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Lack of association of *IDH1*, *IDH2*, and *DNMT3A* mutations with outcome in older patients with AML treated with hypomethylating agents

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The discovery of somatic mutations within genes primarily involved in epigenetic regulation has been one of the major success stories of the genomics era. Within the past 5 years, this novel class of recurrent mutations has been elucidated, which consists of critical regulators of post-translational events including DNA methylation and histone modification. The updated list of mutated epigenetic modifiers in myeloid malignancies now includes the genes isocitrate dehydrogenase 1 (*IDH1*)(1), *IDH2*(2), tet methylcytosine dioxygenase 2 (*TET2*)(3), enhancer of zeste homologue 2 (*EZH2*)(4), mixed lineage leukemia (*MLL*)(5), additional sex combs-like 1 (*ASXL1*)(6) and DNA methyltransferase 3A (*DNMT3A*)(7, 8)

Importantly, many of these epigenetic mutations have subsequently been identified to have prognostic importance for AML patients, particularly within the sizeable cohort of patients with diploid karyotype or otherwise intermediate-risk cytogenetics, and specifically within those intermediate-risk patients with wild-type FMS-related tyrosine kinase 3 (*FLT3*) status. *DNMT3A* mutations, for instance, identified in approximately 20% of adult AML patients, have been shown to lead to both an increased risk of relapse and decreased overall survival (OS), correlate with advanced age and a higher white blood cell count at diagnosis, and are

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CD reviewed and analyzed data and wrote the manuscript.

KP and RL performed the mutational analysis.

SP collected and analyzed the data.

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associated with mutations in *FLT3*, nucleophosmin 1 (*NPM1*) and *IDH1*.(7–10) *TET2* mutations, with a frequency of 7 to 20% in adult AML, also appear to confer an adverse prognosis in intermediate-risk AML.(11, 12) *IDH1* and *IDH2* mutations, taken together, occur in AML with an estimated prevalence of 10–30%, also occurring at an increased frequency in patients with normal diploid karyotype (CN-AML), older age and *NPM1* mutations.(1, 2, 13) The prognostic significance of *IDH* mutations has been less apparent, perhaps related to a difference in prognosis attributed to the three different allelic mutations observed in AML; namely *IDH1*-R132, *IDH2*-R140 and *IDH2*-R172. While *IDH1*-R132 and *IDH2*-R172 mutations appear to have a negative or null clinical impact within the CN-AML patient cohort,(14) *IDH2*-R140 mutations or any *IDH* mutation in conjunction with an *NPM1* mutation rather unexpectedly confers a favorable outcome in otherwise intermediate-risk AML patients.(10, 15)

Despite the clinically significant prognostic information gained through identification of epigenetic mutations, less insight is currently available to determine how these mutations should affect therapeutic decisions. Recent retrospective data in adults aged 18–60 with *de novo* AML has notably suggested that the dose-intensity of induction therapy may be especially important for AML patients with *DNMT3A*, *NPM1* or 11q23 abnormalities involving *MLL*.(10) Nonetheless, AML is primarily a disease of the elderly, with a median age at diagnosis above 65 and with a median survival of less than one year in elderly patients receiving treatment.(16) Given the poor overall response and often increased toxicity associated with intensive chemotherapy in elderly AML, much attention has been paid to the potential role of the hypomethylating agents (HMAs), 5-azacitidine and decitabine, as front-line therapy for elderly patients with AML. With this approach, an improved median survival of 24.5 (versus 16 months with conventional care) was seen in the subgroup of 113 elderly patients with AML receiving azacitidine in the AZA-001 trial.(17) An improved remission rate (CR or CRp) of 18% and median survival of 8 months was seen in a multicenter Phase III study of front-line decitabine versus standard of care (supportive care or low-dose cytarabine) for older patients with AML,(18) suggesting that therapy with HMAs may be beneficial for elderly patients with AML.

Whether there is a relationship between patient response to HMA therapy based on the presence or absence of mutations with epigenetic impact is currently unknown. In one retrospective review by the Group Francophone des Myelodysplasies, the presence of *TET2* mutations in 13 of 86 patients (15%) with MDS or AML with 20–30% marrow blasts treated with azacitidine correlated with a higher overall response rate (ORR).(19) This improved ORR, however, did not translate into a benefit in either response duration or overall survival. As most epigenetic mutations are rare in pediatric AML but occur with increased frequency with increased age, this relationship is particularly important to define among the HMA-treated elderly patients.

To investigate the utility of epigenetic mutations as a marker for the effectiveness of epigenetically-targeted therapy in elderly AML, we herein evaluate the association of *IDH1*, *IDH2*, and *DNMT3A* mutations with clinical outcome, in AML patients over 60 years of age who were treated with front-line HMA therapy.

From 2000 to 2010, 68 patients aged sixty years or older with a diagnosis of AML treated with front-line hypomethylating agent therapy out of an eligible cohort of 110 patients were included. Patients were chosen based on the availability of remaining research samples for epigenetic mutation analysis. Patients received therapy on one of seven clinical protocols of HMA-therapy, including decitabine alone (n=24), decitabine + valproic acid (n=14), azacitidine + ATRA + valproic acid (n=20), azacitidine + vorinostat (n=4), azacitidine + valproic acid (n=3), azacitidine + low-dose cytarabine (n=2) and decitabine + vorinostat (n=1). All patients signed informed consent following institutional guidelines and in accordance with the Declaration of Helsinki. This study was approved by the University of Texas MD Anderson Cancer Center Institutional Review Board. Clinical information of all patients is provided in Table 1, including patient age, laboratory parameters at diagnosis, cytogenetic analysis and presence of FLT3, NPM1 and RAS mutations at AML diagnosis.

IDH1, *IDH2*, *DNMT3A*, *NPM1*, *FLT3* and *RAS* mutation analysis: Exon 4 mutations of codon R132 of *IDH1* and codon R172 and R140 of *IDH2* were detected using polymerase chain reaction (PCR) amplification followed by Sanger sequencing using previously described methodology and PCR primers from Integrated DNA Technologies, Coralville, IA.(20) Mutations in exon 12 of *NPM1* were detected using PCR amplification followed by capillary gel electrophoresis using previously published methodology.(20) Mutation testing of codons 12, 13 and 61 of *KRAS* and *NRAS* were performed using pyrosequencing and FLT3 by PCR followed by capillary gel electrophoresis as previously described.(20, 21) *DNMT3A* mutations were evaluated by high-resolution melting (HRM) analysis of exons 9, 10, 15 to 19, and 21 to 23, and positive results were confirmed by Sanger sequencing.

Patient characteristics are summarized using median (range) for continuous variables and frequency (percentage) for categorical variables. Categorical variables were compared using the χ^2 or Fisher's exact test, and continuous variables using the Wilcoxon Rank-Sum, and statistical significance was defined as a *p* value of < 0.05. Event-free survival (EFS) and overall survival (OS) were based on the Kaplan-Meier method, with differences compared between groups by the long-rank test. OS was measured as the time from date of treatment initiation to death or date of last follow-up (censored). EFS was defined as the time interval between date of initiation of treatment and date of treatment failure, relapse, death, or date of last follow-up (censored). The predictive effects of mutation status and other patient characteristics on OS and EFS were examined using univariate and multivariate Cox proportional hazards models. All statistical analyses were conducted in SAS 9.0.

Clinical and disease-specific characteristics of the patients are detailed in Table 1. The median age of the treated patients was 72 years of age, with a range of 60 to 83 years. 36 patients (53%) had a diagnosis of *de novo* AML; the remainder had secondary or therapy-related AML. By European LeukemiaNet (ELN) cytogenetic and molecular classification system(22), 29 patients (43%) had unfavorable risk, 20 patients (29%) were of intermediate risk, one had ELN favorable risk disease, and 18 (26%) were unknown. Of note, nineteen patients (28%) had diploid cytogenetics (CN-AML).

Overall, 11 patients (16%) had *IDH1* or *IDH2* mutations; no patient had both an *IDH1* and *IDH2* mutation present. Of the 11 *IDH* mutant patients, 3 were *IDH1*-R132 mutated, 7 were

IDH2-R140 mutated, and one patient had an *IDH2*-R172 mutation. Ten patients (15%) had *DNMT3A* mutations, and seven of these 10 patients had mutations affecting arginine codon 882 (R882-*DNMT3A*). Five patients (7%) had both an *IDH1/2* and *DNMT3A* mutation present. The presence of *IDH1/2* mutations were associated with a diploid karyotype ($p=0.03$) and the presence of *NPM1* mutations ($p=0.02$). *DNMT3A* mutations were not associated with specific cytogenetics, *FLT3*, or *RAS* mutations, but were also significantly correlated with *NPM1* mutations ($p=0.006$). Overall, eight patients had *FLT3* mutations (seven with *FLT3*-ITD and one with *FLT3*-D835) and four patients had *RAS* mutations.

All patients were treated with hypomethylating agent therapy, decitabine in 39 (57%) and azacitidine in 29 (43%). 42 patients (62%) also received concomitant histone deacetylase inhibitor (HDACI) therapy with either vorinostat ($n=5$) or valproic acid ($n=37$). Overall, 17 patients (25%) achieved a CR; neither the presence of *IDH1/2* mutations nor of *DNMT3A* mutations (or both) were associated with the achievement of CR (Table 2). With a median duration of follow-up of 60 months, median EFS was 3 months for all patients and median OS was 7 months. *IDH1/2* mutations were not associated with an impact on either EFS ($p=0.29$) or OS ($p=0.14$) [Supplemental Figure 1]. Similarly, *DNMT3A* mutations were not associated with an effect on EFS ($p=0.21$) or OS ($p=0.58$) [Supplemental Figure 2]. When any epigenetic mutation (*IDH1*, *IDH2* or *DNMT3A* mutation, $n=16$) was compared to the lack of an epigenetic mutation ($n=52$), there was likewise no association with EFS ($p=0.6$) or OS ($p=0.97$) [Figure 1].

Overall, 16 patients (24%) died during induction, which was defined as a death before the protocol-defined response assessment (induction death, Table 2). Since increased toxicity from histone deacetylase therapy has been described in the clinical setting, we additionally examined patient outcome based on whether a patient did or did not receive HDACI during HMA-therapy. There was no difference in EFS ($p=0.51$) or OS ($p=0.96$) in our cohort based on the addition of HDACI therapy, with a median EFS of 13 weeks in patients receiving HDACI and 15 weeks in those receiving HMA-therapy alone [Supplemental Figure 3].

Treatment of the older AML patient requires a personalized and risk-adapted approach.(23) Age, comorbidities, cytogenetics, and emerging molecular data need to be analyzed and incorporated into a therapeutic decision that is most appropriate for each individual patient, especially as there is no standard of care for this population. In some patients, intensive therapy with a cytarabine and anthracycline-based regimen is appropriate, and as reported by Juliusson et al., in a selected population of elderly patients, intensive chemotherapy can improve both short and long-term survival compared with palliative approaches.(24) HMA therapy with 5-azacitidine and decitabine is frequently used for older patients with AML who are deemed unfit to receive standard chemotherapy, and decitabine has recently been approved by the European Medicines Agency (EMA) for this front-line indication. As therapy with HMAs is generally well-tolerated with predominantly myelosuppressive adverse events, HMAs are an important therapeutic alternative for elderly patients with AML who are unable to tolerate standard intensive chemotherapy regimens.

We evaluated the utility of front-line HMA therapy in older patients with AML, based on the presence or absence of relevant mutations with known epigenetic impact. This is a

fundamental research question, for it follows that if these mutations were found to confer a particularly favorable response to HMA-therapy, epigenetic therapy could be offered to patients using a personalized therapeutic approach to maximize patient response. Unfortunately, our study failed to identify a direct correlation between the presence of various epigenetic mutations and response, event-free or overall survival in this cohort of older patients treated with front-line HMA therapy.

Certain limitations should be taken into account during interpretation of this study. First, this was a retrospective review including several different treatment regimens, several of which additionally incorporated the use of histone deacetylase inhibitors (HDACIs). While HDACIs are thought to have a similar epigenetic mechanism of action that may also be beneficial to patients with epigenetically active mutations, their clinical utility has been limited by their significant toxicity, particularly the first generation HDACIs. Valproic acid, one such first generation HDACI, was received by the majority (37/68, or 54%) of patients in this analysis. However, there was no evidence that patients receiving HDACI therapy had increased toxicity or worse outcomes [Figure 4]. Death during induction was observed in 16 of 68 patients (24%), of whom 10 (62.5%) received HDACI therapy. This corresponds with the 62% of patients receiving HDACI therapy overall. Thus, we believe it unlikely that increased therapy-related toxicity from the combination of HMAs and HDACIs was responsible for the limited benefit seen in our cohort.

Complete molecular characterization using the currently updated list of mutations with epigenetic importance was not possible in this retrospective study, which most conspicuously lacks *TET2* mutation analysis. While this is a *bona fide* limitation to our analysis, our study includes the largest cohort of elderly AML patients treated with HMA-therapy described to date, and provides important information to clinician and researcher alike with regards to epigenetic therapy.

Furthermore, several important conclusions and topics for future research are raised. The lack of an inferior overall survival in patients with a *DNMT3A* mutation treated with HMAs is noteworthy. This is counter-intuitive as it could be hypothesized that such mutations may interfere with response to HMA therapy. However, our data may provide support to the notion that *DNMT3A* mutations can mechanistically lead to impaired DNA methyltransferase activity, and therefore lead to an improved response to HMA therapy.(25) Additional prospective analysis of *DNMT3A* mutations and HMA-therapy in larger cohorts is both warranted and ongoing at our institution.

As AML is fundamentally a heterogeneous disease arising from the acquisition of diverse clonal alterations leading to a malignant phenotype, and somatic mutations in genes essential to epigenetic regulation have been recurrently identified in AML patients, it follows that aberrant epigenetic patterns are likely an important mechanism of leukemic transformation. Ultimately, however, our study supports the increasingly evident principle that epigenetic mutations, methylation profiles and hypomethylating agents in AML are not related by any simple algorithm. There appears to be a particular link between methylation signatures and certain mutations, such as the hyper-methylated phenotype reported with *IDH1/2* and *TET2* mutations, which subsequently leads to decreased gene expression of genes critical for

myeloid development.(26) Other aberrations such as *DNMT3A* mutations are not obviously correlated with any robust changes in gene expression or specific methylation profiles.(7, 27) Whether effective HMA therapy is ultimately related to re-expression of critically silenced genes, and whether this response is conditional upon the presence of specific epigenetic mutations, remains a clinically and scientifically crucial question that will require larger prospective studies for a definitive answer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Table 1

Clinicopathologic characteristics of patient cohort (n=68)

Patient Characteristics	Total	IDH1/IDH2 WT	IDH1/IDH2 Mutated	P value	DNMT3A WT	DNMT3A Mutated	P value
Number	68	57	11		58	10	
Median age, yrs [Range]	72 [60–83]	72 [60–83]	74 [63–79]	NS	72 [60–83]	73 [63–77]	NS
Median WBC x 10 ⁹ /L [Range]	7.2 [1.2–79.1]	6.4 [1.2–79.1]	8.1 [1.2–30.5]	NS	7.2 [1.2–79.1]	6.2 [1.9–37.0]	NS
Median Plt x 10 ⁹ /L [Range]	52 [2–470]	61 [3–470]	26 [2–61]	0.004	59 [2–470]	36 [19–113]	NS
AML type				NS			NS
De Novo	36	30	6		32	4	
Secondary	17	15	2		15	2	
Therapy-related	15	12	3		11	4	
Cytogenetics				0.026			NS
+8	5	5	0		5		
–5/5q–	5	4	1		5		
–5 and –7	12	11	1		12		
–7/7q–	10	8	2		7	3	
Diploid	19	13	6		14	5	
IM	3	2	1		3		
Miscellaneous	14	14			12	2	
FLT3				NS			NS
ITD	7	1	2		6	1	
D835	1	5			1		
Wild-type	56	47	9		47	9	
Not done	4	4			4		
NPM1				0.023			0.006
Mutated	9	5	4		5	4	
Wild-type	53	46	7		48	5	
Not done	6	6			5	1	

Patient Characteristics	Total	IDH1/IDH2 WT	IDH1/IDH2 Mutated	P value	DNMT3A WT	DNMT3A Mutated	P value
RAS				NS			NS
Mutated	4	1	3		3	1	
Wild-type	52	7	45		44	8	
Not done	12	3	9		11	1	
Cytogenetics				0.007			0.041
ELN Favorable	1	0	1		0	1	
ELN Int-1	8	4	4		6	2	
ELN Int-2	12	11	1		9	3	
ELN Adverse	29	26	3		27	2	
Unknown	18	16	2		16	2	

Table 2

Response by mutational status in patient cohort (n=68)

Response to therapy	Total	IDH WT	IDH Mutated	P value	DNMT3A WT	DNMT3A Mutated	P value
	N (%)	N (%)					
CR	17 (25)	16 (28)	1 (9)	NS	13 (22)	4 (40)	NS
CRp	1 (1.5)	1 (2)			1 (2)		
PR	1 (1.5)	1 (2)			1 (2)		
ID	16 (24)	12 (21)	4 (36)		14 (24)	2 (20)	
Resistant	33 (49)	27 (47)	6 (55)		29 (50)	4 (40)	

CR indicates complete remission; CRp indicates CR with incomplete platelet recovery; PR indicates partial response; ID indicates induction death; WT indicates wild-type; NS indicates not significant.