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Case series of AML patients receiving hypomethylation therapy and retrospectively found to have *IDH1* or *IDH2* mutations

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Specific genetic profiles significantly affect prognosis in acute myeloid leukemia (AML) [1]. Several groups identified somatic mutations in *isocitrate dehydrogenase 1 (IDH1)* and *IDH2*; at least one of these genes is mutated in up to 20–30% of patients with AML [2]. *IDH1* and *IDH2* are cytosolic and mitochondrial enzymes that convert isocitrate to α-ketoglutarate as part of the energy-producing Krebs cycle. When they acquire neomorphic enzymatic properties in a mutated form, *IDH1* and *IDH2* instead avidly convert α-ketoglutarate to 2-hydroxyglutarate (2-HG) [3,4]. The accumulation of 2-HG leads to cellular changes that in part lead to hypermethylation of genomic DNA and inhibition of cellular differentiation [5,6]. AML patients with a *FLT3* wild-type, *NPM1* mutant molecular profile have a worse prognosis if they have an *IDH* mutation [7–9].

Given that *IDH1/2*-mutated AML cells demonstrate global hypermethylation [5], our objective was to determine whether treatment with hypomethylating agents is effective against this AML subtype. *IDH1* and *IDH2* were not previously routinely tested in mutation analysis, however bone marrow aspirates from multiple clinical trials were preserved in the leukemia tissue bank for patients treated at The University of Texas MD Anderson Cancer Center from June 2001 to June 2012. A total of 406 samples were available for retrospective testing, and detection of a mutation in *IDH1* or *IDH2* was carried out using Sanger sequencing over the regions of interest. We reviewed corresponding clinical information including treatments administered. Inclusion and exclusion criteria for treatments varied, depending on the protocol in which each patient participated. For each patient who had an *IDH* mutation and was treated with either azacitidine or decitabine (hypomethylating

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agents), alone or in combination with other therapeutics, we further reviewed each medical record in detail to obtain additional demographic, laboratory, treatment, and clinical data.

Of the 406 AML patients tested, 51 (12.6%) had an *IDH1* mutation alone, 47 (11.6%) had an *IDH2* mutation alone, and 6 (1.5%) had both. Among the 104 patients carrying any *IDH1* or *IDH2* mutation, 9 patients had been treated with azacitidine or decitabine, alone or combined with other chemotherapy agents. We evaluated the following endpoints for these 9 patients: types and durations of responses to therapy, complete blood counts, change in bone marrow (BM) blasts by percentage, change in peripheral blood (PB) blasts by percentage and absolute count, length of follow-up, and survival. Responses were classified according to standard International Working Group definitions of responses as previously described [10]. Morphologic complete remission with incomplete platelet recovery (CRp) included all criteria for complete remission except residual thrombocytopenia ($<100 \times 10^9/L$). Hematologic improvement (HI) was defined as all criteria for partial remission (PR) except residual neutropenia ($<1 \times 10^9/L$) and/or thrombocytopenia ($<100 \times 10^9/L$).

Of the 9 *IDH* mutation-carrying patients treated with hypomethylating agents, 4 had an R132C mutation in *IDH1* and 5 had an R140Q mutation in *IDH2*. Patient characteristics, including age and gender, cytogenetics, any additional mutation analysis, prior therapies, treatment regimen, and outcomes including duration of response, are shown in Table I. Median age at the time of treatment was 74 years (range, 57–79 years). All patients had diploid cytogenetics except for patient 5, who had pseudodiploid cells with a deletion of chromosome 7. Available mutation status is shown; all patients had wild-type *FLT3* and *NPM1* except for patient 2, who had an internal tandem duplication *FLT3* (*FLT3*-ITD), and patient 6, who had an *NPM1* mutation. All the patients had been treated with one of four regimens that included azacitidine or decitabine (see Table I) [11–13]. The patients were all newly diagnosed and had not received prior therapies except for patient 5, who had received 2 prior therapies, and patient 9, who had received 1 prior therapy. Patient 6 was initially diagnosed with MDS, and transformed into AML prior to any treatment.

The median follow-up from initiation of treatment was 9 months (range, 1–12 months). Notable responses occurred in patients 1, 4, and 6, all of whom had a CRp. These patients had been treated with decitabine + VPA or azacitidine + VPA + ATRA. Patient 6 achieved a CRp after only one cycle of azacitidine + VPA + ATRA, but died of methicillin-resistant *Staphylococcus aureus* bacteremia prior to additional courses of therapy. Three patients who had received decitabine alone or azacitidine + VPA + ATRA had a PR or HI. Of the three patients who had no response (NR), two had been treated with azacitidine + ara-C, and one had been treated with azacitidine + VPA + ATRA.

Most patients had an initial decrease in the percentage of BM blasts within the first 2 months of therapy (Figure 1A and 1B). Many had a notable reduction in percentage PB blasts and absolute PB blast count after the first cycle (data not shown). Patients 5 and 7, who had NR, had little or no reduction in BM or PB blasts, and treatment with azacitidine + ara-C was discontinued in both patients within 2 months. In patients who had a CRp, PR, or HI, the median duration of response was 3 months (range, 1–6 months) (Table I). Patient 3 had a significant reduction in BM blasts followed by an increasing BM blast percentage over

several months. Because the initial reduction of BM blasts was not >50%, the outcome was classified as NR, despite temporary stabilization of disease. Patient 8, who received decitabine alone, had a sustained PR lasting 5 months. This patient had a measured, gradual reduction of BM blasts over 5 cycles of therapy (87% → 17%, Figure 1A). However, she developed leukemia cutis, her only sign of disease progression, and therapy was changed.

Patient 9 was initially treated with fludarabine plus ara-C, however he did not respond, with increasing peripheral blood blasts within two weeks of cycle 1. He was next treated with decitabine alone for one cycle and achieved PR for 4 months. He refused further therapy until 2 months later when he received an additional cycle of decitabine. He had HI and was next treated with high-dose cytarabine and achieved PR. He was unable to receive any additional therapy with infectious complications, and he died 12 months after diagnosis.

We compared responses of AML patients carrying an *IDH* mutation who were treated with hypomethylating agents with *IDH* mutation-carrying patients treated with any other therapy ($n = 96$) and with patients who did not carry an *IDH* mutation and were treated with hypomethylation ($n = 18$) (Figure 1C). Comparison populations of patients included treated and untreated *de novo* and MDS-related AML. The total percentage of *IDH* mutation-carrying patients who achieved a CR, CRp, or PR was not significantly different in the hypomethylation-treated group and the group treated with other therapies (55.6% vs. 57.3%, p -value = 0.92, Figure 1C). Likewise, the total percentage of *IDH* mutation-carrying patients receiving hypomethylation therapy and achieving CR, CRp, or PR was equivalent to similarly treated *IDH* wild-type-carrying patients (55.6% vs. 55.6%, Figure 1C).

Kaplan-Meier curves for overall survival for the three groups were compared and found to be similar up to 12 months, and not significantly different overall (p -value = 0.09, Figure 1D). The *IDH* mutation–hypomethylation group had a median age of 74 years (range, 57–79 years), the *IDH* mutation–other treatment group had a median age of 60 years (range, 18–86 years), and the *IDH* wild-type–hypomethylation group had a median age of 69 years (range, 36–81 years). The younger ages of the patients in the two comparison groups is a potential selection bias and may in part account for the difference in survival after 12 months. For this reason we also compared survival curves among the three groups for patients ≥ 57 years (the age of the youngest patient in the *IDH* mutation–hypomethylation group). We found survival rates were comparable in all groups (p -value = 0.9, Figure 1E). We further performed a matched pair analysis for the *IDH* mutation–hypomethylation group vs. *IDH* mutation–other treatment group using the following criteria: age ± 5 years, cytogenetics (favorable vs. intermediate vs. unfavorable), and *IDH* gene mutated (*IDH1* vs. *IDH2*). We were unable to match based on *FLT3* or *NPM1* status. Survival rates for the 9 matched patients in the *IDH* mutation–other treatment group trended towards worse overall survival (p -value = 0.06, Figure 1F). We performed a similar matched pair analysis for the *IDH* wild-type–hypomethylation group based on age ± 7 years, cytogenetics (favorable vs. intermediate/unfavorable), and, where possible, exact regimen. We did not see a difference in survival curves in this matched comparison (data not shown, p -value = 0.72). A stratified log rank test was also used for comparing groups in the two matched comparisons, and also yielded non-significant results (p -values = 0.32 and 0.74 respectively). A significant limitation of matched pair analysis was the small number of potential matches.

Two of the *IDH*-mutation patients treated with hypomethylation agents who had NR were treated with a azacitidine plus ara-C, a combination that may be antagonistic in certain contexts [14]. Several patients had treatment that included valproic acid, a histone modifying therapy that also targets epigenetic regulation [11]. Overall, the patients in this series had widely variable treatment courses, and it is not possible to draw any conclusions by the comparison with the other groups. Additionally, there were small numbers of patients in each group, especially *IDH* mutation-carrying patients receiving hypomethylating treatment. Finally, the mutational status of other genes associated with hypermethylation in AML, including *TET2* (which is mutated in a mutually exclusive fashion from *IDH1/2* mutations) were unknown for our samples [5]. Notably, in one report for chronic myelomonocytic leukemia, mutations in *TET2* were not predictive of survival in patients treated with decitabine [15].

While this retrospective case series does not demonstrate any significant differences between groups compared, there were individual *IDH* mutation-carrying patients who had meaningful benefit from hypomethylating therapy. With recent discoveries linking *IDH* mutations to DNA hypermethylation, and in light of the anti-leukemic activity observed in individual patients carrying *IDH* mutations, it is worthwhile to prospectively test the use of hypomethylating agents in AML patients carrying *IDH* mutations. Most recently, small molecule *IDH* inhibition has been shown to induce cellular differentiation in AML cells [16], and combination with hypomethylating agents may present new therapeutic avenues in the ongoing transition into tailored therapy for AML patients.

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Figure 1.

(A) Percent BM blasts for each *IDH* mutation-carrying AML patient treated with hypomethylating agents. (B) Change in the percentage of BM blasts from baseline over the first 2 months of therapy for each patient. (C) Percentage of patients achieving CR, CRp, or PR for three groups: *IDH*-mutated AML treated with hypomethylation, *IDH*-mutated AML treated with other therapy (not treated with hypomethylation), and *IDH* wild-type AML treated with hypomethylation. Significance determined using a comparison of binomial proportions and defined as $p < 0.05$. *IDH*-mut, *IDH* mutation-carrying; *IDH*-wt, *IDH* wild type. (D) Kaplan-Meier curves for overall survival rates for the three mutation-treatment groups. (E) Overall survival rates for patients 57 years and older. (F) Overall survival rates for matched pairs between *IDH*-mutated AML treated with hypomethylation vs. *IDH*-mutated AML treated with other therapy. Curve comparisons performed using a Mantel-Cox test.

Table 1

Patient characteristics and responses.

Patient	Age/Sex	Cytogenetics	Molecular diagnostics tested	Prior therapies	Treatment	Response (cycles to response)	Initial change (best % improvement)	Duration of ↓blasts (mos)
1	66M	46XY[20]	FLT3-, Ras-, NPM1-	None	Decitabine + VPA	CRp (2)	↓BM blasts (BM: 30→1%)	6
2	69F	46, XX[20]	FLT3-ITD, Ras-, NPM1-	None	Azacitidine + VPA + ATRA	HI (1)	↓BM blasts ↓PB blasts (BM: 36→14%)	2
3	76M	45, X-, Y[4] 46, XY[27]	FLT3-, Ras-, NPM1-	None	Azacitidine + VPA + ATRA	NR	↓BM blasts (BM: 64→40%)	2
4	77F	46, XX[20]	FLT3-, Ras-, cKit-, NPM1-	None	Decitabine + VPA	CRp (2)	↓BM blasts ↓PB blasts (BM: 52→1%)	2
5	57F	46, XX, del(7)(q11.2q36)[19]	FLT3-, Ras-, PML-RARA-	1	Ida + Ara-c + Pravastatin.			
				2	Ida + high-dose Ara-C + EL625 (p53 antisense oligo)	NR	↓PB blasts (PB: 98→22%)	0
6	77M	46XY[20]	FLT3-, Ras-, NPM1+	None	Azacitidine + VPA + ATRA	CRp (1)	↓BM blasts (BM: 35→2%)	1
7	74M	46XY[20]	FLT3-, Ras-, NPM1-	None	Azacitidine + Ara-C	NR	↓PB blasts (PB: 54→8%)	0
8	79F	46, XX[20]	FLT3-, Ras-, cKit-	None	Decitabine	PR (3)	↓BM blasts ↓PB blasts (BM: 87→17%)	5
9	65M	46XY[20]	FLT3-, Ras-, NPM1-, CEBPA-, cKit-	Fludarabine + Ara-C	Decitabine	PR (1)	↓BM blasts ↓PB blasts (BM: 82→6%; PB: 79→0%)	6

Decitabine + VPA [11]: decitabine 20mg/m² IV over 1 hour daily, days 1–5; valproic acid 50mg/kg daily by mouth divided twice daily, days 1–7. Azacitidine + VPA + ATRA [12]: azacitidine 75mg/m² subcutaneously daily, days 1–7; valproic acid 50mg/kg daily by mouth divided twice daily, days 1–7; all-trans retinoic acid 45mg/m² daily by mouth divided twice daily, days 1–5. Azacitidine + Ara-C [13]: azacitidine 37.5mg/m² IV over 20–30min daily, days 1–7; cytarabine 1g/m² continuous IV daily, days 1–3. Decitabine: 20mg/m² IV over 1 hour daily, days 1–5.