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ORIGINAL ARTICLE

Evaluation of *IDH1*G105 polymorphism as prognostic marker in intermediate-risk AML

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Abstract Germline polymorphisms in genes mutated in acute myeloid leukemia (AML) may have prognostic impact. Therefore, the relevance of the polymorphism *IDH1*G105 (*IDH1*105^{GGT} minor allele) was evaluated in the context of concomitant molecular mutations in a cohort of 507 AML cases with intermediate-risk cytogenetics. In addition, a cohort of 475 healthy controls was analyzed for this polymorphism. *IDH1*105^{GGT} minor allele was found in 10 % of AML patients and 9 % of healthy controls. While no differences were seen with regard to cytomorphology or cytogenetics, immunophenotyping revealed significantly reduced expression of the progenitor marker CD34 in AML cases harboring *IDH1*105^{GGT} minor allele. Cases with *IDH1*105^{GGT} minor allele as compared to those with the *IDH1*105^{GGC} major allele had significantly longer event-free survival (EFS) (median 16 vs 11 months, p=0.013) which was most pronounced in the age group >60 years (median 14 vs 9 months, p=0.007) and in the *NPM1* mutated/*FLT3*-ITD/*FLT3*wt ratio <0.5 group (median 61 vs 13 months, p=0.012). However, this association is not independent of other prognostic parameters, and we conclude that *IDH1*105^{GGT} minor allele has to be considered in the context of the genetic background of the individual AML analyzed.

Keywords *IDH1* · *IDH2* · Polymorphism · AML · Prognosis

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Introduction

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder with regard to underlying cellular and molecular biology, acquired genetic lesions, and associated clinical responses to treatment. To date, recurrent cytogenetic aberrations provide the most important prognostic parameter [18, 42]. However, cytogenetically normal AML (CN-AML) comprises the largest subgroup with approximately 45 % of adult AML cases. These cases can be further characterized by different gene mutations including partial tandem duplication in the *MLL* gene [14, 39, 43], *FLT3* tyrosine kinase mutations (*FLT3*-TKD) [2, 44], internal tandem duplications in *FLT3* (*FLT3*-ITD) [40], *NPM1* mutations [15, 41, 46], and *CEBPA* mutations [16, 31, 32]. The latter three parameters are used in clinical practice and affect diagnosis, risk assessment, and also guidance of therapy. However, for the majority of patients, novel markers for risk stratification and new therapeutic approaches are still desirable.

One further potential marker is the mutational status of isocitrate dehydrogenase 1 (*IDH1*). IDH1, a citric acid cycle enzyme encoded by the *IDH1* gene, converts isocytrate to α-ketoglutarate in an $NADP^+$-dependent manner and is supposed to control redox status in cells. Missense mutations at the arginine 132 codon in exon 4 of *IDH1* have been shown to generate

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the putative oncogenic metabolite 2-hydroxyglutarate and may contribute to leukemogenesis via the induction of DNA hypermethylation. Acquired mutations of *IDH1* have been described with a frequency of approximately 5–10 % in AML and are associated with inferior event-free survival (EFS) and possible adverse overall survival (OS) [38]. IDH2 has the same enzymatic activity as IDH1 but is located in the mitochondrial matrix. In AML missense mutations at the arginin, codons 140 and 172 in exon 4 of *IDH2* have been reported [8, 29]. The newly acquired and distinct enzyme activity gained on mutation of IDH1 and IDH2 proteins provides an attractive and novel therapeutic target. A number of IDH inhibitors are in various stages of development and have been evaluated in preclinical studies [23].

In addition to acquired gene mutations, the impact of germline single nucleotide polymorphisms (SNPs) on diseases came into focus [9, 28, 34]. Effects on RNA stability, splicing, or messenger RNA (mRNA) folding have been suggested as potential mechanisms to explain how SNPs can affect biologic functions or drug sensitivity [35, 45]. Germline polymorphisms in *IDH1* exon 4 have been described, the most common of these being SNP rs11554137, representing a GGC to GGT transversion at the glycine residue 105 (minor allele *IDH1*105GGT). In adult AML patients, the minor allele *IDH1*105GGT is described to be present in about 11 % of cases and was found to be an adverse prognostic marker [21, 47].

We focused on the impact of the *IDH1*105GGT minor allele in a cohort of 507 AML patients with intermediate-risk karyotype and compared the incidence of *IDH1*105GGT minor allele to a cohort of 475 healthy controls.

Materials and methods

Patient cohorts

Bone marrow (n=429) or peripheral blood (n=78) samples from 507 AML patients with intermediate-risk karyotype defined according to MRC [17] were screened for *IDH1*105^{GGT} minor allele. All 507 patient samples were referred to our laboratory for first diagnosis of AML between August 2005 and July 2010. AML was diagnosed according to the FAB and WHO classifications [1, 5].

Two hundred patients were female and 283 male. Median age was 68 years (range 18–100 years). Bone marrow blast percentages ranged from 20 to 100 % (median 68 %) in 495 patients with non-M6 AML. Twelve patients with AML M6 subtype had blast percentages below 20 % (3–17 %, median 10 %), as characteristic for the AML M6 subtype. Data on other molecular markers was available in all 507 cases for *ASXL1*, *CEBPA*, *FLT3*-ITD, *FLT3*-TKD, *NPM1*, *IDH1*R132, *IDH2*R140, *IDH2*R172, *MLL*-PTD, *RUNX1*, and *WT1*. Data on *DNMT3A* was available in 412/507 cases. As it has been shown, only a *FLT3*-ITD/*FLT3*wt ratio ≥0.5 has a significant adverse prognostic impact [36]. *FLT3*-ITD negative patients and patients with *FLT3*-ITD/*FLT3*wt ratio <0.5 are combined and designated as *FLT3*-ITD/*FLT3*$wt^{ratio<0.5}$.

Patients (380/507) received intensive therapy based on different treatment schedules and were in part included into controlled trials of German study groups. Prior to therapy, all patients gave their informed consent for molecular genetic studies. The study design adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review board before its initiation.

Healthy controls

The controls for the present study were selected from The KORA (Cooperative Health Research in the Region of Augsburg, Germany) study, a series of population-based epidemiological surveys of participants living in or near the city of Augsburg, Southern Germany [22, 50]. The study sample was drawn from the F4 study (2006–2008), a follow-up study to the KORA Survey S4 (1999–2001), which comprises 3080 participants. Altogether, 475 individuals from KORA matched by age to the AML cohort were analyzed.

Cytomorphology, cytogenetics, immunophenotyping

Cytomorphologic assessment was based on May-Grünwald-Giemsa stains, myeloperoxidase reaction, and non-specific esterase using alpha-naphtyl-acetate as described before and was performed according to the criteria defined in the FAB and the WHO classifications [1, 4, 20]. Cytogenetic studies were performed after short-term culture. Karyotypes, analyzed after G-banding, were described according to the International System for Human Cytogenetic Nomenclature [24]. AML cases with intermediate-risk karyotypes were selected according to the refined MRC criteria [17]. Cytogenetic results were available for all patients in the study. Immunophenotyping was performed as described previously and was available in 263 cases [25].

Molecular genetic analysis

Isolation of mononuclear cells, DNA extraction, and mRNA extraction as well as random primed cDNA synthesis were performed as described previously [41]. Analyses for mutations in *ASXL1*, *NPM1*, *FLT3*-TKD, *RUNX1*, *WT1*, *IDH1*, *IDH2*, *CEBPA* as well as *MLL*-PTD and *FLT3*-ITD were described previously [2, 3, 13, 19, 27, 37–41].

Screening for *IDH1*105^{GGT} minor allele was performed using a LightCycler-based melting curve assay (Roche Diagnostics GmbH, Penzberg, Germany) in all 507

AML patients using the following primers: forward primer IDH1-F: GCTTGTGAGTGGATGGGTAA, reverse primer: IDH1-R: TATGTACCAGGTATGTCACCTT and hybridization probes IDH1-F anchor probe IDH1-FL: TTTTCCAGGCCCAGGAACAACAAAATCAGTT-FL and sensor probe IDH1-640: LCRed640-TCTGTATTGATCCCCATAAGCATGACGAC-p (Fig. 1a). Screening for *IDH1*105^{GGT} minor allele in the KORA control cohort was done by Sanger sequencing using the primers mentioned above (Fig. 1b). Sequencing data was analyzed using Mutation Surveyor Software Version 4.0.8 (Softgenetics, State College, Pennsylvania, USA).

Statistics

Survival curves were calculated for OS and EFS according to Kaplan-Meier and compared using the two-sided log-rank test. OS was the time from diagnosis to death or last follow-up. EFS was the time from diagnosis to treatment failure, relapse, death, or last follow-up in complete remission. Relapse was defined according to Cheson et al. [7]. Cox regression analysis was performed for OS and EFS with different parameters as covariates. Median follow-up was calculated taking into account the respective last observations in surviving cases and censoring non-surviving cases at the time of death. Parameters which were significant in univariate analyses were included into multivariate analyses. Dichotomous variables were compared between different groups using the χ^2 test and continuous variables by Student's *t* test. Results were considered significant at $p<0.05$. All reported *p* values are two-sided. No adjustments for multiple comparisons were performed. SPSS (version 19.0.1) software (IBM Corporation, Armonk, NY) was used for statistical analysis.

Results

Frequency of IDH1105^{GGT} in AML and healthy controls

The *IDH1*105^{GGT} minor allele was detected in 10 % (53/507) of AML and in 9 % (42/475) of the KORA control cohort. This slight difference (odds ratio of 1.18) does not reach statistical significance ($p=0.25$). Also, the frequency of a homozygous *IDH1*105^{GGT} minor allele was not different between the AML cohort (2/53, 4 %) and the KORA cohort (2/42, 5 %, n.s.).

Furthermore, 5/53 patients with *IDH1*105^{GGT} minor allele (9 %) had concomitant *IDH1*R132 mutation; 11/53 patients with *IDH1*105^{GGT} minor allele (21 %) had concomitant *IDH2* mutation.

Patient characteristics in relation to IDH1105^{GGT} minor allele

There was no association of *IDH1*105^{GGT} minor allele with AML FAB subtype, age, WBC, hemoglobin levels, or platelet count (Table 1). Immunophenotyping was done in 263 cases. Patients with *IDH1*105^{GGT} minor allele had significantly lower expression of CD34 (mean positive cells 16±20 vs 30± 30 %, $p=0.001$).

With regard to cytogenetics, there were no differences between patients with *IDH1*105^{GGT} minor allele and patients with *IDH1*105^{GGC} major allele (Table 1). Eighty one of 90 cases (90 %) harboring *IDH1*105^{GGT} minor allele had normal karyotype. Three patients (3 %) had trisomy 8, and the remaining six patients (7 %) had different other aberrations.

Furthermore, we did not detect any correlation of the *IDH1*105^{GGT} minor allele to any other mutation analyzed

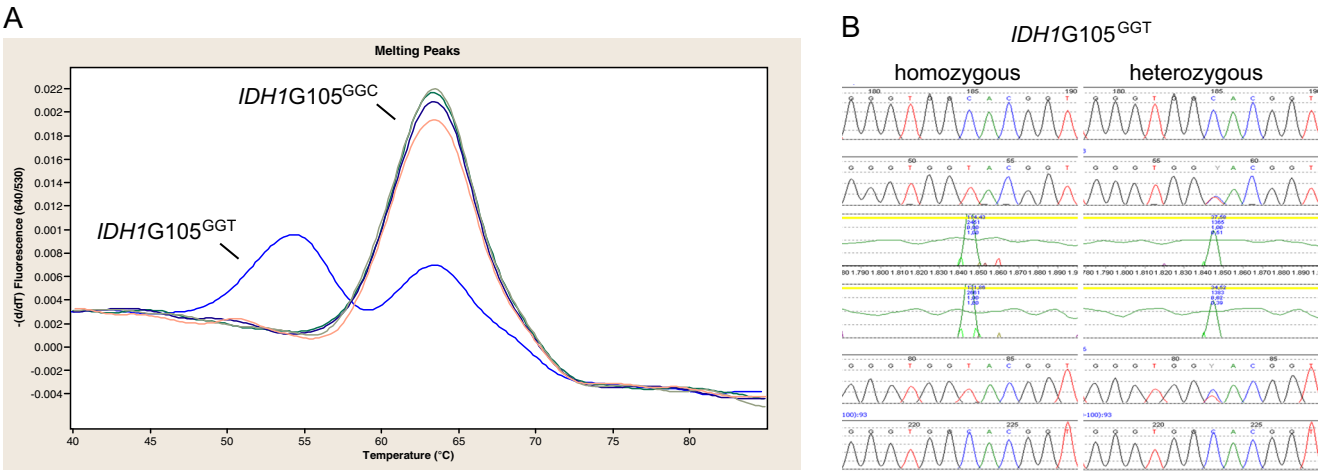


Fig. 1 **a** LightCycler®-based melting curve assay to identify *IDH1*105^{GGT} minor allele. **b** Sanger sequence analysis of *IDH1* as evaluated by Mutation Surveyor Software. *Left panel* shows homozygous base change C > T; *right panel* shows heterozygous base change C > T

Table 1 Patient demographics and clinical and molecular characteristics of AML patients according to *IDH1*G105 status

All patients	Total cohort n=507	*IDH1*105^{GGC} major allele n=454	*IDH1*105^{GGT} minor allele n=53	p
Sex				
Female	224 (44 %)	207 (46 %)	17 (32 %)	0.079
Male	283 (56 %)	247 (54 %)	36 (68 %)	
Age (years)				
Median	68	63	64	0.508
Range	18–100	18–100	26–88	
WBC count (×10^9/L)				
Median	24.9	24.4	29.4	0.755
Range	0.7–600	1.0–600	0.7–177	
Platelet count (×10^9/L)				
Median	64	64	64	0.325
Range	3.0–950	3.0–950	10.0–392	
Hemoglobin (g/dL)				
Median	9.2	9.2	9.5	0.613
Range	4.7–17.5	4.7–17.5	5.1–13.3	
FAB subtype				
M0	18 (4 %)	15 (3 %)	3 (6 %)	0.420
M1	158 (31 %)	142 (31 %)	16 (30 %)	1.000
M2	153 (30 %)	139 (31 %)	14 (26 %)	0.636
M4	133 (26 %)	116 (26 %)	17 (32 %)	0.323
M5	18 (4 %)	17 (4 %)	1 (2 %)	0.709
M6	17 (3 %)	16 (4 %)	1 (2 %)	1.000
nd	10 (2 %)	9 (2 %)	1 (2 %)	1.000
History of disease				
De novo AML	473 (93 %)	426 (95 %)	47 (89 %)	0.152
Secondary AML	25 (5 %)	20 (4 %)	5 (9 %)	0.167
Therapy-related AML	9 (2 %)	8 (0.4 %)	1 (2 %)	1.000
Cytogenetics [17]				
Intermediate-risk NK	447 (88 %)	399 (88 %)	48 (91 %)	0.822
Intermediate-risk AK	60 (12 %)	55 (12 %)	5 (9 %)	0.822

WBC white blood cells, *NK* normal karyotype, *AK* aberrant karyotype

(Table 2). When focusing the analysis to the >60-year-old and ≤60-year-old age groups, we also found no differences in composition or amount of concomitant mutations between patients with *IDH1*105^{GGT} minor allele and patients with *IDH1*105^{GGC} major allele. Restricting the cohort to intensively treated patients (n=380) revealed a mutual exclusiveness of *IDH1*105^{GGT} minor allele and *ASXL1* mutations (p=0.037) (data not shown).

Prognostic impact of IDH1105^{GGT} minor allele

Survival analysis was restricted to 380 patients having received intensive therapy. Cases with *IDH1*105^{GGT} minor allele had significantly longer EFS (median 16 vs 11 months, p=0.013) compared to those with the *IDH1*105^{GGC} major allele in the total cohort (Fig. 2a, b). When focusing the analysis to two separate age groups of >60-year-old and ≤60-year-old, the favorable impact on EFS was restricted to the age group >60 years (median 14 vs 9 months, p=0.007) (Fig. 2c, d). Regarding OS, significant differences in outcome were seen neither in the total cohort nor in the age-restricted analyses.

The favorable prognostic effect of the *IDH1*105^{GGT} minor allele was also detectable in the *NPM1* mutated/*FLT3*-ITD/*FLT3*wt$^{ratio<0.5}$ group with a median EFS of median 61 vs 13 months as compared to those with the *IDH1*105^{GGC} major allele (p=0.012) (Fig. 2e, f).

Further statistical analyses on prevalence and prognostic influence were performed with the combined mutation status of *IDH1* and *IDH2*, as both *IDH1* and *IDH2*

Table 2 Correlation of *IDH1*G105 status to molecular mutations

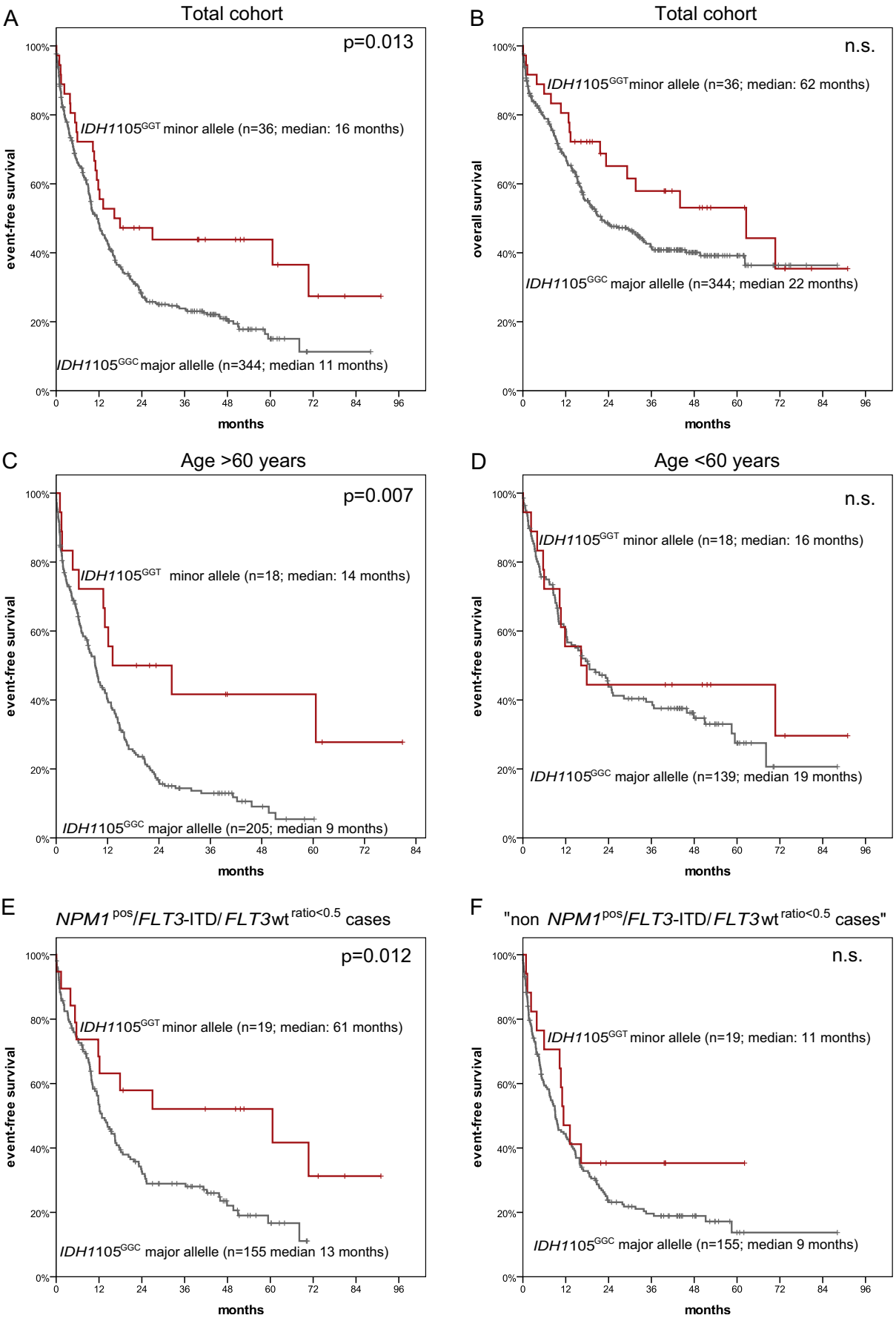
Mutation (*n*=cases analyzed)	*IDH1*105^{GGC} major allele, *n* (%)	*IDH1*105^{GGT} minor allele, *n* (%)	*p*
ASXL1 (*n*=507)			
wt	380 (84)	48 (90)	0.309
mut	74 (16)	5 (10)	
CEBPA (*n*=507)			
wt	409 (90)	50 (94)	0.456
mut	45 (10)	3 (6)	
DNMT3A (*n*=410)			
wt	216 (59)	27 (63)	0.438
mut	151 (41)	16 (37)	
FLT3-ITD (*n*=507)			
wt	396 (87)	43 (81)	0.207
mut	58 (13)	10 (19)	
FLT3-TKD (*n*=507)			
wt	405 (89)	49 (93)	0.636
mut	49 (11)	4 (7)	
NPM1 (*n*=507)			
wt	227 (50)	20 (38)	0.110
mut	227 (50)	33 (62)	
*IDH1*R132 (*n*=507)			
wt	406 (89)	48 (91)	0.626
mut	48 (11)	5 (9)	
*IDH2*R140 or *IDH2*R172 (*n*=507)			
wt	379 (83)	42 (79)	0.441
mut	75 (17)	11 (21)	
MLL-PTD (*n*=507)			
neg	420 (93)	53 (98)	0.240
pos	33 (7)	1 (2)	
RUNX1 (*n*=507)			
wt	381 (84)	42 (78)	0.311
mut	73 (16)	11 (22)	
WT1 (*n*=507)			
wt	424 (93)	51 (96)	0.560
mut	30 (7)	2 (4)	

wt wild type, *mut* mutated

mutants cause the loss of the physiologic enzyme function resulting in elevated 2-hydroxyglutarate levels [12, 49, 52]. No differences were found in outcome between patients harboring the *IDH1*105^{GGT} minor allele with and without an additional mutation in *IDH1* or *IDH2*. Also within patients with *IDH1*105^{GGC} major allele, no differences in outcome were detected between cases with mutated *IDH* and those without. When considering four groups according to the presence of the *IDH1*105^{GGT} minor allele and *IDH* mutations, the sole significant difference in outcome was detected between patients harboring the *IDH1*105^{GGT} minor allele and *IDH* wild type and patients with *IDH1*105^{GGC} major allele and additional *IDH1*/*2* mutation (Fig. 3a, b).

Univariate and multivariate analysis

In univariate Cox regression analysis of 380 intensively treated AML patients, the *IDH1*105^{GGT} minor allele (p=0.014) and *NPM1* mutations and *FLT3*-ITD/*FLT3*wt$^{ratio<0.5}$ were associated with better prognosis (p=0.024). Higher age (p<0.001), higher WBC count (p<0.001), *FLT3*-ITD/*FLT3*wt ratio ≥0.5 (p<0.001), *ASXL1* mutations (p<0.001), *DNMT3A* mutations (p=0.023), *RUNX1* mutations (p<0.001), and *WT1* mutations (p=0.042) were associated with worse EFS. In multivariate analysis, age (p<0.001), WBC count (p<0.001), and *ASXL1* mutations (p=0.004) had independent relevance for EFS. Investigating OS in univariate analysis, *NPM1* mutations were associated with better


A
Total cohort
p=0.013
IDH1105GGT minor allele (n=36; median: 16 months)
IDH1105GGC major allele (n=344; median 11 months)
event-free survival
months
B
Total cohort
n.s.
IDH1105GGT minor allele (n=36; median: 62 months)
IDH1105GGC major allele (n=344; median 22 months)
overall survival
months
C
Age >60 years
p=0.007
IDH1105GGT minor allele (n=18; median: 14 months)
IDH1105GGC major allele (n=205; median 9 months)
event-free survival
months
D
Age <60 years
n.s.
IDH1105GGT minor allele (n=18; median: 16 months)
IDH1105GGC major allele (n=139; median 19 months)
event-free survival
months
E
NPM1pos/FLT3-ITD/FLT3wtratio<0.5 cases
p=0.012
IDH1105GGT minor allele (n=19; median: 61 months)
IDH1105GGC major allele (n=155 median 13 months)
event-free survival
months
F
"non NPM1pos/FLT3-ITD/FLT3wtratio<0.5 cases"
n.s.
IDH1105GGT minor allele (n=19; median: 11 months)
IDH1105GGC major allele (n=155; median 9 months)
event-free survival
months

◀ **Fig. 2** Kaplan-Meier survival analysis according to *IDH1*105 allele status. *IDH1*105^{GGC} major allele (*gray*) compared to *IDH1*105^{GGT} minor allele (*red*). **a** Event-free and **b** overall survival analyzed in the total cohort of 479 patients. **c** Event-free survival in patients >60 years of age and **d** ≤60 years; **e** event-free and **f** overall survival in the subgroup with *NPM1* mutated/*FLT3*-ITD/*FLT3*wt$^{ratio<0.5}$

prognosis ($p<0.001$), whereas higher age ($p<0.001$), higher WBC count ($p<0.001$), *FLT3*-ITD ratio ≥0.5 ($p<0.001$), *ASXL1* mutations ($p<0.001$), *DNMT3A* mutations ($p=0.026$), *MLL*-PTD ($p=0.004$), and *RUNX1* mutations ($p=0.003$) were associated with inferior outcome. In multivariate analysis, age ($p<0.001$), WBC count ($p=0.001$), and *ASXL1* mutations ($p=0.023$) had independent prognostic impact (Table 3).

Discussion

In this study, we analyzed the *IDH1*105^{GGT} minor allele located in the 5′ region of the same exon as the *IDH1*R132 mutation in a large cohort of 507 patients with intermediate-risk karyotype AML in the context of other known prognostic markers. The *IDH1*105^{GGT} minor allele was detected in 10 % of AML patients. Furthermore, we analyzed a large healthy control cohort of 475 individuals. *IDH1*105^{GGT} minor allele was detected at approximately the same frequency (9 %), which is in line with previously published data [47].

Ho et al. [21] analyzed frequency and prognostic impact of *IDH1*105^{GGT} minor allele in a cohort of 274 adult de novo AML patients not selected for cytogenetics. They report the majority of patients with normal karyotype harboring *IDH1*105^{GGT} minor allele to have unfavorable risk features according to *FLT3*-ITD status. We were not able to confirm this, as we saw no differences in frequency of *FLT3*-ITD within the intermediate-risk cytogenetics group between *IDH1*105^{GGT} minor allele and *IDH1*105^{GGC} major allele cases (Tables 1 and 2) or in the cytogenetically normal subgroup of cases (data not shown). Furthermore, in our cohort, *IDH1*105^{GGT} minor allele and *IDH1*R132 mutation are not mutually exclusive as described by Ho et al. as we detected five cases (10 %) with coincident *IDH1*105^{GGT} minor allele and *IDH1*R132 mutation. We also were not able to confirm the mutually exclusiveness of *IDH1*105^{GGT} minor allele and

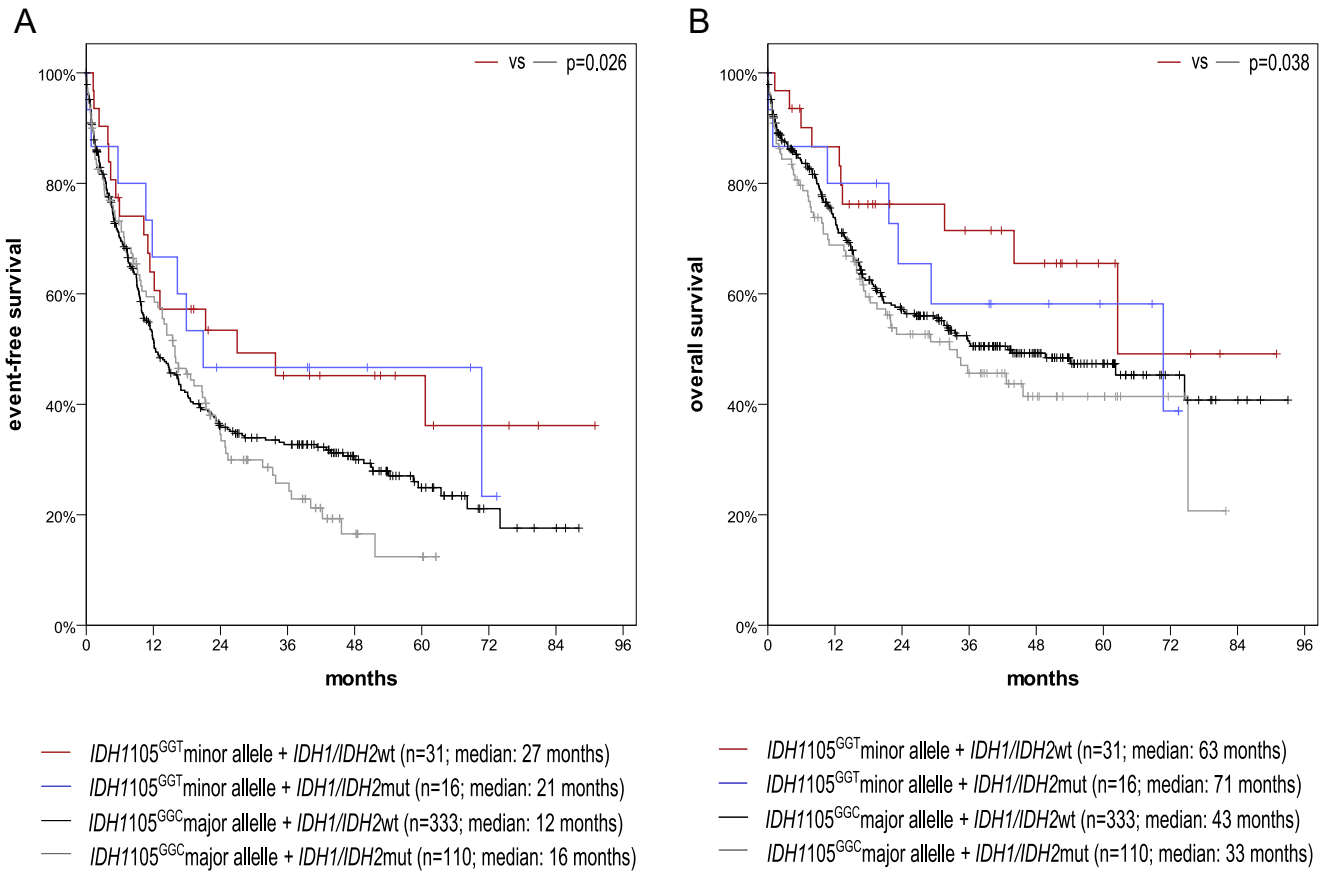


Fig. 3 Kaplan-Meier survival analysis according to *IDH1* and *IDH2* mutation status. Survival within the total cohort of 380 intensively treated patients. Kaplan-Meier plot showing **a** overall and **b** event-free survival of *IDH110*^{5GGT} minor allele + *IDH1*/*IDH2*wt (*red*) compared to *IDH1*105^{GGT} minor allele + *IDH1*/*IDH2*mut (*blue*), *IDH1*105^{GGC} major allele + *IDH1*/*IDH2*wt (*black*), and *IDH1*105^{GGC} major allele+*IDH1*/*IDH2*mut (*grey*)

Table 3 Influence of different biological and leukemia-associated parameters on OS and EFS in 380 AML patients in univariate and multivariate analysis

Parameter	EFS univariate		EFS multivariate		OS univariate		OS multivariate	
	p	RR	*p*	RR	*p*	RR	*p*	RR
Age*	<0.001	1.292	<0.001	1.301	<0.001	1.387	<0.001	1.374
WBC count#	<0.001	1.075	<0.001	1.079	<0.001	1.069	<0.001	1.089
*IDH1*G105	0.014	0.575	NS	–	NS	–	NS	–
*IDH1*R132	NS	0.954	–	–	NS	1.020	–	–
*IDH2*R140	NS	0.915	–	–	NS	0.988	–	–
*IDH2*R172	NS	0.892	–	–	NS	0.948	–	–
NPM1^{+}/*FLT3*-ITD/*FLT3*wt$^{\text{ratio<0.5}}$	0.024	0.761	NS	–	<0.001	0.600	NS	–
FLT3-ITD/*FLT3*wt$^{\text{ratio ≥0.5}}$	0.002	1.654	NS	–	<0.001	2.048	NS	–
ASXL1	<0.001	1.946	0.004	2.351	<0.001	2.406	0.023	2.077
DNMT3A	0.023	1.340	NS	–	0.026	1.402	NS	–
MLL-PTD	NS	–	NS	–	0.004	1.974	NS	–
RUNX1	0.018	1.457	NS	–	<0.001	1.884	NS	–
WT1	0.042	1.502	0.044	1.773	NS	–	NS	–

Age and peripheral blood cell counts were considered as continuous parameters

EFS event-free survival, *NS* not significant, *OS* overall survival, *RR* relative risk

*Per 10 years of increase, # per 10×10^{9}/L

CEBPA mutations as described by Wagner et al. [47], as we found coincident *IDH1*105^{GGT} minor allele and *CEBPA* mutation in three cases (6 %). Overall, we observed a random distribution of the *IDH1*105^{GGT} minor allele independent of all genetic subgroups suggesting that there is no preponderance for carriers to acquire any specific alteration. This corresponds with data published for *WT1* SNP rs16754 indicating a similar distribution of the SNP in molecular genetic subgroups of CN-AML [10].

Wagner et al. [47] reported the *IDH1*105^{GGT} minor allele to be a negative prognostic factor in a cohort of 275 adult CN-AML. We were not able to confirm these data. Even when restricting survival analysis to our CN-AML patients (n= 337), no negative prognostic impact of *IDH1*105^{GGT} minor allele was seen (data not shown). The cohort of Wagner et al. is limited to a maximum of 60 years of age, and thus, mean age is 10 years younger than in our cohort. In our cohort, neither a negative nor a positive prognostic impact was detectable in CN-AML patients ≤60 years of age (n=139). However, we detected a favorable impact of *IDH1*105^{GGT} minor allele on EFS in patients >60 years of age. Since we detected a mutual exclusiveness of *IDH1*105^{GGT} minor allele and *ASXL1* mutations in the subcohort of intensively treated patients, it is likely that the better prognosis observed in patients with *IDH1*105^{GGT} minor allele compared to cases with *IDH1*105^{GGC} major allele is not caused by the polymorphism itself but rather by the absence of *ASXL1* mutations. The lacking prognostic effect of minor allele *IDH1*105GGT per se is supported by two studies. Ravandi et al. [33] found no association with achievement of complete response (CR), remission duration, EFS and OS, and *IDH*1 SNP in a cohort of 170 de novo AML patients. Damm et al. [11] investigated a cohort of 460 pediatric AML cases and observed no effect of *IDH1*105^{GGT} minor allele on EFS or OS.

Several studies reported on effects of synonymous variants. Kimchi-Sarfaty et al. [26] found that synonymous SNPs alter the interaction of the ABC transporter ABCB1 with its substrates and inhibitors. Capon et al. [6] showed that a synonymous SNP in the corneodesmosin gene leads to increased mRNA stability. A study of Nackley et al. [30] gave evidence for synonymous SNPs being capable of affecting protein expression by alteration of mRNA stability. *IDH1*105^{GGT} minor allele is reported to cause elevated levels of the oncometabolite 2-hydroxyglutarate (2-HG) in the plasma, similar to somatic mutations in *IDH1* and *IDH2* [51]. However, levels of cellular 2-HG production depend on subcellular localization of *IDH1* and *IDH2* proteins. Ward et al. [48] demonstrated that mutations in *IDH1*, which is located in the cytosol, result in less cellular 2-HG accumulation compared to mutations in *IDH2*, which is located in the mitochondria. Moreover, the extent of 2-HG production from mitochondrial *IDH2* mutations depends on the particular site that is mutated. *IDH2*R140 mutations result in less cellular 2-HG accumulation than *IDH2*R172 mutations, which also correlates with the weaker impact of *IDH2*R140 mutations regarding impairment of cell differentiation relative to *IDH2*R172 mutations. Furthermore, mutations in cytosolic *IDH1*R132, structurally analogous to mutations in mitochondrial

*IDH2*R172, do not produce as much 2-HG when overexpressed in cells at comparable levels. Finally, also subcellular compartmentalization of metabolic flux can affect the ability of *IDH* mutations to result in cellular 2-HG accumulation. As Wiseman et al. [51] demonstrated, *IDH1*105^{GGT} minor allele also results in only moderate elevation of cellular 2-HG compared to the somatic mutations *IDH1*R132, *IDH2*R140, and *IDH2*R172, which also might lead to only a moderate impairment of cell differentiation. The significantly reduced expression of the progenitor marker CD34 in cases harboring *IDH1*105^{GGT} minor allele in the present series may also be considered in line with a weaker impact of *the SNP* regarding impairment of cell differentiation. However, this finding was not reflected in blast count or cell morphology.

In conclusion, we detected the *IDH1*105^{GGT} minor allele in intermediate-risk AML at the same frequency as in healthy controls. However, studies on clinical relevance of the *IDH1*105^{GGT} minor allele are controversial. We demonstrate the *IDH1*105^{GGT} minor allele to be associated with favorable prognosis in intensively treated AML patients with intermediate-risk karyotype in patients >60 years of age. However, this association is not independent on other prognostic parameters. The consideration of our data in relation to already published studies implies that *IDH1*105^{GGT} minor allele per se has no independent prognostic relevance but has to be considered in the context of the genetic background of the individual AML analyzed.

Conflict of interest CH, WK, TH, and SuS have equity ownership of MLL Munich Leukemia Laboratory GmbH. AF, TA, and CE are employed by MLL Munich Leukemia Laboratory GmbH.

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