Final Report

Randomized Phase II trial with safety run-in phase evaluating low-dose azacitidine, all-trans retinoic acid and pioglitazone versus standard dose azacitidine in patients ≥60 years with acute myeloid leukemia (AML) who are refractory to standard induction chemotherapy

AMLSG 26-16 / AML-ViVA

Sponsor Name and Address:	University Hospital Regensburg Represented by Prof. Dr. Simone Thomas (LKP) Franz-Josef-Strauß-Allee 11 93053 Regensburg, Germany
Investigational Medicinal Products:	Azacitidine (Vidaza®) All-trans retinoic acid (ATRA) (Vesanoid®) Pioglitazone (Actos®)
Indication	Acute myeloid leukemia (AML)
EudraCT-No	2016-000421-39
NCT-No	NCT02942758
Start of Trial	May 31, 2017 (first-patient-in)
End of Trial	March 25, 2020 (premature termination)
Version / Date	V2.0 / April 13, 2021
Written by	Prof. Dr. Simone Thomas, Dr. Daniel Heudobler

Signature Page

By signing this report, I agree to the content of this final results report. The reported clinical trial was carried out in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice (GCP) and applicable regulatory requirements.

Prof. Dr. med. Simone Thomas
Coordinating Investigator,
Sponsor Representative

signature

15-APR-2021
date

Dr. med. Daniel Heudobler
Deputy Coordinating Investigator

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Florian Zeman
Trial Statistician

signature

13 APR 2021
date

TABLE OF CONTENTS

1 Name of Sponsor/Company

Universitätsklinikum Regensburg
Represented by Prof. Dr. Simone Thomas (LKP)
Franz-Josef-Strauß-Allee 11
93053 Regensburg

2 Name of Finished Product

Vidaza®
Vesanoid®
Actos®

3 Name of Active Ingredient

Azacitidine
All-trans retinoic acid (ATRA)
Pioglitazone

4 Individual Study Table

Not applicable

5 Title of Study

Randomized Phase-II trial with safety run-in phase evaluating low-dose azacitidine, all-trans retinoic acid and pioglitazone versus standard dose azacitidine in patients ≥60 years with acute myeloid leukemia (AML) who are refractory to standard induction chemotherapy

Version 2.0 – 01.03.2017

6 Publication (reference)

Abstract Annual Meeting of the American Society of Hematology:

Heudobler D, Klobuch S, Lueke F et al. Low-Dose Azacitidine, Pioglitazone and All-Trans Retinoic Acid Versus Standard-Dose Azacitidine in Patients ≥ 60 Years with Acute Myeloid Leukemia Refractory to Standard Induction Chemotherapy (AMLSG 26-16/AML-ViVA): Results of the Safety Run-in Phase I. In: *Blood* 2019*;* 134 (Supplement_1), 1382. DOI: 10.1182/blood-2019-129977

7 Study period

First patient in: 31. May 2017, **Last patient out (LPLV)**: 16. March 2020

On March 25, 2020, the study trial was prematurely terminated after completion of the safety-run-in-phase owing to financial reasons (Appendix 22.6 “Early Termination”).

8 Phase of development

Randomized Phase II with dose-finding safety run-in phase

9 Objectives

According to the study protocol, the primary objective of the safety-run-in-phase was to assess the safety (as measured by the incidence and intensity of adverse events) of the combination of low-dose azacitidine, ATRA, and pioglitazone in patients with refractory AML.
Primary objective of the randomized phase II part (no patient enrolled due to premature termination) was efficacy as measured by overall survival.

Secondary objectives were:

- To evaluate the rate of complete remission (CR), CR with incomplete blood count recovery (CRi), partial remission (PR), hematological improvement (HI) of the combination of low-dose azacitidine, ATRA, and pioglitazone.
- To evaluate cumulative incidence of relapse (CIR) and death (CID), relapse-free survival (RFS), event-free survival (EFS) of the combination of low-dose azacitidine, ATRA, and pioglitazone.

10 Methodology

The AMSLG 26-16 / AML-ViVA trial is a national, multicentre, prospective, open-label, randomized phase II trial with dose-finding safety run-in phase in patients with AML refractory to standard induction chemotherapy.

In the initial dose-finding safety run-in phase patients were treated in a modified dose-de-escalation 3+3 design (see Table) to evaluate the safety of the combination of azacitidine, ATRA, and pioglitazone. Patients were enrolled in dose level 0 at an ATRA dose of 45 mg/m²/day from day 1 to day 28 and 15 mg/m² thereafter in combination with azacitidine (75 mg/m² from day 1 to day 7 per 28-day treatment cycle) and pioglitazone (45 mg/day continuously starting at day 1). The dose-limiting toxicities (DLT) period was 28 days. Initially three patients were enrolled and treated in dose level 0 without DLTs. If 0/3 or 1/6 enrolled patients experience dose-limiting toxicities (DLTs) attributable to ATRA, an additional 6 patients could be treated at the same dose level. However, if DLTs are recorded in more than 1 out of 6 patients, it was planned to de-escalate the dose of ATRA to dose level n - 1, as shown in the table below. Patients continued treatment for unlimited treatment cycles, as long as clinically appropriate, until AML progression or AML relapse.

Dose Level	ATRA	AZA	Pioglitazone
Dose level 0	45 mg/m²/day from day 1-28 15 mg/m²/day from day 29	fixed dose of 75 mg/day for 7 days, starting with day 1	fixed dose of 45 mg/day from day 1
Dose level -1	45 mg/m²/day from day 1-14 15 mg/m²/day from day 15		
Dose level -2	45 mg/m²/day from day 1-5 15 mg/m²/day from day 6		

In total, 10 patients were enrolled at dose-level 0, with 9 patients providing evaluable toxicity data (one patient withdrew consent very early and consequently had only a short therapy duration prior to drop-out). Based on data collected from the 9 patients treated at dose level 0, it was not necessary to de-escalate the ATRA dose to dose level -1 or -2.

According to study protocol it was planned to proceed with a randomized (1:1 ratio) phase II part of the study to treat 76 patients with low-dose azacytidine, ATRA and and pioglitazone (experimental

arm; n=38) or with standard-dose azacitidine (control arm; n=38). Due to financial reasons the study was prematurely terminated after completion of the safety run-in phase on March 16, 2020.

Patients

Eligible patients were patients ≥60 years of age with confirmed diagnoses of acute myeloid leukemia (AML) who are refractory to standard induction chemotherapy and are not eligible for further intensive induction therapy based on medical reasons (e.g. disease or patient characteristics) or not immediate candidates for allogeneic hematopoietic stem cell transplantation, respectively. Patients had to have an ECOG performance status of ≤ 2. Patients with acute promyelocytic leukemia exhibiting t(15;17(q22;q12), PML-RARA, or with variant translocations as well as patients with AML with isocitratdehyrogenase (IDH) 1 or mutations were excluded.

The institutional review boards and ethic committees of all participating centers approved the protocol (positive vote from the ethics committee of the University of Regensburg, February, 2nd, 2017, approval No.: 16-356-112).

Safety Run-In Phase

In the initial dose-finding safety run-in phase patients were treated in a modified dose-de-escalation 3+3 design to receive ATRA in three different dose levels, low-dose azacitidine and pioglitazone in 28-day cyles. Following dose levels were planned:

Dose Level	ATRA	AZA	Pioglitazone
Dose level 0	45 mg/m²/day from day 1-28 15 mg/m²/day from day 29 continuously	fixed dose of 75 mg/day for 7 days, starting with day 1, next cycle day 29	fixed dose of 45 mg/day from day 1, continuously
Dose level -1	45 mg/m²/day from day 1-14 15 mg/m²/day from day 15 continuously		
Dose level -2	45 mg/m²/day from day 1-5 15 mg/m²/day from day 6 continuously		

Starting with treatment cycle 1 the dose-limiting toxicities (DLT) period was 28 days. Initially three patients were treated in dose level 0 without DLTs. According to the protocol additional six patients were treated within dose level 0. Patients continued treatment for unlimited treatment cycles, as long as clinically appropriate, until AML progression or AML relapse.

Randomized Phase II part

According to study protocol it was planned to proceed with a randomized (1:1 ratio) phase II part of the study to treat 76 patients with low-dose azacytidine, ATRA and and pioglitazone (experimental arm; n=38) or with standard-dose azacitidine (control arm; n=38). Due to premature termination of the study, no patients were treated within the randomized part of the study protocol.

11 Number of patients (planned and analyzed)

Planned: 85 patients; safety run-in phase: 9 patients, randomized part: 76 patients

Enrolled/Treated: 10 patients; safety run-in phase: 10 patients, randomized phase: no patients

Drop-outs: 1 patient (due to very early withdrew of consent)

Analyzed: 9 patients

(Appendix 22.3 Flowchart)

The first patient was enrolled on 31st May 2017 and recruitment to the safety run-in phase was successfully completed by April 2019. In total, 10 patients were enrolled, with 9 patients providing evaluable toxicity data (one patient withdrew consent very early and consequently had only a short therapy duration prior to drop-out). On March 16, 2020, the study trial was prematurely terminated after completion of the safety run-in phase owing to financial reasons (Appendix 22.6 "Early Termination").

Since July 23, 2019 no patient received study medication anymore. Until the end of the study, 10 patients (7 males, 3 females) were enrolled in the safety run-in part of the protocol and received study medication at dose level 0. One patient with withdrew consent very early (day 9 of treatment cycle 1) and consequently had only a short therapy duration prior to drop-out. This patient was replaced. Overall, 10 patients are counted as patients treated with study medication from which 9 patients provided evaluable toxicity data.

All 10 patients, who were treated with study medication terminated study treatment due to different reasons:

- Progression of the underlying disease (7), thereof
  - with (Serious) adverse event (5), thereof
    - Death due to infections (3)
    - Death due to bleeding (2)
- Allogeneic hematopoietic stem cell transplantation (2)
  - Alive (1) days
  - Death due to relapse (1)
- Withdraw of consent during treatment cycle 1 (1)

Relationship of Serious adverse event (5) to study medication

- unlikely (2)
- not related (3)

12 Diagnosis of main criteria for inclusion

We enrolled patients ≥ 60 years who met the following main criteria:

1. Patients with confirmed diagnosis of acute myeloid leukemia (AML) who are refractory* to induction therapy and not eligible for further intensive induction therapy based on documented medical reasons (e.g. disease characteristics or patient characteristics), or
2. Patients with confirmed diagnosis of acute myeloid leukemia (AML) who are refractory* to induction therapy and not immediate candidates for allogeneic HSCT (bridge to transplant is allowed)

   *refractory to induction therapy is defined as no CR, no CRi and no PR (according to standard criteria) after at least one intensive induction therapy including at least 5 days of cytarabine 100-200 mg/m² continuously or an equivalent regimen with cytarabine with total dose not less than 500 mg/m² per cycle and at least 2 days of an anthracycline (e.g. daunorubicin, idarubicin)
3. Age ≥ 60; no upper age limit
4. ECOG performance status of ≤ 2 at screening
5. To control hyperleukocytosis or extramedullary involvement, medication with hydroxyurea is allowed up to 24h before start of study treatment. In case of hyperleukocytosis hydroxyurea

should be given and start of study treatment should be delayed until leukocyte counts are ≤ 15 x 109/L

6. Signed written informed consent

13 Test product, close and mode of administration

Patients in the safety run-in part of the study received a combined biomodulatory treatment consisting of:

- **ATRA**, per os, 45 mg/m²/day from day 1 to 28, 15 mg/m² from day 29, the total dose had to be split up into two doses per day, daily therapy until progression or relapse
- **Azazitidine**, subcutaneously, fixed dose of 75 mg/day from day 1 to 7 per 28-day treatment cycle, repeated cycles until progression or relapse
- **Pioglitazone**, per os, fixed dose of 45 mg/day, once daily until progression or relapse

Patients were allowed to continue treatment for unlimited cyclses, as long as clinically appropriate, until progressive or relapsing disease or until the patient was no longer deriving clinical benefit.

14 Duration of treatment

See section 13

15 Reference substance

In the randomized part patients were planned to be randomly assigned to the control arm (standard-dose azazitidine, 75 mg/m² for 7 days per 28-day treatment cycle). Due to the premature termination of the study no patient was randomized and no received reference substance.

16 Criteria for evaluation (Efficacy and Safety)

The AMLSG 26-16 / AML-ViVA trial addressed the medical need for low-toxic therapies in frequently comorbid patients with refractory AML who are considered to be ineligible for further intensive remission induction therapy. In the safety-run-in phase of this trial we evaluated the safety of a biomodulatory approach in patients ≥ 60 years who failed standard induction chemotherapy.
Primary Endpoint of the safety-run-in phase was “safety” defined as incidence and intensity of adverse events (AEs) according to CTCAE grading. Due to the premature termination of the study, the primary efficacy endpoint (overall survival) of the randomized study phase could not be evaluated. Secondary endpoints were “response rates” (including complete remission, complete remission with incomplete blood count recovery, partial remission, and hematological improvement), cumulative incidence of relapse (CIR) and death (CID), relapse-free survival (RFS), and event-free-survival (EFS).

17 Statistical methods

Dose finding safety run-in phase:

A modified 3+3 design was used in the dose finding run-in phase of the trial. If 0/3 or 1/6 enrolled patients have DLT attributable to ATRA additional 6 patients were planned to be treated with dose the dosage of 45 mg/m² from day 1 to day 28 and 15 mg/m² continuously thereafter (dose level 0). In the case of DLT in more than 1 of 6 patients, the dose of ATRA was planned to be de-escalated as illustrated in the following table:

Dose Level	ATRA	AZA	Pioglitazone
Dose level 0	45 mg/m²/day from day 1-28 15 mg/m²/day from day 29 continuously	fixed dose of 75 mg/day for 7 days, starting with day 1, next cycle day 29	fixed dose of 45 mg/day from day 1, continuously
Dose level -1	45 mg/m²/day from day 1-14 15 mg/m²/day from day 15 continuously		
Dose level -2	45 mg/m²/day from day 1-5 15 mg/m²/day from day 6 continuously		

Randomized Phase II Part:

Due to premature termination no patients were enrolled in the randomized phase II part of the study.

18 Summary – conclusions

Patient characteristics

Between May 2017 and March 2020, ten patients were enrolled in the safety-run-in phase (one patient withdrew informed consent on day 9 of cycle 1). Among all treated patients, the median age was 68 years (range, 60-76 years), and the majority of patients (70%) had an ECOG Performance status of 1 (Table 1). Two patients had secondary AML; another two patients had therapy-related AML (t-AML). Eight patients had a complex aberrant karyotype. Eight patients were enrolled after pre-treatment with one cycle of standard induction chemotherapy, two patients received two cycles or standard induction therapy before enrollment.

Safety Results

After a median follow-up of 131 days, the mean treatment duration was 126 days (range, 27-426 days). A total of N=368 adverse events (AEs) were documented throughout the trial (grade 1: N=176, grade 2: N=82, grade 3: N=60, grade 4: N=34, grade 5: N=4, grade missing: N=12). Table 2 listed the reported AEs on a patient basis. N=13 of the 368 reported AEs were classified as serious adverse events (SAEs) (Table 3). Almost half of all reported SAEs were categorized under the primary MedDRA SOC “infections and infestations”. Since infections are an expected risk for patients undergoing treatment of AML, these events were unremarkable from a safety monitoring perspective. All infection SAEs were assessed unrelated to study medication and instead related to the underlying disease. Only one SAE (“pancytopenia”) was assessed as related to a study drug. There have been no SUSARs.

Out of the 368 AEs, none was classified as dose limiting toxicity (DLT). Thus, no DLT occurred throughout the safety run-in phase and it was not necessary to de-escalate the dose of ATRA to a lower level. Out of 368 AEs, three were rated definitely related to study drug (N=2 for azacitidine, terms “injection site reaction” and “Platelet count decreased”; N=1 for ATRA, term “Skin and

subcutaneous tissue disorders (Hyperkeratosis) (Table 2) "). 35 AEs were rated to be probably related to study drug. In sum, the safety profile was tolerable.

Efficacy Results
According to the primary efficacy endpoint (overall survival) of the randomized part of the trial, overall survival of the safety-run-in population was analyzed (Figure 2). A total of 7 patients died during treatment/follow-up. All deaths were either directly attributable to AML or attributable to SAEs arising from the underlying AML. The median follow-up time as well as the median overall survival time was 131 days. The 1-year overall survival rate was: 45% (95%-CI: 22% - 93%). These final data do not suggest that the study treatment was any less effective than standard care in this group of elderly patients.

All secondary endpoints were part of the randomized part of the trial, which did not come off. Three patients achieved a complete remission (CR), one patient a partial remission (PR) and four a stable disease (SD) (Table 1). Two patients underwent allogeneic hematopoietic stem cell transplantation after end of treatment.

Conclusion
The dose-finding safety run-in phase of the study was successfully completed. In summary, the low-intensity biomodulatory regimen of low-dose azacitidine, pioglitazone, and ATRA demonstrated a tolerable safety profile and encouraging signals for efficacy in patients with AML refractory to standard induction chemotherapy warranting further investigation.

19 Date of Report

13. April 2021

20 Appendix

APPENDIX

20.1 Table 1: Study population, treatment and response

Patient	Sex	Age	WHO-ECOG	AML type	Pre-treatment 1/2	Treatment cycles	Best response	Reason for discontinuation	Comment
1	male	68	1	de novo	Thio/Cyt/Dauno	1	NA	patient wish	study stopped on day 9 of cycle 1
2	male	60	2	sec.	Cyt/Dauno, Mito/Cyt	4	SD	PD	
3	male	75	1	de novo	Cyt/Dauno	10	CR	PD	
4	female	65	0	t-AML	Cyt/Dauno	2	CR	allo-HSCT	allo-HSCT after 2nd cycle
5	male	66	0	t-AML	Cyt/Dauno	2	CR	allo-HSCT	allo-HSCT after 2nd cycle, alive after 434 days
6	male	76	1	de novo	Cyt/Dauno	2	SD	PD	
7	male	62	1	de novo	Cyt/Dauno	14	PR	PD	
8	male	68	1	de novo	Cyt/Dauno	3	SD	PD	
9	female	76	1	de novo	Cyt/Dauno	1	SD	PD	
10	female	65	1	sec.	Thio/Cyt/Dauno, Mito/Cyt	1	NA	PD	

sec. = secondary, t-AML=therapy-related AML, Thio=Thioguanin, Cyt=Cytarabine, Dauno=Daunorubicin, Mito=Mitoxantron, HSCT=hematopoietic stem cell transplantation
NA=not analyzed, SD= stable disease, CR=complete remission, PR=partial remission, PD=progressive disease

20.2 Table 2: Adverse events on patient basis

	Safety population (N=10)	
	Any Grade	Grade 3 or 4
	Number of patients with event (percent)	
Anemia	8 (80%)	6 (60%)
Platelet count decreased	6 (60%)	4 (40%)
White blood cell decreased	6 (60%)	5 (50%)
Neutrophil count decreased	5 (50%)	5 (50%)
Fever	2 (20%)	2 (20%)
Chills	1 (10%)	
Lung infection	4 (40%)	4 (40%)
Urinary tract infection	3 (30%)	2 (20%)
Sepsis	1 (10%)	1 (10%)
Nail infection	1 (10%)	
Infections and infestations - Other	3 (30%)	1 (10%)
Epistaxis	2 (20%)	
Urinary urgency	1 (10%)	1 (10%)
Renal and urinary disorders – Other (urinary tract bleeding)	1 (10%)	1 (10%)
Gastric hemorrhage	1 (10%)	1 (10%)
Eye disorders – Others (retinal bleeding)	1 (10%)	
Cough	4 (40%)	
Dyspnea	3 (30%)	
Hypokalemia	4 (40%)	1 (10%)
Fibrinogen decreased	1 (10%)	
Investigations - Other (CRP increase): 12.38.1	1 (10%)	
Iron overload	1 (10%)	1 (10%)
Hyperthyroidism	1 (10%)	
Pain	7 (70%)	2 (20%)
Bone pain	1 (10%)	
Arthralgia	1 (10%)	
Headache	1 (10%)	
Hypotension	1 (10%)	
Atrial flutter	1 (10%)	
Thromboembolic event	2 (20%)	2 (20%)
Edema limbs	3 (30%)	
Edema trunk	1 (10%)	
Diarrhea	2 (20%)	
Constipation	2 (20%)	1 (10%)
Gastrointestinal pain	1 (10%)	
Gastrointestinal disorders – Other (decrease of appetite)	2 (20%)	
Nausea	1 (10%)	
Vomiting	1 (10%)	
Weight loss	1 (10%)	
Fatigue	1 (10%)	
General disorders and administration site conditions - Other	2 (20%)	

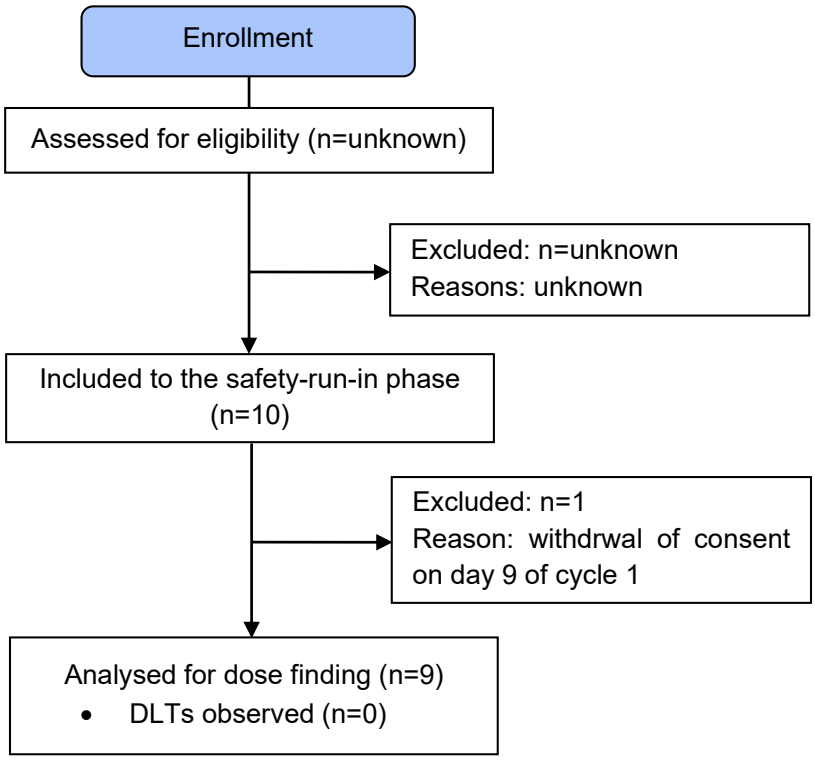
Insomnia	2 (20%)	1 (10%)
Skin and subcutaneous tissue disorders	3 (30%)	
Skin ulceration	1 (10%)	
Wound complication	1 (10%)	1 (10%)
Allergic reaction	1 (10%)	
Injection site reaction	1 (10%)	
Dizziness	2 (20%)	
Nervous system disorders - Other	1 (10%)	1 (10%)
Depression	1 (10%)	
Psychiatric disorders – Others (panic attack)	1 (10%)	

20.3 Table 3: Serious adverse events

MedDRA System Organ Class (SOC)* MedDRA Preferred Term (PT)	**Safety run-in DOSE LEVEL 0**	**Severity**	**Outcome**	**Related to azacitidine**	**Related to ATRA**	**Related to Pioglitazone**
Blood and lymphatic system disorders						
Anaemia	1	Grade 3	unknown	not related	not related	not related
Pancytopenia	1	Grade 4	not recovered/resolved	possible	not related	not related
Gastrointestinal disorders						
Gastric haemorrhage	1	Grade 5	fatal	unlikely	unlikely	unlikely
General disorders and administration site conditions						
Pyrexia	1	Grade 3	recovered/resolved	not related	not related	not related
Infections and infestations						
Bacterial pyelonephritis	1	Grade 3	recovered/resolved	not related	not related	not related
Catheter site infection	1	Grade 3	recovered/resolved	not related	not related	not related
Infection	1	Grade 5	fatal	not applicable	not applicable	not applicable
Pneumonia	1	Grade 5	fatal	not related	not related	not related
Pneumonia	1	Grade 5	fatal	unlikely	unlikely	unlikely
Pneumonia fungal	1	Grade 3	not recovered/resolved	not related	not related	not related
Urinary tract infection	1	Grade 3	recovered/resolved	not related	not related	not related
Psychiatric disorders						
Panic attack	1	Grade 2	recovered/resolved	not related	not related	not related
Renal and urinary disorders						
Haemorrhage urinary tract	1	Grade 4	fatal	not related	not related	not related
TOTAL:	**13**					

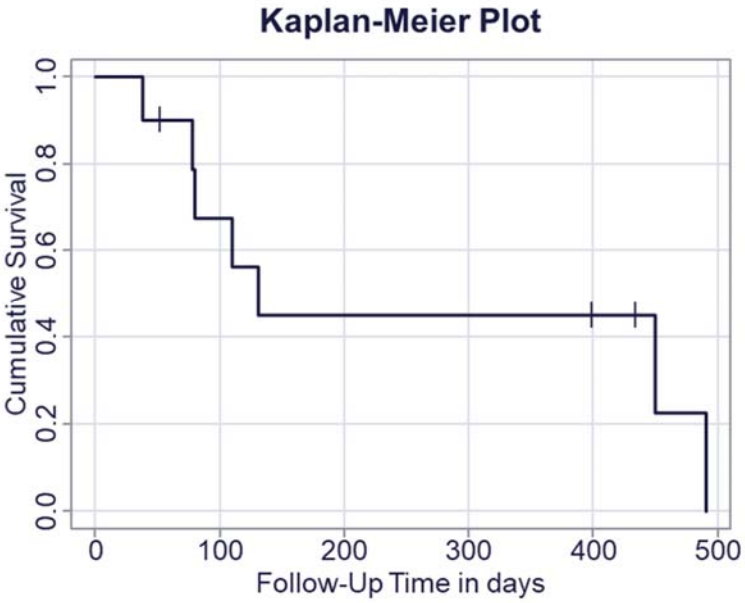
20.4 Flowchart

Figure 1



20.5 Kaplan-Meier Representation of the Overall Survival

Figure 2



median OS: 131 days
1-year OS: 45% (95%-CI: 22% - 93%)

20.6 Substantial Amendments

No substantial amendments were submitted.

20.7 Early termination

On **March 25, 2020**, the study trial was **prematurely terminated** due to financial reasons. At this timepoint the safety-run-in phase of the trial was completed.