SYNOPSIS CLINICAL STUDY REPORT

according to ICH E3 guideline

version 1-0, 09.02.2024

Randomized comparison between two dose levels of daunorubicin and between one versus two cycles of induction therapy for adult patients with acute myeloid leukemia ≤65 years

Sequential two-part, two-arm unblinded open-label multicenter randomized-controlled phase-III treatment optimization trial

DaunoDouble

Trial Protocol version 5.0, 31.03.2017

including protocol version 4.0, 27.01.2014, protocol version 3.0, 19.11.2013,

protocol version 2.0, 15.08.2013 and protocol version 1.0, 08.08.2013

Sponsor	**Technische Universität Dresden** 01062 Dresden
Principal Coordinating Investigator	**Prof. Dr. med. Christoph Röllig** Medizinische Fakultät Carl Gustav Carus der TU Dresden Medizinische Klinik und Poliklinik I Fetscherstraße 74, 01307 Dresden
Sponsor Code:	TUD-2DAUNO-058
EudraCT-Number:	2013-003191-12
ClinicalTrials.gov Identifier:	NCT02140242
Name of Finished Product and Active Substance	Finished Product: Daunorubicin hydrochloride Active Substance: Daunorubicin hydrochloride

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1 SUMMARY OF TRIAL INFORMATION

Sponsor	**Technische Universität Dresden** 01062 Dresden
Principal Coordinating Investigator	**Prof. Dr. med. Christoph Röllig**
Full Title	Randomized comparison between two dose levels of daunorubicin and between one versus two cycles of induction therapy for adult patients with acute myeloid leukemia ≤65 years
Short Title	DaunoDouble
Trial Protocol	Trial protocol version 1.0, 08.08.2013 (not submitted to authorities) Trial protocol version 2.0, 15.08.2013 (conditional approval by ethic comitee) Trial protocol version 3.0, 19.11.2013 (objection of BfArM; ethic comitee – conditions fulfilled) essential changes: • Addition of the exclusion criteria *"central nervous system manifestation of AML"*. • Addition of description of the process of randomization and induction therapy of part II after required sample size for part I has been reached. ➔ All patients will be treated with the standard treatment DA60 and good responders will be randomized in part II of the trial. Trial protocol version 4.0, 27.01.2014 (study start) essential changes: • The restriction of documentation to CTCAE grade ≥3 in the eCRF has been removed again due to an objection from the higher federal authority. All SARs must be recorded in the patient record and in the CRF (Section 7.2). Trial Protocol version 5.0, 31.03.2017 essential changes: • Randomization in trial part I suspended after results of preplanned interim analysis and offer all patients the standard dose of 60 mg/m^2 daunorubicin in both induction cycles (part I and II of the trial) • Study treatment will be changed – DA90 will be removed, all patients receive the standard dose of 60mg/m^2 daunorubicin (DA60) • According to low study-specific risk due to the reduced daunorubicin dose in part I of the trial, all trial-related risks have been removed from the protocol and there are no intervention-related cardiac risks associated with trial participation. Therefore, visits 2-6; 9-12 and the drop out visit are not necessary and have been removed from visit schedule. In visit 7,8 and 13, the study specific assessments (such as echocardiography, ECG, cardiac markers, bilirubine and creatinine measures) were deleted. • Inclusion age raised to 65 years based on the current German treatment guidelines in which patients up to the age of 65 are considered eligible for intensive induction chemotherapy with DA60 [Onkopedia-Leitlinie 2017]

	• Based on the results of interim analysis of part I of the trial, statistics were updated to reflect the changes in protocol version 5.0 (section 11.0). Protocol version 5.0 includes all amendments to the trial protocol.
Indication	Newly diagnosed or secondary acute myeloid leukemia in adult patients ≤ 65 years of age.
Phase of development	III
Study design	Sequential two-part, two-arm unblinded open-label multicenter randomized-controlled phase-III treatment optimization trial
Objective(s) of the clinical trial	Primary objective(s): **Part I:** To investigate whether a higher dose of daunorubicin in induction chemotherapy leads to an increase in hematological good responders defined as having <5% myeloid blasts on day 15 after start of induction therapy **Part II:** To investigate whether the rate of complete remissions (CR/CRi) after single induction is similar to that after double induction in patients with good response to induction I. Secondary objectives: To investigate whether a higher dose of daunorubicin in induction chemotherapy will lead to an increase in cytogenetic and molecular complete remissions. To investigate whether a higher dose of daunorubicin will lead to improved event-free survival (EFS), relapse-free survival (RFS) and overall survival (OS). To investigate whether EFS, RFS and OS are similar after single versus double induction in patients with good response to induction I. To correlate the level of cytogenetic and molecular minimal residual disease after induction treatment with survival outcomes EFS, RFS and OS.
Endpoints of the clinical trial	Primary Endpoint(s): **Part I:** Rate (percentage) of good responders two weeks after start of induction defined by the presence of <5% myeloid blasts on day 15 after start of IT. **Part II:** Rate (percentage) of complete hematological remissions (CR/CRi) as defined by standard criteria [Döhner 2010] after induction treatment. Secondary Endpoints: Efficacy • Rate of complete molecular and cytogenetic remissions • Event-free survival • Relapse-free survival • Overall survival Safety • Rate of early deaths (2 weeks) and induction deaths (until day 60 or beginning of consolidation treatment – whichever occurs first) • Incidence of serious infectious complications (Grades 3-4 CTCAE V4.0) • Incidence of CTCAE grade ≥3 cardiac complications
Number of patients	Patient registered for trial: 861

	Part I: planned sample size: 436 patients enrolled: 317 patients analysed: 317 Part II: planned sample size: 360 patients enrolled: 376 patients analysed: 376
Studied period	First patient in: 16-Apr-2014 Last patient in: 25-Mar-2022 Last patient last visit: 25-Apr-2022
Inclusion criteria	• Newly diagnosed AML other than acute promyelocytic leukemia (APL) according to WHO criteria, i.e. bone marrow aspirate or biopsy must contain ≥20% blasts of all nucleated cells or differential blood count must contain ≥20% blasts. In acute erythroid leukemia, ≥20% blasts in all non-erythroid bone marrow cells. In AML defined by cytogenetic aberrations, the rate of blasts may be <20%. Secondary AMLs are eligible for inclusion. • Age 18- incl. 65 years • ECOG performance status 0-2 • Adequate liver and renal function as assessed by the following laboratory requirements to be conducted within 7 days prior to screening: o Total bilirubin ≤ 1.5 times the upper limit of normal o ALT and AST ≤ 2.5 times upper limit of normal o Creatinine ≤ 1.5 times upper limit of normal • Adequate cardiac function, i.e. left ventricular ejection fraction (LVEF) of ≥ 50% as assessed by transthoracal two-dimensional echocardiography (“M Mode”) or MUGA scan • Signed Informed Consent • Women must fulfill at least one of the following criteria in order to be eligible for trial inclusion: o Post-menopausal (12 months of natural amenorrhea or 6 months of amenorrhea with Serum FSH > 40 U/ml) o Postoperative (i.e. 6 weeks) after bilateral ovariectomy with or without hysterectomy o Continuous and correct application of a contraception method with a Pearl Index of <1% (e.g. implants, depots, oral contraceptives, intrauterine device – IUD). o Sexual abstinence o Vasectomy of the sexual partner
Exclusion criteria	• Patients who are not eligible for standard chemotherapy as assessed by the treating physician • Cardiac disease: i.e. heart failure NYHA III or IV; unstable coronary artery disease (MI more than 6 months prior to study entry is permitted); serious cardiac ventricular arrhythmias requiring anti-arrhythmic therapy • Central nervous system manifestation of AML

	• Patients undergoing renal dialysis • Chronic pulmonary disease with clinical relevant hypoxia • Known HIV or Hepatitis infection • Uncontrolled active infection • Medical conditions other than AML with an estimated life expectancy below 6 months • Previous treatment of AML except hydroxyurea up to 5 days • Relapsed or primary refractory AML • Acute promyelocytic leukemia • Previous anthracycline-containing chemotherapy • Treatment with any known non-marketed drug substance or experimental therapy within 4 weeks prior to enrollment • Incapability of understanding purpose and possible consequences of the trial • Pregnant or breastfeeding women • Evidence suggesting that the patient is not likely to follow the study protocol (e.g. lacking compliance)
Test product(s)	**Daunorubicin** Dose of administration: Part I - 60 mg/m^2 or 90 mg/m^2 Part II – single or double induction cycle Mode of administration: intravenous infusion Batch number(s): NA, defined only by active substance, commercially available daunorubicin hydrochloride was used in this trial
Concomittant medication	Cytarabine Dose of administration: 100 mg/m^2 Mode of administration: continuous intravenous infusion Batch number(s): NA
Duration of treatment	**Part I** Treatment arm “DA60” Daunorubicin **60** mg/m^2 BSA infusion over 60 minutes days 3-5 Cytarabine 100 mg/m^2 BSA cont. infusion over 24 hours days 1-7 Treatment arm “DA90” Daunorubicin **90** mg/m^2 BSA infusion over 60 minutes days 3-5 Cytarabine 100 mg/m^2 BSA cont. infusion over 24 hours days 1-7 **Part II** Treatment arm “S” **S**ingle Induction: no further induction cycle Treatment arm “D” **D**ouble Induction: second cycle of induction with DA

	○ Treatment arm „DA60“ ➔ Daunorubicin **60** mg/m² BSA infusion over 60 minutes days 3-5 + Cytarabine 100 mg/m² cont. infusion over 24 hours days 1-7 ○ Treatment arm „DA90“ ➔ Daunorubicin **45** mg/m² BSA infusion over 60 minutes days 3-5 because of + Cytarabine 100 mg/m² cont. infusion over 24 hours days 1-7

2 INDIVIDUAL STUDY TABLE

Not applicable.

3 INVESTIGATORS AND TRIAL SITES

No. of Trial Site	**Trial Site**	**Investigator(s)**
Germany		
030	Universitätsklinikum Carl Gustav Carus Dresden Medizinische Klinik und Poliklnik I Fetscherstr. 74 01307 Dresden	Prof. Dr. med. Christoph Röllig
003	Klinikum Nürnberg Nord Medizinische Klinik 5 Prof.-Ernst-Nathan-Str. 1 90419 Nürnberg	Dr. med. Kerstin Schäfer-Eckart
036	Universitätsklinikum Gießen und Marburg GmbH, Standort Marburg Klinik für Innere Medizin Baldinger Straße 35032 Marburg	Prof. Dr. med. Andreas Neubauer
046	Charite Campus Benjamin Franklin Universitätsmedizin Berlin Medizinische Klinik III, Hämatologie/ Onkologie Hindenburgdamm 30 12203 Berlin	Dr. med. Kathrin Rieger
010	Universitätsklinikum Erlangen Medizinische Klinik 5 – Hämatologie und Internistische Onkologie Ulmenweg 18 91054 Erlangen	Prof. Dr. med. Stefan Krause
072	Johann Wolfgang Goethe-Universität Frankfurt am Main Medizinische Klinik II Theodor-Stern-Kai 7 60590 Frankfurt am Main	Dr. med. Björn Steffen
068	Uniklinik RWTH Aachen Medizinische Klinik IV Klinik für Onkologie, Hämatologie und Stammzelltransplantation	Dr. med. Edgar Jost

No. of Trial Site	Trial Site	Investigator(s)
	Pauwelsstr. 30 52074 Aachen	
013	Klinikum Altenburger Land GmbH Klinik für Innere Medizin/ Hämatologie/ Onkologie/ Nephrologie/ Endokrinologie/ Diabetologie Am Waldessaum 10 04600 Altenburg	Dr. med. Armin Schulz-Abelius
012	Sozialstiftung Bamberg Klinikum am Bruderwald Medizinische Klinik V Buger Str. 80 96049 Bamberg	Dr. med. Martina Teichmann
048	Klinikum Bielefeld Klinik für Hämatologie, Onkologie und Palliativmedizin Teutoburger Str. 50 33604 Bielefeld	Dr. med. Martin Görner
205	Augusta Kliniken Bochum Hattingen Klinik für Hämatologie, Onkologie & Palliativmedizin Bergstr. 26 44791	Prof. Dr. med. Dirk Behringer
039	Ev. Diakonie-Krankenhaus gGmbH Medizinische Klinik II Abteilung Hämatologie und Onkologie Gröpelinger Heerstr. 406/408 28239 Bremen	Dr. med. Johannes Kullmer
014	Klinikum Chemnitz GmbH Küchwald Krankenhaus Klinik für Innere Medizin III Bürgerstr. 2 09113 Chemnitz	PD Dr. med. Mathias Hänel
118	Krankenhaus Düren gem. GmbH Klinik für Hämatologie und Internistische Onkologie, Palliativmedizin Roonstr. 30 52351 Düren	PD Dr. med. Michael Flaßhove
671	Marienhospital Düsseldorf GmbH Klinik für Onkologie, Hämatologie und Palliativmedizin Rochusstr. 2 40479 Düsseldorf	Prof. Dr. med. Aristoteles Giagounidis
054	Universitätsklinikum Essen Klinik für Hämatologie Hufelandstr. 55 45122 Essen	PD Dr. med. Richard Noppeney
066	Universitätsklinikum Halle (Saale) Klinik und Poliklinik für Innere Medizin IV	Prof. Dr. med. Christine Dierks

No. of Trial Site	Trial Site	Investigator(s)
	Onkologie/ Hämatologie/ Hämostaseologie Ernst-Grube-Str. 40 06120 Halle (Saale)	
016	Universitätsklinikum Heidelberg Medizinische Klinik, Abteilung Innere Medizin V Im Neuenheimer Feld 410 69120 Heidelberg	Prof. Dr. med. Alwin Krämer
002	Westpfalz-Klinikum GmbH INN 1 Hellmut-Hartert-Str. 1 67655 Kaiserslautern	Prof. Dr. med. Gerhard Held
227	Gemeinschaftsklinikum Mittelrhein GmbH Klinik für Innere Medizin Johannes-Müller-Str. 7 56068 Koblenz	Dr. med. Dirk Niemann
007	Universitätsklinikum Münster Medizinische Klinik und Poliklinik A Albert-Schweitzer-Str. 33 48149 Münster	Prof. Dr. med. Christpoh Schliemann
018	Diakonie-Klinikum Schwäbisch Hall gGmbH Klinik für Innere Medizin III Stammhausstr. 8 74523 Schwäbisch Hall	Dr. med. Thomas Geer
008	Robert-Bosch-Krankenhaus Innere Klinik II, Hämatologie/ Onkologie Auerbachstr. 110 70376 Stuttgart	Dr. med. Martin Kaufmann
117	Rems-Murr-Klinikum Winnenden Klinik für Hämatologie, Onkologie und Palliativmedizin Am Jakobsweg 1 71364 Winnenden	Dr. med. Julia Glück-Wolf
067	Universitätsklinikum Jena Klinik für Innere Medizin II, Hämatologie und internistische Onkologie Erlanger Alle 101 07740 Jena	Prof. Dr. med. Sebastian Scholl
032	St. Bernward Krankenhaus Medizinische Klinik III Onkologie/ Hämatologie/ Immunologie Treibstr. 9 31134 Hildesheim	Prof. Dr. med. Ulrich Kaiser
128	Städtisches Klinikum Kiel GmbH 2. Medizinische Klinik Hämatologisch-onkologische Ambulanz Chemnitzstr. 33 24116 Kiel	Dr. med. Sebastian Buske

No. of Trial Site	Trial Site	Investigator(s)
095	Klinikum Augsburg II. Medizinische Klinik Stenglinstr. 2 86156 Augsburg	PD Dr. med. Andreas Rank
071	Helios Klinikum Berlin-Buch Klinik für Hämatologie und Stammzelltransplantation Schwanebecker Chaussee 50 13125 Berlin	Dr. med. Judith Niederland
005	Evangelisches Krankenhaus Hamm gGmbH (EVK Hamm) Facharztzentrum (FAZ) Werler Str. 110 59063 Hamm	Dr. med. Heinz Albert Dürk
009	Asklepios Klinik St. Georg Hamburg Hämatologie, Onkologie und Stammzelltransplantation Lohmühlenstr. 5 20099 Hamburg	Dr. med. Holger Hauspurg
775	Carl-Thiem-Klinikum Cottbus gGmbH Klinik für Hämatologie und Onkologie Thiemstr. 111 03048 Cottbus	PD MD Martin Schmidt-Hieber
Czechia		
040	LF Masarykovy univerzity a Fakultni nemocnice Brno Interni hematologicka onkologicka klinika Jihlavska 20 62500 Brno	Prof. MD Jiri Mayer
747	Fakultní nemocnice Olomouc I.P. Pavlova 185/6 779 00 Olomouc	Doc. MD Tomas Szotkowski
043	Fakultní nemocnice Královské Vinohrady (FN), Praha Dept. of Clinical Hematology Srobarova 50 10034 Praha 10	MD Jan Novak
622	Ústav hematologie a krevní transfuze (ÚHKT), Praha U Nemocnice 2094/1, 12820 Praha 2	MU MD Jolana Mertova
392	Faculty Hospital Hradec Králové II. Clinic of international medicine, Department of Hematology Sokolska 581 50005 Hradec Králové	Doc. MUDr. Pavel Zak

4 METHODOLOGY

It was a prospective, sequential two-part, two-arm unblinded open-label multicenter randomized-controlled phase-III treatment optimization trial with one adaptive interim analysis in each part.

The DaunoDouble-trial was designed to investigate whether a higher dose of daunorubicin in induction chemotherapy leads to an increase in hematological good responders defined as having <5% myeloid blasts on day 15 after start of induction therapy and whether the rate of complete remissions (CR) after single induction is similar to that after double induction in patients with good response to induction I. The Treatment takes place in 2 parts of study and 2 parallel groups in each part.

4.1 COURSE OF CLINICAL TRIAL

4.1.1 STUDY INCLUSION / RANDOMIZATION

Younger patients between the ages of 18 and 65 years with newly diagnosed acute myeloid leukemia except acute promyelocytic leukemia (APL) should have been treated in the trial.

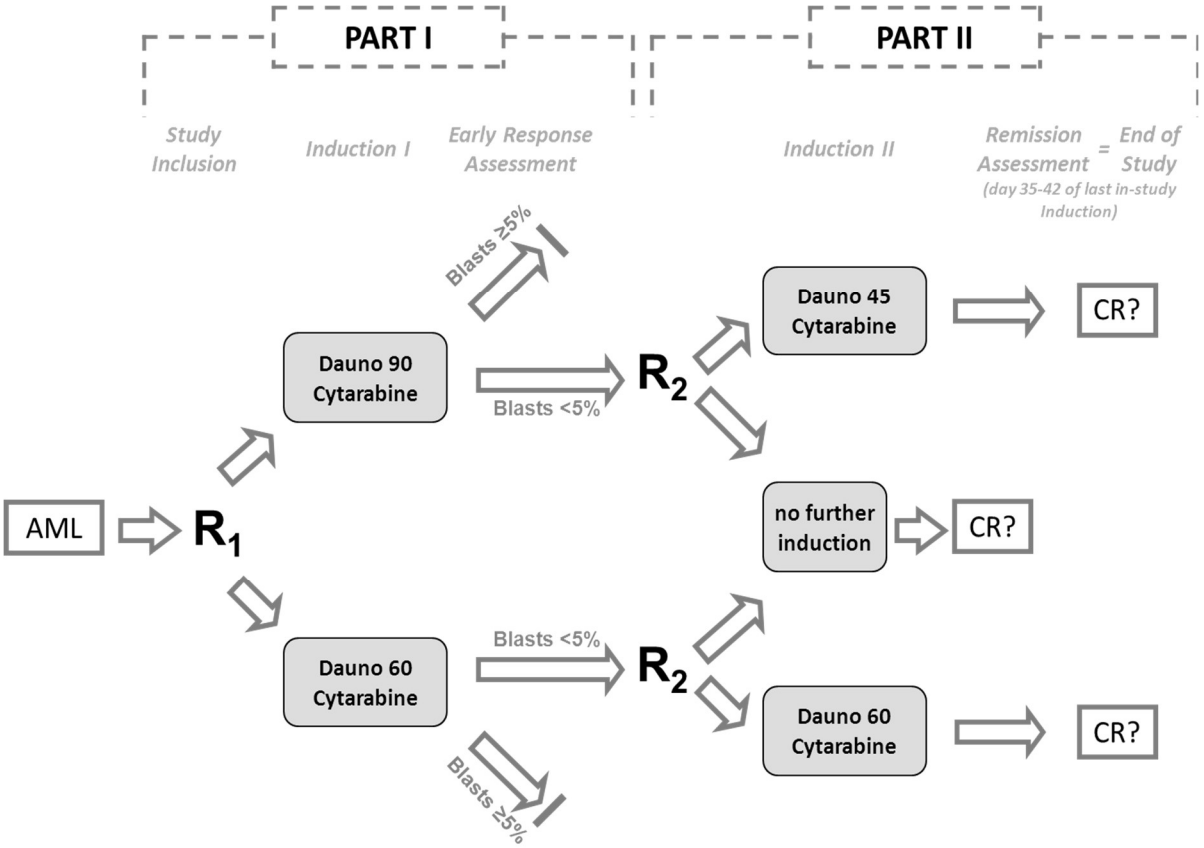


Figure 1 Study design and Flow Chart

Patient recruitment

Study inclusion occurred after the initial diagnosis of AML, the inclusion and exclusion criteria were checked and the study was included with subsequent randomization/registration in part I of the trial.

Trial Part I:

All patients that were eligible to the trial were randomized into the trial by faxing the patient registration form to the SAL Studienzentrale from the participating study centers and later directly in the electronical case report form (eCRF).

After the results of the interim analysis of part I and suspension of randomization to that trial part there was only a registration in part I after check of the inclusion and exclusion criteria.

Trial Part II:

After response evaluation to induction I was done by marrow assessment on day 15, all patients displaying <5% myeloid blasts were qualified for part II of the trial and proceeding to randomization. This was also done by faxing the patient registration form to the SAL Studienzentrale from the participating study centers and later directly in the electronical case report form (eCRF).

Randomization

Trial Part I: Randomization I

Allocation to the two study arms took place in a ratio of 1:1 after checking eligibility criteria for the study and patient registration. Patients were randomly assigned to one of the treatment arms, using a block randomization scheme with variable block length. The use of randomization blocks guarantees roughly equal numbers of patients in each treatment arm at any timepoint in trial conduct. The Data Center of the SAL Studienzentrale was responsible for the randomization process. Eligible study patients were randomized by consecutive entry into the randomization list.

Trial Part II: Randomization II

Response to induction I was assessed by marrow assessment on day 15. All patients displaying <5% myeloid blasts qualified for part II of the trial and proceeding to randomization II.
Randomization II was stratified according to the daunorubicin dose received in induction I and adverse cytogenetic risk defined by the presence of one or more of the following criteria:

- inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1
- t(6;9)(p23;q34); DEK-NUP214
- t(v;11)(v;q23); MLL rearranged
- -5 or del(5q); -7; abnl(17p); complex karyotype (Three or more chromosome abnormalities in the absence of one of the WHO designated recurring translocations or inversions, that is, t(15;17), t(8;21), inv(16) or t(16;16), t(9;11), t(v;11)(v;q23), t(6;9), inv(3) or t(3;3)).

Treatment assignment was not blinded. The randomization result was noted in the patient file and in the trial documentation forms.

4.1.2 STUDY TREATMENT

Part I

- Experimental Arm – Induction with high-dose daunorubicin (**DA90**)

Induction treatment should commence within 24 hours after reception of the randomization fax. Induction consisted of cytarabine in combination with 90 mg daunorubicin:

Daunorubicin	**90** mg/m^2 infusion over 60 minutes	days 3-5

Cytarabine	100 mg/m^2 cont. infusion over 24 hours	days 1-7

- Control Arm – Induction with standard-dose daunorubicin (**DA60**)

Induction treatment should commence within 24 hours after reception of the randomization fax. Induction I consisted of cytarabine in combination with 60 mg daunorubicin:

Daunorubicin	**60** mg/m^2 infusion over 60 minutes	days 3-5
Cytarabine	100 mg/m^2 cont. infusion over 24 hours	days 1-7

Part II

Patients defined as good responders to IT I (<5% blasts in bone marrow, see 5.4.2) were eligible for continuation of study treatment and proceeding to part II of the trial. These patients were randomized 1:1 into the experimental arm S or the control arm D.

- Experimental Arm – Single Induction (Arm **S**)

Patients did not receive a second cycle of induction.

Remission assessment was performed after regeneration of peripheral blood count latest on day 42 after start of the induction I.

- Control Intervention – Double Induction (Arm **D**)

Patients received a second cycle of induction if no signs of significant cardiac damage or cardiac insufficiency were present. Cytarabine dose was identical to induction I. The daunorubicin dose depended on induction I.

Second induction was to commence earliest on day 22 after the start of the first induction. It was allowed to postpone the second induction course if the patient had an uncontrolled infection or transitory contraindications against chemotherapy. The second course of induction could be started once these problems had been resolved, but not later than on day 35 of induction I.

- **Second cycle of induction with DA45**

Patients received **DA90** as first induction and received therefore **DA45 as IT II**:

Daunorubicin	**45** mg/m^2 infusion over 60 minutes	days 3-5
Cytarabine	100 mg/m^2 cont. infusion over 24 hours	days 1-7

- **Second cycle of induction with DA60**

Patients received **DA60** as first induction and received therefore **DA60 as IT II**:

Daunorubicin	**60** mg/m^2 infusion over 60 minutes	days 3-5
Cytarabine	100 mg/m^2 cont. infusion over 24 hours	days 1-7

Remission assessment was performed after regeneration of peripheral blood count latest on day 42 after start of the induction II.

Study treatment ended with the last dose of cytarabine of the last induction cycle according the allocated treatment arm. Further treatment (conventional cytarabine-based consolidation, myeloablative or dose-reduced conditioning followed by allogeneic or autologous stem cell transplantation) was performed at the discretion of the treating physician outside the DaunoDouble trial.

4.1.3 STUDY TREATMENT AFTER INTERIM ANALYSIS PART I (PROTOCOL VERSION 5-0, 31. MARCH 2023)

Results of interim analysis part I

The results of a preplanned interim analysis of randomization part I of the trial showed a difference of 42% (95%-CI, 33-52) good early responses after DA60 versus 47% (95%-CI, 39-59) after DA90 (p=0.341). Based on the observe data, the sample size for stage 2 of part I was recalculated. A total number of 1936 patients (968 per arm) would have to be recruited to stage 2 to be able to reject the null hypothesis, given the observed effect. Providing a recruitment capacity of the trial sites of around 120 patients per year, this would result in a remaining enrollment period of 16 years.

These results and the safety reporting of the trial up to the stage of interim analysis were discussed with the protocol committee and the coordinating investigator. The results from safety assessment for the comparison of the two trial arms (DA60 vs. DA90) showed no difference in the incidence of SAE and no differences in kinetics of cardiac markers. There was no difference in the overall incidence of deaths between both study arms (DA60: 9 vs. DA90: 10). The early death rate (8 weeks) was very low (3.4%) with slightly more cases in the DA90 arm (DA60: 3 patients (2.3%) vs. DA90: 6 patients (4.6%)). The protocol committee and coordinating investigator agreed that

1. There is no obvious relevant excess of risk for the experimental arm (DA90).
2. The observed difference in early response between the two arms is neither significant nor clinically meaningful. Given the current recruitment rate, it would require another 194 months (16 years) to recruit the number of patients needed to show statistical significance for the difference of 7%.

As a result of the discussion, the sponsor decided to suspend randomization in trial part I and to offer all patients the standard dose of 60 mg/m^2 daunorubicin in both induction cycles (part I and II of the trial). This protocol version contains the respective amended changes.

Treatment in Part I

Due to results of the interim analysis I (see above/section 4.1.3) the 90 mg/m^2 daunorubicin dose in part I of the trial was suspended.

All patients received the standard treatment with a 60 mg/m^2 dose of daunorubicin after the registration in the DaunoDouble-trial.

Induction I consisted of cytarabine in combination with 60 mg daunorubicin:

Daunorubicin	**60** mg/m^2 infusion over 60 minutes	days 3-5
Cytarabine	100 mg/m^2 cont. infusion over 24 hours	days 1-7

Treatment in Part II

- Experimental Intervention – Single Induction (Arm **S**):

Patients did not receive a second cycle of induction.

Remission assessment was performed after regeneration of peripheral blood count latest on day 42 after start of the induction I.

- Control Intervention – Double Induction (Arm **D**):

Patients received a second cycle of induction if no signs of significant cardiac damage or cardiac insufficiency were present. Cytarabine and daunorubicine dose was identical to induction I.

Second induction was to commence earliest on day 22 after the start of the first induction. It was allowed to postpone the second induction course if the patient had an uncontrolled infection or transitory contraindications against chemotherapy. The second course of induction could be started once these problems had been resolved, but not later than on day 35 of induction I.

Daunorubicin	60 mg/m^2 infusion over 60 minutes	days 3-5
Cytarabine	100 mg/m^2 cont. infusion over 24 hours	days 1-7

Remission assessment was performed after regeneration of peripheral blood count latest on day 42 after start of the induction II.

Study treatment ends with the last dose of cytarabine of the last induction cycle according the allocated treatment arm. Further treatment (conventional cytarabine-based consolidation, myeloablative or dose-reduced conditioning followed by allogeneic or autologous stem cell transplantation) was performed at the discretion of the treating physician outside the DaunoDouble trial.

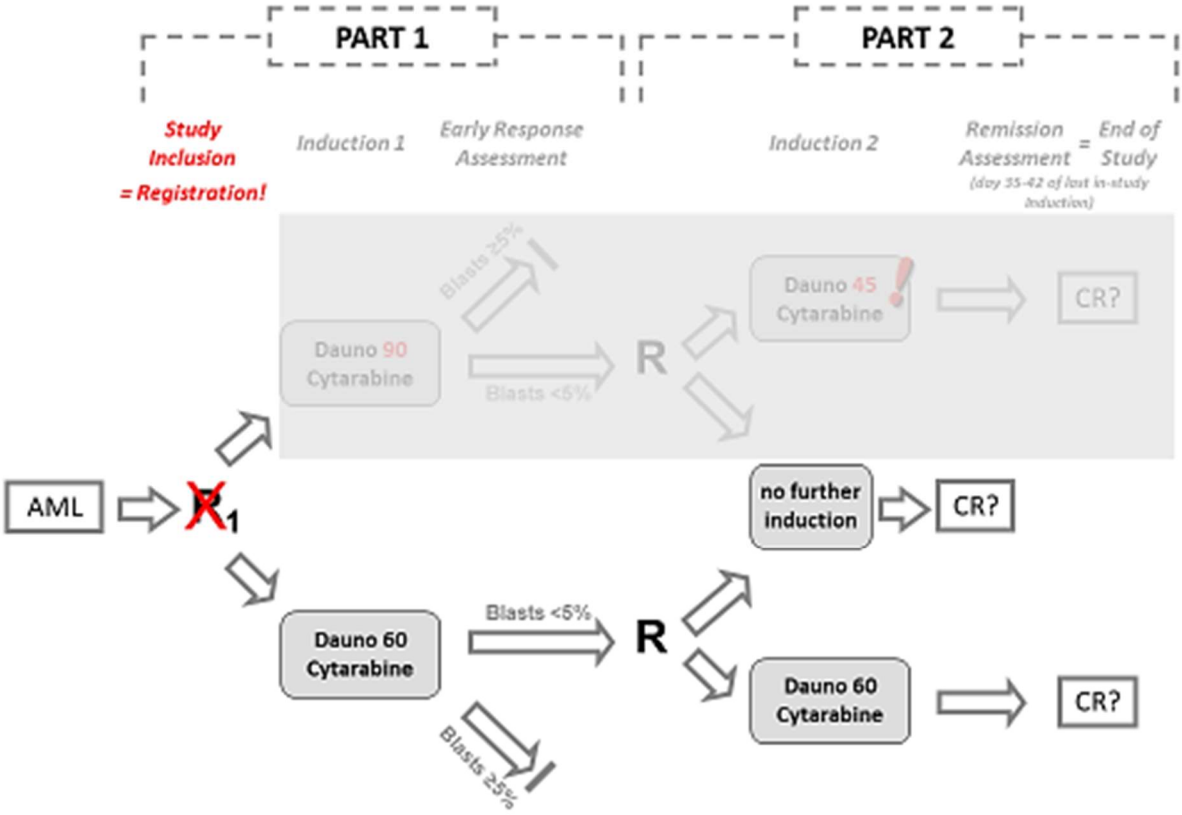


Figure 2 Study Design and Flow Chart after Interim Analysis Part I

4.2 ASSESSMENT OF EFFICACY

4.2.1 EARLY RESPONSE ASSESSMENT

The early response assessment was done on day 15 of the induction I and defined as follows.

Good response:

Reduction in bone marrow blast count to below 5% (aspirate with marrow spicules)

Moderate response:

Reduction in bone marrow blast count, blast count ≥5% (aspirate with marrow spicules)

Refractory disease:

- Increase in bone marrow blast count compared to baseline or
- No change in bone marrow cellularity with unchanged blast count

For the conduct of this study, the response categories moderate response and refractory disease were put in the category "**suboptimal response**" characterizing patients not eligible to continue the trial.

4.2.2 REMISSION ASSESSMENT

Remission assessment followed standard criteria according to Döhner et al., 2010 and was done 26-42 days after start of final induction.

For **remission assessment**, a bone marrow aspirate (or bone marrow biopsy) was performed 35 days after the beginning of the final in-study induction. If platelet and ANC counts were in regeneration but below the CR threshold on day 35 (ANC ≥ 1000/µL and platelets ≥ 100,000/µL, local laboratory), a postponement of remission assessment was possible to day 42 the latest.

If remission assessment showed **< 5%** myeloid blasts in bone marrow and no blasts in peripheral blood and if Auer Rods were absent and if there were no signs of extramedullary disease and if there was no need for red cell transfusions, the patient had achieved a CR/CRi.

If remission assessment revealed **≥ 5%** myeloid blasts, the patient was classified as treatment failure displaying resistant disease.

4.3 ASSESSMENT OF SAFETY

Regular safety assessment ensured that patients had no or minimal study related risk. The following parameters were used for safety assessment:

- Rate of early deaths (2 weeks) and induction deaths (until day 60 or until the beginning of consolidation treatment – whichever occurs first)
- Incidence of serious infectious complications (Grades 3-4 CTCAE V4.0)
- Incidence of CTCAE grade ≥3 cardiac complications

Severity of adverse events and serious adverse events had to be graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events V4.0 (CTCAE) for Cancer Clinical Trials.

4.3.1 DOCUMENTATION OF AES

AEs needed to be documented from signature of the informed consent until 28 days after the last dose of daunorubicin administered in the context of this trial.
All adverse events had to be documented in the participant's chart (source data) and in the eCRF.

Protocol-specific clarifications to the definition of an adverse event:

Therapy-related cytopenia as sign of an intended antileukemic activity was by definition no adverse event. However, complications of such cytopenia (i.e. neutropenic infection, thrombocytopenic bleeding) did constitute an AE.

In order to monitor the safety of the trial participants throughout the trial, untoward medical occurrences between signature of the informed consent form and first administration of the investigational medicinal product also had to be documented as adverse events.

A pathological finding, improved or unchanged in comparison to baseline, did not constitute an adverse event.

Symptoms of the disease under study should not be classified as AEs as long as they were within the normal day-to-day fluctuation. Worsening of the underlying disease or other pre-existing conditions were recorded as an AE.

Abnormal laboratory values without therapeutic consequences were not documented on the AE form. Instead, they were documented on the laboratory values form in the eCRF only.

4.3.2 DOCUMENTATION OF SAES

Any SAE had to be reported immediately (within 24 hrs.) to the sponsor's safety desk by use of a separate SAE form and had to be documented on the AE page, in the eCRF.

As mentioned above, hematological toxicities as signs of an effective antileukemic treatment and an intended myelosuppression did not fulfill the definition of an AE and therefore cannot constitute an SAE.

Study specific reporting rules:

Death of any cause including death from AML progression or relapse constituted an SAE in this trial and must have been reported within the time lines of expedited reporting.

Leukemia-associated serious adverse events were excluded from expedited reporting on this protocol, but must have been documented in the source data and the CRF and were extracted from there into the SAE data base. The events excluded from the time lines of expedited reporting are:

- fever resulting from AML progression
- infections resulting from AML progression
- bleeding resulting from AML progression
- hospitalization resulting from AML progression

The following **signs of hematotoxicity/myelosuppression** were intended and expected events during treatment of AML, did therefore not fulfill criteria of adverse events and did not need to be documented as AEs or SAEs:

- leukopenia
- thrombocytopenia
- anemia.

In contrast to events related to AML progression and signs of hematotoxicity/myelosuppression, **myelosuppression-associated complications** and related hospitalizations must have been documented as SAE, but were excluded from expedited reporting. These complications can be: Grade ≥3 fever and infections resulting from leukopenia or bleeding resulting from thrombopenia following chemotherapy administered in the context of this trial.

4.4 END OF TRIAL AND FURTHER TREATMENT OF THE PARTICIPANTS

4.4.1 PART I

Patients with **≥5%** marrow blasts or no evaluable bone marrow at early response assessment on day 15 of induction I were classified as **suboptimal** responders and went off study. For these patients and other patients who dropped out during trial part I for any reason, either early response assessment was constituted the regulatory end of study or the time point they went off. Further treatment was continued outside this clinical trial at the discretion of the treating physician.

All patients with marrow blasts **<5%** at early response assessment on day 15 of induction I were classified as **good** responders and should have been randomized into treatment arms S or D. The time point for the end-of-study visit was defined according to the treatment arms.

4.4.2 PART II

Treatment arm S

Study treatment was completed by day 7 of induction I. The assessment of remission 26-42 days after the start of induction I constituted the regulatory end of study (visit 13) and should have been done as previously described (see 4.2.2).

For patients who left the trial or who removed from study after randomization but before remission assessment on day 26-42 of induction I, further induction treatment outside the trial should have been documented in the SAL-AML registry (provided that patient gave corresponding written informed consent) and remission assessment should have been done at the end of induction treatment according to routine practice. The final remission assessment was documented in the eCRF (Drop out visit) for evaluation of the primary endpoint of all enrolled patients.

Treatment arm D

Study treatment was completed by day 7 of induction II. The assessment of remission 26-42 days after the start of induction II constituted the regulatory end of study (end of study visit) and should done as previously described (see 4.2.2).

All patients received further treatment (e.g. consolidation, allogeneic stem cell transplantation) outside this trial according to the discretion of the treating physician, either inside or outside a clinical trial. Consolidation and other further treatment should have been followed evidence-based treatment guidelines, preferentially of the SAL study group. This should be documented in the SAL-AML registry (provided that patient gave corresponding written informed consent).

5 STATISTICAL METHODS

5.1 ANALYSIS POPULATIONS

Statistical analyses were conducted in the following analysis sets:

5.1.1 TRIAL PART 1

Full analysis set (FAS): The FAS consisted of all patients that were randomised in part 1 and not excluded according to principles outlined in the ICH E9 guideline[4] section 5.2.1.

Safety evaluation set (SES): The SES consisted of all patients of the FAS that received at least one dose of daunorubicin.

Per protocol set (PPS): The PPS consisted of all patients of the FAS that received at least 80% of the planned cumulative dose of daunorubicin and were evaluated for early response at days 14, 15, or 16 of the first induction cycle.

Deviating from the statistical analysis plan an additional ITT population was defined post-hoc to be able to analyse all patients who received their first induction within the trial. This ITT population consists of all FAS patients (randomized) plus patients who received DA60 after the protocol amendment following the interim analysis for trial part 1 (non-randomized). After the interim analysis the DA90 arm was stopped and standard treatement was declared DA60 for all subsequent patients.

5.1.2 TRIAL PART 2

Full analysis set (FAS): The FAS consisted of all patients that were randomised in part 2 and not excluded according to principles outlined in the ICH E9 guideline[4] section 5.2.1.

Safety evaluation set (SES): The SES consisted of all patients of the FAS that received at least one dose of daunorubicin.

Per Protocol Set (PPS): The PPS consisted of all patients of the FAS that met all of the following criteria:

- D15 blast count < 5% (good response after induction 1)
- No second induction cycle in arm S before remission control
- Second induction cycle in arm D before remission control
- Application of cytarabine and daunorubicin in the second cycle of arm D
- Applied doses of daunorubicine not lower than 90% and not higher than 110% of the planned doses in second cycle of arm D (except patients with dose-capping due to body surface of > $2m^2$, which is defined as valid deviation)
- Remission control not earlier than d26 of last induction cycle and no missing result of remission control
- No application of liposomal daunorubicine

5.2 EVALUATION OF PRIMARY VARIABLE

5.2.1 TRIAL PART 1

The final primary analysis was conducted in the FAS.

Absolute number of subjects with good response and percentage was presented for the entire FAS and the subgroup of subjects who were not part of the interim analysis. Two-sided 95% Clopper-Pearson confidence intervals were calculated for the proportion of good responders per treatment arm.

The proportions in the subgroup of patients who were not part of the interim analysis were compared with the uncorrected Chi-squared test. The p-value of this analysis was multiplied by the p-value of the interim analysis (p = 0.341) and compared to the Fisher criterion of 0.0087, according to the planned procedure of Bauer and Köhne.

The null hypothesis of no difference between the treatment arms would have been rejected, if the product of both p-values is < 0.0087.

This analysis was the only confirmatory analysis of this part of the trial.

As sensitivity analysis a multivariable logistic regression model was fitted with dependent variable 'good response' and independent variables:

- 'randomized treatment arm',
- 'cytogenetic risk group',
- 'age',
- 'NPM1-mutation',
- 'FLT3-ITD mutation low/high',
- 'interaction of NPM1-mutation and FLT-ITD mutation low/high'.

A supportive analysis was conducted in the PPS.

Absolute number of subjects with good response and percentage was presented for the entire PPS. Two-sided 95% Clopper-Pearson confidence intervals was calculated for the proportion of good responders per treatment arm.

The proportions in the PPS was compared with the uncorrected Chi-squared test.

A logistic regression was conducted as described above in the FAS-sensitivity analysis.

5.2.2 TRIAL PART 2

The primary analysis was conducted in the PPS, since the primary test is a non-inferiority test.

To test the null hypothesis H_0: $\varepsilon \leq d$ (d is the non-inferiority margin of – 0.075; ε is the observed difference of the CR/CRi proportions in arm S and arm D ($p_S - p_D$)), the test proposed by Farrington and Manning (1990) to compare binomial trials with null hypotheses of non–zero risk difference was applied to the subgroup of patients that were not part of the interim analysis. The p-value of this analysis is multiplied by the p-value of the interim analysis (p = 0.145) and compared to the Fisher criterion of 0.0087. The null hypothesis of difference between the treatment arms could have be rejected, if the product of both p-values is < 0.0087. This corresponded to a local significance level of 0.059984 for this hypothesis test.

This analysis was the only confirmatory analysis of this part of the trial.

The test was calculated in the R statistical environment using the testBinomial-function of the gsDesign package.

Code for conduct of the hypothesis test:

```
Z <- testBinomial(x1=…,n1=…,x2=…,n2=…, delta0 = 0.075, chisq = 0)
```

Calculation of p-value:

```
pval <- pnorm(Z)  # calculate p-value
```

Calculation of proportions, difference of proportions and confidence interavals:

```
p1 <- x1/n1

p2 <- x2/n2

pdiff <- p1 – p2 # difference of proportions

se <- sqrt((p1 * (1-p1)/n1) + (p2 * (1-p2)/n2)) # standard error

ci.lo <- pdiff – 1.96*se   # lower confidence interval limit

ci.up <- pdiff + 1.96*se   # upper confidence interval limit
```

With n1 = number of patients in reference arm D, n2 = number of patients in experimental arm S, x1 = number of patients who failed to achieve CR/CRi in reference arm D, x2 = number of patients who failed to achieve CR/CRi in experimental arm S.

5.3 EVALUATION OF SECONDARY VARIABLES

5.3.1 OVERALL HEMATOLOGIC REMISSION RATE (ORR)

This endpoint was analysed in the FAS. Absolute and relative frequencies and two-sided 95% Clopper-Pearson confidence intervals were presented for the following groups:

- Patients randomized to receive 60 mg daunorubicin in induction 1 (arm 60)
- Patients randomized to receive 90 mg daunorubicin in induction 1 (arm 90)
- Patients randomized to receive 2 induction cycles (arm D)
- Patients randomized to receive only 1 induction cycle (arm S)
- And all groups created by crossing the two randomization results daunorubicin dose and number of induction cycles (only patients randomized for both trial parts)
- Patients who received 60 mg daunorubicin in induction 1 after stop of randomization to trial part 1 and that were randomized to receive 2 induction cycles (includes only patients with good response)
- Patients who received 60 mg daunorubicin in induction 1 after stop of randomization to trial part 1 and that were randomized to receive 1 induction cycle (includes only patients with good response)

A logistic regression model was fitted to estimate the odds ratio for achieving a CR/CRi for 90 mg daunorubicin compared to 60 mg daunorubicin. The following adjusting variables were also included in the model:

- Induction response (blast count < 5%) (yes / no)
- Age in years
- Cytogenetic risk group (fav, int, adv)
- NPM1 mutation (no, yes)
- FLT3-ITD mutation (no, yes)
- NPM1 by FLT3-ITD interaction term
- Response by treatment arm (60 vs. 90) interaction term
- Single vs. double induction

The model was fitted with the patients randomized in part 1 and as sensitivity analysis also including patients that received 60 mg daunorubicin without randomization after termination of the first study part (for investigation whether the randomized sample differs from patients included without randomization after closing of trial part 1). Cases with missing values in adjusting variables were excluded from this analysis.

Another logistic regression model was fitted to estimate the odds ratio for achieving a CR/CRi after a single induction cycle vs. double induction. Only patients randomized in part 2 were included in this analysis. The model included the following adjusting variables:

- Age in years
- Cytogenetic risk group (fav, int, adv)

- NPM1 mutation (no, yes)
- FLT3-ITD mutation (no, yes)
- NPM1 by FLT3-ITD interaction term
- 60 mg vs. 90 mg daunorubicin in induction 1
- 60 mg vs. 90 mg daunorubicin in induction 1 by single vs. double induction interaction term

5.3.2 SURVIVAL

Overall survival (OS):

Overall survival was analysed using the Kaplan-Meier method. Median survival and survival probabilities at 1, 2, 3, and 5 years with two-sided 95% confidence intervals were estimated for the following groups:

- Patients randomized to receive 60 mg daunorubicin in induction 1 (arm 60)
- Patients randomized to receive 90 mg daunorubicin in induction 1 (arm 90)
- Patients randomized to receive 2 induction cycles (arm D)
- Patients randomized to receive only 1 induction cycle (arm S)
- And all groups created by crossing the two randomization results daunorubicin dose and number of induction cycles (only patients randomized for both trial parts)
- Patients who received 60 mg daunorubicin in induction 1 after stop of randomization to trial part 1 and that were randomized to receive 2 induction cycles (includes only patients with good response)
- Patients who received 60 mg daunorubicin in induction 1 after stop of randomization to trial part 1 and that were randomized to receive 1 induction cycle (includes only patients with good response)

A Cox regression model was fitted to estimate the hazard ratio for OS for 90 mg daunorubicin compared to 60 mg daunorubicin. The following adjusting variables were also included in the model:

- Response (blast count < 5%) (yes / no)
- Age in years
- Cytogenetic risk group (fav, int, adv)
- NPM1 mutation (no, yes)
- FLT3-ITD mutation (no, yes)
- NPM1 by FLT3-ITD interaction term
- Response by treatment arm (60 vs. 90) interaction term

The model was fitted with the patients randomized in part 1 and as sensitivity analysis also including patients that received 60 mg daunorubicin without randomization after termination of the first study part (for investigation whether the randomized sample differs from patients included without randomization after closing of trial part 1). Cases with missing values in adjusting variables were excluded from this analysis.

Another Cox regression model was fitted to estimate the hazard ratio for OS for single vs. double induction. Only patients randomized in part 2 were included in this analysis. The following adjusting variables were also included in the model:

- Age in years
- Cytogenetic risk group (fav, int, adv)
- NPM1 mutation (no, yes)
- FLT3-ITD mutation (no, yes)
- NPM1 by FLT3-ITD interaction term
- 60 mg vs. 90 mg daunorubicin in induction 1
- 60 mg vs. 90 mg daunorubicin in induction 1 by single vs. double induction interaction term

Relapse-free survival (RFS):

Relapse-free survival was analyzed as described for overall survival.

Event-free survival (EFS):
Event-free survival was analyzed as described for overall survival.

5.4 HANDLING OF DROP-OUTS AND MISSING VARIABLES

5.4.1 PRIMARY VARIABLES

Trial part 1 - Number of good responders after induction 1

Number of good responders after induction 1 was a dichotomous endpoint. Patients who achieve a good response as defined by marrow blasts <5% at early response assessment on day 15 of induction are defined as good responders.

The following clinical scenarios constituted failures to achieve a good response:

- D15 marrow blast count >= 5%
- Patients who die during induction or before day 15
- Patients with no evaluable marrow material, i.e. no puncture done or no aspiration possible (punctio sicca) or no marrow spicules and no evaluable histology or puncture material not evaluable for technical reasons
- Patients excluded from study before response assessment on day 15

Blasts of d15 could be recorded in visit 7 or, in case of early termination of study treatment in the dropout visit. Blast counts were analyzed locally and in the central laboratory. The decision about response was usually done by the investigators based on local values.

If d15 local blast count was missing, it was imputed with the central d15 blast count. If d15 blast count was still missing it was imputed with the d15 blast count value recorded in dropout visit, if the patient terminated study treatment before visit 7. If the d15 local blast count at dropout visit was missing it was imputed with the central blast count at dropout visit.

For a sensitivity analysis and for comparisons with historical data a response variable using a threshold of <10% was derived in the above described manner.

Trial part 2 – Rate of complete remissions after induction

Rate of complete remission after induction was a dichotomous endpoint. It was defined as rate of patients who achieved a complete remission (CR/CRi, as defined in section 6.2 of study protocol) at any time point during study participation, but not before day 26 of last induction cycle.

The following clinical scenarios constituted failures to achieve a CR/CRi:

- Patients who do not meet criteria for CR/CRi
- Patients who die before earliest remission assessment time point on day 26

- Patients with no evaluable marrow material, i.e. no puncture done, or no aspiration possible (punctio sicca), or no marrow spicules and no evaluable histology, or puncture material not evaluable for technical reasons
- Patients excluded from the study before remission assessment time point on day 26 of the last induction cycle

5.4.2 SECONDARY VARIABLES

Complete cases were analysed for ORR. Time-to-event endpoints were censored at time of last available information, if no event was observed.

5.5 INTERIM ANALYSIS

5.5.1 TRIAL PART 1

The planned interim analysis was conducted as stated in the protocol. The null hypothesis could not be rejected. Sample size recalculation resulted in an infeasible large number, due to a smaller effect than initially assumed, and due to higher variability (Statistical report of interim analysis trial part 1, 14/12/2016).

It was decided to stop trial part 1 and to amend the protocol to correctly reflect the trial after stopping trial part 1.

During conduct of the interim analysis until amendment of the protocol recruitment was not stopped.

The primary analysis combined the results of the interim analysis with the results of the patients who contributed to the interim analysis and the patients who did not, according to the approach proposed by Bauer and Köhne[1], as described in the statistical analysis plan.

5.5.2 TRIAL PART 2

The planned interim analysis was conducted on November 11th 2020. Differing from the protocol, this analysis was conducted later than stated in the protocol. Therefore, differing numbers of patients were included in this analysis (119 in arm S, 105 in arm D as opposed to 90 per arm).

The null hypothesis could not be rejected. Sample size recalculation resulted in a larger number of required study participants, due to a smaller effect than initially assumed. Nevertheless it was decided to recruit the initially planned number of patients. The recalculated type-1 and type-2 errors for the final analysis were 0.059984 and 0.2727, respectively.

5.6 MULTIPLE COMPARISONS

One primary endpoint per trial part was analysed. A trial part was considered an experiment. With only one confirmatory tested endpoint per experiment no additional control measures for experiment-wise error control were required.

5.7 SUBGROUPS

Subgroups for analyses were:

- Male patients
- Female patients
- Patients with favourable cytogenetic risk
- Patients with intermediate cytogenetic risk

- Patients with adverse cytogenetic risk
- Patient groups resulting from crossing NPM1 with FLT3-ITD high/low according to Döhner et al. (2017)[2]
- Patients treated with allogeneic HSCT in CR1
- Patients treated without allogeneic HSCT in CR1

5.8 CHANGES IN THE PLANNED ANALYSIS

In the protocol section describing the sample size calculation was stated that the analysis of the primary endpoint should be stratified. Contradictory to that, the analysis section of the trial protocol describes the analysis of the primary endpoint as unstratified. The interim analysis was conducted unstratified.

Because cytogenetic analysis is time-consuming, the results usually are not available at time of randomization. A stratified randomization for trial part 1 thus was not feasible. To correctly reflect the mechanism of treatment allocation and because of the sufficient sample size the primary analysis was conducted unstratified.

Definition of the primary endpoint of trial part 2 was slightly changed. Time frame for determination of CR was relaxed from ‘day 35-42’ to not before day 26 after last induction cycle.

Definition of response was slightly relaxed by also accepting response control later than d18. The later the early response control is conducted, the higher is the probability of detecting residual blasts. Patients with late response control and randomization into trial part 2 were therefore accepted for the PPS. It was assumed that the population is shifted slightly towards more favourable patients compared to the initial strict definition of early response control. As delay of early response control by few days does not seem to be uncommon, inclusion of these patients better reflects clinical practice.

Patients with dose capping due to body surface of > $2m^2$ qualify for the PPS too, even if it was a deviation from protocol. It was decided to accept those patients, because practice of dose capping seems to be common practice in some trial sites. Exclusion of those patients would reduce power and generalizability of results. It is possible, that this change introduces some bias favouring the experimental arm by reducing complete remission rate of the D arm. Also, it is possible that toxicity is reduced in the D arm, which, on the other hand, may increase chance for complete remission. Nevertheless, these effects are expected to be small, because the proportion of patients with dose-capping was to be small. This deviation from protocol was addressed by a sensitivity analysis.

Overall survival and event-free survival definitions were changed to start from day of randomization and not from day 1 of study treatment.

An additional ITT population was defined to be analysed for trial part 1. That population was not pre-specified in the statistical analysis plan.

6 RESULTS

6.1 ANALYSIS POPULATIONS

Table 1 Disposition of subjects trial part 1

	Total	Arm60	Arm90
Treated in part 1	864	707	157
Randomized to part 1	317	160	157
FAS part 1	317	160	157
Not FAS part 1	0	0	0
SES part 1	306	154	152
Not SES part 1	11	6	5
PPS part 1	302	152	150
Not PPS part 1	15	8	7
CR data available from SAL registry	300	152	148
OS data available from SAL registry	299	151	148
RFS data available from SAL registry	268	137	131
EFS data available from SAL registry	299	151	148

Table 2 Disposition of subjects trial part 2

	Total	ArmD	ArmS
Randomized to part 2	377	188	189
FAS part 2	377	188	189
Not FAS part 2	0	0	0
SES part 2	377	188	189
Not SES part 2	0	0	0
PPS part 2	328	153	175
Not PPS part 2	49	35	14

	Total	ArmD	ArmS
OS data available from SAL registry	304	139	165
RFS data available from SAL registry	298	138	160
EFS data available from SAL registry	304	139	165

6.2 BASELINE CHARACTERISTICS

6.2.1 TRIAL PART 1

Full Analysis Set (FAS)

Table 3 FAS

	Total (N=317)	60 (N=160)	90 (N=157)	p value
Age (years)				0.1289
- Mean (SD)	48.0 (10.7)	47.3 (10.5)	48.6 (10.8)	
- Median	51.0	50.0	52.0	
- Q1, Q3	43.0, 56.0	40.8, 55.0	44.0, 56.0	
- Range	18.0 - 60.0	18.0 - 60.0	19.0 - 60.0	
Gender				0.2861
- female	159 (50.2%)	85 (53.1%)	74 (47.1%)	
- male	158 (49.8%)	75 (46.9%)	83 (52.9%)	
Height (cm)				0.8720
- N-Miss	3	3	0	
- Mean (SD)	173.6 (9.8)	173.6 (9.5)	173.5 (10.0)	
- Median	172.0	172.0	172.0	
- Q1, Q3	167.0, 181.0	167.0, 180.0	167.0, 181.0	
- Range	150.0 - 203.0	155.0 - 202.0	150.0 - 203.0	
Weight (kg)				0.9386

	Total (N=317)	60 (N=160)	90 (N=157)	p value
- N-Miss	3	3	0	
- Mean (SD)	80.3 (18.8)	80.3 (18.1)	80.4 (19.7)	
- Median	78.0	78.0	78.0	
- Q1, Q3	65.8, 91.0	66.0, 92.0	65.2, 91.0	
- Range	43.0 - 165.2	44.2 - 139.0	43.0 - 165.2	
BSA (m^2)				0.9173
- N-Miss	3	3	0	
- Mean (SD)	1.9 (0.2)	1.9 (0.2)	1.9 (0.2)	
- Median	1.9	1.9	1.9	
- Q1, Q3	1.8, 2.1	1.8, 2.1	1.8, 2.1	
- Range	1.4 - 2.7	1.4 - 2.6	1.4 - 2.7	
ECOG				0.5018
- N-Miss	3	3	0	
- 0	150 (47.8%)	79 (50.3%)	71 (45.2%)	
- 1	150 (47.8%)	70 (44.6%)	80 (51.0%)	
- 2	14 (4.5%)	8 (5.1%)	6 (3.8%)	
Type of AML				0.4922
- N-Miss	9	5	4	
- de novo	265 (86.0%)	134 (86.5%)	131 (85.6%)	
- sAML	36 (11.7%)	19 (12.3%)	17 (11.1%)	
- tAML	7 (2.3%)	2 (1.3%)	5 (3.3%)	
FAB				0.2631
- N-Miss	48	23	25	
- M0	26 (9.7%)	18 (13.1%)	8 (6.1%)	
- M1	66 (24.5%)	34 (24.8%)	32 (24.2%)	
- M2	82 (30.5%)	40 (29.2%)	42 (31.8%)	

	Total (N=317)	60 (N=160)	90 (N=157)	p value
- M4	37 (13.8%)	15 (10.9%)	22 (16.7%)	
- M4Eo	12 (4.5%)	6 (4.4%)	6 (4.5%)	
- M5	32 (11.9%)	18 (13.1%)	14 (10.6%)	
- M6	8 (3.0%)	5 (3.6%)	3 (2.3%)	
- RAEB-t	6 (2.2%)	1 (0.7%)	5 (3.8%)	
Extramedullary manifestation				0.3986
- N-Miss	3	3	0	
- Not done	3 (1.0%)	1 (0.6%)	2 (1.3%)	
- No signs	295 (93.9%)	148 (94.3%)	147 (93.6%)	
- Suspected	5 (1.6%)	4 (2.5%)	1 (0.6%)	
- Yes, histologically proven	11 (3.5%)	4 (2.5%)	7 (4.5%)	
WBC (G/L)				0.6103
- N-Miss	4	3	1	
- Mean (SD)	29.1 (45.7)	25.3 (36.7)	32.9 (53.0)	
- Median	10.1	9.4	10.5	
- Q1, Q3	2.4, 35.8	2.5, 35.5	2.3, 37.1	
- Range	0.5 - 294.8	0.5 - 218.0	0.7 - 294.8	
Hb (g/dL)				0.2949
- N-Miss	4	3	1	
- Mean (SD)	9.1 (1.8)	9.2 (1.8)	9.0 (1.9)	
- Median	8.9	9.0	8.7	
- Q1, Q3	7.8, 10.1	7.9, 10.3	7.8, 10.0	
- Range	4.6 - 14.6	4.6 - 14.0	4.9 - 14.6	
PLT (G/L)				0.5212
- N-Miss	4	3	1	
- Mean (SD)	86.4 (82.9)	84.1 (87.4)	88.7 (78.3)	

	Total (N=317)	60 (N=160)	90 (N=157)	p value
- Median	56.3	54.0	59.9	
- Q1, Q3	34.0, 113.0	34.0, 113.0	34.0, 115.2	
- Range	4.0 - 791.0	4.0 - 791.0	5.0 - 496.0	
LDH (µmol/s*L)				0.5254
- N-Miss	286	143	143	
- Mean (SD)	496.1 (452.5)	550.1 (526.0)	430.6 (351.6)	
- Median	380.0	406.0	298.7	
- Q1, Q3	197.4, 550.8	202.8, 570.0	201.0, 461.1	
- Range	150.0 - 1979.4	152.4 - 1979.4	150.0 - 1306.0	
NPM1				0.6833
- N-Miss	28	15	13	
- N	172 (59.5%)	88 (60.7%)	84 (58.3%)	
- Y	117 (40.5%)	57 (39.3%)	60 (41.7%)	
FLT3I				0.2119
- N-Miss	48	25	23	
- N	215 (79.9%)	112 (83.0%)	103 (76.9%)	
- Y	54 (20.1%)	23 (17.0%)	31 (23.1%)	
FLT3-ITD ratio				0.0115
- N-Miss	268	141	127	
- high	24 (49.0%)	5 (26.3%)	19 (63.3%)	
- low	25 (51.0%)	14 (73.7%)	11 (36.7%)	
CEBPA				0.5738
- N-Miss	81	41	40	
- N	224 (94.9%)	112 (94.1%)	112 (95.7%)	
- Y	12 (5.1%)	7 (5.9%)	5 (4.3%)	
MYH11				0.4435

	Total (N=317)	60 (N=160)	90 (N=157)	p value
- N-Miss	91	46	45	
- N	211 (93.4%)	105 (92.1%)	106 (94.6%)	
- Y	15 (6.6%)	9 (7.9%)	6 (5.4%)	
Bcr-ABL				0.5606
- N-Miss	125	64	61	
- N	189 (98.4%)	95 (99.0%)	94 (97.9%)	
- Y	3 (1.6%)	1 (1.0%)	2 (2.1%)	
Cytogenetic risk (ELN 2017)				0.7705
- N-Miss	17	10	7	
- intermediate	140 (46.7%)	72 (48.0%)	68 (45.3%)	
- favourable	110 (36.7%)	52 (34.7%)	58 (38.7%)	
- adverse	50 (16.7%)	26 (17.3%)	24 (16.0%)	
Peripheral blasts (%)				0.6124
- N-Miss	67	32	35	
- Mean (SD)	34.4 (32.3)	34.9 (31.6)	33.8 (33.2)	
- Median	25.0	26.0	24.0	
- Q1, Q3	3.2, 63.0	4.0, 64.2	2.5, 61.2	
- Range	0.0 - 97.0	0.0 - 97.0	0.0 - 97.0	
Bone marrow blasts (%)				0.9474
- N-Miss	9	6	3	
- Mean (SD)	59.5 (24.4)	59.6 (23.3)	59.3 (25.5)	
- Median	60.0	60.0	59.5	
- Q1, Q3	39.2, 80.0	40.0, 80.0	39.0, 81.8	
- Range	3.0 - 100.0	20.0 - 99.0	3.0 - 100.0	

6.2.2 TRIAL PART 2

Per Protocol Set (PPS)

Table 4 PPS

	Total (N=328)	D (N=153)	S (N=175)	p value
Age (years)				0.1952
- Mean (SD)	48.9 (11.3)	47.9 (12.1)	49.9 (10.6)	
- Median	52.0	50.0	53.0	
- Q1, Q3	42.0, 58.0	40.0, 58.0	43.0, 58.0	
- Range	18.0 - 65.0	18.0 - 65.0	19.0 - 65.0	
Gender				0.0304
- female	172 (52.4%)	90 (58.8%)	82 (46.9%)	
- male	156 (47.6%)	63 (41.2%)	93 (53.1%)	
Height (cm)				0.5943
- Mean (SD)	172.9 (9.3)	172.6 (8.5)	173.2 (10.0)	
- Median	172.0	172.0	172.0	
- Q1, Q3	165.8, 180.0	166.0, 178.0	165.0, 180.0	
- Range	151.0 - 196.0	158.0 - 195.0	151.0 - 196.0	
Weight (kg)				0.0759
- Mean (SD)	82.2 (19.8)	80.1 (19.2)	84.0 (20.1)	
- Median	80.4	78.0	82.4	
- Q1, Q3	67.0, 93.5	66.0, 90.0	70.0, 96.0	
- Range	43.0 - 151.0	43.0 - 142.0	44.0 - 151.0	
BSA (m^2)				0.1832
- Mean (SD)	2.0 (0.3)	1.9 (0.2)	2.0 (0.3)	
- Median	1.9	1.9	2.0	
- Q1, Q3	1.8, 2.1	1.8, 2.1	1.8, 2.1	
- Range	1.4 - 2.8	1.4 - 2.7	1.4 - 2.8	

	Total (N=328)	D (N=153)	S (N=175)	p value
ECOG				0.7495
- N-Miss	2	1	1	
- 0	143 (43.9%)	70 (46.1%)	73 (42.0%)	
- 1	169 (51.8%)	76 (50.0%)	93 (53.4%)	
- 2	14 (4.3%)	6 (3.9%)	8 (4.6%)	
Type of AML				0.6328
- N-Miss	1	0	1	
- de novo	299 (91.4%)	140 (91.5%)	159 (91.4%)	
- sAML	19 (5.8%)	10 (6.5%)	9 (5.2%)	
- tAML	9 (2.8%)	3 (2.0%)	6 (3.4%)	
FAB				0.8561
- N-Miss	31	15	16	
- M0	18 (6.1%)	9 (6.5%)	9 (5.7%)	
- M1	60 (20.2%)	27 (19.6%)	33 (20.8%)	
- M2	73 (24.6%)	37 (26.8%)	36 (22.6%)	
- M4	69 (23.2%)	30 (21.7%)	39 (24.5%)	
- M4Eo	19 (6.4%)	7 (5.1%)	12 (7.5%)	
- M5	46 (15.5%)	24 (17.4%)	22 (13.8%)	
- M6	4 (1.3%)	2 (1.4%)	2 (1.3%)	
- M7	1 (0.3%)	0 (0.0%)	1 (0.6%)	
- RAEB-t	7 (2.4%)	2 (1.4%)	5 (3.1%)	
Extramedullary manifestation				0.6628
- Not done	3 (0.9%)	1 (0.7%)	2 (1.1%)	
- No signs	300 (91.5%)	143 (93.5%)	157 (89.7%)	
- Suspected	15 (4.6%)	5 (3.3%)	10 (5.7%)	
- Yes, histologically proven	10 (3.0%)	4 (2.6%)	6 (3.4%)	

	Total (N=328)	D (N=153)	S (N=175)	p value
WBC (G/L)				0.1314
- Mean (SD)	29.0 (43.4)	36.0 (53.6)	22.9 (31.0)	
- Median	10.5	11.7	9.5	
- Q1, Q3	3.2, 36.2	3.2, 45.0	3.1, 29.4	
- Range	0.5 - 297.0	0.5 - 297.0	0.6 - 208.0	
Hb (g/dL)				0.4277
- Mean (SD)	9.0 (1.9)	8.9 (1.9)	9.1 (1.8)	
- Median	8.7	8.6	8.8	
- Q1, Q3	7.7, 10.1	7.6, 10.1	7.7, 10.0	
- Range	3.7 - 15.3	3.7 - 14.8	5.2 - 15.3	
PLT (G/L)				0.9386
- Mean (SD)	77.5 (81.9)	77.3 (86.1)	77.6 (78.3)	
- Median	53.5	57.0	52.0	
- Q1, Q3	31.0, 96.2	29.0, 99.7	34.0, 92.5	
- Range	3.0 - 836.0	4.0 - 836.0	3.0 - 630.0	
LDH (µmol/s*L)				0.2207
- N-Miss	317	150	167	
- Mean (SD)	302.4 (124.9)	244.3 (95.0)	324.1 (133.1)	
- Median	248.0	202.8	323.0	
- Q1, Q3	203.9, 402.0	189.9, 277.9	222.2, 410.6	
- Range	152.4 - 531.6	177.0 - 353.0	152.4 - 531.6	
NPM1				0.4870
- N-Miss	16	7	9	
- N	139 (44.6%)	62 (42.5%)	77 (46.4%)	
- Y	173 (55.4%)	84 (57.5%)	89 (53.6%)	
FLT3I				0.0937

	Total (N=328)	D (N=153)	S (N=175)	p value
- N-Miss	28	14	14	
- N	223 (74.3%)	97 (69.8%)	126 (78.3%)	
- Y	77 (25.7%)	42 (30.2%)	35 (21.7%)	
FLT3-ITD ratio				0.2779
- N-Miss	257	116	141	
- high	34 (47.9%)	20 (54.1%)	14 (41.2%)	
- low	37 (52.1%)	17 (45.9%)	20 (58.8%)	
CEBPA				0.4785
- N-Miss	60	26	34	
- N	248 (92.5%)	116 (91.3%)	132 (93.6%)	
- Y	20 (7.5%)	11 (8.7%)	9 (6.4%)	
MYH11				0.9855
- N-Miss	64	30	34	
- N	236 (89.4%)	110 (89.4%)	126 (89.4%)	
- Y	28 (10.6%)	13 (10.6%)	15 (10.6%)	
Bcr-ABL				0.8058
- N-Miss	88	44	44	
- N	235 (97.9%)	107 (98.2%)	128 (97.7%)	
- Y	5 (2.1%)	2 (1.8%)	3 (2.3%)	
Cytogenetic risk (ELN 2017)				0.7536
- N-Miss	11	5	6	
- intermediate	107 (33.8%)	53 (35.8%)	54 (32.0%)	
- favourable	178 (56.2%)	80 (54.1%)	98 (58.0%)	
- adverse	32 (10.1%)	15 (10.1%)	17 (10.1%)	
Peripheral blasts (%)				0.7766
- N-Miss	50	24	26	

	Total (N=328)	D (N=153)	S (N=175)	p value
- Mean (SD)	32.2 (29.5)	31.0 (28.3)	33.2 (30.6)	
- Median	24.8	25.0	24.0	
- Q1, Q3	4.0, 55.4	5.0, 48.0	4.0, 59.0	
- Range	0.0 - 98.0	0.0 - 94.0	0.0 - 98.0	
Bone marrow blasts (%)				0.3130
- N-Miss	1	0	1	
- Mean (SD)	60.4 (24.0)	61.9 (23.4)	59.2 (24.5)	
- Median	62.6	66.0	60.0	
- Q1, Q3	40.0, 81.0	40.0, 81.0	40.0, 80.0	
- Range	3.0 - 100.0	20.0 - 100.0	3.0 - 100.0	

6.3 STUDY TREATMENT AND COMPLIANCE

6.3.1 TRIAL PART 1 – EXTEND OF EXPOSURE

Table 5 Extend of Exposure trial part I

	Total (N=317)	60 (N=160)	90 (N=157)	p value
Treatment arm IT1				< 1e-04
- 60	160 (50.5%)	160 (100.0%)	0 (0.0%)	
- 90	157 (49.5%)	0 (0.0%)	157 (100.0%)	
Randomized daunorubicin dose IT1				< 1e-04
- 60	160 (50.5%)	160 (100.0%)	0 (0.0%)	
- 90	157 (49.5%)	0 (0.0%)	157 (100.0%)	
Daunorubicin days cycle 1				0.5517
- N-Miss	3	3	0	
- Mean (SD)	2.9 (0.5)	2.9 (0.4)	2.9 (0.6)	
- Median	3.0	3.0	3.0	
- Q1, Q3	3.0, 3.0	3.0, 3.0	3.0, 3.0	

	Total (N=317)	60 (N=160)	90 (N=157)	p value
- Range	0.0 - 3.0	0.0 - 3.0	0.0 - 3.0	
Daunorubicin dose cycle 1 (mg/m^2)				< 1e-04
- N-Miss	3	3	0	
- Mean (SD)	216.5 (57.8)	175.0 (27.0)	258.1 (50.1)	
- Median	183.5	179.7	269.6	
- Q1, Q3	179.7, 269.6	179.2, 180.4	268.5, 270.4	
- Range	0.0 - 286.7	0.0 - 192.5	0.0 - 286.7	
Cytarabin days cycle 1				0.2490
- N-Miss	3	3	0	
- Mean (SD)	6.8 (1.0)	6.9 (0.9)	6.8 (1.1)	
- Median	7.0	7.0	7.0	
- Q1, Q3	7.0, 7.0	7.0, 7.0	7.0, 7.0	
- Range	0.0 - 7.0	0.0 - 7.0	0.0 - 7.0	
Cytarabin dose cycle 1 (mg/m^2)				0.4503
- N-Miss	3	3	0	
- Mean (SD)	680.4 (101.1)	684.3 (94.5)	676.4 (107.4)	
- Median	700.0	700.0	700.0	
- Q1, Q3	697.8, 700.0	700.0, 700.0	696.0, 700.0	
- Range	0.0 - 742.4	0.0 - 742.4	0.0 - 738.9	

6.3.2 TRIAL PART 2 – EXTEND OF EXPOSURE

Table 6 Extend of Exposure trial part 2

	Total (N=328)	D (N=153)	S (N=175)	p value
Treatment arm IT1				0.6409
- 60	263 (80.2%)	121 (79.1%)	142 (81.1%)	
- 90	65 (19.8%)	32 (20.9%)	33 (18.9%)	

	Total (N=328)	D (N=153)	S (N=175)	p value
Randomized daunorubicin dose IT1				0.6994
- N-Miss	204	94	110	
- 60	59 (47.6%)	27 (45.8%)	32 (49.2%)	
- 90	65 (52.4%)	32 (54.2%)	33 (50.8%)	
Daunorubicin days cycle 1				NaN
- Mean (SD)	3.0 (0.0)	3.0 (0.0)	3.0 (0.0)	
- Median	3.0	3.0	3.0	
- Q1, Q3	3.0, 3.0	3.0, 3.0	3.0, 3.0	
- Range	3.0 - 3.0	3.0 - 3.0	3.0 - 3.0	
Daunorubicin dose cycle 1 (mg/m^2)				0.3039
- Mean (SD)	196.0 (37.1)	197.9 (37.7)	194.4 (36.5)	
- Median	180.0	180.0	180.0	
- Q1, Q3	179.0, 183.2	179.3, 183.9	178.7, 183.0	
- Range	92.0 - 286.7	115.4 - 286.7	92.0 - 279.0	
Cytarabin days cycle 1				0.1854
- Mean (SD)	7.0 (0.1)	7.0 (0.0)	7.0 (0.2)	
- Median	7.0	7.0	7.0	
- Q1, Q3	7.0, 7.0	7.0, 7.0	7.0, 7.0	
- Range	5.0 - 7.0	7.0 - 7.0	5.0 - 7.0	
Cytarabin dose cycle 1 (mg/m^2)				0.4812
- Mean (SD)	698.5 (49.2)	702.5 (61.8)	695.0 (34.4)	
- Median	700.0	700.0	700.0	
- Q1, Q3	696.1, 700.0	696.4, 700.0	692.2, 700.0	
- Range	452.3 - 1400.0	452.3 - 1400.0	502.5 - 869.6	

6.4 PRIMARY ENDPOINT

6.4.1 TRIAL PART 1

Subgroup of patients in interim analysis

Table 7 Good response proportions

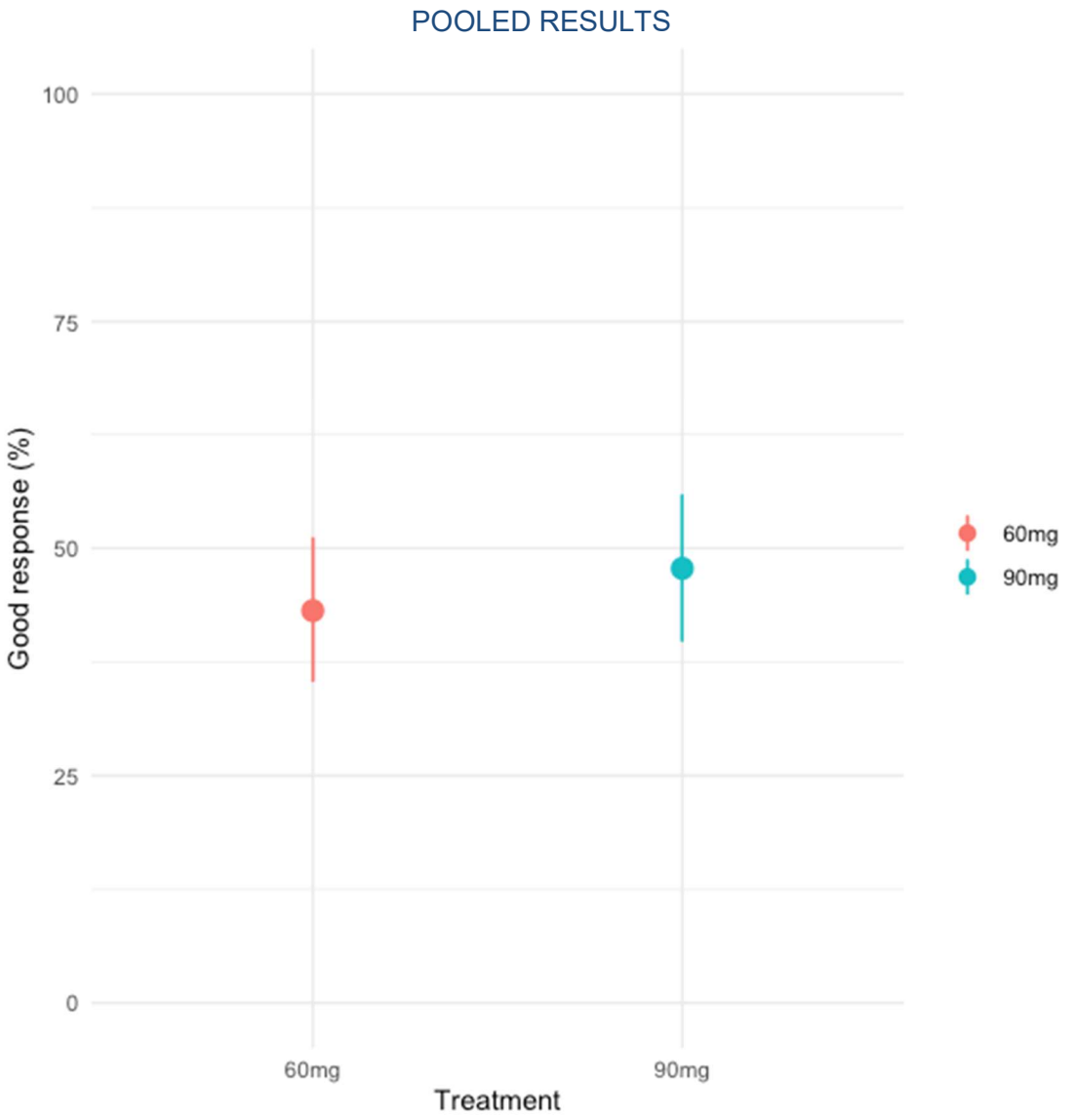
Treatment arm	Good response	n	Proportion (%)	lower 95% CI	upper 95% CI	p-value
60mg	46	109	42.20	32.80	52.04	0.3410
90mg	53	109	48.62	38.94	58.39	

Subgroup of patients not part of interim analysis

Table 8 Good response proportions

Treatment arm	Good response	n	Proportion (%)	lower 95% CI	upper 95% CI	p-value
60mg	23	51	45.10	31.13	59.66	0.9415
90mg	22	48	45.83	31.37	60.83	

The p-values of the interim analysis (p1 = 0.341) and the subgroups of patients not part of the interim analysis (p2 = 0.941) are multiplied according to the Bauer and Koehne procedure (p_combined = 0.321) and compared to the Fisher criterion of 0.0087. The combined p-value is > 0.0087, therefore the null hypothesis of equal good response rates between 60 and 90mg cannot be rejected.



Proportions of good responders per treatment arm

Table 9 Good response proportions

Treatment arm	Good response	n	Proportion (%)	lower 95% CI	upper 95% CI	p-value
60mg	69	160	43.12	35.33	51.18	0.4062
90mg	75	157	47.77	39.75	55.88	

Table 10 Multivariable logistic regression model for good response

Parameter	Estimate	Std. error	Odds ratio	95% CI lower	95% CI upper	p-value
(Intercept)	-0.2547	0.7279	0.7751	0.1861	3.2284	0.7264
Dauno 90mg	0.3286	0.2875	1.3890	0.7906	2.4404	0.2532
Favourable risk	1.0675	0.4584	2.9082	1.1842	7.1420	0.0199

Parameter	Estimate	Std. error	Odds ratio	95% CI lower	95% CI upper	p-value
Adverse risk	-0.3951	0.4610	0.6736	0.2729	1.6627	0.3914
Age (per year)	-0.0190	0.0138	0.9812	0.9550	1.0080	0.1680
NPM1 mutated	0.7590	0.4889	2.1362	0.8194	5.5692	0.1205
FLT3IY	0.3527	0.5173	1.4229	0.5163	3.9220	0.4953
Interaction NPM1 and FLT3-ITD	0.4157	0.7725	1.5154	0.3334	6.8876	0.5905

6.4.2 TRIAL PART 2

Subgroup of patients in interim analysis

Table 11 Results of test for non-inferiority

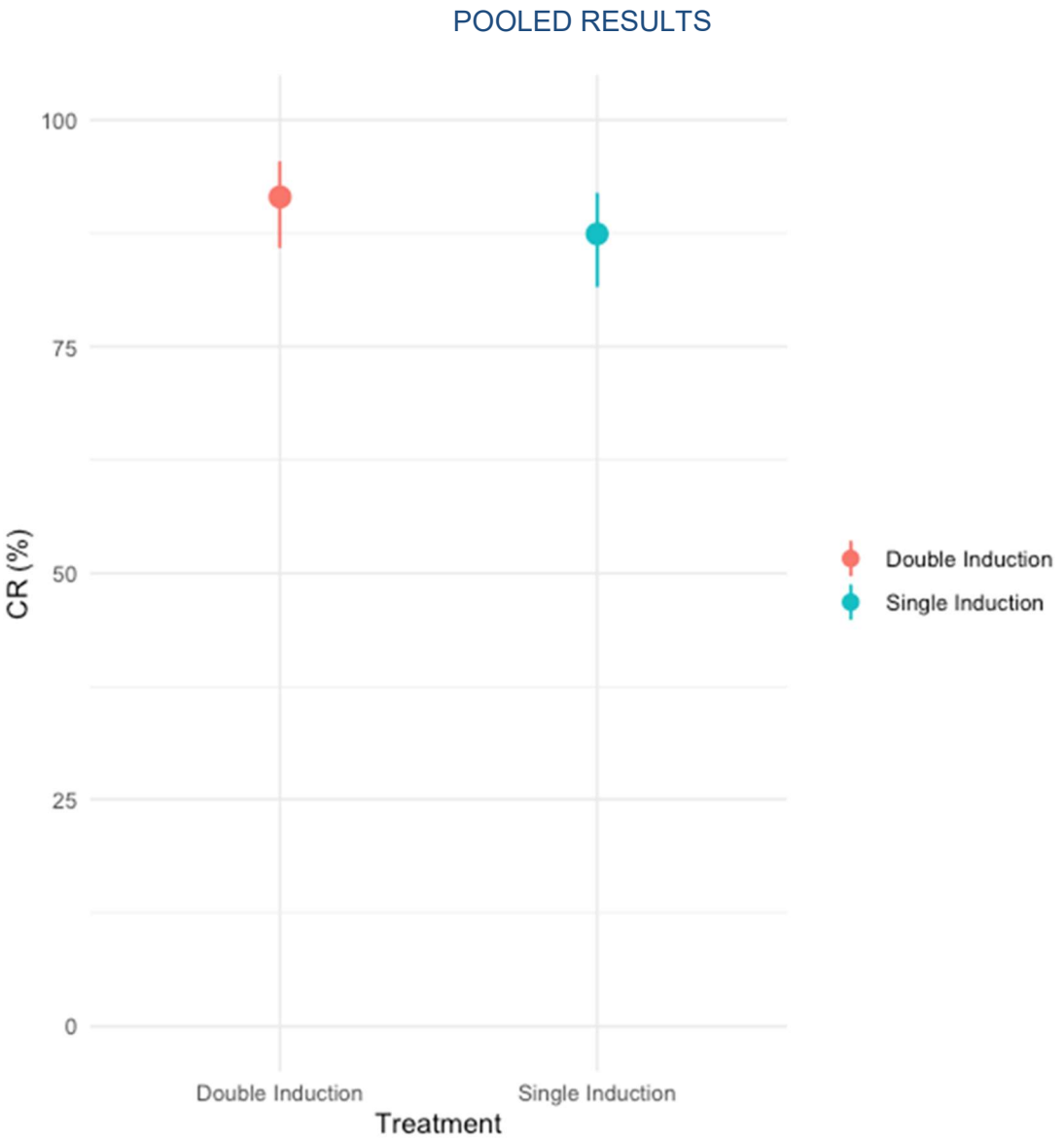
n double induction	CR double induction	n single induction	CR single induction	CR proportion double induction	CR proportion single induction	Difference of CR proportions	lower 95%-CI	upper 95%-CI up	p-value for non-inferiority
105	96	119	105	0.9143	0.8824	0.0319	-0.0469	0.1108	0.145

Subgroup of patients not part of interim analysis

Table 12 Results of test for non-inferiority

n double induction	CR double induction	n single induction	CR single induction	CR proportion double induction	CR proportion single induction	Difference of CR proportions	lower 95%-CI	upper 95%-CI up	p-value for non-inferiority
48	44	56	48	0.9167	0.8571	0.0595	-0.0609	0.1800	0.4008

The p-values of the interim analysis (p1 = 0.145) and the subgroups of patients not part of the interim analysis (p2 = 0.4008) are multiplied according to the Bauer and Koehne preocedure (p_combined = 0.0581) and compared to the Fisher criterion of 0.0087. The combined p-value is > 0.0087, therefore the null hypothesis of different complete remission rates between double and single induction cannot be rejected.



Proportions of complete remission per treatment arm

Table 13 Complete remission proportions

Treatment arm	CR	n	Proportion (%)	lower 95% CI	upper 95% CI
Double Induction	140	153	91.50	85.91	95.40
Single Induction	153	175	87.43	81.59	91.95

Table 14 Results of test for non-inferiority

n double induction	CR double induction	n single induction	CR single induction	CR proportion double induction	CR proportion single induction	Difference of CR proportions	lower 95%-CI	upper 95%-CI up	p-value for non-inferiority
153	140	175	153	0.9150	0.8743	0.0407	-0.0253	0.1068	0.1566

6.5 SECONDARY ENDPOINTS OF EFFICACY

6.5.1 TRIAL PART 1

6.5.1.1. OVERALL HEMATOLOGIC REMISSION

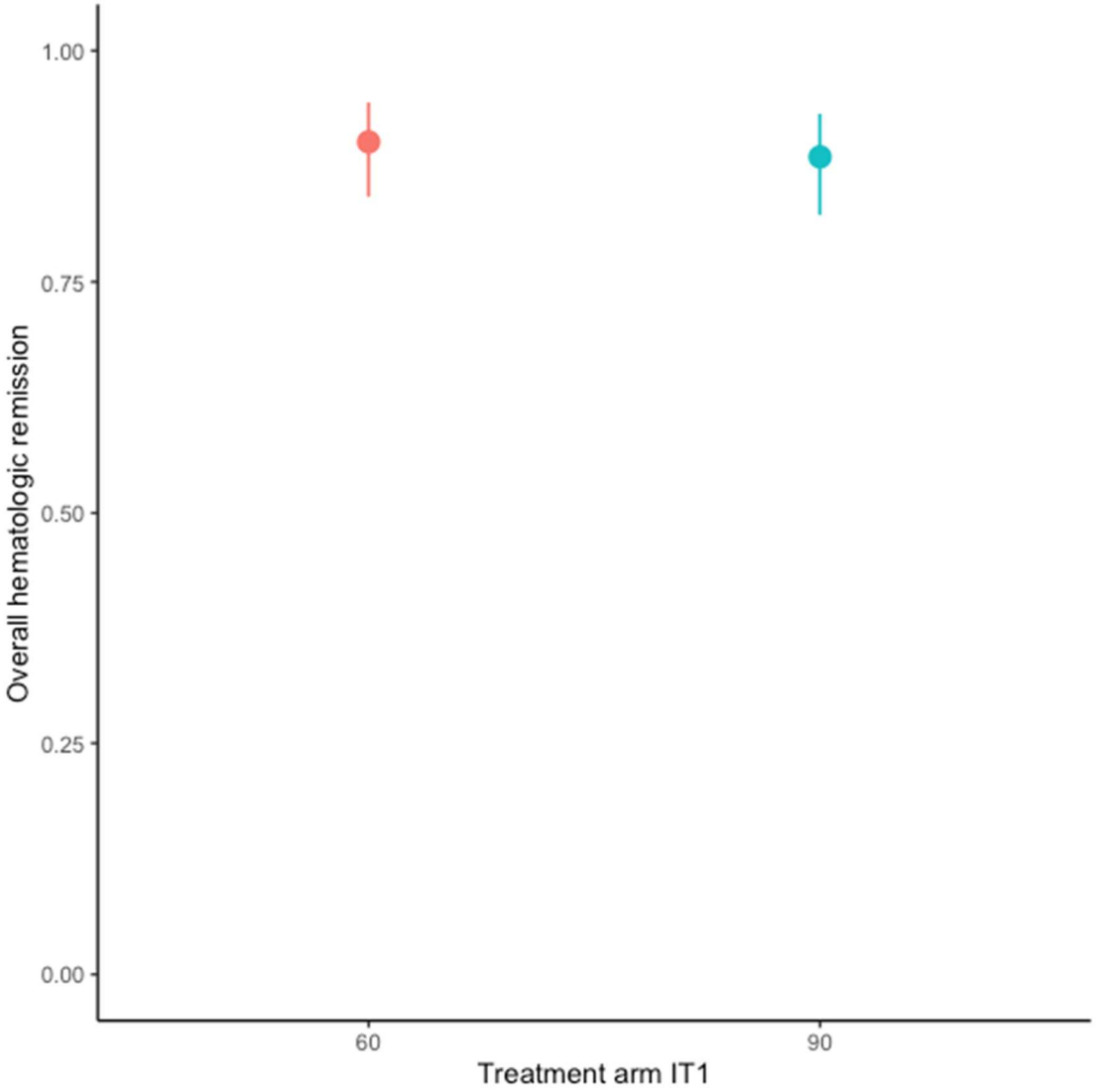


Table 15 Estimates

strata	Overall hematologic remission	n	proportion	LCL	UCL
60	137	152	0.901	0.842	0.944
90	131	148	0.885	0.822	0.932

Table 16 Univariate Logistic regression model for Overall hematologic remission, global p = 0.65

Parameter	log.OR	OR	ci.OR	se.OR	z	p
(Intercept)	2.212	9.133	(5.36 to 15.564)	0.272	8.134	0
Treatment arm IT190	-0.17	0.844	(0.405 to 1.759)	0.375	-0.454	0.65

Table 17 Multiple Logistic regression model for Overall hematologic remission

Parameter	log.OR	OR	ci.OR	se.OR	z	p
(Intercept)	4.275	71.889	(3.261 to 1584.681)	1.578	2.709	0.007
RESPONSE1Y	1.604	4.971	(0.573 to 43.093)	1.102	1.455	0.146
`Age (years)`	-0.054	0.948	(0.896 to 1.003)	0.029	-1.864	0.062
`Cytogenetic risk (ELN 2017)`favourable	0.766	2.151	(0.238 to 19.467)	1.124	0.682	0.495
`Cytogenetic risk (ELN 2017)`adverse	-0.442	0.643	(0.219 to 1.884)	0.549	-0.806	0.42
NPM1Y	1.031	2.804	(0.148 to 52.961)	1.499	0.688	0.492
FLT3IY	-0.197	0.821	(0.203 to 3.315)	0.712	-0.277	0.782
`Treatment arm IT1`90	0.123	1.131	(0.417 to 3.066)	0.509	0.243	0.808
NPM1Y:FLT3IY	-0.529	0.589	(0.017 to 19.911)	1.796	-0.295	0.768
RESPONSE1Y:`Treatment arm IT1`90	0.136	1.146	(0.056 to 23.332)	1.537	0.089	0.929

6.5.1.2. OVERALL SURVIVAL

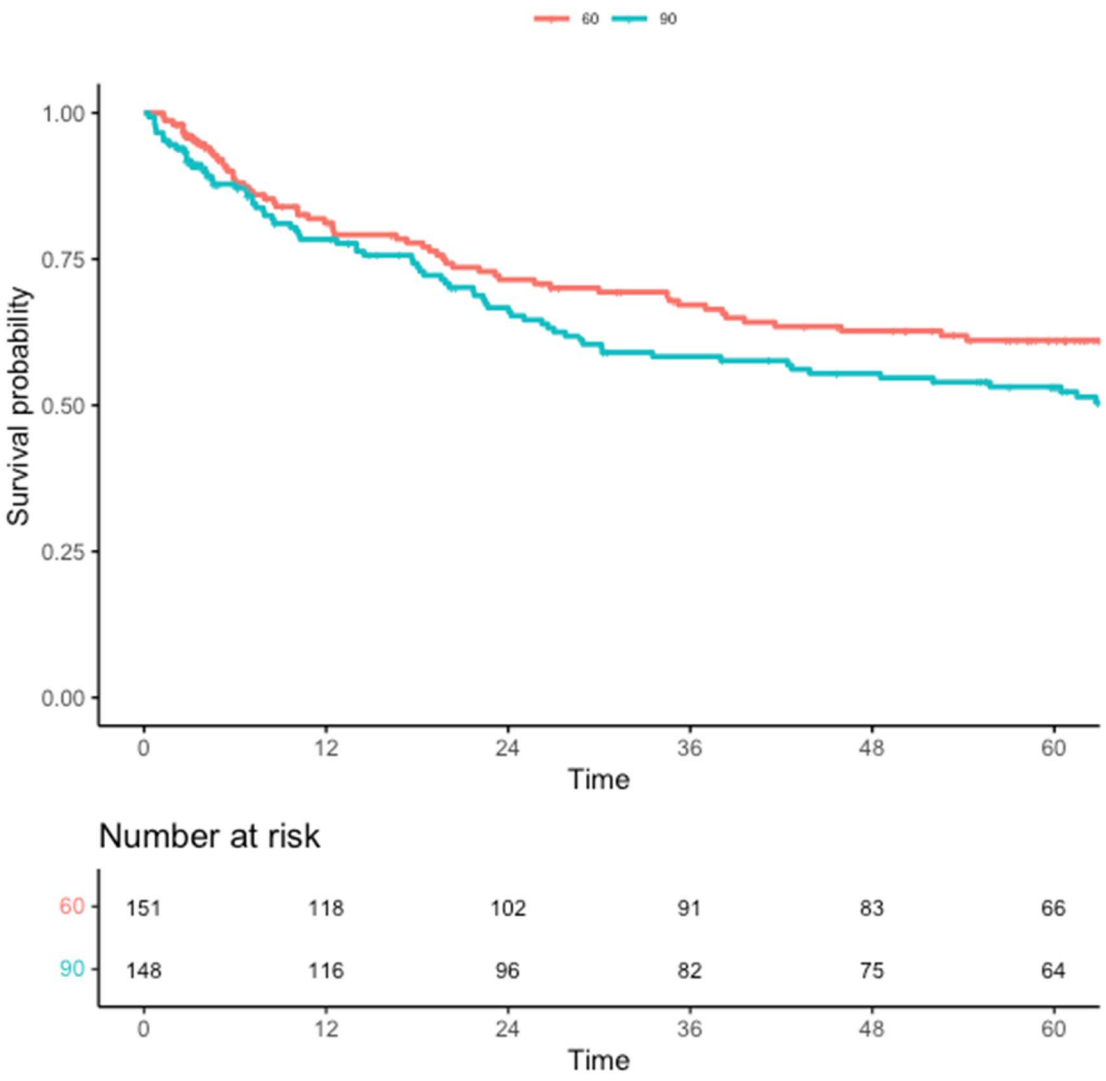


Table 18 Median survival

strata	records	n.max	n.start	events	median	LCL	UCL
60	203	151	151	59		70.424	
90	206	148	148	74	71.405	42.425	

Table 19 Survival probabilities

strata	time	n.risk	survival	LCL	UCL
60	12	118	0.812	0.752	0.877
60	24	102	0.715	0.645	0.792
60	36	91	0.672	0.599	0.753
60	60	66	0.611	0.536	0.697
90	12	116	0.784	0.720	0.853

strata	time	n.risk	survival	LCL	UCL
90	24	96	0.667	0.595	0.748
90	36	82	0.583	0.509	0.669
90	60	64	0.532	0.456	0.620

Table 20 Cox regression model, global p = 0.109

Parameter	log.HR	HR	ci.HR	se.HR	z	p
Treatment arm IT190	0.279	1.322	(0.939 to 1.861)	0.175	1.594	0.111

Table 21 Multiple Cox regression model

Parameter	log.HR	HR	ci.HR	se.HR	z	p
RESPONSE1Y	-0.775	0.46	(0.227 to 0.936)	0.362	-2.141	0.032
`Age (years)`	0.016	1.016	(0.997 to 1.036)	0.01	1.6	0.11
`Cytogenetic risk (ELN 2017)`favourable	-0.792	0.453	(0.207 to 0.992)	0.4	-1.98	0.048
`Cytogenetic risk (ELN 2017)`adverse	0.835	2.306	(1.391 to 3.821)	0.258	3.236	0.001
NPM1Y	0.317	1.373	(0.563 to 3.347)	0.455	0.697	0.486
FLT3IY	0.016	1.017	(0.519 to 1.99)	0.343	0.047	0.963
`Treatment arm IT1`90	0.128	1.137	(0.706 to 1.83)	0.243	0.527	0.598
ALSCTCR1	-0.067	0.935	(0.604 to 1.447)	0.223	-0.3	0.764
NPM1Y:FLT3IY	0.612	1.844	(0.613 to 5.549)	0.562	1.089	0.276
RESPONSE1Y:`Treatment arm IT1`90	0.37	1.447	(0.61 to 3.432)	0.441	0.839	0.401

6.5.1.3. RELAPSE FREE SURVIVAL

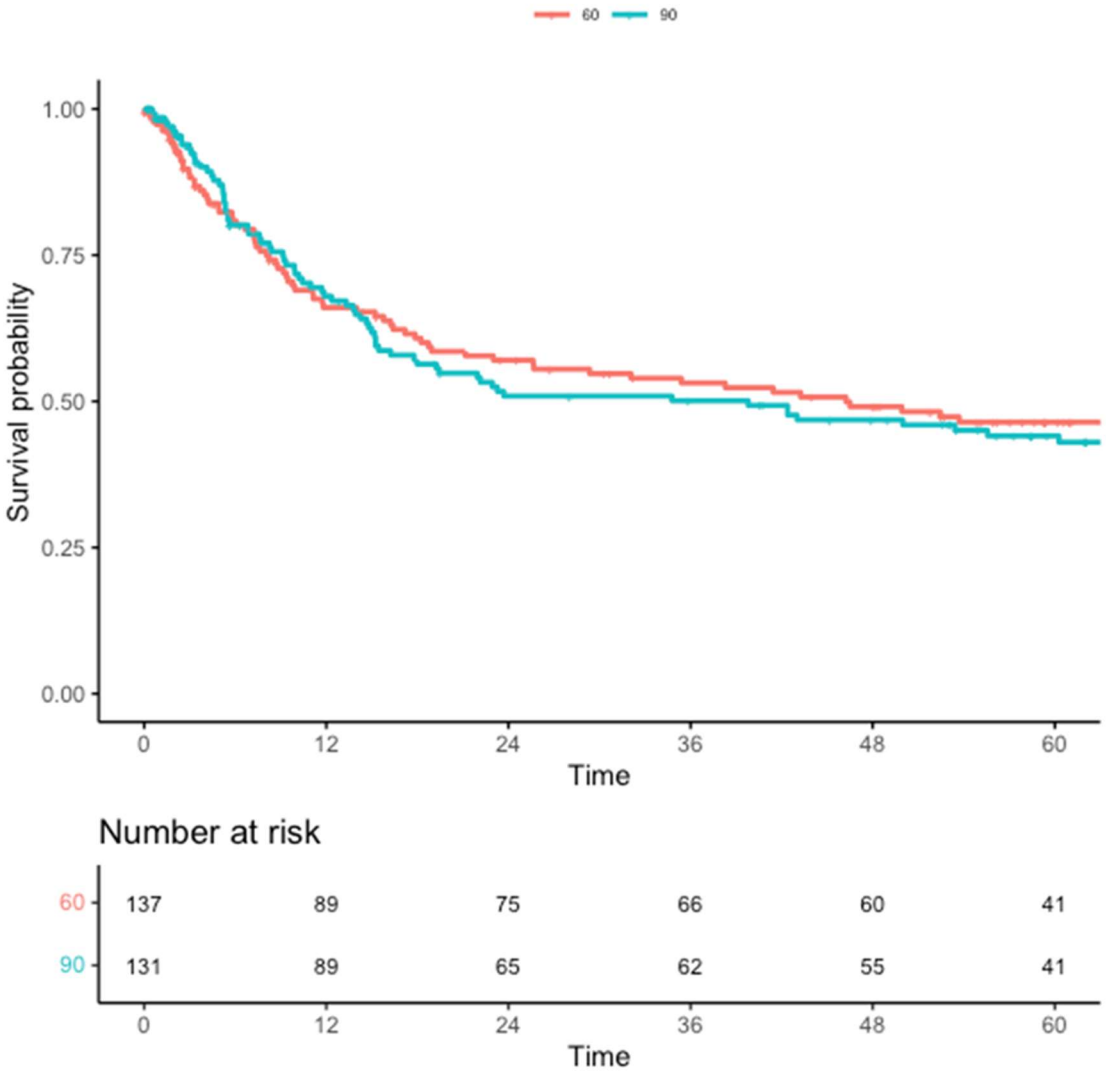


Table 22 Median survival

strata	records	n.max	n.start	events	median	LCL	UCL
60	189	137	137	74	46.237	23.004	
90	189	131	131	74	39.796	17.779	

Table 23 Survival probabilities

strata	time	n.risk	survival	LCL	UCL
60	12	89	0.660	0.585	0.745
60	24	75	0.570	0.493	0.660
60	36	66	0.531	0.453	0.623
60	60	41	0.464	0.386	0.559
90	12	89	0.679	0.604	0.764

strata	time	n.risk	survival	LCL	UCL
90	24	65	0.509	0.430	0.603
90	36	62	0.501	0.422	0.595
90	60	41	0.441	0.362	0.537

Table 24 Cox regression model, global p = 0.837

Parameter	log.HR	HR	ci.HR	se.HR	z	p
Treatment arm IT190	0.034	1.034	(0.749 to 1.428)	0.164	0.207	0.836

Table 25 Multiple Cox regression model

Parameter	log.HR	HR	ci.HR	se.HR	z	p
RESPONSE1Y	-0.202	0.817	(0.474 to 1.409)	0.278	-0.727	0.467
`Age (years)`	0.015	1.015	(0.997 to 1.033)	0.009	1.667	0.096
`Cytogenetic risk (ELN 2017)`favourable	-0.621	0.537	(0.277 to 1.042)	0.338	-1.837	0.066
`Cytogenetic risk (ELN 2017)`adverse	0.555	1.742	(1.046 to 2.901)	0.26	2.135	0.033
NPM1Y	0.246	1.279	(0.615 to 2.658)	0.373	0.66	0.51
FLT3IY	0.41	1.507	(0.824 to 2.756)	0.308	1.331	0.183
`Treatment arm IT1`90	0.01	1.01	(0.618 to 1.65)	0.251	0.04	0.968
ALSCTCR1	-0.159	0.853	(0.575 to 1.266)	0.202	-0.787	0.431
NPM1Y:FLT3IY	0.298	1.347	(0.549 to 3.308)	0.458	0.651	0.515
RESPONSE1Y:`Treatment arm IT1`90	0.149	1.16	(0.563 to 2.391)	0.369	0.404	0.686

6.5.1.4. EVENT FREE SURVIVAL

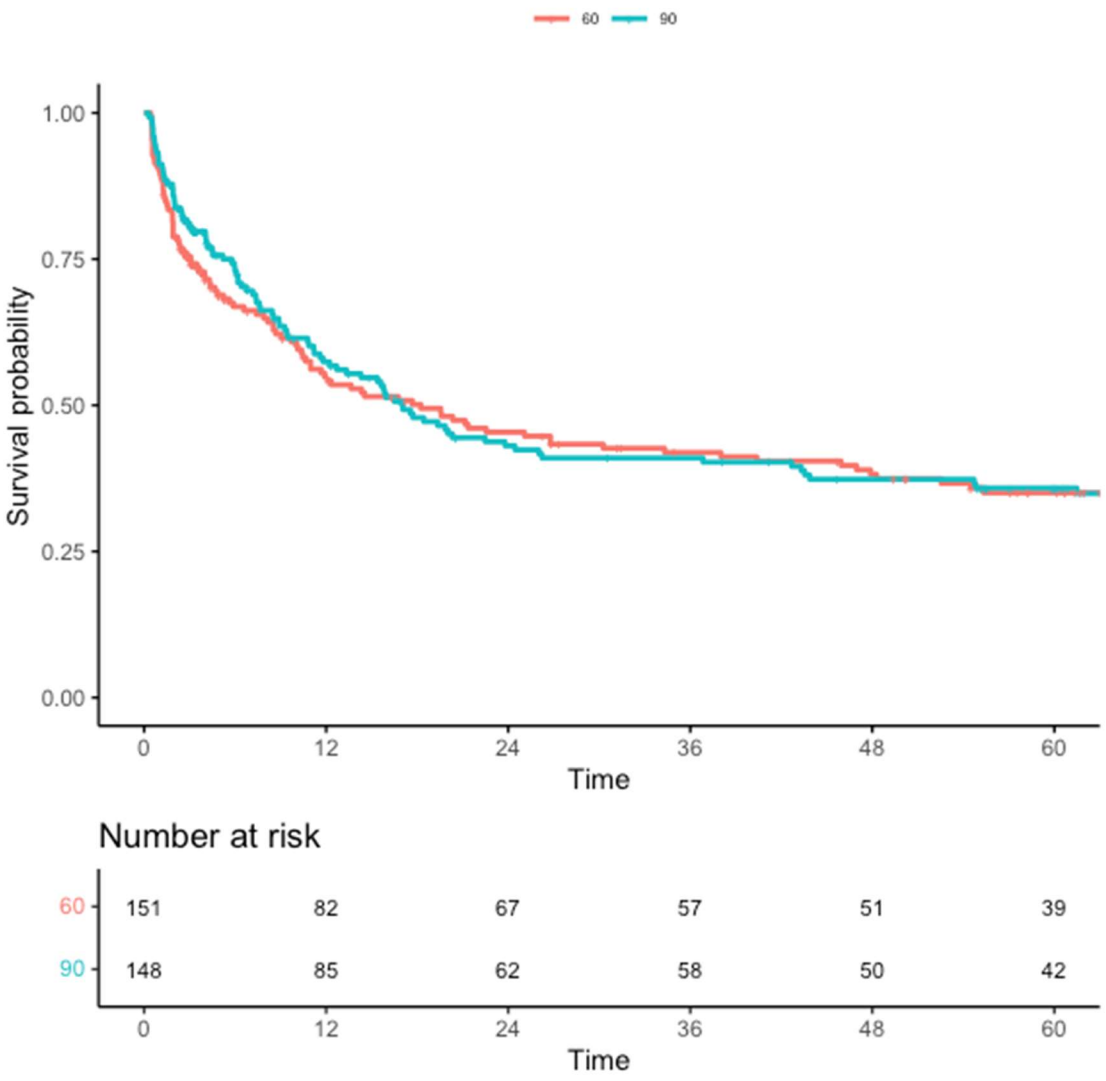


Table 26 Median survival

strata	records	n.max	n.start	events	median	LCL	UCL
60	203	151	151	99	18.239	10.976	38.022
90	206	148	148	96	17.056	11.798	26.224

Table 27 Survival probabilities

strata	time	n.risk	survival	LCL	UCL
60	12	82	0.548	0.474	0.634
60	24	67	0.454	0.381	0.541
60	36	57	0.419	0.347	0.506
60	60	39	0.350	0.281	0.438
90	12	85	0.574	0.500	0.660
90	24	62	0.431	0.358	0.519

strata	time	n.risk	survival	LCL	UCL
90	36	58	0.410	0.338	0.498
90	60	42	0.359	0.288	0.446

Table 28 Cox regression model, global p = 0.726

Parameter	log.HR	HR	ci.HR	se.HR	z	p
Treatment arm IT190	-0.05	0.951	(0.718 to 1.259)	0.143	-0.35	0.727

Table 29 Multiple Cox regression model

Parameter	log.HR	HR	ci.HR	se.HR	z	p
RESPONSE1Y	-0.564	0.569	(0.347 to 0.932)	0.252	-2.238	0.025
`Age (years)`	0.018	1.018	(1.002 to 1.034)	0.008	2.25	0.024
`Cytogenetic risk (ELN 2017)`favourable	-0.722	0.486	(0.263 to 0.897)	0.313	-2.307	0.021
`Cytogenetic risk (ELN 2017)`adverse	0.593	1.809	(1.171 to 2.796)	0.222	2.671	0.008
NPM1Y	0.328	1.388	(0.71 to 2.714)	0.342	0.959	0.338
FLT3IY	0.079	1.082	(0.632 to 1.854)	0.275	0.287	0.774
`Treatment arm IT1`90	-0.067	0.935	(0.625 to 1.4)	0.206	-0.325	0.745
ALSCTCR1	-0.36	0.698	(0.461 to 1.056)	0.212	-1.698	0.089
NPM1Y:FLT3IY	0.271	1.312	(0.565 to 3.047)	0.43	0.63	0.529
RESPONSE1Y:`Treatment arm IT1`90	0.052	1.054	(0.548 to 2.024)	0.333	0.156	0.876

6.5.2 TRIAL PART 2

6.5.2.1. OVERALL SURVIVAL

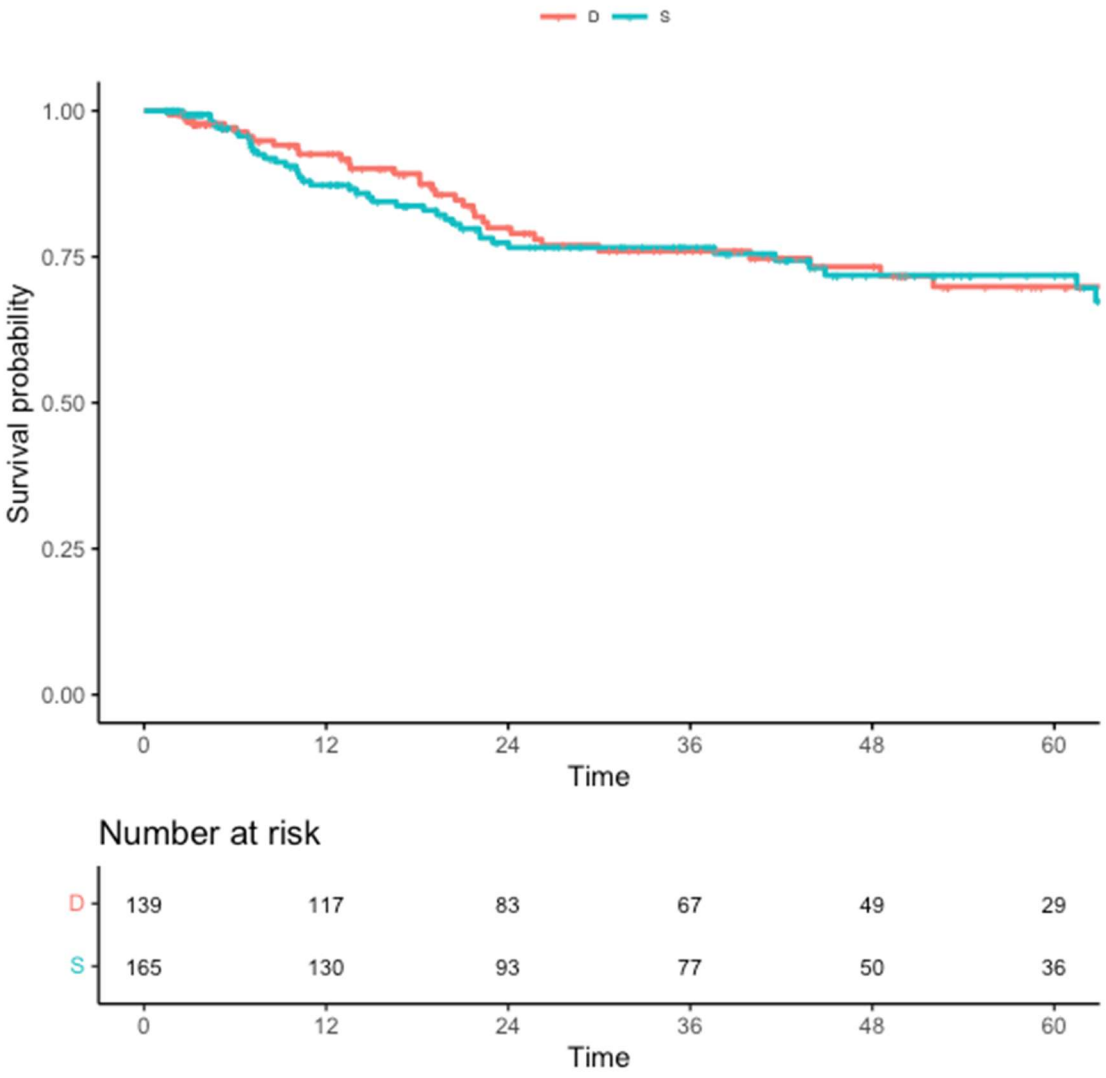


Table 30 Median survival

strata	records	n.max	n.start	events	median	LCL	UCL
D	187	139	139	33			
S	218	165	165	42		71.405	

Table 31 Survival probabilities

strata	time	n.risk	survival	LCL	UCL
D	12	117	0.926	0.882	0.971
D	24	83	0.799	0.730	0.875
D	36	67	0.760	0.685	0.843

strata	time	n.risk	survival	LCL	UCL
D	60	29	0.699	0.612	0.798
S	12	130	0.873	0.822	0.927
S	24	93	0.774	0.708	0.846
S	36	77	0.766	0.699	0.839
S	60	36	0.718	0.643	0.803

Table 32 Cox regression model, global p = 0.625

Parameter	log.HR	HR	ci.HR	se.HR	z	p
Randomized single vs. double inductionS	0.113	1.12	(0.71 to 1.767)	0.233	0.485	0.628

Table 33 Multiple Cox regression model

Parameter	log.HR	HR	ci.HR	se.HR	z	p
`Age (years)`	0.008	1.008	(0.983 to 1.033)	0.012	0.667	0.505
`Cytogenetic risk (ELN 2017)`favourable	-0.537	0.584	(0.276 to 1.237)	0.383	-1.402	0.161
`Cytogenetic risk (ELN 2017)`adverse	0.367	1.443	(0.636 to 3.275)	0.418	0.878	0.38
NPM1Y	-0.223	0.8	(0.343 to 1.865)	0.432	-0.516	0.606
FLT3IY	-0.595	0.552	(0.184 to 1.65)	0.559	-1.064	0.287
`Randomized single vs. double induction`S	-0.028	0.972	(0.571 to 1.656)	0.272	-0.103	0.918
ALSCTCR1	0.241	1.273	(0.716 to 2.263)	0.294	0.82	0.412
NPM1Y:FLT3IY	0.844	2.325	(0.577 to 9.361)	0.711	1.187	0.235

6.5.2.2. RELAPSE FREE SURVIVAL

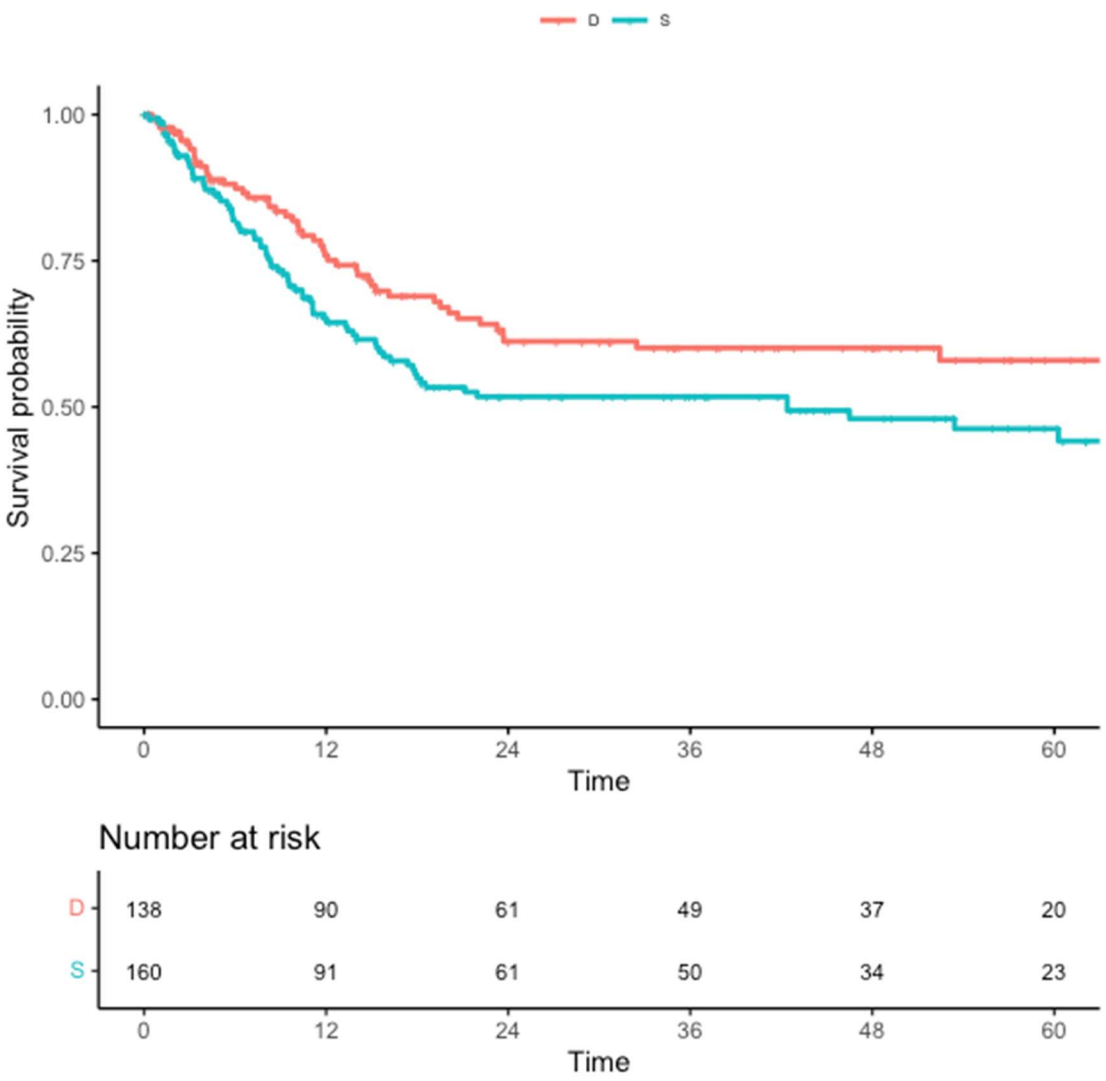


Table 34 Median survival

strata	records	n.max	n.start	events	median	LCL	UCL
D	186	138	138	50		52.448	
S	213	160	160	76	42.392	17.384	

Table 35 Survival probabilities

strata	time	n.risk	survival	LCL	UCL
D	12	90	0.759	0.689	0.837
D	24	61	0.612	0.530	0.707
D	36	49	0.601	0.518	0.697
D	60	20	0.580	0.491	0.684
S	12	91	0.652	0.580	0.732

strata	time	n.risk	survival	LCL	UCL
S	24	61	0.517	0.442	0.606
S	36	50	0.517	0.442	0.606
S	60	23	0.463	0.381	0.562

Table 36 Cox regression model, global p = 0.049

Parameter	log.HR	HR	ci.HR	se.HR	z	p
Randomized single vs. double inductionS	0.356	1.427	(0.999 to 2.04)	0.182	1.956	0.05

Table 37 Multiple Cox regression model

Parameter	log.HR	HR	ci.HR	se.HR	z	p
`Age (years)`	0.007	1.007	(0.989 to 1.026)	0.009	0.778	0.437
`Cytogenetic risk (ELN 2017)`favourable	-0.289	0.749	(0.434 to 1.292)	0.278	-1.04	0.299
`Cytogenetic risk (ELN 2017)`adverse	0.279	1.321	(0.655 to 2.668)	0.358	0.779	0.436
NPM1Y	0.052	1.053	(0.588 to 1.886)	0.297	0.175	0.861
FLT3IY	-0.11	0.896	(0.404 to 1.986)	0.406	-0.271	0.786
`Randomized single vs. double induction`S	0.331	1.393	(0.935 to 2.075)	0.203	1.631	0.103
ALSCTCR1	-0.198	0.821	(0.517 to 1.302)	0.236	-0.839	0.401
NPM1Y:FLT3IY	0.447	1.564	(0.589 to 4.153)	0.498	0.898	0.369

6.5.2.3. EVENT FREE SURVIVAL

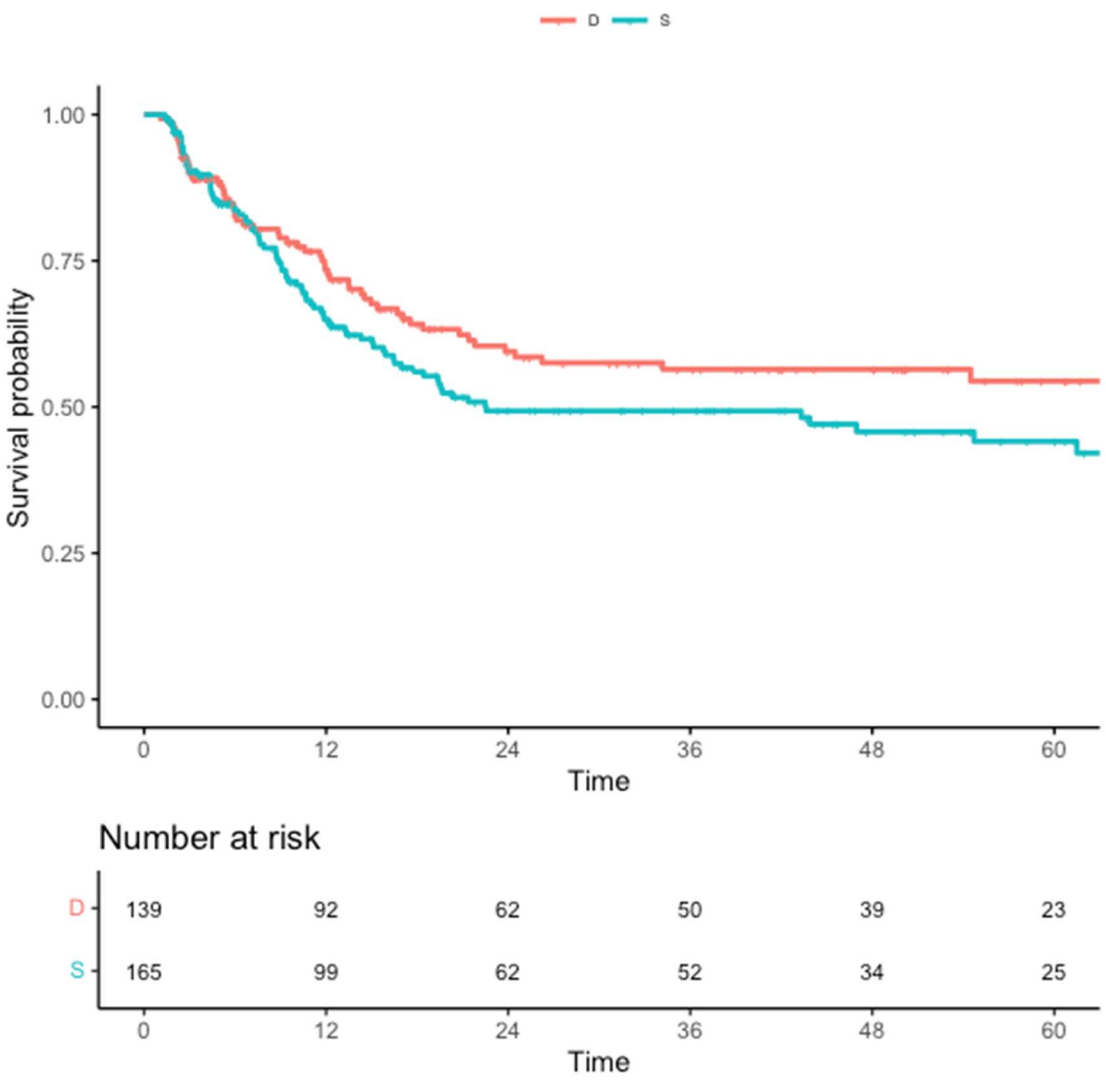


Table 38 Median survival

strata	records	n.max	n.start	events	median	LCL	UCL
D	187	139	139	57		26.224	
S	218	165	165	83	22.545	16.957	

Table 39 Survival probabilities

strata	time	n.risk	survival	LCL	UCL
D	12	92	0.734	0.663	0.813
D	24	62	0.595	0.515	0.688
D	36	50	0.564	0.482	0.660
D	60	23	0.544	0.458	0.647
S	12	99	0.650	0.580	0.728

strata	time	n.risk	survival	LCL	UCL
S	24	62	0.493	0.419	0.580
S	36	52	0.493	0.419	0.580
S	60	25	0.441	0.362	0.537

Table 40 Cox regression model, global p = 0.125

Parameter	log.HR	HR	ci.HR	se.HR	z	p
Randomized single vs. double inductionS	0.262	1.3	(0.928 to 1.821)	0.172	1.523	0.128

Table 41 Multiple Cox regression model

Parameter	log.HR	HR	ci.HR	se.HR	z	p
`Age (years)`	0.009	1.009	(0.992 to 1.027)	0.009	1	0.317
`Cytogenetic risk (ELN 2017)`favourable	-0.236	0.79	(0.47 to 1.327)	0.265	-0.891	0.373
`Cytogenetic risk (ELN 2017)`adverse	0.504	1.655	(0.864 to 3.168)	0.331	1.523	0.128
NPM1Y	0.129	1.138	(0.649 to 1.993)	0.286	0.451	0.652
FLT3IY	-0.043	0.958	(0.465 to 1.975)	0.369	-0.117	0.907
`Randomized single vs. double induction`S	0.205	1.227	(0.845 to 1.782)	0.19	1.079	0.281
ALSCTCR1	-0.226	0.798	(0.502 to 1.269)	0.237	-0.954	0.34
NPM1Y:FLT3IY	0.429	1.536	(0.63 to 3.743)	0.454	0.945	0.345

6.6 SECONDARY ENDPOINTS OF SAFETY

6.6.1 TRIAL PART 1

Table 42 AE summary

	Total (N=306)	60 (N=154)	90 (N=152)	p value
AE				0.2442
- N	7 (2.3%)	2 (1.3%)	5 (3.3%)	
- Y	299 (97.7%)	152 (98.7%)	147 (96.7%)	
AE_IT1				0.7756
- N	11 (3.6%)	6 (3.9%)	5 (3.3%)	

	Total (N=306)	60 (N=154)	90 (N=152)	p value
- Y	295 (96.4%)	148 (96.1%)	147 (96.7%)	
AEgrade3				0.7435
- N	124 (40.5%)	61 (39.6%)	63 (41.4%)	
- Y	182 (59.5%)	93 (60.4%)	89 (58.6%)	
AEgrade3_IT1				0.8942
- N	130 (42.5%)	66 (42.9%)	64 (42.1%)	
- Y	176 (57.5%)	88 (57.1%)	88 (57.9%)	
ED_IT1				0.1409
- N-Miss	10	4	6	
- N	288 (97.3%)	148 (98.7%)	140 (95.9%)	
- Y	8 (2.7%)	2 (1.3%)	6 (4.1%)	
ED60				0.1832
- N-Miss	12	5	7	
- N	284 (96.6%)	146 (98.0%)	138 (95.2%)	
- Y	10 (3.4%)	3 (2.0%)	7 (4.8%)	

6.6.2 TRIAL PART 2

Table 43 AE summary

	Total (N=377)	D (N=188)	S (N=189)	p value
AE				0.0556
- N	10 (2.7%)	2 (1.1%)	8 (4.2%)	
- Y	367 (97.3%)	186 (98.9%)	181 (95.8%)	
AEgrade3				0.1489
- N	144 (38.2%)	65 (34.6%)	79 (41.8%)	
- Y	233 (61.8%)	123 (65.4%)	110 (58.2%)	
ED60				0.9839

	Total (N=377)	D (N=188)	S (N=189)	p value
- N-Miss	26	15	11	
- N	349 (99.4%)	172 (99.4%)	177 (99.4%)	
- Y	2 (0.6%)	1 (0.6%)	1 (0.6%)	

7 CONCLUSION

To date, intensive chemotherapy remains a prerequisite and backbone of curative AML treatment. In the presented trial, we intended to answer two fundamental questions in relation to the most commonly used intensive chemotherapy protocol, commonly named 7+3.

The results of TUD-2DAUNO-058 demonstrate that three doses of 90 mg/m2 daunorubicin leads to similar response and remission rates as 60 mg/m2, with no significant differences in tolerability. We did not observe excess toxicity in the 90 mg arm. This was consistent for all survival types, i.e. EFS, RFS and OS.

With respect to previous trials showing significant improvements in remission and survival for 90 mg/m2 versus 45 mg/m2, these findings indicate a non-linear dose-response relationship of daunorubicin, with a dose around 60 mg/m2 representing the best risk-benefit ratio. Of note, our results do not indicate short-term excess toxicity for 90 mg/m2, suggesting that the higher dose does not seem to cause harm while being equally effective. However, it seems most desirable to always aim for the lowest effective dose in treatment, in particular with respect to the cumulative toxicity threshold of anthracyclines and possible combination therapies with new targeted agents such as tyroskine kinase or bcl2 inhibitors.

In the DaunoDouble trial, we used early response assessment two weeks after commencement of induction both as primary endpoint to detect a difference between the two daunorubicin doses, but also as a requirement to undergo the second randomization between a second 7+3 induction or no further induction. This consideration was based on extensive data showing that early response is predictive for both remission and long-term outcomes. Furthermore, the concept of double induction is based on the start of the second induction cycle on day 21 without the necessity to wait for blood count recovery. Several blast thresholds for early response assessment around day 14 have been evaluated in the past decades, ranging between 5 to 40%. In the context of double induction with the second cycle starting before blood count recovery, we considered it most appropriate and safe from a clinical and ethical perspective, to choose the 5% cut-off and to treat only those patients with a single induction who had a good blast clearance below 5% before second randomization.

An additional second induction cycle did not result in a significant or clinically relevant increase in the rates of first CR in good responders to first induction who were treated as randomized and without relevant protocol deviations (PPS). The 5% difference in CR after induction did not reach the predefined statistical significance for non-inferiority since the upper limit of the 95% confidence interval was 10.8% and not <7.5%, as required for formal non-inferiority. In all randomized patients (FAS), the CR rate after single induction was 2% higher than for double induction, but the risk of selection bias in favor of the single arm must be considered. Notably, a trend for longer RFS in the double arm was observed in the univariable analysis. This difference disappeared after accounting for standard prognostic factors in the multivariable analysis. In addition, we did not observe differences in OS between the two treatment arms, indicating that the overall prognosis including potential relapses and salvage treatments as well as potential late toxicity effects was not affected by the number of induction cycle. The most likely explanation for this constellation is the high efficacy of allogeneic HCT as salvage treatment.

The safety evaluation of this second part of the trial did not show any novel AEs occurring specifically during the second induction. As expected, the number of AEs from beginning of treatment until the final remission assessment was significantly higher when patients had to undergo two induction cycles instead of one. Together with a small difference in CR after single

over double induction, we can conclude that double induction is not necessary in good early responders and should be omitted from treatment plans to spare the patients the side effects of a second induction cycle.

In summary, the results of the DaunoDouble trial show in a prospective randomized setting that a dose of 60 mg/m2 daunorubicin is as efficient as 90 mg/m2, and seems to be high enough for remission induction. Patients responding well to the first induction and achieving a blast count <5% do not benefit from a second induction cycle and should proceed with the appropriate post-remission treatment.

8 PUBLICATIONS

Röllig C, Steffen B, Schliemann C, et al. Single Versus Double Induction with "7+3" Containing 60 Versus 90 Mg Daunorubicin for Newly Diagnosed AML: Results from the Randomized Controlled SAL Dauno-Double Trial. Blood. 2022;140(Supplement 1):523–525.

9 SIGNATURES

The signing persons approve the report presented here by their signature. The described clinical trial was conducted according to the Declaration of Helsinki, Good Clinical Practice (GCP) as well as the applicable legal regulations.

Sponsor

Prof. Dr. Christoph Röllig	Dresden, 9.2.24	
Name in block letters	Place, Date	Signature

Biostatistics

MICHAEL KRAMER	DRESDEN, 12.02.2024	
Name in block letters	Place, Date	Signature

10 LIST OF ABBREVIATIONS

AE	adverse event
AESI	adverse event of special interest
AMG	Arzneimittelgesetz
AR	adverse reaction
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte
BSA	Body Surface Area
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DA60	Daunorubicin+Cytarabin 60mg/m^2
DA90	Daunorubicin+Cytarabin 90mg/m^2
eCRF	Electronic Case Report Form
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FAS	Full analysis set
FPFV	First patient first visit
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
ISF	Investigator Site File
IT	Induction Therapy
ITT	Intention to treat
IV	Intravenous infusion
KKS	Koordinierungszentrum für Klinische Studien
LPLV	Last patient last visit
NA	not applicable
ND	not done
PEI	Paul-Ehrlich-Institut
PPS	Per protocol set
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SAS	Safety Analysis Set
SDV	Source Data Verification
SOP	Standard Operating Procedure
SPSS	Statistical Package for the Social Sciences
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
UAR	Unexpected Adverse Reaction