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

Hartmut Döhner, Daniela Weber, Julia Krzykalla, Walter Fiedler, Michael W M Kühn, Thomas Schroeder, Karin Mayer, Michael Lübbert, Mohammed Wattad, Katharina Götze, Lars Fransecky, Elisabeth Koller, Gerald Wulf, Jan Schleicher, Mark Ringhoffer, Richard Greil, Bernd Hertenstein, Jürgen Krauter, Uwe M Martens, David Nachbaur, Maisun Abu Samra, Sigrid Machherndl-Spandl, Nadezda Basara, Claudia Leis, Anika Schrade, Silke Kapp-Schwoerer, Sibylle Cocciardi, Lars Bullinger, Felicitas Thol, Michael Heuser, Peter Paschka, Verena I Gaidzik, Maral Saadati, Axel Benner, Richard F Schlenk, Konstanze Döhner, Arnold Ganser, on behalf of the German–Austrian AML Study Group

Summary

Background Acute myeloid leukaemia with mutated *NPM1* is associated with high CD33 expression and intermediate-risk cytogenetics. The aim of this study was to evaluate intensive chemotherapy with or without the anti-CD33 antibody–drug conjugate gemtuzumab ozogamicin in participants with newly diagnosed, *NPM1*-mutated acute myeloid leukaemia.

Methods This open-label, phase 3 trial was conducted at 56 hospitals in Germany and Austria. Eligible participants were 18 years or older and had newly diagnosed *NPM1*-mutated acute myeloid leukaemia and an Eastern Cooperative Oncology Group performance status of 0–2. Participants were randomly assigned, using age as a stratification factor (18–60 years *vs* >60 years), 1:1 to the two treatment groups using allocation concealment; there was no masking of participants and investigators to treatment groups. Participants received two cycles of induction therapy (idarubicin, cytarabine, and etoposide) plus all-trans retinoic acid (ATRA) followed by three consolidation cycles of high-dose cytarabine (or an intermediate dose for those older than 60 years) and ATRA, without or with gemtuzumab ozogamicin (3 mg/m² administered intravenously on day 1 of induction cycles 1 and 2, and consolidation cycle 1). The primary endpoints were short-term event-free survival and overall survival in the intention-to-treat population (overall survival was added as a co-primary endpoint after amendment four of the protocol on Oct 13, 2013). The secondary endpoints were event-free survival with long-term follow-up, rates of complete remission, complete remission with partial haematological recovery (CRh), and complete remission with incomplete haematological recovery (CRi), cumulative incidences of relapse and death, and number of days in hospital. This trial is registered with ClinicalTrials.gov (NCT00893399) and has been completed.

Findings Between May 12, 2010, and Sept 1, 2017, 600 participants were enrolled, of which 588 (315 women and 273 men) were randomly assigned (296 to the standard group and 292 to the gemtuzumab ozogamicin group). No difference was found in short-term event-free survival (short-term event-free survival at 6-month follow-up, 53% [95% CI 47–59] in the standard group and 58% [53–64] in the gemtuzumab ozogamicin group; hazard ratio [HR] 0·83; 95% CI 0·65–1·04; p=0·10) and overall survival between treatment groups (2-year overall survival, 69% [63–74] in the standard group and 73% [68–78] in the gemtuzumab ozogamicin group; 0·90; 0·70–1·16; p=0·43). There was no difference in complete remission or CRi rates (n=267 [90%] in the standard group *vs* n=251 [86%] in the gemtuzumab ozogamicin group; odds ratio [OR] 0·67; 95% CI 0·40–1·11; p=0·15) and complete remission or CRh rates (n=214 [72%] *vs* n=195 [67%]; OR 0·77; 0·54–1·10; p=0·18), whereas the complete remission rate was lower with gemtuzumab ozogamicin (n=172 [58%] *vs* n=136 [47%]; OR 0·63; 0·45–0·80; p=0·0068). Cumulative incidence of relapse was significantly reduced by gemtuzumab ozogamicin (2-year cumulative incidence of relapse, 37% [95% CI 31–43] in the standard group and 25% [20–30] in the gemtuzumab ozogamicin group; cause-specific HR 0·65; 0·49–0·86; p=0·0028), and there was no difference in the cumulative incidence of death (2-year cumulative incidence of death 6% [4–10] in the standard group and 7% [5–11] in the gemtuzumab ozogamicin group; HR 1·03; 0·59–1·81; p=0·91). There were no differences in the number of days in hospital across all cycles between treatment groups. The most common treatment-related grade 3–4 adverse events were febrile neutropenia (n=135 [47%] in the gemtuzumab ozogamicin group *vs* n=122 [41%] in the standard group), thrombocytopenia (n=261 [90%] *vs* n=265 [90%]), pneumonia (n=71 [25%] *vs* n=64 [22%]), sepsis (n=85 [29%] *vs* n=73 [25%]). Treatment-related deaths were documented in 25 participants (4%; n=8 [3%] in the standard group and n=17 [6%] in the gemtuzumab ozogamicin group), mostly due to sepsis and infections.

Interpretation The primary endpoints of the trial of event-free survival and overall survival were not met. However, an anti-leukaemic efficacy of gemtuzumab ozogamicin in participants with *NPM1*-mutated acute myeloid leukaemia is shown by a significantly lower cumulative incidence of relapse rate, suggesting that the addition of gemtuzumab

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Department of Internal Medicine III, Ulm University Hospital, Ulm, Germany (H Döhner MD, D Weber MSc, C Leis BSc, A Schrade PhD, S Kapp-Schwoerer MD, S Cocciardi PhD, P Paschka MD, V I Gaidzik MD, K Döhner MD); **Division of Biostatistics, German Cancer Research Center, Heidelberg, Germany** (J Krzykalla PhD, A Benner MSc); **Hubertus Wald University Cancer Center, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany** (W Fiedler MD); **Department of Hematology, Medical Oncology & Pneumology, University Medical Center, Johannes Gutenberg-University Mainz, Mainz, Germany** (M W M Kühn MD); **Department of Hematology, Oncology, and Clinical Immunology, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany** (T Schroeder MD); **Department of Hematology, Oncology, University Hospital Bonn, Bonn, Germany** (K Mayer MD); **Department of Medicine I, Medical Center, University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany** (M Lübbert MD); **Klinik für Hämatologie, Internistische Onkologie & Stammzelltransplantation, Evang. Krankenhaus Essen-Werden, Essen-Werden, Germany** (M Wattad MD);

Klinikum Hochsauerland, Meschede, Germany (M Wattad); Department of Medicine III, Hematology and Medical Oncology, Technical University of Munich, Munich, Germany (K Götze MD); Department of Internal Medicine II, University Hospital Schleswig Holstein, Campus Kiel, Kiel, Germany (L Fransecky MD); Department of Internal Medicine III, Hanusch Krankenhaus Wien, Wien, Austria (E Koller MD); Department of Hematology and Medical Oncology, University Medicine Göttingen, Göttingen, Germany (G Wulf MD); Klinik für Hämatologie, Onkologie, Stammzelltransplantation und Palliativmedizin, Klinikum Stuttgart, Stuttgart, Germany (J Schleicher MD); Department of Internal Medicine III, Städtisches Klinikum Karlsruhe, Karlsruhe, Germany (M Ringhoffer MD); 3rd Medical Department Paracelsus Medical University Salzburg, Salzburg, Austria (R Greil MD); Salzburg Cancer Research Institute Center for Clinical Trials and Immunology Trials, Salzburg, Austria (R Greil); Cancer Cluster Salzburg, Salzburg, Austria (R Greil); Department of Hematology and Oncology, Klinikum Bremen-Mitte, Bremen, Germany (B Hertenstein MD); Medizinische Klinik III, Städtisches Klinikum Braunschweig, Braunschweig, Germany (J Krauter MD); Klinik für Innere Medizin III, SLK-Kliniken Heilbronn, Heilbronn, Germany (U M Martens MD); Universitätsklinik für Innere Medizin V, Medizinische Universität Innsbruck, Innsbruck, Austria (D Nachbaur MD); Medizinische Klinik IV, Universitätsklinikum Gießen, Gießen, Germany (M A Samra MD); Department of Hematology and Oncology, Ordensklinikum Elisabethinen Linz, Linz, Austria (S Machherndl-Spandl MD); Medizinische Klinik I, Malteser Krankenhaus St Franziskus-Hospital Flensburg, Flensburg, Germany (N Basara MD); Department of Hematology, Oncology and Cancer Immunology, Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt

ozogamicin might reduce the need for salvage therapy in these participants. The results from this study provide further evidence that gemtuzumab ozogamicin should be added in the standard of care treatment in adults with *NPM1*-mutated acute myeloid leukaemia.

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Introduction

Mutations of the nucleophosmin 1 (*NPM1*) gene are common genetic alterations in adults with newly diagnosed acute myeloid leukaemia and are present in 25–30% of those with the disease, with a decreasing prevalence with older age.[1,2] On the basis of its characteristic clinicopathological findings, acute myeloid leukaemia with mutated *NPM1* is recognised as a distinct disease entity, in the category of acute myeloid leukaemia with recurrent genetic abnormalities.[3] Acute myeloid leukaemia with mutated *NPM1* has been associated with high response rates and favourable outcomes, in particular in people without the concomitant presence of an *FLT3* internal tandem duplication (ITD) or those with lower ITD allelic ratios.[4] One of the phenotypic characteristics of *NPM1*-mutated acute myeloid leukaemia is that most individuals have a high expression of the CD33 antigen.[5]

Gemtuzumab ozogamicin is a humanised CD33-targeted IgG4 antibody conjugated to a calicheamicin derivative, a natural tumour antibiotic.[6] Several randomised trials were performed combining gemtuzumab ozogamicin with intensive chemotherapy in different trial designs and in different age groups that reported varying outcome results.[7–11] A meta-analysis of these randomised trials showed that the addition of gemtuzumab ozogamicin significantly reduced the risk of relapse and improved 5-year overall survival.[12]

Several predictive biomarkers for the response to gemtuzumab ozogamicin-based therapy have been described, among them CD33 expression concentrations, favourable risk cytogenetics, and molecular genetic alterations such as *NPM1* mutations and *FLT3* ITDs.[12,13] A subgroup analysis within the ALFA-0701 trial showed a significantly beneficial effect of gemtuzumab ozogamicin in *NPM1*-mutated acute myeloid leukaemia.[14,15]

Research in context

Evidence before this study

A specific database search was not conducted for this investigation. Our data are discussed in the context of all published data on randomised trials (including a meta-analysis) with intensive chemotherapy combined with the anti-CD33 antibody–drug conjugate gemtuzumab ozogamicin in individuals with acute myeloid leukaemia. Our clinical trial was designed and initiated before these data were peer reviewed and published. Four randomised trials of intensive chemotherapy plus gemtuzumab ozogamicin were conducted with different trial designs and in different age groups. All trials showed a benefit of adding gemtuzumab ozogamicin, either overall or in participant subsets. A meta-analysis of the randomised trials involving 3325 participants showed that the addition of gemtuzumab ozogamicin did not increase the response rate, but reduced the risk of relapse, and improved 5-year overall survival; this effect was evident for acute myeloid leukaemia with favourable cytogenetics, and to a lesser extent in patients with intermediate-risk cytogenetics, but not for patients with adverse cytogenetics. The subset of patients with acute myeloid leukaemia with mutated *NPM1* in most studies was too small to draw any meaningful conclusions.

Added value of this study

This study is, to our knowledge, the first randomised clinical trial that has been conducted specifically in the molecular subgroup of acute myeloid leukaemia with mutated *NPM1*. One major rationale to focus on this disease entity was that individuals with *NPM1*-mutated disease have high CD33 expression and, in most individuals, have intermediate-risk cytogenetics. The primary endpoints of short-term event-free survival and overall survival were not met. Nevertheless, an anti-leukaemic efficacy of gemtuzumab ozogamicin was shown by significantly reducing the cumulative incidence of relapse and by a better mutant *NPM1* transcript clearance as assessed by a quantitative PCR. The fact that the endpoint of event-free survival was not met was primarily because of an increased early death rate in the gemtuzumab ozogamicin group in participants older than 70 years for whom the chemotherapy backbone and the repetitive administration of gemtuzumab ozogamicin might have been too intensive.

Implication of all the available evidence

In Europe and the USA, gemtuzumab ozogamicin is widely used in core-binding factor acute myeloid leukaemia, but in many countries it is not used in intermediate-risk acute myeloid leukaemia. The data from our trial provide further evidence for a beneficial effect of gemtuzumab ozogamicin by significantly reducing the risk of relapse in people with *NPM1*-mutated acute myeloid leukaemia, which represents a large subgroup of people. Adding gemtuzumab ozogamicin to the treatment regimen significantly reduced the need for salvage treatment, which is a major benefit to those with acute myeloid leukaemia. The addition of gemtuzumab ozogamicin should be restricted to induction therapy, and caution should be taken in older people with acute myeloid leukaemia.

The AMLSG 09–09 trial evaluated induction and consolidation therapy plus all-trans retinoic acid (ATRA) with or without gemtuzumab ozogamicin in patients with *NPM1*-mutated acute myeloid leukaemia. The rationale for the use of ATRA was based on findings from predictive marker studies in two previous AMLSG trials that suggested a benefit of ATRA in *NPM1*-mutated acute myeloid leukaemia.[16,17] We previously reported the results of the event-free survival analysis in the short-term follow-up of this trial.[18] Here, we report the final results of the trial, including data on overall survival and event-free survival with long-term follow-up.

Methods

Study design and participants

AMLSG 09–09 was a randomised, open-label, phase 3 study conducted at 56 study hospitals in Germany and Austria (appendix pp 1–2; study protocol available in the appendix from p 32). Participants aged 18 years or older with newly diagnosed *NPM1*-mutated acute myeloid leukaemia (according to WHO classification) and with an Eastern Cooperative Oncology Group performance status of 0–2 were eligible. A full list of inclusion and exclusion criteria is available in the appendix (p 3). All participants provided written informed consent. The clinical trial was approved by the ethics committees at all sites.

Randomisation and masking

Participants were randomly assigned 1:1 to the two treatment groups using allocation concealment. An allocation sequence scheme was generated by Ulm University Hospital using the statistic software R version 4.1.2 on the basis of age as a stratification factor (18–60 years *vs* >60 years) and a block length of 4. For each stratum, a separate allocation list was generated and implemented into the web-based registration system. Participants were registered at each of the sites via this system and automatically allocated to the next assignment in the sequence dependent on the actual age stratum. There was no masking of participants and investigators to the treatment groups.

Procedures

Molecular screening for an *NPM1* mutation, *FLT3* ITDs and *FLT3* tyrosine kinase domain mutations (codons Asp835 and Ile836), and *DNMT3A* mutations was performed centrally within the AMLSG BiO Registry study (clinical trial number NCT01252485). Genetic risk was categorised as favourable-risk, intermediate-risk, and adverse-risk categories, according to the 2017 European LeukemiaNet (ELN) recommendations.[4]

Participants received induction therapy with two cycles of 12 mg/m² idarubicin hydrochloride intravenously (on days 1, 3, and 5 of the first cycle [days 1 and 3 only in participants older than 60 years]; and on days 1 and 3 of the second cycle in all participants), 100 mg/m² cytarabine administered continuously intravenously (days 1–7), and 100 mg/m² etoposide intravenously (on days 1–3 of the first cycle [days 1 and 3 only in participants older than 60 years]; and on days 1 and 3 of the second cycle in all participants), 45 mg/m² ATRA orally (days 6–8), and 15 mg/m² orally (days 9–21), with or without 3 mg/m² gemtuzumab ozogamicin (manufactured by Wyeth Pharmaceutical Division of Wyeth Holdings, a subsidiary of Pfizer, Pearl River, NY, USA) intravenously on day 1. Because of the long-term haematological recovery in both treatment groups, etoposide was reduced from 3 to 2 days in the second induction cycle after 141 participants were recruited. Participants with no response after the first induction cycle went off protocol treatment but were followed up for survival.

Participants with a complete remission or complete remission with incomplete haematological recovery (CRi) by the end of induction cycle 2 were assigned to consolidation therapy with three cycles of higher doses of cytarabine plus ATRA (ie, cytarabine at a dose of 3000 mg/m² every 12 h on days 1–3 [1000 mg/m² in participants older than 60 years], 15 mg/m² ATRA per day orally on days 4–21; and 6 mg pegfilgrastim subcutaneously on day 10). Participants in the investigational group received 3 mg/m² gemtuzumab ozogamicin intravenously on day 1 in first consolidation therapy. In participants with thrombocytopenia during the first or second induction cycle lasting more than 35 days after the start of the cycle (without signs of leukaemia), gemtuzumab ozogamicin was omitted in the first consolidation cycle. Allogeneic haematopoietic stem-cell transplantation (HSCT) was performed at the discretion of the investigator.

Safety was assessed by reporting adverse events, defined by the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). Toxicity was monitored continuously, between the first dose of study drug up to 28 days after the last dose. Blood samples were taken for haematology and clinical chemistry values at least once a week during induction and consolidation cycles. Remission status was assessed de-centrally and not evaluated independently. Assessment of measurable residual disease (MRD) in bone marrow and blood was performed centrally after each treatment cycle, thereafter at approximately 6-month intervals for another 3 years. Participants could be withdrawn from the study by investigators because of concomitant disease, unacceptable adverse events, and participant non-compliance with protocol requirements.

Outcomes

Initially, the primary efficacy endpoint of the trial was short-term event-free survival only (ie, with 6 months follow-up after inclusion of the last participant). After amendment four (on Oct 13, 2013), overall survival was added as a second primary efficacy endpoint and the sample size increased from the initial 276 to 588 participants, on the basis of data emerging during the

Universität zu Berlin, Berlin, Germany (L Bullinger MD); **Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany** (F Thol MD, M Heuser MD, A Ganser MD); **Freelance Statistician, Saadati Solutions, Ladenburg, Germany** (M Saadati PhD); **National Center of Tumor Diseases, German Cancer Research Center, Heidelberg, Germany** (R F Schlenk MD); **Department of Internal Medicine V, Heidelberg University Hospital, Heidelberg, Germany** (R F Schlenk)

Correspondence to:
Dr Hartmut Döhner, Department of Internal Medicine III, Ulm University Hospital, Ulm 89081, Germany
hartmut.doehner@uniklinik-ulm.de

See **Online** for appendix

course of the study that gemtuzumab provides a significant overall survival benefit for people without adverse cytogenetic characteristics.[8,12] All five amendments of the protocol are presented in the appendix (p 4). Secondary endpoints were event-free survival (with long-term follow-up until end of the study), rates of complete remission, complete remission and complete remission with partial haematological recovery (CRh), and complete remission and CRi, cumulative incidence of relapse, cumulative incidence of death, and number of days in hospital. These secondary endpoints were analysed in an exploratory manner. The definition of secondary endpoints deviated from the protocol in that we also included the rates of complete remission and CRh and complete remission and CRi in the response evaluation. All outcome endpoints were defined using 2022 ELN criteria;[19] event-free survival was also analysed according to the initial protocol definition by the 2017 ELN recommendations.[7]

Safety endpoints were 30-day and 60-day mortality rates and haematological recovery times (ie, absolute neutrophil counts $>0{\cdot}5\times10^9$ per L *vs* $1{\cdot}5\times10^9$ per L, and platelet counts $>20\times10^9$ per L *vs* $>50\times10^9$ per L *vs* $>100\times10^9$ per L after each treatment cycle).

Statistical analysis

The final required number of participants was established for overall survival; the sample size was estimated for testing the null hypothesis of equal overall survival distributions in the two treatment groups via a log-rank test at a two-sided significance level of 5%. Assuming 4-year overall survival of 40% in the standard group (without gemtuzumab ozogamicin; based on historical data of the AMLHD98B,[16] AMLSG 07–04,[17] and AMLSG 06–04 trials[20]) and of 50% in the experimental group (with gemtuzumab ozogamicin), an expected recruitment period of 5·5 years, a follow-up time of 4 years after the inclusion of the last participant, and equal loss to follow-up of 5% in both groups, a total of 588 participants would have to be randomly assigned equally into the study groups to achieve a power of 80%. A futility interim analysis for event-free survival was planned after the accrual of 276 participants. The study was not stopped at the interim analysis (Oct 15, 2013), since the conditional power at the interim (assuming the original design effect) was 39·7% and therefore exceeded the stopping criterion of 30%. The two primary endpoints were evaluated sequentially by using the fallback procedure of Wiens to account for multiple testing[21] (ie, the significance level $\alpha=5\%$ was split in $\alpha=\alpha1+\alpha2$, with $\alpha1=2\%$ for event-free survival and $\alpha2=3\%$ for overall survival). The primary analysis for event-free survival and overall survival was a log-rank test stratified for age group. In addition to the test results, the estimates for the hazard ratio (HR) for event-free survival and overall survival were derived from a stratified univariate Cox model, including 95% CIs. Multivariate Cox proportional hazards models were fitted using age, sex, acute myeloid leukaemia type (de novo *vs* secondary acute myeloid leukaemia or therapy-related acute myeloid leukaemia), white blood cell count, and serum lactate dehydrogenase concentration (both $\log_{10}$ transformed), as well as the mutational status of *FLT3* (ITD and tyrosine kinase domain mutations) and *DNMT3A* as covariates. Additional models were fitted including allogeneic HSCT in first remission as a time-dependent covariate. For multivariate models, multiple imputation was used to address missing information in the covariates.[22] The intention-to-treat population was the primary population for the reporting of baseline characteristics and efficacy variables and included all participants randomised to each group regardless of their eventual treatment.

For response rates, Cochran-Mantel-Haenszel tests and logistic regression were used. Event-free survival was analysed using log-rank tests and Cox proportional hazards regression, and cause-specific Cox regression was applied for cumulative incidence of relapse and death. All statistical tests and models used age groups (18–60 years *vs* >60 years) as a stratification factor to account for the stratified randomisation. Descriptive statistics for overall survival and event-free survival included median survival times and survival rates using Kaplan-Meier estimates with 95% CIs; cumulative incidences for relapse and death were estimated using the method of Aalen-Johansen, again with 95% CIs.

For the primary endpoint of overall survival and all secondary efficacy endpoints, unplanned post-hoc subgroup analyses were done for the two age groups (18–60 years *vs* >60 years) using a logistic model or (cause-specific) Cox model with treatment, age group, and the interaction of both. An additional post-hoc subgroup analysis was done for participants aged 60–70 years. A Wald test for the coefficient of the interaction term in this model was done to test whether the effect of gemtuzumab ozogamicin differed between the age groups. The effect of gemtuzumab ozogamicin on overall survival and event-free survival with long-term follow-up was investigated in subgroups using stratified Cox proportional hazards models including treatment, the covariate defining the respective subgroup, and an interaction term. Outcome after relapse was investigated using a multistate model (appendix p 4). Furthermore, we evaluated the reduction of mutant *NPM1* transcripts after each treatment cycle by treatment group and age group, and analysed the cumulative incidence of relapse by *NPM1* MRD positivity in the blood after two cycles of intensive chemotherapy.

Missing recovery times for lower criteria were imputed by recovery times for higher criteria if available. Recovery times were analysed separately for each cycle in a competing risk setting with death or the start of new treatment without recovery as the competing event. All participants receiving at least one study dose were included in the safety analysis.

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For testing differences in baseline characteristics, Fisher's exact test was used for nominal data and a Cochran-Armitage test was used for ordinal data; and a Mann-Whitney test was used for two-group comparisons of continuous data and a Kruskal-Wallis test was used for the comparison of continuous data for more than two groups. For all secondary analyses, a p value of less than 0·05 was considered statistically significant. Statistical analyses were performed with the statistical software R version 4.1.2, using the R packages survival version 3.2.13, etm version 1.1.1, smcfcs version 1.6.2, mitools version 2.4, mice version 3.14.0, and riskRegression version 2021.10.10. This trial is registered at ClinicalTrialsRegistry.eu (Eudra-CT 2009–011889–28) and ClinicalTrials.gov (NCT00893399).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between May 12, 2010, and Sept 1, 2017, 600 participants were enrolled. 12 participants were excluded from the analysis, six as a result of violation of inclusion or exclusion criteria and six as a result of early (within 5 days) withdrawal of informed consent (figure 1). Of 588 included participants, 296 were randomly assigned to the standard group, and 292 to the gemtuzumab ozogamicin group. Baseline participant and disease characteristics are shown in table 1.

Both the primary endpoints of short-term event-free survival and overall survival were not met. The results from the analysis of the short-term event-free survival (ie, 6 months after the completion of participant recruitment; the database was locked for the short-term analysis on Sept 28, 2018) were previously reported.[18] There was no statistically significant difference between treatment groups for short-term event-free survival (event-free survival at 6-month follow-up, 53% in the standard group [95% CI 47–59] and 58% in the gemtuzumab ozogamicin group [53–64]; HR 0·83; 95% CI 0·65–1·04); the corresponding stratified log-rank test resulted in a p value of 0·10, which was larger than the corresponding significance level α1 of 0·02. Hence, the null hypothesis could not be rejected and overall survival had to be tested at a significance level α2 of 0·03.

After a median follow-up of 64 months (IQR 52–91; the database was locked on July 1, 2022), there was no statistically significant difference in overall survival between the two treatment groups (2-year overall survival, 69% in the standard group [95% CI 63–74] and 73% in the gemtuzumab ozogamicin group [68–78]; HR 0·90; 95% CI 0·70–1·16; p=0·43; table 2; figure 2A). There were 126 deaths in the standard group and 112 in the gemtuzumab ozogamicin group. 2-year and 5-year overall survival are given in table 2. The results

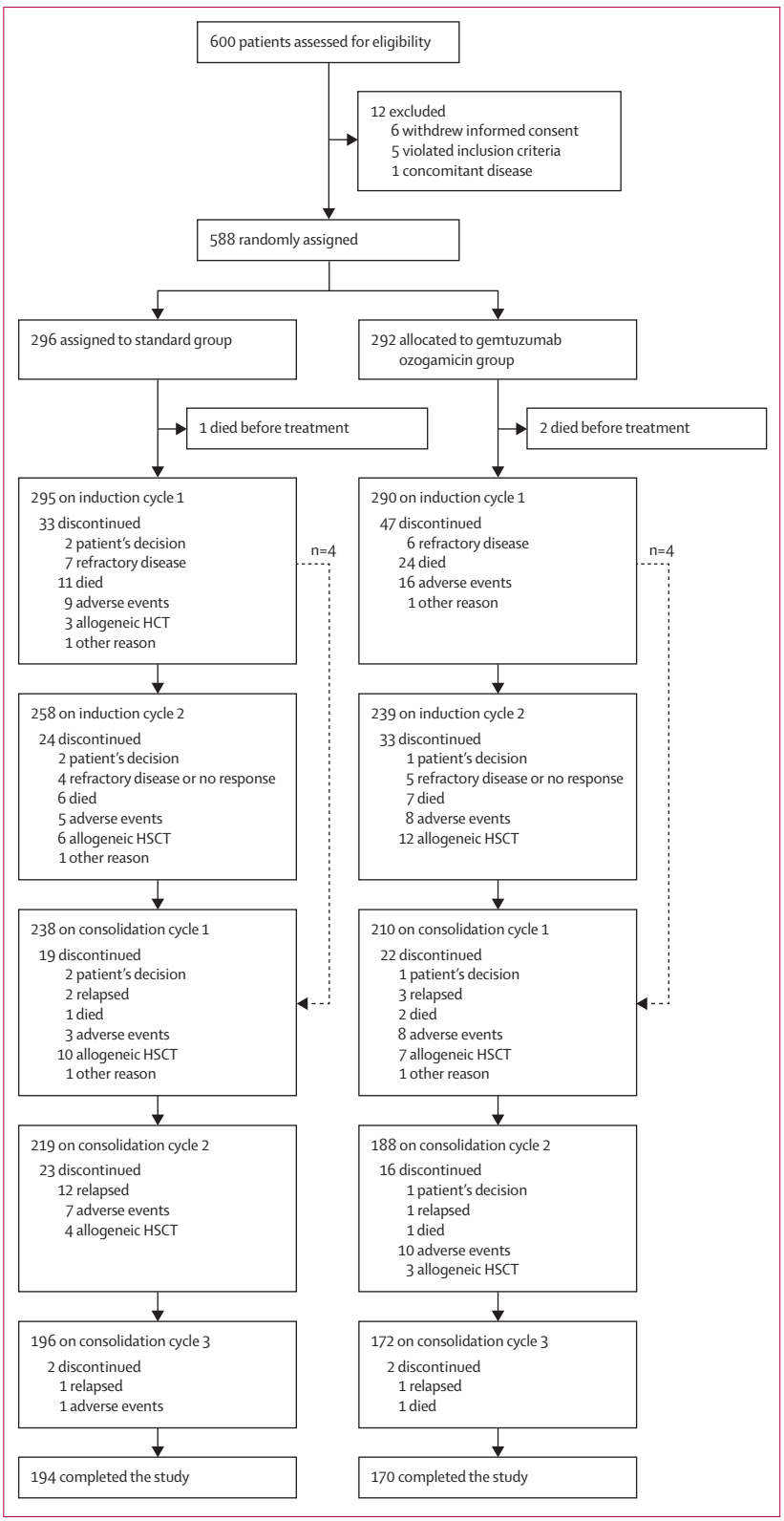

Figure 1: **Consort diagram**
ATRA=all-trans retinoic acid. HSCT=haematopoietic stem-cell transplantation.

	All participants (N=588)	Standard group (n=296)	Gemtuzumab ozogamicin group (n=292)
Age, years			
Median	58·7 (50·7–68·2)	58·8 (50·1–68·2)	58·7 (51·3–68·1)
Sex			
Male	273 (46%)	144 (49%)	129 (44%)
Female	315 (54%)	152 (51%)	163 (56%)
Ethnicity			
White	576 (98%)	291 (98%)	285 (98%)
Asian	1 (<1%)	1 (<1%)	0
North African, Arabian, or Turk	9 (2%)	4 (1%)	5 (2%)
Other African	1 (<1%)	0	1 (<1%)
Other	1 (<1%)	0	1 (<1%)
Acute myeloid leukaemia history			
De novo	547 (93%)	276 (93%)	271 (93%)
Secondary	10 (2%)	3 (1%)	7 (2%)
Therapy related	31 (5%)	17 (6%)	14 (5%)
White blood cell count, 10^9 cells per L			
Median	19·1 (4·5–49·9)	20·5 (4·3–58·1)	16·9 (4·9–40·0)
Missing	3 (1%)	2 (1%)	1 (<1%)
Haemoglobin g/dL			
Median	9·2 (8·1–10·5)	9·4 (8·2–10·6)	9·1 (7·9–10·5)
Missing	3 (1%)	2 (1%)	1 (<1%)
Platelets, 10^9 cells per L			
Median	73·0 (40·8–117·0)	69·5 (42·0–116·0)	74·0 (40·0–118·0)
Missing	4 (1%)	2 (1%)	2 (1%)
Bone marrow blasts, %			
Median	74% (45–90)	71% (41–90)	77% (45–90)
Missing	23 (4%)	11 (4%)	12 (4%)
Peripheral blood blasts, %			
Median	26% (6–65)	25% (5–65)	26% (6–64)
Missing	29 (5%)	13 (4%)	16 (5%)
Lactate dehydrogenase, U/L			
Median	437 (296–668)	439 (305–683)	426 (281–634)
Missing	1 (<1%)	1 (<1%)	0

(Table 1 continues on next page)

from the multivariate Cox model with adjustment for allogeneic HSCT supported the results (HR 0·95; 95% CI 0·73–1·24; p=0·73; appendix p 5). Variables associated with a significantly inferior overall survival were age, *FLT3* ITD, and a *DNMT3A* mutation. Allogeneic HSCT had no notable effect on overall survival. Post-hoc analysis also showed no significant difference in overall survival between treatment groups within the younger participants (18–60 years; 2-year overall survival, 76% in the standard group [69–82] and 80% in the gemtuzumab ozogamicin group [80–91]; HR 0·91; 95% CI 0·61–1·36; p=0·64) and older participants (>60 years; 2-year overall survival, 59% in the standard group [50–67] and 64% in the gemtuzumab ozogamicin group [55–72]; HR 0·90; 0·65–1·25; p=0·54; appendix p 21). The test for the interaction term of age group and treatment with gemtuzumab ozogamicin did not indicate a differential treatment effect of gemtuzumab ozogamicin between the age groups (p=0·99).

Event-free survival was defined both by the original protocol definition according to 2017 ELN and by the updated 2022 ELN criteria.[4,19] Since the results were similar, those according to 2022 ELN criteria are reported here. There was no statistically significant difference in event-free survival between treatment groups (2-year event-free survival, 50% in the standard group [95% CI 44–55] and 55% in the gemtuzumab ozogamicin group [49–61]; HR 0·83; 95% CI 0·67–1·03; p=0·078; table 2; figure 2B). In terms of event-free survival events, there were 29 induction failures in the standard group and 41 in the gemtuzumab ozogamicin group, 114 relapses in the standard group and 76 in the gemtuzumab ozogamicin group, 23 deaths in complete remission in the standard group and 25 in the gemtuzumab ozogamicin group, and 15 starts of new treatment in the standard group and 10 in the gemtuzumab ozogamicin group. Results from the multivariate Cox model supported this finding (HR 0·81; 95% CI 0·65–1·02; p=0·077; appendix p 5). Similar to overall survival, factors associated with significantly inferior event-free survival were an older age, *FLT3* ITD, and a *DNMT3A* mutation. An analysis by type of *DNMT3A* mutation (R882 *vs* non-R882) showed that both types were detrimental, but the effect was even stronger with the R882 hotspot mutation (1·87 [1·43–2·43], p<0·0001 for R882 and non-R882 mutations *vs* 1·32 [0·99–1·77], p=0·059 for wildtype *DNMT3A*; appendix p 6). Subgroup analysis by age showed that participants aged 18–60 years in the gemtuzumab ozogamicin group had a statistically significant better event-free survival (2-year event-free survival, 53% [45–61] for the standard group and 63% [55–70] for the gemtuzumab ozogamicin group; HR 0·71; 95% CI 0·52–0·98; p=0·036). There was no beneficial effect in participants older than 60 years (2-year event-free survival 45% [37–54] for the standard group and 46% [37–54] for the gemtuzumab ozogamicin group; HR 0·95; 0·71–1·28; p=0·75; appendix p 21). Data on participants aged 60–70 years are shown in the appendix (p 23). The p value for the interaction term of age group and treatment with gemtuzumab ozogamicin in the Cox model was p=0·19 for event-free survival defined by 2022 ELN criteria, and p=0·076 for event-free survival defined by 2017 ELN criteria.

The interpretation of response rates (in particular the rate of complete remission) was compromised by the fact that absolute neutrophil and platelet counts were documented in more than 90% of participants 2 days before or after bone marrow assessment, which was scheduled between day 21 and 28, not taking into account continuing haematological recovery thereafter. The complete remission or CRi rate (odds ratio [OR] 0·67; 95% CI 0·40–1·11; p=0·15) and the complete remission or CRh rate (0·77; 0·54–1·10; p=0·18) by the end of induction showed no significant difference between treatment groups (table 2). The response rates by sex are shown in

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the appendix (p 6). The complete remission rate was significantly lower in the gemtuzumab ozogamicin group (OR 0·63; 95% CI 0·45–0·80; p=0·0068; table 2), reflecting increased haematological toxicity. In the younger participants (18–60 years), the complete remission or CRi rate did not differ between the two treatment groups (151 [92%] for the standard group *vs* 146 [91%] for gemtuzumab ozogamicin group; OR 0·97; 95% CI 0·45–2·10; p=0·93). In older participants (>60 years), the rate of complete remission or CRi was significantly lower in the gemtuzumab ozogamicin group (116 [89%] in the standard group and 105 [80%] in the gemtuzumab ozogamicin group; OR 0·50; 95% CI 0·25–1·00; p=0·049). A multivariate logistic regression model for complete remission or CRi revealed age and white blood cell count counts to significantly contribute to not reaching complete remission or CRi (appendix p 7).

Gemtuzumab ozogamicin significantly reduced the hazard of relapse after complete remission or CRi compared with the standard group (table 2; figure 2C). This finding was true for the univariate model (37% [95% CI 31–43] for the standard group and 25% [20–30] for the gemtuzumab ozogamicin group; cause-specific HR 0·65; 95% CI 0·49–0·86; p=0·0028) and the multivariate model (cause-specific HR 0·59; 0·44–0·80; p=0·0010; appendix p 5). There were 118 relapses in the standard group and 79 in the gemtuzumab ozogamicin group, and 24 deaths in complete remission or CRi in the standard group and 25 in the gemtuzumab ozogamicin group. The significant effect of gemtuzumab ozogamicin on cumulative incidence of relapse was driven by a benefit in participants aged 18–60 years (2-year cumulative incidence of relapse, 35% [28–43] for the standard group and 18% [13–26] for the gemtuzumab ozogamicin group; cause-specific HR 0·50; 95% CI 0·33–0·77; p=0·0015). There was no significant effect in participants older than 60 years (2-year cumulative incidence of relapse, 39% [31–49] for the standard group and 34% [25–44] for the gemtuzumab ozogamicin group; cause-specific HR 0·80; 0·54–1·19; p=0·27; appendix p 22). Data on participants aged 60–70 years are shown in the appendix (p 23). There was no difference in the hazard of death in complete remission (1·03; 0·59–1·81; p=0·91). The test for the interaction term of age group and treatment with gemtuzumab ozogamicin did not indicate a differential treatment effect of gemtuzumab ozogamicin between the age groups (cumulative incidence of relapse, p=0·111; cumulative incidence of death, p=0·36).

The response to salvage therapy was similar in both groups, with a complete remission or CRi rate of 52% (62 of 118) in the standard group and 57% (45 of 79) in the gemtuzumab ozogamicin group. To investigate why the benefit of gemtuzumab ozogamicin in the event-free survival of younger participants did not translate into better overall survival, outcome after relapse was investigated using a multistate model including the states alive, relapse or refractory, and death (appendix p 4).

	All participants (N=588)	Standard group (n=296)	Gemtuzumab ozogamicin group (n=292)
(Continued from previous page)			
2017 European LeukemiaNet risk*			
Favourable	532 (90%)	265 (90%)	267 (91%)
Intermediate	56 (10%)	31 (10%)	25 (9%)
***FLT3* internal tandem duplication**			
Positive	99 (17%)	49 (17%)	50 (17%)
<0·5 allelic ratio	43/96 (45%)	18 (37%)	25/47 (53%)
≥0·5 allelic ratio	53/96 (55%)	31 (63%)	22/47 (47%)
Missing	3 (1%)	0	3 (1%)
Negative	489 (83%)	247 (83%)	242 (83%)
***FLT3* tyrosine kinase mutation domain**			
Positive	76 (13%)	34 (11%)	42 (14%)
Negative	512 (87%)	262 (89%)	250 (86%)
Mutated *DNMT3A*			
Positive	299/581 (51%)	141/294 (48%)	158/287 (55%)
R882	157 (57%)	78/131 (60%)	79/146 (54%)
Non-R882	120 (43%)	53/131 (40%)	67/146 (46%)
Missing	22 (7%)	10 (7%)	12 (8%)
Negative	282 (49%)	153 (52%)	129 (45%)
Missing	7 (1%)	2 (1%)	5 (1%)

Data are shown as median (IQR) or n (%). *Genetic risk categorisation according to 2017 European LeukemiaNet recommendations.[4]

Table 1: **Patient and disease characteristics**

For the transition from a relapse or refractory state to death, the hazard in the gemtuzumab ozogamicin group was higher than in the standard group, indicating a worse prognosis for relapsed or refractory participants in the gemtuzumab ozogamicin group (appendix p 24).

We previously reported the results of MRD analyses for mutant *NPM1* transcripts using quantitative PCR (qPCR) in the entire study cohort.[23] The appendix (p 25) illustrates the dynamics of mutant *NPM1* transcript levels by treatment group and age group. A consistent, statistically significant *NPM1* mutant transcript reduction by gemtuzumab ozogamicin was only found in participants aged 18–60 years, but not in participants older than 60 years, corroborating the results from the cumulative incidence of relapse analyses. MRD data in the blood after two cycles were available in 175 (66%) of 267 responding participants in the standard group and 170 (68%) of 251 in the gemtuzumab ozogamicin group. MRD positivity after two cycles of therapy was associated with a significantly increased hazard of relapse after reaching complete remission or CRi during induction, which was supported by multivariate analysis (appendix pp 8, 26). The effect of other factors on the hazard of relapse, such as treatment group, age, and *DNMT3A* mutational status, were statistically significant.

Further exploratory subgroup analyses showed that gemtuzumab ozogamicin was associated with a beneficial

	All patients (N=588)	Standard group (n=296)	Gemtuzumab ozogamicin group (n=292)
Response to induction therapy			
Complete remission or CRi	518 (88%)	267 (90%)	251 (86%)
Complete remission	308 (52%)	172 (58%)	136 (47%)
Complete remission or CRh	409 (70%)	214 (72%)	195 (67%)
Refractory disease	14 (2%)	7 (2%)	7 (2%)
30-day mortality rate	6% (95% CI 3·7–7·5)	4% (95% CI 1·8–6·3)	7% (95% CI 4·2–10·1)
60-day mortality rate	7% (95% CI 5·2–9·4)	6% (95% CI 3·1–8·4)	9% (95% CI 5·6–12·1)
Allogeneic HSCT			
HSCT in complete remission or CRi*	35 (6%)	19 (6%)	16 (5%)
Matched related donor	7 (1%)	5 (2%)	2 (1%)
Matched unrelated donor	28 (5%)	14 (5%)	14 (5%)
Median time to HSCT†	126 (108–150)	139 (114–150)	125 (103–142)
Any HSCT during disease course	194 (33%)	110 (37%)	84 (29%)
Follow-up time			
Median, months	63·9 (51·9–90·8)	65·4 (52·2–95·7)	62·9 (51·4–87·1)
Outcomes			
Median event-free survival, months	29·3 (8·2–NR)	23·8 (8·0–NR)	44·8 (8·6–NR)
2-year event-free survival	52% (95% CI 48–56)	50% (95% CI 44–55)	55% (95% CI 49–61)
5-year event-free survival	41% (95% CI 36–45)	37% (95% CI 31–43)	45% (95% CI 38–51)
Median overall survival, months	NR (16·3–NR)	NR (16·0–NR)	NR (19·7–NR)
2-year overall survival	71% (95% CI 67–74)	69% (95% CI 63–74)	73% (95% CI 68–78)
5-year overall survival	61% (95% CI 57–65)	60% (95% CI 54–65)	62% (95% CI 56–68)
2-year cumulative incidence of relapse	31% (95% CI 27–35)	37% (95% CI 31–43)	25% (95% CI 20–30)
5-year cumulative incidence of relapse	39% (95% CI 35–43)	45% (95% CI 39–52)	32% (95% CI 27–39)
2-year cumulative incidence of death	7% (95% CI 5–9)	6% (95% CI 4–10)	7% (95% CI 5–11)
5-year cumulative incidence of death	9% (95% CI 7–12)	8% (95% CI 5–12)	10% (95% CI 6–14)

Data are n (%), median (IQR), or Kaplan-Meier or Aalen-Johansen estimates (95% CI). For response to induction chemotherapy, rates of allogeneic HSCT, and 2-year and 5-year overall survival by sex, see the appendix (pp 6–7). HSCT=haematopoietic stem-cell transplantation. CRi=complete remission with incomplete haematological recovery. CRh=complete remission with partial haematological recovery. NR=not reached. *Allogeneic HSCT performed within 180 days after random assignment. †Time from treatment start to HSCT.

Table 2: **Response to induction therapy, rates of allogeneic HSCT, and efficacy outcomes**

effect on event-free survival and overall survival in participants without concomitant *FLT3* ITD, whereas those with *FLT3* ITD did worse with gemtuzumab ozogamicin (*FLT3* ITD negative: median event-free survival, 24·9 months [18·0–39·8] in the standard group *vs* 59·8 months [38·2–not reached (NR)] in the gemtuzumab ozogamicin group, HR 0·72 [0·56–0·92]; median overall survival, NR [97·9–NR] in the standard group *vs* NR [NR–NR] in the gemtuzumab ozogamicin group, HR 0·77 [0·57–1·03]; and *FLT3* ITD positive: median event-free survival, 12·8 months [8·7–69·0] in the standard group *vs* 8·4 months [2·3–NR] in the gemtuzumab ozogamicin group, HR 1·54 [0·97–2·45]; median overall survival, 31·0 months [14·9–NR] in the standard group *vs* 11·5 [8·0–27·9] in the gemtuzumab ozogamicin group, HR 1·65 [1·01–2·67]; appendix pp 27–28). In addition, gemtuzumab ozogamicin had a beneficial effect on event-free survival in participants with AML with an *NPM1* and *DNMT3A* co-mutation (*DNMT3A* wildtype: median event-free survival, 58·6 months [38·3–NR] in the standard group *vs* 64·2 months [25·9–NR] in the gemtuzumab ozogamicin group, HR 1·06 [0·76–1·48]; *DNMT3A* mutated: median event-free survival, 13·6 months [9·9–16·4] in the standard group *vs* 30·5 months [16·0–59·8] in the gemtuzumab ozogamicin group, HR 0·64 [0·48–0·85]) that also translated into an overall survival advantage (*DNMT3A* wildtype: median overall survival NR [97·9–NR] in the standard group *vs* NR [73·6–NR] in the gemtuzumab ozogamicin group, HR 1·14 [0·78–1·68]; *DNMT3A* mutated: median overall survival 68·2 [26·5–NR] in the standard group *vs* NR [66·6–NR] in the gemtuzumab ozogamicin group, HR 0·72 [0·51–1·01]). Regarding clinical variables, there was a beneficial effect of gemtuzumab ozogamicin on event-free survival in female participants, but not on overall survival (appendix p 27).

Overall, early mortality rates were higher in the gemtuzumab ozogamicin group than in the standard group (table 2). Early deaths in most participants were due to infections (29 [69%] of 42 participants). Marked differences were found by age group: in participants aged 18–60 years, 30-day mortality rates were 2%

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(95% CI 0–5) in the standard group and 3% (0–6) in the gemtuzumab ozogamicin group; and the 60-day mortality rates were 4% (0–6) in the standard group and 6% (2–9) in the gemtuzumab ozogamicin group. In participants older than 60 years, the 30-day mortality rates were 6% (2–10) in the standard group and 12% (6–18) in the gemtuzumab ozogamicin group; and the 60-day mortality rates were 8% (4–13) in the standard group and 13% (7–18) in the gemtuzumab ozogamicin group. The higher mortality rates in participants older than 60 years were mainly caused by a high mortality rate in the 99 participants that were older than 70 years (30-day mortality, 8% (0–15) *vs* 60-day mortality, 10% (1–18) in the standard group; 30-day mortality, 20% (8–31) *vs* 60-day mortality, 20% (8–31) in the gemtuzumab ozogamicin group). Overall, there were no differences in days in hospital between treatment groups (induction cycle 1, median 30 days [IQR 27–35] in the standard group *vs* 30 days [27–35] in the gemtuzumab ozogamicin group; induction cycle 2, median 25 days [22–29] in the standard group *vs* 27 days [23–32] in the gemtuzumab ozogamicin group; consolidation cycle 1, median 20 days [16–23] in the standard group *vs* 21 days [18–25] in the gemtuzumab ozogamicin group; consolidation cycle 2, median 19 days [16–22] in the standard group *vs* 19 days [17–22] in the gemtuzumab ozogamicin group; consolidation cycle 3, median 19 days [16–22] in the standard group *vs* 19 days [14–22] in the gemtuzumab ozogamicin group).

The appendix (pp 29–31) illustrates haematological recovery (ie, an absolute neutrophil count of $>0\cdot5\times10^9$ per L and platelet count of $>50\times10^9$ per L after all treatment cycles). In the first induction cycle, there was no difference in haematological recovery times; the median time to an absolute neutrophil count of more than $0\cdot5\times10^9$ per L was 28 days in the standard group and 27 days in the gemtuzumab ozogamicin group, and to a platelet count of more than 50×10^9 per L, it was 25 days in the standard group and 26 days in the gemtuzumab ozogamicin group. In the second induction cycle, there was again no significant difference in the recovery of the absolute neutrophil count (25 days in the standard group and 26 days in the gemtuzumab ozogamicin group); however, the recovery of the platelet count was delayed in the gemtuzumab ozogamicin group (24 days in the standard group and 34 days in the gemtuzumab ozogamicin group). In consolidation cycle 1, a similar pattern was found with delayed platelet recovery (27 days in the standard group and 32 days in the gemtuzumab ozogamicin group). In consolidation cycles 2 and 3, in which gemtuzumab ozogamicin was not administered, haematological recovery was similar between the two treatment groups.

Table 3 lists all grade 1–2 adverse events that occurred in at least 10% of participants, grade 3–4 in at least 5% of participants, and all grade 5 adverse events. All adverse events by sex are presented in the

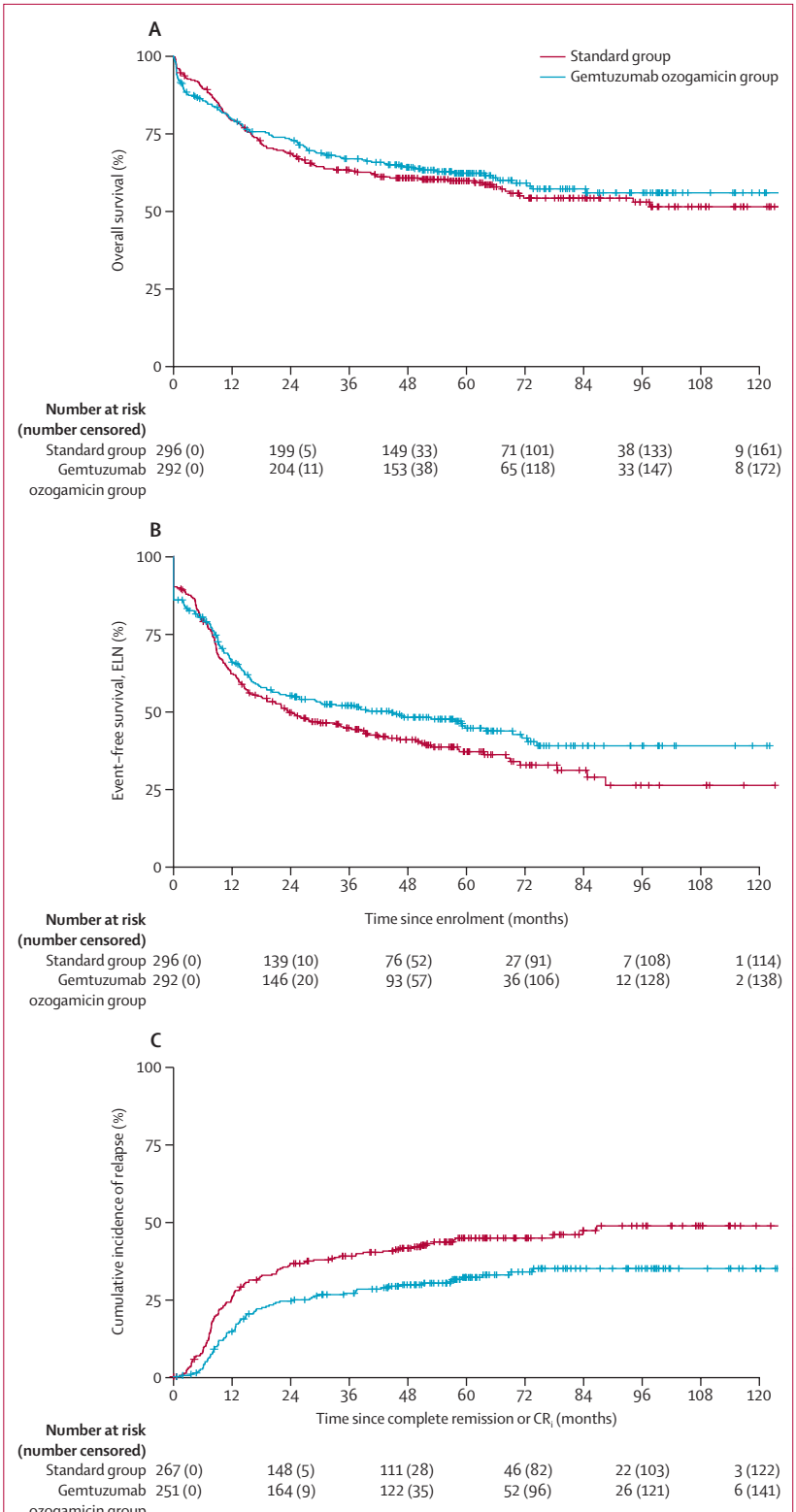

***Figure 2*: Overall survival, event-free survival, and cumulative incidence of relapse**
(A) Overall survival in the intention-to-treat population. (B) Event-free survival in the intention-to-treat population. (C) Cumulative incidence of relapse in participants who achieved complete remission or complete remission with incomplete haematological recovery.

	Standard group (n=295)				Gemtuzumab ozogamicin group (n=290)			
	Grade 1–2	Grade 3	Grade 4	Grade 5	Grade 1–2	Grade 3	Grade 4	Grade 5
Blood and lymphatic system disorders								
Febrile neutropenia	13 (4%)	117 (40%)	5 (2%)	1 (<1%)	8 (3%)	127 (44%)	8 (3%)	..
Leukopenia	2 (1%)	5 (2%)	227 (77%)	..	2 (1%)	3 (<1%)	217 (75%)	..
Lymphopenia	1 (<1%)	1 (<1%)	11 (4%)	..	..	2 (<1%)	13 (5%)	..
Cardiac disorders								
Arrhythmia supraventricular	36 (12%)	7 (2%)	2 (1%)	..	32 (11%)	8 (3%)	2 (1%)	..
Atrial fibrillation	1 (<1%)	..	..	..	..	..	..	1 (<1%)
Atrioventricular block	3 (1%)	..	..	..	..	..	..	1 (<1%)
Cardiac arrest	..	..	1 (<1%)	1 (<1%)	..	..	..	..
Cardiac failure	..	1 (<1%)	..	..	3 (1%)	..	1 (<1%)	2 (1%)
Hypertension	48 (16%)	18 (6%)	2 (1%)	..	56 (19%)	28 (10%)	..	..
Gastrointestinal disorders								
Abdominal pain	65 (22%)	8 (3%)	1 (<1%)	..	74 (26%)	10 (3%)	.	..
Abdominal pain upper	39 (13%)	4 (1%)	..	..	37 (13%)	6 (2%)	..	..
Colitis	9 (3%)	15 (5%)	1 (<1%)	..	13 (5%)	17 (6%)	2 (1%)	..
Constipation	112 (38%)	3 (1%)	..	..	111 (38%)	2 (1%)	..	..
Diarrhoea	123 (42%)	35 (12%)	..	..	110 (38%)	40 (14%)	2 (1%)	..
Gastrointestinal inflammation	100 (34%)	33 (11%)	5 (2%)	..	92 (32%)	50 (17%)	3 (1%)	..
Ileus	2 (1%)	3 (1%)	4 (1%)	1 (<1%)	2 (1%)	5 (2%)	3 (1%)	..
Intussusception	..	..	..	1 (<1%)	..	..	..	..
Nausea	146 (50%)	33 (11%)	..	..	158 (55%)	38 (13%)	1 (<1%)	..
Neutropenic colitis	1 (<1%)	1 (<1%)	..	..	..	..	1 (<1%)	1 (<1%)
Vomiting	45 (15%)	5 (2%)	..	..	92 (32%)	10 (3%)	..	..
General disorders and administration site conditions								
Chills	23 (8%)	..	..	..	60 (21%)	2 (1%)	..	..
Fatigue	78 (26%)	5 (2%)	..	..	78 (27%)	11 (4%)	3 (1%)	..
Injection site reaction	55 (19%)	6 (2%)	..	..	62 (21%)	9 (3%)	..	..
Multiple organ dysfunction syndrome	..	..	..	1 (<1%)	..	..	..	..
Pain	64 (22%)	17 (6%)	1 (<1%)	..	44 (15%)	9 (3%)	..	..
Pyrexia	146 (50%)	28 (10%)	3 (1%)	..	139 (48%)	38 (13%)	1 (<1%)	..
Sudden death	..	..	..	2 (1%)	..	..	..	..
Hepatobiliary disorders								
Hepatic failure	2 (1%)	1 (<1%)	..	..	1 (<1%)	1 (<1%)	1 (<1%)	1 (<1%)
Hepatitis toxic	..	..	..	..	..	..	..	1 (<1%)
Immune system disorders								
Hypersensitivity	53 (18%)	7 (2%)	..	..	55 (19%)	14 (5%)	..	..
Infections and infestations								
Bronchopulmonary aspergillosis	..	1 (<1%)	..	1 (<1%)	..	..	..	..
Clostridial infection	2 (1%)	2 (1%)	..	..	..	..	..	1 (<1%)
Device related infection	18 (6%)	15 (5%)	..	..	13 (5%)	27 (9%)	..	..
H1N1 influenza	..	..	..	1 (<1%)	..	..	..	..
Infection	12 (4%)	44 (15%)	..	..	16 (6%)	29 (10%)	..	..
Influenza	1	..	..	..	3 (1·0)	1 (<1%)	..	1 (<1%)
Neutropenic infection	..	8 (3%)	4 (1%)	..	1 (<1%)	7 (2%)	1 (<1%)	1 (<1%)
Opportunistic infection	..	..	..	..	1 (<1%)	..	..	1 (<1%)
Pneumonia	21 (7%)	56 (19%)	8 (3%)	6 (2%)	25 (9%)	54 (19%)	17 (6%)	5 (2%)
Pseudomonas infection	1	..	..	..	..	2 (1%)	..	1 (<1%)
Sepsis	15 (5%)	53 (18%)	20 (7%)	8 (3%)	18 (6%)	61 (21%)	24 (8%)	18 (6%)
Septic shock	..	..	..	..	..	..	1 (<1%)	1 (<1%)
Urinary tract infection	12 (4%)	14 (5%)	..	..	17 (6%)	13 (5%)	..	..

(Table 3 continues on next page)

	Standard group (n=295)				Gemtuzumab ozogamicin group (n=290)			
	Grade 1–2	Grade 3	Grade 4	Grade 5	Grade 1–2	Grade 3	Grade 4	Grade 5
(Continued from previous page)								
Investigations								
Blood bilirubin	5 (2%)	15 (5%)	2 (1%)	..	13 (5%)	13 (5%)	4 (1%)	..
Blood creatinine	5 (2%)	..	..	1 (<1%)	21 (7%)	3 (1%)	..	..
C-reactive protein increased	34 (12%)	36 (12%)	3 (1%)	..	31 (11%)	26 (9%)	3 (1%)	..
γ-glutamyltransferase	10 (3%)	21 (7%)	5 (2%)	..	16 (6%)	27 (9%)	6 (2%)	..
Glomerular filtration rate	..	..	..	1 (<1%)	1 (<1%)	..	..	..
Haemoglobin	15 (5%)	206 (70%)	40 (14%)	..	14 (5%)	205 (71%)	39 (13%)	..
Neutrophil count	7 (2%)	1 (<1%)	116 (39%)	..	3 (1%)	8 (3%)	111 (38%)	..
Platelet count decreased	3 (1%)	10 (3%)	255 (86%)	..	2 (1%)	9 (3%)	252 (87%)	..
Weight increased	46 (16%)	1 (<1%)	..	..	51 (18%)	1 (<1%)	..	..
Metabolism and nutrition disorders								
Decreased appetite	38 (13%)	5 (2%)	..	..	44 (15%)	7 (2%)	..	..
Hypokalaemia	100 (34%)	44 (15%)	6 (2%)	..	103 (36%)	59 (20%)	18 (6%)	..
Musculoskeletal and connective tissue disorders								
Arthralgia	28 (10%)	3 (1%)	..	..	28 (10%)	2 (1%)	..	..
Back pain	54 (18%)	5 (2%)	..	..	45 (16%)	8 (3%)	..	..
Bone pain	38 (13%)	2 (1%)	..	..	32 (11%)	2 (1%)	..	..
Pain in extremities	25 (9%)	3 (1%)	..	..	28 (10%)	3 (1%)	..	..
Nervous system disorders								
Cerebral haemorrhage	..	..	2 (1%)	1 (<1%)	2 (1%)	..	3 (1%)	2 (1%)
Cerebrovascular accident	..	..	..	..	..	..	..	1 (<1%)
Dizziness	45 (15%)	2 (1%)	..	..	50 (17%)	1 (<1%)	..	..
Headache	89 (30%)	3 (1%)	..	..	106 (37%)	10 (3%)	..	..
Syncope	5 (2%)	15 (5%)	..	..	3 (1%)	15 (5%)	..	..
Psychiatric disorders								
Agitation	29 (10%)	4 (1%)	..	..	31 (11%)	1 (<1%)	..	..
Insomnia	89 (30%)	3 (1%)	..	..	107 (37%)	..	..	..
Renal and urinary disorders								
Acute kidney injury	..	2 (1%)	..	..	1 (<1%)	..	..	1 (<1%)
Fluid retention	121 (41%)	10 (3%)	..	..	117 (40%)	8 (3%)	..	..
Renal failure	2 (1%)	6 (2%)	1 (<1%)	1 (<1%)	5 (2%)	4 (1%)	3 (1%)	2 (1%)
Renal injury	..	..	..	..	..	..	..	1 (<1%)
Cough	62 (21%)	1 (<1%)	..	..	61 (21%)	4 (1%)	..	..
Dyspnoea	26 (9%)	15 (5%)	4 (1%)	..	30 (10%)	16 (6%)	6 (2%)	..
Oropharyngeal pain	32 (11%)	2 (1%)	..	..	31 (11%)	1 (<1%)	..	..
Pleural effusion	9 (3%)	4 (1%)	1 (<1%)	..	13 (5%)	6 (2%)	1 (<1%)	1 (<1%)
Pulmonary haemorrhage	70 (24%)	6 (2%)	..	..	75 (26%)	12 (4%)	..	2 (1%)
Pulmonary oedema	1 (<1%)	..	..	2 (1%)	2 (1%)	1 (<1%)	1 (<1%)	..
Skin and subcutaneous tissue disorders								
Petechiae	47 (16%)	6 (2%)	..	..	68 (23%)	4 (1%)	..	..
Pruritus	43 (15%)	3 (1%)	..	..	24 (8%)	1 (<1%)	..	..
Rash	94 (32%)	16 (5%)	1 (<1%)	..	92 (32%)	11 (4%)	..	..
Vascular disorders								
Haematoma	22 (8%)	3 (1%)	..	..	34 (12%)	2 (1%)	..	..
Phlebitis	38 (13%)	1 (<1%)	5 (2%)	..	39 (13%)	1 (<1%)	1 (<1%)	..

Data shown as n (%). Ordered by MedDRA System Organ Class.

Table 3: **All grade 1–2 adverse events that occurred in at least 10% of patients, grade 3–4 adverse events in at least 5% of patients, and all grade 5 adverse events**

For the **MedDRA System Organ Class** see https://www.meddra.org/

appendix (pp 9–12), as are all adverse events (appendix pp 13–20). The following adverse events grade 3 or worse were reported more frequently in the gemtuzumab ozogamicin group: gastrointestinal disorders (140 [48%] in the gemtuzumab ozogamicin group *vs* 114 [39%] in the standard group); metabolism and nutrition disorders (103 [36%] in the gemtuzumab ozogamicin group *vs* 79 [27%] in the standard group); cardiac disorders (53 [18%] in the gemtuzumab ozogamicin group *vs* 41 [14%] in the standard group); and respiratory, thoracic, and mediastinal disorders (57 [20%] in the gemtuzumab ozogamicin group *vs* 40 [14%] in the standard group). There was a higher incidence of sepsis in the gemtuzumab ozogamicin group, in particular in induction cycle 2 and in the first consolidation cycle. There were two cases of veno-occlusive disease (and both occurred in the second induction cycle, one of which was lethal).

Serious adverse events were reported in 55% (160/290) of participants in the gemtuzumab ozogamicin group and 42% (125/295) of participants in the standard group. There were 67 participants (11%) of 588 who prematurely discontinued the study because of adverse events (39 [7%] were drug-related); 40 (14%) of which were of the 292 in the gemtuzumab ozogamicin group (12 [4%] were drug-related toxicities) and 27 (9%) were of the 296 in the standard group (six [2%] were drug-related toxicities). There were 57 deaths (10%) reported during study treatment; more participants died during study treatment in the gemtuzumab ozogamicin group (35 [12%]) compared with the standard group (22 [7%]). Treatment-related deaths were documented in 25 participants (4%; eight [3%] in the standard group and 17 [6%] in the gemtuzumab ozogamicin group). The main causes of death in the standard group were sepsis (six [2%]), pneumonia (one [<1%]), cardiac arrest (one [<1%]); and in the gemtuzumab ozogamicin group they were sepsis (eight [3%]), pneumonia (two [1%]), pulmonary haemorrhage (two [1%]), neutropenic colitis, opportunistic infection, hepatic toxicity, cerebral haemorrhage, and atrial fibrillation (n=1 each). Overall, dose reductions were required in 156 participants (27%), and more participants required dose reduction in the standard group (85 [14%] of 588) compared with the gemtuzumab ozogamicin group (71 [12%] of 588).

Discussion

In this randomised trial conducted in participants with newly diagnosed *NPM1*-mutated acute myeloid leukaemia, the addition of a single dose of gemtuzumab ozogamicin at 3 mg/m² to intensive induction and consolidation therapy plus ATRA did not lead to a statistically significant improvement of the two primary endpoints: short-term event-free survival and overall survival. Nevertheless, an anti-leukaemic efficacy of gemtuzumab ozogamicin is shown by a significantly reduced relapse rate and by a better mutant *NPM1* transcript clearance in the gemtuzumab ozogamicin group. Post-hoc subgroup analysis by age showed that in participants aged 18–60 years, there was a statistically significant improvement of both event-free survival and cumulative incidence of relapse, thereby reducing the need for salvage therapy, whereas this effect was not found in participants older than 60 years. A multistate model indicated that once a participant relapsed in the gemtuzumab ozogamicin group, the prognosis was worse than in the standard group. One possible explanation is that the rate of allogeneic HSCT as part of the salvage treatment was higher in the standard group. Consistent with previous trials, gemtuzumab ozogamicin did not have an effect on the overall response rate.[7–9,12]

The 30-day and 60-day mortality rates were increased by gemtuzumab ozogamicin; however, this finding was mainly because of a high early mortality rate of 20% in participants older than 70 years in the gemtuzumab ozogamicin group. One limitation of the trial might be that both the chemotherapy backbone—namely, that etoposide is no longer considered a standard of care in induction therapy—and the repetitive administration of gemtuzumab ozogamicin, including in the first consolidation cycle, was too intensive in participants older than 70 years, in large part contributing to the overall negative result. Consistent with data from the ALFA-0701 trial,[9] the repeated administration of gemtuzumab ozogamicin led to delayed platelet count recovery in the second induction and first consolidation cycles. The incidence of sepsis was higher in the gemtuzumab ozogamicin group, in particular in the second induction cycle and in the first consolidation cycle in which gemtuzumab ozogamicin was administered.

The results from our trial are difficult to interpret in the context of previous trials combining gemtuzumab ozogamicin with intensive chemotherapy, since eligibility was restricted to the molecular subset of participants with *NPM1*-mutated acute myeloid leukaemia. In a meta-analysis of randomised trials,[12] subgroup analysis revealed a survival benefit of gemtuzumab ozogamicin for participants with favourable-risk or intermediate-risk cytogenetics, but not for participants with adverse-risk cytogenetics. No effect of gemtuzumab ozogamicin was found by subgroup analysis with respect to age, sex, *FLT3* ITD, and *NPM1* mutation status. With regard to the younger participants, the data from the MRC AML15 trial that randomly assigned patients to gemtuzumab ozogamicin in induction (for one cycle) or in consolidation (for one cycle), or both, showed a trend for better overall survival in the participant group with intermediate-risk cytogenetics, in which almost all our participants are classified.[7] However, in the subgroup of 138 participants with *NPM1*-mutated acute myeloid leukaemia, there was no effect on the risk of relapse or on overall survival. With regard to the older participants, our data differ from those of the NCRI AML16 trial and the ALFA-0701 trial.[8,9,11] The AML16 trial, in which participants were randomly

assigned to gemtuzumab ozogamicin (3 mg/m² on day 1 of cycle 1 of therapy) reported a significantly improved cumulative incidence of relapse and overall survival for older participants in all cytogenetic subgroups, including intermediate-risk participants.[8] The subset of patients with *NPM1*-mutated acute myeloid leukaemia (n=56) was too small to draw any meaningful conclusions. The ALFA-0701 trial, conducted in 280 participants aged 50–70 years with de novo acute myeloid leukaemia, showed an improved event-free survival in participants with favourable-risk and intermediate-risk cytogenetics, including participants with *NPM1*-mutated disease.[9,14] The beneficial effect of gemtuzumab ozogamicin in *NPM1*-mutated acute myeloid leukaemia in this trial was corroborated by data from MRD analyses using qPCR analysis, showing that participants in the gemtuzumab ozogamicin group more frequently had MRD negativity compared with those treated in the standard group.[15]

Differences in trial design might explain some of the varying results in the outcomes observed. The ALFA-0701 trial used fractionated doses of gemtuzumab ozogamicin (3 mg/m²) on days 1, 4, and 7, and thereafter a single dose in consolidation cycles one and two;[9] however, the MRC AML15 and NCRI AML16 trials used only a single dose of gemtuzumab ozogamicin (3 mg/m²), similar to our trial.[7,8] Thus, this alternative schedule of gemtuzumab ozogamicin is rather unlikely to provide a plausible explanation. A potentially important difference between our trial and those conducted by the MRC and NCRI and ALFA groups, in particular in participants older than 60 years, relates to the intensity of the chemotherapy backbone, with two induction cycles and three consolidation cycles with higher doses of cytarabine administered in our trial that might have abrogated the beneficial effect of gemtuzumab ozogamicin observed in the AML16 and ALFA-0701 trials. Of note in this context, older participants with *NPM1*-mutated AML have been shown to respond favourably to intensive chemotherapy.[24] Another potential disease-modifying factor is the use of ATRA in our study, for which we had previously shown, in a randomised trial, a statistically significant beneficial effect in participants older than 60 years with genotype mutant *NPM1* without *FLT3* ITD.[16]

The finding in our study that the beneficial effect of gemtuzumab ozogamicin for cumulative incidence of relapse was confined to younger participants was corroborated by data from our MRD analysis using sensitive qPCR. A consistent significant decrease of mutant *NPM1* transcript levels was found in younger participants, whereas this was not evident in older participants. Since the chemotherapy backbone did not substantially differ between the two age groups, except for the use of intermediate-dose instead of high-dose cytarabine in the consolidation of older participants, this finding might point to age-related differences in disease biology, for example, different co-mutation patterns. The targeted DNA sequencing of diagnostic samples from the trial is in progress, and data will be reported separately.

MRD positivity in blood after two cycles of intensive chemotherapy is known to be a predictor of high relapse rate and inferior outcome.[22,25] In the current analysis, MRD positivity after two cycles of therapy was a highly significant factor for an increased relapse rate; treatment with gemtuzumab ozogamicin as well as age and *DNMT3A* mutation retained their significant effect for cumulative incidence of relapse in a multivariate analysis.

Consistent with previous data,[26] in our study, the *DNMT3A* co-mutation was a significant factor for inferior outcome. This effect was evident for both R882 and non-R882 mutations, but the negative effect was even more pronounced for the R882 hotspot mutation. Subgroup analyses identified the *DNMT3A* co-mutation as a potential predictive biomarker for response to therapy with gemtuzumab ozogamicin. Gemtuzumab ozogamicin exerted a strong beneficial effect on event-free survival (and to a lesser degree also on overall survival) in patients with a *DNMT3A* co-mutation. Gemtuzumab ozogamicin nearly abrogated the significant negative prognostic effect of the *DNMT3A* co-mutation that was evident in the multivariate Cox model. In the ALFA-0701 trial,[14] this effect by *DNMT3A* co-mutational status was not observed. Acute myeloid leukaemia cell lines with the $DNMT3A^{R882}$ mutation compared with $DNMT3A^{WT}$ have been shown to be less sensitive to daunorubicin; however, *DNMT3A* mutational status might not influence the sensitivity of acute myeloid leukaemia cells to other DNA-damaging agents with other mechanisms of action.[27] Whether *DNMT3A*-mutated acute myeloid leukaemia cells might be particularly sensitive to the tumour antibiotic calicheamicin, resulting in DNA double-strand cleavage, is unknown.

The presence of *FLT3* ITD and other signal gene mutations have been proposed as biomarkers for the response to gemtuzumab ozogamicin in the ALFA-0701 trial,[9,14] but not in the MRC AML15 and NCRI AML16 trials.[7,8] Subgroup analyses in our trial did not provide support for a benefit of gemtuzumab ozogamicin in acute myeloid leukaemia with co-occurring *FLT3* ITD, whereas patients without *FLT3* ITD had a significant benefit. However, this finding needs to be interpreted with caution, since the number of patients with concurrent *FLT3* ITD was low in this trial. Overall, *FLT3* ITD was a significant independent prognostic factor for event-free survival and overall survival. The *FLT3* inhibitor midostaurin has been shown to improve outcome in all *NPM1* and *FLT3* ITD genotypes, as defined by 2017 ELN criteria.[28] Whether gemtuzumab ozogamicin might exert an additional beneficial effect in the context of treatment with *FLT3* inhibitors has yet to be shown and is currently being explored (eg, NCT04385290). CD33 expression could not be assessed since they were not systematically measured in our trial. In the initial analysis of the trial, we reported sex-specific differences,

with gemtuzumab ozogamicin showing a beneficial effect on event-free survival only in female but not male participants.[17] This effect on event-free survival was confirmed in the current analysis, whereas no difference in overall survival was found. This differential effect was not observed in previous studies and is elusive.[9,12] No difference in the pharmacokinetics of the components of gemtuzumab ozogamicin based on sex or age have been described.[29]

The development of new precision medicines for *NPM1*-mutated acute myeloid leukaemia is an active field of research.[30] *NPM1*-mutated acute myeloid leukaemia has been shown to be sensitive to chromatin complex inhibition including menin, similar to *KMT2A*-rearranged acute myeloid leukaemia.[31] Based on preclinical results, several menin inhibitors have entered early clinical development with a focus on *KMT2A*-rearranged and *NPM1*-mutant disease. Inhibition of the spleen tyrosine kinase appears to be another approach. Sensitivity to spleen tyrosine kinase inhibition has been linked to *HOXA9* and *MEIS1* overexpression in preclinical studies, a gene signature also found in *NPM1*-mutated acute myeloid leukaemia.

Although the primary endpoints of this trial were not met, the anti-leukaemic effect of gemtuzumab ozogamicin is clearly shown by significantly reducing the relapse rate. The results provide further evidence that the addition of gemtuzumab ozogamicin to intensive chemotherapy should be a standard of care in younger patients with *NPM1*-mutated acute myeloid leukaemia. Considering all available evidence, the tolerability of the combination can be improved by restricting the addition of the antibody–drug conjugate to induction therapy.

Contributors

HD, DW, JK, RFS, AB, and AG contributed to the study conception and design. HD, DW, JK, MS, and AB analysed the data. HD wrote the first draft of the manuscript. HD, DW, JK, AB, and AG wrote the manuscript. All authors collected and interpreted the data, and edited and approved the manuscript. The coordinating investigator (HD) and the project management and statistical team (DW, JK, MS, and AB) have accessed and verified the data, and were responsible for the decision to submit the manuscript. The German–Austrian AML Study Group (AMLSG) Clinical Trial Office and the AMLSG statistical team collected and analysed data in conjunction with all authors.

Declaration of interests

HD declares being in an advisory role for Abbvie, Agios, Amgen, Astellas, AstraZeneca, Berlin-Chemie, Bristol Myers Squibb, Celgene, Daiichi Sankyo, GEMoaB, Gilead, Janssen, Jazz Pharmaceuticals, Novartis, Servier, Stemline, and Syndax; and research funding from Abbvie, Agios, Amgen, Astellas, Bristol Myers Squibb, Jazz Pharmaceuticals, Kronos-Bio, Novartis, and Pfizer. WF declares a membership on an entity's board of directors or advisory committee for AbbVie, Amgen, ARIAD/Incyte, Celgene, Jazz Pharmaceuticals, MorphoSys, Stemline, Clinigen, Novartis, and Pfizer; patents and royalties from Amgen; support for meeting attendance from Amgen, Daiichi Sankyo, Gilead, Jazz Pharmaceuticals, and Servier; and research funding from Amgen and Pfizer. MWMK declares being in an advisory role for Abbvie, Bristol Myers Squibb, Jazz Pharmaceuticals, Kura-Oncology, and Pfizer; speakers honoraria from Abbvie and Gilead; travel support from Abbvie, Celgene, and Daiichi Sankyo; and research funding from Kura-Oncology. TS declares being in an advisory role for Abbvie, Astellas, Celgene, Janssen, Jazz Pharmaceuticals, Novartis, Takeda, Pfizer Bristol Myers Squibb, and Telix Pharma; speakers honoraria from Abbvie, Astellas, Celgene, Janssen, Jazz Pharmaceuticals, and Novartis; research funding from Jazz Pharmaceuticals and Bristol Myers Squibb; and travel support from Jazz Pharmaceuticals and Medac. KM declares being on an advisory role and paid for lectures for Bristol Myers Squibb; and travel support from Amgen, Astellas, Bristol Myers Squibb, Celgene, Jazz Pharmaceuticals, Novartis, Pfizer, and Roche. ML declares being in an advisory role for AbbVie, Astex Pharmaceuticals, Janssen, Pfizer, Otsuka, and Syros; and research funding from Aristopharm, Cheplapharm, Janssen, and TEVA. KG declares being in an advisory role for Abbvie, BMS, and Servier. LF declares being in an advisory role for AbbVie, Amgen, Medac, Novartis, and Takeda; and research funding from Abbvie, Kite, and Speaker´s Bureau for Celgene. EK reports speaker honoraria from Abbvie, Astellas, Celgene/BMS, Jazz, Novartis, and Servier; and being in an advisory or consultancy role for Abbvie, Astellas, Celgene/BMS, Jazz, and Servier. GW reports consultancy for Clinigen and Novartis; and honoraria from Novartis, Gilead, and Takeda. JS reports being in an advisory role and honoraria from AbbVie, Bayer, BMS, Janssen, Novartis, Pfizer, and Sanofi. RG reports being in an advisory role for Abbvie, Astra Zeneca, BMS, Celgene, Daiichi Sankyo, Gilead, Janssen, Merck, MSD, Novartis, Roche, Sanofi, and Takeda. BH reports being in an advisory role for Bristol Myers Squibb, Celgene, Novartis, and Sanofi. UMM reports being in an advisory role for BMS and Sanofi. SM-S reports being in an advisory role for Amgen, BMS/Celgene, Jazz Pharmaceuticals, and Novartis. SK-S reports consultancy for Abbvie, Jazz Pharmaceuticlas, and Pfizer. LB reports being in an advisory role for Abbvie, Amgen, Astellas, Bristol-Myers Squibb, Celgene, Daiichi Sankyo, Gilead, Hexal, Janssen, Jazz Pharmaceuticals, Menarini, Novartis, Pfizer, Sanofi, and Seattle Genetics; and research funding from Bayer and Jazz Pharmaceuticals. FT reports being in an advisory role for Abbvie, Astellas, Bristol Myers Squibb/Celgene, Jazz Pharmaceuticals, Novartis, and Pfizer. MH reports being in an advisory role for Abbvie, BMS/Celgene, Daiichi Sankyo, Jazz Pharmaceuticals, Novartis, Pfizer, Roche, and Tolremo; honoraria from Jazz Pharmaceuticals, Janssen, and Novartis; and research funding to institution from Astellas, Bayer Pharma, BergenBio, Daiichi Sankyo, Jazz Pharmaceuticals, Karyopharm, Novartis, Pfizer, and Roche. PP reports being in an advisory role for Abbvie, Agios, Astellas, Astex Pharmaceuticals, Bristol Myers Squibb, Celgene, Jazz Pharmaceuticals, Novartis, Otsuka Pharma, Pfizer, and Sunesis; speakers bureau for Abbvie, Agios, Astellas, Bristol Myers Squibb, Celgene, Jazz Pharmaceuticals, Novartis, and Pfizer; and travel support for Abbvie, Bristol Myers Squibb, Celgene, Janssen, Novartis, and Takeda. VIG reports being in an advisory role for Abbvie and Pfizer; and speakers bureau for Pfizer and Janssen. RFS reports consulting for or an advisory board membership with Astellas, Daiichi Sankyo, Novartis, and Pfizer; research funding from AstraZeneca, Boehringer Ingelheim, Daiichi Sankyo, Pfizer, PharmaMar, and Roche; and travel, accommodations, and expenses covered by Daiichi Sankyo. KD reports being in an advisory role for Amgen, Bristol Myers Squibb, Celgene, Daiichi Sankyo, Janssen, Jazz Pharmaceuticals, Novartis, and Roche; and research funding from Agios, Astex, Astellas, Bristol Myers Squibb, Celgene, and Novartis. AG reports being in an advisory role for Celgene, JAZZ Pharmaceuticals, and Novartis. All other authors declare no competing interests.

Data sharing

This clinical trial data can be requested (by contacting aml.sekretariat@uniklinik-ulm.de) by qualified researchers who perform rigorous, independent research; data will be provided after the review and approval of a research proposal and statistical analysis plan and execution of a data sharing agreement. Data will be accessible for 12 months, with possible extensions considered.

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