Center for Internal Medicine
Department of Internal Medicine III
University of Ulm
Director: Prof. Dr. med. H. Döhner

Condensed versus standard schedule of high-dose cytarabine consolidation therapy with pegfilgrastim growth factor support in acute myeloid leukemia

A dissertation presented to the medical faculty of the
Ulm University
In fulfillment of the requirements for the degree of
Doctor in Medicine

Sonia Jaramillo Segura
Born in Bogotá, Colombia

2016

Acting Dean:	Prof. Dr. rer. nat. Thomas Wirth
First rapporteur:	Prof. Dr. Richard F. Schlenk
Second rapporteur:	Prof. Dr. Gabriele Nagel
Thesis defense:	October 26, 2017

Teile dieser Dissertation wurden bereits in dem folgenden Fachartikel veröffentlicht:

Sonia Jaramillo, Axel Benner, Jurgen Krauter, Hans Martin, Thomas Kindler, Martin Bentz, Helmut R. Salih, Gerhard Held, Caus-Henning Köhne, Katharina Götze, Michael Lübbert, Andrea Kündgen, Peter Brossart, Mohammad Amen Wattad, Hans-Juergen Salwender, Bernd Hertenstein, David Nachbaur, Gerald Wulf, Heinz A. Horst, Heinz Kirchen, Walter Fiedler, Aruna Raghavachar, Gudrun Russ, Stephan Kremers, Elisabeth Koller, Volker Runde, Gerhard Heil, Daniela Weber, Brigitte Schlegelberger, Konstanze Döhner, Arnold Ganser, Hartmut Döhner and Richard F. Schlenk "Condensed versus standard schedule of high-dose cytarabine consolidation therapy with pegfilgrastim growth factor support in acute myeloid leukemia" Blood Cancer J. 2017.

Index

Abbreviations

AML	Acute myeloid Leukemia
AMLSG	German-Austrian AML Study Group
ATRA	All-trans retinoic acid
b.i.d.	Bis in die (twice a day)
CALGB	Cancer and Leukemia Group B
CI	Confidence interval
CID	Cumulative incidence of death
CIR	Cumulative incidence of relapse
CR	Complete remission
ECOG	Eastern Cooperative Oncology Group
ELN	European LeukemiaNet
FAB	French-American-British
FLT3	FMS-like tyrosine kinase 3
FLT3-ITD	FLT3 internal tandem duplication
FLT3-TKD	FLT3 tyrosine kinase domain
FMS	Feline McDonough sarcoma
g	Gram
G-CSF	Granulocyte colony-stimulating factor
HDAC	High-dose cytarabine
HLA	Human leukocyte antigen
HR	Hazard ratio
HSCT	Hematopoietic stem cell transplantation
ICE	Idarubicin, cytarabine, etoposide
IV	Intravenous
L	Liter
MDS	Myelodysplastic Syndrome
mg/m²	milligram per square meters of body surface
n	Sample size
NCCN	National Comprehensive Cancer Network
OS	Overall survival
PEG	Polyethylene glycol
PR	Partial remission
RFS	Relapse-free survival
sAML	Secondary AML
s/tAML	Secondary or treatment-related AML
tAML	Treatment-related AML
VPA	Valproate or valproic acid
vs.	Versus
WBC	White blood cell
WHO	World Health Organization
WLW	Wein-Lin-Weissfeld

1. Introduction

1.1 The acute myeloid leukemia

Acute myeloid leukemia (AML) is an aggressive hematological malignancy with poor prognosis, and it is according to the American Cancer Society the second most common type of leukemia in adults in the United States. Between 2008 and 2012 according to the surveillance program of the National Cancer Institute in the United States, the incidence of AML in the population under 65 years of age was of 1.9 per 100,000 per year and in the population over 65 years of age was of 18.3 per 100,000 per year (SEER, 2015). According to this surveillance program, the rate for new AML cases has been rising on average by 3.4% each year over the last ten years. Despite the latest efforts in therapy, only 26.6% of all the patients diagnosed with this disease are alive after five years, and death rates have been stable since 2004. The highest incidence is among the ages 80 to 85 years. Patients over 65 years of age have a median overall survival (OS) of only 2 to 8 months (Thein, Ershler, Jemal, Yates, & Baer, 2013). According to the Swedish Acute Leukemia Registry, the new cases in patients of ages 65 years and 85 years were of 73 and 154 per 100 000 inhabitants respectively (Juliusson et al., 2009).

AML is characterized by rapid proliferation of abnormal cells in the bone marrow and interferes with the production of normal blood cells. The World Health Organization (WHO) AML classification defines unique clinical and biologically essential subgroups (Vardiman, 2010; Arber DA, Vardiman JW, & Brunning RD, 2015). The WHO classification from 2001, its update from 2008 and the last version from 2016 have now replaced the formerly used French-American-British (FAB) classification, and a risk-score was developed according to molecular and cytogenetic status (Dohner et al., 2010; Papaemmanuil et al., 2016). Based on the WHO classification, the blast threshold for the diagnosis of AML was reduced from 30% to 20% blasts (Vardiman et al., 2009).

AML can emerge *de novo*, be treatment-related after chemotherapy or radiation, or be a transformation of an existing myelodysplastic or myeloproliferative disorder into AML. These last two conditions are also reflected in the WHO 2008 classification with separate entities for therapy-related neoplasms including therapy-related AML and AML with myelodysplasia-related changes either defined via distinct cytogenetic aberrations including −7, −5 and a complex karyotype or a history of preceding myelodysplastic syndrome (MDS) or typical morphological changes such as multilineage dysplasia. In most patients who are fit for intensive chemotherapy the primary goal of the first intensive

chemotherapy, also called induction therapy, is to achieve a complete remission (CR). A CR is defined as less than 5% blasts in the bone marrow, an absolute neutrophil count of 1.000/µl or more, a platelet count of 100.000/µl or more, no blasts in the peripheral blood and no extramedullary leukemia (Cheson et al., 2003; Dohner et al., 2010). There are several factors with a prognostic value with regard to the achievement of a CR of which genetic markers are the most important ones (Schlenk & Dohner, 2013). After successful intensive induction therapy, a subsequent consolidation therapy is mandatory to prevent a disease relapse (Schlenk, 2014).

1.2 Consolidation therapy

The concept of intensive post-remission chemotherapy in AML is based on the observation that despite induction of a first CR by intensive induction therapy, virtually all patients relapse in the absence of further treatment (Cassileth et al., 1988). Furthermore, randomized studies showed that intensive post-remission chemotherapy was superior to prolonged low-dose maintenance therapy in younger patients (Cassileth et al., 1992). With regard to post-remission chemotherapy, the landmark study conducted by the Cancer and Leukemia Group B (CALGB) established the current standard for patients aged 60 years and younger (Mayer et al., 1994). In the prospective up-front randomized study, four repeated cycles of high-dose cytarabine (HDAC) (3 g/m² b.i.d., days 1, 3 and 5) had been superior to intermediate- (400 mg/m² continuous IV, days 1−5) or standard-dose cytarabine (100 mg/m² continuous IV, days 1−5) with respect to relapse-free survival (RFS) and OS. Of note, the design of the schedule of the HDAC regimen with b.i.d dosing on days 1, 3 and 5 was mostly attributable to the wish of comparable regimens concerning the total length of 5 days rather than to a strong scientific background. Cytarabine has a short plasma half-life (Herzig et al., 1987) and is known to be an S-phase specific agent. For some leukemic cells, progress through this phase is possible in the period where there is no exposure to cytotoxic substances (Leclerc & Momparler, 1984).

Several alternative intensive combinations chemotherapy regimens have been evaluated in randomized trials in the last years. A single agent HDAC (3 g/m², b.i.d., days 1, 3 and 5) remains the preferable post-remission chemotherapy in younger adults with core binding factor AML and intermediate-risk AML including cytogenetically normal AML, whereas combination post-remission therapy may be considered in high-risk patients (Dombret & Gardin, 2016; Schlenk, 2014).

1.3 Aplasia and complications during the consolidation therapy

Chemotherapy-induced neutropenia represents a major risk factor for infection-related morbidity and mortality during AML treatment (O'Donnell et al., 2012; Heil et al., 1997). The incidence of neutropenic fever after a consolidation therapy ranges from 50% to 90% (Ottmann, Bug, & Krauter, 2007), influenced mainly by depth and duration of neutropenia, which correlate with the risk and severity of infections. Reducing the severity and duration of neutropenia is a considerable relevant clinical endpoint.

According to the meta-analysis performed by Smith et al. the use of prophylactic granulocyte colony-stimulating factor (G-CSF) decreases the incidence of infections and diminishes the likelihood of hospitalizations after the initial induction chemotherapy or after the completion of the consolidation chemotherapy for patients in complete remission (Smith et al., 2006). The analyzed randomized studies compared patients treated during consolidation therapy with or without G-CSF. Most of them report a statistically significant reduction in the rate of infections. These are defined as microbiologically documented infections, febrile neutropenia, days of antibiotic therapy and the duration of neutropenia with the use of G-CSF (Archimbaud et al., 1999; Braess et al., 2009; Heil et al., 2006).

Sung et al. obtained similar results in a meta-analysis published in 2007, where 148 randomized clinical trials were evaluated. These included patients with solid and hematological malignancies, treated with chemotherapy or stem cell transplantation (Sung, Nathan, Alibhai, Tomlinson, & Beyene, 2007). In the subgroup analysis focused on AML, the risks of microbiologically documented infections (HR: 0.86; CI: 0.77−0.96) and febrile neutropenia (HR: 0.71; CI: 0.63−0.80) were found to be lower in the group randomized to G-CSF (Sung et al., 2007). However, the infection-related mortality was not significantly different with and without G-CSF ($p = 0.44$). In terms of disease relapse, the clinical trial of the *Groupe Ouest-Est Leucémies Aigues Myeloblastiques* showed that the CR rate, the number of treatment failures and early relapses were not different in the patients treated with G-CSF in comparison with patients who received placebo. However, the duration of neutropenia was seven days shorter in the G-CSF group (Harousseau et al., 2000). Nonetheless, different results came from a systematic review published by Gurion et al., in which 19 randomized clinical trials in patients with AML were examined. They found no survival benefit and no difference in the infection rates in the group of patients treated with G-CSF to the placebo group after chemotherapy (Gurion et al., 2012). The work performed

by Sung et al. included 17 out of 19 of the studies reviewed by Gurion et al. For fever in neutropenia, the publication of Gurion et al. analyzed nine studies. No differences were found regarding infections between the two groups of therapy (HR: 0.98; CI: 0.94−1.03). These nine studies and 17 more were analyzed by Sung et al., where an HR of 0.71 for fever in neutropenia was found. This suggests that in the meta-analysis by Gurion et al. no difference could be seen due to a smaller sample size. Thus, the two large systematic meta-analyses indicate that the usage of G-CSF during consolidation therapy could lead to a reduction in neutropenia duration and decrease the rate of infection as well as febrile neutropenia, however, without an effect on non-relapse mortality and OS. Due to these inconclusive findings, the last actualization of the National Comprehensive Cancer Network (NCCN) AML guidelines supported the use of G-CSF in AML only within clinical trials or after salvage therapy (O'Donnell et al., 2012).

Pegfilgrastim is the PEGylated formulation of G-CSF that allows a one-time administration compared to daily administration with filgrastim, which is possible because of the different routes of clearance. Filgrastim is mainly eliminated renally and pegfilgrastim by the internalization via cell surface G-CSF-receptors on neutrophils (Molineux et al., 1999). In a randomized phase II trial, Sierra et al. found no clinically meaningful difference between a single dose of pegfilgrastim and daily dosing of filgrastim regarding the duration of severe neutropenia after induction and consolidation therapy (Sierra et al., 2008).

1.4 Research questions

The primary objectives of my study were to analyze the effects of a condensed regimen of HDAC on days 1, 2 and 3 (HDAC-123) compared to the standard regimen with HDAC on days 1, 3 and 5 (HDAC-135). As well as evaluating the effect of pegfilgrastim administered after consolidation therapy with regard to hematological reconstitution, supportive care, infectious complications and days in hospital.

My secondary objective was to determine if HDAC-123 had at least an equivalent efficacy in terms of RFS and OS to HDAC-135.

2. Materials and methods

2.1 AMLSG 07-04 study

In 2004 an up-front randomized four- arm study was started. The purpose was to evaluate in a 2 × 2 factorial design all-trans retinoic acid (ATRA) and valproic acid (VPA) as an adjunct to intensive induction and consolidation therapy (Schlenk et al., 2016).

From August 2004 to January 2006 patients were randomized to receive induction chemotherapy with or without ATRA and with or without VPA resulting in four arms, ATRA, ATRA-VPA, VPA and STANDARD (HDAC + pegfilgrastim). The induction therapy consisted of 2 cycles ICE (idarubicin: 12 mg/m² IV, days 1, 3 and 5; cytarabine: 100 mg/m² continuous IV, days 1−7; etoposide: 100 mg/m² IV, days 1−3) or the same chemotherapy plus ATRA (45 mg/m² orally, days 5−7 and 15 mg/m², days 8−21). Patients achieving a CR or partial remission (PR) after the first induction received a second cycle according to their initial randomization with a reduced dosage of idarubicin (12 mg/m², days 1 and 3) (Schlenk et al., 2016).

The consolidation therapy consisted of three cycles of HDAC, from August 2004 to November 2006 with cytarabine: 3 g/m² b.i.d. on days 1, 3 and 5 (HDAC-135). Starting in November 2006 with a condensed schedule with the application of cytarabine: 3 g/m² b.i.d. on days 1, 2 and 3 (HDAC-123) (Jaramillo et al., 2017).

2.2 Patients and consolidation therapy

Patients aged between 18 and 60 years with newly diagnosed AML including *de novo* AML, secondary AML with a preceding history of myelodysplastic or myeloproliferative disorder (sAML), and therapy-related AML following treatment of a primary malignancy (tAML), as defined by the WHO 2001 classification, were eligible for the trial (Jaffe ES, Harris NL 2001). Patients with acute promyelocytic leukemia (APL), patients with concomitant renal (creatinine > 1.5 × upper normal serum level), liver (bilirubin, aspartate aminotransferase or alkaline phosphatase > 2 × upper normal serum level) or cardiac dysfunction class III or IV (New York Heart Association), uncontrolled infectious disease, primary coagulation disturbance, Eastern Cooperative Oncology Group (ECOG) performance status > 2 or active concomitant malignant disease, were excluded. We obtained written informed consent at study entry. The protocol was approved by the local Ethics Review Committee (Number: 108/2004) and registered at clinicaltrialsregister.eu

(European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number: 2004-004321-95) and clinicaltrials.gov (NCT00151242) (Jaramillo et al. 2017).

Patients were recruited from August 2004 to August 2009. A first up-front 1:9 randomization was performed between the standard German AML Intergroup arm and the German-Austrian AML Study Group (AMLSG) 07-04 study (Büchner et al., 2012). The remaining patients were randomized in a 2×2 factorial design to receive induction chemotherapy. In 2006 the protocol was amended, and the treatment with VPA and its randomization was terminated based on an excessive hematological toxicity in combination with chemotherapy, which was similarly noted by Tassara et al. in older patients (Tassara et al., 2014). Patients with high-risk AML defined by high-risk cytogenetics, or induction failure (Schlenk et al., 2010), were assigned to receive allogeneic hematopoietic stem cell transplantation (HSCT) from a matched related donor or a matched unrelated donor. If a matched related donor was available, an allogeneic HSCT was intended in first complete remission (CR) in all patients except those with core-binding factor AML. Starting from December 2006, AML patients exhibiting a FLT3-ITD were also categorized as high-risk (Jaramillo et al., 2017).

All other patients were assigned to three repetitive cycles of consolidation chemotherapy with HDAC. From August 2004 to November 2006 patients were treated with HDAC-135 and 6 mg of pegfilgrastim on day 10. Starting on November 2006 patients were treated with HDAC-123 and 6 mg of pegfilgrastim on day 8. Patients randomized to the German AML Intergroup arm (Büchner et al., 2012) were treated according to the standard regimen with HDAC-135 without prophylactic growth-factor support (Jaramillo et al., 2017)

2.3 Molecular and cytogenetic

Chromosome banding analysis was performed centrally in the AMLSG Laboratory for Cytogenetic and Molecular Diagnosis. Karyotypes were designated according to the International System for Human Cytogenetic Nomenclature (Mitelman F, 1995). Leukemia samples were analyzed for mutations in *FLT3* (*FLT3* internal tandem duplication [ITD], FLT3 tyrosine kinase domain [TKD] mutations at codons D835/I836), *CEBPA* and *NPM1* as described in a previous publication (Schlenk et al., 2008).

2.4 ELN classification

The risk stratification was performed according to the European LeukemiaNet (ELN) recommendations (Döhner et al., 2010).

2.5 Study endpoints

Hematological recovery was determined in each patient in every cycle of consolidation therapy. Hematological reconstitution was defined as an absolute leukocyte count equal or above 1.0×10^9/l, an absolute neutrophil count of 0.5×10^9/l or more and a platelet count equal or above 20×10^9/l. Time to leukocyte, neutrophil and platelet recovery was defined as the duration in days from the first day of chemotherapy of each cycle until the first day of achievement of the above defined cut-offs. I did not perform an intention-to-treat analysis of platelet reconstitution because the time to platelet reconstitution was not fully collected in the German AML Intergroup arm (Büchner et al. 2012). The data on the number of units of packed red blood cells (RBC) and platelets were collected in each therapy cycle. In the statistical analysis, one HLA class I compatible single donor platelet unit was considered equal to four platelet units from random donors. Infection was defined as microbiologically documented infection or febrile neutropenia. The duration of hospitalization was defined as the time from the first day of therapy till discharge. Secondary outcomes were OS and cumulative incidence of relapse (CIR) and death (CID), which were defined as recommended (Döhner et al., 2010).

2.6 Statistical analysis

Pairwise comparisons between patient subgroups were performed by the Mann-Whitney or Kruskal Wallis test for continuous variables and by Fisher's exact test for categorical variables. Significance was defined as a p-value < 0.05 as recommended. Cumulative incidences and differences between groups of recovery times of white blood cells (WBCs) and neutrophils were calculated using the method described by Gray (Gray, 1988). A p-value Bonferroni correction was applied in three-way comparisons leading to a significance level of <0.016 (Jaramillo et al., 2017).

Multivariable analyses with the endpoint time to WBC recovery were performed using a Wei-Lin-Weissfeld (WLW) extend Cox regression model. This statistical test was chosen to analyze the time to hematological reconstitution allowing us to adjust for multiple times to events integrating all applied consolidations (Harrell FE., 2001). This model permitted us to analyze a disease process consisting of a recurring outcome, which in this case is the hematological recovery after repetitive consolidation cycles.

I performed the multivariable analysis with the subgroup of the patients who received the three consolidations. This way I was able to reduce some confounding variables. The independent variables included in all the regressions were those which did not change

during the therapy, such as molecular characteristics of the disease, age, gender and type of therapy.

A stratified conditional logistic regression model with the 07-04 patients was used to analyze infectious complications during consolidation therapy (Michell H Gail., Jay H Lubin, & Lawrence V Rubinstein, 1980). Here I included all consolidation cycles in a stratified manner. To reduce confounding variables, I performed this analysis just with the subgroup of patients who received all three consolidation cycles.

For survival analyses, I only included patients treated in the 07-04 study. Survival distributions were compared using the log-rank test. I performed the Kaplan Meier curves for all the patients of the 07-04 study who received at least one consolidation therapy. A Cox regression model was set up using survival times with censoring at the time of allogeneic HSCT for patients transplanted in first CR to account for the changing criteria used over time to select these patients for allogeneic HSCT performed in first CR (Jaramillo et al., 2017).

2.7 R packages

All statistical analyses were performed with the statistical software environment R (version 3.0.1), using the R packages: rms (version 3.3-1) and cmprsk (version 2.2-2).

3. Results

3.1 Patients and baseline characteristics

568 Patients received at least one consolidation cycle within the 07-04 protocol or the German AML Intergroup protocol (Figure 1). In the 07-04 protocol 135 patients (Cohort-1) were assigned between August 2004 and December 2006 to the standard consolidation therapy with HDAC-135.After that, the remaining 392 patients (Cohort-2) were assigned from January 2006 to August 2009 to HDAC-123 therapy. In the German AML Intergroup protocol, 41 patients were assigned to the HDAC-135 therapy (Jaramillo et al., 2017).

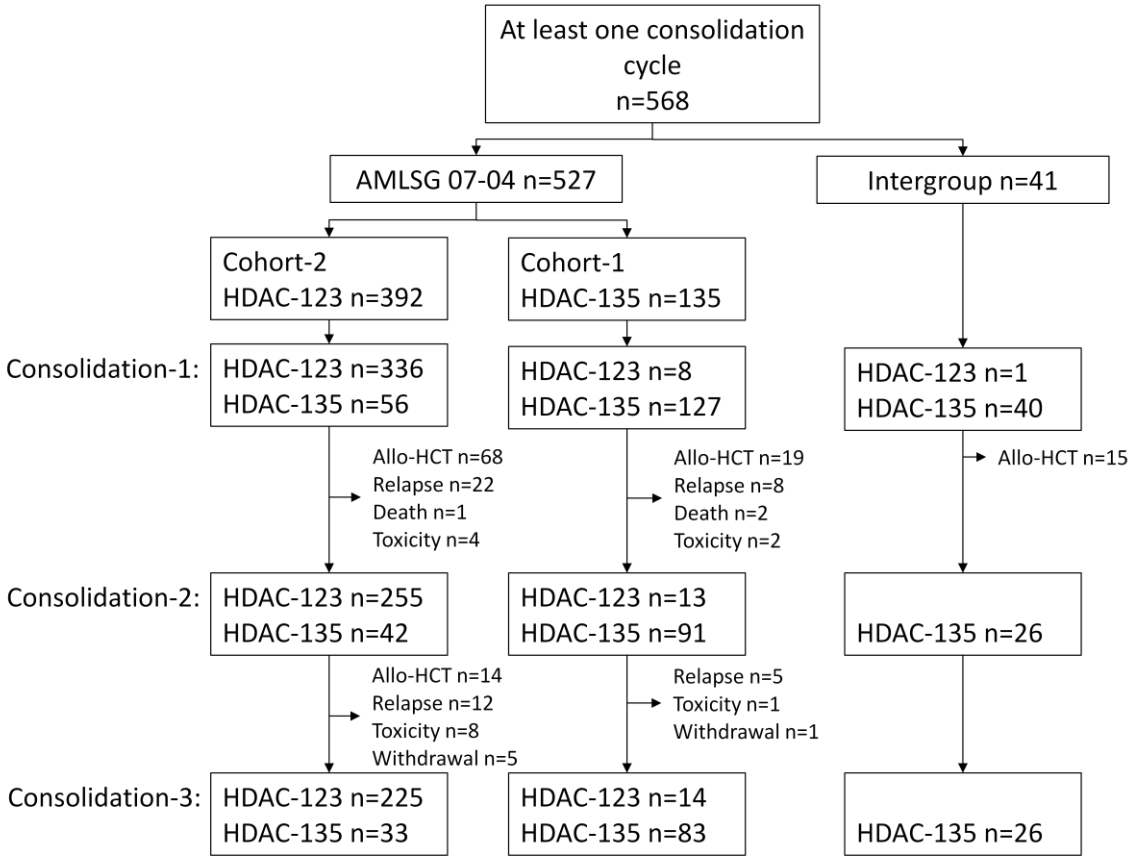

Figure 1: Flowchart showing received therapy and drop-outs according to the assigned treatment. Abbreviations: AMLSG 07-04, Akute Myeloische Leukämie Studiengruppe 07-04 study; HDAC-135, High-dose cytarabine on days 1, 3 and 5; HDAC-123, High-dose cytarabine on days 1, 2 and 3; Intergroup, German AML Intergroup studies; Allo-HCT, Allogeneic hematopoietic stem cell transplantation; n, sample size. Figure from Jaramillo et al., Condensed versus standard schedule of high dose cytarabine consolidation therapy with pegfilgrastim growth factor in acute myeloid leukemia. Blood Cancer J. 2017.

There were no significant differences in patient characteristics at diagnosis between the three groups (Table 1). A first consolidation cycle was applied in 568 patients, n = 41 in the Intergroup, n = 135 in Cohort-1 and n = 392 in Cohort-2; 8 patients in Cohort-1 and 56 patients in Cohort-2, were treated with the vice versa schedule. After the first consolidation

cycle, 102 patients proceeded to an allogeneic HSCT, 30 patients relapsed, 6 patients received no further treatment due to toxicity, and 3 patients died treatment related. A second consolidation cycle was administered in 427 patients, 26 in the Intergroup, 104 in Cohort-1 and 297 in Cohort-2; 13 patients in Cohort-1 and 42 patients in Cohort-2, were treated with the vice versa schedule. After the second consolidation cycle, 14 patients proceeded to an allogeneic HSCT, 17 patients relapsed and 15 patients received no further treatment. A third consolidation cycle was applied in 381 patients, in the Intergroup, n = 97 in Cohort-1 and 258 in Cohort-2; 14 patients in Cohort-1 and n = 33 patients in Cohort-2, were treated with the vice versa schedule. In total 1376 cycles of consolidation therapy could be analyzed (Jaramillo et al., 2017).

Table 1: Patient characteristics at initial diagnosis according to assigned treatment

	German AML Intergroup n = 41	Cohort-1 HDAC-135 n = 135	Cohort-2 HDAC-123 n = 393	p-value
Age [years], median (range)	41.6 (19−60)	47.6 (18−61)	47.7 (18−61)	0.55
Gender [male], No (%)	20 (48.8)	65 (48.2)	207 (52.8)	0.61
WBC [10^9/l], median (range)	20.2 (0.2−210)	19.3 (0.9−217)	13.2 (0.3−394)	0.21
Missing	0	0	4	
Platelets [10^9/l], median (range)	55 (8−380)	53 (14−511)	52 (5−574)	0.91
Missing	0	1	3	
Hemoglobin [g/dL], median (range)	9.4 (2.7−16.2)	9.2 (5.2−14.4)	9.3 (3.8−16.0)	0.48
Missing	0	0	3	
LDH [U/l], median (range)	400 (149−2639)	492 (167−4566)	435 (94−5438)	0.42
Missing	0	0	5	
BM-blasts [%], median (range)[(1)]	29 (0−94)	31 (0−97)	37 (0−99)	0.94
Missing	5	18	77	
PB-blasts [%], median (range)	32 (0−94)	30 (0−97)	37 (0−99)	0.71
Missing	4	17	57	
Type of AML, No (%)				0.91
de novo	40 (97.6)	124 (92.6)	355 (92.4)	
sAML		3 (2.2)	12 (3.1)	
tAML	1 (2.4)	7 (5.2)	18 (5.6)	
ELN risk group, No (%)				0.22
Low	16 (42)	65 (54)	171 (48.5)	
Intermediate-1	14 (37)	36 (30)	97 (27.5)	
Intermediate-2	4 (10.5)	17 (14)	56 (16)	
High	4 (10.5)	3 (2)	28 (8)	
Missing	3	14	40	

[(1)]In case of BM-blasts < 20% diagnosis of AML was established based on extramedullary disease or PB-blast > 20%.

Abbreviations: AML, acute myeloid leukemia; HDAC 135, High-dose cytarabine on days 1, 3 and 5; HDAC 123, High-dose cytarabine on days 1, 2 and 3; Intergroup, German AML Intergroup studies; No, number; WBC, white blood cell; LDH, lactate-dehydrogenase; BM, bone marrow; PB, peripheral blood; sAML, secondary AML after a preceding myelodysplastic syndrome; tAML, treatment-related AML; ELN, European LeukemiaNet. Table from Jaramillo et al., Condensed versus standard schedule of high dose cytarabine consolidation therapy with pegfilgrastim growth factor in acute myeloid leukemia. Blood Cancer J. 2017.

3.2 Influence of HDAC schedules on time to hematological recovery

Within the 07-04 protocol, initial up-front randomization into four treatment arms (ATRA, ATRA-VPA, VPA, and STANDARD) was stopped for VPA in July 2006 due to hematotoxicity, in particular, after the second induction therapy (Schlenk et al., 2016). No influence of VPA or ATRA in Cohort-1 (neutrophils: $p = 0.78$; WBCs: $p = 0.49$; platelets: $p = 0.67$) and of ATRA in Cohort-2 (neutrophils: $p = 0.65$; WBCs: $p = 0.40$; platelets: $p = 0.30$) on hematological recovery was evident. Therefore, patients treated in the 07-04

protocol according to up-front randomization were combined for the analyses of hematological recovery after consolidation therapy and grouped into Cohort-1 and Cohort-2 for further analysis as described above (Jaramillo et al., 2017).

On an intention-to-treat analysis evaluating the three different schedules (German AML Intergroup, HDAC-123, and HDAC-135 of the 07-04 protocol) for the three consolidation cycles, I noted consistently shorter recovery times in HDAC-123 compared to the other two regimens. Overall, I observed a reduction of WBCs recovery by 4 days with HDAC-123 in all three consolidation cycles ($p = 0.0008$, $p = 0.0003$ and $p = 0.001$ respectively) (Table 2). Time to neutrophil recovery was also shorter in all three consolidation cycles in HDAC-123 compared to HDAC-135 and the German AML Intergroup again with an average of 4 days with a significant and in trend difference in the first and second consolidation cycle (Table 2).

Table 2: Hematological recovery according to intention-to-treat arm allocation

	German AML Intergroup n = 41	Cohort-1 HDAC-135 n = 135	Cohort-2 HDAC-123 n = 393	p-value
Consolidation 1				
WBC ≥ 1.0×10^9/l median (days)	20	19	16	0.0008[(1)] 0.0003[(2)] 0.32[(3)]
Neutrophils ≥ 0.5×10^9/l median (days)	23	22	17	0.008[(1)] 0.002[(2)] 0.52[(3)]
Consolidation 2				
WBC ≥ 1.0×10^9/l median (days)	22	20	16	0.0003[(1)] <0.0001[(2)] 0.59[(3)]
Neutrophils ≥ 0.5×10^9/l median (days)	25	22	17	0.09[(1)] 0.03[(2)] 0.76[(3)]
Consolidation 3				
WBC ≥ 1.0×10^9/l median (days)	20	20.5	16	0.001[(1)] 0.0004[(2)] 0.74[(3)]
Neutrophils ≥ 0.5×10^9/l median (days)	22	21.5	18	0.17[(1)] 0.06[(2)] 0.62[(3)]

[(1)]Comparisons were performed overall with three groups.
[(2)]Comparisons between two groups defined by HDAC intended on days 1, 3 and 5 vs. days 1, 2 and 3.
[(3)]Comparisons between two groups defined by HDAC intended on days 1, 3 and 5 given in the AMLSG 07-04 and the German AML Intergroup studies.
Abbreviations: AML, acute myeloid leukemia; HDAC 135, High-dose cytarabine on days 1, 3 and 5; HDAC 123, High-dose cytarabine on days 1, 2 and 3; WBC, white blood cell; HDAC, high-dose cytarabine; No, number; n, sample size. Table from Jaramillo et al., Condensed versus standard schedule of high dose cytarabine consolidation therapy with pegfilgrastim growth factor in acute myeloid leukemia. Blood Cancer J. 2017.

Exemplarily, in Figure 2 and Figure 3 WBC and neutrophil recovery is illustrated after the first consolidation cycle according to the designated treatment group. In a subgroup analysis, I compared the up-front randomized patients between the German AML Intergroup and the 07-04 study. Both schedules were assigned to receive HDAC on days 1, 3 and 5 with the intended prophylactic pegfilgrastim at day 10 of therapy in the 07-04 study compared to no prophylactic growth factor support in the German AML Intergroup study. This analysis revealed on average shorter recovery times for WBCs and neutrophils of one day in the Cohort-1 in all three consolidation cycles without reaching statistical significance (Table 2). 381 patients received all three consolidation cycles, median time intervals between cycles 1 and 2 were 40 days (range: 28−140 days), and between cycles 2 and 3 were 41 days (range: 26−129 days). Interestingly, I did not identify any cumulative hematological toxicity with increasing number of applied consolidation cycles with regard to WBC and neutrophil recovery for HDAC-135 ($p = 0.26$ and $p = 0.90$ respectively; $n = 97$), HDAC-123 ($p = 0.17$ and $p = 0.61$ respectively; $n = 258$) and German AML Intergroup ($p = 0.78$ and $p = 0.74$ respectively; $n = 26$) (Jaramillo et.al., 2017).

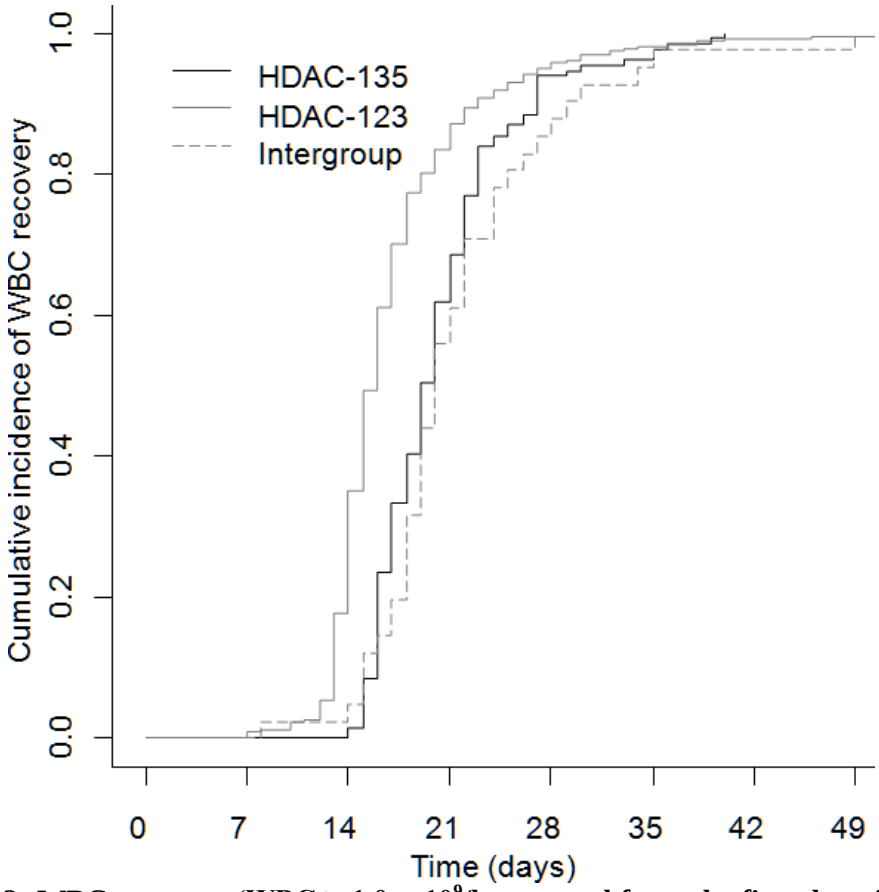

Figure 2: WBC recovery (WBC ≥ 1.0 × 10^9/l measured from the first day of chemotherapy) after the first consolidation cycle. Abbreviations: HDAC-135: cytarabine intended on days 1, 3 and 5; HDAC-123: cytarabine intended on days 1, 2 and 3; Intergroup: German AML Intergroup studies. Figure from Jaramillo et al., Condensed versus standard schedule of high dose cytarabine consolidation therapy with pegfilgrastim growth factor in acute myeloid leukemia. Blood Cancer J. 2017.

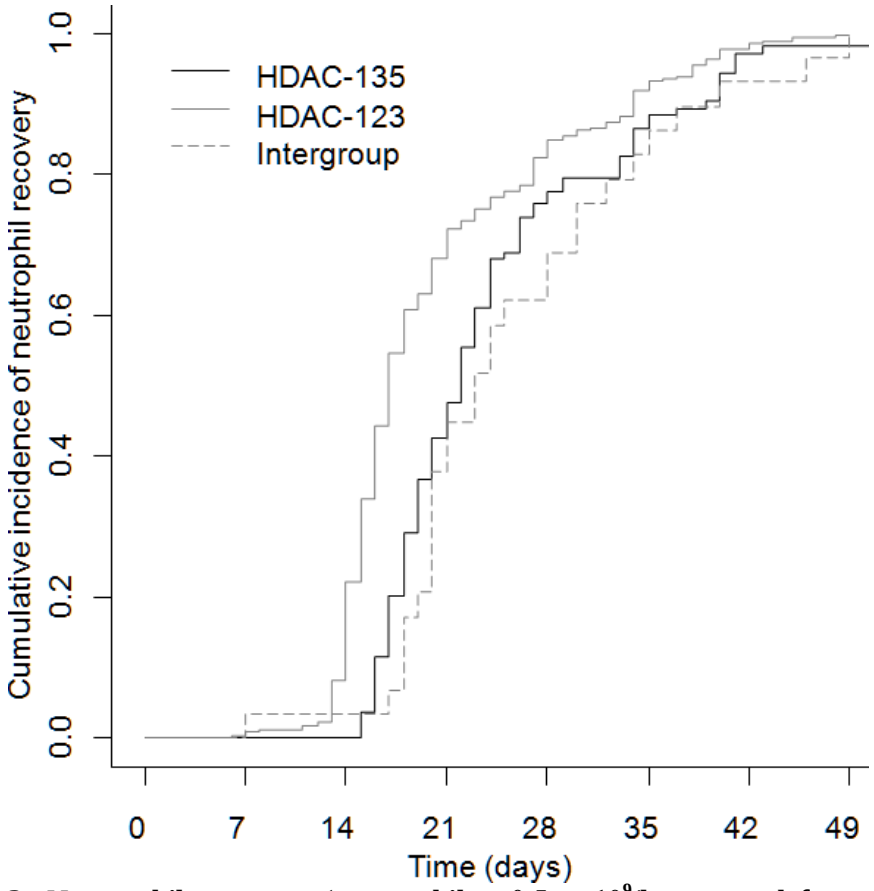

Figure 3: Neutrophil recovery (neutrophil ≥ 0.5 × 10^9/l measured from the first day of chemotherapy) after the first consolidation cycle. Abbreviations: HDAC-135: cytarabine intended on days 1, 3 and 5; HDAC-123: cytarabine intended on days 1, 2 and 3; Intergroup: German AML Intergroup studies. Figure from Jaramillo et al., Condensed versus standard schedule of high dose cytarabine consolidation therapy with pegfilgrastim growth factor in acute myeloid leukemia. Blood Cancer J. 2017.

3.3 Influence of pegfilgrastim on time to hematological recovery

Within the 07-04 study pegfilgrastim 6 mg single subcutaneous administration was intended to be applied at day 10 in the standard HDAC-135 and day 8 in the condensed HDAC-123 schedules. In an as-treated analysis patients receiving pegfilgrastim were compared to those not receiving pegfilgrastim (Table 3). There was an overall reduction of the duration of leukopenia of 3 days each, in both schedules ($p < 0.0001$ each). There was also an overall reduction of neutropenia of 5 days for HDAC-135 and 3 days for HDAC-123 ($p = 0.03$ and $p = 0.003$ respectively) without impact on duration of thrombocytopenia in both schedules ($p = 0.77$ and $p = 0.70$ respectively).

Table 3: Hematological recovery according to treatment arm and pegfilgrastim administration

	HDAC-135 Peg-day 10	HDAC-135 no Peg	p-value	HDAC-123 Peg-day 8	HDAC-123 no Peg	p-value
Consolidation 1						
WBC $\geq 1.0\times10^9$/l	n = 104	n = 31	0.16	n = 310	n = 80	0.01
median (days)	19	21		15	23	
Neutrophils $\geq 0.5\times10^9$/l	n = 83	n = 24	0.35	n = 227	n = 43	0.04
median (days)	21	24		17	18	
Consolidation 2						
WBC $\geq 1.0\times10^9$/l	n = 68	n = 35	0.0005	n = 192	n = 97	0.009
median (days)	19	23		15	17	
Neutrophils $\geq 0.5\times10^9$/l	n = 55	n = 28	0.13	n = 134	n = 53	0.13
median (days)	20	29		17	18.5	
Consolidation 3						
WBC $\geq 1.0\times10^9$/l	n = 55	n = 42	0.18	n = 171	n = 82	0.04
median (days)	20	21		15	18	
Neutrophils $\geq 0.5\times10^9$/l	n = 41	n = 36	0.41	n = 122	n = 53	0.71
median (days)	20	22		18	19	
Stratified and adjusted comparison including all applied consolidation cycles						
WBC $\geq 1.0\times10^9$/l	n = 227	n = 108	<0.0001[(1)]	n = 673	n = 259	<0.0001[(1)]
median (days)	19	22		15	18	
Neutrophils $\geq 0.5\times10^9$/l	n = 179	n = 88	0.03[(1)]	n = 483	n = 149	0.003[(1)]
median (days)	20	25		17	20	

[(1)]Adjusted p-values using a stratified and clustered approach for three consolidation cycles and patients with repetitive observations, respectively.

Abbreviations: Peg: pegfilgrastim; HDAC 135, High-dose cytarabine on days 1, 3 and 5; HDAC 123, High-dose cytarabine on days 1, 2 and 3; WBC: white blood cell; HDAC: high-dose cytarabine; n, sample size. Table from Jaramillo et al., Condensed versus standard schedule of high dose cytarabine consolidation therapy with pegfilgrastim growth factor in acute myeloid leukemia. Blood Cancer J. 2017.

3.4 Multivariable Wei-Lin-Weissfeld model on WBC recovery

Multivariable analysis based on the WLW method for multiple times to events integrating all applied consolidation cycles in patient receiving three consolidation cycles in a stratified manner revealed that HDAC-123 (HR: 1.94; $p < 0.0001$) and treatment with pegfilgrastim (HR: 1.58; $p < 0.0001$) were significantly associated with shorter WBC recovery times. Interestingly older age was associated with longer WBC recovery times (HR of a ten years difference: 0.89; $p = 0.001$). Factors without significant impact were gender, risk group according to ELN recommendations and type of AML (*de novo* vs. s/tAML) (Table 4) (Jaramillo et al., 2017).

Table 4: Wei-Lin-Weissfeld model on WBC recovery including three consolidation cycles per patient

	HR	CI	p-value
HDAC-123[(1)]	1.94	1.67−2.24	<0.0001
Pegfilgrastim[(2)]	1.58	1.37−1.84	<0.0001
Age (10 years difference)	0.89	0.83−0.95	0.001
ELN risk group[(3)]			
Low	1.06	0.89−1.26	0.51
Intermediate-2	0.95	0.75−1.22	0.70
High	1.32	0.95−1.85	0.10
Male gender[(4)]	1.12	0.96−1.32	0.15
s/tAML[(5)]	0.90	0.64−1.24	0.51

Reference group: [(1)]HDAC-135, [(2)] without pegfilgrastim, [(3)]intermediate-1, [(4)]female gender, [(5)]*de novo* AML

Abbreviations: AML, acute myeloid leukemia; HDAC 135, High-dose cytarabine on days 1, 3 and 5; HDAC 123, High-dose cytarabine on days 1, 2 and 3HR, hazard ratio; CI, confidence interval; ELN, European LeukemiaNet; s/tAML, secondary AML after a proceeding myelodysplastic syndrome or treatment-related AML. Table from Jaramillo et al., Condensed versus standard schedule of high dose cytarabine consolidation therapy with pegfilgrastim growth factor in acute myeloid leukemia. Blood Cancer J. 2017.

3.5 Infection rates, days of hospitalization and number of platelet transfusions with HDAC-135 and HDAC-123

The overall infectious complications including infection with clinical focus (mostly pneumonia) and febrile neutropenia were 37.3%, 40.0% and 41.3% in the three consecutive consolidation cycles respectively. Overall, the infectious complication rates were highest in the German AML Intergroup arm after HDAC-135 without prophylactic growth-factor support ranging from 74% to 83 % and lowest in the HDAC-123 schedule of the 07-04 protocol with the administration of prophylactic pegfilgrastim ranging from 30% to 36% (Table 5).

The probability of experiencing an infection during the second consolidation cycle was 3.32 (CI: 1.68−6.68; $p < 0.001$) times higher for those patients with a manifested infection during the first cycle. Nearly the same was true for patients with an infection during the second consolidation cycle, where they had a 4.33 (CI: 2.21−8.67; $p < 0.001$) higher probability of developing an infection during the third cycle.

Table 5: Infections, platelet transfusion needs, hospitalization according to treatment arm, and pegfilgrastim administration

	HDAC-135[2] Intergroup	HDAC-135 AMLSG 07-04	p-value	HDAC-135 AMLSG 07-04 Peg-day 10	HDAC-135 AMLSG 07-04 no Peg	p-value	HDAC-123 AMLSG 07-04 Peg-day 8	HDAC-123 AMLSG 07-04 no Peg	p-value
Consolidation 1[1]	n = 39	n = 135		n = 104	n = 31		n = 310	n = 81	
Days in Hospital, median (range)	23 (5−39)	23 (5−70)	0.68	22 (5−70)	24 (6−47)	0.03	17 (3−55)	19 (3−44)	0.04
ITT Cohort-1 vs. Cohort-2[3]				23 (5−70)			17 (3−55)		<0.0001
Hospital discharge									
≤10 days, No (%)	8/38 (21)	12/135 (9)	0.05	9/104 (9)	3/31 (10)	0 99	87/310 (28)	26/81 (32)	0.99
Readmission, No (%)	4/8 (50)	4/12 (33)	0.65	3/9 (33)	1/3 (33)	0.99	28/87 (32)	6/20 (30)	0.99
Infection, No (%)	29/39 (74)	57/135 (42)	0.0004	43/104 (41)	14/31 (45)	0.83	93/310 (30)	33/81 (41)	0.08
Platelet transfusions, median				8	6	0.62	4	2	0.01
ITT Cohort-1 vs. Cohort-2[3]				8			4		<0.0001
Consolidation 2[1]	n = 24	n = 103		n = 68	n = 35		n = 193	n = 99	
Days in Hospital, median (range)	20 (5−29)	23 (5−51)	0.03	22 (5−51)	26 (5−39)	0.02	15 (2−40)	20 (3−45)	<0.0001
ITT Cohort-1 vs. Cohort-2[3]				23 (3−50)			17 (2−45)		<0.0001
Hospital discharge									
≤10 days, No (%)	8/24 (33)	19/103 (18)	0.16	15/68 (22)	4/35 (11)	0.12	76/193 (39)	22/99 (22)	0.004
Readmission, No (%)	3/8 (38)	10/19 (53)	0.68	8/15 (53)	2/4 (50)	0.99	27/76 (36)	5/22 (23)	0.31
Infection, No (%)	20/24 (83)	43/103 (42)	0.0002	31/68 (46)	12/35 (34)	0.30	67/193 (35)	39/99 (39)	0.44
Platelet transfusions, median				8	9	0.63	4	3	0.87
ITT Cohort-1 vs. Cohort-2[3]				8			4		<0.0001
Consolidation 3[1]	n = 25	n = 97		n = 55	n = 42		n = 171	n = 85	
Days in Hospital, median (range)	21 (5−38)	22 (5−42)	0.35	20 (5−42)	25 (5−42)	0.0009	16 (3−60)	20 (3−56)	<0.0001
ITT Cohort-1 vs. Cohort-2[3]				27 (5−42)			17 (3−60)		<0.0001
Hospital discharge									
≤10 days, No (%)	6/23 (26)	16/97 (16)	0.59	14/55 (25)	2/42 (5)	0.001	69/171 (40)	19/85 (22)	0.005
Readmission, No (%)	2/6 (33)	6/16 (38)	0.99	6/14 (43)	0	0.50	21/69 (30)	2/19 (11)	0.13
Infection, No (%)	20/25 (80)	41/97 (42)	0.001	23/55 (42)	18/42 (43)	0.99	62/171 (36)	34/85 (40)	0.03
Platelet transfusions, median				6	12	0.16	4	4	0.71
ITT Cohort-1 vs. Cohort-2[3]				8			4		<0.0001

[1]Adjusted p-values using a stratified and clustered approach for three consolidation cycles and patients with repetitive observations, respectively.
[2]Platelet transfusions were not regularly recorded in the German AML Intergroup.
[3]Intention-to-treat analysis: HDAC-135 vs. HDAC-123 (Cohort-1 vs. Cohort-2).
Abbreviations: AMLSG 07-04, HDAC 135, High-dose cytarabine on days 1, 3 and 5; HDAC 123, High-dose cytarabine on days 1, 2 and 3; Akute Myeloische Leukämie Studiengruppe 07-04 study; Intergroup, German AML Intergroup studies; Peg, pegfilgrastim; ITT, intention-to-treat; No, number. Table from Jaramillo et al., Condensed versus standard schedule of high dose cytarabine consolidation therapy with pegfilgrastim growth factor in acute myeloid leukemia. Blood Cancer J. 2017.

A conditional logistic regression model based on all consolidation cycles and stratified for them revealed that HDAC-123 (OR, 0.58; $p < 0.0001$) and the prophylactic administration of pegfilgrastim (OR, 0.68; $p = 0.002$) were associated with a reduction in infectious complications. Patients with a secondary AML had a higher risk of experiencing infectious complications (OR, 1.62; $p = 0.05$). Age, gender and ELN risk category had no significant impact (Table 6). In the HDAC-123 schedule with prophylactic administration of pegfilgrastim, the lower rate of infectious complications and shorter hematological recovery times probably led to the significantly shorter duration of hospitalization (Table 5). This was mainly due to a substantial proportion of patients being discharged within ten days of 28%, 39% and 40% after consolidation cycles 1, 2 and 3 respectively. About one-third of these early discharged patients were readmitted due to infectious complications necessitating IV antibiotic treatment. None of the early discharged patients died during this phase due to infectious complications. The need of platelet transfusions was markedly reduced in the condensed schedule from in median 8 units in the HDAC-135 schedule to in median 4 units in the HDAC-123 schedule ($p < 0.0001$). As expected the platelets transfusion needs were not affected by the administration of pegfilgrastim (Table 5) (Jaramillo et al., 2017).

Table 6: Conditional Logistic Regression Model on the endpoint infection including all consolidation cycles per patient

	OR	CI	p-value
HDAC-123[(1)]	0.58	0.45 – 0.74	<0.0001
Pegfilgrastim[(2)]	0.68	0.54 – 0.87	0.002
Age (10 years difference)	1.07	0.96 – 1.19	0.22
ELN risk group[(3)]			
Low	1.06	0.80 – 1.39	0.70
Intermediate-2	0.99	0.67 – 1.39	0.94
High	1.10	0.62 – 1.92	0.75
Male gender[(4)]	1.04	0.83 – 1.31	0.71
s/tAML[(5)]	1.62	1.01 – 2.60	0.05

Reference group: [(1)]HDAC-135, [(2)] without pegfilgrastim, [(3)]intermediate-1, [(4)]female gender, [(5)]*de novo* AML

Abbreviations: OR, odds ratio; CI, confidence interval; HDAC 135, High-dose cytarabine on days 1, 3 and 5; HDAC 123, High-dose cytarabine on days 1, 2 and 3; ELN, European LeukemiaNet; s/tAML, secondary AML after a proceeding myelodysplastic syndrome or treatment-related AML. Table from Jaramillo et al., Condensed versus standard schedule of high dose cytarabine consolidation therapy with pegfilgrastim growth factor in acute myeloid leukemia. Blood Cancer J. 2017.

3.6 Survival Analyses

For survival analyses, only patients treated in the 07-04 study were included.

In the analysis of all the patients who underwent at least one consolidation no difference (p = 0.90) between HDAC-135 (n = 135) and HDAC-123 (n = 392) in terms of OS was evident (Figure 4). This was also true for OS censored at the date of allogeneic HSCT performed in first CR (p = 0.84) (Figure 5). We also found no differences in RFS (p = 0.48) (Figure 6) and RFS censored at the date of allogeneic HSCT performed in first CR (p = 0.84) (Figure 7). In addition, there was no difference in CIR and CID between the two HDAC schedules (p = 0.90 and p = 0.33 respectively). In the subgroup receiving all three consolidation cycles, again no difference between the two schedules was evident.

The multivariable analysis was concordant with the univariable analyses results. To account for confounding variables, I performed a stratified multivariable Cox regression analysis (n = 479). The model was stratified by the ELN risk group to allow for different baseline hazards in the strata. Also, patients receiving an allogeneic HSCT in first CR were censored at this time point to eliminate potential bias introduced by this treatment which generally has an enormous impact on the endpoint OS. This model revealed neither an impact of consolidation treatment schedule (HDAC-123 vs. HDAC-135) (p = 0.61) nor the addition of pegfilgrastim (p = 0.39) on the endpoint OS (Table 7) (Jaramillo et al., 2017).

Table 7: OS Cox regression stratified by risk group according to ELN classification

	HR	CI	p-value
HDAC-123[(1)]	0.91	0.64 − 1.30	0.61
Pegfilgrastim[(2)]	0.84	0.58 − 1.24	0.39
Age (10 years difference)	1.20	1.01 − 1.42	0.03
Male gender[(3)]	0.90	0.65 − 1.25	0.53

Reference group: [(1)]HDAC-135, [(2)]without pegfilgrastim, [(3)]female gender

Abbreviations: OS, overall survival; HR, hazard ratio; CI, confidence interval; HDAC 123, High-dose cytarabine on days 1, 2 and 3; ELN, European LeukemiaNet. Table from Jaramillo et al., Condensed versus standard schedule of high dose cytarabine consolidation therapy with pegfilgrastim growth factor in acute myeloid leukemia. Blood Cancer J. 2017.

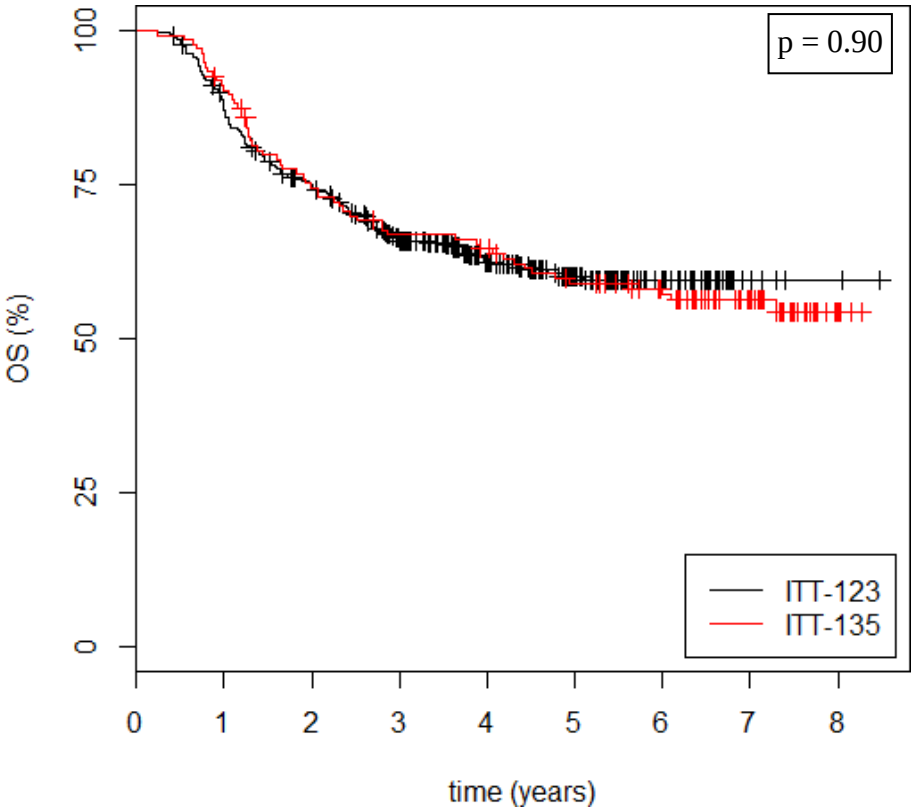

Figure 4: Overall survival of 07-04 patients according to consolidation schedule who received at least one consolidation cycle. Abbreviations: OS, overall survival; ITT, intention-to-treat; ITT-135, cytarabine intended on days 1, 3 and 5; ITT-123, cytarabine intended on days 1, 2 and 3.

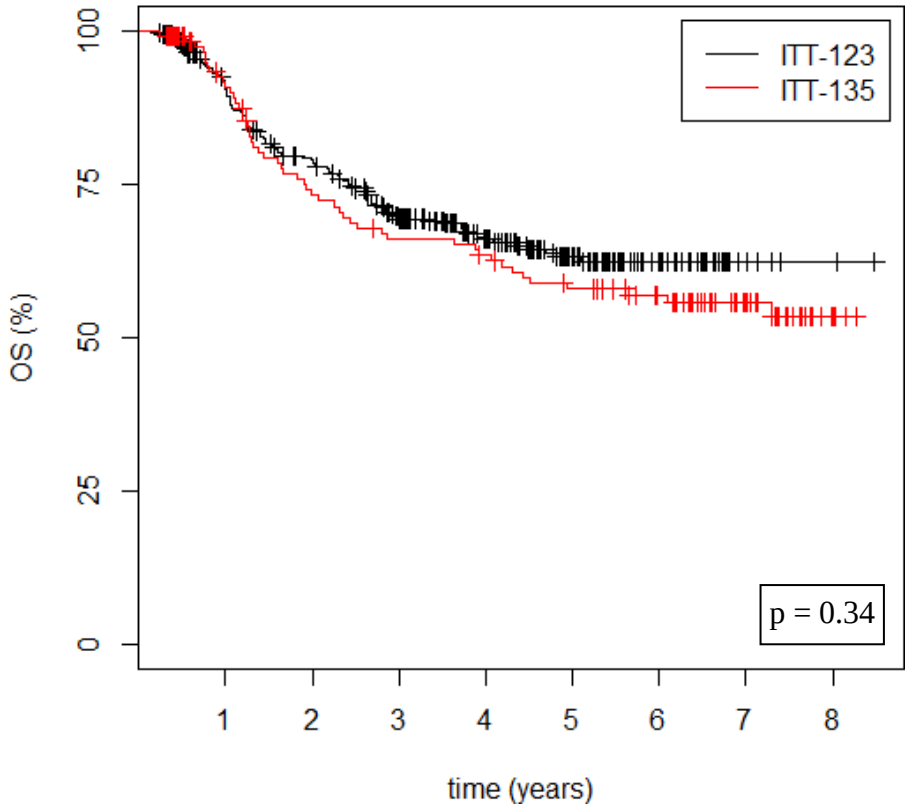

Figure 5: Overall survival of 07-04 patients according to consolidation schedule censored at allogeneic HSCT date in first CR. Abbreviations: OS, overall survival; HSCT, hematopoietic stem cell transplantation; ITT, intention-to-treat; ITT-135, cytarabine intended on days 1, 3 and 5; ITT-123, cytarabine intended on days 1, 2 and 3; CR, complete remission.

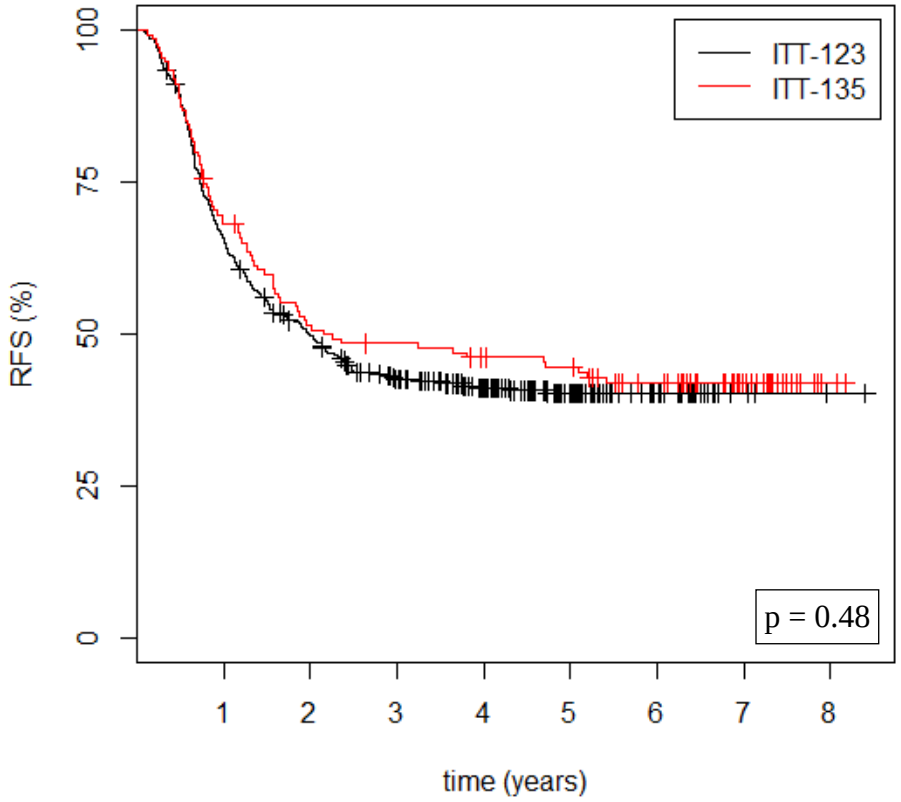

Figure 6: Relapse-free survival of 07-04 patients according to consolidation schedule who received at least one consolidation cycle. Abbreviations: RFS, relapse-free survival; ITT, intention-to-treat; ITT-135, cytarabine intended on days 1, 3 and 5; ITT-123, cytarabine intended on days 1, 2 and 3.

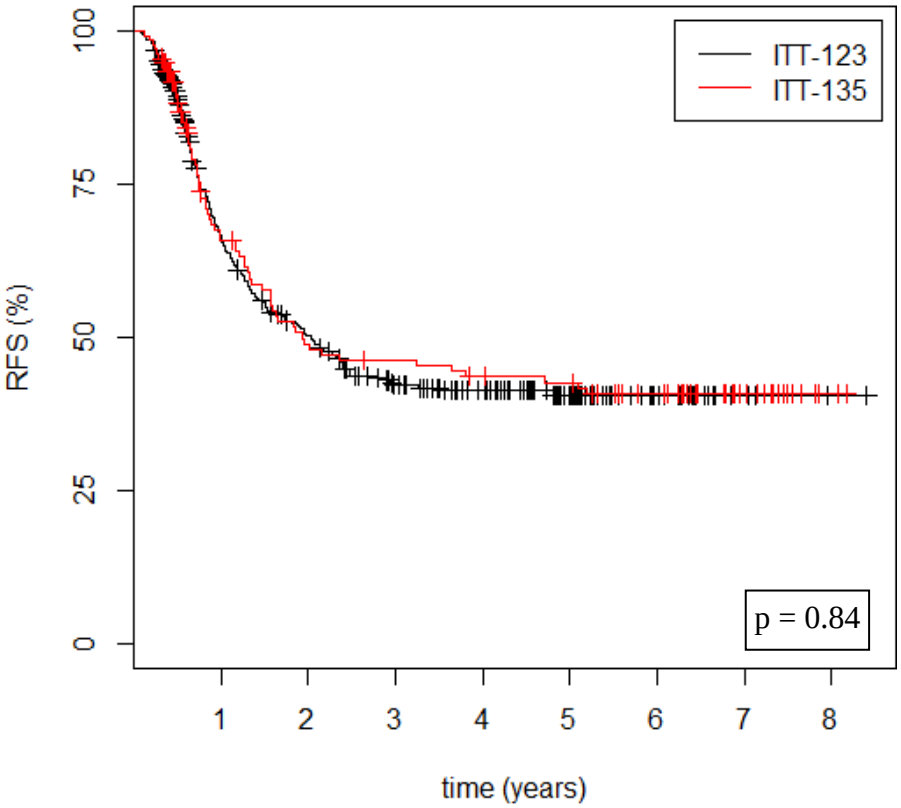

Figure 7: Relapse-free survival of 07-04 patients according to consolidation schedule censored at allogeneic HSCT date in first CR. Abbreviations: RFS, relapse-free survival; HSCT, hematopoietic stem cell transplantation; ITT, intention-to-treat; ITT-135, cytarabine intended on days 1, 3 and 5; ITT-123, cytarabine intended on days 1, 2 and 3; CR, complete remission.

4. Discussion

In this prospective study integrating the 07-04 protocol (Schlenk et al., 2016) and the German AML Intergroup common standard arm (Buchner et al., 2012), I evaluated a condensed schedule of HDAC consolidation therapy on days 1, 2 and 3 compared to the standard on days 1, 3 and 5 (Mayer et al., 1994) and the prophylactic use of pegfilgrastim. Overall, I was able to show a significantly and clinically meaningful reduction of in median 4 days of time to leukocyte and neutrophil recovery measured from the first day of chemotherapy with the HDAC-123 schedule compared to the HDAC-135 schedule. In addition, this significant reduction in leukocyte and neutrophil recovery in the HDAC-123 schedule was associated with a significantly lower rate of infections, a lesser amount of platelet transfusions and fewer days in hospital. Interestingly in an upfront randomized manner, I was not able to show a difference between the German AML Intergroup standard arm using HDAC-135 without prophylactic use of pegfilgrastim and the HDAC-135 arm within the 07-04 protocol with the intended use of pegfilgrastim at day 10 in terms of leukocyte and neutrophil recovery. This may be related to the limited protocol adherence with only 68% of the patients receiving the intended pegfilgrastim.

In per protocol analysis pegfilgrastim was effective in reducing the time to WBC and neutrophil recovery of in median 3 days in the HDAC-135 and 2 days in the HDAC-123 schedule, which is in accordance with previous studies demonstrating in randomized approaches a significant reduction in the duration of severe neutropenia (Archimbaud et al., 1999; Braess et al., 2009; Heil et al., 2006). These observations were supported by the multivariable analysis in which the condensed schedule of cytarabine as well as the administration of pegfilgrastim were significantly associated with shorter WBC recovery times. Furthermore, my multivariable analyses revealed that WBC recovery times were longer with increasing age. This points out to the blood stem cell aging described in *in vivo* and *in vitro* studies, where older stem cells have been found to be less effective in contributing to the hematopoiesis (Morrison, Wandycz, Akashi, Globerson, & Weissman, 1996). This finding was paralleled by the results of the univariable and multivariable analysis with the endpoint infectious complications including fever in neutropenia and infections with a clinical focus.

Consistently, I observed that both the condensed schedule with HDAC-123 and the prophylactic administration of pegfilgrastim were associated with a significant reduction in the rates of infectious complications. These results were concordant with the findings of

Sung et al. (Sung, Nathan, Alibhai, Tomlinson, & Beyene, 2007) based on a systematic meta-analysis showing that the usage of G-CSF during consolidation therapy led to a reduction in the duration of neutropenia and decreased the rate of infections as well as febrile neutropenia. Thus my results strongly argue for the usage of a condensed schedule of HDAC-123 combined with prophylactic pegfilgrastim at day 8 in consolidation therapy in terms of shorter WBC and neutrophil recovery, a lower rate of infectious complications, shorter hospital stays and fewer platelet transfusions.

According to my results, there was no cumulative hematological toxicity in any of the consolidation therapy schedules. This kind of toxicity was expected because repeated administration of chemotherapy should have produced an increased time to hematological reconstitution in comparison to the first dose of therapy.

Importantly, no difference in any survival endpoint analyzed was evident between the HDAC-123 and HDAC-135 schedule in the 07-04 protocol. In the multivariable analysis stratified by the ELN risk group, no differences were seen in terms of OS between the HDAC-123 and HDAC-135 schedule or between therapy with pegfilgrastim and without it. This strongly argues for the at least equivalent efficacy of the HDAC-123 schedule compared to the current standard.

One limitation of my study is having used a non-randomized sequential cohort design instead of an up-front randomized study. Therefore, my results have to be interpreted with caution and further randomized trials have to show the equivalent efficacy of the HDAC-123 schedule regarding RFS and OS. However, the overall favorable results of the 07-04 study (Schlenk et al., 2016) with an OS after four years of 54% (95%-CI: 50−58%) did not support a suspicion of inferiority of the HDAC-123 schedule.

In conclusion, data from my study suggest that during the consolidation therapy the condensed schedule of cytarabine given on days 1, 2 and 3 is superior to the standard HDAC therapy in terms of hematological reconstitution, infection and days of hospitalization. Furthermore, it is not inferior to the standard therapy according to OS and RFS. The use of pegfilgrastim leads to shortening of the hematological reconstitution time reduces the incidence of infections in neutropenia and the duration of hospitalization after consolidation therapy in AML.

5. Summary

The concept of intensive post-remission chemotherapy in acute myeloid leukemia (AML) is based on the observation that despite achievement of a first complete remission (CR) after intensive induction therapy virtually all patients relapse in the absence of further treatment. Moreover, randomized studies showed that intensive post-remission consolidation chemotherapy was superior to prolonged low-dose maintenance therapy in younger patients. Concerning consolidation therapy, the landmark study conducted by the Cancer and Leukemia Group B (CALGB) established the current standard for patients aged 60 years and younger with high-dose cytarabine (HDAC) 3 g/m² b.i.d. on days 1, 3 and 5. The study aimed to compare a condensed schedule of HDAC on days 1, 2 and 3 with the standard HDAC given on days 1, 3 and 5 as well as to evaluate the prophylactic use of pegfilgrastim after chemotherapy in patients in first CR receiving repetitive consolidation cycles for AML. We included patients aged 18 to 60 years between 2004 and 2009. They were randomized up-front 1:9 between the standard German AML Intergroup arm and the AMLSG 07-04 study (NCT00151242). Induction therapy in the 07-04 study consisted of two cycles of idarubicin, cytarabine and etoposide ± All-trans retinoic acid (ATRA) and ± valproic acid (VPA) in a 2 × 2 factorial design. After recruitment of 392 patients, due to toxicity, we stopped the randomization for VPA. For consolidation therapy, patients with high-risk AML, defined either by high-risk cytogenetics or induction failure, were assigned to receive allogeneic hematopoietic cell transplantation from a matched related or unrelated donor. All other patients were assigned to three cycles of HDAC from 2004 to November 2006 with cytarabine 3 g/m² b.i.d. on days 1, 3 and 5 and pegfilgrastim on day 10 (HDAC-135). From December 2006 to 2009 patients were treated with a condensed schedule with cytarabine 3 g/m² b.i.d. on days 1, 2 and 3 and pegfilgrastim on day 8 (HDAC-123). Patients randomized to the German AML Intergroup arm were treated for consolidation therapy with cytarabine 3 g/m² b.i.d. on days 1, 3 and 5 (HDAC-135) without prophylactic growth-factor support. Overall 568 patients receiving 1376 consolidation cycles were included in the study. According to up-front randomization 41 were treated with HDAC-135 without prophylactic growth factor support in the German AML Intergroup protocol, 135 with HDAC-135 and 392 with HDAC-123 with intended prophylactic pegfilgrastim at day 10 and 8, respectively, in the 07-04 protocol. Time from start to chemotherapy until hematological recovery (leukocytes $> 1.0 \times 10^9$/l and neutrophils $> 0.5 \times 10^9$/l) was significantly ($p < 0.0001$ each) and in median four days

shorter in patients receiving HDAC-123 compared to HDAC-135. By adding pegfilgrastim, I was able to further reduce the hematologic recovery by two days ($p < 0.0001$). Treatment with ATRA and VPA according to initial randomization had no impact on hematological recovery times. Rates of infections were significantly reduced by HDAC-123 compared to HDAC-135 ($p < 0.0001$) and pegfilgrastim yes versus no ($p = 0.002$). Days in hospital and platelet transfusions were also significantly reduced in patients receiving HDAC-123 compared to HDAC-135. Relapse-free and overall survival were similar with HDAC-123 and HDAC-135 ($p = 0.48$ and $p = 0.90$ respectively).Data from this study suggest that consolidation therapy with a condensed schedule of HDAC-123 is superior to that of standard HDAC-135 regarding faster hematological recovery, lower infection rate and fewer days in hospital. In addition, the administration of one dose of pegfilgrastim after chemotherapy further shortened hematological recovery and reduced infection rate. Importantly, similar efficacy concerning relapse-free and overall survival rates after HDAC-123 and HDAC-135 was observed.

6. References

1. Arber DA, Vardiman JW, & Brunning RD. Acute myeloid leukaemia with recurrent genetic abnormalities. Swerdlow (Ed.), WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues. Fourth Edition Geneva, Switzerland WHO PRESS 2008, (2015).

2. Archimbaud E, Ottmann OG, Yin JA, Lechner K., Dombret H, Sanz MA, Heil G, Fenaux P, Brugger W, Barge A, O'Brien-Ewen C, Matcham J, Hoelzer DA. Randomized, double-blind, placebo-controlled study with pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuMGDF) as an adjunct to chemotherapy for adults with de novo acute myeloid leukemia. Blood, 94: 3694-3701, (1999).

3. Braess J, Spiekermann K, Staib P, Gruneisen A, Wormann B, Ludwig WD, Serve H, Reichle A, Peceny R, Oruzio D, Schmid C, Schiel X, Hentrich M, Sauerland C, Unterhalt M, Fiegl M, Kern W, Buske C, Bohlander S, Heinecke A, Baurmann H, Beelen D, Berdel WE, Büchner T, Hiddemann W. Dose-dense induction with sequential high-dose cytarabine and mitoxantrone (S-HAM) and pegfilgrastim results in a high efficacy and a short duration of critical neutropenia in de novo acute myeloid leukemia: a pilot study of the AMLCG. Blood, 113: 3903-3910, (2009).

4. Buchner T, Schlenk RF, Schaich M, Dohner K, Krahl R, Krauter J, Heil G, Krug U, Sauerland MC, Heinecke A, Späth D, Kramer M, Scholl S, Berdel WE, Hiddemann W, Hoelzer D, Hehlmann R, Hasford J, Hoffmann VS, Döhner H, Ehninger G, Ganser A, Niederwieser D, Pfirrmann M. Acute Myeloid Leukemia (AML): different treatment strategies versus a common standard arm-combined prospective analysis by the German AML Intergroup. J.Clin.Oncol., 30: 3604-3610, (2012).

5. Cassileth PA, Harrington DP, Hines JD, Oken, MM, Mazza, JJ, McGlave P, Bennett JM, O'Connell MJ. Maintenance chemotherapy prolongs remission duration in adult acute nonlymphocytic leukemia. J Clin Oncol., 6: 583-587, (1998).

6. Cassileth PA, Lynch E, Hines JD, Oken MM, Mazza JJ, Bennett JM, McGlave PB, Edelstein M, Harrington DP, O'Connell MJ. Varying intensity of postremission therapy in acute myeloid leukemia. Blood, 79:1924-1930, (1992).

7. Cheson, BD, Bennett JM, Kopecky KJ, Buchner T, Willman CL, Estey EH, Schiffer CA, Doehner H, Tallman MS, Lister TA, Lo-Coco F, Willemze R, Biondi A, Hiddemann W, Larson RA, Löwenberg B, Sanz MA, Head DR, Ohno R, Bloomfield

CD, International Working Group for Diagnosis. Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Onco., 21: 4642-4649, (2003).

8. Dohner H, Estey EH, Amadori S, Appelbaum FR, Buchner T, Burnett AK, Dombret H, Fenaux P, Grimwade D, Larson RA, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz MA, Sierra J, Tallman MS, Löwenberg B, Bloomfield CD; European LeukemiaNet. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood, 115:453-474, (2010).
9. Dohner H., Weisdorf DJ., & Bloomfield CD. Acute Myeloid Leukemia. N.Engl.J Med., 373: 1136-1152, (2015).
10. Dombret H, & Gardin C. An update of current treatments for adult acute myeloid leukemia. Blood, 127: 53-61, (2016).
11. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. The Annals of Statistics, 16: 1141-1154, (1998).
12. Gurion R, Belnik-Plitman Y, Gafter-Gvili A, Paul M, Vidal L, Ben-Bassat I, Shpilberg O, Raanani P. Colony-stimulating factors for prevention and treatment of infectious complications in patients with acute myelogenous leukemia. Cochrane.Database.Syst.Rev., 6, (2012).
13. Harrell FE. Regression Modeling Strategies: With Applications to Linear Models, Logistic Regression, and Survival Analysis. New York, NY: Springer, (2001).
14. Harousseau JL, Witz B, Lioure B, Hunault-Berger M, Desablens B, Delain M, Guilhot F, Le Prise PY, Abgrall JF, Deconinck E, Guyotat D, Vilque JP, Casassus P, Tournilhac O, Audhuy B, Solary E. Granulocyte colony-stimulating factor after intensive consolidation chemotherapy in acute myeloid leukemia: results of a randomized trial of the Groupe Ouest-Est Leucemies Aigues Myeloblastiques. J Clin Oncol., 18: 780-787, (2000).
15. Heil G, Hoelzer D, Sanz MA, Lechner K, Liu Yin JA, Papa G, Noens L, Szer J, Ganser A, O'Brien C, Matcham J, Barge A. A randomized, double-blind, placebo-controlled, phase III study of filgrastim in remission induction and consolidation therapy for

adults with de novo acute myeloid leukemia. The International Acute Myeloid Leukemia Study Group. Blood, 90: 4710-4718, (1997).

16. Heil G, Hoelzer D, Sanz MA, Lechner K, Noens L, Szer J, Ganser A, Matcham J, Renwick J, International Acute Myeloid Leukemia Study Group. Long-term survival data from a phase 3 study of Filgrastim as an adjunct to chemotherapy in adults with de novo acute myeloid leukemia. Leukemia, 20: 404-409, (2006).
17. Herzig RH, Hines JD, Herzig GP, Wolff SN, Cassileth PA, Lazarus HM, Adelstein DJ, Brown RA, Coccia PF, Strandjord S. Cerebellar toxicity with high-dose cytosine arabinoside. J Clin Oncol., 5:927-932, (1987).
18. Hoffman RF. Clinical manifestations of acute myeloid leukemia (Hematology: Basic Principles and Practice. 5th Edition ed.), (2008).
19. Jaramillo S, Benner A, Krauter J, Martin H, Kindler T, Bentz M, Salih HR, Held G, Köhne CH, Götze K, Lübbert M, Kündgen A, Brossart P, Wattad M, Salwender H, Hertenstein B, Nachbaur D, Wulf G, Horst HA, Kirchen H, Fiedler W, Raghavachar A, Russ G, Kremers S, Koller E, Runde V, Heil G, Weber D, Göhring G, Döhner K, Ganser A, Döhner H, Schlenk RF. Condensed versus standard schedule of high-dose cytarabine consolidation therapy with pegfilgrastim growth factor support in acute myeloid leukemia. Blood Cancer J. 2017 May 26;7(5):e564. doi:10.1038/bcj.2017.45.
20. Juliusson G, Lazarevic V, Horstedt AS, Hagberg O, Hoglund M. Acute myeloid leukemia in the real world: why population-based registries are needed. Blood, 119: 3890-3899, (2012).
21. Leclerc JM, Momparler RL. Effect of the interval between exposures to cytarabine on its cytotoxic action on HL-60 myeloid leukemic cells. Cancer Treat.Rep., 68:1143-1148, (1984).
22. Mayer, R. J., Davis, R. B., Schiffer, C. A., Berg, D. T., Powell, B. L., Schulman, P., Omura GA, Moore JO, McIntyre OR, Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. N.Engl.J Med., 331:896-903,(1994).
23. Michell H Gail., Jay H Lubin, Lawrence V Rubinstein. Likelihood calculations for matched case-control studies and survival studies with tied death times. Biometrika,68:703-707, (1980).
24. Mitelman F. An International System for Human Cytogenetic Nomenclature (ISCN ed.) Basel, Switzerland, Karger, (1995).

25. Molineux G, Kinstler O, Briddell B, Hartley C, McElroy P, Kerzic P, Sutherland W, Stoney G, Kern B, Fletcher FA, Cohen A, Korach E, Ulich T, McNiece I, Lockbaum P, Miller-Messana MA, Gardner S, Hunt T, Schwab G. A new form of Filgrastim with sustained duration in vivo and enhanced ability to mobilize PBPC in both mice and humans. Exp.Hematol., 27:1724-1734, (1999).

26. Morrison SJ, Wandycz AM, Akashi K, Globerson A, Weissman IL. The aging of hematopoietic stem cells. Nat.Med., 2: 1011-1016, (1996).

27. Mrozek, K., Marcucci, G., Nicolet, D., Maharry, K. S., Becker, H., Whitman, S. P., Becker H, Schwind S, Metzeler KH, Powell BL, Carter TH, Kolitz JE, Wetzler M, Carroll AJ, Baer MR, Caligiuri MA, Larson RA, Bloomfield CD. Prognostic significance of the European LeukemiaNet standardized system for reporting cytogenetic and molecular alterations in adults with acute myeloid leukemia. J Clin Oncol., 30: 4515-4523, (2012).

28. O'Donnell MR, Abboud CN, Altman J, Appelbaum FR, Arber DA, Attar E, Borate U, Coutre SE, Damon LE, Lancet J, Maness LJ, Marcucci G, Martin MG, Millenson MM, Moore JO, Ravandi F, Shami PJ, Smith BD, Stone RM, Strickland SA, Wang ES, Gregory KM, Naganuma M; National Comprehensive Cancer Network. Acute myeloid leukemia. J.Natl.Compr.Canc.Netw., 10: 984-1021, (2012).

29. Ottmann OG, Bug G, Krauter J. Current status of growth factors in the treatment of acute myeloid and lymphoblastic leukemia. Semin.Hematol., 44: 183-192, (2007).

30. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, Potter NE, Heuser M, Thol F, Bolli N, Gundem G, Van Loo P, Martincorena I, Ganly P, Mudie L, McLaren S, O'Meara S, Raine K, Jones DR, Teague JW, Butler AP, Greaves MF, Ganser A, Döhner K, Schlenk RF, Döhner H, Campbell PJ. Genomic Classification and Prognosis in Acute Myeloid Leukemia. N.Engl.J Med., 374: 2209-2221, (2016).

31. SEER. (2015). Surveillance Epidemiology and End Results (SEER) Program. Limited use-data (1973 – 2004). Website: http://www.seer.cancer.gov. Accessed Aug.2016.

32. Schlenk RF. Post-remission therapy for acute myeloid leukemia. Haematologica, 99: 1663-1670, (2014).

33. Schlenk RF, Dohner K, Krauter J, Frohling S, Corbacioglu A, Bullinger L, Habdank M, Späth D, Morgan M, Benner A, Schlegelberger B, Heil G, Ganser A, Döhner H; German-Austrian Acute Myeloid Leukemia Study Group. Mutations and treatment

outcome in cytogenetically normal acute myeloid leukemia. N.Engl.J Med., 358:1909-1918, (2008).

34. Schlenk RF, Dohner K, Mack S, Stoppel M, Kiraly F, Gotze K, Hartmann F, Horst HA, Koller E, Petzer A, Grimminger W, Kobbe G, Glasmacher A, Salwender H, Kirchen H, Haase D, Kremers S, Matzdorff A, Benner A, Döhner H. Prospective evaluation of allogeneic hematopoietic stem-cell transplantation from matched related and matched unrelated donors in younger adults with high-risk acute myeloid leukemia: German-Austrian trial AMLHD98A. J Clin.Oncol., 28: 4642-4648, (2010).
35. Schlenk RF, Dohner H. Genomic applications in the clinic: use in treatment paradigm of acute myeloid leukemia.Hematology.Am.Soc.Hematol.Educ.Program., 2013: 324-330, (2013).
36. Schlenk RF, Lübbert M, Benner A, Lamparter A, Krauter J, Herr WH, Salih HR, Kündgen A, Horst HA, Brossart P, Götze K, Nachbaur D, Wattad M, Köhne CH, Fiedler W, Bentz M, Wulf G, Held G, Hertenstein B, Salwender H, Gaidzik VI, Schlegelberger B, Weber D, Döhner K, Ganser A, Döhner H; German-Austrian Acute Myeloid Leukemia Study Group. All-trans retinoic acid as adjunct to intensive treatment in younger adult patients with acute myeloid leukemia: results of the randomized AMLSG 07-04 Study. Ann Hematol., 95:1931-1942, (2016).
37. Sierra J, Szer J, Kassis J, Herrmann R, Lazzarino M, Thomas X, Noga SJ, Baker N, Dansey R, Bosi A. A single dose of pegfilgrastim compared with daily filgrastim for supporting neutrophil recovery in patients treated for low-to-intermediate risk acute myeloid leukemia: results from a randomized, double-blind, phase 2 trial. BMC.Cancer, 8: 195, (2008).
38. Smith, TJ, Khatcheressian J, Lyman GH, Ozer H, Armitage JO, Balducci L, Bennett CL, Cantor SB, Crawford J, Cross SJ, Demetri G, Desch CE, Pizzo PA, Schiffer CA, Schwartzberg L, Somerfield MR, Somlo G, Wade JC, Wade JL, Winn RJ, Wozniak AJ, Wolff AC. 2006 update of recommendations for the use of white blood cell growth factors: an evidence-based clinical practice guideline. J.Clin.Oncol., 24: 3187-3205, (2006).
39. Sung L, Nathan PC, Alibhai SM, Tomlinson GA, Beyene J. Meta-analysis: effect of prophylactic hematopoietic colony-stimulating factors on mortality and outcomes of infection. Ann.Intern.Med., 147: 400-411, (2007).

40. Tassara M, Dohner K, Brossart P, Held G, Gotze K, Horst H, Ringhoffer M, Köhne CH, Kremers S, Raghavachar A, Wulf G, Kirchen H, Nachbaur D, Derigs HG, Wattad M, Koller E, Brugger W, Matzdorff A, Greil R, Heil G, Paschka P, Gaidzik VI, Göttlicher M, Döhner H, Schlenk RF. Valproic acid in combination with all-trans retinoic acid and intensive therapy for acute myeloid leukemia in older patients. Blood, 123: 4027-4036, (2014).

41. Thein MS, Ershler WB, Jemal A, Yates JW, Baer MR. Outcome of older patients with acute myeloid leukemia: an analysis of SEER data over 3 decades. Cancer, 119: 2720-2727, (2013).

42. Vardiman JW. The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues: an overview with emphasis on the myeloid neoplasms. Chem.Biol.Interact., 184: 16-20, (2010).

43. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, Harris NL, Le Beau MM, Hellström-Lindberg E, Tefferi A, Bloomfield CD. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood, 114: 937-951, (2009).

44. Vardiman JW, Harris NL, Brunning, RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood, 100: 2292-2302, (2002).

Acknowledgements

Die Danksagung wurde aus Gründen des Datenschutzes entfernt.

Curriculum vitae

Der Lebenslauf wurde aus Gründen des Datenschutzes entfernt.