Mutations in the isocitrate dehydrogenase 2 gene and IDH1 SNP 105C > T have a prognostic value in acute myeloid leukemia

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Abstract

Background: The isocitrate dehydrogenase (IDH1/IDH2) genes are metabolic enzymes, which are frequently mutated in acute myeloid leukemia (AML). The enzymes acquire neomorphic enzymatic activity when they mutated.

Methods: We have investigated the frequency and outcome of the acquired IDH1/IDH2 mutations and the IDH1 SNP 105C > T (rs11554137) in 189 unselected de novo AML patients by polymerase chain reaction amplification followed by direct sequencing. The survival are presented in Kaplan Meier curves with log rank test. Multivariable survival analysis was conducted using Cox regression method, taking age, risk group, treatment, IDH1/2 mutations and IDH1 SNP105 genotype into account.

Results: Overall, IDH1/2 mutations were found in 41/187 (21.7%) of the AML patients. IDH1 codon 132 mutations were present in 7.9%, whereas IDH2 mutations were more frequent and mutations were identified in codon 140 and 172 in a frequency of 11.1% and 2.6%, respectively. The SNP 105C > T was present in 10.5% of the patients, similar to the normal population. A significantly reduced overall survival (OS) for patients carrying IDH2 codon 140 mutation compared with patients carrying wild-type IDH2 gene (p < 0.001) was observed in the intermediate risk patient group. Neither in the entire patient group nor subdivided in different risk groups, IDH1 mutations had any significance on OS compared to the wild-type IDH1 patients. A significant difference in OS between the heterozygous SNP variant and the homozygous wild-type was observed in the intermediate risk FLT3 negative AML patients (p = 0.004).

Conclusions: Our results indicate that AML-patients with IDH2 mutations or the IDH1 SNP 105C > T variant can represent a new subgroup for risk stratification and may indicate new treatment options.

Keywords: AML, IDH1, IDH2, SNP, Prognostic markers

Background

Acute myeloid leukemia (AML) is a hematological malignancy caused by acquired genetic alterations in genes affecting the normal proliferation and terminal differentiation of myeloid progenitor cells. Based on cytogenetic abnormalities, cases of AML are usually classified into three groups, with favorable, intermediate and adverse prognosis [1]. The largest group is the intermediate risk group in which patients with cytogenetically normal karyotype (CN-AML) constitute about 45% of de novo AML [2,3]. These patients form a heterogeneous group where some achieve complete remission and become long term survivors, while others rapidly relapse, often with a more aggressive or resistant disease. The overall 5-year survival is 35-40%, but less than 15% in AML patients above the age of 60 [4]. During the last decades, several new mutations with prognostic impact have been identified in AML. These include internal tandem duplications (ITDs) in the fms-like tyrosine kinase 3 (FLT3)
gene, conferring an adverse prognosis, and nucleophosmine 1 (NPM1) gene mutations, which in the absence of FLT3-ITD confer a favorable prognosis [5-8]. Both these genes have become clinically established prognostic markers in CN-AML. However, there is still a large group of intermediate risk patients without FLT3-ITD/NPM1 mutations or other reliable prognostic markers, highlighting the need for additional markers that could explain the differential outcome in this heterogeneous patient group.

Genome-wide analysis in patients with AML have identified further genetic markers, thus extending the possibilities for more accurate prognostic distinctions between subgroups, and might aid the clinicians in treatment decisions such as choice of chemotherapy regime or early stem cell transplantation (SCT).

The isocitrate dehydrogenase (IDH) 1 and 2 genes were identified to be mutated in AML [9]. The IDH family consists of three isoforms, IDH1, IDH2 and IDH3 where IDH1 is located in the cytosol, while IDH2 and IDH3 are located in the mitochondrion and are normally involved in citrate metabolism in the tricarboxylic acid cycle [10]. The IDH1 and IDH2 enzymes are encoded by the IDH1 gene at chromosome 2q33 and the IDH2 gene resides at chromosome 15q26. The enzymes are NADP⁺-dependent to catalyze isocitrate oxidation to α-ketoglutarate (α-KG) and the cofactor NADPH is generated. Mutations in the IDH1 genes were first identified in malignant gliomas [11,12] and subsequently IDH1 mutations were frequently found in AML [9] and later also recurrent IDH2 mutations were found in AML [13-15]. No mutations have been reported in the IDH3 gene. IDH1/2 mutations are usually heterozygous with one wild-type allele and one mutant allele, affecting the arginine at codon 132 in exon 4 in the IDH1 gene, codon 140 and codon 172 in exon 4 in the IDH2 gene. The mutants acquire neomorphic enzymatic activity by converting α-KG to 2-hydroxyglutarate (2-HG) [16,17]. Studies have shown that IDH1/2 mutations are associated with epigenetic alterations, by inhibiting the function of TET2, a DNA demethylase enzyme which is dependent on α-KG and essential for DNA demethylation. Mutations in the IDH1 or IDH2 genes favour 2-HG production and decrease the amount of α-KG, resulting in a hypermethylation phenotype and impaired hematopoietic differentiation [18,19]. Further, a synonymous single nucleotide polymorphism (SNP) (rs11554137) located in codon 105 in exon 4 in the IDH1 gene, was recently reported to be of prognostic value in both adult and paediatric AML patients [20,21].

In this study we aimed to investigate the frequency of the acquired IDH1 and IDH2 mutations and the SNP 105C>T (rs11554137) located in the IDH1 gene and correlate the different genotypes to the outcome in AML patients.

Results

**IDH1 and IDH2 mutation analysis**

All patients were successfully genotyped for IDH1 codon 132 mutations, IDH2 codon 140 and codon 172 mutations, and for the IDH1 codon 105 SNP (rs11554137) (Table 1). Mutational data distributions in the entire cohort and in patient subgroups are presented in Table 2. In total, IDH1/2 mutations were found in 41/189 (21.7%) of the AML patients. Fifteen patients (7.9%) had mutations in codon 132 in the IDH1 gene resulting in four different amino acid exchanges, arg > his (7/15), arg > cys (6/15), arg > leu (1/15) and arg > gly (1/15). IDH2 mutations were found in exon four at codon 140 in 21 (11.1%) of the patients and at codon 172 in 5 (2.6%) of the patients. For IDH2 codon 140 mutations, two amino acid exchanges were detected: arg > gln (20/21) and arg > gly (1/21). For IDH2 codon 172 mutations all were arg > lys exchanges (5/5). Mutations in the IDH1 gene were mutually exclusive with mutations in the IDH2 gene (Table 1).

No significant differences between IDH genotype groups in terms of median age at diagnosis, gender, treatment regime, or distribution of FLT3/NPM1 mutations were found in the patient cohort. However, the median age at diagnosis appear to be higher in patients with mutated IDH gene (IDH1 or IDH2) than in patients with wild-type IDH gene (69 vs. 62 years, respectively, p = 0.036, Table 2).

**Impact of IDH1 and IDH2 mutations on treatment response and overall survival**

We found no significant difference on OS for patients with IDH1 codon 132 mutations, neither in the entire group nor when stratified in different risk groups.

Mutations in the IDH2 gene codon 140 revealed a significant increased risk for shorter OS in the whole patient group in relation to the wild type IDH2 codon 140, (HR = 1.94; 1.07-3.53; 95% confidence interval, p = 0.03) (Cox regression Table 3; 15 patients with missing karyotype data where excluded from the analysis). This was most pronounced among the intermediate risk group

| Gene | Nucleotide change | Amino acid change | Number of patients |
|------|------------------|-------------------|--------------------|
| IDH1 | CGT > TGT        | R132C             | 7                  |
| IDH1 | CGT > CAT        | R132H             | 6                  |
| IDH1 | CGT > GGT        | R132G             | 1                  |
| IDH1 | CGT > CTT        | R132L             | 1                  |
| IDH2 | CGG > CAG        | R140Q             | 20                 |
| IDH2 | CGG > GGG        | R140G             | 1                  |
| IDH2 | AGG > AAG        | R172K             | 5                  |
| IDH1 | GGC > GGT        | G105G             | 20                 |
patients with a median OS 6 vs. 18 months, for mutated and wild type patients, respectively, p = 0.001, (Figure 1A; entire cohort presented in Figure 1B). Patients with \textit{IDH2} codon 172 mutations showed an improved survival in the entire patient group compared to patients with wild type \textit{IDH2} codon 172 in cox regression analysis (HR = 0.22; 0.07-0.74; 95% confidence interval, p = 0.014) (Table 3) and Kaplan Meier analysis, (p = 0.09, Figure 2).

### Table 2 Patient characteristics and distributions of \textit{IDH} mutations for all patients and within groups

| CHARACTERISTIC | All | \textit{IDH1} codon 132: | \textit{IDH1} codon 105 (synonymous SNP): | \textit{IDH2} codon 140: | \textit{IDH2} codon 172: |
|---------------|-----|-------------------------|------------------------------------------|-------------------------|-------------------------|
|               | Total n = 189 | Wild type | Mutation | Wild type | Variant | Wild type | Mutation | Wild type | Mutation |
| Age median (range), years | | 64 (19–88) | 63 (19–85) | 70 (30–88)* | 64 (19–88) | 66 (29–84) | 63 (19–88) | 66 (37–83)* | 64 (19–88) | 72 (46–74)* |
| Gender | | | | | | | | | | |
| Male | 95 | 90 | 5 | 85 | 10 | 85 | 10 | 92 | 3 |
| Female | 94 | 84 | 10 | 84 | 10 | 83 | 11 | 92 | 2 |
| Karyotype | | | | | | | | | |
| Normal | 108 | 99 | 9 | 95 | 13 | 96 | 12 | 106 | 2 |
| Aberrant | 75 | 69 | 6 | 68 | 7 | 66 | 9 | 72 | 3 |
| Missing data | 6 | 6 | | 6 | | 6 | | 6 | |
| Risk group | | | | | | | | | |
| Low | 32 | 31 | 1 | 30 | 2 | 29 | 3 | 31 | 1 |
| Intermediate | 87 | 80 | 7 | 72 | 15 | 75 | 12 | 86 | 1 |
| High | 55 | 51 | 4 | 52 | 3 | 51 | 4 | 52 | 3 |
| Missing data | 15 | 12 | 3 | 15 | 13 | 2 | 14 | 1 |
| FLT3 status | | | | | | | | | |
| FLT3 wild type | 116 | 109 | 7 | 104 | 12 | 101 | 15 | 114 | 2 |
| FLT3-ITD | 37 | 34 | 3 | 32 | 5 | 33 | 4 | 36 | 1 |
| NPM1 status | | | | | | | | | |
| NPM1 wild type | 99 | 92 | 7 | 87 | 12 | 88 | 11 | 96 | 3 |
| NPM1 mutation | 52 | 49 | 3 | 47 | 5 | 44 | 8 | 52 | 0 |
| Missing data** | 38 | 33 | 5 | 35 | 3 | 36 | 2 | 36 | 2 |
| Induction treatment response | | | | | | | | | |
| Complete remission | 132 | 122 | 10 | 119 | 13 | 119 | 13 | 127 | 5 |
| Not complete remission | 49 | 44 | 5 | 43 | 6 | 43 | 6 | 49 | 0 |
| Missing information | 8 | 8 | 0 | 7 | 1 | 6 | 2 | 8 | 0 |
| \textit{IDH1} codon 132 | | | | | | | | | |
| Wild type | 174 | 157 | 17 | 153 | 21 | 169 | 5 |
| Mutation | 15 | 12 | 3 | 15 | 0 | 15 | 0 |
| \textit{IDH1} codon 105 (synonymous SNP) | | | | | | | | | |
| Wild type | 169 | | 151 | 18 | 165 | 5 |
| Variant | 20 | | 17 | 3 | 19 | 1 |
| \textit{IDH2} codon 140 | | | | | | | | | |
| Wild type | 168 | | | | 163 | 5 |
| Mutation | 21 | | | | 21 | 0 |
| \textit{IDH2} codon 172 | | | | | | | | | |
| Wild type | 184 | | | | |
| Mutation | 5 |

*Mann-Whitney test for difference in age distribution between patients with \textit{IDH} mutation (\textit{IDH1} or \textit{IDH2}) and \textit{IDH} wildtype patients, median 62 vs 69 years; p = 0.036.

**FLT3-ITD and NPM1 mutations were not routinely analyzed in all non-normal karyotype patients.
There were no significant differences in the distribution of IDH1 or IDH2 genotypes among patients with CR and no CR.

The IDH1 SNP variant influences overall survival
All patients were successfully genotyped for IDH1 codon 105 SNP (rs11554137) (Table 1) that was not associated with the IDH mutations (only 7 overlapping cases; 3 in IDH1 and 4 in IDH2). The synonymous SNP (GGC > GGT) was found in 20 patients (10.6%) in the entire cohort. Kaplan Meier curves with log rank tests also revealed a significant difference in OS between the IDH1 codon 105 SNP variants, where heterozygous carriers of the T allele displayed a shorter survival compared to patients with homozygous wild-type C alleles. This was significant only in the intermediate risk FLT3-ITD negative AML patients. In this risk group, the median OS was 20 vs. 6 months for codon 105 wild-type C/C and variant T/C patients, respectively, (p = 0.004, Figure 3). It should be noted that all the intermediate risk FLT3-ITD negative patients with the codon 105 T allele were also negative for NPM1 mutations. However, in cox regression analysis the codon 105 SNP did not display independent significance due to other stronger factors affecting the outcome in the entire cohort (Table 3).

Discussion
Mutations in the IDH1 and IDH2 genes in AML are reported as being associated to diverse outcomes by different groups [22]. Mardis et al. [9] was the first to identify mutations in the IDH1 gene as a new recurrent mutation associated with CN-AML. Further, Marcucci et al. [13] reported two different mutations in the IDH2 gene (R140 and R172) in AML. In the present study we have investigated the frequency and impact of IDH mutations on outcome according to the different clinical risk groups, normal/aberrant cytogenetics, and also according to the FLT3 and NPM1 mutation status in 189 unselected AML patients. In our study cohort we found IDH1 and IDH2 mutated in 21.7% of the cases. IDH2 mutations were more common than IDH1 mutations (13.8% vs 7.9%). The frequency of IDH1 mutations (7.9%) in our AML cohort is similar to previous reports of unselected AML patients (5.5%-10.4%) [9,14,15,17,23-26]. The IDH2 mutations have been reported to have a prevalence of 6.1%-17.7% in unselected AML [15,17,24-28], as compared to 13.8% in our study group. Investigation of the influence on OS in the entire study population (no selection in karyotypes, risk groups or FLT3/NPM1 status) for IDH1 mutations conferred no significant difference compared to wild-type IDH1, nor when statistical stratification was applied. In some studies, an influence on OS is seen with IDH1 mutations for patients with CN-AML or intermediate risk group according to the FLT3/NPM1 status [13-15,23,28], while other groups could not detect any impact on survival with mutated IDH1 gene, which is in accordance with our results [9,20,24,26]. Furthermore, in our cohort we also found a slightly higher median age at diagnosis in patients with mutated IDH1 gene than in patients with wild type IDH1. Our cohort covered a wide age span including both younger and older patients, but future studies investigating the impact of IDH mutations specifically in older AML patients could be warranted.

Two hotspot mutations are found in exon 4 in the IDH2 gene, R140 and R172. In our cohort we found R140 to be altered with a frequency of 21/189 (11.1%) and R172 in 5/189 (2.6%) patients. We found prognostic significance on OS for the IDH2 codon 140 mutations, where the intermediate risk patients with codon 140 mutations revealed a significantly shorter OS than codon 140 wildtype. IDH2 codon 172 mutations were identified in a low frequency, only in 5 individuals, and were provided with a favorable outcome in our study cohort. However, Ward et al. noted a trend toward improved survival for patients with IDH2 codon 140 mutations and also Green et al. reported an unexpected favorable outcome associated with IDH2 R140 mutations and an unfavorable outcome for patients with IDH2 R172 mutation [17,27]. Patel et al. also found a favorable effect for patients with mutant IDH2 codon 140 [29]. However, in the study of Green et al., there was a

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**Table 3 Cox regression of overall survival, forced entry method**

| Covariates                     | N  | HR  | 95% CI     | p   |
|--------------------------------|----|-----|------------|-----|
| Age                           | 1,022 | 1.002-1.042 | 0.033 |
| Risk group                    |    |     |            |     |
| Low risk                      | 32 | 1   |            |     |
| Intermediate risk             | 87 | 2.980 | 1.495-5.942 | 0.002 |
| High risk                     | 55 | 5.993 | 2.912-12.333 | <0.001 |
| Treatment                     |    |     |            |     |
| Chemotherapy                  | 118 | 1   |            |     |
| Chemotherapy + allo-SCT       | 56 | 0.231 | 0.118-0.450 | <0.001 |
| IDH1 codon 132                |    |     |            |     |
| Wild type                     | 162 | 1   |            |     |
| Mutated                       | 12 | 0.816 | 0.390-1.708 | 0.59 |
| IDH2 codon 140                |    |     |            |     |
| Wild type                     | 155 | 1   |            |     |
| Mutated                       | 19 | 1.942 | 1.068-3.530 | 0.030 |
| IDH2 codon 172                |    |     |            |     |
| Wild type                     | 169 | 1   |            |     |
| Mutated                       | 5  | 0.222 | 0.067-0.738 | 0.014 |
| IDH1 SNP codon 105 GGC > GGT  |    |     |            |     |
| Wild type                     | 154 | 1   |            |     |
| Variant                       | 20 | 1.496 | 0.812-2.756 | 0.196 |

95% CI = 95% Confidence interval; HR = Hazard ratio. Significant P-values (P < 0.05) in boldface.
difference in patient median age at diagnosis compared to our study cohort, 43 vs. 64 years respectively. In the study of Patel et al., the patients’ median age at diagnosis also was much lower than in our cohort, 48 vs. 64 years respectively. These may indicate that the effect of the IDH2 mutations is seen in elderly patients.

Figueroa et al. [18] have shown that IDH mutant enzymes may result in a global DNA hypermethylation profile,
Figure 2 Kaplan-Meier curve of OS, IDH2 codon 172. The low frequency of AML-patients with mutated IDH2 codon 172 showed a tendency towards improved OS survival compared to wildtype IDH2, OS 30 vs. 12 months, p = 0.09.

Figure 3 Kaplan-Meier curve of OS, IDH1 codon 105 SNP. Significant differences in OS between codon 105 genotypes in FLT3-ITD negative intermediate risk patients; median OS 6 vs. 20 months (p = 0.004) for codon 105 variant allele and wild-type patients, respectively.
also identified to be mutated in de novo AML (7-23%) and 
IDH2 and sufficient to promote leukemogenesis when mutated 
demethylation of 5-MeC and, other studies have demon-
gene, was also analyzed in our study.

The synonymous SNP 105C > T, located in the same 
exon, but only overlapping in three cases, as R132 in the 
IDH1 gene, was also analyzed in our study. IDH2 mutations 
simultaneously with the codon 105 variant were found in 
three patients with codon 140 mutation and in one case 
with codon 172 mutation. The frequency of the SNP was 
20/189 (10.6%) in the entire cohort, and almost the same 
frequency (11.7%) has been reported among healthy volun-
tees by a German group [20]. The same group also re-
ported this SNP to correlate to an inferior prognosis in 
CN-AML [20]. In accordance with this, we found a pro-
nounced significant inferior overall survival in intermediate 
risk FLT3-ITD negative patients carrying the variant codon 
105 allele. The biologic effect of the silent SNP remains to 
be investigated in AML. One speculative explanation with a 
synonymous SNP is that it will cause a change in the rate 
of the protein translation resulting in affected protein fold-
ing and altered function of the protein [32], or cause a new 
splicing site. Potentially the T variant enables a new splice 
variant was detected.

In summary, our results identified in total 21.7% 
IDH1/IDH2 mutations in the study population. Our re-
results indicate that the IDH2 codon 140 mutation have 
the highest potential as a prognostic marker, further stratifying intermediate risk patients.

In addition, the synonymous SNP 105C > T in the 
IDH1 gene may be a novel prognostic marker in AML 
of intermediate risk FLT3 negative patients however, 
this has to be confirmed through future studies. These 
markers may be especially useful in this heterogeneous 
group of AML patients, where other prognostic markers 
are absent and the outcomes vary widely. Further, stud-
ies have been carried out on possible new drugs by tar-
geting the mutant IDH enzyme on leukemia cells, 
resulting in inhibition of accumulation of the 2-HG 
oncometabolite and subsequently differentiation of the 
AML blasts [33,34].

Conclusions

IDH mutational status and/or IDH1 SNP 105C > T vari-
ant may represent a new subgroup of AML patients and 
have the potential as tools for selecting patients expected 
benefit the most from the new treatment alternatives.

Methods

Patients

This study included 189 Swedish patients diagnosed with de 
 novo AML at Linköping University Hospital and Karolinska 
University Hospital in Huddinge between 1988 and 2010. 
The inclusion of the patients in this study was not con-
successively included. Median age at diagnosis was 64 years, 
range 19–88 years. Patient characteristics are summarized 
in Table 2. Bone marrow or peripheral blood samples 
collected at diagnosis were used to isolate DNA for further 
genetic analysis. Risk group assignment at diagnosis was 
based on cytogenetic and molecular genetic findings as 
defined by ELN (European Leukemia Net) [35] and Inter-
national Working Group recommendations [36], and 
other prognostic factors such as age, performance status 
and comorbidity, with minor modifications (see Swedish 
Hematology Association guidelines, http://www.sfhem.se/
Filarkiv/Nationella-riktlinjer accessed 2013-05-28). Swedish 
AML patients diagnosed in 2004 or later have been treated 
according to nationwide AML treatment guidelines (http:// 
www.sfhem.se/Filarkiv/Nationella-riktlinjer accessed 2013-05-28). Thus, the majority of the patients received induc-
tion treatments including daunorubicin 60 mg/m² once a 
day for three days combined with Cytarabine (AraC) as 
1000 mg/m² twice a day in 2 h i.v. infusions for 5 days. Be-
fore 2004, regional guidelines most commonly included AraC doses of 200 mg/m² as 24 h i.v. infusions for 7 days 
combined with three days either daunorubicin or idarubi-
cin [37]. Some patients also received other drugs in com-
bination with daunorubicin/idarubicin and/or AraC, such

Table 4 Induction treatment regimes

| Regime | N (%) |
|--------|------|
| Daunorubicine and Cytarabine (n = 116) or Daunorubicine, cytarabine and mitoxantrone (n = 2) | 118 (62.4) |
| Idarubicine and Cytarabine (n = 26) or Idarubicine, Cytarabine and Etoposide (n = 3) | 29 (15.3) |
| Idarubicine, Cytarabine and Cladribine | 20 (10.6) |
| Mitoxantrone, cytarabine and Etoposide (n = 7) or Mitoxantrone and Cytarabine (n = 2) | 9 (4.8) |
| Daunorubicine, Cytarabine and 6-Thioguanine | 8 (4.2) |
| Other/unknown¹ | 5 (2.6) |

¹Including 2 in clinical trial of combination therapy with Tipifarnib, and 1 with Fludarabine, Cytarabine and G-CSF. 2 patients with unknown treatment but known curative intent.
as Mitoxantrone, Etoposide, 6-Thioguanine and Cldarbine. For further treatment details, see Table 4. Treatment response was evaluated as non-complete remission (no CR) or morphologic complete remission (CR) [36]. Patients treated by allogeneic stem cell transplantation (allo-SCT) (n = 59) were censored at the time of transplantation in the survival analysis. Informed consent was obtained from the patients and the study was approved by the local ethical committees and conducted in accordance with the Helsinki declaration.

**IDH1 and IDH2 genotyping analysis**

Mononuclear cells from either peripheral blood or bone marrow were enriched by Ficoll-Paque density centrifugation at the time of diagnosis and genomic DNA was extracted. For *IDH1* and *IDH2* genotyping analysis, a PCR reaction was performed in a total volume of 20 μl containing 10–50 ng DNA, 0.5 μl Taq DNA polymerase, 2 mM MgCl₂, 0.2 mM dNTPs, 1xPCR buffer, 1 μM each of *IDH1* forward primer (5'ctcgagctcctcgcttctg) and reverse primer (5'cacatacaagttggaaatttctgg) and of *IDH2* forward primer (5'ggtttcaatctgttggta) and reverse primer (5'cttgccgag-gagctccagt). The terminal cycling conditions for both *IDH1* and *IDH2* were an initial denaturation at 94°C for 2 min followed by 35 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 30 s and an end extension at 72°C for 5 min. The PCR product was purified by using ExoSAP-IT and direct sequencing was performed by using BigDye Terminator v3.1 Cycle Sequencing Kit (AB Applied Biosystems). The *IDH1/2* sequences were compared to the wild type *IDH1/2* to detect the genetic variations (NM_005896.2 and NM_002168.2 respectively).

**Statistical analysis**

Fisher's exact test was used to compare differences in genotype distribution between patients with CR and no CR. Mann Whitney Test or Fisher’s exact test was used to investigate differences between genotype groups in terms of age, gender and karyotype distributions, or other characteristics. Kaplan Meier survival analysis with log rank test for significance was used to evaluate the impact of *IDH1* and *IDH2* genotype on overall survival (OS) (calculated as time from diagnosis until death, date of the latest follow-up, or date of allo-SCT). Multivariable survival analysis was conducted using Cox regression with a forced entry method, taking age, risk group, treatment, *IDH1/2* mutations and *IDH1 SNP*105 genotype into account. The impact of *IDH* genotype was evaluated in the entire patient material and in subgroups stratified by risk group and *FLT3* status. A p-value of 0.05 was considered significant, and all analyses were performed using IBM SPSS Statistics v.20.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

KW: Research, laboratory work, data compilation, manuscript writing; UF: Research, clinical data compilation and statistical analysis, manuscript writing; R.C: Clinical data compilation and consultation; E.P: Patient material and clinical data collection; MH: FLT3/NPM1 analysis, data collection; H.G: Data and statistical analysis; KL: Research, patient material and clinical data collection; P.S: Conception and study design, research. All authors critically reviewed the manuscript. All authors read and approved the final manuscript.

**Authors’ information**

Kourosh Lotti and Peter Söderkvist share last authorship.

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