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## Molecular alterations of the *IDH1* gene in AML: a Children's Oncology Group and Southwest Oncology Group study

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## Abstract

Recent whole-genome sequencing efforts led to the identification of *IDH1*<sup>R132</sup> mutations in AML patients. We studied the prevalence and clinical implications of *IDH1* genomic alterations in pediatric and adult AML. Diagnostic DNA from 531 AML patients treated on Children's Oncology Group trial COG-AAML03P1 (N=257), and Southwest Oncology Group trials SWOG-9031, SWOG-9333, and SWOG-9500 (N=274), were tested for *IDH1* mutations. Codon R132 mutations were absent in the pediatric cohort, but were found in 12/274 adult patients (4.4%, 95% CI 2.3-7.5%). *IDH1*<sup>R132</sup> mutations occurred most commonly in patients with normal karyotype, and those with *FLT3/ITD* and *NPMc* mutations. Patients with *IDH1*<sup>R132</sup> mutations trended towards higher median diagnostic WBC counts ( $59.2 \times 10^9/L$  vs.  $29.1 \times 10^9/L$ , P=0.19) than those without mutations, but the two groups did not differ significantly in age, bone marrow blast percentage, overall survival, or relapse-free survival. Eleven patients (2.1%) harbored a novel V71I sequence alteration, which was found to be a germline polymorphism. *IDH1* mutations were not detected in pediatric AML, and are uncommon in adult AML.

## Keywords

Acute Myeloid Leukemia; *IDH1*; isocitrate dehydrogenase; pediatric AML; *NPM*; *FLT3*

## INTRODUCTION

The *IDH1* gene encodes isocitrate dehydrogenase 1, a citric acid cycle enzyme that catalyzes the oxidative decarboxylation of isocitrate to alpha-ketoglutarate. Function-altering mutations of *IDH1* codon 132 occur frequently (>70%) in low grade gliomas and secondary glioblastoma multiforme.<sup>1-5</sup> Screening for *IDH1*<sup>R132</sup> mutations has been performed in a variety of cancers; such mutations are rare in tumors outside the CNS.<sup>3,4,6</sup> Although *IDH1* mutations were found in 1.7% of patients with B-cell ALL, no mutations were reported in 145 AML patients screened.<sup>4,6</sup> Recently, whole-genome sequencing performed on tumor as well as germline DNA from an AML patient with known *NPMc+* and *NRAS* mutations led to the discovery of an *IDH1*<sup>R132</sup> mutation;<sup>7</sup> subsequent analyses of 187 patients with AML treated on multiple clinical protocols detected *IDH1*<sup>R132</sup> mutations in 8.5% (16/188) of adult patients, corresponding to 16.3% (13/80) of those with normal cytogenetics. The study further suggested a possible adverse outcome for those with mutated *IDH1* and wild-type *NPM*. To define the prevalence and potential clinical implications of *IDH1* mutations in unselected AML patients, we genotyped diagnostic marrow DNA obtained from 1 pediatric and 3 adult AML trials for identification of *IDH1* exon 4 mutations.

## PATIENTS, MATERIALS, AND METHODS

Newly diagnosed patients with de novo AML enrolled on either the pediatric AML trial COG-AAML03P1, or 1 of 3 SWOG adult AML trials (SWOG-9031, SWOG-9333, and SWOG-9500), were eligible for this study. SWOG-9333 patients randomized to induction chemotherapy with mitoxantrone and etoposide were excluded, as the results of that trial demonstrated somewhat poorer outcomes for the mitoxantrone / etoposide treatment arm.<sup>8</sup> COG-AAML03P1 enrolled patients aged 0 to 21 years; SWOG-9031 and SWOG-9333 enrolled “older” adult AML patients (> 55 years), while SWOG-9500 enrolled “younger” adult AML patients (18-55 years). Patients with acute promyelocytic leukemia (M3 AML) were excluded. Details of these clinical trials have been published.<sup>8-11</sup> Genomic DNA extracted from diagnostic marrow specimens was available from 257 COG and 274 SWOG patients. In accordance with the Declaration of Helsinki, consent was obtained from all study participants for treatment on their respective clinical trials and evaluation of tissue samples. Institutional review board approval was obtained from the Fred Hutchinson Cancer Research Center prior to mutation analysis, and this study was approved by the Myeloid Disease Biology Committees of the COG and SWOG.

### *IDH1* Molecular Analysis

We amplified the entire coding region of *IDH1* exon 4, containing codon R132, by performing polymerase chain reaction (PCR) using the following primer pair: IDH1F (5'-CTCAGAGCCTTCGCTTTCTG-3') and IDH1R (5'-GCAAAATCACATTATTGCCAAC-3'). Thermocycler conditions were as follows: 94°C for 5 minutes; 35 cycles at 94°C for 30 seconds, 58°C for 45 seconds, and 72°C for 1 minute; and a final extension step at 72°C for 7 minutes. Purified PCR products were sequenced using the BigDye<sup>®</sup> Terminator sequencing reaction and run on an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA).

### Statistical Methods

This study included all eligible patients on studies COG-AAML03P1, SWOG-9031, SWOG-9333, and SWOG-9500 for whom DNA was available for analysis. Data regarding clinical characteristics and treatment outcomes were collected and evaluated according to the standard practices of the COG and SWOG for their respective studies. Overall survival (OS) was calculated as time from study entry until death, with observation censored at the date of last contact for patients last known to be alive. Relapse-free survival (RFS) was calculated as time from complete remission (CR) until relapse or death, whichever occurred first, with observation censored at the date of last contact for patients last known to be alive without report of relapse.

## RESULTS

### Patient Population

Cryopreserved diagnostic specimens were available from 257 (76%) of the 340 eligible pediatric patients enrolled on COG-AAML03P1, and 274 (56%) of the 487 eligible adult patients enrolled on SWOG-9031, SWOG-9333 and SWOG-9500. Demographics,

laboratory and clinical characteristics, and outcome for those with and without available specimens were compared. SWOG patients included in this study had significantly higher ( $P < 0.001$ ) median diagnostic white blood cell (WBC) count ( $29.8 \times 10^9/L$  vs.  $4.6 \times 10^9/L$ ), peripheral blood blast percentage (42% vs. 12%), and marrow blast percentage (71% vs. 59%) than the 213 patients without available samples. The two groups did not differ significantly in induction CR rate, although SWOG patients included in this study had somewhat improved OS (17% vs. 9%,  $P = 0.094$ ) and RFS (22% vs. 8%,  $P = 0.012$ ) compared to adult patients whose samples were not studied.

### **IDH1 Mutation Analysis**

We aimed to determine the prevalence and clinical implications of *IDH1* mutations in both pediatric and adult AML. Of the 531 (257 COG, 274 SWOG) diagnostic AML specimens screened, 70 contained nucleotide substitutions involving 3 distinct codons (Figure 1). R132 missense mutations were identified in 12 patients, in which arginine was replaced with 1 of 4 different amino acids (8 patients with R132H, 2 with R132L, 1 with R132G and 1 with R132C). In addition, 58 patients had silent G105G polymorphisms; 11 patients with G105G harbored an additional, novel V71I sequence alteration (Figure 1). Overall, *IDH1*<sup>R132</sup> mutations were found in 12/531 (2.3%, 95% CI 1.2-3.9%) patients in our study cohort, while *IDH1*<sup>V71I,G105G</sup> was present in 11/531 patients (2.1%, 95% CI 1.0-3.7%).

**Pediatric AML**—In the COG cohort (median age 9.8 years), 37 nucleotide substitutions were identified in 30/257 (11.7%; 95% CI, 8.0%-16.2%) patients tested. G105G silent mutations accounted for 30 of these, and 7 patients (2.7%, 95% CI 1.1%-5.5%) harbored the additional V71I alteration. Previously described *IDH1*<sup>R132</sup> mutations were not detected (0.0%, 95% CI 0.0%-1.4%). Cytogenetic data were available for 235 (91%) patients, of whom 47 (20%) lacked abnormalities, 62 (26%) had core-binding factor (CBF) translocations, and 49 (21%) had 11q23 abnormalities. Since function-altering mutations in neoplasia have only been previously described at codon R132 of *IDH1*, we sought to determine whether V71I might represent a novel germline polymorphism. Remission specimens from 3 patients with *IDH1*<sup>V71I,G105G</sup> were available and were examined by direct sequencing. In 3/3 cases, *IDH1*<sup>V71I,G105G</sup> was detected in paired germline DNA obtained from remission specimens, confirming that *IDH1*<sup>V71I,G105G</sup> is a naturally occurring polymorphism. Thus, disease-associated mutations were not identified in childhood AML.

**Adult AML**—In the 274 adult AML patients (median age 63 years) treated on the 3 SWOG clinical trials, we detected 44 nucleic acid substitutions in 40 patients; 12 patients (4.4%, 95% CI 2.3-7.5%) harbored R132 mutations, 4 patients (1.5%, 95% CI 0.4-3.7%) had both V71I and G105G, and 24 patients (8.8%, 95% CI 5.7-12.8%) had G105G alone. Characteristics of the adult AML patients are shown by R132 mutation status in Table 1. Evaluation of *IDH1* mutations by age category in adult AML demonstrated R132 mutations were detected in 3/82 (3.7%, 95% CI 0.8-10.3%) of younger adults enrolled on SWOG-9500, compared to 9/192 (4.7%, 95% CI 2.2-8.7%) of older adults enrolled on SWOG-9031 and SWOG-9333.

Cytogenetic data were available for 203 (74%) SWOG patients, 85 (41.9%) of whom had normal karyotype. Six of ten *IDH1*<sup>R132</sup> patients with known cytogenetic data had normal karyotype. The prevalence of *IDH1*<sup>R132</sup> mutations in patients with a normal karyotype was 7.1% (6/85, 95% CI 2.6-14.7%). Clonal cytogenetic abnormalities in patients with *IDH1*<sup>R132</sup> included +8, +13, and inv(3) in one patient each (Table 1). We evaluated 255 adult patients for *FLT3/ITD*,<sup>13, 14</sup> which was detected in 6/12 (50.0%) patients with *IDH1*<sup>R132</sup> mutations, and in 83/243 (34.2%) patients without such mutations. We then screened patients with mutated *IDH1*<sup>R132</sup> for other AML-associated mutations. *NPM* exon 12 mutations were common, occurring in 9/12 (75.0%) of patients with *IDH1*<sup>R132</sup> mutations; *NRAS* exon 1 mutations were detected in 2/12 (16.7%) patients, while *CEBPA* bZip mutations were detected in 1/12 patients (8.3%), and *WT1* mutations were not detected. Nine (75.0%) of the 12 adult AML patients with *IDH1*<sup>R132</sup> achieved CR; 7 of these patients have relapsed or died and 2 remain in long-term remission, (9+ years), for an estimated median RFS of 19 months (95% CI 8-36 months). Two patients (16.7%) with *IDH1*<sup>R132</sup> had resistant disease (RD) and died within 30 days, while one died at day 19 of induction toxicity and had indeterminate response to induction.

## DISCUSSION

In summary, *IDH1*<sup>R132</sup> mutations were detected in 12/531 (2.3%) of all AML specimens screened, while another 11 patients (2.1%) harbored the *IDH1*<sup>V71I,G105G</sup> polymorphism. The R132 mutation was not detected in the pediatric cohort; its prevalence was 4.4% in adult AML patients. *IDH1*<sup>R132</sup> mutations occurred most frequently in patients with normal karyotype, and showed significant overlap with *FLT3/ITD* and *NPMc*. Interestingly, *IDH1*<sup>R132</sup> mutations were not detected in any of the 257 pediatric patients tested, and were in fact observed only in patients 34 years of age or older. A similar phenomenon has been observed in brain tumors, where, despite the high mutation rate in adult gliomas and supratentorial PNETs, *IDH1*<sup>R132</sup> mutations are almost never observed in pediatric tumors of similar histology.<sup>2, 4, 12</sup> These somatically acquired mutations may uncover a biologically distinct, temporally-acquired pathway to neoplasia that is exclusive to adult patients.

Given the low prevalence of *IDH1* mutations, our study did not have adequate power to determine either significant differences in outcome between patients with and without such mutations, or the interaction between *IDH1* mutations and other disease-associated mutations. It is notable that 7 of 12 patients with *IDH1*<sup>R132</sup> mutations died of refractory disease or after relapse from CR; one additional patient died of induction toxicity, and two patients died in CR (one of consolidation toxicity, the other of stroke after 8 years). Nonetheless, the small number of patients with *IDH1* mutations in our study appeared to have higher rates of CR, OS, and RFS than their wild-type counterparts. As *IDH1* mutations are highly associated with *NPM* exon 12 mutations, any improvement in outcome in the *IDH1*-mutated cohort may be related to the favorable prognostic effect of harboring *NPMc*. Larger prospective studies will need to be performed to define the prognostic relevance of *IDH1* mutations in AML, as well as their interaction with other known molecular markers of prognosis.

Finally, the role of *IDH1* mutations in AML pathogenesis is not clear. Recent data showed that *IDH1*<sup>R132</sup> mutations result in dominant-negative inhibition of isocitrate dehydrogenase catalytic activity, resulting in the induction of the HIF-1 $\alpha$  pathway.<sup>15</sup> HIF signalling has been linked to the regulation of myeloid cell survival,<sup>16</sup> and HIF-1 $\alpha$  expression is increased by activation of the PI3K/Akt pathway,<sup>17</sup> a signaling cascade known to be dysregulated in AML. It remains to be determined whether *IDH1* mutations contribute to myeloid pathogenesis via HIF-1 $\alpha$  activation, or whether these mutations are simply secondary events unrelated to the leukemic process. If the former is true, then antineoplastic agents currently in development that target HIF-1 $\alpha$ <sup>18</sup> may someday provide a novel therapeutic approach for AML patients with *IDH1*<sup>R132</sup> mutations.

The discovery by Mardis et al<sup>7</sup> of an AML-associated mutation in *IDH1*, a metabolic enzyme not previously implicated in leukemia, highlights the tremendous potential of whole-genome sequencing to further elucidate the molecular biology of AML. As next-generation sequencing technologies become more readily available, their application to the various cytogenetic and molecular subtypes of this heterogeneous disease will allow the discovery of novel genomic alterations. These discoveries should improve disease classification and risk stratification, enhance our understanding of leukemogenesis, and provide novel targets of therapy. Cooperative groups such as COG and SWOG will be provided the opportunity to determine the prevalence and prognostic significance of mutations in newly-recognized genes through retrospective studies, with the ultimate goal of identifying clinically-relevant mutations for upfront risk identification and therapy allocation in future trials.

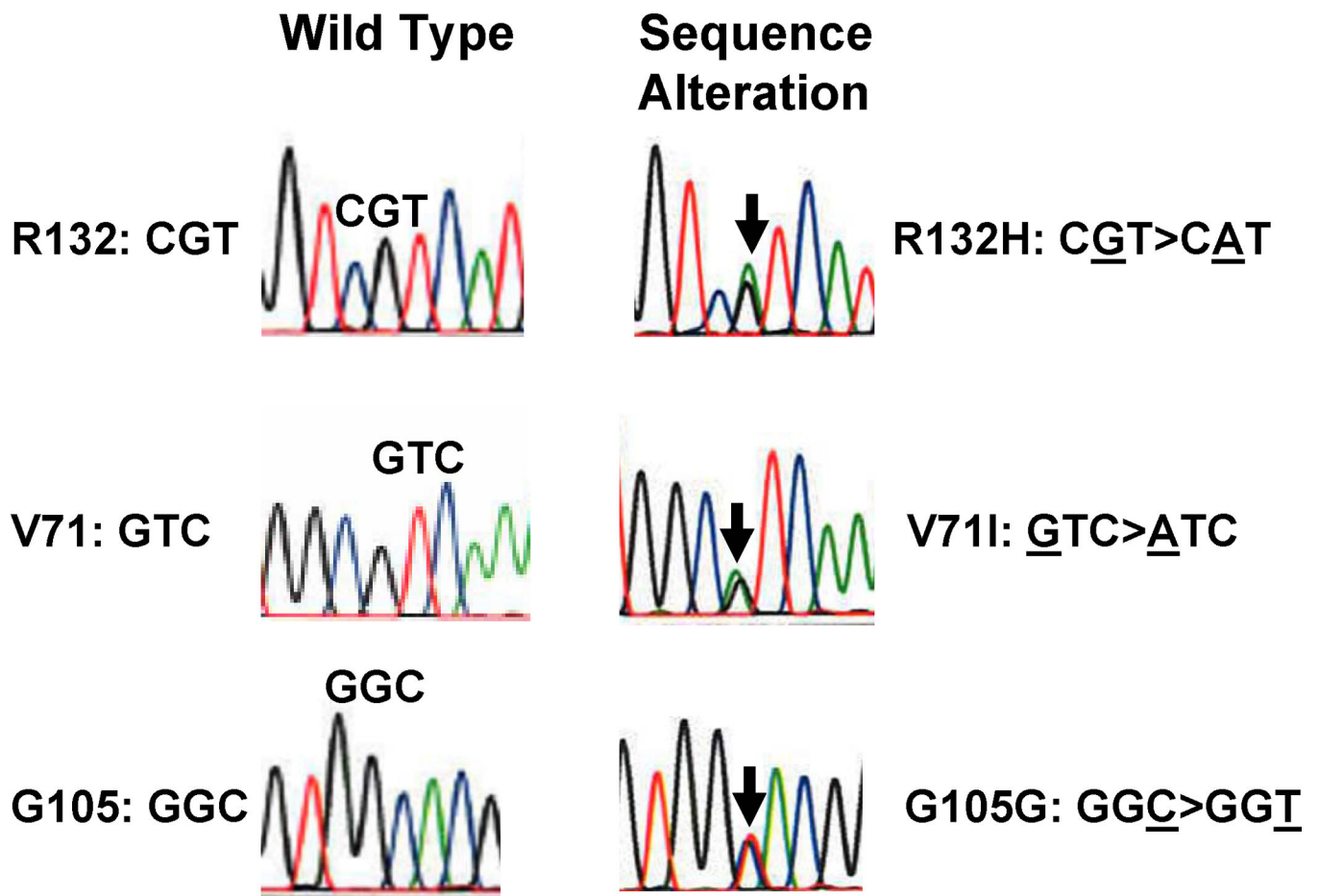
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**Figure 1. Representative sequence alterations of the *IDH1* gene, as shown on electropherogram**  
 Wild-type and heterozygous mutant sequences are shown for the affected amino acid residues: R132, V71, and G105.



**Table 1**

Characteristics of 274 adult AML patients, by R132 mutation status.

	R132 Mutation (N =12)			R132 Wildtype (N = 262)			
	Patients	%	Patients	%	Patients	%	<i>P a</i>
Gender							
Female	4	33%	122	47%			0.56
Male	8	67%	140	53%			
Race							
Asian	1	8%	6	2%			0.34
Black	0	0%	26	10%			
Native American/ Alaskan	0	0%	1	0.4%			
White	11	92%	226	86%			
Unknown	0	0%	3	1%			
Normal	6	60%	79	41%			0.33
+8	1	10%	27	14%			1.00
-7/del(7q)	0	0%	16	8%			1.00
CBF abnormality	0	0%	22	11%			0.61
Abn(11q23)	0	0%	9	5%			1.00
Other clonal abnormalities	2	20%	44	23%			1.00
Nonclonal abnormality	1	10%	6	3%			0.30
FLT3/ITD (12 R132+, 243 WT)							
Present	6	50%	83	34%			0.35
Absent	6	50%	160	66%			
	Median	Min-Max	Median	Min-Max			<i>P a</i>
Age (yrs)	61	34-81	63	18-88			0.59
BM Blasts (%; 11 R132+, 246 WT)	80%	38-99%	71%	0-99%			0.59
WBC ( $\times 10^9/L$ )	59.2	1.2-98.2	29.1	0.7-298.0			0.19
Circ. Blasts (%; 10 R132+, 255 WT)	48%	4-99%	42%	0-99%			0.52
Platelets ( $\times 10^9/L$ ; 11 R132+, 262 WT)	53	10-189	57	2-1052			0.98
Hemoglobin (g/dL; 12 R132+, 256 WT)	9.5	6.0-12.2	9.2	4.3-14.3			0.67

		R132 Mutation (N =12)		R132 Wildtype (N = 262)			
		Patients	%	Patients	%	95% CI	p <sup>a</sup>
		Estimate	95% CI	Estimate	95% CI		
Response to induction chemotherapy (%)	Complete response	75%	43-95%	51%	45-57%		0.14
	Resistant disease	17%	2-48%	29%	24-35%		0.52
	Other, not evaluable	8%	---	20%	---		
Overall survival at 5 years (%)		25%	5-57%	17%	12-21%		0.55
Relapse-free survival at 5 years (%)		33%	7-70%	22%	14-29%		0.51

<sup>a</sup>P-values based on Fisher's exact test (sex, karyotypes, FLT3/ITD, response); Pearson chi-squared test (race; exact calculation); Wilcoxon rank-sum test (continuous variables); or logrank test (overall and relapse-free survival). Results based on all 274 patients except where indicated.

<sup>b</sup>Total of karyotype categories in R132 Wildtype column exceeds 193 because some patients have multiple clonal abnormalities.

TABLE 2

Clinical profile of 12 adult AML patients with *IDH1/R132* mutations.

Patient	1	2	3	4	5	6	7	8	9	10	11	12
Mutation	R132H	R132H	R132H	R132H	R132L	R132H	R132H	R132H	R132G	R132L	R123H	R132C
Sex	F	M	M	M	F	F	F	M	M	M	M	M
Age at diagnosis	65	60	64	40	57	62	34	71	64	58	81	52
WBC ( $\times 10^9/L$ )	31.9	98.2	81.7	56.3	62.1	21.9	68	63.4	55	94	1.2	6.3
BM blasts (%)	80	82	94	82	NA	38	99	44	71	64	80	40
Cytogenetics <sup>1</sup>	Nonclonal abn <i>t</i>	Normal	NA	Normal	NA	inv(3)	Normal	Normal	Normal	Normal	+13	+8
<i>NPM</i> exon 12 mutation	+	+	+	+	+	+	+	+	+	+		
<i>FLT3/ITD</i>	+	+	+	+	+					+		
<i>NRAS</i> exon 1 mutation						+	+					
<i>CEBPA</i> bZip mutation	+											
Response to induction chemotherapy	CR	CR	Ind <sup>2</sup>	CR	CR	CR	CR	CR	CR	RD	RD	CR
Relapse-free survival <sup>3</sup>	Relapse d14	Relapse d399	NA <sup>2</sup>	Died in CR d91	Alive CCR d3696	Relapse d255	Alive CCR d3451	Died in CR d2900	Relapse d568	NA <sup>2</sup>	NA <sup>2</sup>	Relapse d1106
Overall survival <sup>4</sup>	Died d113	Died d688	Died d19	Died d139	Alive d3727	Died d496	Alive d5497	Died d2923	Died d1203	Died d20	Died d22	Died d1518

<sup>1</sup> Normal cytogenetics is defined by the absence of clonal abnormalities. Patient #2 had a single nonclonal abnormality: +18 in one of 50 metaphase cells examined.

<sup>2</sup> Ind = indeterminate (patient died before response assessment); NA = not applicable.

<sup>3</sup> For relapse-free survival, time to event (relapse or death in CR) or last contact is measured from date of CR.

<sup>4</sup> For overall survival, time to death or last contact is measured from date of entry into study.