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# CPX-351 versus daunorubicin, cytarabine plus gemtuzumab ozogamicin in older adults with non-adverse risk AML: NCRI AML18

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**Abstract:**
We compared daunorubicin/AraC plus fractionated gemtuzumab (DAGO2) with CPX-351 (CPX) (1:2 randomisation) in 439 patients ≥60yrs (median age 68yrs) without known adverse-risk cytogenetics entering the NCRI AML18 version2 trial (NCT02272478). Median follow-up was 35 months. Patients not in MRD-negative remission after course-1 could enter a second randomization between standard versus intensified chemotherapy. Post course-1 response rates were greater after DAGO2 (CR+CRi, 60% vs 47.5%, OR 0.61 95%CI 0.41-0.91, p=0.016). Following course-2 the overall response was not significantly different, 85% for DAGO2 vs 78% for CPX (OR 0.64, 95%CI 0.39-1.09, P=0.095). More patients attained CR with MRD negativity post course-1 in the DAGO2 arm (47% vs 29% for CPX, OR 0.46 95%CI 0.29-0.72, p=0.004). We observed better 3yr EFS (34% vs 27%, HR 0.73 95%CI 0.57-0.93, P=0.012) and OS (52% vs 35%, HR 0.62, 95%CI 0.46-0.83, P=0.001) with DAGO2. In a stratified analysis, CPX did not provide a survival benefit in patients with MDS-related mutations (HR 1.40, 95%CI 0.97-2.03) and was associated with poorer survival in patients with NPM1 (HR 2.83 95%CI 1.17-6.82) and FLT3 mutations (HR 2.14, 95%CI 0.98-4.68). 37% of patients were transplanted in CR1 and this did not differ by randomization. Survival post-transplant did not differ between arms. For patients entering the course-2 randomisation (n=107) survival was equivalent between standard versus intensified CPX doses (P=0.565).In this population of older patients without known adverse-risk cytogenetics, DAGO2 resulted in superior survival compared to CPX. CPX did not benefit those with MDS-related mutations over DAGO2.

**Conflict of interest:** COI declared - see note

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# Cover page

Title

# CPX-351 versus daunorubicin, cytarabine plus gemtuzumab ozogamicin in older adults with non-adverse risk AML: NCRI AML18 trial

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CPX versus DA and Gemtuzumab in older AML patients

**Key Points**

**1.** In fit older AML patients >60yrs without known adverse-risk cytogenetics, we randomised DA chemotherapy plus Gemtuzumab against CPX-351.

**2.** Response and survival was better with DAGO including in those patients with MDS-related mutations.

**Abstract.**

We compared daunorubicin/AraC plus fractionated gemtuzumab (DAGO2) with CPX-351 (CPX) (1:2 randomisation) in 439 patients ≥60yrs (median age 68yrs) without known adverse-risk cytogenetics entering the NCRI AML18 version2 trial (NCT02272478). Median follow-up was 35 months. Patients not in MRD-negative remission after course-1 could enter a second randomization between standard versus intensified chemotherapy. Post course-1 response rates were greater after DAGO2 (CR+CRi, 60% vs 47.5%, OR 0.61 95%CI 0.41-0.91, p=0.016). Following course-2 the overall response was not significantly different, 85% for DAGO2 vs 78% for CPX (OR 0.64, 95%CI 0.39-1.09, P=0.095). More patients attained CR with MRD negativity post course-1 in the DAGO2 arm (47% vs 29% for CPX, OR 0.46 95%CI 0.29-0.72, p=0.004). We observed better 3yr EFS (34% vs 27%, HR 0.73 95%CI 0.57-0.93, P=0.012) and OS (52% vs 35%, HR 0.62, 95%CI 0.46-0.83, P=0.001) with DAGO2. In a stratified analysis, CPX did not provide a survival benefit in patients with MDS-related mutations (HR 1.40, 95%CI 0.97-2.03) and was associated with poorer survival in patients with *NPM1* (HR 2.83 95%CI 1.17-6.82) and *FLT3* mutations (HR 2.14, 95%CI 0.98-4.68). 37% of patients were transplanted in CR1 and this did not differ by randomization. Survival post-transplant did not differ between arms. For patients entering the course-2 randomisation (n=107) survival was equivalent between standard versus intensified CPX doses (P=0.565).

In this population of older patients without known adverse-risk cytogenetics, DAGO2 resulted in superior survival compared to CPX. CPX did not benefit those with MDS-related mutations over DAGO2.

## Introduction

The UK NCRI AML Working Group has run a sequence of randomized trials aiming to improve the outcome for older adults with Acute Myeloid Leukaemia (AML) fit for intensive chemotherapy.[1-3] The AML16 trial showed that the addition of a single dose of Gemtuzumab ozogamicin (GO) with daunorubicin/Ara-C (DA) improved overall survival (OS) and that this benefit was primarily seen in patients with favourable or intermediate risk cytogenetics. In version 1 (v1) of the NCRI AML18 trial we demonstrated that, in older adults, a fractionated schedule of two doses of Gemtuzumab ozogamicin (GO2) combined with course 1 DA induction had greater efficacy than a single dose of GO based on better measurable residual disease (MRD) reduction and superior OS for patients with non-adverse risk cytogenetics.[2] This survival benefit was apparent in those with myelodysplasia (MDS)-related gene mutations as well as those with *de novo* AML and was dependent upon delivery of transplant in first remission (CR1). We also demonstrated that, for patients with evidence of residual disease following a first course of DA-GO induction, survival was improved by course 2 chemotherapy intensification.[4]

In the last few years evidence has emerged that CPX-351 (CPX), a liposomal formulation of daunorubicin and AraC improves survival in older patients with newly diagnosed secondary AML (therapy-related AML and AML with myelodysplasia-related changes, AML-MRC) compared to DA chemotherapy.[5] In v2 of the AML18 trial (June 2019-Dec 2022) we compared DAGO2 with CPX-351 for up to 3 courses in AML patients >60 years without known adverse risk cytogenetics. Following the EMA approval of CPX for clinically-defined secondary AML, these patients were also excluded from trial entry. As persistence of measurable disease following first induction is associated with an adverse outcome in older AML patients,[6] patients who were not in MRD-negative remission by flow cytometry following course 1 CPX could enter a randomization comparing intensified (3 doses) versus standard (2 doses) CPX in course 2.

Here we report outcomes from this study that closed after accrual of 439 patients, with recruitment affected by the COVID pandemic. Although the approved use of CPX is for patients with clinical secondary AML or with myelodysplasia-related cytogenetic abnormalities, it might also benefit additional AML patients with myelodysplasia-related mutations as suggested by an exploratory analysis of the AML19 comparison of CPX with FLAG-Ida in high risk younger adults.[7] MDS-related mutations were frequent in v1 of AML18 (~50% in patients without adverse risk cytogenetics).[2] We therefore also evaluated for any differential efficacy between CPX and DA-GO2 across relevant molecular subgroups by molecular profiling together with flow cytometric MRD testing.

## Methods

### Patients and Trial Treatments

The UK NCRI AML18 protocol (ISRCTN-31682779, EudraCR-2013-002730-21) involved several therapeutic questions in previously untreated AML patients aged ≥60 years without known adverse risk cytogenetics who were fit for intensive chemotherapy and did not have acute promyelocytic leukaemia or blast transformation of chronic myeloid leukaemia. Patients with high-risk myelodysplastic syndrome (defined as >10% marrow blasts at diagnosis) were also eligible. Clinical secondary AML was defined as resulting from either an antecedent haematologic disorder or prior chemotherapy for a non-haematological malignancy. Following the EMA approval of CPX for secondary AML, these patients were also excluded from entry into the CPX randomisation.

Full treatment schedules and trial schema for the CPX randomisation is displayed in Figure 1. Patients entering were assigned on a 1:2 basis between chemotherapy course 1 comprising DA (Daunorubicin [60mg/$m^2$ days 1,3,5], AraC [100mg/$m^2$ bd days 1-10]) with 2 doses of Gemtuzumab Ozogamicin (DAGO2)[2] or CPX-351 (100 units/$m^2$, 100 mg/m AraC and 44 mg/$m^2$ daunorubicin) administered on days 1, 3, and 5 (CPX 300). Patients with abnormal liver function could enter the randomization and receive DA alone if so randomized. In both arms, patients who were not in MRD-negative remission could enter a second course randomization for treatment intensification or not. Results of treatment intensification in the DAGO2 arm with FLAG-Ida have been reported.[4] The daily AraC dose in FLAG-Ida was limited to 1g/$m^2$ and FLAG-Ida was further dose-reduced for patients over 70yrs and in course-3 for all patients (Fludarabine from 30mg/$m^2$ days 1-5 to 25mg/$m^2$ days 1-4, Idarubicin from 8mg/$m^2$ days 3-5 to 5mg/$m^2$ days 2-4). In the CPX-351 arm, the intensification randomisation compared a second 3-day course on days 1, 3 and 5 (intensified CPX, CPX 300) with a standard 2-day course on days 1 and 3 (100 units/$m^2$) (CPX 200) (Figure 1). If MRD-negative, patients in the CPX-351 arm received 100 units/$m^2$ on days 1 and 3 as course 2; those randomised to the DAGO2 arm received DA(3+8) without GO. CPX was reduced to 65 units/$m^2$ on days 1 and 3 (CPX 130) for the 3$^{rd}$ course. Patients in the DAGO2 arm who were *FLT3* mutated could also enter a post course-1 randomisation to receive quizartinib (AC220) or not, the results of this randomisation have been reported elsewhere.[8] Only 7 patients received quizartinib. Patients were enrolled from 81 centers in the United Kingdom and 6 in Denmark. The study was approved by the ethics committees (All Wales Research Ethics Committee, approved by Danish national and regional Ethics bodies for sites in Denmark) and conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. All patients provided written informed consent for trial entry and for the separate randomisations.

### Laboratory studies

Cytogenetic analyses, performed locally, were reviewed and coded centrally according to Grimwade 2010 criteria.[9] Mutation analysis of *FLT3* and *NPM1* was performed in a single reference lab. Banked diagnostic DNA was analysed for variants in 75 recurrently mutated myeloid genes (Supplementary Methods). AML with secondary-type mutations (myelodysplasia-related mutations) was defined by presence of one or more mutations in *ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, or *ZRSR2*.

Measurable residual disease was assessed by flow cytometry in a single reference laboratory as previously described.[2,4] Results were entered into the trial database within 24-48hrs of sample receipt, blinded to investigator reported remission status to allow independent refinement of clinical remission assessments. Post course-1 results were then issued immediately to investigators by the trials unit. Flow MRD testing combined detection of diagnostic leukemic aberrant immunophenotypes (LAIP) and different from normal aberrant immunophenotypes (DfN) as per consensus recommendations[10,11] with any measurable level of MRD considered positive (above sensitivity threshold of 0.02-0.05%). An MRD- result required negativity in an adequate bone marrow (BM) by both DfN and LAIP analysis (prerequisite of LAIP target(s) identified at baseline). Patients were categorised as MRD unassessable post course-1 if no adequate BM was received prior to course-2 assignment or in the absence of a baseline LAIP to confirm MRD negativity. Post course 2 BMs for MRD were only requested in patients entering the course 2 intensification randomisation.

**Statistical Considerations and End Points**

The randomisation opened to recruitment in June 2019, with a primary endpoint of overall survival (OS), based on a total of 700 patients and approximately 440 events. Due to the impact of COVID on recruitment (which fell from approximately 30 patients per month in 2019 to fewer than 10 patients per month throughout the pandemic), the primary endpoint was amended in December 2021 to event-free survival (EFS), incorporating relapse and resistance to treatment as well as death into the definition of an event,[12,13] with a target of 506 patients. The randomisation closed in December 2022, under authorisation from the Independent Data Monitoring Committee, having accrued 439 patients.

The analyses are by intention to treat. End points were defined according to the revised International Working Group criteria.[14] Responses were based on investigator assessment of bone marrows. CR and CRi (up to 50 days for course 1 response and up to 100 days for induction response) have been combined for outcome analyses. Characteristics of the patients are summarized across the group using frequency and percentage for categorical data, and median and quartile range for quantitative data. Comparisons of patient characteristics were made using $\chi 2$ tests, 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. Outcomes are reported as effect sizes with 95% confidence intervals (CIs); significance was set at $P < 0.05$. Event free survival (EFS) was measured in all patients and was defined as time from randomisation to treatment failure (refractory disease or partial response by the end of course 2, relapse or death from any cause). If treatment failure was due to refractory disease or partial response, the event was recorded on cycle 1 day 1. Overall survival (OS) was defined as the time from randomisation to death from any cause with those still alive censored at the date last seen. Relapse-free survival (RFS) was calculated only for patients who achieved complete remission (CR) or CR with incomplete hematological recovery (CRi), and was measured from the date of CR/CRi until the date of relapse or death from any cause. Competing-risk analysis was performed for the cumulative incidence of relapse and cumulative incidence of death in remission, with nonrelapse mortality and hematological relapse respectively as the competing risk, using the Gray's test and Fine and Gray model. For the exploratory analyses of key subgroups, HRs were calculated using Cox proportional hazards models and represented in forest plots, with a test for trend of heterogeneity across the subgroups, wherever applicable.

Toxicity (hematologic recovery times and non-hematologic toxicity) was scored using the National Cancer Institute Common Toxicity Criteria, version 3.[15] Resource use data (blood product support, days on antibiotics and hospitalisation) were collected.

Data Sharing Statement: Applications to access the dataset can be made by contacting the trial Sponsor on AML18@cardiff.ac.uk. The Sponsor is supportive of collaborative use of clinical trial data - applications are assessed on the basis of planned analyses; complex planned datasets and analyses may require additional resource to support data preparation and extraction.

## Results

### Patient Characteristics

From June 2019-Dec 2022 439 patients were randomised 2:1 in favour of CPX-351 (CPX-351, n=295, DAGO2, n=144, including 7 with DA alone)(CONSORT, Figure 2). Clinical baseline characteristics of the patients are shown in Table 1 and were generally balanced between the treatment arms. There was no significant difference between arms for ELN genetic risk and mutation subgroups (P NS for all). Failed/unknown cytogenetic results were more frequent in the DAGO2 arm (P=0.029). The median age was 68 years with 33% of patients >70 years; 81% had clinical *de novo* AML, 7% clinical secondary AML (entered prior to the amendment that excluded these from randomization) and 12% high-grade MDS. Because some patients in this study were randomized before their cytogenetic results were known, the cohort eventually included 8% with adverse

cytogenetics. In total, 404 patients had mutation panel data (Supplementary Figure S1), *FLT3* mutations were present in 16%, *NPM1* mutations in 24%, *TP53* mutations in 4% and MDS-related gene mutations (secondary AML mutations) were present in 60%. *DDX41* mutations were present in 9%. Median follow-up was 35 months.

**Response and Outcome**

Following course 1, overall (CR+CRi) and CR response rates were higher after DAGO2 (CR+CRi, 60% vs 47.5%, OR 0.61 95%CI 0.41-0.91, p=0.016; CR 50.5% vs 39%, OR 0.62 0.42, 0.93 p=0.020). Following course 2 the overall response was not significantly different, 85% for DAGO2 vs 78% for CPX (OR 0.64, 95%CI 0.39-1.09, P=0.095) although there was a higher frequency of CR with DAGO2, 78.5% vs 68% (OR 0.59 95%CI 0.37-0.94, P=0.024) (Table 2). Next, we assessed the impact on MRD reduction measured by flow cytometry. Significantly lower MRD levels were observed in DAGO2 patients compared to CPX (Supplementary Figure S2A) in all patients evaluable for response by flow cytometric MRD (Supplementary Figure S2B). Of the randomized patients, 404 were assessable for composite response that included MRD for those in CR and CRi after first induction (Table 2). Among these, CR without MRD based on ELN criteria (MRD <0.1%) was achieved by 47% and 29% of patients assigned to DAGO2 and CPX respectively (P =0.004; Table 2). MRD response data were available in 84.5% of patients in CR/CRi post course 1 (CPX, 114 of 140; DAGO 73 of 86, Table 2). Of these, more attained MRD <0.1% in the DAGO2 arm (85% vs 68% for CPX-351, OR 0.37 95%CI 0.16-0.82 p=0.008) (Table 2). Distribution of flow cytometric MRD responses by treatment arm in specific mutation subgroups are displayed in Supplementary Figure S2C. We observed increased efficacy by MRD for DAGO2 compared to CPX across mutation groups, including MDS-related mutations.

Both EFS (HR 0.73 95%CI 0.57-0.93, p=0.012) and OS (HR 0.62, 95% CI 0.46-0.83, p=0.001) were better with DAGO2. With a median follow up of 35 months, EFS and OS at 3yrs was 34% and 52% for DAGO2 compared to 27% and 35% for CPX. (Figure 3A and B). Relapse-free survival (RFS) was also higher with DAGO2 although this did not reach significance (HR 0.75 95%CI 0.57-1.01, p=0.058). There was no significant difference between treatment arms in survival from remission (HR 0.74 95%CI 0.48-1.13, p=0.162), cumulative incidence of relapse (SHR 0.87 95%CI 0.56-1.31, p=0.52) or cumulative incidence of death in complete remission (SHR 1.05 95%CI 0.43-2.54, p=0.92) for those patients who attained CR/CRi by day 50 (Supplementary Figure S3).

A sensitivity analysis that excluded patients with adverse cytogenetics/TP53-mutated AML confirmed better survival with DAGO2 in patients with favourable and intermediate risk cytogenetics (Supplementary Figure S4).

The results for EFS and OS at 3 years from the time of randomization favoured DAGO2 in all subgroups based on clinically defined baseline characteristics (age, white blood cell count, disease type, and cytogenetics) (Figure 4, Supplementary Figure S5.A). In a stratified analysis, CPX-351 did not provide an OS benefit over DAGO2 in patients with MDS-related mutations (HR 1.40, 95%CI 0.97-2.03), and was associated with worse OS in patients with *NPM1* (HR 2.83 95%CI 1.17-6.82) and *FLT3* mutations (HR 2.14, 95%CI 0.98-4.68) (Figure 4B, EFS results shown in Supplementary Figure S5.B). OS at 3 years in patients with *NPM1* mutations was 78% with DAGO2 compared to 51% with CPX. Similar results were observed when the 7 patients in the DAGO2 arm receiving quizartinib from course 2 were excluded from the analysis (Supplementary Figure S6). In exploratory subgroup analysis there was less OS benefit from DAGO2 compared to CPX for patients with MDS related mutations and wildtype *NPM1* / *FLT3* (HR 1.09 95%CI 0.73-1.62) versus other patients (HR 2.08 95%CI 1.29-3.37) (P value for heterogeneity 0.038) (Figure 5).

OS at 3yrs was 49% for patients with *DDX41* mutations with no detectable difference by randomization.

In total, 162 (37%) patients received an allogenic stem cell transplant (ASCT); most transplants (137, 85%) were undertaken in first remission (CR1 transplants: 44/142 (36%) in the DAGO2 arm and 93/230 (40%) in the CPX-351 arm) (Table 2). Survival following CR1 transplant did not differ by randomisation (OS, HR 0.98, 95% CI 0.52-1.85). OS and RFS at 3yrs post transplant was 57% and 55% for DAGO2 compared to 64% and 61% for CPX. (Supplementary Figure S7).

**Treatment toxicity and resource usage**

Haematological recovery was slower with CPX-351 than DAGO2 (Supplementary Table S1). Following course-1 the time to neutrophil recovery to $1x10^9$/L was 31 and 34 days for DAGO2 and CPX-351 respectively (P= 0.033). Likewise platelet count was delayed with CPX, the time to platelet recovery to 100 x $10^9$/L was 31 and 34 days for DAGO2 and CPX respectively (P=0.02). With respect to supportive care, days of receiving antibiotics or hospitalization, or the time to the start of course 2 did not differ between the CPX and DAGO2 arms. There was greater gastrointestinal toxicity with DAGO2 (Supplementary Figure S8). Early all-cause mortality was not different at day 30 between DAGO2 and CPX (at 4% and 7%, HR 1.67, 95% CI 0.66-4.26, p=0.27) but was higher with CPX-351 at day 60 (4% vs 11%, HR 2.80, 95% CI 1.14-6.86, p=0.019). The increased early mortality was mainly related to infection and disease. (Supplementary Figure S8).

**Outcomes of high risk randomisation in CPX arm**

After course 1 a total of 157 patients entered the high risk randomisations for patients not in an MRD negative remission (107 after CPX-351 induction and 50 after DAGO2 induction) (CONSORT Figure 2). Baseline characteristics of the patients are shown in Supplementary Table S2 and were

balanced between the treatment arms. Outcomes for patients randomised to DAGO2 have previously been reported.[4] There was no evidence that intensified CPX increased rates of conversion to CR from refractory disease or increased MRD negativity for those patients in an MRD+ CR post course 1 (Supplementary Table S3). Likewise there was no evidence of a survival benefit (Supplementary Figure S9).

Of all patients who were eligible for the high risk course 2 randomization (excluding early deaths), 126 did not enter (CPX 88 of 195 eligible, DAGO2 38 of 88 eligible); these included 102 not in remission after course 1. Of these, 43% (33/76) attained CR/CRi in the CPX arm and 27% (7/26) in the DA GO arm) within 100 days.

**Discussion.**

Previous studies with CPX have primarily focused on patients with high risk AML where a survival benefit has been shown when compared with standard DA induction in patients with clinically defined secondary disease or MDS-related cytogenetic abnormalities.[5] This survival benefit was ascribed to a favourable toxicity profile, higher rates of transplantation and superior post-transplant survival.[5] We considered that these benefits might also extend to older patients without these adverse risk factors however, based on our recent encouraging experience in the NCRI AML18v1 trial, we compared CPX with DA plus fractionated GO (DAGO2) rather than with DA alone.[2] The randomization recruitment target was not reached due to the impact of the COVID pandemic (Supplementary Figure S10). Nonetheless, the results of this comparison show that in fit older patients, primarily with clinical *de novo* AML and intermediate risk cytogenetics, CPX did not improve survival outcomes in comparison with DAGO2, overall or in any subgroup, including those with MDS-related mutations. DAGO2 induction resulted in higher rates of hematologic and flow-MRD response from first induction and lower 60-day mortality. There was less hematological toxicity with DAGO2 although greater grade 1-2 gastro-intestinal toxicity. The risks of relapse and death in remission were similar between the two treatment arms and the improvement in OS and EFS was primarily due to the superior and deeper early response and better tolerability. The advantage for DAGO2 was particularly apparent in patients with *de novo* AML type mutations, specifically *NPM1* and *FLT3*. There was only a trend to a survival benefit with DAGO2 in the MDS-related mutation group, which was not present when patients with co-mutations in *NPM1* and *FLT3* were excluded. The more advantageous DAGO2 response observed in the MDS-related mutation group with co-mutated *NPM1*, as opposed to MDS-related mutations without *NPM1*, is consistent with other studies indicating that the *NPM1* mutation is the primary determinant of outcome in patients

with mutations in both *NPM1* and MDS-related genes and therefore should guide induction decisions.[16-19] In the NCRI AML19 trial we had previously observed a superior outcome with CPX compared to FLAG-Ida in high risk patients with MDS-related mutations.[7] This benefit was primarily due to less hematological toxicity and lower mortality following the second course of CPX compared to FLAG-Ida with more patients transplanted following CPX and superior post-transplant outcome. In AML18 we did not observe a benefit for CPX in patients with MDS-related mutations even when we excluded patients with co-existing *NPM1* and *FLT3* mutations from the analysis, suggesting that DAGO2 was better tolerated in this older adult population than was FLAG-Ida in our younger adult AML19 trial. Furthermore, in the current study, transplant rates in first remission with DAGO2 and CPX were comparable with no difference in post-transplant survival by randomisation.

Interestingly, although remission rates after course 1 were significantly lower in the CPX arm, overall remission rates were not. Furthermore, the inferior MRD response post-course 1 in CPX patients did not appear to reduce post-transplant overall and relapse free survival compared to GO2. This differs from the previous observed benefit of early efficacy on post transplant outcomes from GO2 compared to GO1 in AML18v1.[2] A limitation of this study is the unavailability of post-course 2 MRD results and treatment information for patients who did not enter the high risk course 2 randomisations. We could not therefore assess whether CPX is associated with slower response kinetics or if off-trial chemotherapy compensated for the poorer cytoreduction following course 1 CPX in patients who proceeded to transplant. However, course 2 intensification with 3 vs 2 doses of CPX did not improve survival in patients without an MRD negative remission post course 1. This is in contrast to our experience of the benefit of post-induction treatment intensification with FLAG-Ida or Cladribine with Daunorubicin/ AraC following initial DAGO2.[4]

Notably the population studied here, consisting primarily of older fit patients with clinical *de novo* AML, was very different to those in the pivotal trial with secondary AML where a survival benefit of CPX compared to DA was reported.[5] Our patients were older and excluded patients with known adverse risk cytogenetics or with a history of antecedent haematological disease, focusing primarily on patients with more chemo-sensitive disease where outcomes with DAGO2 are very encouraging with survival of over 50% at 3 years. In this cohort, compared with reported results in patients with high risk/secondary AML receiving CPX, overall response rates with CPX were improved.[5,20] Indeed, the survival of patients receiving CPX here was comparable to those seen with DA and a single dose of GO (DAGO1) in the first version of AML18.[2] The improved survival of patients receiving DAGO2 compared to that seen in AML18v1 may reflect both reduced early mortality and the increased number of patients transplanted in first remission over the course of the trial reaching 36% for DAGO2 compared to 25% in AML18v1. We note that FLT3 inhibitors were not available as

standard of care during the study and the combination with CPX is currently under investigation ([21], NCT04293562). Further possible limitations to this study include the possible impact that the COVID pandemic may have had on outcomes (although no early deaths were attributed to COVID) and that GO could not be included in the CPX arm precluding direct comparison of CPX GO with DAGO. Potentially the addition of GO to CPX might achieve higher early response rates to those reported here; this has been explored however[22,23] including a recent randomized paediatric AML study in which CPX-GO (in comparison to DA-GO) was associated with inferior 2-year EFS and relapse incidence along with greater associated risk of greater haematological toxicity.[23]

In summary in this study of older patients, primarily with clinical *de novo* AML and intermediate-risk cytogenetics, DAGO2 resulted in superior survival compared to CPX including in patients with MDS-related mutations. This was consistent with the observed difference in MRD measured early leukemia clearance, thus supporting MRD as an early clinical endpoint in AML.

## Author contributions

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## Disclosure of Conflicts of Interest

NHR, Honoraria: Jazz Pharmaceuticals, Pfizer, Astellas Pharma, Research Funding: Jazz Pharmaceuticals (Inst), Travel, Accommodations, Expenses: Jazz Pharmaceuticals
RD, research support from Abbvie, Amgen, Jazz and Pfizer, travel support from Servier and Jazz, consultancy with Abbvie, Astellas, Jazz, Pfizer and Servier and membership of a Data Safety and Monitoring Board with AvenCell.
CSH, research support from Illumina, consultancy with Astellas.
PM,Honoraria: Astellas Pharma, Pfizer, Jazz Pharmaceuticals, AbbVie, Servier Consulting or Advisory Role: Jazz Pharmaceuticals, AbbVie Speakers' Bureau: Jazz Pharmaceuticals, AbbVie, Astellas Pharma Travel, Accommodations, Expenses: Jazz Pharmaceuticals.
JB, Advisory Role, : Jazz Pharmaceuticals,
MD, Research Funding: Celgene (Inst), Daiichi Sankyo (Inst), Bio-Cancer Treatment International
SDF, Consulting or Advisory Role: MPAACT, Speakers' Bureau: Novartis, Jazz Pharmaceuticals Research Funding: Jazz Pharmaceuticals (Inst), Bristol Myers Squibb/Celgene (Inst), AstraZeneca (Inst), Cytek (Inst).
SK, research support from Novartis, travel support from Servier and consultancy with Abbvie, Astellas, Jazz, Novartis,Pfizer and Servier.
LWD, Honoraria: Bio-Rad Laboratories

## References


1. Burnett AK, Russell NH, Hills RK, et al: Addition of gemtuzumab ozogamicin to induction chemotherapy improves survival in older patients with acute myeloid leukemia. J Clin Oncol 30:3924-31, 2012
2. Freeman SD, Thomas A, Thomas I, et al: Fractionated vs single-dose gemtuzumab ozogamicin with determinants of benefit in older patients with AML: the UK NCRI AML18 trial. Blood 142:1697-1707, 2023
3. Burnett AK, Milligan D, Goldstone A, et al: The impact of dose escalation and resistance modulation in older patients with acute myeloid leukaemia and high risk myelodysplastic syndrome: the results of the LRF AML14 trial. Br J Haematol 145:318-32, 2009
4. Russell NH, Thomas A, Hills RK, et al: Treatment Intensification With Either Fludarabine, AraC, G-CSF and Idarubicin, or Cladribine Plus Daunorubicin and AraC on the Basis of Residual Disease Status in Older Patients With AML: Results From the NCRI AML18 Trial. Journal of Clinical Oncology:JCO. 24.00259, 2024
5. Lancet JE, Uy GL, Cortes JE, et al: CPX-351 (cytarabine and daunorubicin) Liposome for Injection Versus Conventional Cytarabine Plus Daunorubicin in Older Patients With Newly Diagnosed Secondary Acute Myeloid Leukemia. J Clin Oncol 36:2684-2692, 2018
6. Freeman SD, Virgo P, Couzens S, et al: Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia. J Clin Oncol 31:4123-31, 2013
7. Othman J, Wilhelm-Benartzi C, Dillon R, et al: A randomized comparison of CPX-351 and FLAG-Ida in adverse karyotype AML and high-risk MDS: the UK NCRI AML19 trial. Blood Adv 7:4539-4549, 2023
8. Knapper S, Thomas A, King S, et al: S131: A RANDOMISED ASSESSMENT OF THE SEQUENTIAL ADDITION OF THE KINASE INHIBITOR QUIZARTINIB TO INTENSIVE CHEMOTHERAPY IN OLDER ACUTE MYELOID LEUKAEMIA (AML) PATIENTS: RESULTS FROM THE NCRI AML18 TRIAL. HemaSphere 7:e0819670, 2023
9. Grimwade D, Hills RK, Moorman AV, et al: Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood 116:354-65, 2010
10. Schuurhuis GJ, Heuser M, Freeman S, et al: Minimal/measurable residual disease in AML: a consensus document from the European LeukemiaNet MRD Working Party. Blood 131:1275-1291, 2018
11. Heuser M, Freeman SD, Ossenkoppele GJ, et al: 2021 Update on Measurable Residual Disease (MRD) in Acute Myeloid Leukemia (AML): A Consensus Document from the European LeukemiaNet MRD Working Party. Blood, 2021
12. Norsworthy KJ, Gao X, Ko CW, et al: Response Rate, Event-Free Survival, and Overall Survival in Newly Diagnosed Acute Myeloid Leukemia: US Food and Drug Administration Trial-Level and Patient-Level Analyses. J Clin Oncol 40:847-854, 2022
13. Dohner H, Wei AH, Appelbaum FR, et al: Diagnosis and Management of AML in Adults: 2022 ELN Recommendations from an International Expert Panel. Blood, 2022
14. Cheson BD, Bennett JM, Kopecky KJ, et al: Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol 21:4642-9, 2003
15. Trotti A, Colevas AD, Setser A, et al: CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. Semin Radiat Oncol 13:176-81, 2003
16. Wright MF, Pozdnyakova O, Hasserjian RP, et al: Secondary-type mutations do not impact prognosis in acute myelogenous leukemia AML with mutated NPM1. Am J Hematol 97:E462-E465, 2022
17. Cocciardi S, Saadati M, Weiss N, et al: Impact of myelodysplasia-related and additional gene mutations in intensively treated patients with NPM1-mutated AML. Hemasphere 9:e70060, 2025

18. Othman J, Potter N, Ivey A, et al: Molecular, clinical, and therapeutic determinants of outcome in NPM1-mutated AML. Blood 144:714-728, 2024
19. Falini B, Dillon R: Criteria for Diagnosis and Molecular Monitoring of NPM1-Mutated AML. Blood Cancer Discov 5:8-20, 2024
20. Mehta P, Campbell V, Maddox J, et al: CREST-UK: Real-world effectiveness, safety and outpatient delivery of CPX-351 for first-line treatment of newly diagnosed therapy-related AML and AML with myelodysplasia-related changes in the UK. Br J Haematol 205:1326-1336, 2024
21. Pullarkat VA, Levis M, McCloskey J, et al: V-FAST: A phase 1b master trial to investigate CPX-351 combined with targeted agents in adults with newly diagnosed AML. Blood Neoplasia:100123, 2025
22. Oliai C, Park S, Damon LE, et al: CPX-351 plus gemtuzumab ozogamicin for relapsed/refractory acute myelogenous leukemia: a University of California Hematologic Malignancies Consortium trial. Leuk Lymphoma:1-7, 2024
23. Pollard JA, Alonzo TA, Gerbing R, et al: A Phase 3 Randomized Trial for Patients with De Novo AML Comparing Standard Therapy Including Gemtuzumab Ozogamicin (GO) to CPX-351 with GO-a Report from the Children's Oncology Group. Blood 144:967, 2024

1 **Tables**

2 **Table 1** Patient Demographics and Clinical Characteristics

| | **Overall** | **CPX** | **DAGO2 or DA** |
|---|---|---|---|
| | **N=439** | **N=295** | **N=144** |
| **Age** median (range) | 68 (64 - 71) | 68 (64 - 70) | 67 (64 - 71) |
| Age ≥ 65yrs | 310 (70.6) | 209 (70.9) | 101 (70.1) |
| Age ≥ 70yrs | 147 (33.5) | 97 (32.9) | 50 (34.7) |
| **Male** | 262 (59.7) | 174 (59.0) | 88 (61.1) |
| **WBC** x $10^9$ / L median (range) | 3.5 (1.7 – 13.6) | 3.3 (1.7 – 13.2) | 4.4 (1.6 – 14.6) |
| <10 | 304 (69.3) | 205 (69.5) | 99 (68.8) |
| ≥ 50 | 31 (7.1) | 21 (7.1) | 10 (6.9) |
| **Diagnosis** | | | |
| Clinical De Novo AML | 357 (81.3) | 240 (81.4) | 117 (81.3) |
| Clinical Secondary AML | 30 (6.8) | 20 (6.8) | 10 (6.9) |
| High Risk MDS | 52 (11.9) | 35 (11.8) | 17 (11.8) |
| **Performance ID** (ECOG) | | | |
| 0 | 211 (48.1) | 143 (48.5) | 68 (47.2) |
| 1 | 198 (45.1) | 132 (44.7) | 66 (45.8) |
| 2 | 30 (6.8) | 20 (6.8) | 10 (6.9) |
| **Small Molecule from Course 2** | | | |
| Long quizartinib | 4 (2.4) | 0 (0.0) | 4 (6.9) |
| Short quizartinib | 3 (5.2) | 0 (0.0) | 3 (5.2) |
| No quizartinib | 158 (95.8) | 107 (100.0) | 51 (87.9) |
| **Genetic risk** | | | |
| **Cytogenetic** (Grimwade2010) | | | |
| Favourable | 4 (1.0) | 2 (0.7) | 2 (1.4) |
| Intermediate | 346 (81.8) | 242 (85.5) | 104 (74.3) |
| Adverse | 32 (7.6) | 20 (7.1) | 12 (8.6) |
| Failed | 20 (4.7) | 8 (2.8) | 12 (8.6) |
| Not reported | 21 (5.0) | 11 (3.9) | 10 (7.1) |
| **ELN 2022** | | | |
| Favourable | 97 (27.2) | 63 (25.8) | 34 (30.1) |
| Intermediate | 71 (19.9) | 48 (19.7) | 23 (20.4) |
| Adverse | 189 (52.9) | 133 (54.5) | 56 (49.6) |
| Unknown/Missing | 82 | 51 | 31 |
| **Mutations** | **N=404** | **N=273** | **N=131** |
| *FLT3*-ITD/TKD | 66 (16.3) | 45 (16.5) | 21 (16.0) |
| *NPM1* | 97 (24.0) | 66 (24.2) | 31 (23.7) |
| MDS-related | 243 (60.1) | 165 (60.4) | 78 (59.5) |
| MDS-related excluding *FLT3/NPM1* | 199 (49.3) | 136 (49.8) | 63 (48.1) |

| | | | |
|---|---|---|---|
| *TP53* | 17 (4.2) | 14 (5.1) | 3 (2.3) |
| Unknown | 35 | 22 | 13 |

1

**Table 2** Response, Early Deaths and Rates of Allogeneic Stem Cell Transplantation

| | CPX | DAGO2 or DA | P-value | OR (95% CI) |
|---|---|---|---|---|
| | N= 295 | N= 144 | | |
| **Response** | | | | |
| CR+CRi | 230 (78.0%) | 122 (84.7%) | 0.095 | 0.64 (0.37, 1.09) |
| CR | 201 (68.1%) | 113 (78.5%) | 0.024 | 0.59 (0.37, 0.94) |
| | | | | |
| CR+CRi after course 1 | 140 (47.5%) | 86 (59.7%) | 0.016 | 0.61 (0.41, 0.91) |
| CR after course 1 | 115 (39%) | 73 (50.7%) | 0.020 | 0.62 (0.42, 0.93) |
| | | | | |
| **Response including flow cytometric MRD status after course 1** | N=273 [a] | N=131 [a] | | |
| CR/CRi MRD <0.1% | 80 (29.3) | 62 (47.3) | 0.004 | 0.46 (0.29, 0.73) |
| CR MRD <0.1% | 78 (28.6) | 61 (46.6) | 0.004 | 0.46 (0.29, 0.72) |
| CR/CRi MRD negative | 68 (24.9) | 50 (38.2) | 0.006 | 0.54 (0.34, 0.86) |
| CR MRD negative | 66 (24.2) | 48 (36.6) | 0.009 | 0.66 (0.34, 0.89) |
| **Post course 1 CR/CRi with MRD result** | N=118 [b] | N=73 [b] | | |
| MRD <0.1% | 80 (67.8) | 62 (84.9) | 0.008 | 0.37 (0.16, 0.82) |
| CR MRD <0.1% | 78 (66.1) | 61 (83.6) | 0.008 | 0.38 (0.17, 0.83) |
| MRD negative | 68 (57.6) | 50 (68.5) | 0.134 | 0.63 (0.34, 1.16) |
| CR MRD negative | 66 (55.9) | 48 (65.8) | 0.180 | 0.66 (0.36, 1.21) |
| | | | | |
| **Early death** | | | | |
| Day 30 | 20 (6.8%) | 6 (4.2%) | 0.276 | 1.67 (0.66, 4.26) |
| Day 60 | 32 (10.9%) | 6 (4.2%) | 0.019 | 2.80 (1.14, 6.86) |
| | | | | |
| **Allogeneic Stem Cell Transplant** | 108 (36.6%) | 54 (37.5%) | 0.856 | 0.96 (0.64,1.45) |
| Allograft in CR1 | 93 (40.4%) | 44 (36.1%) | 0.424 | 1.20 (0.76, 1.89) |
| Time to allograft in CR1 median (range) days [c] | 127 (84- 190) | 137 (80 – 190) | 0.478 | - |

MRD measured by flow cytometry, MRD <0.1%, MRD negative or detectable but <0.1% .

[a] All evaluable patients = patients in CR or CRi with MRD data (+ all patients not attaining CR/CRi including day 30 deaths)

[b] evaluable patients in CR or CRi = patients in CR/CRi with MRD data,

[c] in patients receiving CR1 transplant

$\chi 2$ or exact test used to generate the P values. OR, odds ratio.

**Figure Legends**

*Figure 1*. Trial Schema.
CPX total doses (units/$m^2$) by course given in brackets (further information in Methods).
Post course 1 randomisation in DA GO arm for patients with residual or unassessable disease is reported by reference 4 (further information on FLAG Ida schedule in Methods).

*Figure 2*. CONSORT diagram.

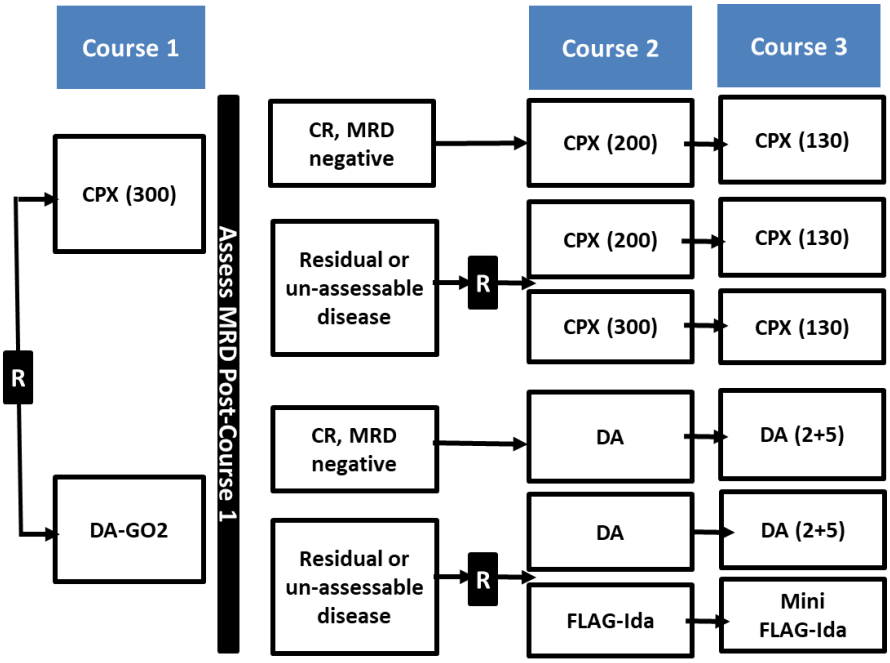
*Figure 3*
A. Event-free Survival B. Overall survival.

*Figure 4*. Subgroup Analysis of Overall Survival
A Patient characteristics. B. Baseline genetics
MDS-related mutation subgroup includes patients with *NPM1* or *FLT3* ITD/TKD mutations

*Figure 5*. Forest Plot of Overall Survival by randomisation of patient subgroup with MDS related mutations excluding *NPM1* or *FLT3* ITD/TKD mutations versus others

Figure 1

**Figure 1. Trial Schema**




Abbreviations: DA – Daunorubicin/AraC; GO2 – Gemtuzumab Ozogamicin, 2 doses; FLAG-Ida – Fludrabine/AraC/G-CSF/Idaribicin; MRD – measurable residual disease; CR – complete remission; R – randomisation

**Course 1: CPX (300)**

- CPX-351 (Vyxeos) 100 units/m² IV infusion, over 90 minutes, days 1, 3, 5 (3 doses)

**Course 1: DA-GO2**

- Daunorubicin 60 mg/m² daily by IV infusion, days 1, 3, 5 (3 doses)
- Cytosine Arabinoside 100 mg/m² 12-hourly IV, days 1–10 (20 doses)
- Mylotarg (GO) 3 mg/m² (maximum 5 mg per dose), days 1 and 4 of DA (2 doses)

**Course 2: CPX (200)**

- CPX-351 100 units/m² IV infusion, over 90 minutes, days 1 and 3 (2 doses)

**Course 2: CPX (300)**

- As course 1 above

**Course 3: CPX (130)**

- CPX-351 65 units/m² IV infusion, over 90 minutes, days 1 and 3 (2 doses)

Figure 2

**Figure 2. Consort**

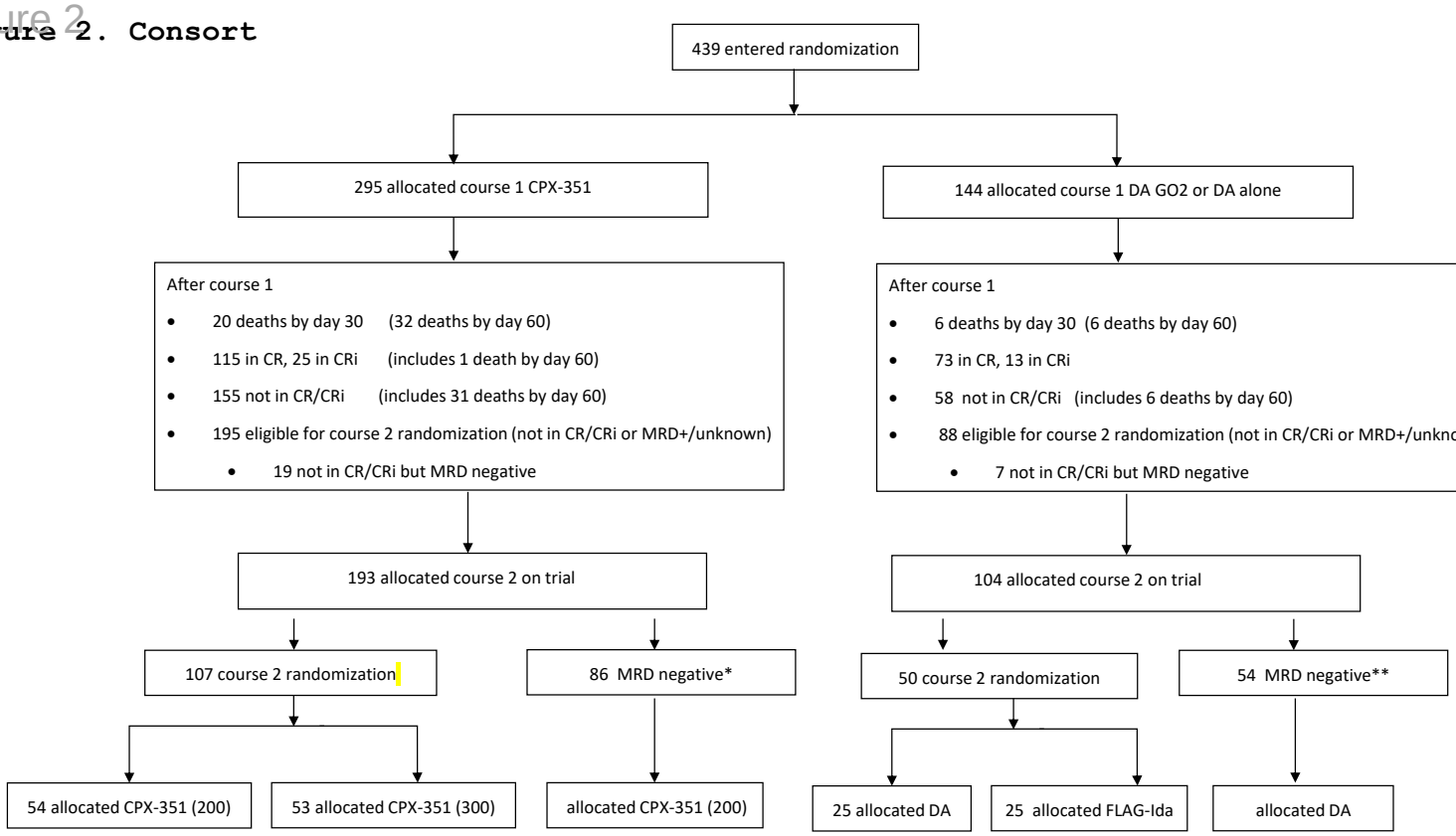
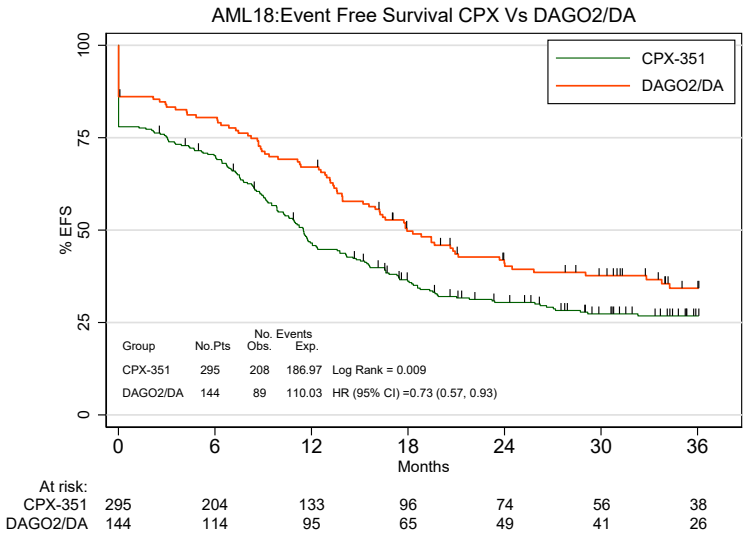


Figure 3

**A** Event Free Survival




**B** Overall Survival

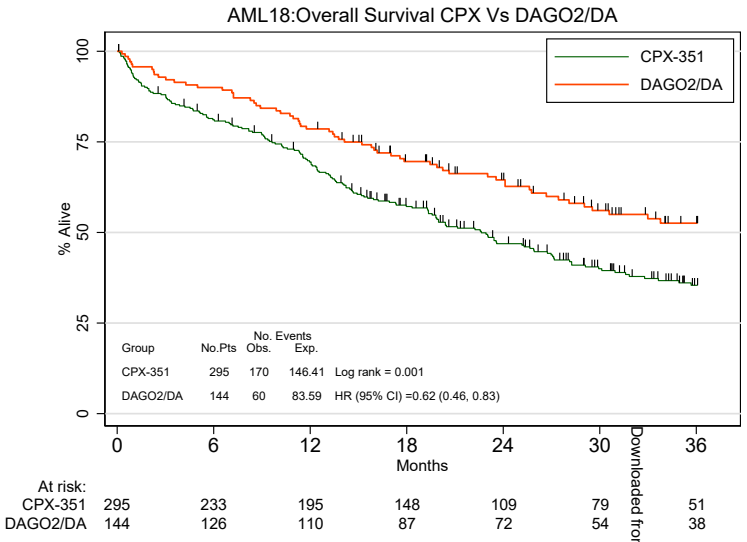


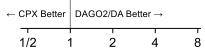
Figure 4

**Figure 4**

**A.**

AML18: Subgroup analysis OS

| Subgroup | DAGO2/DA | CPX-351 | (O-E) | Var. | HR (95% CI) (CPX:DAGO2/DA) |
|---|---|---|---|---|---|
| **Age Group** | | | | | |
| <65 Yrs | 14/43 | 44/86 | -6.96 | 13.31 | 1.78 ( 0.98, 3.26) |
| 65 - 69 Yrs | 19/51 | 65/112 | -13.15 | 19.68 | 2.13 ( 1.28, 3.56) |
| ≥ 70 Yrs | 27/50 | 61/97 | -2.43 | 19.54 | 1.14 ( 0.72, 1.79) |
| Test for Trend: Chi Sq=1.95 P value=0.162 | | | | | |
| **Disease Type** | | | | | |
| De novo AML | 48/117 | 138/240 | -19.32 | 42.82 | 1.63 ( 1.18, 2.27) |
| Secondary AML | 4/10 | 16/20 | -4.09 | 4.68 | 2.79 ( 0.92, 8.43) |
| High Risk MDS | 8/17 | 16/35 | -0.19 | 5.39 | 1.04 ( 0.44, 2.42) |
| Test for Heterogeneity: Chi Sq=0.43 P value=0.513 | | | | | |
| **ECOG ID** | | | | | |
| 0 | 28/68 | 75/143 | -6.44 | 22.86 | 1.35 ( 0.87, 2.08) |
| 1 | 28/66 | 81/132 | -14.66 | 25.78 | 1.87 ( 1.21, 2.87) |
| 2 | 4/10 | 14/20 | -2.93 | 4.24 | 2.20 ( 0.72, 6.70) |
| Test for Heterogeneity: Chi Sq=1.36 P value=0.244 | | | | | |
| **SEX** | | | | | |
| Male | 36/88 | 97/174 | -12.00 | 30.60 | 1.52 ( 1.04, 2.23) |
| Female | 24/56 | 73/121 | -11.68 | 22.40 | 1.78 ( 1.12, 2.82) |
| Test for Heterogeneity: Chi Sq=0.25 P value=0.615 | | | | | |
| **WBC Group** | | | | | |
| <10 | 43/99 | 113/205 | -10.78 | 35.17 | 1.38 ( 0.97, 1.97) |
| 10 - 49.9 | 13/35 | 43/69 | -10.51 | 13.36 | 2.43 ( 1.30, 4.54) |
| 50+ | 4/10 | 14/21 | -1.94 | 3.96 | 1.73 ( 0.57, 5.27) |
| Test for Trend: Chi Sq=1.33 P value=0.249 | | | | | |




**B.**

AML18: Subgroup analysis OS

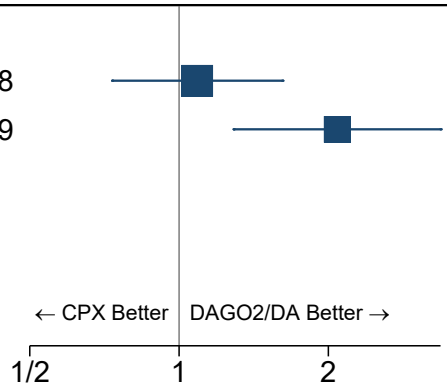
| Subgroup | DAGO2/DA | CPX-351 | (O-E) | Var. | HR (95% CI) (CPX:DAGO2/DA) |
|---|---|---|---|---|---|
| **Cytogenetics** | | | | | |
| Favorable | 2/2 | 1/2 | 0.33 | 0.72 | 0.62 ( 0.05, 7.00) |
| Intermediate | 42/104 | 138/242 | -20.07 | 40.47 | 1.73 ( 1.23, 2.45) |
| Adverse | 7/12 | 17/20 | -2.63 | 5.68 | 1.64 ( 0.68, 3.98) |
| **ELN 2022** | | | | | |
| Favorable | 14/34 | 34/63 | -5.34 | 11.49 | 1.64 ( 0.88, 3.06) |
| Intermediate | 7/23 | 30/48 | -6.79 | 8.54 | 2.57 ( 1.13, 5.88) |
| Adverse | 30/56 | 83/133 | -4.92 | 24.09 | 1.24 ( 0.82, 1.88) |
| **Molecular Marker** | | | | | |
| FLT3 all | 8/21 | 30/45 | -5.73 | 8.67 | 2.14 ( 0.98, 4.68) |
| NPM1 all | 6/31 | 29/66 | -6.88 | 8.09 | 2.83 ( 1.17, 6.82) |
| MDS Related Mutations | 39/78 | 103/165 | -9.52 | 31.87 | 1.40 ( 0.97, 2.03) |

Figure 5

## AML18: Subgroup analysis MDS-OS

| Subgroup | DAGO2/DA | CPX-351 | (O-E) | Var. | HR (95% CI) (CPX:DAGO2/DA) |
|---|---|---|---|---|---|
| **MDS Related Mutation** | | | | | |
| Yes w/o NPM1 or FLT3 | 35/63 | 83/136 | -2.11 | 25.38 | 1.09 ( 0.73, 1.62) |
| No or NPM1 or FLT3 | 22/68 | 78/137 | -16.19 | 23.39 | 2.08 ( 1.29, 3.37) |

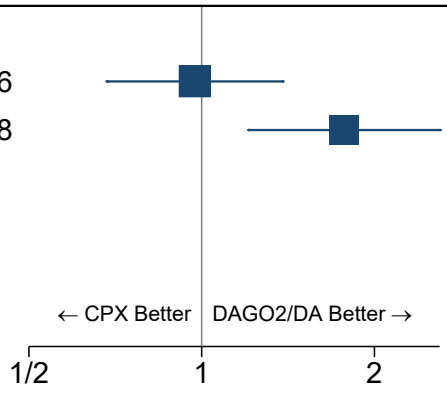
Test for Heterogeneity: Chi Sq=4.18 P value=0.041




## AML18: Subgroup analysis MDS-EFS

| Subgroup | DAGO2/DA | CPX-351 | (O-E) | Var. | HR (95% CI) (CPX:DAGO2/DA) |
|---|---|---|---|---|---|
| **MDS Related Mutation** | | | | | |
| Yes w/o NPM1 or FLT3 | 46/63 | 95/136 | 0.88 | 28.86 | 0.97 ( 0.68, 1.38) |
| No or NPM1 or FLT3 | 36/68 | 98/137 | -16.59 | 29.48 | 1.77 ( 1.21, 2.60) |

Test for Heterogeneity: Chi Sq=5.08 P value=0.024



# Comparing CPX-351 With DA-GO2 (Daunorubicin, Cytarabine and Fractionated Gemtuzumab Ozogamicin) in Older Adults With Non-Adverse Risk AML

## ClinicalTrials.gov NCT02272478

**UK NCRI AML18**
June 2019 – Dec 2022
AML and high-risk MDS
Aged ≥60 years
Fit for intensive chemotherapy

↓

Not known to have adverse risk cytogenetics at trial entry
(Patients with secondary AML excluded after EMA approval of CPX-351)

**n=439**

↓

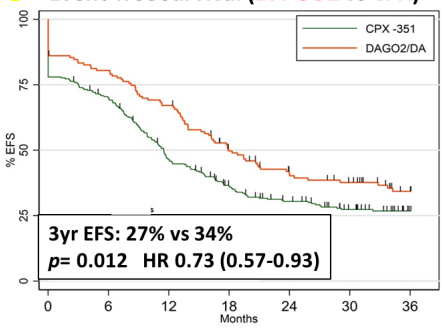
**R**

**295** → **CPX-351**

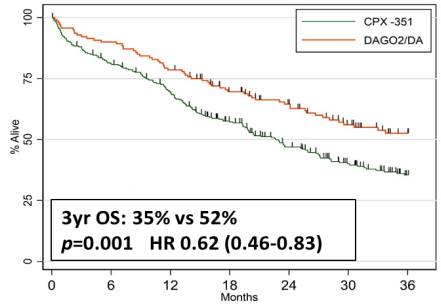
**144** → **DA-GO2**

## Main Findings

**Event-free survival (DA-GO2 vs CPX)**



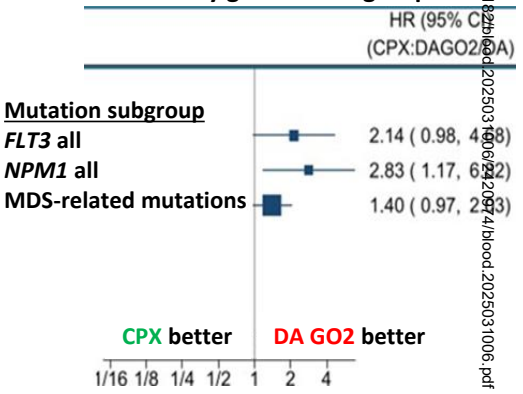

**Overall survival (DA-GO2 vs CPX)**




**Early Responses and Deaths**

| | CPX-351 | DA-GO2 | |
|---|---|---|---|
| CR post course 1 | 44% | 57% | p=0.020 |
| CR/CRi post course 1 | 48% | 60% | p=0.015 |
| Day 60 mortality | 11% | 4% | p=0.049 |

**Overall survival by genetic sub-group**




**Conclusions:** **In fit, older AML patients without adverse-risk cytogenetics, DA-GO2 was associated with deeper post-course 1 responses and superior event-free and overall survival compared with CPX-351. CPX-351 was associated with poorer survival in patients with *NPM1* and *FLT3* mutations and did not benefit those with MDS-related mutations.**

*Knapper et al. DOI: 10.xxxx/**blood**.2025xxxxxx*

blood Visual Abstract