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Mcl-1 dependence predicts response to vorinostat and gemtuzumab ozogamicin in acute myeloid leukemia⋆

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ABSTRACT

Older adults with acute myeloid leukemia (AML) are commonly considered for investigational therapies, which often only benefit subsets of patients. In this study, we assessed whether BH3 profiling of apoptotic functionality could predict outcomes following treatment with vorinostat (histone deacetylase inhibitor) and gemtuzumab ozogamicin (GO; CD33-targeted immunoconjugate). Flow cytometry of BH3 peptide priming with Noxa (anti-apoptotic protein Mcl-1 modulator) correlated with remission induction ($p = .026$; AUC = 0.83 [CI: 0.65–1.00; $p = .00042$]: AUC = 0.88 [CI:0.75–1.00] with age adjustment) and overall survival ($p = .027$ logistic regression; AUC = 0.87 [0.64–1.00; $p = .0017$]). This Mcl-1-dependence suggests a pivotal role of Bcl-2 family protein-mediated apoptosis to vorinostat/GO in AML patients.

1. Introduction

The outcome of older adults with acute myeloid leukemia (AML) with standard curative-intent chemotherapeutics remains dismal because of increased risks of both treatment-related mortality as well as therapeutic resistance associated with advancing patient age, accumulating medical comorbidities, and changing disease biology [1–3]. As a result, current expert guidelines, e.g. by the National Comprehensive Cancer Network (NCCN), recommend that these patients should receive investigational therapies whenever possible [4].

Considerable effort has focused on the integration of antibody-based therapeutics, most notably the CD33-targeted immunoconjugate, gemtuzumab ozogamicin (GO), alone or in combination with other agents, into the treatment scheme of older adults with AML [5,6]. One such approach has explored whether histone deacetylase (HDAC) inhibitors such as vorinostat could augment the anti-AML efficacy of GO. Initial in vitro studies suggested this possibility by demonstrating that HDAC inhibitors lead to chromatin remodeling that facilitates DNA intercalation of the toxic moiety of GO, a calicheamicin-γ_1 derivative, and enhance GO-induced DNA degradation and cellular apoptosis [7,8]. As a result of these findings, we conducted a phase 2 trial and studied vorinostat as chemosensitizer with GO in 31 older adults with untreated AML; however, while the treatment regimen was well tolerated, only 7 patients achieved either a complete remission (CR) or CR with incomplete platelet recovery (CRp) [9].

Undoubtedly, pre-treatment biomarkers that accurately predict response and eventual outcome of a treatment regimen would greatly facilitate personalized decision-making. Herein, we investigated whether BH3 profiling, a method for assessing mitochondrial functionality in apoptosis signaling [10–12], could serve as such a biomarker for patients receiving vorinostat/GO for untreated AML. The underlying principle of BH3 profiling is that mitochondrial depolarization following exposure to BH3 domain containing peptides serves as a functional biomarker for a cell's ability to respond to pro-apoptotic cues. As a result of aberrant phenotypes, cancer cells may develop blocks in cell death/apoptosis pathways [13]. BH3

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Table 1
Baseline characteristics of study population.

	Responders ($N=7$)	Non-responders ($N=16$)	All patients ($N=23$)	p-Value
Median age (range), years	68.7 (61.1–74.4)	76.3 (64.7–80.7)	73.8 (61.1–80.7)	0.022
Male gender, *n* (%)	4 (57.1%)	10 (62.5%)	14 (60.9%)	0.824
Cytogenetic risk group, *n* (%)				0.378
Favorable	0 (0%)	1 (6.2%)	1 (4.3%)	
Intermediate	6 (85.7%)	9 (56.2%)	15 (65.2%)	
Unfavorable	1 (14.3%)	6 (37.5%)	7 (30.4%)	
NPM1 mutation, *n* (%)				0.084
Negative	4 (57.1%)	11 (68.8%)	15 (65.2%)	
Positive	3 (42.9%)	0 (0%)	3 (13%)	
FLT3/ITD, *n* (%)				0.497
Negative	7	9	16	
Positive	0	2	2	
ND	0	5	5	
Antecedent hematologic disorder, *n* (%)	3 (42.9%)	9 (56.2%)	12 (52.2%)	0.890

profiling determines if such a dependence on certain apoptosis-regulating proteins occurs in any given cancer cell, and identifies the dependent protein [14]. In turn, this understanding then provides insight into the likelihood of a cancer cell to respond to treatment. The scientific rationale for our study was provided by the fact that members from the calicheamicin family of cytotoxins involve mitochondrial pathways of apoptosis [15], and that HDAC inhibitors have been suggested to exert anti-leukemic cytotoxic effects largely through Bcl-2 family proteins, most notably Mcl-1 [16,17].

2. Materials and methods

2.1. Study population and treatment

Details of the phase 2 trial investigating vorinostat/GO (NCT00673153) have been described previously [9]. Patients aged ≥60 years were eligible if they had untreated primary or secondary AML (other than acute promyelocytic leukemia) according to the 2008 World Health Organization classification, provided they had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–3 and adequate organ function. Subjects were ineligible if they were previously diagnosed with another malignancy (unless they were disease-free for >6 months), received prior AML-like systemic therapy, GO or HDAC inhibitors, had central nervous system disease involvement, had a known HIV infection, or had an uncontrolled systemic infection. Patients received vorinostat 400 mg orally once daily on Days 1–9 and GO 3 mg/m^2 on Day 8; hydroxyurea was given to reduce the WBC to less than 10×10^9/L before beginning vorinostat. Those achieving either CR or CRp after 2–3 cycles of therapy (the protocol was amended after 8 enrolled patients to allow a third induction course before response assessment) were eligible to receive one cycle of consolidation treatment with vorinostat/GO at the same doses. Patients could then proceed with vorinostat maintenance therapy as long as CR/CRp was maintained or were removed from study treatment to receive more intensive consolidation therapy including hematopoietic cell transplantation (HCT). Cytogenetic risk-group assignment was according to the Southwest Oncology Group (SWOG)/ECOG criteria. Treatment responses were according to standard criteria by international working groups [3,18]. The study was approved by the institutional review board of participating institutions, and patients gave informed consent for the clinical trial and associated correlative laboratory studies in accordance with the Declaration of Helsinki.

2.2. BH3 profiling

Thawed aliquots of pretreatment peripheral blood- and bone marrow aspirate-derived mononuclear cells containing leukemic blasts were stained with the antibodies CD45-V450, CD3-Biotin (BD Bioscience, San Jose, CA), and CD20-Biotin (eBiosciences, San Diego, CA) followed by incubation with Streptavidin-APC. Specimens were permeabilized with digitonin and incubated with JC-1 mitochondrial dye and 100 μM BH3 peptides (Bim, Puma, Noxa, Bad, Hrk; Bim and Puma. were also assayed at 0.1 μM and 10 μM, respectively); these peptide sequences have been described previously [14] and were synthesized by New England Peptide (Gardner, MA). Specimens were also incubated individually with dimethyl sulfoxide (DMSO [(1%]) or Carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP [10 μM]); the latter serves as an uncoupling reagent control and induces mitochondrial depolarization to 100% completion. The JC-1 signal, proportional to mitochondrial charge, is lost during mitochondrial outer membrane permeabilization; i.e. the full signal is retained with DMSO treatment but lost with CCCP. Peptide induced depolarization is then calculated as a percent relative to the CCCP control which is normalized at 100% priming. Samples were run in duplicate, except in cases where insufficient viable cells were available, on a BD FACS Canto II cytometer (BD Bioscience), and data were analyzed using FACS Diva software. The blast population was identified as CD45 dim, CD3$^-$ and CD20$^-$ [10–12]. The quantifiable propensity of a pro-apoptotic peptide to induce mitochondrial depolarization relative to an uncoupling reagent control was calculated using the median signal intensity of the phycoerythrin channel normalized for DMSO as background (negative control) and CCCP served as 100% priming (positive control) [12]:

$$\%\,\text{priming} = \left(1 - \left(\frac{\text{Peptide} - \text{CCCP}}{\text{DMSO} - \text{CCCP}}\right)\right) \times 100$$

2.3. Statistical analysis

Univariate testing association between biomarker status (% priming) and responder or non-responder classification was by logistic regression analysis. We pre-determined a statistical analysis plan with significance set at $p < 0.05$. Marker predictive ability was assessed using the area under the receiver operator characteristic curve (AUC). Multivariate analyses were performed using logistic regression and significant adjustment variables from patient clinicopathologic data. Overall survival (OS) was tested for correlation with % priming by logistic regression and AUC. Missing values were treated as imputed data for statistical analyses. Analyses utilized SAS software, version 9.2 (Cary, NC), R version 2.14.2 (Vienna, Austria), and/or Graphpad Prism version 5.04 (La Jolla, CA).

3. Results

3.1. Characteristics of study cohort

The original phase 2 study of vorinostat and GO in older adults with untreated AML enrolled 31 patients [9]. Of these, pre-treatment bone marrow (BM) or peripheral blood (PB) specimens were available from 26 patients (83.9%); their pertinent baseline characteristics, stratified by response to induction therapy (i.e. achievement of CR/CRp vs. not) are summarized in Table 1. Patients achieving a CR/CRp were younger than those who failed therapy with vorinostat/GO ($p = .022$); age was therefore included as covariate in our multivariate analyses of BH3 profiling data. No statistically significant differences were found for any of the other variables in this small patient cohort, although there was a trend toward higher incidence of *NPM1* mutations in responders ($p = .084$).

3.2. BH3 profiling of pre-treatment patient specimens

From 26 study participants (median age of 73.8 years [range: 61.1–80.7 years]), aliquots of pre-treatment specimens were thawed for the purpose of BH3 profiling. Upon thawing, these specimens yielded cells with excellent viability (median of 82.1% [range: 62.2–97.9%] live cells). They were then subjected to in vitro exposure to individual BH3 peptides, including an activator (Bim) and several sensitizers (Noxa, Puma, Bad, Hrk) as surrogates for the function of Bcl-2 family proteins. Twenty-three of 26 tested