Low-dose azacitidine, pioglitazone and all-*trans* retinoic acid is safe in patients aged ≥60 years with acute myeloid leukemia refractory to standard induction chemotherapy (AMLSG 26-16/AML-ViVA): results of the safety run-in phase

Patients with acute myeloid leukemia (AML) refractory to intensive induction therapy (primary induction failure) have an unfavorable outcome. In elderly patients not fit for further intensive salvage treatment due to age and comorbidities, effective treatment options are lacking.[1,2] The median overall survival (mOS) ranges from 1.6 to 3.1 months for non-intensive/palliative treatment.[3] In retrospective studies, response rates (complete remission [CR], CR with incomplete recovery of neutrophils or platelets [CRi]) for monotherapy with hypomethylating agents [HMA], i.e., 5-azacytidine [azacitidine] and 2-deoxy-5-azactidine [decitabine]) were low (up to 16%) in patients with relapsed/refractory (r/r) AML[4] indicating the high unmet medical need. During the last years, we have developed a biomodulatory treatment for AML with low-dose azacitidine, all-*trans* retinoic acid (ATRA) and pioglitazone, a peroxisome proliferator-activated receptor (PPAR) γ agonist with the aim to overcome treatment resistance, induce differentiation of leukemic blasts and reduce toxicity. Besides preclinical data, this approach was also supported by our clinical results on five chemorefractory AML patients, who developed CR associated with strong myeloid differentiation upon treatment with low-dose azacitidine, ATRA and pioglitazone.[5]

We here report the final analysis of the safety run-in part of the AMLSG 26-16/AML-ViVA trial (*clinicaltrials gov. Identifier: NCT02942758; EudraCT number 2016-000421-39*). The AMLSG 26-16/AML-ViVA trial is a multicenter, prospective, open-label, randomized phase II trial with dose-finding safety run-in phase in patients ≥60 years of age with AML refractory to at least one standard induction chemotherapy and not eligible for further intensive induction therapy based on medical reasons or not immediate candidates for allogeneic hematopoietic stem cell transplantation, respectively. Patients with acute promyelocytic leukemia (APL) were not eligible. All patients gave written informed consent in accordance with the Declaration of Helsinki. Approval was obtained from the ethics committees of all participating trial centers.

Between May 2017 and March 2020, ten patients were treated in the dose-finding safety run-in phase at five of 19 participating sites of the German-Austrian AML study group (AMLSG) in Germany in a modified dose de-escalation 3+3 design to evaluate the safety of the combination of azacitidine, ATRA, and pioglitazone. Patients were enrolled in dose level 0 at an ATRA dose of 45 mg/m²/day from day 1 to day 28 and 15 mg/m² thereafter (per os) in combination with subcutaneously administered azacitidine (75 mg fixed dose from day 1 to day 7 per 28-day treatment cycle) and pioglitazone (45 mg/day, per os, continuously starting at

Table 1. Baseline and disease characteristics of patients.

Variable	N=10
Sex, N (%)	
Male	7 (70)
Female	3 (30)
Age in years, median (range)	67 (60-76)
Type of AML, N (%)	
de novo AML	6 (60)
secondary AML	2 (20)
treatment-related AML	2 (20)
ECOG performance score, N (%)	
0	2 (20)
1	7 (70)
2	1 (10)
Cytogenetics at baseline, N (%)	
complex karyotype	9 (90)
del(9)	1 (10)
Molecular genetics (mutations) at baseline, N (%)	
none*	6 (60)
TP53	1 (10)
ASXL1, TP53	1 (10)
TP53, NF1	1 (10)
ASXL1, IDH2, SF3B1, U2AF1	1 (10)
ELN 2017 genetic risk category, N (%)	
adverse	10 (100)
N and type of pretreatment, N (%)	
1 cycle	8 (80)
cytarabine/daunorubicin	7 (70)
thioguanine/cytarabine/daunorubicin	1 (10)
2 cycles	2 (20)
cytarabine/daunorubicin; mitoxantrone/cytarabine	1 (10)
thioguanine/cytarabine/daunorubicin; mitoxantrone/cytarabine	1 (10)
Hemoglobin g/dL, median (range)	9.0 (6.5-10.6)
Platelets x10⁹/L, median (range)	50.5 (5-175)
White blood count x10⁹/L, median (range)	0.99 (0.38-4.8)
Neutrophils x10⁹/L, median (range)	0.2 (0-2.7)
Bone marrow blasts %, median (range)	56 (10-90)
Periperal blood blasts %, median (range)	0 (0-18)

*Analyzed in standard panel: *MLLT3/MLL, CEBPA, PML/RARA, RUNX1, FLT3-ITD, FLT3-TKD, NPM1*). AML: acute myeloid leukemia; ECOG: Eastern Cooperative Oncology Group; ELN: European LeukemiaNet.

day 1). Patients continued treatment as long as clinically appropriate, until AML progression or relapse. According to the study protocol, it was planned to proceed with a randomized (1:1 ratio) phase II part of the study to treat 76 patients with low-dose azacitidine, ATRA and pioglitazone or with standard-dose azacitidine. Due to a slow accrual rate, the study was prematurely terminated after completion of the safety run-in phase. All ten patients received at least one dose of the study treatment and are thus part of the safety population. Baseline clinical and disease characteristics are summarized in Table 1. Among the patient cohort, the median age was 67 years (range, 60-76 years), and 70% of patients had an Eastern Cooperative Oncology Group (ECOG) performance status of 1. Six patients had *de novo* AML, two patients had secondary AML, and another two patients had therapy-related AML (t-AML). Nine patients had a complex karyotype. All patients suffered from AML categorized as adverse risk according to European LeukemiaNet (ELN) recommendations of 2017.[1] Most patients exhibited severe cytopenia at baseline with a median neutrophil count of $0.2x10^9$/L (range, 0-2.7). Nine patients were analyzed for dose finding since one patient withdrew informed consent on day 9 of cycle 1.

After a median follow-up of 131 days, the mean treatment duration was 126 days (range, 27-426 days). Reported AE (grade 3 and 4) on a patient basis are listed in *Online Supplementary Table S1*. Seven of 13 reported serious adverse events (SAE) were infections, followed by only one-time occurrences of anemia, pancytopenia, gastric or urinary tract hemorrhage, fever, and panic attack. Four SAE were reported with fatal outcome. Since infections are an expected risk for patients undergoing treatment of AML, these events were unremarkable from a safety monitoring perspective. All infection SAE were assessed unrelated to study medication and instead related to the underlying disease. No DLT were reported throughout the safety run-in phase and it was not necessary to de-escalate the dose of ATRA. Three AE were rated definitely related to study drug, N=2 for azacitidine ("injection site reaction" and "platelet count decreased"); N=1 for ATRA "hyperkeratosis")]. Thirty-five AE were rated to be probably related to study drug. Overall, the safety profile was tolerable, and the observed toxicities were comparable in type and frequency to those described in other clinical trials with r/r AML patients.

OS as the primary objective of the phase II part was analyzed exploratory for the safety run-in population (*Online Supplementary Figure S1*). A total of seven patients died during treatment/follow-up. All deaths were either directly attributable to AML or attributable to SAE arising from the underlying AML. The mOS was 131 days, i.e., 4.3 months. All secondary endpoints were part of the randomized part of the trial, which was not performed. Patients received a median of two cycles of study treatment (range, 1-14)

Table 2. Treatment and best overall response.

Outcome	N=10
CR, N (%)	3 (30)
CRi, N (%)	0
HI-N, N (%)	2 (20)
PR, N (%)	1 (10)
SD, N (%)	4 (40)
PD, N (%)	6 (38)
NA	1 (10)*
Treatment cycles, median (range)	2 (1-14)
Reason for discontinuation, N (%)	
PD	7 (70)
allo-HSCT	2 (20)
patient wish	1 (10)

*Study treatment stopped on day 9 of cycle 1 due to patient's wish. CR: complete remission; CRi: complete remission with incomplete hematologic recovery; HI-N: hematologic improvement of neutrophils; PR: partial remission; SD: stable disease; PD: progressive disease; NA: not analyzed; allo-HSCT: allogeneic hematopoietic stem cell transplantation.

(Table 2). Three patients achieved a CR, one patient a PR and four a SD (Table 2). CR occurred fast after one (2 patients) or two (1 patient) treatment cycles. Two patients underwent allogeneic hematopoietic stem cell transplantation after the second cycle of treatment.

Interestingly, morphologic review of bone marrow smears showed signs of differentiation of AML blasts in responding patients (Figure 1). Besides the patients developing CR or PR, hematologic improvement of neutrophils (HI-N) was observed in two additional patients. In line with this, one patient demonstrated resolution of fungal pneumonia during the study (*Online Supplementary Figure S2*).

Primary induction failure still poses a major therapeutic challenge in AML. With the AMLSG 26-16/AML-ViVA trial we investigated a novel, low-intensity, biomodulatory regimen. Common grade 3/4 AE included hematologic cytopenias and infections. Since most patients were enrolled with severe baseline cytopenia, most likely due to refractory AML and high toxicity burden of previous induction therapy, many hematological AE were attributed to underlying disease or previous treatment. Piccini *et al*. recently reported in a retrospective study on venetoclax-based HMA combinations in r/r AML neutropenia grade ≥4 in 100%, febrile neutropenia in 45%, thrombocytopenia grade ≥4 in 95,7% and infections in 36% of patients. In this context, the AML-ViVA treatment seems to be less myelosuppressive.[6]

In our safety run-in population, we observed a median OS of 4.3 months. The overall response rate (ORR) was 40% (4/10) with three CR (30%) and one long-lasting PR in this elderly patient cohort consisting of exclusively ELN adverse risk AML. Among the three patients harboring mutations in *TP53*, two developed CR, one patient a PR. In general, in patients with r/r AML remissions are hardly achieved. A large international, retrospective study com-

prising 655 patients from 12 centers demonstrated a CR/CRi-rate of 16% for azacitidine or decitabine monotherapy with a median OS from the time of initiation of HMA of 6.7 months (95% confidence interval [CI]: 6.1-7.3).[4] Several retrospective studies and one prospective study on the use of venetoclax + HMA in r/r-AML described CR/CRi rates of 12-55% with median OS from 3.4 to 10.7 months.[6-8] These data suggest that the AML-ViVA treatment was at least not less effective than standard care in this elderly patient group, with the major limitation of a small patient cohort. In terms of HMA + ATRA combinations, decitabine + ATRA resulted in an improved ORR and survival compared

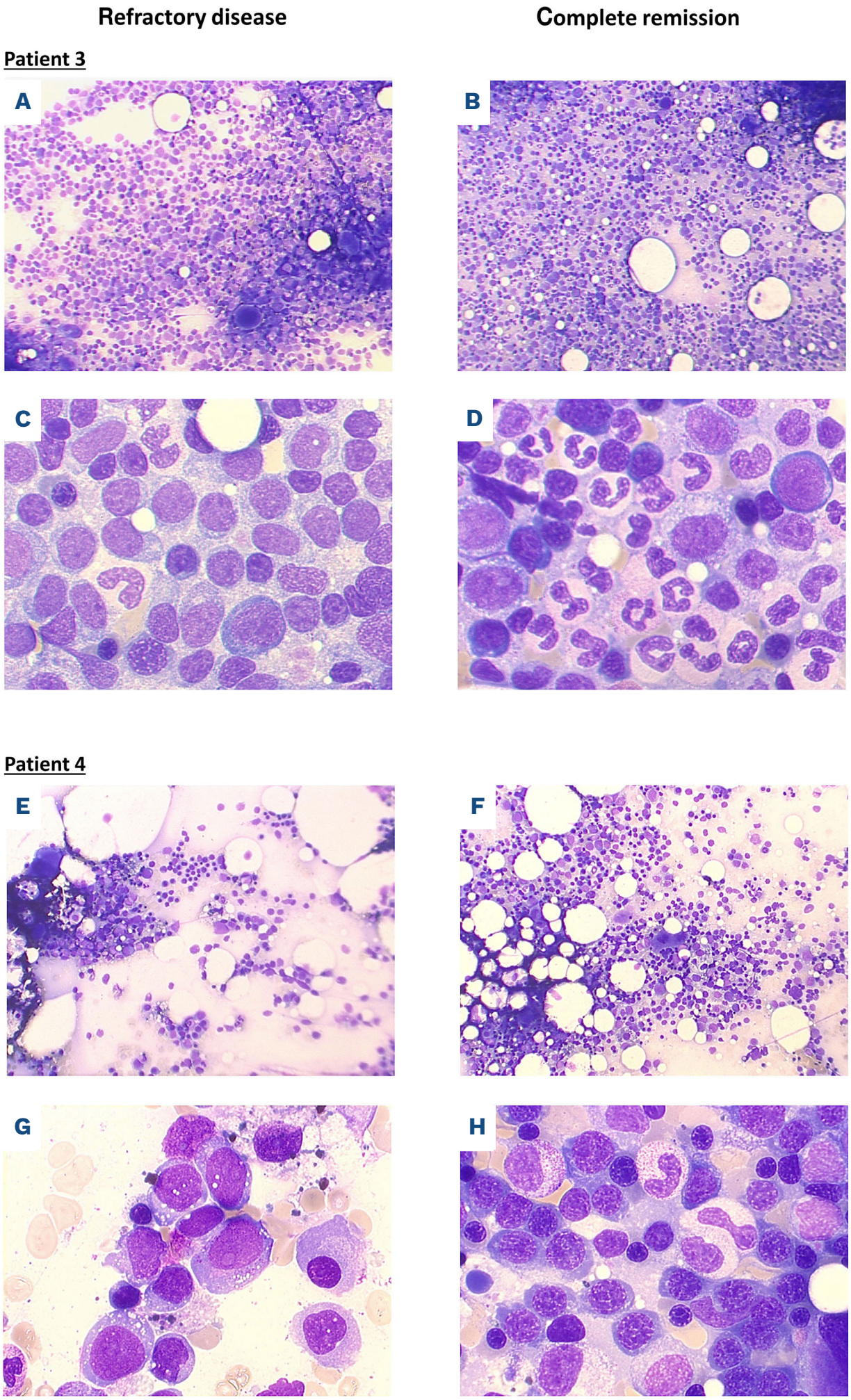

Figure 1. Bone marrow cytomorphology. Bone marrow smears of patient 3 (A-D) and patient 4 (E-H) were analyzed by light microscopy after May-Grünwald and Giemsa staining at the time point of study enrollment (refractory disease after 1 cycle of cytarabine/daunorubicin therapy each) (A, C, E, G) and at the time point of complete response after the first cycle of study treatment (B, D, F, H); magnification 100x (A, B, E, F) and 630x oil immersion (C, D, G, H).

to decitabine alone highlighting the potential efficacy of HMA + ATRA combinations.[9]

Interestingly, CR in our phase I study were achieved early after only one or two cycles. Responding patients showed improvement/normalization of cell counts. This clinical observation has already been made in a previously treated patient series of five patients and two other cases of refractory high-risk AML.[5,10,11] Thereby, we already showed that the early increase of neutrophils upon AML-ViVA treatment included a fraction of differentiated leukemic blasts still harboring leukemia-specific genetic alterations.[5] *In vitro* studies on primary AML blasts confirmed the differentiation promoting ability of AML-ViVA treatment. AML blast derived neutrophils were functionally capable of reactive oxygen species production and phagocytosis.[12] ATRA-induced differentiation has also been shown in another pilot trial using LSD1 inhibition combined with ATRA in r/r AML patients.[13] As neutropenic infections represent a major cause of mortality in AML patients, differentiation-inducing therapies are highly relevant as they diminish leukemic blasts along with improving immunity. With the underlying molecular mechanism of action being still mostly unclear, further preclinical investigations are needed to enhance its clinical activity.

We acknowledge the small sample size of ten patients as a major limitation of our study. Also considering the great heterogeneity of r/r AML the extrapolation of clinical efficacy in a greater population has to be done with great caution requiring larger clinical trials. Still, the AML-ViVA treatment was well-tolerated and yielded encouraging results in an elderly, adverse-risk patient population.

Authors

Daniel Heudobler,[1,2] Florian Lüke,[1,3] Joachim Hahn,[1] Matthias Grube,[1] Pavla Schlosser,[1] Stephan Kremers,[4] Thomas Südhoff,[5] Jörg Westermann,[6] Marie Luise Hütter-Krönke,[6,7] Richard F. Schlenk,[8,9] Daniela Weber,[7] Peter Paschka,[7] Florian Zeman,[10] Hartmut Döhner,[7] Wolfgang Herr,[1] Albrecht Reichle[1#] and Simone Thomas[1,11#]

[1]University Hospital Regensburg, Department of Internal Medicine III, Hematology and Oncology, Regensburg; [2]Bavarian Center for Cancer Research, University Hospital Regensburg, Regensburg; [3]Division of Personalized Tumor Therapy, Fraunhofer Institute for Toxicology and Experimental Medicine, Regensburg; [4]Caritaskrankenhaus Lebach, Department of Hematology/Oncology, Lebach; [5]Klinikum Passau, Department Hematology and Oncology, Passau; [6]Charité - University Medical Center Berlin - Department of Hematology, Oncology and Tumor Immunology, Campus Virchow-Klinikum, Berlin; [7]University Hospital Ulm, Department of Internal Medicine III, Ulm; [8]National Center of Tumor Diseases Trial Center, German Cancer Research Center and Heidelberg University Hospital, Heidelberg; [9]Heidelberg University Hospital, Department of Internal Medicine V, Heidelberg; [10]Center for Clinical Studies, University Hospital Regensburg, Regensburg and [11]Leibniz Institute for Immunotherapy, Division of Genetic Immunotherapy, Regensburg, Germany

[#]AR and ST contributed equally as senior authors.

Correspondence:
D. HEUDOBLER - daniel.heudobler@ukr.de

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Contributions

ST, AR, RFS, and HD developed the concept and design of the study and provided administrative support. ST and PS performed trial setup. DH, FL, JH, PS, MG, SK, TS, JW, JH, MHK, PP, HD, WH,

ST and AR treated the patients and collected the epidemiological and clinical data. DH, DW, FZ, ST and AR analyzed the data. DH, ST, and AR drafted the manuscript. All authors critically revised the final manuscript and are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data-sharing statement

The data supporting the conclusions of this article will be made available by the authors upon reasonable request.

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