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# Safety and Efficacy of Combining Midostaurin and Gemtuzumab Ozogamicin with Induction Chemotherapy in FLT3 mutated AML

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**Abstract:**
Despite the use of FLT3 inhibitors, outcomes for patients with FLT3 mutated (FLT3mut) AML remain suboptimal because of high rates of relapse. We evaluated the safety and efficacy of the combination of daunorubicin, cytarabine (DA), gemtuzumab ozogamicin (GO) and midostaurin (DAGO+m) for younger patients with newly diagnosed FLT3mut AML in the UK NCRI AML19 trial. 195 patients were randomised to receive DA with either one or two doses of GO (DAGO1 and DAGO2). 77 had a FLT3 mutation (60 had FLT3-ITD) and received midostaurin for two weeks after each chemotherapy course and then as maintainance for one year unless transplanted. 39 patients received midostaurin with DAGO1 (DAGO1+m) and 38 with DAGO2 (DAGO2+m). Their median age was 51y (range 20-74) and 16 (20%) were aged >60y. The overall response rate (CR + CRi) was 91%. Day 30 and day 60 mortality was 0% with no increase in toxicity compared to patients treated contemporaneously with DAGO1 and DAGO2 without midostaurin. 2y overall survival was 77%. 2y event-free survival and cumulative incidence of relapse were 62% and 31% respectively. MRD clearance was enhanced compared to patients with FLT3-mutated AML treated with DAGO1 and DAGO2 without midostaurin. 81% of evaluable patients were NPM1 MRD negative by RT-qPCR in the peripheral blood after course 2 (76% with DAGO1+m and 86% with DAGO2+m), 79% were MRD negative in the bone marrow by FLT3-ITD NGS, and all patients had FLT3-MRD levels below 0.01%. DAGO+m appears safe and effective . DAGO2+m will now be evaluated in a randomised study (OPTIMISE-FLT3, ISRCTN 34016918). Trial: ISRCTN78449203

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

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**Clinical trial registration information (if any):** ISRCTN78449203

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**Running Title:**
Midostaurin, Gemtuzumab Ozogamicin and Induction Chemotherapy for *FLT3* mutated AML

**Key Points:**

1. Midostaurin can be safely combined with Gemtuzumab Ozogamicin (GO) and intensive induction chemotherapy in *FLT3*$^{mut}$ AML.
2. The addition of midostaurin to DA+GO increased the clearance of molecular MRD assessed by RTqPCR (for NPM1 mutation) and NGS (for FLT3 ITD).

**Data Sharing Statement:**

Access to de-identified data and supporting information can be requested by qualified researchers who engage in rigorous, scientific research. Data may be provided following review and approval of a research proposal, Statistical Analysis Plan, and execution of a Data Sharing Agreement. For requests, please contact the trial sponsor canhamj@cardiff.ac.uk.

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**Abstract**

Despite the use of FLT3 inhibitors, outcomes for patients with *FLT3* mutated (*FLT3*$^{mut}$) AML remain suboptimal because of high rates of relapse. We evaluated the safety and efficacy of the combination of daunorubicin, cytarabine (DA), gemtuzumab ozogamicin (GO) and midostaurin (DAGO+m) for younger patients with newly diagnosed *FLT3*$^{mut}$ AML in the UK NCRI AML19 trial. 195 patients were randomised to receive DA with either one or two doses of GO (DAGO1 and DAGO2). 77 had a *FLT3* mutation (60 had *FLT3*-ITD) and received midostaurin for two weeks after each chemotherapy course and then as maintainance for one year unless transplanted. 39 patients received midostaurin with DAGO1 (DAGO1+m) and 38 with DAGO2 (DAGO2+m). Their median age was 51y (range 20-74) and 16 (20%) were aged >60y. The overall response rate (CR + CRi) was 91%. Day 30 and day 60 mortality was 0% with no increase in toxicity compared to patients treated contemporaneously with DAGO1 and DAGO2 without midostaurin. 2y overall survival was 77%. 2y event-free survival and cumulative incidence of relapse were 62% and 31% respectively. MRD clearance was enhanced compared to patients with *FLT3*-mutated AML treated with DAGO1 and DAGO2 without midostaurin. 81% of evaluable patients were *NPM1* MRD negative by RT-qPCR in the peripheral blood after course 2 (76% with DAGO1+m and 86% with DAGO2+m), 79% were MRD negative in the bone marrow by *FLT3*-ITD NGS, and all patients had *FLT3*-MRD levels below 0.01%. DAGO+m appears safe and effective . DAGO2+m will now be evaluated in a randomised study (OPTIMISE-*FLT3*, ISRCTN 34016918). Trial: ISRCTN78449203

## Introduction

Activating somatic mutations in the gene encoding the FLT3 receptor tyrosine kinase (*FLT3*) are present in approximately one-third of patients with Acute Myeloid Leukaemia (AML). Despite the incorporation of FLT3 inhibitors alongside intensive chemotherapy, outcomes remain suboptimal: 4-year overall survival (OS) was 51% in patients treated with daunorubicin, cytarabine (DA) and midostaurin in the RATIFY study[1] and 48% in patients treated with DA + quizartinib in the QUANTUM-First study[2]. In both, relapse was the leading cause of treatment failure, occurring in 42% and 34% of patients respectively[2,3]. Therefore, improved treatment strategies are still clearly needed.

Gemtuzumab Ozogamicin (GO) has been shown to improve survival in patients with favourable and intermediate risk cytogenetics although the original studies did not evaluate *FLT3* mutation status[4]. Nevertheless, blasts from patients with *FLT3* mutated (*FLT3*$^{mut}$) AML express high levels of CD33[5], and sub-group analyses from randomised studies suggest that patients with *FLT3*$^{mut}$ AML benefitted from the addition of GO to induction chemotherapy[6,7].

Despite this, GO and FLT3 inhibitors are not routinely used together with intensive chemotherapy because of limited data regarding the safety and efficacy of these combinations.

To address this issue, we evaluated the combination of DA + midostaurin with either one or two doses of GO within the UK NCRI AML19 trial. The aims were to evaluate safety and efficacy in terms of overall survival, event-free survival and clearance of measurable residual disease (MRD).

## Methods

*Trial Design and Treatments*

The NCRI AML19 trial (ISRCTN78449203) enrolled younger adults aged <60y with newly-diagnosed AML between November 2015 and November 2021. Older patients could enter if judged fit and after discussion with a trial co-ordinator. The results of the primary randomisations, in which no FLT3 inhibitors were used, have already been reported[8,9].
In November 2020 we amended the protocol to evaluate the combination of GO and midostaurin alongside DA chemotherapy (called AML19 version 2, AML19v2). From November 2020 to November 2021 we enrolled only patients without known adverse karyotype, and they were randomised 1:1 to receive induction chemotherapy with DA 3+10 (Daunorubicin 60mg/$m^2$ on days 1, 3 and 5 and cytarabine 100mg/$m^2$ twice a day on days 1-10) with either a single dose of GO (3mg/$m^2$ on day 1, DAGO1) or 2 doses (3mg/$m^2$ capped at 5mg on days 1 & 4, DAGO2). Patients underwent rapid centralised screening for *FLT3* mutations and if these were detected, patients were offered entry into a sub-study (called Midotarg); those who consented received midostaurin for 14 days from day 11. After blood count recovery the bone marrow was assessed, and if this showed complete or partial remission they then received a second induction (DA 3+8, daunorubicin 50mg/$m^2$ on days 1, 3 and 5 plus cytarabine 100mg/$m^2$ twice daily on days 1-8) without GO and with midostaurin given for 14 days from day 9. Consolidation therapy was 2 courses of high-dose cytarabine (HDAC, 3g/$m^2$ twice daily on days 1, 3 and 5, reduced to 1.5g/$m^2$ in patients aged >60y) with midostaurin for 14 days from day 6, followed by midostaurin maintenance for one year, except in patients proceeding to allogeneic stem cell transplant (alloSCT). Patients without a *FLT3* mutation or who did not consent to the Midotarg substudy received the same chemotherapy but without midostaurin. Patients with refractory disease after course 1 (>15% blasts and <50% reduction in blasts) were recommended for salvage therapy with FLAG-Ida (without midostaurin) as course 2. The trial schema is shown in figure S1.

Patients could receive alloSCT at any time at the discretion of the treating team, however alloSCT in 1$^{st}$ complete remission (CR1) was recommended for patients with a *FLT3*-ITD allelic ratio (AR) >0.05 without *NPM1* mutation or core binding factor (CBF) translocation,

for patients with *NPM1* mutation who were MRD positive by RT-qPCR in the peripheral blood (PB) following course 2 and for patients found to have adverse risk cytogenetics after trial entry or who failed to achieve a CR/CRi after two courses of induction[10]. There was no protocol-specified post-transplant maintenance therapy. The primary endpoint was overall survival. Secondary endpoints included response (CR and CRi), MRD response and toxicity (haematological and non-haematological).

Written informed consent was required for trial entry and for entry into the Midotarg substudy. The trial was approved by the Wales Multicentre Research Ethics Committee 3 (14/WA/1056) and conducted in accordance with the Declaration of Helsinki.

*Molecular and Cytogenetic Testing*

Patients underwent screening for *FLT3* and *NPM1* mutations in a central laboratory. Cytogenetic testing was performed in accredited regional laboratories and reviewed centrally according to the MRC cytogenetic classification[11]. RNA sequencing using a targeted panel (TruSight RNA Fusion, Illumina, Cambridge UK) was performed centrally for patients with *FLT3* ITD without an *NPM1* mutation or common fusion gene, and where fusion genes were identified, these were confirmed by PCR. Testing for *UBTF* tandem duplication (*UBTF*-TD) was performed retrospectively by PCR as previously described[12].

*Measurable residual disease*

Molecular MRD assessment was performed prospectively for patients with *NPM1* mutations or fusion genes using RT-qPCR at a central reference laboratory as previously described[13]. Assessments were performed after each course of therapy and then every 3 months for 2 years with investigators informed of the results. Additional treatment was recommended for patients with MRD relapse according to the European Leukaemia Network definitions[14], but was not protocol specified.

*FLT3* ITD MRD was assessed retrospectively by next generation sequencing (NGS) using a modified getITD assay as previously described[15,16]. Briefly, this assay uses 500ng genomic DNA and has a sensitivity of 0.001%.

To assess the effect of adding midostaurin, we compared MRD measurements against those from patients with the same genotype treated with DAGO without midostaurin in the first part of AML19 prior to the protocol amendment (AML19v1). Further details of the MRD testing and analysis methods are provided in the Supplementary Appendix.

*Enhanced Safety Monitoring*

In addition to data on serious adverse events (SAE), we collected toxicity data for liver, kidney and cardiac adverse events (at any grade), and bleeding events (at grade 3 or 4) on a weekly basis for four weeks after the first dose of midostaurin for patients joining the Midotarg substudy, and this was reviewed by the trial team and by the independent data monitoring committee after 25 and 50 patients had been treated. The rate of SAEs was compared to that seen in contemporaneous patients not entering the substudy, who received the same induction therapy without midostaurin.

*Statistical Analysis*

Response endpoints were defined according to the revised International Working Group criteria[17]. 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, morphological or MRD 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 (molecular or haematological) or death from any cause. Cumulative incidence of relapse (CIR), included molecular relapse and was calculated using cumulative incidence

functions with non-relapse mortality as a competing risk. Primary analyses were by intention to treat.and the final data cutoff was in January 2024. Survival outcomes were compared using Cox regression. Competing-risk analysis was performed for the cumulative incidence of relapse with nonrelapse mortality as the competing risk, using the Gray's test and Fine and Gray model. Median follow-up was determined by reversing the censor indicator of Kaplan-Meier analysis for overall survival.

## Results

*Patients*

From November 2020 to November 2021, 195 patients were randomised: 97 to DAGO1 and 98 to DAGO2. *FLT3* mutations were detected in 80 patients, of whom 77 consented to enter the Midotarg substudy and were allocated to receive midostaurin (39 DAGO1 + midostaurin, DAGO1+m, 38 DAGO2 + midostaurin, DAGO2+m). Of these, 55 had *FLT3*-ITD, 17 *FLT3*-TKD and 5 had both mutations. The baseline characteristics of those patients in the Midotarg substudy and those who received DAGO1 or DAGO2 without midostaurin are shown in Table 1.

Of the 77 patients who were allocated to receive midostaurin, the median age was 51y with 16 (21%) >60y. After the first course, 66/77 patients received DA as course 2 and 57/77 received at least one course of HDAC consolidation. After the second course 8/77 patients were designated high-risk on the basis of *NPM1* MRD positivity in the PB. Overall 18/77 patients (23%) received CR1 alloSCT of whom 11 had received DAGO1+m and 7 DAGO2+m. A CONSORT diagram is shown in Figure 1.

*Molecular and Cytogenetic Characteristics*

*NPM1* mutations were present in 49/77 patients (64%). MRC cytogenetic risk group was favorable in 6 patients, intermediate in 64 (including 1 *KMT2A*::*MLLT3* and 1 *DEK*::*NUP214*), unknown in 6 and adverse in 1. Of 20 patients without *NPM1* mutation or a common fusion gene, 16 patients underwent RNA sequencing, revealing *KMT2A* partial tandem duplication (PTD) in 5 patients and *ETV6::MECOM* fusion in 1. *UBTF*-TD was detected in 5 patients.

*Compliance and Toxicity*

Sixty-four of the 77 patients (83%) received all 28 doses of prescribed midostaurin in course 1 or missed no more than 1 dose, the remaining patients missed between 3 and 20 doses. Two patients randomised to DAGO1+m did not receive midostaurin in course 1 due to gastrointestinal intolerance and one patient randomised to DAGO2+m did not receive midostaurin due to a pre-existing QTc prolongation. Seventeen SAEs (CTC grade 3 or greater) were reported (DAGO1+m, n=11, DAGO2+m n= 6). No cases of VOD were reported.

We could not find evidence that blood count recovery was delayed in patients receiving midostaurin compared to those receiving DAGO alone. In course 1 time to neutrophil recovery to $\geq 1 \times 10^9$/L was 33 and 33 days with DAGO1+m and DAGO2+m compared to 32 and 32 days in patients receiving DAGO1 and DAGO2 alone without midostaurin in AML19v2 (table 2). Likewise, time to platelet recovery to $\geq 100 \times 10^9$/L was not delayed with DAGO+m compared to DAGO alone (table 2).

There was no significant difference in non-haematological toxicity between patients who did and did not receive midostaurin in AML19v2 (figure 2), nor was there a significant difference in non-haematological toxicity between DAGO1+m and DAGO2+m (Supplementary Figure 2). Day 30 and day 60 mortality for both DAGO1+m and DAGO2+m was 0%.

Midostaurin maintenance was administered in 18/39 (46%) patients randomised to DAGO1+m and 17/38 (45%) randomised to DAGO2+m, with a median of 12 cycles administered in both groups (Figure 1).

*Response*

The overall response rate (including CR and CRi) after the first course of induction was 87%: 85% for DAGO1+m and 89% for DAGO2+m. After 2 courses of induction CR/CRi was achieved in 91% and did not differ between DAGO1+m (90%) and DAGO2+m (92%, table 3). There were 10 patients not in remission after course 1: 6 in the DAGO1+m and 4 in the DAGO2+m arm. Of these, 4 had achieved PR (<15% blasts in BM) and received course 2 of

DA+m as per protocol. The 6 patients with refractory disease post course 1 were treated off protocol with FLAG-Ida (n=3), giliteritinib (n=2) or azacytidine (n=1).

*Measurable Residual Disease*

Bone marrow (BM) MRD levels after each course of chemotherapy for patients with *NPM1* mutation are shown in figure 3a. For comparison, we identified 55 patients with both *NPM1* and *FLT3* mutations treated with DAGO1 and DAGO2 without midostaurin in the preceeding AML19v1 protocol. The characteristics of the patients are shown in supplementary table 1. The two groups were generally comparable although there was a higher proportion of patients with *FLT3*-ITD and a higher allelic ration in the midostaurin treated group. Bone marrow *NPM1* MRD levels at the end of treatment (post course 4) were lower in patients receiving midostaurin: 72% became MRD negative with DAGO+m compared to 56% for DAGO without midostaurin in AML19v1.

We previously showed that PB *NPM1* MRD status after course 2 (PC2) provides more powerful prognostic information than BM[10], and PC2 PB *NPM1* MRD status was used to allocate CR1 alloSCT in this study. PC2 PB MRD negativity was 80% in patients receiving DAGO+m compared to 68% in those receiving DAGO without midostaurin in AML19v1.

We next analysed the effect of GO dose on MRD levels in patients receiving midostaurin. More patients receiving DAGO2+m were PB MRD negative after course 2 (75% and 86% for DAGO1+m and DAGO2+m respectively, figure 3b, table 3). For patients receiving DAGO1 and DAGO2 without midostaurin in AML19v1 these figures were 61% and 74% respectively.

Next-generation sequencing was used to detect *FLT3* ITD MRD (figure 3c-d, supplementary figure 3). For comparison we selected 32 patients with *FLT3* ITD treated with DAGO without midostaurin in AML19v1 who had available samples. The characteristics of all patients in the *FLT3* ITD MRD analysis are shown in supplementary table 2. *FLT3* ITD MRD negativity in BM after cycle 2 was attained in 30/38 (79%) patients treated with midostaurin, this was 68% and 89% in those patients treated with DAGO1+m and DAGO2+m repectively, and no patient had a *FLT3* ITD VAF ≥0.01%.

Although the rates of BM *FLT3* MRD negativity were similar in patients treated without midostaurin (73% and 75% for DAGO1 and DAGO2 respectively), more of these patients were MRD positive above a level of 0.01% (17% vs. 0% for DAGO vs DAGOm, p = 0.017).

*Survival Outcomes*

With a median follow-up of 28.9 months, 2 year event free survival (EFS) and overall survival (OS) among all patients in the Midotarg substudy was 62% and 78% respectively (figure 4a,b) and did not differ between DAGO1+m and DAGO2+m (figure 4c,d, table 3). EFS at 2 years was 59% and 66% for DAGO1+m and DAGO2+m respectively (HR 0.86, 95%CI 0.41-1.79, p=0.68). Likewise there was no difference in OS (HR 0.90, 95%CI 0.35-2.35, p=0.83) which at 2 years was 76% for DAGO1+m and 78% for DAGO2+m (figure 4). The cumulative incidence of relapse (CIR) was 31% at 2 years and did not vary by GO dose (HR 0.94 95%CI 0.40 – 2.20, p= 0.88, supplementary figure 4a,b). Likewise there was no difference in relapse free survival (RFS) by GO dose (HR 0.88, 95%CI 0.38 – 2.04; P = 0.77) which was 66% vs 71% for DAGO1+m and DAGO2+m respectively (supplementary figure 4c,d). For patients transplanted in first remission (n=18) OS at 2y was 73% and 100% for DAGO1+m and DAGO2+m respectively (supplementary figure 5).

*Exploratory Analyses of Clinical and Molecular Subgroups*

Age did not significantly affect survival: in the 16 patients aged >60y 2y OS was 69% compared to 80% in younger patients (p=0.6, figure 5a). There was no difference in EFS or OS between patients with *FLT3*-ITD and those with *FLT3*-TKD or by *FLT3* allelic ratio (figure 5b,c). In contrast there were major differences in survival amongst different genomic groups. Patients with *NPM1* mutation and CBF AML had excellent outcomes with 2y OS of 88% and 100% respectively; patients without either of these lesions (n=22) had poorer survival (p<0.001, figure 5d). This group included patients with *UBTF* tandem duplication (n=5), *KMT2A::MLLT3* (n=1), *DEK::NUP214* (n=1), *ETV6::MECOM* (n=1), monosomy 7 (n=1) and *KMT2A* partial tandem duplication (n=3), as well as 6 patients with normal and 4 patients with other intermediate karyotypes who could not be further subclassified.

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## Discussion

Previous studies have shown that both a single dose and fractionated dosing schedule of GO can safely be combined with DA chemotherapy in adult patients fit for intensive therapy including older patients[8,18-20]. We now show that midostaurin can safely be added to that combination. The triplet was generally well tolerated in both younger and older patients with no day 60 mortality and no increase in haematological or non-haematological toxicity compared to patients receiving the same chemotherapy without midostaurin. Compliance was good and over 80% of patients were able to complete Midostaurin in course 1. Furthermore, there was no additional toxicity with DAGO2+m compared to DAGO1+m. The safety of the DAGO + midostaurin combination is supported by a number other smaller studies. The Study Alliance Leukaemia (SAL) co-operative group recently reported a phase 1 study[21] (MOSAIC) of 11 patients combining DAGO with midostaurin, confirming the safety and feasibility of delivering the triplet using a fractionated GO schedule (3mg/m$^2$ on days 1 and 4). A Czech AML group study also reported on 11 patients combing midostaurin with a fractionated 3 dose schedule of GO (3mg/m$^2$ on days 1, 4 and 7) and DA chemotherapy[22]. In this study GO was also given in consolidation, a high response rate over 90% was reported but some liver toxicity was reported including a case of VOD.

In *NPM1* mutated AML, addition of GO to intensive chemotherapy has been reported to increase *NPM1* MRD negativity[23]. Our results show that the addition of midostaurin further increases this effect. We found enhanced clearance of *NPM1*$^{mut}$ transcripts compared to the cohort of *NPM1*$^{mut}$ patients receiving DAGO without midostaurin in AML19v1. For patients receiving DAGO and midostaurin in AML19v2, 81% were MRD negative in the peripheral blood after cycle 2 compared with 68% for DAGO alone in AMLv1. Similarly, the proportion of patients achieving *FLT3* MRD clearance below 0.01% was significantly greater in patients receiving DAGO + midostaurin compared to DAGO alone.

Regarding the question of the optimal dose of GO, there was no difference in toxicity and fewer patients receiving the fractionated schedule (DAGO2) tested PB *NPM1* MRD positive following course 2 compared to those receiving DAGO1. This is in keeping with previous observations of a benefit of fractionated GO in reducing MRD in *NPM1*$^{mut}$ AML compared to

a single dose[20,24]. In AML19v1, the proportion of patients testing PB PC2 MRD negative increased from 69% with DAGO1 to 84% with DAGO2. As we have recently reported that the benefit of transplant in *NPM1*$^{mut}$ AML is confined to those testing MRD positive at this time point, this represents a substantial diminution in the proportion of patients recommended for allogeneic SCT in first remission[10]: indeed fewer patients receiving DAGO2 in this study were transplanted in CR1. Of note, we recently reported that patients treated with DAGO2 in the AML18 trial had improved post-transplant survival compared to those treated with DAGO1[20].

It is difficult to compare our results with those reported with for induction chemotherapy with a FLT3 inhibitor but without GO due to differences in the patient populations enrolled. Crudely, the 2y OS of 78% reported here compares favourably with the reported 2y OS of 62% for DA + midostaurin in the RATIFY study[1] (which included more adverse risk patients but was limited to those aged <60y) and 55% for DA + quizartinib in the QUANTUM-first study[2] (which was limited to patients with *FLT3* ITD but included older patients). We observed particularly encouraging results in subgroups including in patients with CBF translocations or *NPM1* mutation (with 2y OS of 100% and 88% resepectively) and in those aged >60y where there was no evidence of increased toxicity. Of note maintenance was generally well tolerated and of the 20 patients with molecular MRD markers (17 with *NPM1*$^{mut}$ and 3 with CBF) who completed 12 cycles all were persistently MRD negative and only 1 patient has relapsed after stopping midostaurin.

A series of trials have suggested that intensification of induction chemotherapy can improve outcomes in *FLT3*$^{mut}$ AML. Both escalated daunorubicin dose of 90mg/m$^2$ in the NCRI AML17 trial[25] and FLAG-Ida-GO in the NCRI AML19v1 trial[8] improved overall survival compared to DA-GO. Excellent results have also been reported in *FLT3*mut AML with the combination of Cladribine, Idarubicin and Cytarabine (CLIA) combined with Sorafenib[26]. Combining venetoclax with chemotherapy and a *FLT3* inhibitor is also being explored (NCT03661307).

These observations clearly warrant the development of randomised studies to definitively assess the effect of GO and intensified chemotherapy regimens when combined with a FLT3 inhibitor. Given our current results, we therefore plan to perform a randomised comparison of DA+midostaurin, DAGO2+midostaurin and FLAG-Ida-GO+midostaurin in the recently opened OPTIMISE-*FLT3* trial (ISRCTN 34016918).

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**Conflicts of Interest:**

RD declares 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. SK declares research support from Novartis, travel support from Servier and consultancy with Abbvie, Astellas, Jazz, Novartis, Pfizer and Servier.

**Authorship Contributions**

J.O., A.T., and O.C., curated data and performed statistical analysis. N.P., S.D.F., N.M., J.J., PA and M.R. coordinated and performed *NPM1* MRD analyses. A.T., optimised, performed, reported and analysed the *FLT3*-ITD NGS MRD analysis. MRD analyses were supervised by RD and SF. J.Canham., L.B., E.H., provided trial coordination. A.G. undertook molecular analyses and coordinated patient samples. J. Cavenagh, P.K., C.A., C.H., D.C., M.D., and S.K., enrolled patients onto the studies. N.H.R. conceived and designed the trial and was chief investigator. S.K., M.D. were clinical coordinators of the trial. NHR., J.O. and R.D., drafted the manuscript, which was revised and approved by all authors.

## Tables

**Table 1.** Demographics of patients enrolled in AML19v2 trial. Including those in the Midotarg substudy receiving DAGO + midostaurin and those receiving DAGO alone.

| | Midotarg substudy | | | Other patients enrolled in AML19v2 | | |
|---|---|---|---|---|---|---|
| | **Total** | **DAGO1 + Mido** | **DAGO2 + Mido** | **Total** | **DAGO1 only** | **DAGO2 only** |
| N | 77 | 39 | 38 | 118 | 58 | 60 |
| Median age (range) | 51 (20 – 74) | 52 (21 - 74) | 50 (20 - 72) | 50 (17 - 65) | 48 (18 - 64) | 50 (17 - 65) |
| Age group (years) | | | | | | |
| <30 | 5 (6.5%) | 2 (5.1%) | 3 (7.9%) | 14 (11.9%) | 8 (13.8%) | 6 (10.0%) |
| 30-39 | 13 (16.9%) | 6 (15.4%) | 7 (18.4%) | 17 (14.4%) | 9 (15.5%) | 8 (13.3%) |
| 40-49 | 19 (24.7%) | 10 (25.6%) | 9 (23.7%) | 25 (21.2%) | 11 (19.0%) | 14 (23.3%) |
| 50-59 | 24 (31.2%) | 13 (33.3%) | 11 (28.9%) | 49 (41.5%) | 23 (39.7%) | 26 (43.3%) |
| 60+ | 16 (20.8%) | 8 (20.5%) | 8 (21.1%) | 13 (11%) | 7 (12.1%) | 6 (10.0%) |
| Gender | | | | | | |
| Male | 37 (48.1%) | 19 (48.7%) | 18 (47.4%) | 58 (49.2%) | 29 (50.0%) | 29 (48.3%) |
| Female | 40 (51.9%) | 20 (51.3%) | 20 (52.6%) | 60 (50.8%) | 29 (50.0%) | 31 (51.7%) |
| Prior haematological disorder | 1 (1.3%) | 1 (2.6%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| Prior chemotherapy or radiotherapy | 1 (1.3%) | 1 (2.6%) | 0 (0.0%) | 3 (2.6%) | 2 (3.6%) | 1 (1.7%) |
| WBC ($x10^9$/l) | | | | | | |
| <10 | 27 (35.1%) | 14 (35.9%) | 13 (34.2%) | 60 (50.8%) | 28 (48.3%) | 32 (53.3%) |
| 10 to <50 | 36 (46.8%) | 18 (46.2%) | 18 (47.4%) | 47 (39.8%) | 24 (41.4%) | 23 (38.3%) |
| 50 to <100 | 7 (9.1%) | 4 (10.3%) | 3 (7.9%) | 9 (7.6%) | 5 (8.6%) | 4 (6.7%) |
| 100+ | 7 (9.1%) | 3 (7.7%) | 4 (10.5%) | 2 (1.7%) | 1 (1.7%) | 1 (1.7%) |
| WHO Performance status | | | | | | |
| Normal activity | 36 (46.8%) | 18 (46.2%) | 18 (47.4%) | 68 (57.6%) | 34 (58.6%) | 34 (56.7%) |
| Restricted activity | 37 (48.1%) | 19 (48.7%) | 18 (47.4%) | 43 (36.4%) | 21 (36.2%) | 22 (36.7%) |
| In bed <50% waking hours | 4 (5.2%) | 2 (5.1%) | 2 (5.3%) | 7 (5.9%) | 3 (5.2%) | 4 (6.7%) |
| *FLT3*-ITD mutation | 60 (77.9%) | 29 (74.4%) | 31 (81.6%) | 3 (2.6%) | 2 (3.5%) | 1 (1.7%) |
| *FLT3*-ITD allelic ratio, median (IQR) | 0.46 (0.16, 0.74) | 0.45 (0.13, 0.71) | 0.48 (0.23, 0.78) | 0.70 (0.36, 0.735) | 0.74 (0.70, 0.77) | 0.02 (0.02, 0.02) |
| *FLT3* TKD mutation | 22 (28.6%) | 12 (30.8%) | 10 (26.3%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| *NPM1* mutation | 49 (63.6%) | 25 (64.1%) | 24 (63.2%) | 32 (27.4%) | 16 (28.1%) | 16 (26.7%) |
| Cytogenetics (Grimwade 2010) | | | | | | |
| Core binding factor | 6 (7.8%) | 2 (5.1%) | 4 (10.5%) | 21 (17.8%) | 11 (19.0%) | 10 (16.7%) |
| Normal | 41 (53.2%) | 18 (46.2%) | 23 (60.5%) | 53 (44.9%) | 30 (51.7%) | 23 (38.3%) |
| Other intermediate | 23 (29.9%) | 15 (38.5%) | 8 (21.1%) | 23 (19.5%) | 10 (17.2%) | 13 (21.7%) |
| Adverse | 1 (1.3%) | 1 (2.6%) | 0 (0.0%) | 17 (14.4%) | 7 (12.1%) | 10 (16.7%) |
| Failed | 6 (7.8%) | 3 (7.7%) | 3 (7.9%) | 4 (3.3%) | 0 (0.0%) | 4 (6.7%) |

**Table 2**. Count recovery times and resource usage.

| | DAGO1 | DAGO1+m | P value | DAGO2 | DAGO2+m | P value |
|---|---|---|---|---|---|---|
| **Recovery Times** | | | | | | |
| Neutrophil recovery to $1x10^9$/L | | | | | | |
| Days, median (95% CI) | 32 (28,34) | 33 (28, 36) | 0.37 | 32 (30, 35) | 33 (30, 38) | 0.40 |
| Platelet recovery to $100x10^9$/L | | | | | | |
| Days, median (95% CI) | 28 (26, 30) | 27 (26, 30) | 0.814 | 29 (27, 30) | 28 (27, 32) | 0.580 |
| | | | | | | |
| **Resource Use** Median (IQR) | | | | | | |
| Units of Blood | 8 (6,13) | 10 (7,11) | 0.445 | 9 (7, 12) | 7 (6, 9) | 0.029 |
| Units of Platelets | 10 (6.5,14) | 11.5 (8,15) | 0.406 | 13 (10, 17) | 9 (6.5,12) | 0.001 |
| Days of IV antibiotics | 17 (13, 27.5) | 17 (12,26) | 0.804 | 21 (15, 28) | 20 (12, 25) | 0.147 |
| Nights in hospital | 36 (30, 40) | 39 (31,50) | 0.103 | 36 (31, 40) | 36 (28,43) | 0.884 |

**Table 3.** Outcomes for patients enrolled in the Midotarg substudy receiving DAGO + midostaurin.

| | DAGO1 + Mido N=39 | DAGO2 + Mido N=38 |
|---|---|---|
| **Response** | | |
| ORR(CR+CRi) | 90% | 92% |
| CR | 77% | 81.5% |
| CRi | 13% | 10.5% |
| $CR/CR_i$ after course 1 | 85% | 89% |
| Post course 2 PB MRD-ve for *NPM1* | 75% (18 of 24) | 86% (19 of 22) |
| **Allogeneic transplant in CR1** | 11 | 7 |
| **Survival** | | |
| 2yr Overall Survival | 76% | 78% |
| 2yr Event Free Survival | 59% | 66% |
| 2yr Cumulative Incidence of Relapse | 33% | 29% |
| 2yr Relapse Free Survival | 66% | 71% |

**Figure Legends**

1. CONSORT diagram showing the number of patients randomised, screened for *FLT3* mutations and treated with and without midostaurin in each arm.

2. Non haematological toxicity of patients entering the Midotarg substudy, compared to patients in AML19v2 who did not enter and were treated with DAGO without midostaurin.

3. Measurable Residual Disease as assessed in the bone marrow. a) *NPM1* MRD measured by RT-qPCR after each treatment cycle. Patients with *NPM1* and *FLT3* mutations treated with DAGO (n=55) without midostaurin in AML19v1 are shown for comparison. b) *NPM1* MRD by GO dose for patients in the Midotarg sub-study.
c) *FLT3* ITD NGS MRD after treatment cycles 1 and 2. Patients with *FLT3* ITD mutations treated with DAGO without midostaurin in AML19v1 are shown for comparison. d) *FLT3* ITD NGS MRD by GO dose for patients in Midotarg substudy.

4. Survival outcomes for patients in AML19v2. a) EFS for all patients in the Midotarg substudy. b) EFS by randomisation (DAGO1+m vs DAGO2+m).
c) OS for all patients in the Midotarg substudy. d) Overall survival by GO randomisation (DAGO1+m vs DAGO2+m).

5. Exploratory analyses of overall survival in clinical and molecular subgroups. a) According to age (above or below 60y). b) According to *FLT3* mutation type. c) According to allelic ratio for patients with *FLT3* ITD mutation. d) According to genomic subgroup (core binding factor AML, *NPM1* mutated AML and others).

## References


1. Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. N Engl J Med 2017;377(5):454-464. DOI: 10.1056/NEJMoa1614359.
2. Erba HP, Montesinos P, Kim HJ, et al. Quizartinib plus chemotherapy in newly diagnosed patients with FLT3-internal-tandem-duplication-positive acute myeloid leukaemia (QuANTUM-First): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet 2023;401(10388):1571-1583. DOI: 10.1016/S0140-6736(23)00464-6.
3. Larson RA, Mandrekar SJ, Huebner LJ, et al. Midostaurin reduces relapse in FLT3-mutant acute myeloid leukemia: the Alliance CALGB 10603/RATIFY trial. Leukemia 2021;35(9):2539-2551. DOI: 10.1038/s41375-021-01179-4.
4. Hills RK, Castaigne S, Appelbaum FR, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: a meta-analysis of individual patient data from randomised controlled trials. Lancet Oncol 2014;15(9):986-96. DOI: 10.1016/S1470-2045(14)70281-5.
5. Khan N, Hills RK, Virgo P, et al. Expression of CD33 is a predictive factor for effect of gemtuzumab ozogamicin at different doses in adult acute myeloid leukaemia. Leukemia 2017;31(5):1059-1068. DOI: 10.1038/leu.2016.309.
6. Fournier E, Duployez N, Ducourneau B, et al. Mutational profile and benefit of gemtuzumab ozogamicin in acute myeloid leukemia. Blood 2020;135(8):542-546. DOI: 10.1182/blood.2019003471.
7. Tarlock K, Alonzo TA, Gerbing RB, et al. Gemtuzumab Ozogamicin Reduces Relapse Risk in FLT3/ITD Acute Myeloid Leukemia: A Report from the Children's Oncology Group. Clin Cancer Res 2016;22(8):1951-7. DOI: 10.1158/1078-0432.CCR-15-1349.
8. Russell NH, Wilhelm-Benartzi C, Othman J, et al. Fludarabine, Cytarabine, Granulocyte Colony-Stimulating Factor, and Idarubicin With Gemtuzumab Ozogamicin Improves Event-Free Survival in Younger Patients With Newly Diagnosed AML and Overall Survival in Patients With NPM1 and FLT3 Mutations. J Clin Oncol 2024;42(10):1158-1168. DOI: 10.1200/JCO.23.00943.
9. 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 2023;7(16):4539-4549. DOI: 10.1182/bloodadvances.2023010276.
10. Othman J, Potter N, Ivey A, et al. Post induction molecular MRD identifies patients with NPM1 AML who benefit from allogeneic transplant in first remission. Blood 2024. DOI: 10.1182/blood.2023023096.
11. 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 2010;116(3):354-65. DOI: 10.1182/blood-2009-11-254441.
12. Harrop S, Nguyen PC, Byrne D, et al. Persistence of UBTF tandem duplications in remission in acute myeloid leukaemia. EJHaem 2023;4(4):1105-1109. DOI: 10.1002/jha2.808.
13. Potter N, Jovanovic J, Ivey A, et al. A Randomised Trial of Molecular Monitoring versus Standard Clinical Care in Younger Adults with Acute Myeloid Leukaemia: Results from the UK NCRI AML17 and AML19 studies. Lancet Haematol 2025;In Press.
14. Heuser M, Freeman SD, Ossenkoppele GJ, et al. 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. Blood 2021;138(26):2753-2767. DOI: 10.1182/blood.2021013626.
15. Othman J, Potter N, Mokretar K, et al. FLT3 inhibitors as MRD-guided salvage treatment for molecular failure in FLT3 mutated AML. Leukemia 2023. DOI: 10.1038/s41375-023-01994-x.
16. Blatte TJ, Schmalbrock LK, Skambraks S, et al. getITD for FLT3-ITD-based MRD monitoring in AML. Leukemia 2019;33(10):2535-2539. DOI: 10.1038/s41375-019-0483-z.

17. 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 2003;21(24):4642-9. DOI: 10.1200/JCO.2003.04.036.
18. Castaigne S, Pautas C, Terre C, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. Lancet 2012;379(9825):1508-16. DOI: 10.1016/S0140-6736(12)60485-1.
19. Burnett AK, Hills RK, Milligan D, et al. Identification of patients with acute myeloblastic leukemia who benefit from the addition of gemtuzumab ozogamicin: results of the MRC AML15 trial. J Clin Oncol 2011;29(4):369-77. DOI: 10.1200/JCO.2010.31.4310.
20. 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 2023;142(20):1697-1707. DOI: 10.1182/blood.2023020630.
21. Rollig C, Schliemann C, Ruhnke L, et al. Gemtuzumab ozogamicin plus midostaurin in combination with standard '7 + 3' induction therapy in newly diagnosed AML: Results from the SAL-MODULE phase I study. Br J Haematol 2024;204(6):2254-2258. DOI: 10.1111/bjh.19436.
22. Weinbergerova B, Cernan M, Kabut T, et al. Gemtuzumab ozogamicin plus midostaurin in conjunction with standard intensive therapy for FLT3- mutated acute myeloid leukemia patients - Czech center experience. Haematologica 2023;108(10):2826-2829. DOI: 10.3324/haematol.2022.282263.
23. Dohner H, Weber D, Krzykalla J, et al. Intensive chemotherapy with or without gemtuzumab ozogamicin in patients with NPM1-mutated acute myeloid leukaemia (AMLSG 09-09): a randomised, open-label, multicentre, phase 3 trial. Lancet Haematol 2023. DOI: 10.1016/S2352-3026(23)00089-3.
24. Russell N, Wilhelm-Benartzi C, Knapper S, et al. FLAG-Ida Combined with Gemtuzumab Ozogamicin (GO) Improves Event Free Survival in Younger Patients with Newly Diagnosed Acute Myeloid Leukaemia (AML) and Shows an Overall Survival Benefit in NPM1 and FLT3 mutated Subgroups. Results from the UK NCRI AML19 Trial. Blood 2022;140:Supplement 1:526-528.
25. Burnett AK, Russell NH, Hills RK, United Kingdom National Cancer Research Institute Acute Myeloid Leukemia Study G. Higher daunorubicin exposure benefits FLT3 mutated acute myeloid leukemia. Blood 2016;128(3):449-52. DOI: 10.1182/blood-2016-04-712091.
26. Kadia TM, Ravandi F, Molica M, et al. Phase II study of cladribine, idarubicin, and ara-C (CLIA) with or without sorafenib as initial therapy for patients with acute myeloid leukemia. Am J Hematol 2023;98(11):1711-1720. DOI: 10.1002/ajh.27054.

**Figure 1**

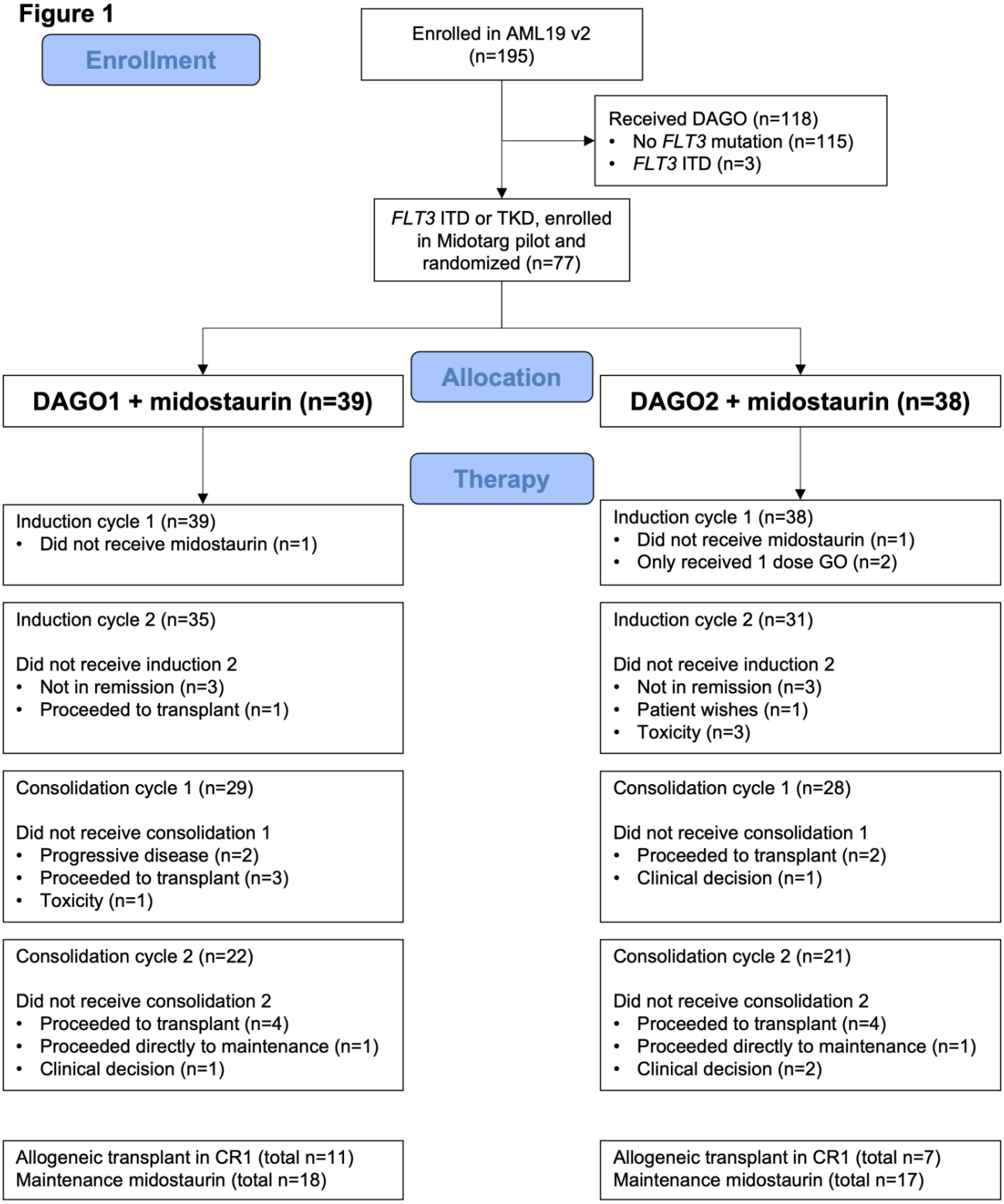
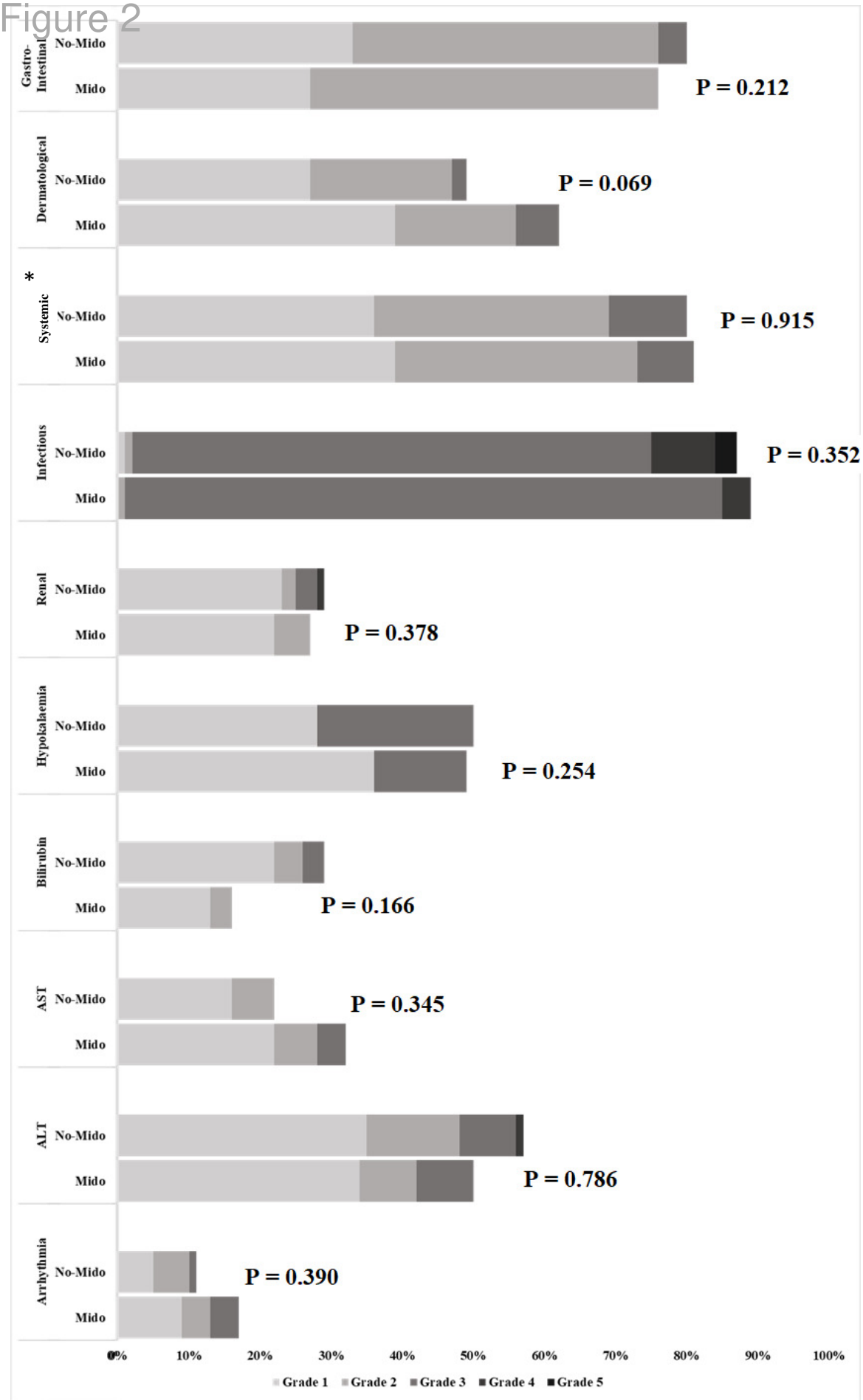


Figure 2




* Systemic symptoms comprised weight loss, fatigue, headache and pain

Figure 3

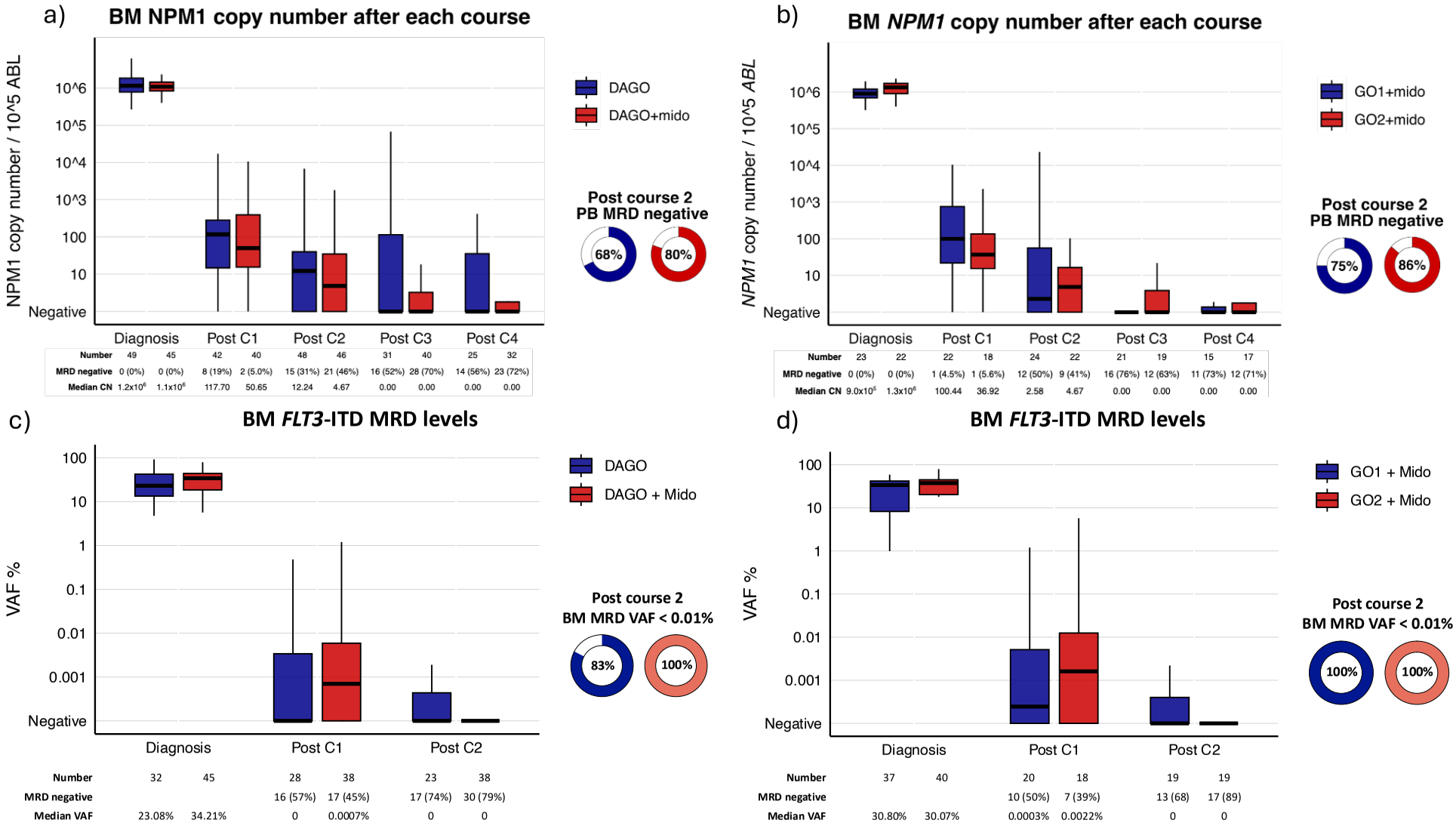


Figure 4

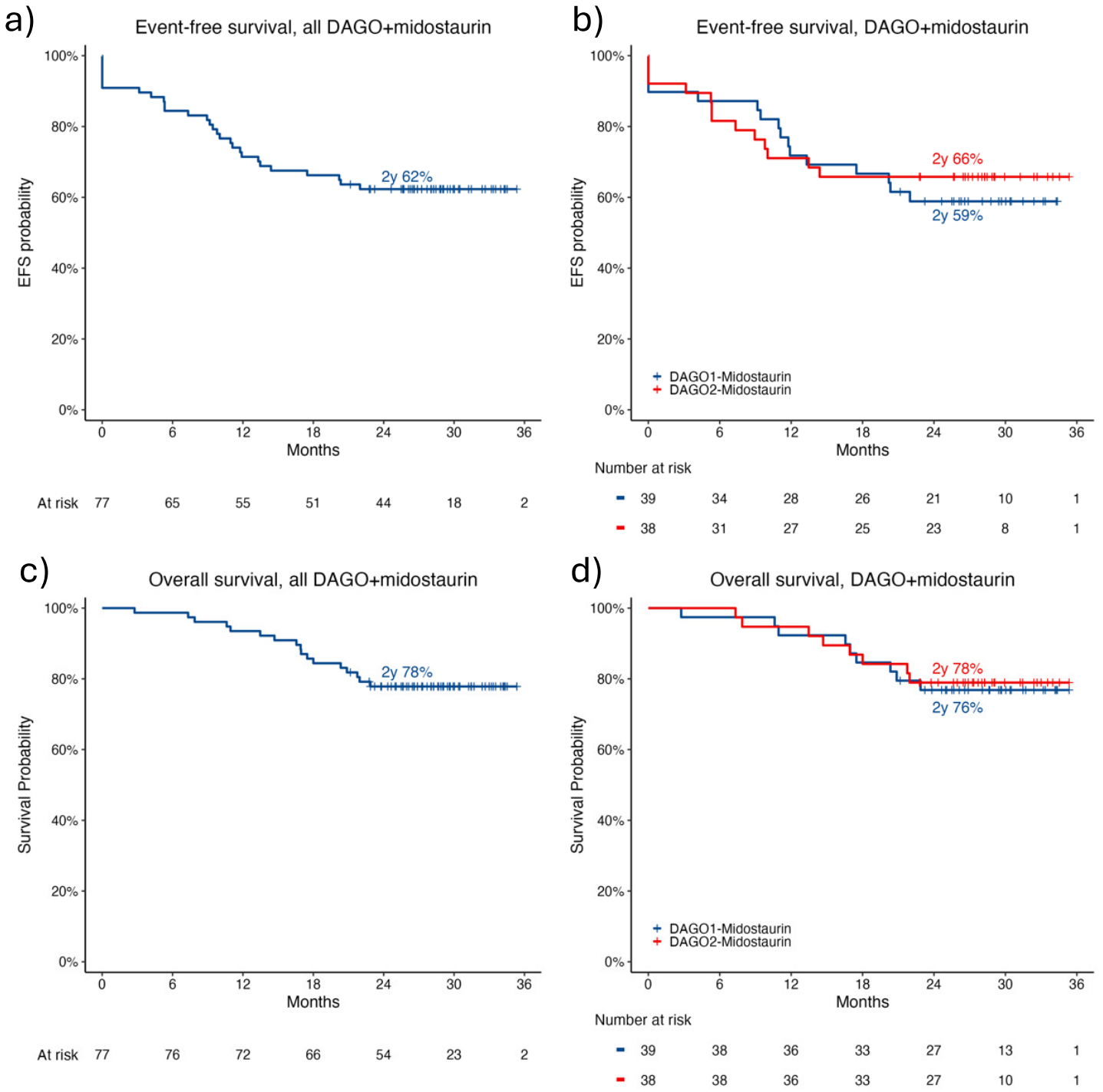


Figure 5