VOLUME 27 · NUMBER 1 · JANUARY 1 2009

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Age-Related Risk Profile and Chemotherapy Dose Response in Acute Myeloid Leukemia: A Study by the German Acute Myeloid Leukemia Cooperative Group

Thomas Büchner, Wolfgang E. Berdel, Claudia Haferlach, Torsten Haferlach, Susanne Schnittger, Carsten Müller-Tidow, Jan Braess, Karsten Spiekermann, Joachim Kienast, Peter Staib, Andreas Grüneisen, Wolfgang Kern, Albrecht Reichle, Georg Maschmeyer, Carlo Aul, Eva Lengfelder, Maria-Cristina Sauerland, Achim Heinecke, Bernhard Wörmann, and Wolfgang Hiddemann

From the Departments of Hematology and Oncology and Medical Informatics and Biomathematics, University of Münster, Münster; Munich Leukemia Laboratory; Department of Internal Medicine III, University of Munich, Munich; Clinic I Internal Medicine, University of Cologne, Cologne; Department of Hematology and Oncology, Municipal Hospital Neukölln, Berlin; Department of Hematology and Oncology, University of Regensburg, Regensburg; Department of Hematology and Oncology, Medical Center Ernst von Bergmann, Potsdam; Department of Hematology and Oncology, St Johannes Hospital, Duisburg; Department of Hematology and Oncology, University of Mannheim, Mannheim; Department of Hematology and Oncology, Municipal Hospital, Braunschweig; and Clinical Cooperative Group "Acute Leukemia" of the Helmholtz Zentrum München–German Research Center for Environmental Health, Neuherberg, Germany.

Submitted November 19, 2007; accepted July 24, 2008; published online ahead of print at www.jco.org on December 1, 2008.

Supported by Grants No. M17/92/Bü1 and 70-2839-Bü4 from Deutsche Krebshilfe, Grant No. 01 GI 9976 from Bundesministerium Bildung und Forschung Competence Network Acute and Chronic Leukemias, Grant No. LSH-2002-2.2.0-3 from European Commission European LeukemiaNet, and an unrestricted grant from AMGEN.

Presented in part at the 43rd Annual Meeting of the American Society of Clinical Oncology, June 1-5, 2007, Chicago, IL.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical Trials repository link available on JCO.org.

Corresponding author: Thomas Büchner, MD, PhD, University of Münster, Department of Hematology/Oncology, Albert-Schweitzer-Str 33, 48129 Münster, Germany; e-mail: buechnr@uni-muenster.de.

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

0732-183X/09/2701-61/$20.00

DOI: 10.1200/JCO.2007.15.4245

ABSTRACT

Purpose
The purpose of the study was to assess the contribution of age and disease variables to the outcome of untreated patients with acute myeloid leukemia (AML) receiving varying intensive induction chemotherapy.

Patients and Methods
Patients 16 to 85 years of age with primary AML, known karyotype, and uniform postremission chemotherapy enrolled onto two consecutive trials were eligible and were randomly assigned to induction either with a standard-dose (cytarabine, daunorubicin, and 6-thioguanine) and a high-dose (cytarabine and mitoxantrone) combination, or with two courses of the high-dose combination. Subgroups were defined by karyotype, nucleophosmin and *FLT3* mutation, WBC count, serum lactate dehydrogenase, and residual blasts.

Results
In 1,284 patients, the overall survival at 4 years in those younger and older than 60 years was 37% versus 16% ($P < .001$) and the ongoing remission duration was 46% versus 22% ($P < .001$). Similar age-related differences in outcome were found for all defined subgroups. No difference in outcome according to randomly assigned treatment regimen was observed in any age group or prognostic subset. Regarding prognostic subgroups, molecular factors were also considered.

Conclusion
Under harmonized conditions, older and younger patients with AML show modest differences in their risk profiles and equally no dose response to intensified chemotherapy. Their observed fundamental difference in outcome across all subgroups remains unexplained. Further molecular investigation may elucidate the age effect in AML and identify new targets.

J Clin Oncol 27:61-69. © 2008 by American Society of Clinical Oncology

INTRODUCTION

In acute myeloid leukemia (AML), some two thirds of patients are now 60 years of age or older.[1,2] Even in multicenter trials, the proportion of older patients has increased. Thus in the 1981 study by the German AML Cooperative Group, patients older than 60 years accounted for 25% of patients;[3] the percentage of patients in this age group reached 53% in the 1999 study.[4] Compared with the gradual improvements achieved in younger patients, however, the therapeutic outcome shows a lack of progress in older patients.[5,6]

After earlier investigations failed to support attenuation strategies,[7] the present project aimed to determine whether outcome in AML can be improved by intensification of induction chemotherapy and whether intensification benefits particular prognostic groups among younger and older patients, such as groups recently defined according to mutations of the nucleophosmin (*NPM1*) gene and the Fms-like tyrosine kinase length mutation in the juxtamembrane domain (*FLT3-LM*).[8-10] To answer these questions, the data of two consecutive prospective randomized trials by the German AML Cooperative Group were evaluated. For maximum homogeneity, only patients with primary AML whose leukemic cell karyotype was known and who were assigned to the uniform prolonged maintenance chemotherapy were considered.

PATIENTS AND METHODS

Patients

In two consecutive trials starting in 1992 and 1999, previously untreated patients 16 years of age and older with no upper age limit who had AML, except for acute promyelocytic leukemia, were eligible. Although patients with AML secondary to cytotoxic treatment, myelodysplastic syndrome, or other antecedent hematologic disorders and patients with high-risk myelodysplasia were also included in the 1999 trial, present analysis is restricted to patients with primary AML in both trials. The trials were approved by the ethics committees of the participating centers and were conducted in accordance with the Declaration of Helsinki. Written informed consent was given by all participants.

Prognostic Factors

At diagnosis, samples of bone marrow aspirates were examined for chromosomal abnormalities using standard banding techniques and classified according to the International System for Human Cytogenetic Nomenclature.[11] The rate of adequate cytogenetics was 66% in the 1992 trial and 97% in the 1999 trial. Only patients with known karyotypes were considered in present analysis. According to the chromosomal findings, the individual leukemias were classified into three cytogenetic groups, with subdivisions into intermediate-normal and intermediate-other karyotype, as well as unfavorable-complex and unfavorable-other karyotype (Table 1). A sample of 396 patients with normal karyotype representative in outcome for this cytogenetic groups was characterized for mutations of the *NPM1* gene and *FLT3-LM* by methods described.[9,12] Other prognostic factors evaluated included WBC count, dichotomized at $20 \times 10^3/\mu L$; serum lactate dehydrogenase (LDH), a proven risk factor in high-grade lymphoma,[13] testicular cancer,[14] and AML,[15,16] dichotomized at 700 U/L; and blasts in the bone marrow 1 week after the first induction course,[16,17] dichotomized at 10%[17] as proving a highly significant independent prognostic factor in a previous study.

Table 1. Patient Characteristics in the Two Age Groups

Characteristic	Age < 60 Years (n = 520)	Age ≥ 60 Years (n = 764)	*P*
Male sex, %	52.9	55.0	.461
Age, years			
Median	45.0	66.00	
Range	16.0-59.0	60.0-85.0	
WBC count			
Median, cells/μL	19,800	12,510	< .001
Range, cells/μL	50-964,000	400-1,017,000	
> $20 \times 10^3/\mu L$, %	49.71	40.84	.0018
Serum LDH			
Median, U/L	491	403	< .001
Range, U/L	88-14,332	7.5-11,150	
> 700 U/L, %	31.57	23.58	.0017
Karyotype, %*			< .001
Favorable	16.0	6.6	
Intermediate, normal	52.5	52.0	
Intermediate, other	13.8	17.0	
Unfavorable, complex	4.6	13.9	
Unfavorable, other	13.1	10.5	
Normal karyotype and mutation†			.0189
NPM1+/*FLT3*−	36.5	33.2	
NPM1+/*FLT3*+	29.9	18.9	
NPM1−/*FLT3*−	26.3	40.2	
NPM1−/*FLT3*+	7.3	7.7	
Day 16 bone marrow blasts			
Median, % blasts	5	5	
Range, % blasts	0-100	0-100	
≥ 10% blasts, % of patients	33.71	41.31	.0120

NOTE. *P* values were calculated by the χ^2 or Wilcoxon test.
Abbreviations: LDH, lactate dehydrogenase; *NPM1*, nucleophosmin 1 gene; *FLT3*, Fms-like tyrosine kinase gene (length mutation).
*Favorable karyotype was defined by the presence of t(8;21) (q22;22), inv(16) (p13q22), or t(16;16) (p13q22). Unfavorable karyotype was defined by the presence of loss or deletions of chromosome 5 or 7 (−5, 5q−, −7, 7q−), abnormal 3q21, q26, abnormal 11q23, or complex aberrant karyotypes with at least three structural and/or numerical abnormalities; intermediate karyotype was defined by the presence of a normal karyotype or abnormalities not considered favorable or unfavorable.
†Sample of 396 patients with complete mutation status.

Study Design and Chemotherapy

The standard version of induction treatment (TAD-HAM) started with cytarabine 100 mg/m^2 per day by continuous intravenous (IV) infusion on days 1 and 2 and by 30-minute IV infusions every 12 hours on days 3 through 8, daunorubicin 60 mg/m^2 by 60-minute IV infusion on days 3, 4, and 5, and 6-thioguanine 100 mg/m^2 orally every 12 hours on days 3 through 9 (TAD). The second induction course combined cytarabine 3 g (in patients < 60 years of age) or 1 g (in patients ≥ 60 years of age)/m^2 by 3-hour IV infusion every 12 hours on days 1 through 3, with mitoxantrone 10 mg/m^2 by 60-minute IV infusions on days 3 through 5 (HAM). The second induction course was given to all patients younger than 60 years and, among patients 60 years and older, to those with 5% or more residual blasts in their bone marrow on day 16. After achieving complete remission, all patients received consolidation by one course of TAD. For maintenance treatment, patients received monthly courses of cytarabine 100 mg/m^2 with subcutaneous injections every 12 hours on days 1 through 5, and as second agent from course to course, either daunorubicin 45 mg/m^2 by 60-minute IV infusion on days 3 and 4, 6-thioguanine 100 mg/m^2 orally every 12 hours on days 1 through 5, or cyclophosphamide 1 g/m^2

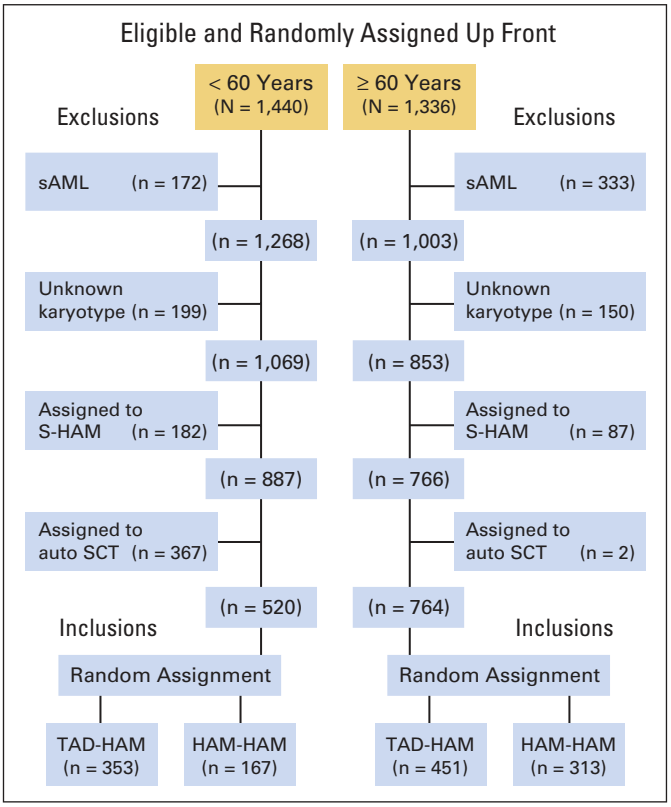

Fig. 1. CONSORT diagram. sAML, secondary acute myeloid leukemia; S-HAM, sequential high-dose cytarabine and mitoxantrone induction; auto SCT, autologous stem-cell transplantation; TAD, standard-dose cytarabine, daunorubicin, and 6-thioguanine induction.

by IV injection on day 3, with the second agent added in a rotating sequence. Maintenance continued for 3 years, and dose reductions by 50% were done after critical nadirs in absolute neutrophils of less than 500/μL or platelets of less than $20 \times 10^3/\mu$L were observed. For the intensified version of induction treatment (HAM-HAM), both induction courses consisted of the high-dose cytarabine/mitoxantrone combination described above, whereas the TAD consolidation and maintenance was as after the standard version of induction. Only patients assigned to the uniform maintenance regimen were considered for the present analysis. Patients randomly assigned to other treatment modalities, such as intensified consolidation instead of maintenance or autologous stem-cell transplantation, were not included in the present analysis (Fig 2). Allogeneic stem-cell transplantation in first remission was applied to patients younger than 60 years with histocompatible siblings in both trials. At 26 of the 47 centers within the 1999 trial, half of the patients were randomly assigned to receive granulocyte colony-stimulating factor by daily subcutaneous injections of 150 μg/m^2 from 48 hours before until the last dose of each chemotherapy course during the first year. Assignment to granulocyte colony-stimulating factor did not affect the outcome[18] and was accepted for present analysis.

Statistical Analysis

The primary objective of the present study was to determine the effect of intensified induction chemotherapy on patient outcome. Among the criteria of outcome, complete remission (CR) was defined as cellular marrow with less than 5% blasts and peripheral blood with at least $1.5 \times 10^3/\mu$L absolute neutrophils and $100 \times 10^3/\mu$L platelets. Survival was measured from treatment initiation to death, remission duration was measured from achievement of complete remission criteria until relapse, and relapse-free survival was measured from achievement of complete remission until relapse or death in remission. As part of the protocol, allogeneic stem-cell transplantation (12% of patients $<$ 60 years of age) remained uncensored, because censoring had no major influence on the results.[4] The outcome criteria were evaluated according to intention-to-treat. Significance were calculated for response rates by χ^2 test and for survival and remission duration by the log-rank test. Potential prognostic factors were tested using the Cox proportional hazards model, including the dichotomized variables of age (≥ 60 *v* < 60 years), karyotype (favorable *v* other; unfavorable *v* other), normal karyotype with presence of nucleophosmin (*NPM1*) mutation in absence of *FLT3-LM* (+/−) versus other combinations of the two mutations (+/+ or −/− or −/+), day 16 bone marrow blasts ($\geq$ 10% *v* $<$ 10%), LDH ($>$ 700 U *v* $\leq$ 700 U), and WBC ($>$ $20 \times 10^3/\mu$L *v* $\leq$ $20 \times 10^3/\mu$L). The comparator groups were the respective other karyotypes and counterparts of the dichotomized variables. The study adhered to the revised recommendations of the International Working Group for Standardization in AML.[19]

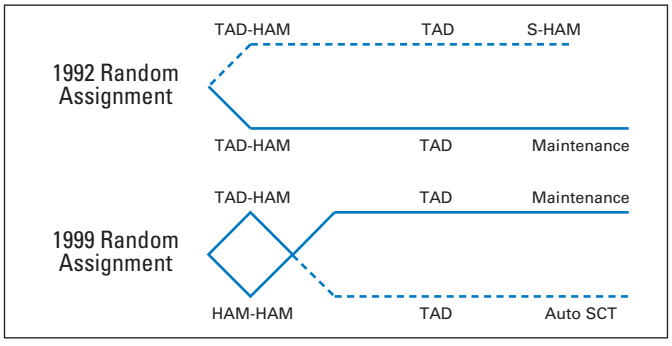

Fig 2. Design and patient selection: 1992 and 1999 trial and present study. Included are 1,284 patients assigned to standard-dose cytarabine, daunorubicin, and 6-thioguanine (TAD) and high-dose cytarabine and mitoxantrone (HAM) induction (TAD-HAM) or HAM-HAM induction, TAD consolidation, and maintenance. Excluded are 638 patients assigned to sequential HAM (S-HAM) or autologous stem-cell transplantation (auto SCT). 505 patients with secondary acute myeloid leukemia (sAML), and 349 patients with unknown karyotype.

RESULTS

Patient Population

A total of 2,776 patients (age $<$ 60 years, n = 1,440; age $\geq$ 60 years, n = 1,336; Fig 1) entered the 1992 and 1999 trials between January 1993 and November 2005. In the entire patient population, the CR rate was 63.0%, the overall survival at 4 years was 25.8%, the ongoing CR rate was 35.2%, and the relapse-free survival rate was 25.5%. To ensure maximum comparability, only patients with primary AML whose karyotype of leukemic bone marrow cells was known and who were assigned to a uniform postremission consolidation and maintenance chemotherapy were evaluated. A total of 505 patients were therefore excluded from present analysis as a result of having secondary AML. From the remaining 2,271 patients, 349 patients (15.4%) were excluded because of unknown karyotype. An additional 269 patients were not considered because they were assigned to intensive consolidation with high-dose cytarabine instead of maintenance,[16] and 369 patients were excluded because they were assigned to autologous stem-cell transplantation (Figs 1 and 2).[4] No

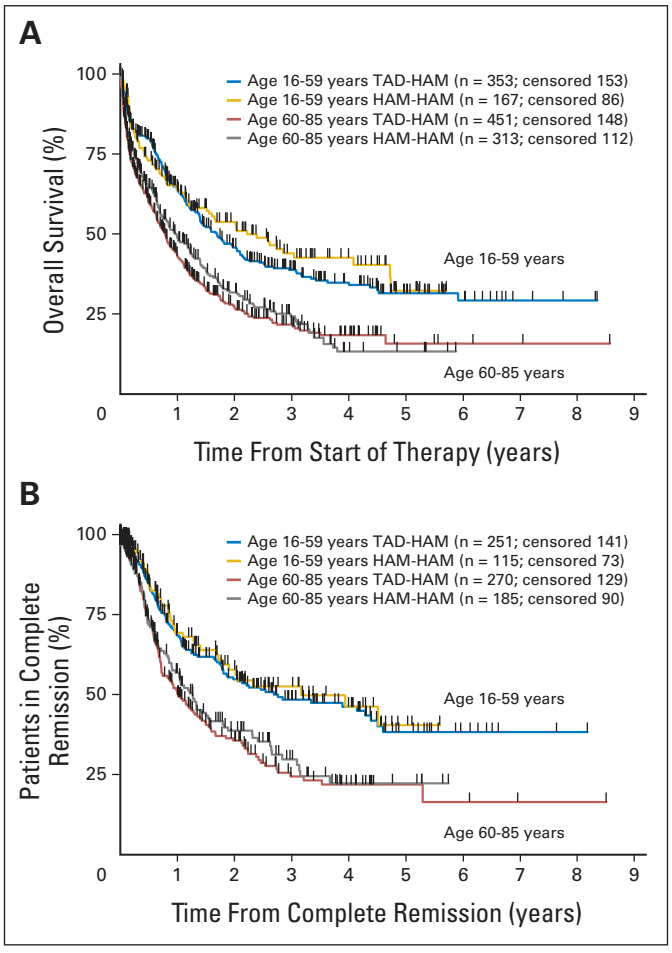

Fig 3. (A) Survival and (B) remission duration by age group and up-front randomization for induction therapy. Cytarabine in high-dose cytarabine and mitoxantrone induction (HAM) was 3 g/m^2 $\times$ 6 in patients $<$ 60 and 1 g/m^2 $\times$ 6 in patients $\geq$ 60 years of age. Younger patients received the second course (HAM) in any case; older patients did so in case of residual bone marrow blasts only. TAD, standard-dose cytarabine, daunorubicin, and 6-thioguanine induction; CR, complete remission.

Table 2. Multivariate Analysis of Prognostic Factors Including All Patients

Prognostic Factor	Complete Remission		Overall Survival		Remission Duration		Relapse-Free Survival	
	Odds Ratio	*P*	Hazard Ratio	*P*	Hazard Ratio	*P*	Hazard Ratio	*P*
Age ≥ 60 years	1.531	< .001	1.633	< .001	1.709	< .001	1.689	< .001
Favorable karyotype	0.747	.181	0.672	.007	0.410	< .001	0.528	< .001
Unfavorable karyotype	2.754	< .001	2.270	< .001	2.588	< .001	2.481	< .001
Day 16 bone marrow blasts ≥ 10%			1.576	< .001	1.255	.046	1.179	.118
WBC count > 20 × $10^3/\mu$L	1.117	.412	1.171	.058	1.196	.120	1.152	.176
Serum LDH > 700 U/L	1.174	.281	1.212	.037	1.291	.043	1.238	.065

NOTE. *P* values were calculated by the logistic or Cox regression analysis (Wald test). Odds and hazard ratios give the probabilities to not achieve complete remission, to die, to experience relapse, and to experience relapse or die in complete remission.
Abbreviation: LDH, lactate dehydrogenase.

patients were excluded for other reasons. The analysis thus included 520 patients younger than 60 years and 764 patients older than 60 years, with no upper age limit.

Among the 1,284 patients included, 804 patients (353 patients younger and 451 patients older than 60 years) were randomly assigned to TAD-HAM induction and subsequent postremission TAD consolidation, followed by prolonged monthly maintenance. The other 480 patients (167 patients younger and 313 patients older than 60 years) were randomly assigned to HAM-HAM induction, equal TAD consolidation, and equal maintenance (Figs 1 and 2).

Table 1 lists patient characteristics. Although in older patients, karyotypes and day 16 blasts were more unfavorable, WBC counts and LDH were lower than in younger patients. Similar frequencies between the two age groups are found in the more favorable (+/−) and the more unfavorable (+/+, −/−, −/+) associations of the *NPM1* mutation and *FLT3-LM* in case of normal karyotype.

Drug Delivery

By the protocol for the induction treatment, HAM as second course was given to 88.1% of all younger patients and 37.3% of those older patients with 5% or more residual bone marrow blasts. Among patients in remission, 82.6% younger and 79.1% older patients received TAD consolidation. Fifty-eight percent of younger and 57.6% of older patients proceeded to maintenance treatment. Exclusions from consolidation or maintenance were due to relapse, toxicity, allogeneic stem-cell transplantation, or other reasons. The delivery of maintenance followed a monthly schedule, with necessary delays and dose reductions according to grade and duration of cytopenia. Thus the adaptions of maintenance were strongly dependent on the stability of remission and development of relapse. Among the patients remaining in remission for 3 years or more, 86% continued with maintenance for at least 30 months.

Outcome of Therapy by Randomization for Induction

Among the older patients, 59.6% achieved CR, 59.9% in the TAD-HAM arm and 59.1% in the HAM-HAM arm. Accordingly, 24.4% and 27.8% of older patients in the TAD-HAM and HAM-HAM arms remained with resistant leukemia, and 15.7% and 13.1% succumbed to early or hypoplastic death, respectively ($P = .412$). Among younger patients, 70.4% achieved CR, 71.1% in the TAD-HAM arm and 68.9% in the HAM-HAM arm. Accordingly, 16.4% and 20.4% of younger patients in the TAD-HAM and HAM-HAM arms remained with resistant leukemia, and 12.4% and 10.8% succumbed to early or hypoplastic death, respectively ($P = .512$).

Figure 3 shows Kaplan-Meier estimates of overall survival and remission duration by randomization for induction in older compared with younger patients. Although older patients show inferior outcome, there is no dose response in either age group.

Multivariate Analysis of Prognostic Factors

Table 2 lists the independent prognostic factors and their significance related to major therapeutic end points. The strongest factors predicting overall survival were unfavorable karyotype, older age, high day 16 blasts, and favorable karyotype.

Table 3 analyzes patients with normal karyotype and their prognostic factors, including mutations of the *NPM1* and *FLT3* genes. In this large subgroup, the most important risk factors predicting overall

Table 3. Multivariate Analysis of Prognostic Factors in Patients With Normal Karyotype and Complete *NPM1/FLT3* Mutation Status

Prognostic Factor	Complete Remission		Overall Survival		Remission Duration		Relapse-Free Survival	
	Odds Ratio	*P*	Hazard Ratio	*P*	Hazard Ratio	*P*	Hazard Ratio	*P*
Age ≥ 60 years	1.581	.067	1.448	.018	1.900	.001	1.759	.002
Mutation *NPM1*+ and *FLT3-ITD*−	0.500	.007	0.496	< .001	0.384	< .001	0.379	< .001
Day 16 bone marrow blasts ≥ 10%	—		1.540	.004	0.951	.817	0.968	.866
WBC count > 20 × $10^3/\mu$L	1.017	.946	1.371	.049	1.126	.562	1.192	.342
Serum LDH > 700 U/L	1.039	.891	1.297	.122	1.426	.111	1.445	.064

NOTE. *P* values were calculated by the logistic or Cox regression analysis (Wald test). Odds and hazard ratios give the probabilities to not achieve complete remission, to die, to experience relapse, and to experience relapse or die in complete remission.
Abbreviations: *NPM1*, nucleophosmin 1 gene; *FLT3*, Fms-like tyrosine kinase gene (length mutation); LDH, lactate dehydrogenase.

survival were the sole mutation of *NPM1*, high day 16 blasts, and older age.

Outcome by Randomization in Prognostic Groups

On the basis of the multivariate analysis, prognostic subgroups were defined according to karyotype, *NPM1/FLT3* mutation status, LDH, WBC, and day 16 bone marrow blasts (Tables 1, 2, and 3). As in the entire population, there was no significant difference in the overall survival and remission duration between the TAD-HAM and the HAM-HAM induction regimen in any subgroup, neither in older nor in younger patients (Appendix Tables A1 and A2, online only).

Outcome by Age in Prognostic Groups

As in the overall patient population (Fig 3), there is an inferior survival and remission duration in the older versus younger patients in all subgroups defined by karyotype (Fig 4), *NPM1/FLT3* mutation status (Fig 5), WBC, LDH, and day 16 bone marrow blasts (Fig 6; Appendix Table A3, online only).

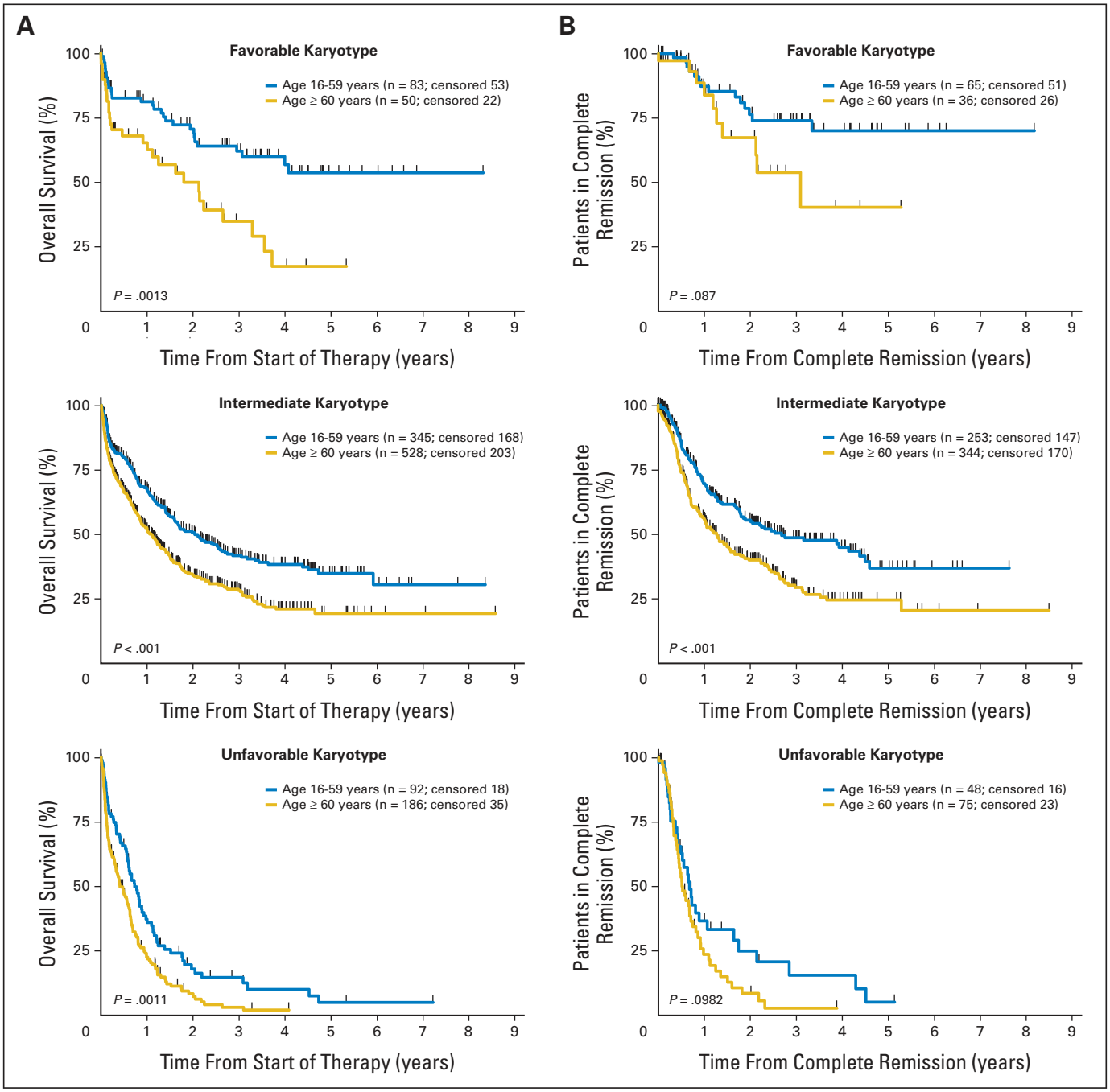

Fig 4. (A) Overall survival and (B) remission duration in younger (age 16 to 59 years) and older (age 60+ years) patients predicted by favorable, intermediate, and unfavorable karyotype.

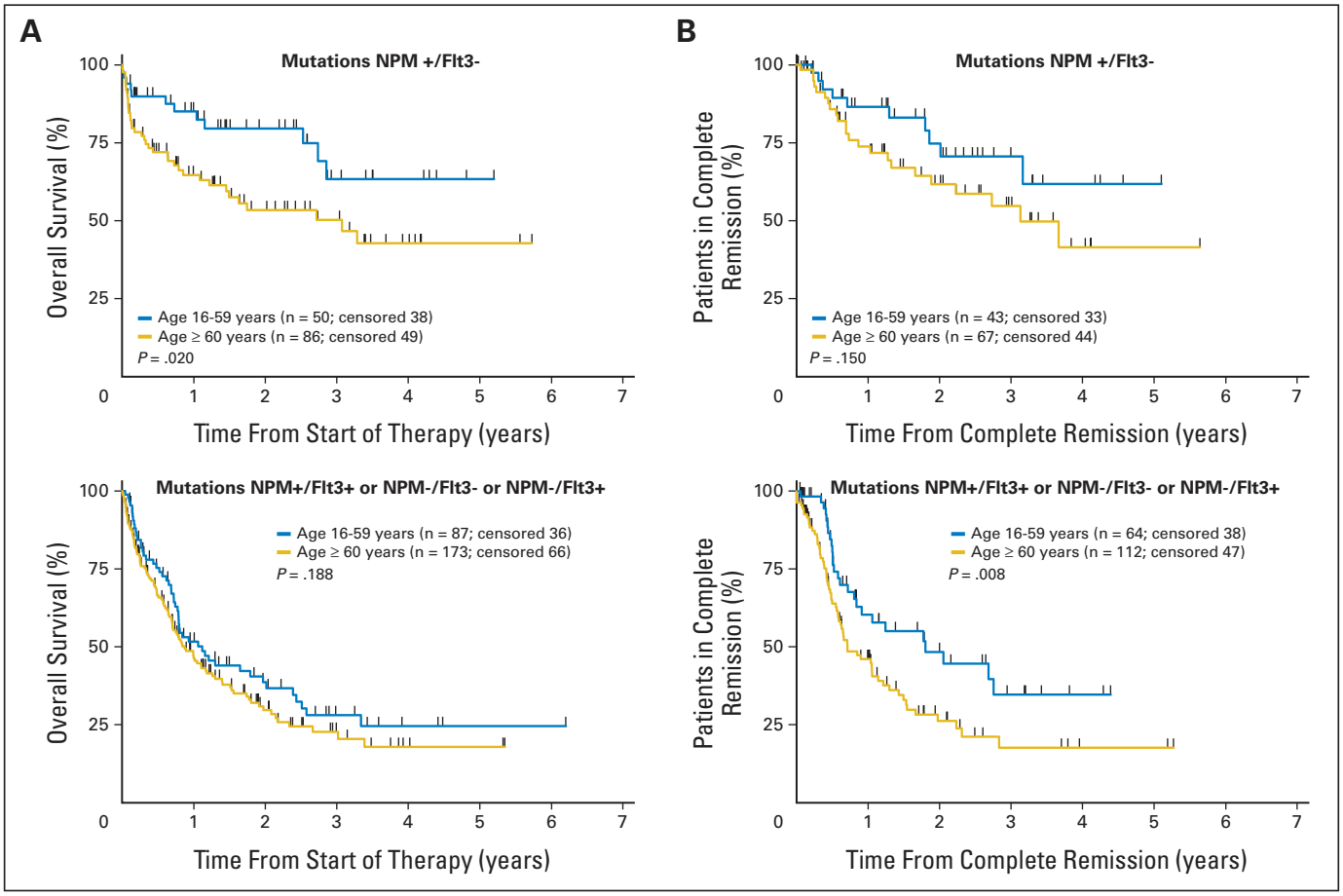

Fig 5. (A) Overall survival and (B) remission duration in younger (age 16 to 59 years) and older (age 60+ years) patients predicted by normal karyotype and NPM1/FLT3 mutation status.

Outcome in Excluded Patients

The outcome by randomization and by age in patients with secondary AML or unknown karyotype was similar to that in the defined prognostic groups (data not shown).

DISCUSSION

The present evaluation of 1,284 patients spanning all ages from 16 to 85 years, restricted to primary AML and identical postremission treatment, confirmed the inferiority of older age in terms of therapeutic outcome. Patients older than 60 years achieved a survival only half that of younger patients as a result of less frequent remissions, more frequent resistant disease, and more frequent and earlier relapses. These differences were equally seen in all prognostic subgroups defined by cytogenetics, *NPM1/FLT3* mutation status, WBC, LDH, and early blast clearance.

The disease seems resistant even against intensification of chemotherapy. In fact, the HAM-HAM version of induction represents a marked intensification against the TAD-HAM version, even taking an age adaption in the patients older than 60 years into account. As we previously reported, double induction by TAD-HAM versus TAD-TAD produced a higher CR rate ($P = .004$) and longer event-free ($P = .012$) and overall survival ($P = .009$) in patients younger than 60 years with poor prognosis.[15] The recovery time of neutrophils and platelets was a median of 16 days after TAD-TAD and 20 days after TAD-HAM ($P = .0001$).[15] Regarding patients older than 60 years, the CR rate after the first induction course was 30% in the TAD-HAM arm and 36% in the HAM-HAM arm ($P = .049$). Older patients with a high LDH showed a trend to longer survival from HAM-HAM induction ($P = .024$).[4] Although the TAD-HAM and the HAM-HAM induction regimens differ markedly in their intensities, the overall survival and remission duration could not be further improved in either age group. This was equally found across all prognostic subgroups, defined by cytogenetics, *NPM1/FLT3* mutation status, WBC, LDH, and early blast clearance. Thus the general absence of a dose response suggests that once a certain intensity has been reached, a further intensification will not further improve the antileukemic potential of chemotherapy.

The inherently poor outcome in older patients with AML is incompletely understood. Prognostic factors commonly discussed, such as a preceding myelodysplastic syndrome or cytotoxic treatment,[20,21] were excluded here. Beyond the negative history, chromosomal abnormalities described as typical for secondary AML[22-24] and ranging in the subset of unfavorable karyotype were only modestly increased in the older compared with the younger patients (24% *v* 18%). In other series, an expression of the multidrug resistance gene or P glycoprotein was shown in 71% of older and 35% of younger patients[25] and was associated with poorer response.[26] A relationship to the relapse rate or relapse-free survival was not found[25] and has not

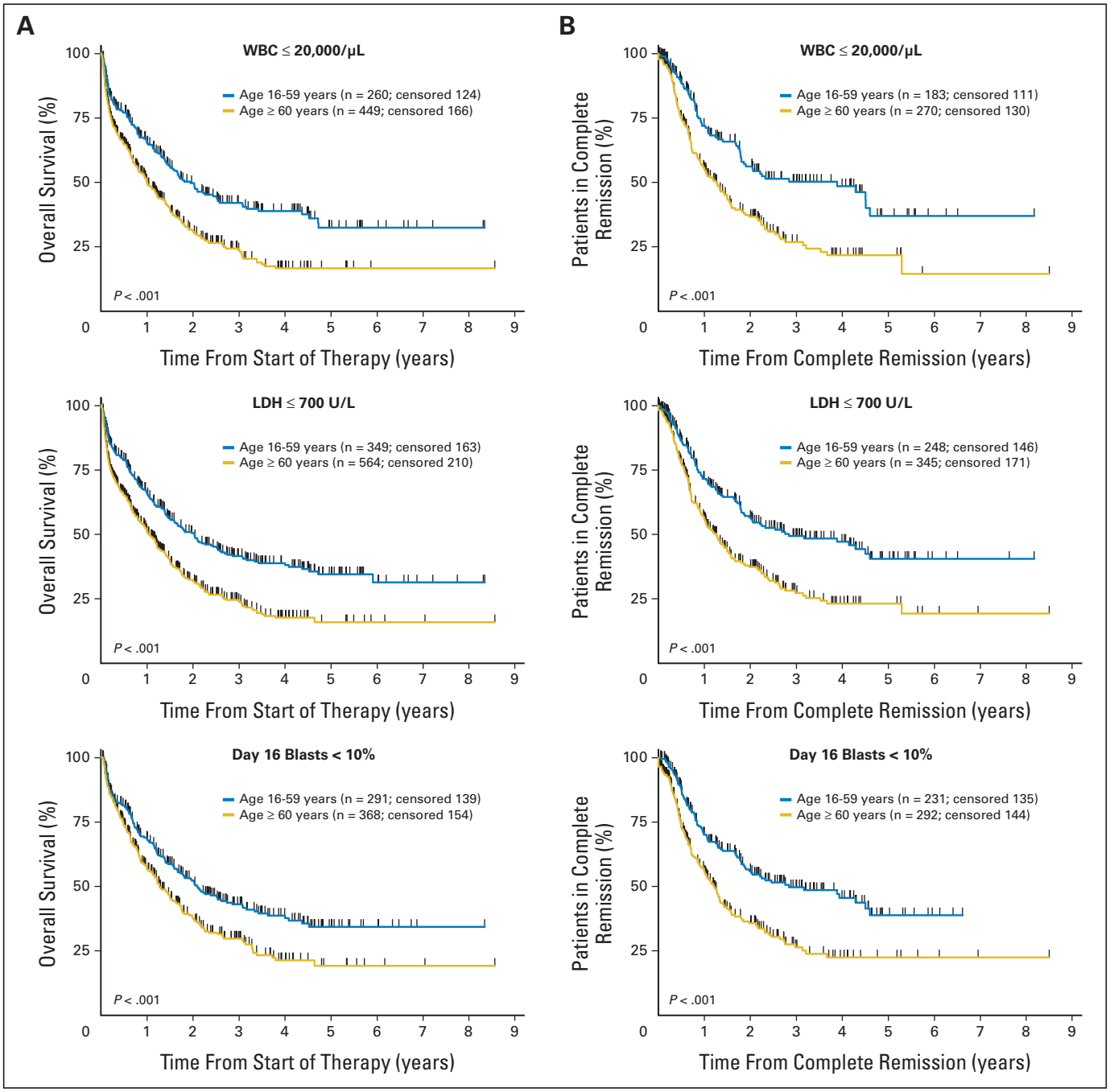

Fig 6. (A) Overall survival and (B) remission duration in younger (age 16 to 59 years) and older (age 60+ years) patients predicted by WBC ≤ 20,000/μL, serum lactate dehydrogenase (LDH) ≤ 700 U/L, and day 16 bone marrow blasts less than 10%.

been substantiated thus far. Moreover, the effect of high-dose cytarabine seems to not be affected by multidrug resistance.[27] Among other risk factors, morphologic dysplasia has not been confirmed as an independent factor in AML.[28,29] The mixed lineage leukemia gene partial tandem duplication was infrequent overall.[30] The frequent *FLT3* gene mutations occurred in 23% to 32% of patients,[12,16,31-33] who were rather younger.[12]

When comparing 1,612 patients younger than 55 years with an older population of 1,065 patients in two consecutive British trials,[34] favorable karyotypes were found in 24% versus 7% and unfavorable karyotypes in 10% versus 19%, respectively, and thus did not characterize the bulk of older patients. Even smaller differences of only 16% versus 7% favorable karyotypes and 18% versus 24% unfavorable karyotypes in younger and older patients, respectively, were found in the present analysis, which, unlike the British trials, divided the age groups at 60 years and excluded children. In five separate trials, two in younger and three in older patients, the Southwest Oncology Group treated 968 patients with primary and secondary AML by differing regimens. In four groups at increasing levels of age, an increasingly poor performance status, unfavorable cytogenetics, and deteriorating outcome within the cytogenetic groups were found.[6] In the context of age-related disease biology, an increased WBC count has commonly

been considered an adverse prognostic factor.[35-39] However, a lower WBC was found in older patients, and the authors assumed older age AML was a less proliferative disease.[6] Even restricted to primary AML, present analysis supports this hypothesis by showing significantly lower WBC as well as LDH in the older than in the younger patients.

Recently, cytoplasmic dislocation of nucleophosmin (NPM) with mutation of the *NPM1* gene has been described as being associated with normal karyotype and responsiveness to induction chemotherapy.[8] In the trials of two AML study groups, mutant NPM was detected in half of the patients with normal karyotype and frequently occurred together with *FLT3* length mutations. *NPM1* mutation significantly predicted for favorable overall survival and relapse-free survival if *FLT3-LM* was absent,[9,10] essentially confirmed by other groups.[40-43] In the two studies including patients younger and older than 60 years, no relation of *NPM1* mutation to age was described.[9,41] Among patients with normal karyotype in the present analysis, the favorable coexpression of mutant *NPM1* and normal *FLT3* was found at comparable frequencies (37% and 33%) in younger and older patients, respectively, and equally predicted for superior survival and remission duration.

Considering postremission treatment, others have shown no benefit from intermediate or high-dose chemotherapy in older as compared with younger patients.[5] Prolonged maintenance as the preferred postremission chemotherapy in the present study produced a relapse-free survival similar to that achieved with intensive consolidation in younger and older patients.[16]

In conclusion, as new findings in the present study restricted to homogeneous and comparable patient populations, the outcome in older (60+ years) patients is inferior to that of younger (16 to 59 years) patients equally across prognostic groups defined by cytogenetics, *NPM1/FLT3* mutation, WBC, LDH, and early blast clearance. Also, there is no dose response to two different intensive induction regimens in either age group. The difference in outcome is not explained by the modest differences in the defined risk profiles between older and younger patients. Recently described mutations in the *FLT3*, *NPM1*, *CEBPA*, and *MLL* genes and expression changes in the *BAALC* and *ERG* genes have not shown age-related differences.[44] Further gene expression profiling may elucidate the age effect in AML and detect new therapeutic targets.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

Conception and design: Thomas Büchner, Wolfgang E. Berdel, Claudia Haferlach, Torsten Haferlach, Carsten Müller-Tidow, Georg Maschmeyer, Eva Lengfelder, Maria-Cristina Sauerland, Bernhard Wörmann, Wolfgang Hiddemann
Administrative support: Thomas Büchner, Wolfgang E. Berdel
Provision of study materials or patients: Thomas Büchner, Wolfgang E. Berdel, Claudia Haferlach, Torsten Haferlach, Susanne Schnittger, Jan Braess, Karsten Spiekermann, Joachim Kienast, Peter Staib, Andreas Grüneisen, Wolfgang Kern, Albrecht Reichle, Carlo Aul, Eva Lengfelder, Bernhard Wörmann, Wolfgang Hiddemann
Collection and assembly of data: Thomas Büchner, Claudia Haferlach, Torsten Haferlach, Maria-Cristina Sauerland, Achim Heinecke
Data analysis and interpretation: Thomas Büchner, Wolfgang E. Berdel, Claudia Haferlach, Torsten Haferlach, Susanne Schnittger, Carsten Müller-Tidow, Jan Braess, Karsten Spiekermann, Joachim Kienast, Peter Staib, Andreas Grüneisen, Wolfgang Kern, Albrecht Reichle, Georg Maschmeyer, Carlo Aul, Eva Lengfelder, Maria-Cristina Sauerland, Achim Heinecke, Bernhard Wörmann, Wolfgang Hiddemann
Manuscript writing: Thomas Büchner, Wolfgang E. Berdel, Torsten Haferlach
Final approval of manuscript: Thomas Büchner, Wolfgang E. Berdel, Claudia Haferlach, Torsten Haferlach, Susanne Schnittger, Carsten Müller-Tidow, Jan Braess, Karsten Spiekermann, Joachim Kienast, Peter Staib, Andreas Grüneisen, Wolfgang Kern, Albrecht Reichle, Georg Maschmeyer, Carlo Aul, Eva Lengfelder, Maria-Cristina Sauerland, Achim Heinecke, Bernhard Wörmann, Wolfgang Hiddemann

REFERENCES

1. Ries LAG, Eisner MP, Kosary CL, et al: SEER Cancer Statistics Review, 1975-2000. Bethesda, MD, National Cancer Institute, 2003. http://seer.cancer.gov/csr/1975_2000/, 2003
2. Taeuber CM: Sixty-Five Plus in America (rev ed): Current Population Reports, Special Studies, P 23-178.RV. Washington, DC, United States Government Printing Office, 1993
3. Büchner T, Urbanitz D, Hiddemann W, et al: Intensified induction and consolidation with or without maintenance chemotherapy for acute myeloid leukemia (AML): Two multicenter studies of the German AML Cooperative Group. J Clin Oncol 3:1583-1589, 1985
4. Büchner T, Berdel WE, Schoch C, et al: Double induction containing two versus one course of high-dose cytarabine/mitoxantrone (HAM), and autologous stem cell transplantation versus prolonged maintenance for acute myeloid leukemia (AML). J Clin Oncol 24:2480-2489, 2006
5. Mayer RJ, Davis RG, Schiffer CA, et al: Intensive postremission chemotherapy in adults with acute myeloid leukemia. N Engl J Med 331:896-903, 1994
6. Appelbaum FR, Gundacher H, Head DR, et al: Age and acute myeloid leukemia. Blood 107:3481-3485, 2006
7. Hiddemann W, Kern W, Schoch C, et al: Management of acute myeloid leukemia in elderly patients. J Clin Oncol 17:3569-3576, 1999
8. Falini B, Mecucci C, Tiacci E, et al: Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med 352:254-266, 2005
9. Schnittger S, Schoch C, Kern W, et al: Nucleophosmin gene mutations are predictors of favorable prognosis in acute myeloegenous leukemia with a normal karyotype. Blood 106:3733-3739, 2005
10. Döhner K, Schlenk RF, Habdank M, et al: Mutant nucleophosmin UNPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: Interaction with other gene mutations. Blood 106:3740-3746, 2005
11. Mitelmann F (ed): An International System for Human Cytogenetic Nomenclature. Basel, Switzerland, Karger, 1995
12. Schnittger S, Schoch C, Dugas M, et al: Analysis of FLT3 length mutations in 1003 patients with acute myeloid leukemia: Correlation of cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. Blood 100:59-66, 2002
13. The International Non-Hodgkin's Lymphoma Prognostic Factors Project: A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med 329:987-994, 1993
14. The International Germ Cell Cancer Collaborative Group: International germ cell consensus classification: A prognostic factor-based staging system for metastatic germ cell cancers. J Clin Oncol 15:594-603, 1997
15. Büchner T, Hiddemann W, Wörmann B, et al: Double induction strategy for acute myeloid leukemia: The effect of high-dose cytarabine with mitoxantrone instead of standard-dose cytarabine with daunorubicin and 6-thioguanine—A randomized trial by the German AML Cooperative Group. Blood 93:4116-4124, 1999
16. Büchner T, Hiddemann W, Berdel WE, et al: 6-thioguanine, cytarabine, and daunorubicin (TAD) and high-dose cytarabine and mitoxantrone (HAM) for induction, TAD for consolidation, and either prolonged maintenance by reduced monthly TAD or TAD-HAM-TAD and one course of intensive consolidation by sequential HAM in adult patients at all ages with de novo acute myeloid leukemia (AML): A

randomized trial of the German AML Cooperative Group. J Clin Oncol 21:4496-4504, 2003

17. Kern W, Haferlach T, Schoch C, et al: Early blast clearance by remission induction therapy is major independent prognostic factor for both achievement of complete remission and long-term outcome in acute myeloid leukemia: Data from the German AML Cooperative Group (AMLCG) 1992 trial. Blood 101:64-70, 2003

18. Büchner T, Berdel WE, Hiddemann W: Priming with granulocyte colony-stimulating factor: Relation to high-dose cytarabine in acute myeloid leukemia. N Engl J Med 350:2215-2216, 2004

19. Cheson B, Bennett JM, Kopecky KJ, et al: Revised recommendations of the international working group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol 21:4642-4649, 2003

20. Hamblin T: The treatment of acute myeloid leukemia preceded by the myelodysplastic syndrome. Leuk Res 16:101-108, 1992

21. Hoyle CR, de Bastos M, Wheatley K, et al: AML associated with previous cytotoxic therapy, MDS or myeloproliferative disorders: Results form the MRC's 9th AML trial. Br J Haematol 72:45-53, 1989

22. Second International Workshop on Chromosomes in Leukemia. Cancer Genet Cytogenet 2:89-113, 1980

23. Rowley J: Annotation: Chromosome changes in acute leukaemia. Br J Haematol 44:339-346, 1980

24. Yunis JJ, Lobell M, Arnesen MA, et al: Refined chromosome study helps define prognostic subgroups in most patients with primary myelodysplastic syndrome and acute myelogenous leukaemia. Br J Haematol 68:189-194, 1988

25. Leith CP, Kopecky KJ, Chen I, et al: Frequency and clinical significance of the expression of the multidrug resistance proteins MDRS1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia: A Southwest Oncology Group study. Blood 94:1086-1099, 1999

26. Leith CP, Kopecky KJ, Godwin J, et al: Acute myeloid leukemia in the elderly: Assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy—A Southwest Oncology Group Study. Blood 89:3323-3329, 1997

27. Van den Heuvel-Eibri MM, van der Holt B, Te Boekhorst PAW, et al: MDR1 expression is an independent prognostic factor for response and survival in de novo acute myeloid leukemia. Br J Haematol 99:76-83, 1997

28. Haferlach T, Schoch C, Löffler H, et al: Morphologic dysplasia in de novo acute myeloid leukemia (AML) is related to unfavorable cytogenetics but has no independent prognostic relevance under the conditions of intensive induction therapy: Results of a multiparameter analysis from the German AML Cooperative Group studies. J Clin Oncol 21:256-265, 2003

29. Wandt H, Schäkel U, Kroschinsky F, et al: MLD according to the WHO classification in AML has no correlation with age and no independent prognostic relevance as analyzed in 1766 patients. Blood 111:1855-1861, 2008

30. Schnittger S, Kinkelin U, Schoch C, et al: Screening for MLL tandem duplication in 387 unselected patients with AML identify a prognostically unfavorable subset of AML. Leukemia 14:796-804, 2000

31. Kiyoi H, Naoe T, Nakano Y, et al: Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood 93:3074-3080, 1999

32. Thiede C, Steudel C, Mohr B, et al: Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: Association with FAB subtypes and identification of subgroups with poor prognosis. Blood 99:4326-4335, 2002

33. Fröhling S, Schlenk RF, Breitruck J, et al: Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: A study of the AML Study Group Ulm. Blood 100:4372-4380, 2002

34. Grimwade D, Walker H, Harrison G, et al: The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood 98:1312-1320, 2001

35. Stone RM, Berg DT, Stephen LG, et al: Postremission therapy in older patients with de novo acute myeloid leukemia: A randomized trial comparing mitoxantrone and intermediate-dose cytarabine with standard-dose cytarabine. Blood 98:548-553, 2001

36. Goldstone AH, Burnett AK, Wheatley K, et al: Attempts to improve treatment outcomes in acute myeloid leukemia (AML) in older patients: The results of the United Kingdom Medical Research Council AML11 trial. Blood 98:1302-1311, 2001

37. Löwenberg B, van Putten W, Theobald M, et al: Effect of priming with granulocyte colony-stimulating factor on the outcome of chemotherapy for acute myeloid leukemia. N Engl J Med 349:743-752, 2003

38. Wheatley K, Brookes CL, Hills RK, et al: Prognostic factors in older AML patients receiving intensive and non-intensive therapy: Analysis of the UK AML11 and AML14 trials. Blood 106:199a, 2005 (abstr 674)

39. Farag SS, Archer KJ, Mrozek K, et al: Pretreatment cytogenetics add to other prognostic factors predicting complete remission and long-term outcome in patients 60 years of age or older with acute myeloid leukemia: Results from Cancer and Leukemia Group B 8461. Blood 108:63-73, 2006

40. Verhaak RGW, Goudswaard CS, van Putten W, et al: Mutations in nucleophosmin (NPM1) in acute myeloid leukemia (AML): Association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. Blood 106:3747-3754, 2005

41. Thiede C, Koch S, Creutzig E, et al: Prevalence and prognostic impact of NPM1 in 1485 adult patients with acute myeloid leukemia (AML). Blood 107:4011-4020, 2006

42. Gale RE, Green C, Allen C, et al: The impact of FLT3 internal tandem duplicaton mutant level, number, size and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood 111:2776-2784, 2008

43. Scholl S, Theuer C, Scheble V, et al: Clinical impact of nucleophosmin mutation and Flt3 internal tandem duplications in patients older than 60 yr with acute myeloid leukemia. Eur J Haematol 80:208-215, 2008

44. Mrózek K, Marcucci G, Paschka P, et al: Clinical relevance of mutations and gene-expression changes in adult acute myeloid leukemia with normal cytogenetics: Are we ready for a prognostically prioritized molecular classification? Blood 109:431-448, 2007

Acknowledgment

We thank Birgit Mayerhoffer for secretarial assistance.

