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Prognostic Significance of Alterations in IDH Enzyme Isoforms in Patients With AML Treated With High Dose Cytarabine and Idarubicin

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Abstract

Background—*IDH1* and *IDH2* gene mutations are novel, recurring molecular aberrations among patients with normal karyotype acute myeloid leukemia (AML).

Methods—Among 358 patients with AML treated on 4 protocols using high dose ara-C plus idarubicin induction, pre-treatment samples were available for 170 [median age 53 years, (range, 17 - 73); 96% ≤ 65] and were evaluated for *IDH1R132*, *IDH2R172* and *IDH2R140* mutations or the codon 105 SNP in *IDH*1.

Results—*IDH1* and *IDH2* mutations were present in 12 (7%) and 24 (14%) patients, and *IDH1* G105 SNP in 24 (14%). Overall, 52 (30%) patients had *IDH* gene alterations. There was no association with complete response (CR), remission duration, overall and event-free survival and any of the *IDH* alterations and no association with a higher CR rate or survival with the 4 regimens for the 52 patients with aberrant *IDH*. Among the patients with diploid karyotype and $NPM1^{mut}FLT3^{WT}$genotype, those with *IDH1* or *IDH2* mutations had an inferior outcome.

Conclusions—*IDH* aberrations and *IDH1* codon 105 SNP occur in about 30% of younger patients with AML, mostly with diploid karyotype. Using high-dose ara-C based induction regimens, we did not detect an association with outcome for any of the aberrations.

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Keywords

AML; IDH; Mutations; SNP; outcome

INTRODUCTION

Isocitrate dehydrogenase (IDH) is a member of the β-decarboxylating dehydrogenase family of enzymes and catalyzes the oxidative decarboxylation of isocitrate into α-ketoglutarate (α-KG) in the tricarboxylic acid (TCA) cycle, an important biochemical pathway in the synthesis of nucleotides, lipids, and amino acids.[1] Three isoforms of the IDH enzymes with different subcellular localization have been described in mammalian cells: IDH1 is localized in the cytoplasm and the peroxisomes, whereas IDH2 and IDH3 are mitochondrial enzymes.[2] These enzymes use nicotanomide adenine dinucleotide (NAD) or NAD phosphate as cofactors to catalyze the conversion of isocitrate to α-KG yielding NADH or NADPH as a result.

Recent interest in this family of enzymes in relation to carcinogenesis is based on the initial observations in colorectal cancer and gliomas with several reports of recurring mutations in the *IDH1* gene on chromosome band 2q33.3 and the *IDH2* gene on chromosome band 15q26.1 in patients with grade II and III gliomas and, in particular, secondary glioblastoma multiforme (GBM) developing from lower grade gliomas.[3-7] Overall, these mutations have been associated with a longer survival in patients with GBM.

The *IDH* mutations identified in brain tumors (and in patients with AML) alter the amino acid residues R132 in *IDH1* and R172 in *IDH2*. The mutated enzyme protein products have been associated with a loss of function in the forward reaction that decorboxylates isocitrate as well as a gain of function in the reverse reaction that reduces α-KG to 2-hydroxyglutarate (2HG).[8] Both these loss and gain of function reactions have significant implications for cellular metabolism with 2HG potentially deregulating several α-KG-dependent enzymes and transcription factors, such as HIF-1α, implicated in carcinogenesis.[9,10]

Initial studies failed to detect mutations of the *IDH* genes in patients with leukemia.[6,11,12] Recently, Mardis and colleagues examined the entire genome sequence of leukemia cells from a single patient with acute myeloid leukemia (AML) with normal cytogenetics (CN-AML) comparing it with the sequence from normal skin cells from the same patient.[13] Among 750 possible mutations, several (including mutations in *NPM1* and *NRAS*, as well as *IDH1*) were recurrent in other AML genomes suggesting their potential role in leukemogenesis.[13] This had led to several groups investigating the incidence and prognostic implications of mutations in *IDH1* and *IDH2* in patients with AML, (particularly those with normal karyotype) in an effort to further improve stratification of these patients and identify potential targets for therapeutic intervention. These studies have reported conflicting associations between the presence of these mutations and clinical outcomes.

In a study by the Cancer and Leukemia Group B (CALGB), leukemia cells from a third of 358 patients examined harbored *the IDH1* or *IDH2* mutation.[14] Among the younger patients (< 60 years) with molecular low-risk group [mutated *NPM1* but without fms-like tyrosine

kinase-3- internal tandem duplication (*FLT3*-ITD)], patients with mutated *IDH*1 (*IDH1* R132 had a significantly worse disease-free survival (DFS) with a trend for worse overall survival (OS).[14] Patients with R140 *IDH2* had no differences in disease outcomes compared to those with wild-type (WT) *IDH1/IDH2*, whereas older patients with R172 *IDH2* had a lower complete remission (CR) rate than those with *IDH1/IDH2* wild-type (WT) gene.[14]

In another study conducted by the German-Austrian AML study group (AMLSG), 129 (16%) of 805 adults (age range, 16-60 years) with AML had one of the *IDH* mutations.[15] Among patients with CN-AML, mutated *NPM1*, and *FLT3*^{WT} AML, *IDH* mutations adversely affected relapse-free survival (RFS) and OS whereas the outcome was not affected in patients lacking this genotype.[15] In a study by the Acute Leukemia French Association (ALFA), *IDH1* and *IDH2* mutations were identified in 9.6% and 3% of adults with AML with an association with a worse outcome for *IDH2* among the patients with CN-AML and for *IDH2* for those patients with CN-AML and a favorable genotype (*NPM1* mutated, *FLT3* WT).[16] Other groups have also reported on an adverse prognostic effect for *IDH* mutations as well as the single nucleotide polymorphism (SNP) rs1554137 located in codon 105 in the same exon as the *IDH*1 R132 mutations in patients with CN-AML, particularly those with *NPM1* mutated, *FLT3* wild type genotype.[17-19] Although this SNP does not result in a change in the amino acid sequence, it has been reported to be associated with a higher mRNA expression compared to patients with two WT alleles.

Here we examined the incidence and prognostic impact of *IDH* mutations as well as the G105 polymorphism among 358 younger patients with AML treated with an induction regimen containing idarubicin, high dose cytarabine with or without tipifarnib, sorafenib, or vorinostat; we also sought to determine whether any of the 4 regimens had an impact on the outcome of the *IDH* mutated patients. Although *IDH1* G105 polymorphism is not a true aberration and has not been to date shown to be associated with generation of 2HG, we decided to include these patients in the analysis due to recent reports suggesting their negative impact.

MATERIALS AND METHODS

Patients and treatment

From October 2004 to February 2010, 358 patients with newly diagnosed AML were treated on 4 consecutive protocols using high dose ara-C plus idarubicin induction therapy (IA alone, IA plus tipifarnib [IAT], IA plus sorafenib [IAS], and IA plus vorinostat [IAV]). The details of the 4 regimens have been published previously.[20-22] All patients reviewed and signed a consent, approved by the M. D. Anderson Cancer Center institutional review board to participate in the clinical trials and to provide bone marrow samples for analysis. The induction regimen in all patients included ara-C 1.5 g/m^2 given by 24 hour continuous infusion daily for 4 days (3 days in patients 60 years or older) and idarubicin 12 mg/m^2 intravenously daily for 3 days. Patients received tipifarnib 300 mg twice daily for 21 days, sorafenib 400 mg twice daily for 7 days, or vorinostat 500 mg 3 times daily for 3 days, with IAT, IAS, or IAV regimens, respectively. Patients could receive up to 2 induction courses and if in CR or CR without platelet recovery (CRp) would proceed to receive consolidation which consisted of up to 5 courses of idarubicin 8 mg/m^2 per day on days 1-2, ara-C 0.75

g/m^2 per day on days 1-3, and tipifarnib, sorafenib or vorinostat as appropriate to the protocol. Maintenance with tipifarnib, sorafenib or vorinostat was given for up to 12 months. Patients treated with the IA regimen would receive no further therapy after the completion of the 5 courses of consolidation chemotherapy with no maintenance (Table 1). It should be further emphasized that cytarabine was administered by continuous infusion during induction in all regimens.

Pre-treatment samples for testing for *IDH1R132*, *IDH2R172* and *IDH2R140* mutations were available for 170 patients and these are the subjects of this report. Their median age was 53 years (range, 17 - 73) and the majority of the patients (96%) were 65 years or younger. Other pre-treatment characteristics of the patients are shown in Table 2.

Molecular analysis for *IDH*1 and *IDH*2 mutations and SNP

For *IDH1*, a 214-bp partial exon 4 sequence containing both G105 and R132 was amplified using a forward primer within exon 4 and a reverse primer within intron 4. For *IDH2*, a 288-bp sequence with the entire exon 4 containing R140 and R172 was amplified using forward primer within intron 3 and a reverse primer within intron 4. All primers were tagged with M13 sequence to allow Sanger sequencing using M13 primers. This design allowed interrogation of all three active site arginine residues for both *IDH1* (R100, R109, R132) and *IDH2* (R140, R149, R172). The primer sequences were as follows: M13-*IDH1* forward, TGTAAAACGACGGCCAGTTGAGAAGAGGGTTGAGGAGTT; M13-*IDH1* reverse, CAGGAAACAGCTATGACCAACATGCAAAATCACATTATTGCC; M13-*IDH2* forward, TGTAAAACGACGGCCAGTGGGTTCAAATTCTGGTTGAA; M13-*IDH2* reverse; CAGGAAACAGCTATGACCTAGGCGAGGAGCTCCAGT; M13 forward, TGTAAAACGACGGCCAGT; M13 reverse, CAGGAAACAGCTATGACC. All Sanger assays were performed bi-directionally using M13 primers. Bidirectional Sanger sequencing is an accepted and more stringent way of confirming the presence of a mutation compared to simply repeating a unidirectional sequencing. For Sanger sequencing performed directly from the DNA aliquot, reaction mixtures of 50 μL contained 1 mM dNTPs, 2 mM $MgCl_2$, 200 nM primers, 1.5 U Taq Polymerase (Applied Biosystems, Carlsbad, CA). For each reaction, 200 ng of genomic DNA was amplified with the following PCR conditions: an initial 10-minute denaturation at 95°C followed by 40 cycles of 30 seconds at 95°C; 30 seconds at 60°C; 30 seconds at 72°C, and a final extension of 7 minutes at 72°C.

PCR products were purified using Agencourt Ampure magnetic beads kit (Beckman Coulter, Brea, CA) prior to quality and quantity assessment by gel electrophoresis. Sequencing analysis was performed using a multi-capillary sequencer (ABI 3130 Genetic Analyzer; Applied Biosystems) following cycle sequencing using dye-terminator chemistry according to manufacturer's protocol (BigDye Terminator v1.1 Ready Reaction Cycle Sequencing Kit; Applied Biosystems). Briefly, 4 L of PCR product in a total volume 20 L containing, 3.2 pmol of either the forward or reverse M13 primer and 6 L of the sequencing mixture were placed in a DNA thermal cycler and amplified at 96°C for 1 minute, 25 cycles at 96°C for 10 seconds, 58°C for 5 seconds, 60°C for 4 minutes, and final hold at 4°C. Sequencing reactions were purified using the Qiagen DyeEx™ purification kit (Qiagen,

Valencia, CA) as per manufacturer's protocol. The resulting data was analyzed by Seqscape software (Applied Biosystems).

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Molecular analysis for *NPM1* and *FLT3* mutations

Mutations in coding regions of exon 12 of *NPM1* were detected using PCR amplification of a 168-base-pair segment followed by capillary gel electrophoresis. PCR primers included forward, 5′-FAM-GATGTCTATGAAGTGTTGTGGT-TCC-3′ and reverse, 5′-GGACAGCCAGATCAACTG-3′. PCR was performed in a 50- L reaction volume that contained 2 L of patient DNA (100 ng/ L), 5 L of 10× ThermoPol Buffer (New England BioLabs, Ipswich, MA) with magnesium sulfate, 5 L of 10 mmol/L dNTP, 1 L of *NPM1* forward primer (10 mol/L), 1 L of *NPM1* reverse primer (10 mol/L), 35.2 L of water, and 0.75 L of Vent DNA polymerase (New England Biolabs) (2 U/ L). PCR conditions included initial denaturing at 95°C for 10 minutes, 40 cycles of 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, and final extension at 72°C for 7 minutes. PCR products were analyzed by capillary electrophoresis on a 3100 or 3130 genetic analyzer (Applied Biosystems).

A multiplex fluorescent-based PCR method was used to detect ITD and D835 point mutations in *FLT3.* DNA was isolated from bone marrow aspirate samples by using a standard phenol-chloroform extraction method. The presence of ITD was assessed by amplification of the juxtamembrane domain using primers from exons 11 and 12 as described by Kiyoi and Naoe.[23] The presence of D835 point mutations was assessed by a restriction fragment length polymorphism–mediated assay using primers flanking the mutation site.15 To facilitate the detection of PCR products by capillary electrophoresis on a 3100 genetic analyzer (Applied Biosystems, Foster City, CA), forward primers for ITD and D835 were labeled with a fluorescent dye, 6-carboxyfluorescein (FAM).16 The presence of any PCR fragment larger than the wild-type allele was considered positive for ITD. D835 PCR products were digested with the *Eco*RV restriction enzyme before capillary electrophoresis. The wild-type allele cut by this enzyme resulted in 2 fragments of 64 and 48 base pairs. In contrast, mutations at D835 alter the *Eco*RV recognition site and result in one 112-base-pair fragment. The sensitivity of these assays is approximately 2%, ie, 1 mutated cell in 50 total cells, as established by dilutional studies.

Statistical methods

Patient characteristics are summarized using median (range) for continuous variables and frequency (percentage) for categorical variables. OS is defined as the time interval between the date of initiation of treatment and the date of death due to any cause; patients alive at last follow-up are censored at the last follow-up date. Event-free survival (EFS) is defined as the time interval between the date of initiation of treatment and date of treatment failure, relapse, or death, whichever occurred first; patients were otherwise censored at the last follow-up date. The probabilities of OS and EFS were estimated using the Kaplan-Meier

method and were compared among subgroups of patients using the log-rank test. The predictive effects of *IDH* mutations and other patient characteristics on OS and EFS were examined using univariate and multivariate Cox proportional hazards models. Univariate and multivariate logistic regression models were also used to assess the association between patient covariates and the probability of response. All statistical analyses were conducted in SAS 9.0.

RESULTS

Incidence of *IDH* mutations and the G105 SNP

Mutations of *IDH1* and *IDH2* were present in 12 (7%) and 24 (14%) patients, respectively, and *IDH1G105* SNP was noted in 24 (14%). Overall, 52 (30%) patients had *IDH* gene mutations or the previously described SNP rs 11554137 in codon 105 of *IDH1*.[17] In concordance with previous reports *IDH1* and *IDH*2 mutations did not commonly occur in the same patient with the exception of a single patient who had *IDH1R132* mutation, *IDH1G105* SNP, and *IDH2R140* mutation. [14-16] Two patients had concomitant *IDH1R132* mutation and *IDH1G105* SNP, 3 patients had *IDH2R140* mutation and *IDH1G105* SNP, 1 patient *IDH2R172* mutation and *IDH1G105* SNP. *IDH1* mutations were most commonly R132H or R132C with the exception of one which was R132G. Twenty four patients had *IDH2* mutations, either *IDH2* R172 or R140. All *IDH2* R172 mutations led to a R172K substitution.

Association of clinical characteristics with *IDH* mutations and SNP

There was a strong association with normal karyotype with 11 of 12 (92%) of *IDH1* mutated, 18 of 24 (75%) of *IDH2* mutated, and 17 of 24 (71%) of *IDH1* SNP being diploid (Table 3). There was no association between any of the *IDH* alterations and patient age, sex, therapy-related vs. de novo AML, presenting WBC, peripheral blood blasts, or FAB subtype.

Among the 52 patients with *IDH* mutation or SNP, 12 had FLT3-ITD, 5 FLT3-TKD (including 2 patients with both ITD, and TKD), and 21 had NPM1 mutation. There was a strong association between *IDH1* and *IDH2* mutations, and *NPM1* mutations (P values 0.0004 and 0.04, respectively). However, 12 of 52 patients with *IDH* alterations also had *FLT3-ITD* compared to 24 of 116 patients with IDH^{WT} (p=NS). The favorable genotype, diploid, $NPM1^{mut}$ and FLT3 WT occurred in 9 patients.

Association of *IDH* mutations with outcome of therapy

There was no association with achievement of complete response (CR), remission duration, overall and event-free survival and either of the *IDH* mutations or *IDH*1 SNP (Figures 1a, b, and c). Similarly, combining all patients with IDH mutations or SNP G105, we were not able show any difference in survival, EFS or CR duration (Figures 2a, b, and c). Furthermore, there was no association with a higher CR rate or survival among any of the 4 different regimens for the 52 patients with *IDH* gene mutations or *IDH1* SNP. On univariate analysis for response, EFS, and OS having *IDH1*, or *IDH2* mutations or any *IDH* aberration was not significantly associated with the outcome and predictors known to be important

such as cytogenetics, *NPM1*^{mut}/*FLT3*^{wt} genotype, and de novo vs, secondary AML were significant on the multivariate model (data not shown).

IDH mutations and outcome in molecular subsets

Among the 104 patients with diploid cytogenetics, *IDH1* mutations occurred in 11 (11%), *IDH2* mutations in 18 (17%), and G105 SNP in 17 (16%) of patients. The presence of *IDH1* or *IDH2* mutations or the *IDH1* SNP did not affect the outcome and was not significant on univariate analysis (data not shown). However, among the 23 patients with diploid karyotype and the favorable genotype of NPM1^{mut}FLT3^{WT}, those with *IDH1*R132, *IDH2*R140, or *IDH2*R172 had a significantly worse survival as compared to those without these mutations (Figures 3a and b). However, the outcome of the patients with the diploid cytogenetics and the favorable genotype NPM1^{mut}FLT^{WT} was not affected by the presence or absence of the G105 SNP. Furthermore, among the patientas with diploid cytogenetics who were FLT3 mutated, presence of *IDH1* mutation (but not *IDH1* SNP G105 or *IDH2* mutations) was associated with an improved outcome although this was based on a small number of patients (Figure 3c).

Association of outcome of therapy with the 4 regimens used in the molecular subsets

We also examined whether any of the 4 regimens used to treat the patients (IA, IAT, IAS, or IAV) had an impact on the response rate or survival of patients with *IDH1*, *IDH2* mutations or G105 SNP and did not find any correlation with a better or worse outcome among the 4 regimens.

DISCUSSION

Identification of *IDH1* mutations by sequencing the whole leukemia genome of a patient with diploid AML has generated significant interest in the *IDH* genes and their potential role in leukemogenesis.[10,13,24] *IDH* mutations were previously described in patients with glioma where they are associated with a more favorable outcome.[4,25] A number of studies have suggested that these mutations may be an early event in the oncogenesis of secondary GBM.[24] Similarly, a number of reports have shown a low incidence of these mutations in myelodysplastic syndromes (MDS) and myeloproliferative disorders but have suggested a possible role for them in transformation into acute leukemia.[26-28]

In AML, a number of groups have examined the incidence and clinico-pathological features of these mutations in patients, particularly those with diploid cytogenetics.[13-19,29,30] These studies have suggested that *IDH1* and *IDH2* mutations each occur in 5% to 15% of patients with a strong association with the diploid karyotype. The *IDH1* SNP in codon G105 occurs in about 12% of cytogenetically normal patients and healthy volunteers and is associated with an inferior outcome with the impact most pronounced among those with the high risk genotype (NPM^{WT} or FLT3-ITD positive).[17]

In the current study, we detected *IDH1* and *IDH2* mutations in 12 (7%) and 24 (14%) of patients (overall 21%) and *IDH*1 G105 in another 24 (14%) of patients. Among the patients with diploid cytogenetics, *IDH1* mutations occurred in 11 (11%), *IDH2* mutations in 18 (17%), and G105 SNP in 17 (16%) of patients. Overall, the presence of the mutations and/or

the G105 SNP was not associated with any impact on the achievement of CR, EFS, and overall survival. Similarly, among the patients with diploid cytogenetics (n=104), the presence of these IDH alterations did not affect any of the outcome measures adversely. However, when we examined the group of patients with diploid karyotype and the favorable genotype of $NPM1^{mut}/FLT3^{WT}$ (n=23), patients with either *IDH1* or *IDH2* mutations had a significantly worse survival than those without the mutations but the presence of G105 SNP did not affect the outcome, although it should be stressed that these findings are in small numbers of patients.

Our results are consistent with those from other investigators reporting the incidence of these mutations as about 5% to 15% (overall up to 30%) of patients with AML with a strong association with those with cytogenetically normal disease. [14-16,19,29,30] The variations amongst the various reports is likely due to different patient populations including differences in the age of the patients evaluated in the studies. We also detected a similar incidence of the G105 SNP among the patients with AML as was previously reported by Wagner et al but failed to show an adverse prognostic impact on survival as suggested by them.[17] Overall, alterations in the *IDH* genes account for a sizable proportion of patients, particularly those with normal cytogenetics and appear to have an impact on survival among those with a favorable genotype of $NPM1^{mut}/FLT3^{WT}$. This is considerable importance in further refining the prognosis of this group of patients and the potential for different forms of post-remission therapy. Importantly, we also were not able to show any impact of the selection of the 4 different treatment regimens on the outcome of patients with *IDH* alterations.

Further studies on the role of IDH enzymes in cellular metabolism is likely to provide further insight into the association of the IDH gene alterations with diploid karyotype AML and their impact on the outcome of the subset with *NPM1* but without *FLT3* ITD mutations. In gliomas, one possible theory of the improved outcome of the patients with *IDH1* mutation is sensitization of the glioma cells to chemotherapy and radiation through the reduction of intracellular pools of NADPH.[31] If this were to be a true mechanism in gliomas, the paradox in AML where, among the patients with favorable genotype, those with IDH mutations appear to have a worse outcome has to be reconciled. Other important effects of the deregulated IDH function such as reduction of decorboxylation of isocitrate to α-KG as well as increased conversion of α-KG to 2-hydroxyglutarate (2HG) may be potentially responsible for their prognostic influence in specific subsets; recent studies have demonstrated the accumulation of 2HG in AML cells.[32,33] A potential hypothesis is that, through its structural similarities to α-KG, 2HG competitively inhibits several α-KG-dependent enzymes important in leukemogenesis. A recent report suggested that *IDH* mutated AML cells display global DNA hypermethylation with a specific signature, and expression of 2HG producing *IDH* alleles in cells induced global hypermethylation. [34] The authors further demonstrated that loss-of-function mutations in *TET2*, an α-KG dependent enzyme, also produced similar methylation defects as IDH mutants and expression of mutatnt *IDH*1/2 or depletion of TET2 resulted in impaired hematopoietic differentiation.[34]

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None

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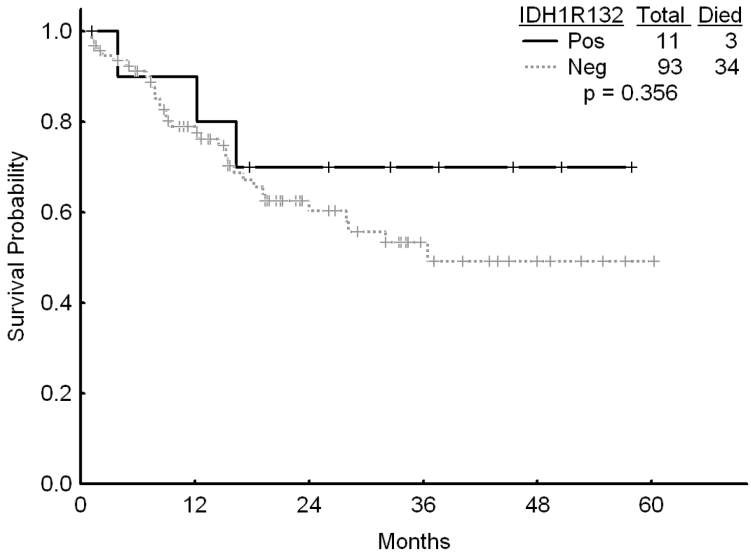
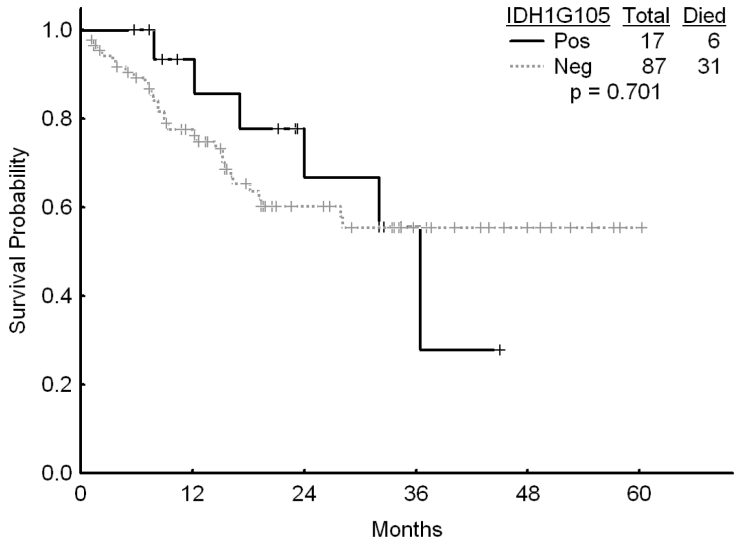




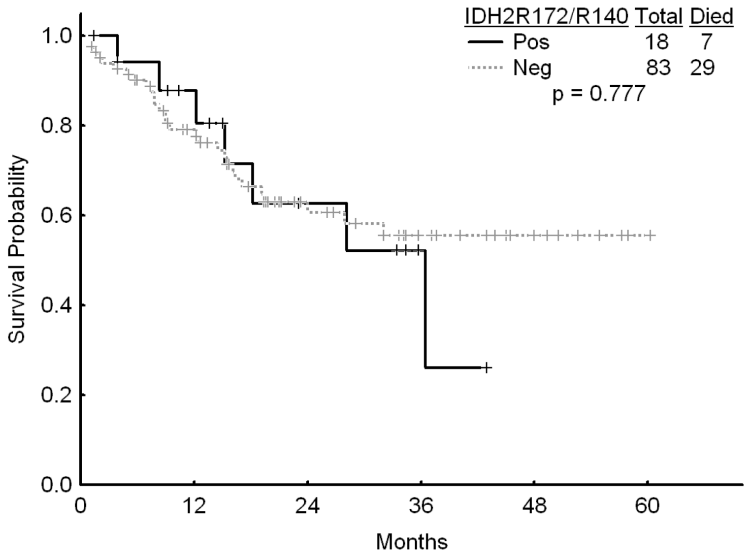
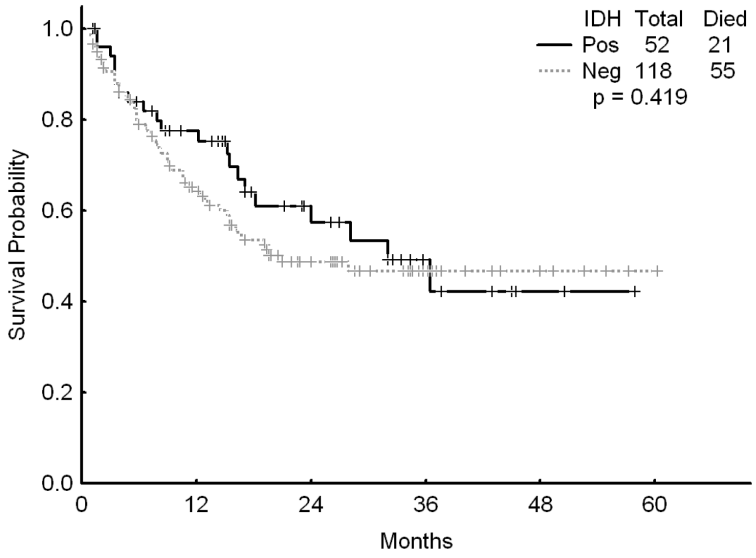


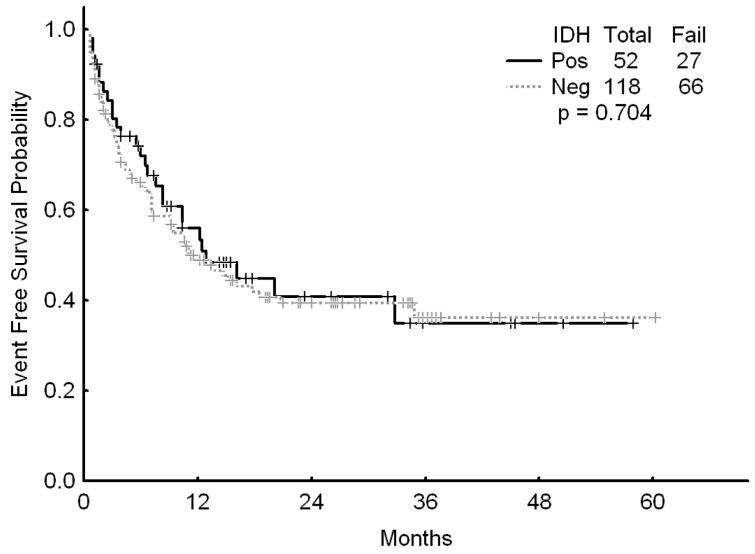
Figure 1.

a Survival among patients with diploid karyotype by the presence of IDH1 mutation

b Survival among patients with diploid karyotype by the presence of *IDH1* G105 SNP

c Survival among patients with diploid karyotype by the presence of *IDH2* mutations





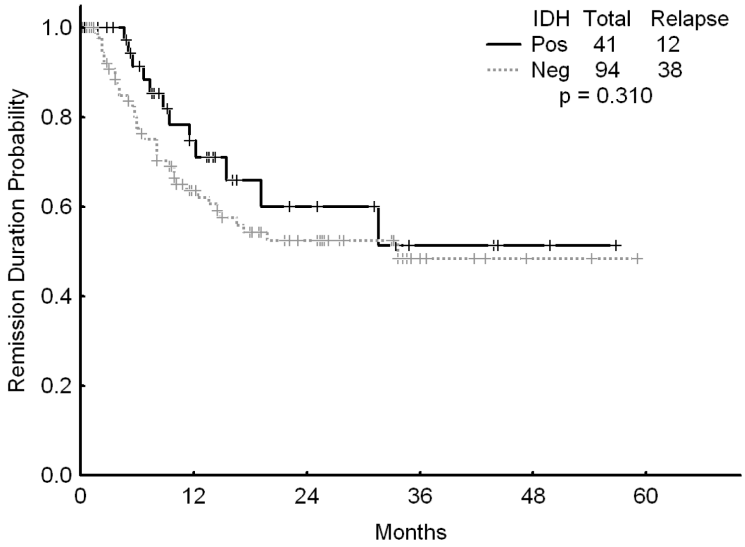
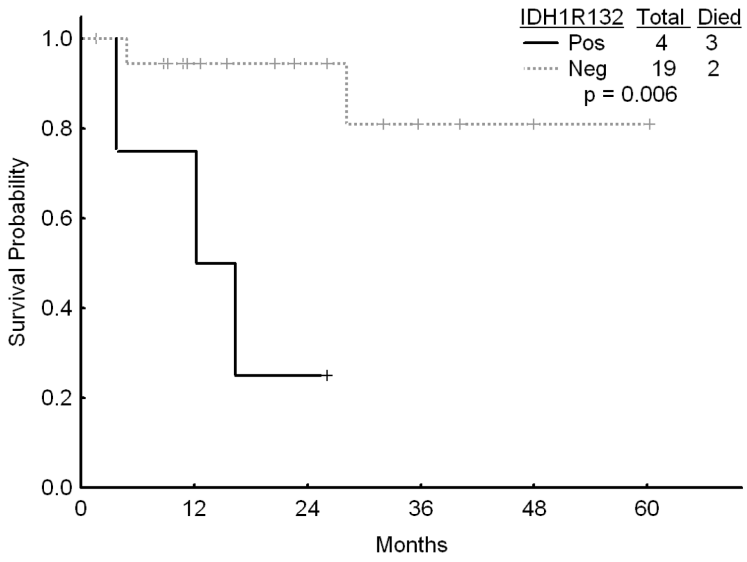


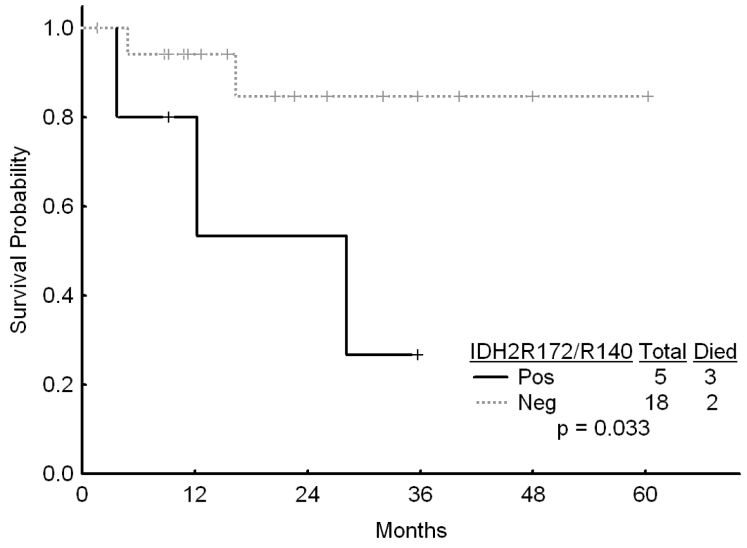
Figure 2.

a Survival for patients with *IDH* mutations or G105 SNP (dubbed "*IDH* positive) versus other

b Event-free survival for patients with *IDH* mutations or G105 SNP (dubbed "*IDH* positive) versus other

c Complete remission duration for patients with *IDH* mutations or G105 SNP (dubbed "*IDH* positive) versus other





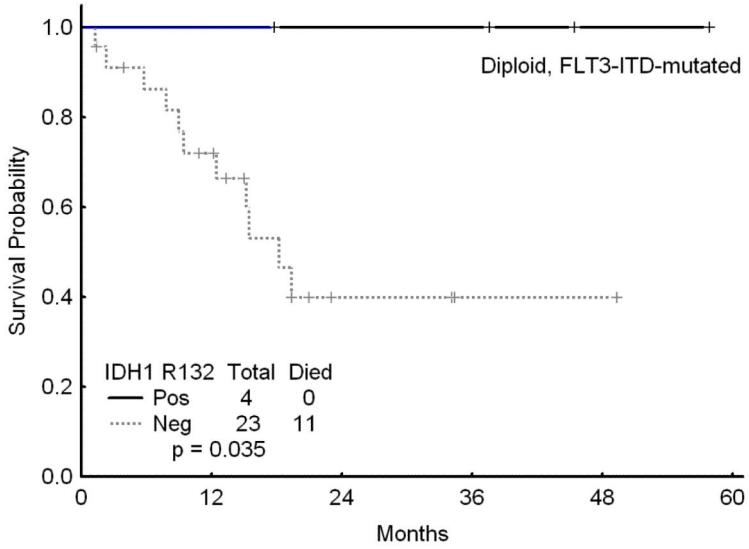


Figure 3.

a Survival in patients with diploid karyotype and *NPM1*^{mut}/*FLT3*^{WT} genotype by the presence of absence of *IDH1* R132

b Survival in patients with diploid karyotype and NPM1^{mut}/FLT3^{WT} genotype by the presence of absence of *IDH2* mutations

c Survival of patients with diploid karyotype and *FLT3*-ITD by the presence or absence of *IDH1* R132

Table 1

Details of the treatment regimens

	IA (From 2006-2009) (n*=78)	**IAT[21] (From 2004-2006) (n*=33)**	**IAS[20] (From 2007-2010) (n*=38)**	**IAV[22] (From 2008-2010)) (n*=21)**
Induction	Ara-C 1.5 g/m^2 by CI daily × 4*	Ara-C 1.5 g/m^2 by CI daily × 4*	Ara-C 1.5 g/m^2 by CI daily × 4*	Ara-C 1.5 g/m^2 by CI daily × 4*
	Idarubicin 12 mg/m^2 IV daily × 3	Idarubicin 12 mg/m^2 IV daily × 3	Idarubicin 12 mg/m^2 IV daily × 3	Idarubicin 12 mg/m^2 IV daily × 3
		Tipifarnib 300 mg po bid × 21 days	Sorafenib 400 mg po bid × 7 days	Vorinostat 500 mg po tid × 3 days
Consolidation	Up to 5 courses Ara-C 0.75 g/m^2 by CI × 3 days	Up to 5 courses Ara-C 0.75 g/m^2 by CI × 3 days	Up to 5 courses Ara-C 0.75 g/m^2 by CI × 3 days	Up to 5 courses Ara-C 0.75 g/m^2 by CI × 3 days
	Idarubicin 8 mg/m^2 daily × 2 days	Idarubicin 8 mg/m^2 daily × 2 days	Idarubicin 8 mg/m^2 daily × 2 days	Idarubicin 8 mg/m^2 daily × 2 days
		Tipifarnib 300 mg po bid × 14 days	Sorafenib 400 mg po bid for up to 28 days per cycle	Vorinostat 500 mg po tid × 3 days per cycle
Maintenance	None	Tipifarnib 300 mg po bid × 21 days every 4-6 weeks, up to 6 months	Sorafenib 400 mg po bid, up to 1 year	Vorinostat 200 mg po tid × 14 every 28 days for 12 cycles

IA: idarubicin plus ara-C; T: tipifarnib; S: sorafenib; V: vorinostat; CI: continuous infusion;

* number of patients with pre-treatment samples included in this study

Table 2

Patient characteristics

Characteristic	Number (%)
Patients	170
Age in years, median [range]	53 (17-73)
De novo AML	150 (88)
Secondary AML	20 (12)
Plt × 10^9/L, median [range]	53 (5-306)
WBC × 10^9/L, median [range]	4.9 (0.3–161.5)
% bone marrow blasts, median [range]	52 (20-98)
Cytogenetics	
Diploid	104 (61)
Chromosome 5/7deletion/complex	32 (18)
Other	34 (20)
***NPM1* mutated**	46 (27)
***NPM1* wild-type**	92 (54)
***NPM1* not done**	32 (19)
***FLT3*-ITD Positive**	36 (21)
***FLT3*-ITD Negative**	132 (78)
***FLT3*-ITD not done**	2 (1)
***NPM1* mutated/ FLT3-ITD negative**	28 (16)

Table 3

Patient characteristics and their association with IDH1 and IDH2 mutations and IDH1 SNP

Character	IDH1 R 132		P value	IDH1 G105		P value	IDH2 R172 & R140		P value
	+	–		+	–		+	–	
Number	12 (7)	158 (93)		24 (14)	146 (86)		24 (15)	141 (85)	
Age, median (range)	53 (36-73)	53 (17-72)	NS	53 (18-72)	53 (17-73)	NS	56 (19-68)	53 (17-73)	NS
Sex, Male	5 (42)	85 (54)	NS	10 (42)	80 (55)	NS	10 (42)	78 (55)	NS
De novo **Secondary**	11 (92) 1 (8)	139 (89) 19 (12)	NS	22 (92) 2 (8)	128 (88) 18 (12)	NS	23 (96) 1 (4)	124 (88) 17 (12)	NS
Plt × 10^9/L, median (range)	99 (7-214)	50 (5-306)	**0.047**	68 (24-242)	46 (5-306)	**0.04**	67 (15-238)	49 (5-306)	NS
WBC × 10^9/L, median, (range)	8.8 (0.6-50.7)	4.9 (0.3-161.5)	NS	4.8 (1.4-84)	5.0 (0.3-161.5)	NS	4.5 (0.5-50.7)	5.1 (0.3-161.5)	NS
% BM blasts, median (range)	73 (24-92)	52 (10-98)	NS	39 (18-90)	54 (10-98)	**0.03**	61 (10-94)	52 (14-98)	NS
% PB blasts, median (range)	65 (0-91)	16 (0-98)	NS	15 (0-95)	17 (0-98)	NS	13 (0-93)	18 (0-98)	NS
Cytogenetics									
Diploid **5/7/comples** **Other**	11 (92) 1 (8)	93 (59) 31 (20) 34 (22)	**0.07**	17 (71) 1 (4) 6 (25)	87 (60) 31 (21) 28 (19)	NS	18 (75) 1 (4) 5 (21)	83 (59) 29 (21) 29 (21)	NS
NPM1									
Pos **Neg**	8 (67) 3 (25)	38 (24) 89 (56)	**0.004**	7 (29) 9 (38)	39 (27) 83 (57)	NS	10 (42) 8 (33)	36 (26) 82 958)	**0.04**
***FLT3*-ITD**									
Pos **Neg**	4 (33) 8 (67)	32 (20) 124 (78)	NS	4 (17) 20 (83)	32 (22) 112 (77)	NS	5 (21) 19 (79)	31 (22) 109 (77)	NS
***NPM1* Pos/**									
FLT3-Neg Otherwise	4 (33) 8 (67)	24 (15) 134 (85)	NS	3 (13) 21 (88)	25 917) 121 (83)	NS	6 (25) 18 (75)	22 (16) 119 (84)	NS
Induction regimen									
IA **IAT** **IAS** **IAV**	3 (25) 6 (50) 1 (8) 2 (17)	75 (47) 27 (17) 37 (23) 19 (12)	**0.032**	10 (42) 6 (25) 6 (25) 2 (8)	68 (47) 27 (18) 32 (22) 19 (103)	NS	10 (42) 6 (25) 5 (21) 3 (13)	65 (46) 27 (19) 32 (23) 17 (12)	NS