Mutational correlates of response to hypomethylating agent therapy in acute myeloid leukemia

Acute myeloid leukemia (AML) is an aggressive malignancy with median age at diagnosis of 67 years,[1] stressing the importance of developing treatments that are both effective and tolerable in elderly patients. Hypomethylating agents (HMAs) have been extensively studied in AML, typically in older adults who are deemed to be unfit for standard induction chemotherapy, demonstrating improved complete response (CR) rate and trends toward improvement in overall survival (OS) compared with conventional care regimens in the phase III setting with less favorable responses in the relapsed/refractory setting.[2,3] Genome-wide studies of AML patients have revealed that 44% of patients with *de novo* AML harbor mutations in genes affecting DNA-methylation, including *DNMT3A* (26%), *IDH1/2* (20%), *TET2* (8%), and *WT1* (6%).[4] The presence of these mutations has been suggested to have therapeutic implications in small, retrospective series.[5-8] Using data from two large referral centers together with previously reported data, we sought to investigate the relationship between somatic gene mutations affecting DNA methylation and HMA response in an expanded AML patient cohort. We did not observe a relationship between response to HMAs and *IDH1/2* and *TET2* mutations. We identified *DNMT3A* mutations to predict response to HMAs in patients treated in the frontline setting [odds ratio (OR), 3.12; *P*=0.001], but not in the total cohort when including relapsed/refractory patients (OR 1.72; *P*=0.23).

This is a dual institution, retrospective study. Permission to review medical records was obtained by the Institutional Review Board of each participating institution. From March 2010 to December 2014, 242 patients were identified at Memorial Sloan Kettering Cancer Center (MSKCC) who had a diagnosis of AML by World Health Organization (WHO) criteria and next-gen-

Table 1. Patients' characteristics in combined MSKCC/MCC cohorts and response assessment by mutation status.

	MSKCC	MCC	Combined
Number of patients	62	21	83
DNMT3A mutants	10	4	14
IDH1/2 mutants	18 *IDH1/2* 7 *IDH1* 11 *IDH2*	7[a] *IDH1/2* 4 with *IDH1* 4 with *IDH2*	25 *IDH1/2* 11 *IDH1* 15 *IDH2*
TET2 mutants	14	4	18
NPM1 mutants	7	1	8
FLT3 mutants	4 with *FLT3*-ITD 2 with *FLT3*-TKD[b]	2 with *FLT3*-ITD 2 with *FLT3*-TKD[b] 3 were not tested	6 with *FLT3*-ITD 4 with *FLT3*-TKD[b] 3 were not tested
NGS performed at time of initial AML diagnosis	53%	81%	60%
NGS performed prior to HMA initiation	68%	76%	70%
%Females	37%	57%	42%
Median age	66	73	70
Median WBC count	5.8	2.5	3.2
Median hemoglobin	9.1	9.2	9.1
Median platelet count	80	64	71
Median bone marrow blast %	42	40	40
Cytogenetic risk	37% (22/59) with poor-risk by NCCN	30% (7/21) with poor-risk by NCCN	36% (29/80) poor-risk by NCCN
Treatments received by cohort	Decitabine only: 40 Azacitidine only: 8 Decitabine alternating with sapacitabine: 4 Decitabine + thioguanine: 3 Decitabine + ruxolitinib: 2 Decitabine + plerixafor: 2 Azacitibine + sorafenib: 2 Azacitidine ± pracinostat: 1	Azacitidine only: 14 Decitabine only: 2 Azacitidine + sorafenib: 2 Azacitidine + lenalidomide: 2 Azacitidine + SGN33: 1	Decitabine only: 42 Azacitidine only: 22 Decitabine alternating with sapacitabine: 4 Azacitidine + sorafenib: 4 Decitabine + thioguanine: 3 Decitabine + ruxolitinib: 2 Decitabine + plerixafor: 2 Azacitidine + lenalidomide: 2 Azacitidine ± pracinostat: 1 Azacitidine + SGN33: 1
Overall rate of CR (CR+CRi)[c]	23%	43%	28%
Median/mean number of HMA cycles (range)	Median: 2 (1-23) Mean: 4.4	Median: 4 (1-12) Mean: 5.6	Median: 3 (1-23) Mean: 4.7
Frontline treatment *vs.* previously treated for AML	Frontline: 34 Relapsed/refractory: 32[d]	Frontline: 11 Relapsed/refractory: 11[e]	Frontline: 45 Relapsed/refractory: 43

NGS: next-generation sequencing; NA: not available; MSKCC: Memorial Sloan Kettering Cancer Center; MCC: Moffitt Cancer Center; AML: acute myeloid leukemia; HMA: hypomethylating agents; WBC: white blood cell counts; CR: complete response; CRi: incomplete blood count recovery; NCCN: National Comprehensive Cancer Network criteria. [a]One patient had both IDH1 and IDH2 mutation. [b]One patient at MSKCC and one patient at MCC had a FLT3-ITD and a FLT3-TKD mutation. [c]Response assessment as per International Working Group criteria. [d]Four patients received HMA in both frontline and relapsed/refractory settings. [e]One patient received HMA in both frontline and relapsed/refractory settings.

Table 2. Patients' characteristics in cohorts from previously published studies examining the relationship between *DNMT3A, IDH1/2*, and/or *TET2* mutations and response to hypomethylating agents.

	Itzykson *et al.*[5]	Metzeler *et al.*[8]	Dinardo *et al.*[7]	Emadi *et al.*[6]
Number of patients	86 total; 23 with AML	46	68	42
DNMT3A analyzed	N	Y	Y	N
IDH1/2 analyzed	N	Y	Y	Y
TET2 analyzed	Y	Y	N	N
%Females	35%[a]	26%	29%	21%
Median age	71[a]	74	72	76
Median WBC count (K/mcL)	3.0[a]	6.4[b]	7.2	12.3
Median hemoglobin (g/dL)	9.5[a]	NA	9.4	9.3
Median platelet count (K/mcL)	64[a]	NA	52	52
Median bone marrow blast %	12[a]	39[b]	33	57
Cytogenetic risk	15 (17%)[a] had poor-risk cytogenetics by IPSS	16 (38%) had ELN-adverse risk; cytogenetics 11 (24%) had complex karyotype	29 (43%) had ELN-adverse risk cytogenetics	12 (29%) had ELN-adverse risk cytogenetics; 11 (26%) had complex karyotype
Treatments received	Azacitidine only	Decitabine only: 39 Decitabine + bortezomib: 9	Decitabine only: 24 Decitabine + valproic acid: 14 Azacitidine + all-trans retinoic acid + valproic acid: 20 Azacitidine + vorinostat: 4 Azacitidine + valproic acid: 3 Azacytidine + low-dose cytarabine: 2 Decitabine + vorinostat: 1	Decitabine only: 32 Decitabine alternating with sapacitabine: 2 Decitabine + bortezomib: 2 Azacitidine only: 6
Overall rate of response to HMA	7/23 (30%) AML patients achieved CR (6 CR and 1 mCR)	19/46 (41%) achieved CR	18/68 (26%) achieved CR (17 CR and 1 CRp)	12/42 (29%) achieved CR (9 CR and 3 CRi)
Frontline treatment *vs.* previously reated for AML	Of 23 AML patients, 3 had previously been treated with LDAC; others treated in frontline setting	Frontline only	Frontline only	Frontline only

Y: yes; N: no; WBC: white blood cell count; IPSS: International Prognostic Scoring System; ELN: European Leukemia Net; HMA: hypomethylating agents; CR: complete response; mCR: major cytogenetic response; CRp: incomplete platelet recovery; CRi: incomplete blood count recovery; LDAC: low-dose cytarabine; NA: not available. [a]Data for the entire cohort, not just patients with acute myeloid leukemia (AML). [b]Mean value.

eration sequencing (NGS) performed. Of these, 62 evaluable patients were treated with HMAs alone or in combination. From May 2013 to October 2014, 82 patients were identified in the Moffitt Cancer Center (MCC) cohort with a diagnosis of AML by WHO criteria and NGS. HMA therapy was administered alone or in combination in 21 evaluable patients (Table 1). NGS was performed as previously described.[9,10]

A systematic search was conducted to identify studies examining the response to HMAs in patients with AML in relation to presence/absence of mutations in *DNMT3A, IDH1/2,* and/or *TET2*. Studies containing both AML and MDS patients were included provided that the number of AML patients exceeded 20 and individual patient level data for AML patients could be obtained. Studies analyzing HMAs in combination with a non-intensive agent were included; however, studies analyzing HMAs in combination with intensive induction chemotherapy were excluded. Two reviewers (CCC and DAS) independently performed literature searches using Pubmed and Ovid in order to assess study eligibility. Search terms included "acute myeloid leukemia" AND "hypomethylating agent" AND "azacitidine" AND "Vidaza" AND "Dacogen" AND "decitabine" AND "*DNMT3A*" AND "*TET2*" AND "*IDH1*" AND "*IDH2*" AND "isocitrate dehydrogenase" as Medical Subject Heading and text terms. Boolean operators (*NOT, AND, OR*) were also used in succession to narrow and widen the search. For included publications,[5-8] references and subsequent citing publications were also reviewed.

We extracted data from selected publications regarding clinical parameters and response to HMA therapy (Table 2).[5-8] For one publication containing data on both MDS and oligoblastic AML,[5] we obtained primary data from study authors to determine individual treatment responses for AML patients. Logistic regression was used to estimate the association between mutation status and CR attainment both overall and among the subset of patients receiving HMA as part of frontline therapy. To account for potential correlation among treatment centers or publications, robust estimates of the variance were obtained using the sandwich estimator when reporting confidence intervals and associated *P*-values. As a sensitivity analysis, each regression model was repeated including site/publication as a fixed effect in the model. All analyses were conducted in R (v.3.2.3, R Foundation for Statistical Computing).

A total of 83 patients from MSKCC and MCC met the

Table 3. Results of pooled analyses of MSKCC/MCC and previously published cohorts.

		Combined frontline and relapsed/refractory				Frontline only			
		N	CR	OR 95% CI	P	N	CR	OR 95% CI	P
MSKCC/MCC									
IDH1/IDH2/TET2	Absent	41	11 (27%)	(reference)		24	8 (33%)	(reference)	
	Present[a]	42	12 (29%)	1.09 (0.89-1.34)	0.41	21	8 (38%)	1.23 (0.64-2.38)	0.54
DNMT3A	Absent	69	20 (29%)	(reference)		40	13 (33%)	(reference)	
	Present	14	3 (21%)	0.67 (0.36-1.23)	0.20	5	3 (60%)	n/a	n/a
IDH1/IDH2	Absent	58	14 (24%)	(reference)		35	10 (29%)	(reference)	
	Present	25	9 (36%)	1.77 (0.41-7.60)	0.44	10	6 (60%)	3.67 (0.84-15.90)	0.08
TET2	Absent	65	20 (31%)	(reference)		34	14 (41%)	(reference)	
	Present	18	3 (17%)	0.45 (0.05-3.72)	0.46	11	2 (18%)	0.32 (0.07-1.40)	0.130
All Sites									
DNMT3A	Absent	165	47 (28%)	(reference)		136	40 (29%)	(reference)	
	Present	32	13 (41%)	1.72 (0.71-4.17)	0.23	23	13 (57%)	3.12 (1.64-5.94)	0.001
DNMT3A and *NPM1*	Absent	186	52 (28%)	(reference)		148	31 (21%)	(reference)	
both mutated	Present	13	8 (62%)	2.57 (1.18-5.94)	0.017	11	8 (73%)	2.82 (1.33-6.00)	0.007
IDH1/IDH2	Absent	189	55 (29%)	(reference)		166	51 (31%)	(reference)	
	Present	50	17 (34%)	1.26 (0.50-3.17)	0.63	35	14 (40%)	1.50 (0.51-4.74)	0.46
TET2	Absent	121	40 (33%)	(reference)		88	34 (39%)	(reference)	
	Present	31	9 (29%)	0.82 (0.30-2.25)	0.71	23	8 (35%)	0.85 (0.27-2.61)	0.77

MSKCC: Memorial Sloan Kettering Cancer Center; MCC: Moffitt Cancer Center; n/a: not available; N: number; CR: complete response; OR: odds ratio; CI: confidence interval.
[a]*One patient had both IDH1 and TET2 mutations.*

study criteria (Table 1). NGS was performed prior to initiation of HMA in 70% of patients. Our cohort was enriched for higher risk disease as therapy-related AML occurred in 17% of patients (n=14), and 36% patients (n=30) had AML that evolved from an antecedent hematologic disorder (4 were also considered as having therapy-related disease). The remaining 52% of patients (n=43) had *de novo* AML.

Among patients treated with HMAs in the frontline setting (n=45), we noted a 60% CR rate [including CR and incomplete blood count recovery (CRi)] in *DNMT3A* mutants compared to 33% of those with wild-type *DNMT3A*, although numbers were insufficient to allow statistical comparisons. We noted a trend toward a higher CR rate in *IDH1/2* mutants compared to wild-type *IDH1/2* (60% *vs.* 29%, odds ratio (OR) 3.67; *P*=0.08). We did not observe an association between *TET2* mutation status and response (18% CR rate in *TET2* mutants *vs.* 41% in wild-type *TET2*, OR 0.33; *P*=0.195). Given the mutual exclusivity of *IDH1/2* and *TET2* mutations, we compared response rates of patients with presence of *IDH1/2* and *TET2* to patients with neither mutation, but could not demonstrate an association with presence of mutation and CR rate (38% CR in mutants compared to 33% in non-mutants; *P*=0.62). When considering all patients (n=83) and treatment setting (frontline and relapsed/refractory), there were no statistically significant associations (Table 1).

We next sought to combine the MSKCC/MCC cohort with previously published cohorts examining the role of mutations and response to HMAs (Table 3). This included 197 patients with *DNMT3A* mutation status, 239 with *IDH1/2* mutation status, and 152 with *TET2* mutation status. In the frontline setting only, there was a statistically significant association between presence of *DNMT3A* mutation and attainment of CR [57% *vs.* 29%, OR 3.12 (1.63-5.94) with *P*=0.001]. Presence of mutation in both *DNMT3A* and *NPM1* demonstrated a CR rate of 73% compared to 21% in patients without co-mutation of these genes [OR 2.82 (1.33-6.00) with *P*=0.007]. There was no correlation between CR rate and mutation status for *IDH1/2* and *TET2*.

When examining all patients treated in both the frontline and relapsed/refractory setting, we noted a significantly higher CR in *DNMT3A/NPM1* co-mutants compared to others [62% *vs.* 28%, OR 2.57 (1.18-5.94) with *P*=0.017]. However, there were no statistically significant associations for *DNMT3A, IDH1/2,* or *TET2* when considered alone.

The mechanistic link between mutations in *DNMT3A* and increased response to HMAs has not been elucidated. The majority of *DNMT3A* mutations seen in AML are a heterozygous substitution at arginine 882, which reduces methyltransferase activity leading to global hypomethylation, making it counterintuitive that further reduction in methylation could predict clinical response.[11] However, the mechanism of action of HMAs is poorly understood, as there is not a clear relationship between the amount of demethylation following HMA therapy and clinical response.[12] Cancer cells are often hypermethylated at promoter regions, leading to down-regulated expression of tumor suppressor genes but hypomethylated at repetitive DNA regions, leading to chromosomal instability and to malignant transformation. Hence, current methods measuring global methylation may not be the optimal approach, as these depict the sum of hyper- and hypomethylation at respective sites.[12]

Limitations of this study include the retrospective nature and cohort heterogeneity of both our series and previously published series, as we included patients from multiple institutions treated in the frontline and relapsed/refractory setting, with both HMAs alone and in combination with additional drugs. While the majority of patients had samples at time of newly diagnosed AML, a subset of patients in our cohort had NGS later in their clinical course with a small subset having NGS performed after initiation of HMA, in which clonal evolution could conceivably have led to acquisition or loss of mutations. Given that *IDH1/2* and *TET2* mutations lead to a hypermethylated state,[13] one would expect an association with

mutation status in these genes and HMA response. We may be underpowered to determine true associations with *IDH1/2*, *TET2*, and *HMA* response.

In conclusion, we have identified a significantly higher CR rate in AML patients harboring *DNMT3A* mutations who were treated in the frontline setting with HMAs. This increased response rate was not observed when our analysis included patients in the relapsed/refractory setting. We also identified a statistically significant improved CR rate in *DNMT3A/NPM1* co-mutants who were treated in both frontline and relapsed/refractory settings. To our knowledge, this is the largest dataset assembled to address the impact of mutations in epigenetic modifiers in AML patients treated with HMAs. Notably, the response rate reported here compares favorably with those reported in elderly AML patients treated with induction chemotherapy.[14] In the context of the relatively favorable side-effect profile of HMAs, this may have important implications for therapeutic decision making when considering older patients who are borderline candidates for induction chemotherapy, especially as *DNMT3A*-mutant AML has been associated with improved responses to daunorubicin dose intensification.[15] This series has not examined whether the increase in CR translates to an OS benefit. Notably, in the HMA phase III trials for AML, comprehensive molecular profiling including *DNMT3A, IDH1/2*, and *TET2* was not reported, so prospective data regarding molecular predictors of HMA response are limited.[2,3] Therefore, a prospective therapeutic trial utilizing HMAs with comprehensive molecular profiling of all patients at baseline is warranted with both CR and OS as end points.

Catherine C. Coombs,[1] David A. Sallman,[2] Sean M. Devlin,[3] Shweta Dixit,[4] Abhinita Mohanty,[5] Kristina Knapp,[4] Najla H. Al Ali,[2] Jeffrey E. Lancet,[2] Alan F. List,[2] Rami S. Komrokji,[2] Eric Padron,[2] Maria E. Arcila,[5] Virginia M. Klimek,[1] Marcel R. M. van den Brink,[6] Martin S. Tallman,[1,7] Ross L. Levine,[1,4] Raajit K. Rampal,[1] and Franck Rapaport[1*]*

[1]Memorial Sloan Kettering Cancer Center, Department of Medicine, Leukemia Service, New York, NY; [2]H. Lee Moffitt Cancer Center and Research Institute, Department of Malignant Hematology, Tampa, FL; [3]Memorial Sloan Kettering Cancer Center, Department of Epidemiology & Biostatistics, New York, NY; [4]Memorial Sloan Kettering Cancer Center, Human Oncology and Pathogenesis Program, New York, NY; [5]Memorial Sloan Kettering Cancer Center, Department of Pathology, New York, NY; [6]Memorial Sloan Kettering Cancer Center, Sloan Kettering Institute, New York, NY; [7]Weill Cornell Medical College, New York, NY, USA

*Contributions: *RKR and FR contributed equally to this work*

Acknowledgments: the authors acknowledge Courtney DiNardo, Ashkan Emadi, and Michaela Fontenay for providing original data from their publications to assist in pooled analyses. We also acknowledge the patients and their families for their participation. The authors thank Peter Todd, PhD, (Tajut Ltd, Kaiapoi, New Zealand) for medical editorial assistance with this article, for which he received financial compensation from Kyowa Kirin Pharmaceutical Development Inc. (Princeton, New Jersey).

Funding: this study was supported in part by NIH/NCI P30 CA008748 (Cancer Center Support Grant) and by the Susan and Peter Solomon Genomics Program at Memorial Sloan Kettering Cancer Center.

Correspondence: rampalr@mskcc.org
doi:10.3324/haematol.2016.148999

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7-30.
2. Kantarjian HM, Thomas XG, Dmoszynska A, et al. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. J Clin Oncol. 2012;30(21):2670-2677.
3. Dombret H, Seymour JF, Butrym A, et al. International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. Blood. 2015;126(3):291-299.
4. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059-2074.
5. Itzykson R, Kosmider O, Cluzeau T, et al. Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. Leukemia. 2011;25(7):1147-1152.
6. Emadi A, Faramand R, Carter-Cooper B, et al. Presence of isocitrate dehydrogenase mutations may predict clinical response to hypomethylating agents in patients with acute myeloid leukemia. Am J Hematol. 2015;90(5):E77-79.
7. DiNardo CD, Patel KP, Garcia-Manero G, et al. Lack of association of IDH1, IDH2 and DNMT3A mutations with outcome in older patients with acute myeloid leukemia treated with hypomethylating agents. Leuk Lymphoma. 2014;55(8):1925-1929.
8. Metzeler KH, Walker A, Geyer S, et al. DNMT3A mutations and response to the hypomethylating agent decitabine in acute myeloid leukemia. Leukemia. 2012;26(5):1106-1107.
9. Sallman DA, Komrokji R, Vaupel C, et al. Impact of TP53 mutation variant allele frequency on phenotype and outcomes in myelodysplastic syndromes. Leukemia. 2016;30(3):666-673.
10. Cheng DT, Cheng J, Mitchell TN, et al. Detection of mutations in myeloid malignancies through paired-sample analysis of microdroplet-PCR deep sequencing data. J Mol Diagn. 2014;16(5):504-518.
11. Russler-Germain DA, Spencer DH, Young MA, et al. The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. Cancer Cell. 2014;25(4):442-454.
12. Voso MT, Santini V, Fabiani E, et al. Why methylation is not a marker predictive of response to hypomethylating agents. Haematologica. 2014;99(4):613-619.
13. Figueroa ME, Abdel-Wahab O, Lu C, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell. 2010;18(6):553-567.
14. Lowenberg B, Ossenkoppele GJ, van Putten W, et al. High-dose daunorubicin in older patients with acute myeloid leukemia. N Engl J Med. 2009;361(13):1235-1248.
15. Patel JP, Gonen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366(12):1079-1089.