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A phase I study using bortezomib with weekly idarubicin for treatment of elderly patients with acute myeloid leukemia

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ABSTRACT

We report the results of a phase I study with four dose levels of bortezomib in combination with idarubicin. Eligible patients were newly diagnosed with acute myeloid leukemia (AML) age ≥60 years, or any adult with relapsed AML. Bortezomib was given twice weekly at 0.8, 1.0, or 1.2 mg/m^2 with once weekly idarubicin 10 mg/m^2 for four weeks. Twenty patients were treated: 13 newly diagnosed (median age 68, range 61–83) and 7 relapsed (median age 58, range 40–77). Prior myelodysplastic syndrome (MDS) was documented in 10/13 (77%) newly diagnosed and 1/7 (14%) relapsed patients; the three newly diagnosed patients *without* prior MDS had dyspoietic morphology. Two dose-limiting toxicities occurred at the initial dose level (bortezomib 0.8 mg/m^2 and idarubicin 10 mg/m^2); idarubicin was reduced to 8 mg/m^2 without observing subsequent dose-limiting toxicities. The maximum tolerated dose in this study was bortezomib 1.2 mg/m^2 and idarubicin 8 mg/m^2. Common adverse events included: neutropenic fever, infections, constitutional symptoms, and gastrointestinal symptoms. No subjects experienced neurotoxicity. Most patients demonstrated hematologic response as evidenced by decreased circulating blasts. Four patients (20%) achieved complete remission. There was one treatment-related death. The combination of bortezomib and idarubicin in this mostly poor-risk, older AML group was well tolerated and did not result in high mortality. This trial was registered at www.clinicaltrials.gov as #NCT00382954.

1. Introduction

Acute myeloid leukemia (AML) is a hematologic cancer with median age at diagnosis of 65 years [1]. Although primarily a disorder of older adults, such patients have been largely excluded from advances in AML therapy. This is due to host-related factors that limit older patients' tolerance of intensive therapies, as well as the frequent occurrence of myelodysplasia (MDS), unfavorable karyotypes, and overexpression of multidrug-resistance gene *MDR1* at diagnosis, which contribute to a poor response to standard cytotoxic chemotherapy. Patients 60 years and older experience nearly half the rate of remission success compared to younger cohorts and are twice as likely to die during induction [2–17]. Age remains one of the most important adverse prognostic factors in AML and the optimal therapy for patients over age 60 is undefined. Regardless of age, at the time of relapse there is no widely accepted standard of care. In patients for whom a hematopoietic stem cell transplant is not an option, durable remissions after relapse are infrequent.

In recent years numerous studies have described the biologic relevance and cellular and molecular properties of normal human hematopoietic stem cells [18–21]. Furthermore, progress has been made in the characterization of malignant stem cells. Multiple groups have identified and characterized a leukemic stem cell (LSC) in patients with AML [22–26]. Prospective identification and isolation of enriched LSCs have allowed investigators to define biologic characteristics of normal *versus* leukemic stem cells, presenting an opportunity for targeted leukemia therapy [27]. Chemotherapeutic agents effectively ablate leukemia blast cells, but may not effectively target LSC due to the generally quiescent state of LSCs. It is plausible that the failure of standard chemotherapy to sustain durable remission in most patients with AML is related to the survival of LSC. Data from our laboratory has shown that nuclear factor kappa B (NF-kB) is constitutively activated in primary AML specimens, including the relatively quiescent LSC population. In

Abbreviations: AML, acute myeloid leukemia; DLT, dose-limiting toxicity; DSMC, Data Safety Monitoring Committee; EMSA, electrophoretic mobility shift assay; FACS, fluorescence-activated cell sorting; iKBa, inhibitor of NF-kB; LSC, leukemic stem cell; MDS, myelodysplastic syndrome; MTD, maximum tolerated dose; NF-kB, nuclear factor kappa B.

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Table 1
Dosing schema.

Dose level	Bortezomib (mg/m^2)	Idarubicin (mg/m^2)
I	0.8	10
II	1.0	10
III	1.2	10
IV	1.2	12

addition, molecular genetic studies using a dominant negative allele of an inhibitor of NF-kB (IkBa) demonstrated that inhibition of NF-kB contributes to apoptosis in AML cells [28]. One action of proteasome inhibition is to block the degradation of IkBa, the NF-kB regulator, resulting in loss of NF-kB activity [29,30]. Moreover, the apoptosis observed when the proteasome inhibitor, bortezomib, is combined with idarubicin appears to be greater than bortezomib alone. This targeted therapy holds significant promise as a low-toxicity treatment in selected groups of AML patients who would otherwise have few treatment options [31,32]. Based on the encouraging results of our preclinical studies with this combination, we initiated a phase I study translating our observations from the laboratory into the clinical setting.

2. Patients and methods

2.1. Patient eligibility

Eligible patients were treated at either the University of Kentucky or the University of Rochester and provided written informed consent according to the respective Institutional Review Board guidelines and in agreement with the Declaration of Helsinki. Eligibility criteria included: diagnosis of AML as defined by the World Health Organization classification [33] and meeting one of two entry criteria: (1) newly diagnosed and age ≥60 years unsuitable for intensive chemotherapy induction – antecedent hematologic disorders, pre-existing MDS, unfavorable cytogenetics, or unacceptable comorbidities; or, (2) age ≥18 with relapsed disease occurring after at least one successful complete remission. Other requirements included Karnofsky performance status >60, cardiac ejection fraction >40% by multiple-gate acquisition scan, and no symptomatic cardiac disease. Exclusion criteria included: prior induction chemotherapy for newly diagnosed AML with the exception of hydroxyurea within the preceding 14 days; prior anthracycline dose of ≥150 mg/m^2 of doxorubicin or >36 mg/m^2 of idarubicin [34,35]; AML-M3; active central nervous system leukemia; or uncontrolled bacterial, fungal, or viral (including HIV) infections.

2.2. Treatment

This trial was conducted under the supervision of the University of Kentucky General Clinical Research Center funded by the National Institutes of Health, National Center for Research Resources (M01 RR02602). The combination of idarubicin and bortezomib was administered once as a remission induction regimen. Idarubicin was commercially available and was given weekly at a dose of 8–12 mg/m^2 in 50 ml of normal saline as a 15-min infusion (days 1, 8, 15, and 22) [36]. Idarubicin at 10 mg/m^2 is associated with peak plasma levels around 9 ng/ml, comparable to the *in vitro* concentrations used in our experiments. Bortezomib was provided by Millennium Pharmaceuticals and was administered twice weekly as an intravenous push over 3 to 5 s on days 1, 4, 8, 11, 15, 18, 22, and 25 at least 2 h after the idarubicin dose when given on the same day. This dosing schema was implemented to optimize the potential interaction between bortezomib and idarubicin. Given 2 h after idarubicin, bortezomib inhibits NF-kB upregulation induced by idarubicin, thwarting an attempt at cell survival and increasing cytotoxicity of idarubicin. The dose escalation schema (Table 1) was based on doses from prior phase I studies of bortezomib [37]. Idarubicin 8 mg/m^2 in a modified dose escalation schema (Table 2) was implemented after review by the Data Safety Monitoring Committee (DSMC) of initial dose-limiting toxicity on dose level one. Selected doses of bortezomib were associated with levels of proteasome inhibition that corresponded to dose-dependent inhibition of tumor growth [37–39]. This schedule of administration was designed to accomplish two important goals: (1) the administration of a tolerable outpatient regimen; and, (2) a schedule that allowed for maximal overlap of bortezomib and idarubicin, optimizing the potential for proteasome inhibition to suppress cellular attempts to resist the toxic effects of idarubicin [40,41]. A bone marrow biopsy and aspirate was obtained on day 18; patients with aplasia (<5% cellularity) did not receive days 22 and 25 of treatment. The International Working Group criteria were used to determine response in AML [42,43].

Table 2
Modified dosing schema. After two dose-limiting toxicities in the first cohort, the dose of idarubicin was reduced and dose escalation followed a modified strategy.

Modified dose level	Bortezomib (mg/m^2)	Idarubicin (mg/m^2)
I-M	0.8	8
II-M	1.0	8
III-M	1.2	8

Table 3
Patient characteristics.

Number of patients	20
Male/Female	15/5
Median age (range)	65 (40–83)
Relapsed	7
Median age (range)	58 (40–77)
Previously untreated	13
Median age (range)	68 (61–83)
Prior AHD/MDS	11
Unfavorable cytogenetics	8
Complex	7
Intermediate cytogenetics	12
Relapsed/M6/M7	6

AHD, antecedent hematologic disease; MDS, myelodysplastic syndrome.

2.3. Study design

Study design was a standard modified Fibonacci, 3+3 design looking at four dose levels of bortezomib in combination with idarubicin. A minimum of three and a maximum of six patients were entered at each dose level. If toxicities exceeded what was defined as tolerable in one of the first three patients, the cohort was expanded to six. Evaluation of toxicity in each cohort was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.0. Drug-related dose-limiting toxicity was defined as any grade 4 hematologic toxicity attributable to treatment and lasting >28 days after the last day of therapy, or any non-hematologic toxicity attributable to treatment of grade 4 or greater except for nausea/vomiting (which may be grade 4). The FACT/GOG-Neurotoxicity Questionnaire, Version 4.0 was administered weekly to specifically assess neurotoxicity. A DSMC was established to review all adverse events semi-annually. Unacceptable toxicities in two of six patients defined the maximum tolerated dose (MTD) as one dose level lower. Once the MTD was established the study enrolled three additional patients at the established MTD.

2.4. Correlative endpoints

We sought to confirm our preclinical observation that demonstrated leukemia-specific induction of apoptosis, including in LSC, with bortezomib and idarubicin in human subjects. Patients had samples collected from peripheral blood before and after initial drug treatment to assess apoptosis in the AML cells using standard fluorescence-activated cell sorting (FACS) assays; samples were also collected for NF-kB electrophoretic mobility shift assay (EMSA). In combination with the FACS assays, we attempted to correlate Nf-kB activity with ablation of AML blasts and LSC.

2.5. Statistical considerations

Based on a 3+3 design, a maximum of 24 patients could be potentially enrolled across the four dose levels. Descriptive statistics were calculated to summarize patient characteristics, frequencies were tabulated to summarize adverse event data and clinical outcomes such as tumor response. The Kaplan–Meier curve was utilized to estimate overall survival among patients enrolled in the trial. Correlative endpoints including white blood count and absolute blast counts were displayed graphically and comparison of change from day 1 to last time point of follow-up was performed using non-parametric paired test statistics.

3. Results

3.1. Patients

From January 2005 through July 2008 a total of 20 eligible and consented patients were treated (Table 3). Thirteen of the 20 patients were newly diagnosed, previously untreated AML (median age 68, range 61–83); the remaining seven patients were relapsed (median age 58, range 40–77). Only 5/20 patients were <60 years (all relapsed patients); six were age 70 or older. More than half of treated patients on this study had prior MDS: 10/13 (77%) newly