REGULAR ARTICLE

blood advances

A randomized comparison of CPX-351 and FLAG-Ida in adverse karyotype AML and high-risk MDS: the UK NCRI AML19 trial

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Key Points

- In high-risk AML and MDS, CPX-351 did not improve response or survival compared with FLAG-Ida but produced better relapse-free survival.
- In the exploratory subgroup of patients defined by the presence of mutations in MDS-related genes, CPX-351 improved OS.

Liposomal daunorubicin and cytarabine (CPX-351) improved overall survival (OS) compared with 7+3 chemotherapy in older patients with secondary acute myeloid leukemia (AML); to date, there have been no randomized studies in younger patients. The high-risk cohort of the UK NCRI AML19 trial (ISRCTN78449203) compared CPX-351 with FLAG-Ida in younger adults with newly diagnosed adverse cytogenetic AML or high-risk myelodysplastic syndromes (MDS). A total of 189 patients were randomized (median age, 56 years). Per clinical criteria, 49% of patients had de novo AML, 20% had secondary AML, and 30% had high-risk MDS. MDS-related cytogenetics were present in 73% of the patients, with a complex karyotype in 49%. *TP53* was the most common mutated gene, in 43%. Myelodysplasia-related gene mutations were present in 75 (44%) patients. The overall response rate (CR + CRi) after course 2 was 64% and 76% for CPX-351 and FLAG-Ida, respectively. There was no difference in OS (13.3 months vs 11.4 months) or event-free survival in multivariable analysis. However, relapse-free survival was significantly longer with CPX-351 (median 22.1 vs 8.35 months). There was no difference between the treatment arms in patients with clinically defined secondary AML or those with MDS-related cytogenetic abnormalities; however, an exploratory subgroup of patients with MDS-related gene mutations had significantly longer OS with CPX-351 (median 38.4 vs 16.3 months). In conclusion, the OS of younger patients with adverse risk AML/MDS was not significantly different between CPX-351 and FLAG-Ida.

Introduction

The treatment of AML with adverse karyotype remains unsatisfactory. These patients have a lower response rate, a higher risk of refractory disease, and a shorter duration of remission in those who do

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Access to deidentified data, and supporting documentation, is available via formal application to Cardiff University from the corresponding author, Nigel H. Russell (Nigel.russell@nottingham.ac.uk). Cardiff University is committed to open access to deidentified clinical trial data.

The full-text version of this article contains a data supplement.

respond.[1-4] Although the only curative treatment is allogeneic stem cell transplantation (SCT), better induction strategies are required to increase the proportion of patients undergoing SCT. Improved induction treatments can potentially improve survival after SCT.

For decades, the induction strategy for these patients has been the standard 7 + 3 chemotherapy with daunorubicin and cytarabine (DA). More recently, because there has been substantial overlap between high-risk cytogenetic abnormalities and those considered to be myelodysplasia-related,[5,6] a relatively high proportion of these patients are eligible for treatment with CPX-351, provided that the karyotype is known at the time of treatment initiation. CPX-351 is a liposomal formulation of cytarabine and daunorubicin encapsulated in a preclinically identified optimally synergistic 5:1 ratio. Following on from a randomized phase 2 study,[7] CPX-351 demonstrated a higher response rate with improved overall survival (OS) compared with 7 + 3 in patients aged 60 to 75 years with previous MDS or chronic myelomonocytic leukemia, therapy-related AML, or an MDS-related karyotype (median OS, 9.56 vs 5.95 months; $P = .003$).[8,9] The rate of SCT was higher in the CPX-351 arm than in the 7 + 3 arm (34% vs 25%), with a landmark survival analysis from the time of SCT also favoring CPX-351 (median OS NR vs 10.25 months; $P = .009$). These findings have led to the approval of CPX-351 in younger and older patients with newly diagnosed secondary AML, although it is important to note that there is currently no randomized evidence of its benefit in patients aged <60 years.

The widespread availability of sequencing technologies has significantly altered AML classification in recent years.[5,6,10,11] Secondary AML has traditionally been used to describe patients whose disease has evolved from a prior myeloid disorder (MDS, MPN, or MDS/MPN) or after exposure to cytotoxic therapy.[2,8,10,11] However a number of studies have demonstrated that mutational status, in particular mutations in *TP53* and "secondary-like" genes, may better define distinct clinicopathological subgroups,[12,13] and these findings have been incorporated into the most recent classification systems. The World Health Organization (WHO) now defines AML, myelodysplasia related as the presence of either a mutation in *ASXL1, BCOR, EZH2, SF3B1, SRSF2, STAG2, U2AF1*, or *ZRSR2,* an MDS-related cytogenetic abnormality, or a history of MDS or MDS/MPN.[6] The International Consensus Classification (ICC) and European Leukemia Net (ELN) further prioritize genomics, with a mutation in the same list of genes or *RUNX1* classifying a patient as AML or MDS/AML with myelodysplasia-related gene mutations, whereas those with MDS-related cytogenetic abnormalities are described as AML or MDS/AML with myelodysplasia-related cytogenetic abnormalities. A clinical history of MDS or MDS/MPN is added only as a diagnostic qualifier.[5,14] Importantly, the potential benefits of CPX-351 in patients with AML/MDS with myelodysplasia-related gene mutations have not been previously analyzed.

We previously reported in the UK National Cancer Research Institute (NCRI) AML17 trial that the FLAG-Ida regimen resulted in superior OS compared with daunorubicin and clofarabine when administered after induction therapy in younger adults with high-risk AML.[15] Furthermore, in an exploratory study of 115 patients enrolled in the Medical Research Council (MRC) AML15 trial with secondary AML and a median age of 52 years who otherwise met the entry criteria for the CPX-351 pivotal trial, survival was improved for patients treated with FLAG-Ida compared with daunorubicin and cytarabine +/− etoposide.[16] This finding was consistent with the favorable effect of FLAG-Ida on relapse observed in AML15, which was apparent in all demographic subgroups, including adverse risk cytogenetics.[17] Therefore, we considered FLAG-Ida as the standard of care for younger patients with high-risk AML and MDS and the appropriate control arm for a randomized comparison against CPX-351 in the high-risk cohort of the NCRI AML19 trial.

Patients and methods

The UK NCRI AML19 trial (ISRCTN78449203) enrolled younger adults with newly diagnosed AML or MDS, with >10% blasts, between November 2016 and November 2020. Patients were generally <60 years but older patients could enter if deemed fit by the treating physician. Patients were eligible for various randomizations depending on their cytogenetic and molecular characteristics (supplemental Figure 1).

Patients with a known adverse karyotype at diagnosis per the MRC 2010 criteria[18] or who had high-risk MDS with ≥10% blasts were eligible for high-risk randomization between CPX-351 and FLAG-Ida. From July 2018, patients with MDS with 5%-9% bone marrow blasts and an IPSS-R very high, high, or intermediate (provided that the IPSS-R was >3.5) were eligible. Patients were randomized 2:1 in favor of CPX-351 and stratified by age group, performance status, and clinical disease type (de novo or secondary AML). The final numbers randomized were less than the 2:1 ratio because of CPX-351 supply issues early in the trial.

In addition to this group (Group 1, n = 189), other patients could also enter the high-risk randomization after induction time points: group 2 (n = 264) was randomized after induction course 1 and were at high-risk based on a validated risk score, had *FLT3*-ITD without an *NPM1* mutation, or had refractory disease; group 3 (n = 178) was randomized after course 2 if they had persistent measurable residual disease (MRD) by flow cytometry or RT-qPCR for *NPM1* transcripts, or at the time of relapse (supplemental Figure 1). Here, we present the results for patients in group 1 with a CONSORT (Consolidated Standards Of Reporting Trials) diagram shown in Figure 1.

FLAG-Ida comprised fludarabine 30 mg/m^2 IV on days 2 to 6 inclusive, cytarabine 2 g/m^2 over 4 hours starting 4 hours after fludarabine on days 2 to 6 (reduced to 1 g/m^2 in patients >60 years), granulocyte colony stimulating factor (lenograstim 263 μg) subcutaneously daily on days 1 to 7, and idarubicin 8 mg/m^2 IV daily on days 4 to 6. Up to 2 courses could be administered, followed by 2 courses of consolidation with MACE (amsacrine, cytarabine and etoposide) and MiDAC (mitoxantrone and cytarabine) chemotherapy[17] if no donor was available. CPX-351 induction course 1 consisted of 100 units/m^2 (100 mg/m^2 cytarabine and 44 mg/m^2 daunorubicin) administered as a 90-minute infusion on days 1, 3, and 5. A second induction course of 100 units/m^2 was administered on days 1 and 3 in all the patients. For patients with complete remission (CR) or CR with incomplete blood count recovery (CRi) after induction course 2, postremission therapy consisted of up to 2 cycles of 65 units/m^2 CPX-351 (65 mg/m^2 cytarabine and 29 mg/m^2 daunorubicin) on days 1 and 3. Allogeneic SCT was recommended for all patients after induction, if an appropriately matched donor was available. The trial

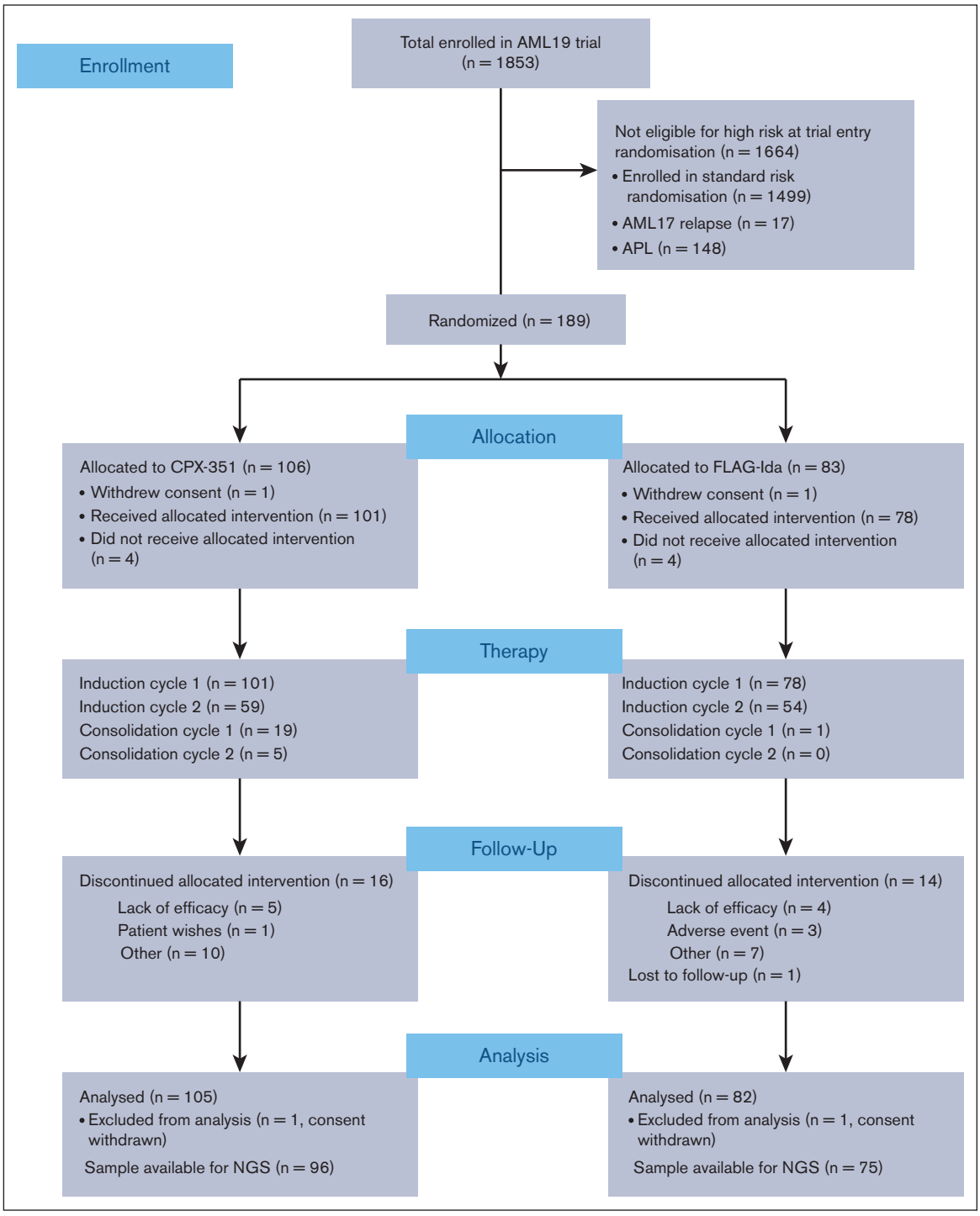

Figure 1. CONSORT diagram. APL (acute promyeloctic leukemia); CONSORT, Consolidated Standards Of Reporting Trials; NGS, next-generation sequencing.

was approved by the Wales Multicenter Research Ethics Committee and each institution's ethics committee in accordance with the Declaration of Helsinki.

Statistical analyses

Full details of the statistical analyses are provided in the supplemental Appendix. The adverse karyotype randomization was not prospectively powered in the original study design; hence, all reported *P* values were nominal. Primary analyses were based on intention to treat, and the primary end point of this randomization was OS. End points were defined per the revised International Working Group criteria.[19] OS was defined as the time from randomization to death from any cause with those still alive censored at the date last seen. The final data cutoff was on 17 May 2022. Relapse-free survival (RFS) was calculated only for patients who achieved CR and was measured from the date of CR to the

date of disease relapse or death from any cause. Event-free survival (EFS) was measured in all patients and was defined as the time from randomization to treatment failure (refractory disease or partial response) by the end of course 2, disease relapse, or death from any cause. For the outcomes of OS, RFS, EFS and CR achievement, multivariable analyses were adjusted for all stratification variables used at the time of randomization (age group, sex, performance status, baseline white blood cell count, and disease type).

The responses were based on the investigator's assessment of bone marrow. Toxicity (hematologic recovery times and nonhematologic toxicity) was scored using the National Cancer Institute Common Toxicity Criteria, Version 3, and resource use data (blood product support, days on antibiotics, and hospitalization) were collected.

Patients characteristics were summarized across the groups using frequency and percentage for categorical data and median and quartile range for quantitative data. Comparisons of patient characteristics were performed using chi-squared, Mantel-Haenszel tests for trend, or Wilcoxon rank sum tests as appropriate. Time-to-event outcomes were compared using log-rank tests and Cox regression or Gray test for cumulative incidence, with competing risk analyses. Outcomes are reported as effect sizes with 95% confidence intervals (CIs).

Cytogenetic and genomic analyses

Karyotype analyses were performed in accredited regional laboratories and the reports were centrally reviewed. Cytogenetic classifications were defined using the MRC 2010 criteria.[18] Following the completion of the trial, banked diagnostic DNA was analyzed for variants in 41 recurrently mutated myeloid genes (supplemental Table 1), including the entire coding regions of all myelodysplasia-related genes according to the 2022 WHO, ICC, and ELN criteria (*ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1,* and *ZRSR2*).[5,6,14] Libraries were prepared using the Agilent SureSelect XT HS2 platform and sequenced on an Illumina NextSeq2000, achieving a median depth of 955×. Further details are provided in the supplemental Appendix.

Results

Patient population

Between November 2016 and November 2020, 189 patients with AML or high-risk MDS were randomized, of whom 2 later withdrew consent. Their median age was 56 years (range, 18-70 years with 51 patients aged >60 years). Patient characteristics based on randomization are shown in Table 1. Of the entire cohort, 49% were classified based on clinical features as de novo AML, 20% as secondary AML, and 30% as high-risk MDS. Eight percent of the patients had a history of chemotherapy or radiotherapy. Myelodysplasia-related cytogenetic abnormalities were present in 73% of the patients, with a complex karyotype of 49%. Next-generation sequencing results were available for 171 of 187 patients (91%). *TP53* was the most commonly mutated gene, identified in 44% of patients, followed by *DNMT3A* (19%), and *ASXL1* (18%) (supplemental Figure 2; supplemental Table 2). A mutation in at least 1 MDS-related gene was present in 75/171 (44%) patients, of whom 59 (35%) were categorized as having AML/MDS with myelodysplasia-related gene mutations, which per ICC 2022 criteria require the absence of *TP53* variants.[5] Most patients had mutations in more than 1 MDS-related gene (Table 1). In this cohort, 35% of patients with a clinical diagnosis of de novo AML were found to have myelodysplasia-related gene mutations (supplemental Figure 3).

Induction response

There was a trend toward a higher overall response rate (ORR, ie, CR + CRi) in patients randomized to FLAG-Ida (Table 2). After cycle 1, the ORR was 51% and 65% for CPX-351 and FLAG-IDA, respectively ($P = .15$). After cycle 2 the ORR was 64% vs 76% (OR [odds ratio] 0.54; 95% CI, 0.28-1.04; $P = .06$). Day 30 and day 60 mortality were not different between the treatment arms (day 30, 5% vs 7%; $P = .46$; day 60; 12% vs 11%; $P = .77$ for CPX-351 and FLAG-Ida, respectively). Seven patients randomized to the CPX-351 received FLAG-Ida as course 2 because of refractory disease, 3 of whom achieved CRi. The median number of courses administered in both arms was 2, with only 1 patient in the FLAG-Ida arm proceeding to consolidation, as compared with 19 patients who received at least 1 consolidation cycle of CPX-351 (Figure 1).

Toxicity

Platelet recovery to $>100 \times 10^9$/L was longer with CPX-351 in course 1, with median days to platelet recovery of 34 for CPX-351 vs 29 for FLAG-Ida ($P < .001$) with no difference in neutrophil recovery to 1.0×10^9/L (32 days for CPX-351 vs 30 for FLAG-Ida; $P = .11$; Table 2). The most important differences were observed after course 2, with significantly fewer patients recovering neutrophils and platelets, and the time to recovery was markedly delayed in those who recovered (31 vs 46 days for neutrophils; $P = .002$, and 31 vs 36 days for platelets; $P = .19$; Table 2). This resulted in longer hospitalization in course 2 with FLAG-Ida (27 vs 35.5 days; $P = .002$), as well as greater requirements for blood transfusion, platelet transfusion, and IV antibiotics (supplemental Table 3). Grade 3 or higher nonhematological toxicities with CPX-351 were comparable, being present in 18% compared with 21% with FLAG-Ida (supplemental Table 4).

Survival outcomes

OS at 3 years was 32% and 25% and median OS was 13.3 months vs 11.4 months for CPX-351 and FLAG-Ida respectively (HR [hazard ratio], 0.85; 95% CI, 0.6-1.21; $P = .36$). EFS was not significantly different (HR, 0.97; 95% CI, 0.69-1.37; $P = .86$) (Figure 2 and Table 2). In patients achieving CR, RFS at 3 years was 39% and 29% and median RFS was 22.1 months vs 8.35 months (HR, 0.66; 95% CI, 0.41-1.06; $P = .08$) for CPX-351 and FLAG-Ida respectively (Figure 2). In a multivariable Cox regression model adjusted for sex, age group, performance status, baseline white blood cell count, disease type, cytogenetic risk, *NPM1* and *FLT3* mutation status, there was no benefit in OS (HR, 0.78; 95% CI, 0.55-1.12; $P = .17$) and EFS (HR 0.9; 95% CI, 0.64-1.27; $P = .55$), whereas RFS was better with CPX-351 (HR, 0.58; 95% CI, 0.36-0.93; $P = .03$). The RFS advantage was predominantly because of the lower cumulative incidence of death in remission in the CPX-351 arm, with the incidence of relapse being similar (Figure 2).

Table 1. Baseline characteristics in each arm

	FLAG-IDA (n = 82)	CPX-351 (n = 105)
Median age, y (range)	55 (18-67)	57 (23-70)
Age group		
<39	14 (17%)	9 (8.6%)
40-49	12 (15%)	16 (15%)
50-59	34 (41%)	51 (48%)
60+	22 (27%)	29 (28%)
Female sex	34 (41%)	45 (43%)
Diagnosis		
De novo AML	42 (51%)	50 (48%)
Secondary AML	17 (21%)	21 (20%)
High-risk MDS	23 (28%)	34 (32%)
Prior history		
History of previous cytotoxic/radiotherapy	9 (11%)	7 (6.8%)
History of MDS/MPN	17 (21%)	16 (16%)
WHO performance status		
0 (Normal activity)	48 (59%)	52 (49%)
1 (Restricted activity)	29 (35%)	46 (44%)
2 (In bed <50% waking hours)	5 (6%)	7 (7%)
Cytogenetics + FISH*		
Complex ≥3 abnormalities	43 (54%)	51 (50%)
Complex ≥4 abnormalities	40 (51%)	49 (48%)
−5 / del5q / add5q	32 (40%)	45 (43%)
−7 / del7q / add7q	36 (45%)	46 (44%)
−17 / abn17p	12 (15%)	25 (24%)
11q23	6 (8%)	8 (7.7%)
3q21	3 (4%)	6 (5.8%)
MDS-related cytogenetics (WHO 2016)	60 (75%)	74 (71%)
Cytogenetic risk group (MRC 2010)		
Adverse	69 (84%)	87 (83%)
Intermediate	11 (13%)	17 (16%)
Missing/failed	2 (2%)	1 (1.0%)
Mutations		
TP53†	32 (43%)	43 (45%)
Mutation in MDS-related gene†,‡	38 (51%)	37 (29%)
AML/MDS with MDS-related gene mutation (without comutation in *TP53*)†,‡	29 (39%)	30 (31%)
1 mutated MDS-related gene†,‡	10 (14%)	8 (8%)
≥2 mutated MDS-related genes†,‡	19 (26%)	22 (23%)
NPM1 mutant	2 (2%)	4 (4%)
FLT3 TKD	1 (1%)	1 (1%)
FLT3 ITD	4 (5%)	4 (4%)
ELN 2022 risk group		
Adverse	78 (95%)	99 (94%)
Intermediate	3 (4%)	5 (5%)
Missing	1 (1%)	1 (1%)

FISH, fluorescence in situ hybridization.
*Missing in 3 patients
†Percentages of those with gDNA for sequencing (171/187 patients)
‡*ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2*

Table 2. Response and outcomes in each group (FLAG IDA/CPX)

	FLAG-IDA (n = 82)	CPX-351 (n = 105)	*P* value
Response after cycle 1			
CR	42 (51%)	42 (40%)	.15
CRi	11 (13%)	12 (11%)	
ORR (CR + CRi)	53 (65%)	54 (51%)	
Best response after 2 cycles			
CR	55 (68%)	63 (60%)	.06
CRi	7 (9%)	4 (4%)	
ORR (CR+CRi)	62 (77%)	67 (64%)	
Early mortality			
Day 30	6 (7%)	5 (5%)	.46
Day 60	9 (11%)	13 (12%)	.77
Count recovery in course 1			
Recovered neutrophils to >1.0 × 10^9/L	71 (88%)	72 (71%)	.01
Median days to neutrophil recovery (IQR)	30 (26-35)	32 (26-39)	.11
Recovered platelets to >100 × 10^9/L	58 (72%)	63 (62%)	.16
Median days to platelet recovery (IQR)	29 (25-33)	34 (28-44)	<.01
Count recovery in course 2			
Recovered neutrophils to >1.0 × 10^9/L	41 (71%)	55 (83%)	.09
Median days to neutrophil recovery (IQR)	46 (32-52)	31 (26-41)	<.01
Recovered platelets to >100 × 10^9/L	21 (36%)	39 (59%)	.01
Median days to platelet recovery (IQR)	36 (35-53)	31 (24-47)	.20
Allogeneic transplant			
Allogeneic transplant at any time	36 (44%)	53 (50%)	.41
Allogeneic transplant in first response*	30 (48%)	43 (64%)	.10
Outcomes at 3 years			
OS	25%	32%	.36
EFS	24%	25%	.86
RFS	29%	39%	.08

IQR, interquartile range.
*Percentage of those achieving CR/Cri.

Numerically a greater number of patients receiving CPX-351 underwent transplantation, although this did not reach statistical significance (53/105 [51%] vs 36/82 [44%]; $P = .41$). More patients receiving CPX-351 underwent transplantation in first remission (43/67, 64%) compared with those receiving FLAG-Ida (30/62 [48%]; $P = .10$). The median number of courses administered before SCT was 2 in both arms, and the median time to SCT was 139 days with CPX-351 and 131 days for FLAG-Ida ($P = .86$). The cumulative incidence of death in remission censored at SCT was higher with FLAG-Ida (supplemental Figure 4). Among the patients who underwent transplantation, survival did not differ per the induction regimen (supplemental Figure 5).

Exploratory subgroup analyses by genomic class

In patients with secondary AML defined based on clinical history only, there was no difference in OS between the treatment groups (HR, 1.0; 95% CI, 0.59-1.69). In patients with high-risk MDS, there was a trend toward longer OS in patients treated with CPX-351 (HR, 0.54; 95% CI, 0.28-1.00) however, the *P* value for heterogeneity was 0.23 for this analysis (Figure 3). When secondary disease was defined by the presence of myelodysplasia-related cytogenetic abnormalities, there was no difference between treatment arms (HR, 0.94; 95% CI, 0.63-1.40) (Figures 3 and 4).

In patients with mutationally defined secondary AML/MDS, those treated with CPX-351 had significantly longer OS, median 38.4 months with CPX-351 and 16.3 months with FLAG-Ida (HR, 0.42; 95% CI, 0.21-0.85; *P* value for heterogeneity = .05) (Figures 3 and 4) despite a similar ORR (70% vs 62%; $P = .5$) and no decrease in relapse (3-year CIR, 19% vs 20%). Outcomes were similar in patients with single mutations and those with ≥2 mutated MDS-related genes (supplemental Figure 6). Patients with MDS-related gene mutations had significantly higher hematologic toxicity after the second course of FLAG-Ida than patients in other genomic groups, whereas this difference was not observed with CPX-351 (supplemental Table 4).

Patients with *TP53* mutations had an adverse prognosis, with a median OS of 7 months compared with 28 months in those with wild-type *TP53*. There was no difference between treatment arms for this group (HR, 0.89; 95% CI, 0.55-1.45). *TP53* mutations were

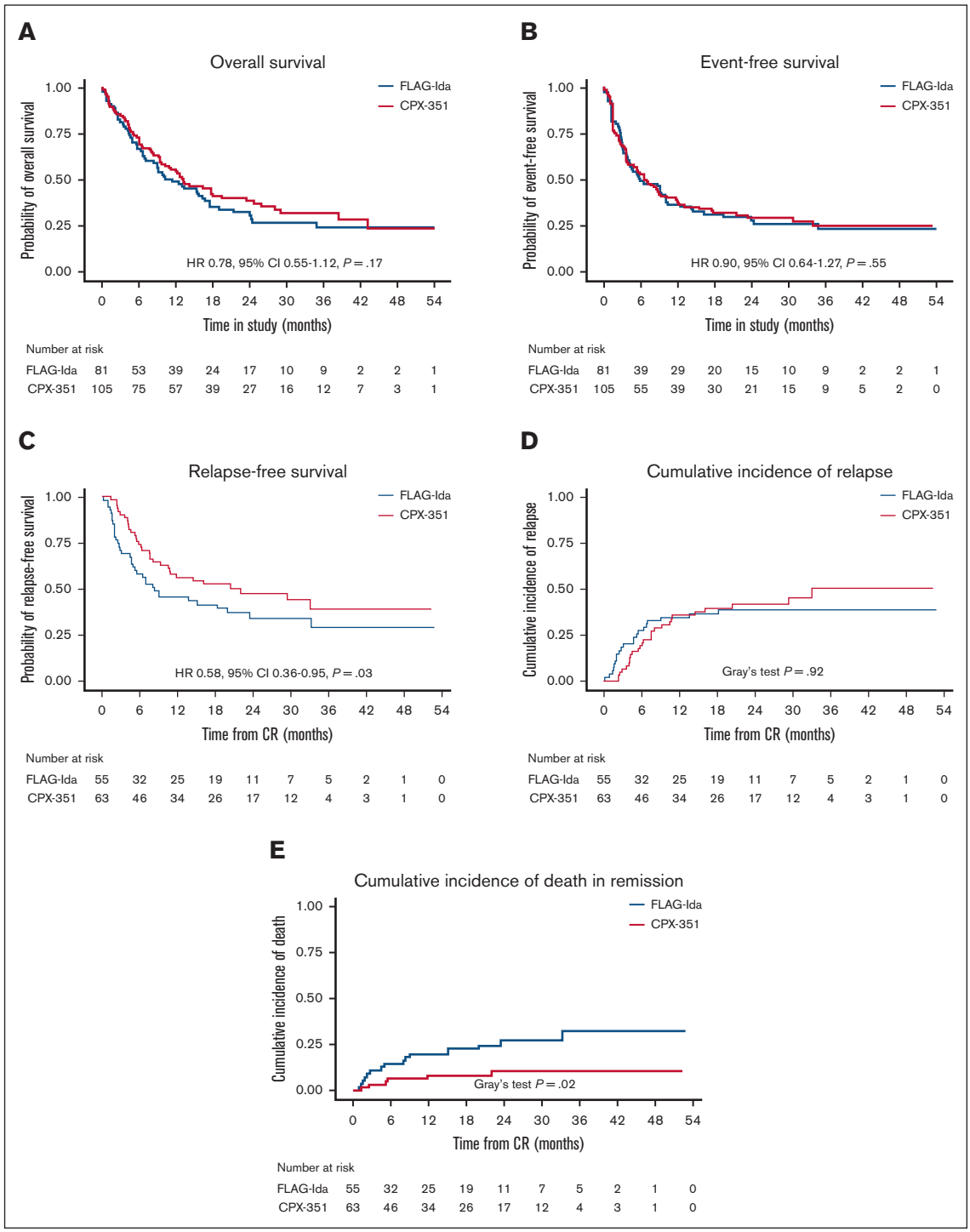

Figure 2. Outcomes based on treatment allocation. (A) OS, (B) EFS, (C) RFS, (D) cumulative incidence of relapse, and (E) cumulative incidence of death during remission.

present in all clinical groups but were enriched in those with MDS-related cytogenetics (supplemental Figure 3).

Measurable residual disease

Bone marrow MRD results were available for 59 patients, either by flow cytometry after cycle 1 (n = 47; CPX-351 31 and FLAG-Ida 16) or RT-qPCR after cycle 2 (n=12, CPX-351 8 and FLAG-Ida 4, comprising 4 *NPM1,* 7 *KMT2A* rearrangements, and 1 *PICALM*::*AF10* fusion). Using a cutoff of <0.1% for MFC MRD and >4 log reduction from the diagnostic result for RT-qPCR, 22 of 59 (37%) patients achieved an MRD response. Patients who achieved an MRD response had a longer OS than those who did not (median 24.3 vs 8.4 months). The MRD response was higher in patients with FLAG-Ida (11/20, 55%) than in those receiving

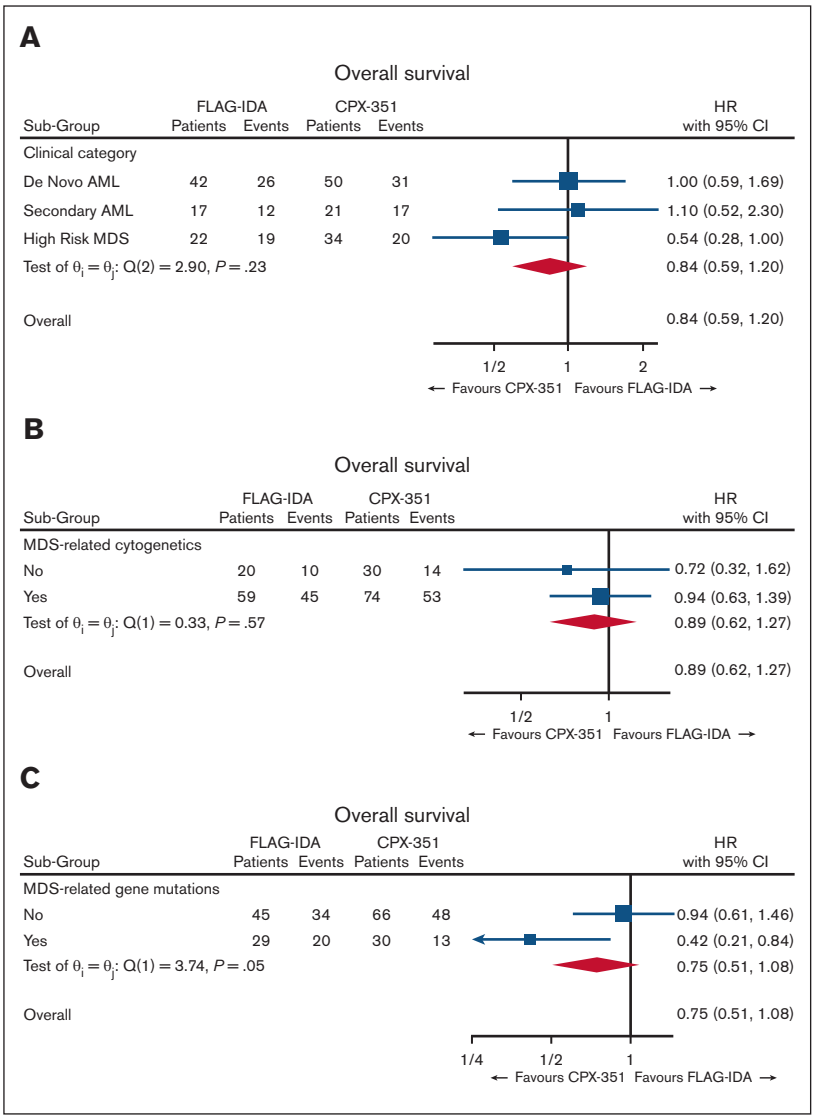

Figure 3. Subgroup analysis of OS. (A) Clinical classification, (B) Cytogenetic classification, and (C) Molecular classification.

CPX-351 (11/39; 28%), with the same trend observed when the MRD was analyzed as a continuous variable (supplemental Figure 6). The results were similar when the analysis was limited to those with flow cytometric MRD results (supplemental Tables 6 and 7). In a small subgroup of patients with MDS-related gene mutations, the rate of MRD response was similar in both arms, 4 of 11 (36%) with CPX-351 and 3 of 9 (33%) with FLAG-Ida.

Discussion

Previous UK NCRI trials established the FLAG-Ida regimen as the preferred regimen for patients aged <60 years with high-risk and secondary AML.[15,16] CPX-351 has been approved for the treatment of adults with newly diagnosed AML with myelodysplasia-related changes (AML-MRC) and therapy-related AML (t-AML), irrespective of age, but it is based on a randomized comparison with 7 + 3 chemotherapy in older patients only.[8]

In this randomized comparison between FLAG-Ida and CPX-351 in younger adults with newly diagnosed AML and high-risk MDS with an adverse karyotype, there was no detectable difference in OS between treatments. Interestingly, despite the trend toward a lower ORR and a lower proportion achieving MRD negativity with CPX-351, there was a trend toward a higher rate of SCT, as was observed in the pivotal CPX-351 trial.[8] Patients who were able to reach SCT had good outcomes irrespective of randomization. In patients who achieved CR, RFS favored CPX-351, although the numerically lower CR rate with CPX-351, and therefore, a smaller proportion of patients included in the RFS calculation should be noted. The RFS advantage may be related to the reduced number of deaths in remission, which allowed more patients to undergo transplantation. Despite enrolling a younger population, the higher rate of death in remission in the control arm was consistent with that observed in the registration study.[8] The second course of FLAG-Ida was associated with delayed count recovery and

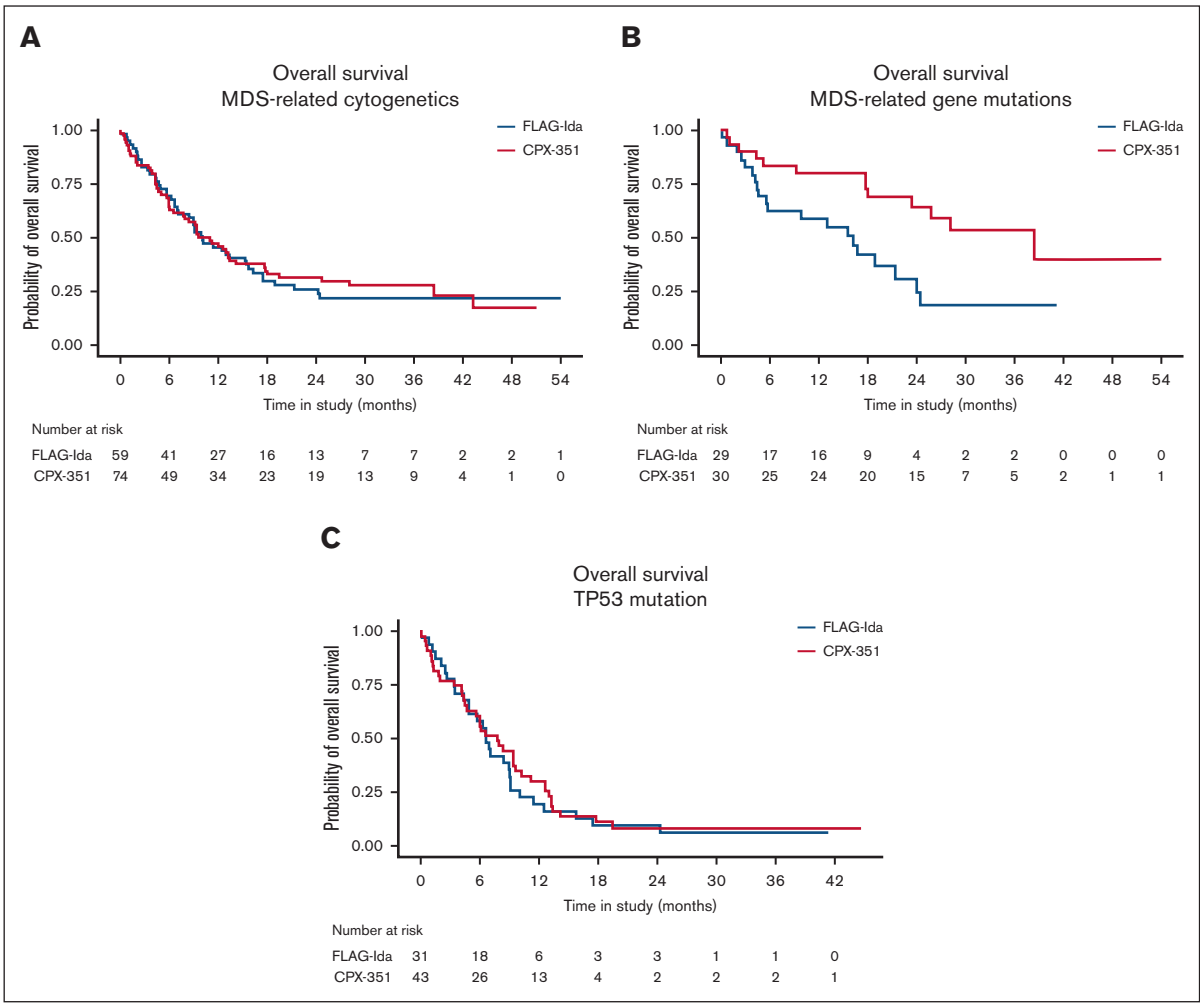

Figure 4. OS by randomization in the genomic subgroups. (A) MDS-related cytogenetic abnormalities, (B) MDS-related gene mutations, and (C) TP53 mutation.

reduced the benefit of a higher response rate, suggesting that in responding patients, earlier SCT or a less intensive second course as bridging to SCT may have improved outcomes. In this context, FLAG-Ida augmented by the addition of Venetoclax has been reported to result in high remission rates following a single course, which can be successfully consolidated by SCT.[20]

In light of the previously demonstrated benefits of CPX-351 in secondary AML, we performed an exploratory analysis of patients with a clinical or cytogenetic diagnosis of secondary AML, in which there was no advantage for CPX-351. However, a significant survival benefit with CPX-351 over FLAG-Ida was observed in patients with secondary AML, as defined by the presence of MDS-related gene mutations, with the important caveat of small numbers and potential unmeasured confounders within this subgroup analysis. We suggest that this benefit was driven by a combination of lower toxicity and a trend toward a higher transplantation rate, findings that are consistent with those from the phase 3 randomized trial of CPX-351 vs 3 + 7 in older patients.[8] It is increasingly recognized that secondary AML may be better defined by mutational profile than clinical history;[5,6,13,14] however, patients with molecularly defined secondary AML were not specifically studied in previous trials. Our finding of a survival benefit in this category supports the genomic definition of secondary AML. A previous study by our group suggested a worse outcome for patients with mutations in 2 or more MDS-related genes, an effect not noted in this study, in which these patients were treated as high-risk and recommended for transplantation.[21] We observed no benefit in patients with *TP53* mutations, consistent with a secondary analysis of a phase 3 randomized trial of CPX-351[22] and a French real-world study,[23] even when these occurred in the presence of secondary AML mutations. Given that almost half of the patients with clinically defined secondary AML and almost 60% of those with MDS-related cytogenetics had *TP53* mutations, the lack of benefit in these groups may be mediated by the coexistence of *TP53* mutations.

Although our observations require validation in other prospective studies, if confirmed these exploratory findings have important implications for the rational use of CPX-351. Consistent with previous reports,[21,24-26] we found that a significant proportion of patients with a clinical diagnosis of de novo AML had mutations in MDS-related genes and benefited from treatment with CPX-351. Conversely, many patients clinically diagnosed with secondary AML do not benefit from this therapy. Therefore, improving outcomes in this group is likely to require the rapid availability of next-generation sequencing results before the initiation of therapy.

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Authorship

Contribution: N.H.R. was the chief investigator and designed the study; C.W.-B. and J.W. provided statistical analyses; L.M.B., J.C., E.L.H., S.B. provided trial coordination; R.D., S.K., S.D.F., U.M.O., P.M., P.K., J.C., C.H., C.A., M.D., and N.H.R. enrolled patients in the study; A.G. performed molecular analyses and coordinated patient samples; C.W.B. and J.O. performed data analysis at the Centre for Trials Research, Cardiff University; R.D., N.P., and N.H.R. were responsible for molecular MRD analyses; S.D.F. performed flow cytometric MRD analyses; J.O., R.D., and W.V. performed and analyzed genomic sequencing; M.K. was responsible for DNA library preparation and sequencing; J.O., C.W.B., R.D., and N.H.R. drafted the manuscript; all authors revised and approved the manuscript.

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