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Association between early promoter-specific DNA methylation changes and outcome in older acute myeloid leukemia patients

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ABSTRACT

Treatment options for older patients with acute myeloid leukemia (AML) range from supportive care alone to full-dose chemotherapy. Identifying factors that predict response to therapy may help increase efficacy and avoid toxicity. The phase II SWOG S0703 study investigated the use of hydroxyurea and azacitidine with gemtuzumab ozogamicin in the elderly AML population and found survival rates similar to those expected with standard AML regimens, with less toxicity. As part of this study, global DNA methylation along with promoter DNA methylation and expression analysis of six candidate genes (*CDKN2A*, *CDKN2B*, *HIC1*, *RARB*, *CDH1* and *APAF1*) were determined before and during therapy to investigate whether very early changes are prognostic for clinical response. Global DNA methylation was not associated with a clinical response. Samples after 3 or 4 days of treatment with azacitidine showed significantly decreased *CDKN2A* promoter DNA methylation in patients achieving complete remission (CR) compared to those who did not. Samples from day 7 of treatment showed significantly decreased *RARB*, *CDKN2B* and *CDH1* promoter DNA methylation in responders compared to nonresponders. Gene-specific DNA methylation analysis of peripheral blood samples may help early identification of those older AML patients most likely to benefit from demethylating agent therapy.

1. Introduction

Treatment of AML in older patients remains a therapeutic challenge. While outcomes for younger patients with AML have improved over time, this trend has not been seen in patients over the age of 60. This population has a higher frequency of medical comorbidities and is more likely to have a suboptimal performance status. Additionally, there is a higher frequency of secondary AML (due to transformation from another hematologic malignancy or from prior therapies), adverse karyotypes and drug resistance in older patients which all contribute to inferior outcomes. There is no accepted standard of care in these patients and current therapeutic options range from supportive care alone to low intensity regimens such as demethylating agents and low dose cytarabine, or full dose chemotherapy [1–5]. While better therapies are badly needed, there would also be an advantage in being able to select from available therapies those to which a given patient would most likely respond.

Gene silencing via methylation of promoter CpG islands appears to play a significant role in the pathogenesis of hematologic malignancies such as myelodysplastic syndromes (MDS) and AML. Studies in MDS have shown epigenetic silencing of tumor suppressor genes such as *CDKN2B* (p15, INK4b), aberrant DNA methylation, and clinical activity of agents that affect DNA methylation, such as 5-azacytidine (azacitine) and 5-azadeoxycytidine (decitabine) [6–12]. In AML, DNA methylation inhibitors have also shown activity [13,14]. Distinct DNA cytosine methylation patterns distinguish AML subgroups [15]. DNA promoter regions of critical genes are

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inactivated through hypermethylation in AML [16,17]. *CDKN2B* and E-cadherin (*CDH1*) are independently associated with poor prognosis in AML when methylated. Patients with both *CDKN2B* and *CDH1* methylation had worse prognosis compared to those with either gene methylated alone [18]. Reactivation of the tumor suppressor p73 by demethylation of its promoter region has been shown to occur after treatment of AML cells with decitabine [19]. This leads to $p21^{WAF1}$ (*CDKN1A*) expression which correlated with AML cell cycle arrest [19]. *APAF1* is another tumor suppressor inactivated in some cases of AML that can be re-expressed after treatment with a hypomethylating agent [20]. *CDH1*, *HIC1*, *CDKN2A* and *CDKN2B* genes can be hypermethylated in patients with AML [21–23]. The degree of DNA methylation has been shown to decrease after treatment with decitabine [14]. Another study measured global methylation status of bone marrow specimens from older patients with AML before and after treatment with decitabine; although post-treatment specimens showed significantly less methylation, it did not correlate with the percentage of bone marrow blasts [24]. Comparison of demethylation status before and after treatment and whether this correlates with clinical outcome has not been firmly established in acute leukemia.

The monoclonal antibody-antineoplastic conjugate Gemtuzumab ozogamicin (GO), which targets CD33, a myeloid antigen expressed on most AML leukemic cells, has been shown to reduce relapse rate and increase survival when added to induction chemotherapy in older adults [25]. Similarly, a recent Phase 3 trial showed that GO added during induction and consolidation may improve outcome of AML patients aged 50–70 years [26]. This drug was withdrawn from the U.S. market in 2010 when a confirmatory trial showed no improvement in survival and a higher fatality rate in the group treated with GO [27]. More recently, mutation analysis of patients enrolled on a Phase 3 clinical trial found that cytogenetically normal (CN)-AML patients had a preferential benefit from GO treatment as compared to AML patients with abnormal cytogenetics [28]. Two recent meta-analyses examined data from five randomized trials and concluded that GO improves overall survival (OS) and reduces relapse [29,30]; it was also shown to reduce the development of disease resistance [29].

The phase 2 Southwest Oncology Group (SWOG S0703) study by Nand et al. investigated the use of hydroxyurea and azacitidine with GO in the elderly AML population [31]. This regimen was shown to be at least as effective as standard therapy but with lower toxicity in poor risk patients (70 years and older, Zubrod performance status [PS] of 2–3). Similar outcomes were seen in the good risk group (60–69 years old or PS 0–1) although data in this subset of patients did not reach predefined significance goals [31].

Here we report the laboratory findings of samples accrued as part of the S0703 study. Global DNA methylation, promoter DNA methylation of six candidate genes chosen because of their previous association with DNA methylation in AML, and expression analysis of the same genes were determined at several time points before and during therapy. Goals of this study were to investigate DNA methylation or gene expression as indicators of clinical response.

2. Materials and methods

2.1. Patients

All patients were enrolled on SWOG phase 2 clinical trial S0703. Patients with a newly diagnosed non-M3 AML under the WHO classification who had reached their 60th birthday and had a performance status of 0–3 were eligible for entry in the study. A total of 142 patients were enrolled in the study, with 83 and 59 in the good-risk and poor-risk cohorts, respectively. Good risk (age between 60 and 69 years or performance status of 0–1) and poor risk (age 70 years or older and performance status of 2 or more) based on prior experience in SWOG with older patients, were as previously defined for this clinical trial [31]. The patients were given hydroxyurea to bring the WBC count to less than $<10{,}000 \times 10^9$/L and started on azacitidine 75 mg/m^2 subcutaneously or intravenously daily for 7 days. Gemtuzumab ozogamicin 3 mg/m^2 intravenously was administered on day 8. Those achieving CR or CRi received an identical treatment as consolidation therapy followed by 4 cycles of azacitidine therapy. The study design and the clinical findings from the study were recently published [31]. For statistical analysis, patients were grouped based on their clinical response. Complete Response (CR) was defined as <5% marrow blasts by morphology, no Auer rods, absolute neutrophil count (ANC) $1{,}000 \times 10^9$/L or higher, platelet count $100{,}000 \times 10^9$/L or higher and no evidence of extramedullary disease. CR with incomplete recovery of blood counts (CRi) was the same as CR but ANC may be $<1000 \times 10^9$/L and/or platelet count $<100{,}000 \times 10^9$/L. Relapse from CR or CRi was defined as reappearance of leukemic blasts 5% or higher in peripheral blood or bone marrow or appearance/reappearance of extra-medullary disease. Written informed consent for treatment and for correlative studies was obtained from all patients in accordance with the Declaration of Helsinki. The study was approved by the institutional review boards of participating institutions.

2.2. Samples

Peripheral blood samples for laboratory correlative studies were collected pre-study, on Day 3 or 4 (range 2–5) and on Day 7 (range 6–9) of induction treatment. Samples were also submitted around Day 30 (range 27–35), after achievement of complete remission, completion of all required therapy and at the time of relapse. All specimens were sent to SWOG central laboratories for DNA and RNA isolation using standard methods. Peripheral blood samples analyzed required a prestudy sample, plus either day 3 or 4, or day 7 samples of sufficient quality from the same patient. DNA methy-

Table 1
Study samples analyzed and promoter methylation changes of *APAF1* and *HIC1* genes.

Samples for analysis	Total	CR(%)	CRI(%)	Remission failure (%)
Total enrolled in S0703	133	35 (26)	19 (14)	79 (59)
DNA methylation analysis[a]	84	25 (30)	12 (14)	47 (60)
RNA expression analysis[a]	82	24 (29)	12 (15)	46 (56)
APAF1 promoter methylation complete cohort				
TIme points	N	Median change (IQR)		*P*-value
Prestudy and day 3–4	84	7.46 (0.34, 6.35)		<0.01
Prestudy and day 7	83	4.22 (0.18, 2.53)		0.095
HIC1 promoter methylation changes at day 3–4				
Good risk group ($N = 51$)	Poor risk group ($N = 31$)			*P*-value
1.56 (0.82, 2.83)	0.96 (0.34, 1.79)			0.021

[a] Those analyzed required prestudy plus either day 3 or 4, or day 7 samples of sufficient quality available for analysis.