of normal, and a cardiac ejection fraction ≥50% assessed by echocardiography or multigated acquisition scan. Patients with prior cumulative anthracycline exposure greater than 368 mg/m^2 of daunorubicin (or equivalent), history of Wilson disease or other copper metabolism disorders, active central nervous system leukemia, active second malignancies, uncontrolled infection, or clinically significant myocardial impairment of any cause were excluded.

End points, assessments, and definitions

The primary end point of the study was the CR/CRi rate after induction. Secondary end points included early mortality, OS, event-free survival (EFS), disease-free survival (DFS), the cumulative incidence of relapse (CIR), duration of response, depth of response as assessed by MFC-MRD testing, rate of allo-HSCT, safety and toxicity measures, feasibility and compliance of the maintenance phase, impact of G-CSF priming, and quality of life as measured using the EuroQol 5-Dimension 5-Level (EQ-5D-5L) questionnaire. Adverse events (AEs) were graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 4.03.

Bone marrow aspirates were performed after each induction and consolidation course, every 3 months during the first 12 months after the start of maintenance therapy, and upon suspicion of relapse or progression. Bone marrow analyses (with the exception of centralized MRD assessments) were done locally. Responses to treatment were defined according to the International Working Group response criteria.[17] Disease risk stratification was done using the 2022 European LeukemiaNet (ELN2022) criteria in the primary trial analysis and the Medical Research Council (MRC) 2010 cytogenetic risk classification in the matched analysis.[18,19]

The EQ-5D-5L questionnaire was administered at screening and on day +36 after induction and consolidation cycles. In this questionnaire, health status is captured through five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, each comprising five severity levels. A severity index (SI) was calculated as $SI = (\sum s_{ij} - 5) \bullet 5$, where s_{ij} are the severity levels j ($j = 1, 2, 3, 4, 5$) of each of the dimensions i ($i = 1, 2, 3, 4, 5$). Therefore, the SI values range between 0 and 100, where 0 denotes an absence of health problems and 100 signifies the most severe health state. The visual analogue scale (EQ-VAS)—which takes values between 0 (worst imaginable health) and 100 (best imaginable health)—was also used.

QUANTIFICATION OF MRD BY MFC

Centralized MFC-MRD testing was performed on bone marrow samples in remission after induction. At least 500,000 viable cells per tube were acquired in eight-color digital FACSCanto II cytometers (Becton Dickinson). Cytometers were calibrated and compensated in accordance with Euroflow recommendations using Diva software (Becton Dickinson), and analyses were performed using Infinicyt 2.0 (Cytognos).[20]

Next-generation sequencing and mutational clearance analysis

Centralized mutational analysis was performed through next-generation sequencing (NGS) on the Ion S5 platform using the Oncomine Myeloid Research Assay panel (Thermo Fisher Scientific). This panel covers mutation hotspots of 23 genes (*ABL*, *BRAF*, *CBL*, *CSF3R*, *DNMT3A*, *FLT3*, *GATA2*, *HRAS*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *KRAS*, *MPL*, *MYD88*, *NPM1*, *NRAS*, *PTPN11*, *SETBP1*, *SF3B1*, *SRSF2*, *U2AF1*, and *WT1*) and full coding regions of 17 genes (*ASXL1*, *BCOR*, *CALR*, *CEBPA*, *ETEV6*, *EZH2*, *IKZF1*, *NF1*, *PHF6*, *PRPF8*, *RB1*, *RUNX1*, *SH2B3*, *STAG2*, *TET2*, *TP53*, and *ZRSR2*). The average coverage obtained was 2000X with >85% uniformity, and the minimum reported variant allele frequency (VAF) was 3%. Variants were filtered using Ion Reporter software (Thermo Fisher Scientific), excluding synonymous, intronic, and polymorphic variants (minor allele frequency ≥0.01 and/or included in the dbSNP filter). NGS was performed in bone marrow samples at diagnosis and subsequently in bone marrow

samples after the first or second chemotherapy cycle in patients achieving CR/CRi.

Ex-vivo sensitivity analysis

Centralized ex-vivo drug sensitivity analyses were performed using the Vivia Biotech automated PharmaFlow platform, as previously described,[21,22] Briefly, bone marrow samples were collected by aspiration before the start of induction chemotherapy. After initial sample processing, cells were dispensed into 96-well plates containing increasing concentrations of CPX-351. The plates were incubated for 72 hours at 37°C in a humidified atmosphere containing 5% CO_2. Red blood cells were lysed after incubation, and the remaining cells were stained with annexin V and a cocktail of antibodies to allow for leukemic blast quantification. A single dose-response inhibitory logistic model based on the Hill equation was fitted for parameter estimation. Drug activity was estimated using the area under the curve (AUC). Samples were classified according to their sensitivity to CPX-351 after a cluster analysis based on the Euclidian distance between AUC values calculated from each dose-response curve.[23]

Statistical considerations

With a two-sided alpha of .05 and 80% power, a sample size of 59 patients was estimated to be sufficient to establish an expected CR/CRi rate in the study population of 48% with a 95% confidence interval (CI) of 40%–56%. A 95% exact CI for the response rate was obtained using the Clopper–Pearson method.

All analyses were performed in the intention-to-treat population. OS, DFS, and EFS were estimated using the Kaplan–Meier method. DFS was defined as the time from the first documentation of remission to the documentation of disease recurrence or death, whereas EFS was defined as the time from diagnosis to death, nonresponse, or relapse. Probabilities of relapse and nonrelapse mortality (NRM) were summarized using cumulative incidence estimates, with NRM and relapse considered as competing risks for relapse and NRM, respectively.

A multivariate logistic regression model for treatment allocation (CPX-351 vs. 3 + 7) was constructed for the estimation of propensity scores (PS). Age, ECOG score, white blood cell count at diagnosis, cytogenetic risk per MRC 2010 criteria, and AML subtype (t-AML vs. mds-AML vs. cmml-AML vs. dn-AML) were included as covariates in the model. The unavailability of NGS data in the majority of patients in the historical cohort precluded the incorporation of molecular risk stratification. Single nearest-neighbor PS matching without replacement was then performed. Individual patients were matched on the logit of the PS by using a caliper of width equal to 0.3 times the standard deviation of the logit of the PS. Changes in the standardized percentage bias were used to assess balance after PS matching. A pair-stratified univariate Cox proportional hazards model was fitted to evaluate the association between treatment and survival outcomes in the matched cohort. Statistical analyses were performed using Stata 13 (StataCorp) and R (R Foundation for Statistical Computing; http://www.r-project.org).

Study oversight

The study protocol was approved by the institutional review boards of the participating centers. All patients provided written informed consent, and the study was conducted in accordance with the principles of the Declaration of Helsinki. The trial was registered at ClinicalTrials.gov (identifier NCT04230239).

RESULTS

Characteristics of the study cohort

Patient and disease characteristics are summarized in Table 1. In total, 59 patients were included in the study between December 2019 and December 2020 from 12 academic medical centers in Spain. The median patient age at the time of screening was 68 years (range, 60–75 years), with a median age of 64 years (range, 60–70 years) in patients who underwent allo-HSCT compared with 69 years (range, 64–73 years) in those who proceeded to the maintenance phase. Twenty-three patients (39%) older than 70 years were included. With the exception of one patient with a baseline ECOG score of 2, all patients had ECOG scores of 0 (68%) or 1 (31%). In terms of the subtype of sAML, 32 patients (54%) had a history of MDS, 10 (17%) had a history of CMML, 7 (12%) had t-AML, and 10 (17%) had dn-AML with MDS-related cytogenetic abnormalities. Twenty-two patients (37%) had received prior treatment with HMAs. Most patients (83%) had adverse cytogenetic/molecular risk AML according to ELN 2022 criteria, 35 (59%) had mutations in myelodysplasia-related genes, eight (14%) had *TP53*-mutated AML without accompanying mutations in myelodysplasia-related genes, four (7%) harbored mutations in both *TP53* and at least one myelodysplasia-related gene, 10 (17%) did not harbor mutations in either of these two categories, and two (3%) did not have available NGS results.

Response to induction and post-remission treatment

All 59 patients completed at least one cycle of induction chemotherapy. Twenty-nine patients (49%; 95% exact CI, 37%–62%) achieved CR ($n = 18$) or CRi ($n = 11$) after one or two cycles of induction. After the first induction cycle, 26 patients (44%) achieved CR ($n = 18$) or CRi ($n = 8$), nine (15%) achieved a PR, 13 (22%) were refractory, one (2%) had a nonevaluable bone marrow in aplasia, and 10 (17%) died before response assessment. Seven patients (12%) who achieved a PR after the first cycle underwent a second induction cycle. Among

TABLE 1 Demographic and clinical characteristics of the study cohort, $n = 59$.

Characteristic	No. (%)
Age: Median [range], years	68 [60–75]
60–65	21 (36.0)
66–70	15 (25.0)
>70 years	23 (39.0)
Men	40 (68.0)
ECOG performance status	
0	40 (68.0)
1	18 (31.0)
2	1 (2.0)
ELN 2022 risk category	
Favorable	2 (3.0)
Intermediate	6 (10.0)
Adverse	49 (83.0)
Missing/not reported	2 (3.0)
Mutational status	
Myelodysplasia-related mutations	35 (59.0)
TP53 mutation	8 (14.0)
Both *TP53* and myelodysplasia-related mutations	4 (7.0)
De novo-like mutations	10 (17.0)
Missing/not reported	2 (3.0)
BM blast %: Median [range]	30 [8–87]
WBC count: Median [range], $\times 10^9$/L	4 [0.5–57]
WBC count ≥20,000/μL, No. (%)	9 (15.0)
Platelet count: Median [range], $\times 10^9$/L	45 [1–1035]
AML type	
Prior CMML	10 (17.0)
Prior MDS	32 (54.0)
Therapy-related	7 (12.0)
De novo with MDS-related cytogenetic abnormalities	10 (17.0)
Prior HMA treatment	22 (37.0)

Abbreviations: AML, acute myeloid leukemia; BM, bone marrow; CMML, chronic myelomonocytic leukemia; ECOG, Eastern Cooperative Oncology Group; ELN, European LeukemiaNet; HMA, hypomethylating agent; MDS, myelodysplastic syndrome; WBC, white blood cells.

TABLE 2 Response after one or two cycles of induction therapy, $n = 59$.

Response	No. (%)
Response after one induction cycle	
CR	18 (31.0)
CRi	8 (14.0)
PR	9 (15.0)
Aplasia/not evaluable	1 (2.0)
Resistant disease	13 (22.0)
Death before BM assessment	10 (17.0)
Response after two induction cycles	
CRi	3 (43.0)
PR	1 (14.0)
Aplasia/not evaluable	0 (0.0)
Resistant disease	1 (14.0)
Death before BM assessment	2 (29.0)
Best response after one or two induction cycles	
CR	18 (31.0)
CRi	11 (19.0)
PR	6 (10.0)
Aplasia/not evaluable	1 (2.0)
Resistant disease	13 (22.0)
Death before BM assessment	10 (17.0)
4-week mortality	7 (12.0)
8-week mortality	13 (22.0)

Abbreviations: BM, bone marrow; CR, complete remission; CRi, complete remission with incomplete hematologic recovery; PR, partial response.

these, three patients (43%) achieved CRi, one (14%) achieved a PR, one (14%) was refractory, and two (29%) died before response assessment (Table 2). By day +36 after the first cycle, 16 of the patients (62%) who achieved CR/CRi had neutrophil recovery (>1000/μL), 13 (50%) had platelet recovery (>100,000/μL), and 12 (46%) had both neutrophil and platelet recovery. By day +36 after the second induction cycle, two patients (67%) had neutrophil recovery, but no patients had platelet recovery. The 4-week and 8-week mortality rates from the start of induction were 12% and 22%, respectively. Causes of early mortality were: disease progression ($n = 3$), coronavirus disease 2019 (COVID-19; $n = 2$), bacterial/fungal pneumonia ($n = 2$), septic shock ($n = 2$), alveolar hemorrhage ($n = 1$), hemothorax ($n = 1$), thrombotic stroke ($n = 1$), and intracranial hemorrhage ($n = 1$). No additional COVID-19–related deaths were reported during follow-up, and no deaths occurred during the consolidation or maintenance cycles.

Overall, 25 patients (42%) received additional consolidation chemotherapy cycles (one cycle in 14 patients and two cycles in 11 patients). Ten patients (17%) underwent allo-HSCT. Nine patients (15%) entered the maintenance phase, and five of them completed the six cycles allowed according to protocol. The median time from diagnosis to either transplantation or the start of maintenance was 5.2 months (range, 4.2–9.1 months) and 4.9 months (range, 4.3–5.5 months), respectively. The patient flow chart is provided in Figure S2.

Survival outcomes

The median follow-up among survivors was 16.8 months (range, 8.7–24.3 months). The median EFS, DFS, and OS were 3.0 months (95% CI, 1.4–7.3 months), 10.6 months (95% CI, 7.3 months to not available [NA]), and 7.4 months (95% CI, 3.7–12.7 months), respectively; and the 1-year EFS, DFS, and OS estimates were 24% (95% CI, 15%–38%), 48% (95% CI, 33%–70%), and 37% (95% CI, 27%–52%), respectively. The 1-year CIR was 38% (95% CI, 20%–55%; Figure 1).

In landmark analyses at day +100 from diagnosis, the 1-year OS and EFS estimates for patients who underwent allo-HSCT were 70% (95% CI, 47%–100%) and 70% (95% CI, 47%–100%), respectively, with medians not reached. The corresponding values were 89% (95% CI, 71%–100%) and 44% (95% CI, 21%–92%), respectively, for patients who entered the maintenance phase. In this latter subgroup, the median OS and EFS were 15.7 (range, 12.7 months to NA) and 11.2 (range, 5.4 months to NA), respectively (Figure 2).

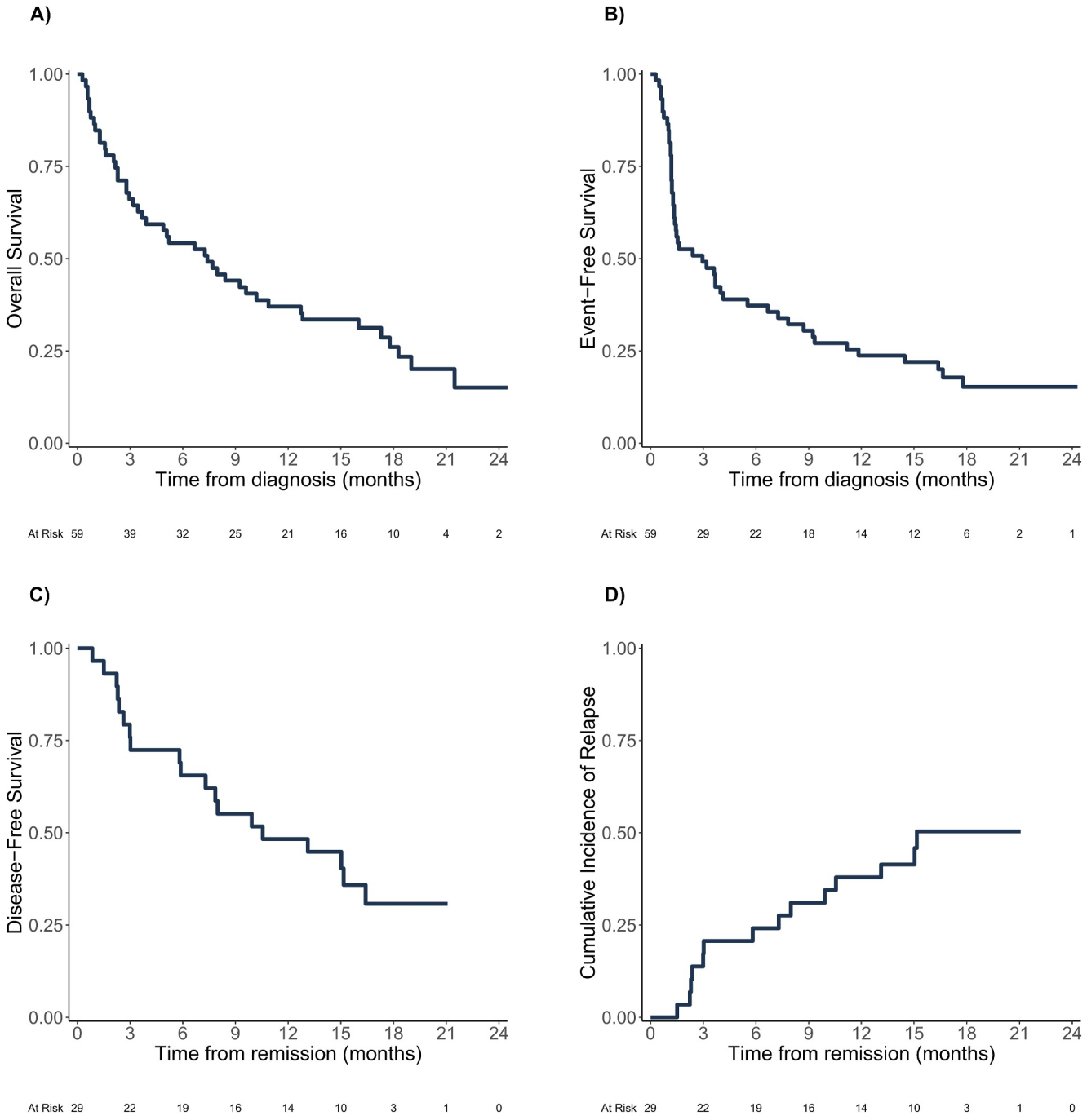

FIGURE 1 Estimates of (A) overall survival, (B) event-free survival, (C) disease-free survival, and (D) the cumulative incidence of relapse are illustrated for the entire study cohort.

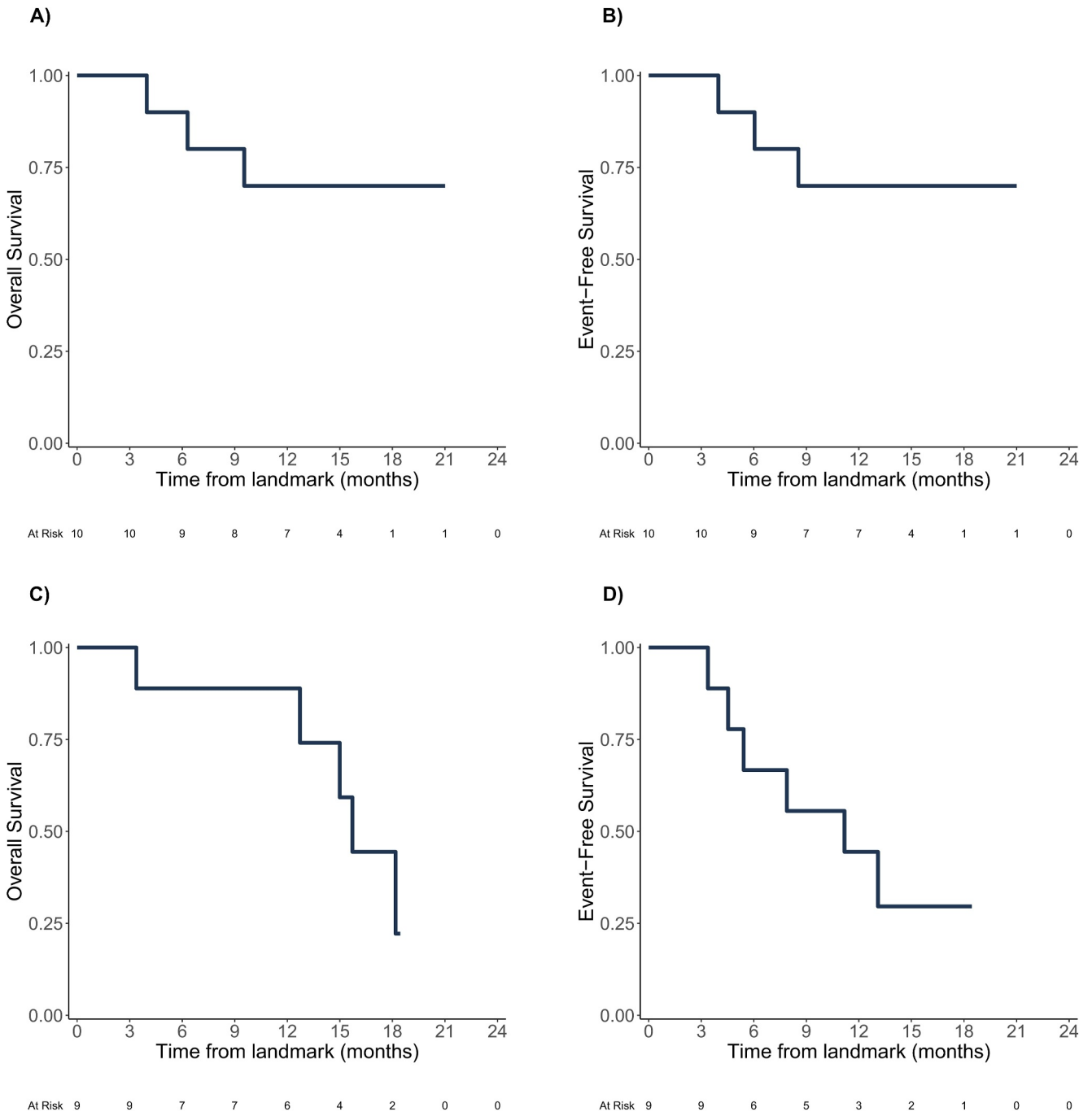

FIGURE 2 Estimates of overall survival and event-free survival in patients who (A,B) underwent allogeneic hematopoietic stem cell transplantation or (C,D) entered the maintenance phase. Landmark analyses from day +100 after diagnosis are shown.

Sensitivity analyses

Outcomes appeared to be particularly unfavorable among patients who had cmml-AML (CR/CRi rate, 10%; median OS, 1.6 months [95% CI, 0.7 months to NA]; median EFS, 1.1 months [95% CI, 0.7 months to NA]) compared with patients who had mds-AML (CR/CRi rate, 50%; median OS, 9.1 months [95% CI, 4.9–19 months]; median EFS, 3.4 months [95% CI, 1.5–11.8 months]), t-AML (CR/CRi rate, 86%; median OS, 9.2 months [95% CI, 3.7 months to NA]; median EFS, 5.5 months [95% CI, 3.0 months to NA]), or dn-AML (CR/CRi rate, 60%; median OS, 7.5 months [95% CI, 2.1 months to NA]; median EFS, 5.5 months [95% CI, 1.2 months to NA]; see Figure S3). Conversely, patients who had a *de novo* mutational profile (CR/CRi rate, 73%; median OS, 18.3 months [95% CI, 17.3 months to NA]; median EFS, 16.4 months [95% CI, 3.0 months to NA]) had better outcomes than those who had myelodysplasia-related mutations (CR/CRi rate, 44%; median OS, 6.0 months [95% CI, 3.2–16.0 months]; median EFS, 2.0 months [95% CI, 1.3–7.3 months]), or *TP53* mutations (CR/CRi rate, 42%; 5.1 months [95% CI, 2.2 months to NA]; median EFS, 1.5 months [95% CI, 1.2 months to NA]; see Figure S4).

Comparison with a matched historical cohort

We compared the outcomes of patients included in the trial with those of a matched historical cohort from the PETHEMA registry comprising patients with AML who were treated intensively with standard induction chemotherapy (i.e., 3 + 7 regimen) between 1994 and 2020.[10] Two hundred forty patients were identified as potentially trial-eligible based on their age, sAML status, ECOG score, renal function, and liver function. There were no significant differences in outcomes between the unmatched trial and control cohorts (see Figure S5). Yet the historical cohort differed from the trial patients with respect to the distributions of age, ECOG score, white blood cell count at diagnosis, cytogenetic risk according to MRC 2010 criteria, and AML subtype. Propensity score matching substantially reduced these imbalances, resulting in matched trial and control cohorts (n = 32 per group) with similar distributions of the aforementioned variables (see Table S1). In the matched analysis, the 1-year OS and EFS estimates were 31% (95% CI, 19%–52%) and 25% (95% CI, 114%–46%) in the trial cohort versus 58% (95% CI, 142%–79%) and 32% (95% CI, 119%–55%) in the historical cohort (stratified log-rank p = .012 and p = .26 for OS and EFS, respectively; Figure 3). There was no difference in the postinduction CR/CRi rate (53%) between groups. Three patients (9%) and six patients (19%) underwent allo-HSCT in the historical and trial cohorts, respectively.

Safety

Treatment-emergent AEs stratified by treatment phase are summarized in Table 3. No unexpected toxicities were noted. Severe hematologic toxicity was universal during the induction and consolidation cycles. A lesser degree of hematologic toxicity was documented during the maintenance cycles (grade 3–4 neutropenia, anemia, and thrombocytopenia in 56%, 22%, and 78% of patients, respectively). The most frequently reported nonhematologic grade ≥2 AEs during the induction cycle were infections (67%; lung infections in 32% and bacteremia/sepsis in 22%; G5 events in 14%). Other frequent toxicities were bleeding (grade ≥2 in 16%, grade 3–4 in 3%, and grade 5 in 5%) and skin rashes (grade 2 in 10%). Similarly, the most common AEs during the consolidation cycles were infections (28%; all grade 3–4 events). Few grade ≥2 nonhematologic AEs were reported during the maintenance phase, and no infection events were documented.

Quality of life

SI and EQ-VAS scores were available in 54 (92%) and 55 (93%) patients, respectively. The median baseline SI and EQ-VAS scores were 5 (range, 0–50) and 68 (range, 0–100), respectively. The median baseline SI was higher in patients who died within the first 4 weeks (28; range, 0–50) compared with survivors beyond this time point (5; range, 0–45). Corresponding EQ-VAS scores were 53 (range, 2–90) and 70 (range, 0–100), respectively. Notably, the baseline SI was associated with the risk of death at 4 weeks (odds ratio [OR] per 5-point increase, 1.38 [95% CI 1.06–1.80; p = .017]; OR for SI >20, 9.52 [95% CI, 1.85–49.16; p = .007]). In contrast, no statistically significant association was observed between baseline EQ-VAS scores and 4-week mortality (OR per 20-point decrease, 1.45 [95% CI, 0.75–2.78; p = .27]; OR for score <50, 1.41 [95% CI, 0.24–8.16; p = .70]; Figure 4A,B). Likewise, no significant longitudinal changes were observed in the SI or EQ-VAS scores after the induction and consolidation chemotherapy cycles in patients who did or did not achieve CR/CRi (Figure 4C,D)

Centralized MFC-MRD testing

Fifteen of 29 patients (52%) had adequate bone marrow samples available for centralized MFC-MRD testing at the time of CR/CRi

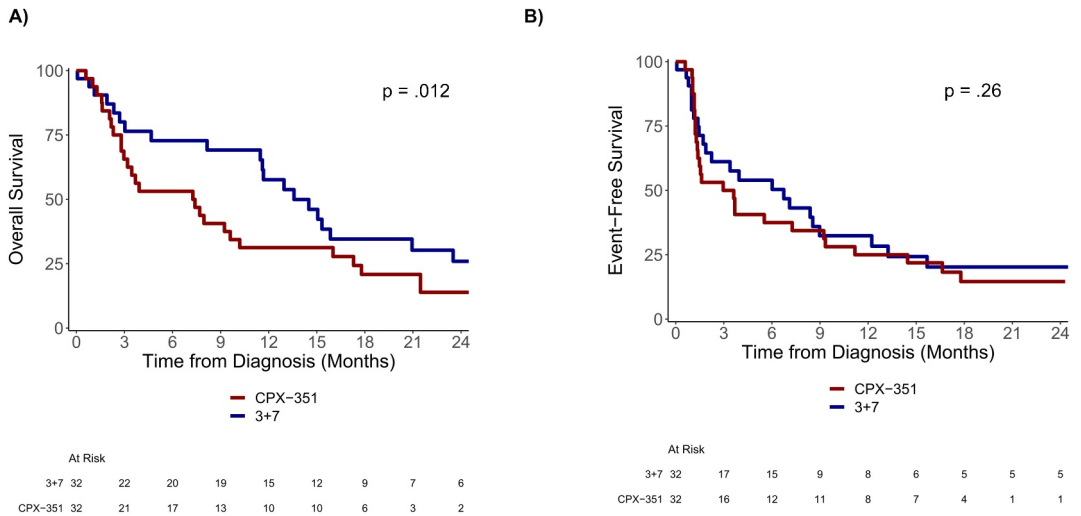

FIGURE 3 Estimates of (A) overall survival, and (B) event-free survival in the propensity score-matched trial and historical cohorts.

TABLE 3 Treatment-emergent adverse events stratified by treatment phase.

	Treatment-emergent adverse events, No. (%)[a]						
	Induction			Consolidation		Maintenance	
Event	G2	G3–G4	G5	G2	G3–G4	G2	G3–G4
Thrombocytopenia	1 (2.0)	58 (98.0)	—	—	25 (100.0)	1 (11.0)	7 (78.0)
Anemia	14 (24.0)	45 (76.0)	—	12 (48.0)	13 (52.0)	4 (44.0)	2 (22.0)
Neutropenia	—	57 (97.0)	—		23 (92.0)	1 (11.0)	5 (56.0)
Infection	8 (14.0)	31 (53.0)	8 (14.0)	—	7 (28.0)	—	—
Lung infection	—	13 (22.0)	6 (10.0)		1 (4.0)		
Bacteremia/sepsis	1 (2.0)	11 (19.0)	2 (3.0)		3 (12.0)		
Catheter-related infection	1 (2.0)	2 (3.0)	—		1 (4.0)		
Other	6 (10.0)	5 (8.0)	—		2 (8.0)		
Fever	7 (12.0)	14 (24.0)	—	1 (4.0)	5 (20.0)	1 (11.0)	—
Bleeding	5 (8.0)	2 (3.0)	3 (5.0)	1 (4.0)	—	—	—
Skin rash	6 (10.0)	—	—	2 (8.0)	—	—	—
Thrombosis	1 (2.0)	2 (3.0)	—	2 (8.0)	1 (4.0)	1 (11.0)	—
Peripheral edema	4 (7.0)	1 (2.0)	—	—	—	1 (11.0)	—
Hyperbilirubinemia	—	5 (8.0)	—	—	—	—	—
Respiratory failure	2 (3.0)	2 (3.0)	2 (3.0)	—	—	—	—
ALT/AST elevation	—	3 (5.0)	—	1 (4.0)	—	—	—
Arrhythmia	2 (3.0)	—	—	1 (4.0)	1 (4.0)	1 (11.0)	—
Constipation	3 (5.0)	—	—	1 (4.0)	—	—	—
Mucositis	2 (3.0)	1 (2.0)	—	1 (4.0)	—	—	—
Renal failure	4 (7.0)	—	—	—	—	—	—
Asthenia	2 (3.0)	—	—	—	—	1 (11.0)	—
Diarrhea	1 (2.0)	—	—	1 (4.0)	—	—	—
Enterocolitis	—	2 (3.0)	—	—	—	—	—
GGT elevation	—	1 (2.0)	—	—	—	—	1 (11.0)
Hypoalbuminemia	2 (3.0)	—	—	—	—	—	—
Hypokalemia	—	1 (2.0)	—	1 (4.0)	—	—	—
Hypomagnesemia	—	—	—	1 (4.0)	—	1 (11.0)	—
Nausea/vomiting	1 (2.0)	—	—	1 (2.0)	—	—	—

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; G, grade; GGT, gamma glutamyl transpeptidase.

[a]All grade 2 or greater treatment-emergent adverse events reported in at least two patients are included. Toxicity grading is based on Common Terminology Criteria for Adverse Events, version 4.03.

achievement. Eight patients (53%) achieved MFC-MRD negativity postinduction when considering any level of MRD as positive, whereas 10 patients (67%) were MFC-MRD–negative if the ELN-recommended positivity threshold at 0.1% was selected.[24]

The median OS and DFS were 21.9 months (95% CI, 8.3 months to NA) and 15.2 months (95% CI, 15.0 months to NA) for MFC-MRD–negative patients and 10.9 months (95% CI, 9.2 months to NA) and 8.0 months (95% CI, 2.6 months to NA) for patients who had evidence of any level of MFC-MRD after induction. The 1-year OS and DFS estimates were 75% (95% CI, 50%–100%) and 75% (95% CI, 50%–100%), respectively, in MFC-MRD–negative patients and 43% (95% CI, 18%–100%) and 29% (95% CI, 9%–92%), respectively, in MFC-MRD–positive patients. Similarly, the 1-year CIR was 25% (95% CI, 3%–58%) and 43% (95% CI, 7%–77%), respectively (see Figure S6).

Analysis of mutational clearance by NGS

Twenty-one of 29 responding patients (72%) had NGS data available from bone marrow samples in remission. Of these, 20 patients had at

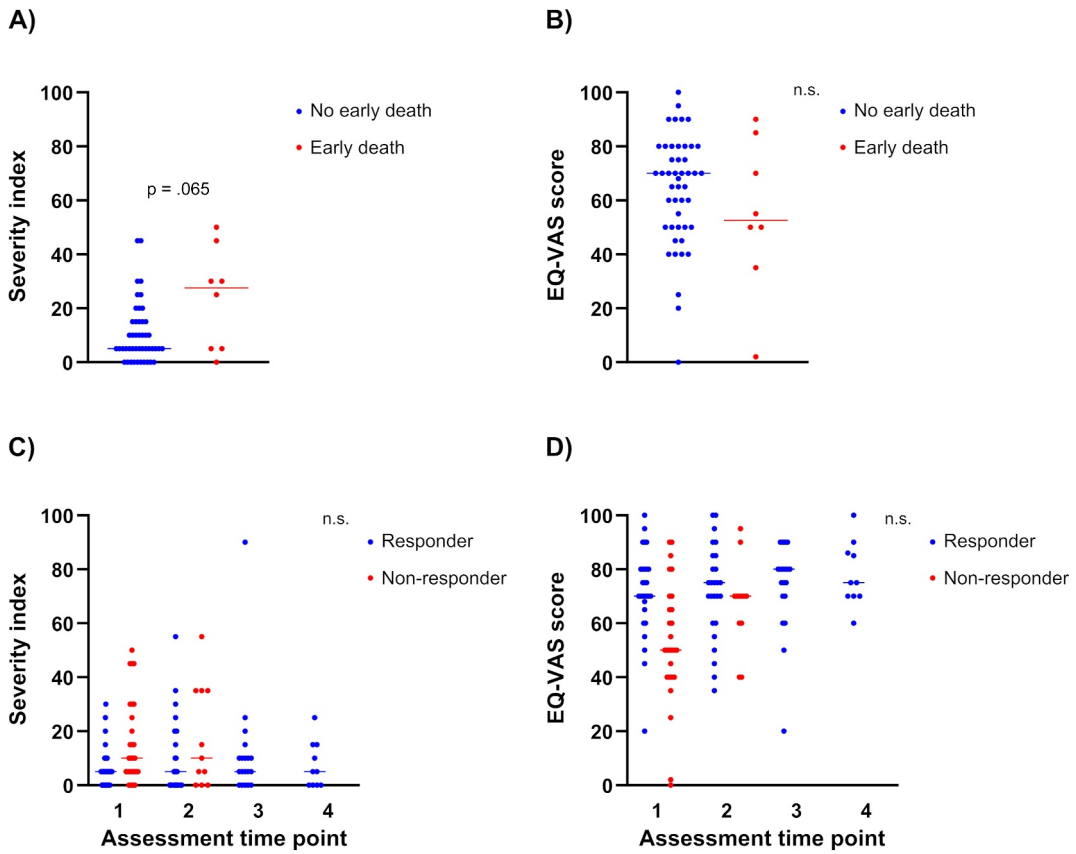

FIGURE 4 Quality-of-life analyses as measured by the EQ-5D-5L questionnaire. (A) The severity index and (B) EQ-VAS scores at baseline stratified by early (4-week) mortality status. (C,D) Longitudinal changes in (C) the severity index and (D) EQ-VAS scores in responders and nonresponders. Quality-of-life metrics were assessed at baseline (time point 1), on day +36 after induction (time point 2), and on day +36 after consolidation cycle 1 (time point 3) and cycle 2 (time point 4). EQ-5D-5L indicates the EuroQol 5-Dimension 5-Level questionnaire; EQ-VAS, the EuroQol visual analog scale; n.s., nonsignificant.

least one mutation detected at diagnosis and thus were included in the mutational clearance analysis. NGS was performed after one cycle of chemotherapy in 17 responders and after two cycles in three patients. Clearance of all mutations present at diagnosis was achieved in seven (35%) patients. Two (10%) additional patients had only one DTA (i.e., mutations in *DNMT3A*, *TET2*, and *ASXL1*) mutation (*ASXL1*) detected in remission, and the remaining 11 patients (55%) had at least one persisting non-DTA mutation. The median number of mutated genes at diagnosis was three (range, from one to five), and the median number of persisting mutations in remission was one (range, from one to three). Across the most frequently mutated genes, clearance rates were 100% for *RUNX1* and *NPM1*, 75% for *STAG2*, 67% for *IDH2*, 60% for *DNMT3A* and *TP53*, and 40% for *SRSF2* (Figure 5). Mutational persistence in CR/CRi at high VAF (≥5%) was common for mutations in *SRSF2* (60%), *TP53* (40%), *IDH2* (33.3%), and *DNMT3A* (20%). Among the patients who had concurrent MFC-MRD assessments available (n = 14), there were four discordant cases in which patients with persisting mutations (*ASXL1* [n = 2], *ETV6* [n = 1], and *SF3B1* [n = 1]) were categorized as MFC-MRD–negative and one case in which a patient who achieved mutational clearance was categorized as MFC-MRD–positive.

The median OS and DFS were 18.3 months (95% CI, 9.6 months to NA) and 15 months (95% CI, 8.0 months to NA), respectively, for patients achieving mutational clearance of non-DTA mutations (mol-MRD–negative) and 17.8 months (95% CI, 9.2 months to NA) and 10.6 months (95% CI, 7.9 months to NA), respectively, for those with persistence of non-DTA mutations (mol-MRD–positive). The 1-year OS and DFS estimates were 67% (95% CI, 42%–100%) and 67% (95% CI, 42%–100%), respectively, in mol-MRD–negative patients and 72% (95% CI, 49%–100%) and 46% (95% CI, 24%–87%), respectively, in mol-MRD–positive patients. Similarly, the 1-year CIR was 22% (95% CI, 3%–53%) and 45% (95% CI, 1%–72%), respectively (see Figure S7).

Ex vivo sensitivity analysis

Ex vivo sensitivity testing was performed in bone marrow samples at diagnosis from 28 patients. Of these, 21 patients were evaluable for postinduction response. A cluster analysis of AUC values identified three major hierarchical subgroups, with high (n = 8; median EC50, 0.08 μM; range, 0.06–0.19 μM), intermediate (n = 7; median EC50,

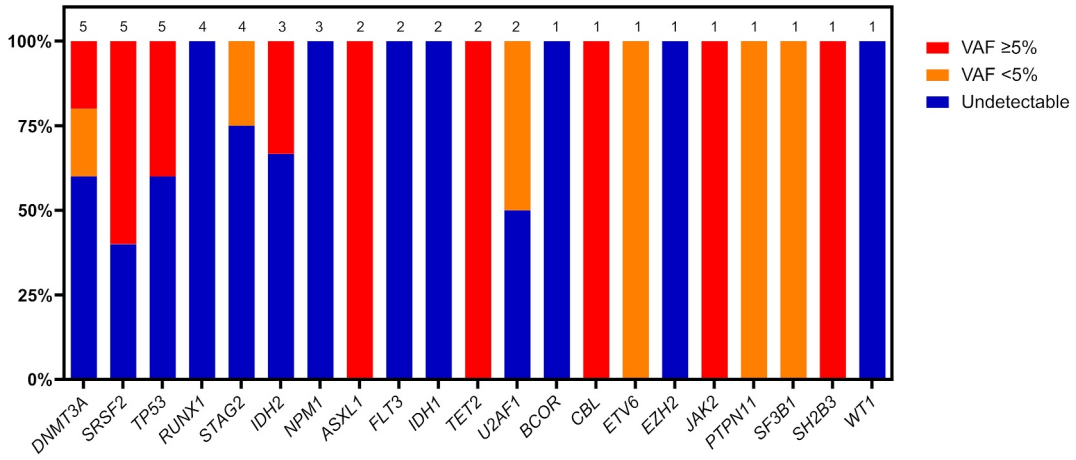

FIGURE 5 Mutational clearance assessed by NGS in bone marrow samples in morphologic remission. The number of patients harboring mutations in each gene is shown on top of the corresponding column. NGS indicates next-generation sequencing; VAF, variant allele frequency.

0.36 μM; range, 0.25–0.48 μM), and low ($n = 6$; median EC50, 1.97 μM; range, 1.06–3.17 μM) sensitivity to CPX-351. There was no statistically significant association between ex-vivo sensitivity as categorized by the cluster analysis and the probability of achieving CR/CRi (OR, 1.94; 95% CI, 0.32–11.76; $p = .47$; with the intermediate and low sensitivity clusters as reference). In addition, receiver operating characteristic (ROC) curves were built to further assess the performance of the sensitivity clusters. The ROC AUC for the sensitivity clusters was 0.58 (95% CI, 0.36–0.79). Finally, ROC curves were constructed to evaluate the performance of the raw AUC from the dose-response curves (ROC AUC, 0.39; 95% CI, 0.12–0.66) and the EC50 values (ROC AUC, 0.35; 95% CI, 0.11–0.60).

DISCUSSION

With a CR/CRi rate of 49% after the induction phase, this study validates the CR/CRi rates from the pivotal CPX-351 trial (48%). Thus, it may be suggested that G-CSF priming did not have a substantial impact on the remission rate. Likewise, EFS (median, 3.0 months) and OS (median, 7.4 months; 1-year OS, 37%) estimates were consistent with those from the CPX-351 arm of the phase 3 study (median, 2.5 and 9.6 months, respectively; 1-year OS, 42%). In contrast, and despite overlapping eligibility criteria in both studies, early mortality rates at 4 weeks (12% vs. 6%) and 8 weeks (22% vs. 14%) were higher in our clinical trial. Also, allo-HSCT rates (17% vs. 34%) were lower in the current study.[13] Although early mortality might have contributed to this latter finding, only a minority of patients in remission (34%) underwent allo-HSCT. Restrictive protocol recommendations on allo-HSCT indication, with transplantation being advised against in patients older than 70 years (representing 39% of our cohort), the availability of an alternative maintenance option for patients not undergoing allo-HSCT, and the conduct of our trial during the lock-down of the severe acute respiratory virus–coronavirus-2 (SARS–CoV-2) pandemic, might have been contributing factors, all of them potentially influencing the OS results in our study cohort. Notably, the median OS and the allo-HSCT rate in patients treated with a later CPX-351 early access program across PETHEMA centers increased to 10.3 months and 34%, respectively.[25] Taken together, our response and survival outcomes compare unfavorably with those of the Italian (CR/CRi rate, 70%; 1-year OS: 69%; median not reached), French (CR/CRi rate, 59%; median OS, 16.1 months), and German (CR/CRi rate, 47%; median OS, 21 months) real-world studies.[25–28] Variable age and disease profile distributions, as well as regional differences in transplantation indication for older patients, might underlie discrepancies between our trial and real-world outcomes. Indeed, allo-HSCT rates were higher in the Italian (28%), French (35%), and German (62%) cohorts, and those three studies included patients younger than 60 years. The reasons for the comparatively higher early mortality in our study remain unclear. Although only two deaths were directly attributed to COVID-19, indirect effects of health care disruption during the early phases of the SARS–CoV-2 pandemic also may have contributed to these results.

As reported here, older patients with sAML frequently will not proceed to allo-HSCT in remission because of comorbidities, clinical deterioration derived from prior chemotherapy, or personal choice. For these patients, the adoption of maintenance strategies could delay leukemia progression. In this regard, maintenance with oral azacitidine has demonstrated an OS benefit—albeit not increasing curative fractions—in this setting.[29] We hypothesized that the outpatient administration of maintenance cycles with low doses of CPX-351 might prolong remission duration with limited toxicity. Although it was demonstrated that this strategy was feasible from a safety and tolerability perspective, EFS and OS were poor, and no survival plateau was observed in this patient subgroup. In contrast, outcomes were substantially better among patients undergoing allo-HSCT, in line with previous reports.[13,25–28] Although limited by a small number of patients and a relatively short follow-up at the time of the analyses, these results underscore the value of allo-HSCT in

older patients with sAML patients who are treated with CPX-351. Accordingly, current PETHEMA guidelines recommend treatment with CPX-351 only for those patients deemed eligible for a subsequent allo-HSCT.

In addition to response and survival end points, quality-of-life considerations are of paramount importance in the management of older patients with AML. Post-hoc analyses from the randomized trial demonstrated significant improvements in quality-adjusted time without symptoms of disease or toxicity (Q-TWiST) in patients treated with CPX-351 compared with those in the 7 + 3 arm, with greater gains reported in responding patients.[30] Here, our analyses failed to detect longitudinal changes in quality-of-life measures as captured by the EQ-5D-5L questionnaire, irrespective of disease response. Of note, early assessments on day +36 of the induction and consolidation cycles could have precluded an adequate assessment of changes in quality of life that might only arise at later time points in patients recovering from intensive therapy. Interestingly, the SI obtained from the EQ-5D-5L questionnaire—but not the EQ-VAS score—was associated with the risk of early mortality after CPX-351. This discriminatory ability is noteworthy because the trial was already restricted to patients who had adequate performance status and clinically nonsignificant organ dysfunction at baseline. Although several scores have been developed to predict early treatment-related mortality after intensive chemotherapy, these only consider the patient's functional status as assessed by the physician.[31,32] Whether the incorporation of patient-reported measures might increase predictive accuracy warrants investigation in future studies.

sAML comprises a heterogeneous spectrum of biologic entities, but the extent to which these might differ in their sensitivity to CPX-351 is not well established.[14,27,28] In sensitivity analyses, response rates and survival outcomes among patients with cmml-AML were dismal in our study, suggesting a marginal role for CPX-351 in this patient subgroup. Conversely, patients with a *de novo* molecular profile showed more favorable outcomes. This latter finding is consistent with prior reports indicating that biologic characteristics should take precedence over clinical descriptors of ontogeny for risk-classification purposes.[33] Alternatively, ex-vivo testing strategies might inform therapy selection by providing a rapid functional read-out of the leukemia's sensitivity to a particular treatment. As part of the current trial, we assessed the performance of a flow cytometry-based ex-vivo sensitivity testing platform at predicting response to CPX-351. In contrast to prior studies that demonstrated a promising ability to predict response to idarubicin and cytarabine conventional induction therapy, the assay was not discriminative in this setting.[21,22] It could be speculated that the pharmacologic properties of the liposomal formulation, which are expected to influence in-vivo responses, might not be appropriately captured by this platform.

MRD assessments were not conducted in the pivotal study, and subsequent analyses in patients who underwent allo-HSCT suggest that the better outcomes in the CPX-351 arm were primarily attributable to decreased treatment-related mortality rather than relapse risk.[34] Here, we provide the first report of centralized MFC-MRD testing after CPX-351 treatment. Our results, producing an MRD negativity rate >50%, are in line with results from decentralized MRD testing previously reported in registry studies.[26–28] Although a deleterious impact of MRD positivity on relapse risk was not apparent, sample size constraints precluded a formal analysis of the prognostic value of MFC-MRD positivity in patients with sAML who were treated with CPX-351. In this regard, conflicting results have been reported on the prognostic significance of MRD in patients with sAML.[28,35–37] MFC-MRD monitoring might be complemented by the analysis of residual disease mutational dynamics.[38] Accordingly, we assessed mutational clearance by NGS in those patients who achieved morphologic remission. Notably, and despite the use of a low-sensitivity assay not designed for MRD assessment purposes, our analyses revealed that a majority of patients (58%) had persistent non-DTA mutations in remission. In addition, certain non-DTA mutations, such as those in *SRSF2*, *TP53*, and *IDH2*, were prevalent at high VAF (≥5%) in remission, which might reflect reversion to a preleukemic or MDS-like state. Although determining the clinical and biological impact of these findings falls beyond the scope of the current study, our data demonstrate a high residual mutational burden among patients who are in remission after CPX-351 treatment.

Several limitations must be acknowledged in the current study. First, the relatively small sample size limited our ability to perform detailed subgroup analyses and draw definite conclusions about prognostic factors. Second, and most important, the nonrandomized design of the study precluded direct comparisons regarding the relative clinical value of CPX-351 versus alternative therapeutic strategies. In this regard, retrospective analyses suggest that comparable OS results might be achieved after treatment with CPX-351 or the combination of azacitidine and venetoclax, with lower infection-related morbidity in patients receiving the latter, albeit without apparent differences in early mortality.[39] However, prospective trials will be necessary to delineate which patient subgroups benefit most from either strategy, particularly as a bridge to allo-HSCT. To contextualize our results, we performed a propensity score-matched, retrospective comparison with a historical cohort of patients with sAML who received 3 + 7 standard chemotherapy. Notably, this analysis not only failed to demonstrate a discernible benefit in CPX-351–treated patients but produced better outcomes in the control cohort. However, caution should be exerted when interpreting these results. Of note, our propensity score analyses could not account for prior exposure to HMAs or differences in molecular profiles between the cohorts. This might be of relevance given the high prevalence of adverse biologic features in the trial cohort, and residual confounding driven by these unmatched variables cannot be ruled out. Finally, our study was conducted during the first year of the SARS–CoV-2 pandemic, resulting in immeasurable deleterious effects in patient management that could have affected overall outcomes. Although these results should not be interpreted to question those from the randomized pivotal trial, they might serve to highlight the persisting unmet need for more effective therapies for patients with sAML. Notwithstanding these limitations,

our data provide novel insights that might inform the clinical positioning and optimal use of CPX-351, complementing previous results from pivotal and registry studies.

AUTHOR CONTRIBUTIONS

Eduardo Rodríguez-Arbolí: Conceptualization, writing–original draft, formal analysis, and investigation. **Rebeca Rodríguez-Veiga**: Writing–review and editing and investigation. **Elena Soria-Saldise**: Investigation and writing–review and editing. **Juan M. Bergua**: Investigation and writing–review and editing. **Teresa Caballero-Velázquez**: Investigation and writing–review and editing. **Montserrat Arnán**: Investigation and writing–review and editing. **Susana Vives**: Investigation and writing–review and editing. **Josefina Serrano**: Investigation and writing–review and editing. **Teresa Bernal**: Investigation and writing–review and editing. **Pilar Martínez-Sánchez**: Investigation and writing–review and editing. **Mar Tormo**: Investigation and writing–review and editing. **Carlos Rodríguez-Medina**: Investigation and writing–review and editing. **Pilar Herrera-Puente**: Investigation and writing–review and editing. **Esperanza Lavilla-Rubira**: Investigation and writing–review and editing. **Blanca Boluda**: Investigation and writing–review and editing. **Evelyn Acuña-Cruz**: Investigation and writing–review and editing. **Isabel Cano**: Investigation and writing–review and editing. **Sara Cáceres**: Investigation and writing–review and editing. **Juan Ballesteros**: Investigation and writing–review and editing. **Jose Falantes**: Investigation and writing–review and editing. **David Martínez-Cuadrón**: Investigation and writing–review and editing. **José A. Pérez-Simón**: Investigation, supervision, writing–review and editing. **Pau Montesinos**: Investigation, conceptualization, funding acquisition, writing–review and editing, supervision, and project administration.

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CONFLICT OF INTEREST STATEMENT

Eduardo Rodríguez-Arbolí reports personal/consulting fees from Astellas Pharma, Laboratories Delbert, and Servier Pharmaceuticals; support for other professional activities from AbbVie and Astellas Pharma Europe; and travel support from AbbVie, Astellas Pharma, Eurocept, Gilead Sciences Inc., and Jazz Pharmaceuticals outside the submitted work. Juan M. Bergua reports travel support from F. Hoffman-La Roche AG outside the submitted work. Susana Vives reports personal/consulting fees from Jazz Pharmaceuticals outside the submitted work. Teresa Bernal reports personal/consulting fees from AbbVie and Jazz Pharmaceuticals; and support for other professional activities from Astellas Pharma outside the submitted work. Mar Tormo reports personal/consulting fees from AbbVie and Sobi; support for other professional activities from Jazz Pharmaceuticals; and travel support from Bristol Myers Squibb Company and Jazz Pharmaceuticals outside the submitted work. David Martínez-Cuadrón reports personal/consulting fees from Astellas Pharma, Laboratories Delbert, and Otsuka Pharmaceutical; support for other professional activities from Servier Pharmaceuticals; and travel support from Otuska Pharmaceutical, Pfizer, and Servier Pharmaceuticals outside the submitted work. José A. Pérez-Simón reports personal/consulting fees from Jazz Pharmaceuticals; and support for other professional activities from Incyte Corporation, Novartis, and Sanofi outside the submitted work. Pau Montesinos reports personal/consulting fees from AbbVie, Celgene, and Servier Pharmaceuticals outside the submitted work. The remaining authors disclosed no conflicts of interest.

DATA AVAILABILITY STATEMENT

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

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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