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A phase I study of decitabine and rapamycin in relapsed/refractory AML

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ABSTRACT

A phase I study utilizing decitabine (DAC) followed by the mammalian target of rapamycin (mTOR) inhibitor, rapamycin, in patients with relapsed/refractory adult AML was undertaken to assess safety and feasibility. Patients received DAC 20 mg/m^2 intravenously daily for 5 days followed by rapamycin from day 6 to day 25 at doses of 2 mg, 4 mg, and 6 mg/day in a standard 3 + 3 dose escalation design. Twelve patients completed treatment for safety evaluation. Maximum tolerated dose (MTD) was not reached, and except for grade 3 mucositis in 4 patients, no other significant unexpected non-hematologic toxicities have occurred indicating safety of this regimen. This trial is registered at clinical trials.gov as NCT00861874.

1. Introduction

While the molecular basis of leukemogenesis is better understood today than previously, most adult patients with AML are not cured and will either fail to enter remission or will relapse after remission is achieved. Therapy of relapsed acute myelogenous leukemia (AML) remains unsatisfactory with low response rates and short survival times in the absence of stem cell transplantation [1,2].

Decitabine (5-aza-2′-deoxycytidine) is an S-phase specific cytosine analog that inhibits DNA methylation and has activity in myelodysplastic syndromes (MDS) and AML. It incorporates into DNA and traps DNA methyltransferase (DNMT) in the form of a covalent protein–DNA adduct [3]. Cellular DNA methyltransferase is then rapidly depleted and genomic DNA is hypomethylated during DNA replication [3].

Low-dose decitabine has been utilized in elderly patients with newly diagnosed AML who were not candidates for standard induction therapy [4,5]. When dosed at 20 mg/m^2/day for 5 days every 4 weeks, the overall response rate in AML was 26% [5]. Blum et al. utilized decitabine at 20 mg/m^2 for 10 days in newly diagnosed older AML patients and found a complete remission rate of 47% [6]. Higher miR-29b levels were associated with complete remission (CR). Decitabine has not been studied extensively in settings of relapsed or refractory AML, but it has been utilized to treat relapsed AML after allogeneic stem cell transplantation [7]. In a trial where bortezomib was combined with decitabine, the CR/CRi rate was 50% in previously untreated patients and only 22% in those with relapsed or refractory disease [8], suggesting that response rates in this group may be inferior and lending rationale to combination of decitabine with other agents in this setting.

mTOR is a member of the phosphatidylinositol 3-kinase (PI3) serine threonine protein kinase family and a key component of the PI3K/AKT/mTOR signaling pathway that regulates multiple cellular functions including transcription, translation, cell cycle progression, and apoptosis induction [9]. Previous studies have shown that the PI3K/AKT/mTOR pathway is constitutively activated in 50–70% of AML cases via multiple mechanisms, including epigenetic modulation [10–13]. Since studies have shown that this pathway is necessary for the survival of AML blasts, and that targeting this pathway with pharmacologic inhibitors may be of benefit in AML therapy, there is rationale for use of mTOR inhibitors in

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AML. Rapamycin is a macrolide fungicide mTOR inhibitor approved for the prevention of allograft rejection following solid organ transplantation. When combined with mitoxantrone, etoposide, cytarabine (MEC) chemotherapy, it did not enhance responses in AML [14], however, in a series of 23 AML cases, 4E-BP1 and p70S6K phosphorylation were inhibited by rapamycin in vitro, clonogenic properties were inhibited, and 4/9 patients treated with single agent rapamycin for refractory/relapsed AML had reduction in peripheral blood and/or marrow blast percentage [15].

Thus, responses to decitabine monotherapy or to rapamycin alone in relapsed/refractory AML would be expected to be minimal. In the study reported upon here, we have examined the safety of combining decitabine with rapamycin in a sequential fashion. This combination has rationale in that other epigenetic modulators such as histone deacetylase inhibitors (HDACi), block mTOR/AKT signaling [16]. There is evidence in AML that expression of an inhibitory member of the mTOR pathway, tuberous sclerosis 2 (TSC2), is downregulated as a result of promoter hypermethylation, suggesting that hypomethylating agents may enhance TSC2 expression and inhibit MTORC1 via a separate mechanism [17,18]. Since both decitabine and rapamycin have been shown to have activity in patients with AML, the use of these agents sequentially was also postulated to allow testing of the benefit of targeting distinct mechanisms that appear aberrant in this disease as has been achieved with other decitabine containing regimens [19]. In this work, we have therefore examined whether the sequence of decitabine followed by rapamycin would prove safe in patients with relapsed/refractory AML.

2. Patients and methods

2.1. Eligibility criteria and study design

This study was designed to determine the safety and feasibility of administering decitabine at 20 mg/m^2 daily for 5 days in sequence with escalating doses of rapamycin in patients with relapsed and refractory acute myeloid leukemia and to define the maximum tolerated dose (MTD) of rapamycin in combination with decitabine for use in a phase II trial. Patients greater than or equal to 18 years of age with a diagnosis of AML according to World Health Organization criteria [20] with refractory or relapsed leukemia were eligible for the study. Acute promyelocytic leukemia was excluded. Refractory AML was defined as failure to achieve CR after 2 cycles of induction chemotherapy or persistence of >40% marrow blasts after one cycle of chemotherapy induction, and relapsed AML was defined as any evidence of disease recurrence after achieving a documented first or greater complete remission, including relapse after allogeneic stem cell transplant >100 days post-stem cell infusion. Those with ECOG performance status <3 were allowed, and patients had to have acceptable renal and hepatic function (creatinine clearance $\geq$ 30 mL/min and bilirubin $\leq$ 2.0 mg/dL and transaminases $\leq$ to 2.5 times the upper limits of normal). Patients could not have active systemic infection or be pregnant or breastfeeding. Those who were post-allogeneic transplantation could not have active GHVD greater than grade 1 of skin, and if relapse had been treated with donor leukocyte infusions, at least 4 weeks had to have elapsed since the last infusion. Informed, written consent was obtained on all patients before entry into the study with approval of the Research Subjects Review Board of the University of Rochester.

This pilot was an open label, single arm, phase I dose escalation study. Decitabine was given intravenously at 20 mg/m^2 for 5 days followed by rapamycin orally once per day on days 6–25 at 3 dose cohorts with cohort progression based on toxicity determinations. Rapamycin, a cytostatic drug was administered after the S-phase specific demethylating agent to avoid antagonism. Rapamycin was orally dosed at 2, 4, or 6 mg/day. Cycles were 28 days in duration. Hydroxyurea could be given before and during the first cycle for white cell count reduction to <30,000/μL, but no other anti-leukemia therapies were allowed.

A marrow aspirate without biopsy was obtained after the first five days (on days 5 or 6) of decitabine in the first cycle. Marrow aspirate and biopsy was obtained between days 22 and 26 of the 1st and 3rd cycles for response evaluation, and a marrow at time of withdrawal from study was recommended, but optional. If there was no overt progression of disease (defined as doubling of the peripheral blood blast count) after the first cycle, the patient could proceed on to two more cycles. If after the third cycle, there was evidence of a PR/CR, the patient could remain on study for 3 more cycles up to a maximum of 6 cycles.

Dose modifications were pre-defined. If the patient experienced a grade 2 non-hematologic toxicity, rapamycin was withheld until the toxicity had resolved to less than or equal to grade 1. Rapamycin was then resumed at the same daily dose. If the grade 2 toxicity recurred, rapamycin was withheld until the toxicity had resolved to less than or equal to grade 1 and restarted at the next lowest dose cohort. In those patients receiving 2 mg/day, dosing was restarted on a 1 mg/day basis. If a patient experienced a grade 3 or 4 toxicity, study drug was withheld until the toxicity had resolved to less than or equal to grade 1 and the daily dose was reduced to 1 mg/day for those on 2 mg, If the grade 3 or 4 toxicity recurred, the drug was stopped. Cycles could be delayed up to a week in the event of stable disease if severe neutropenia and active infection were present until the infection was controlled.

Dosage adjustments were also allowed for elevated rapamycin levels. If the rapamycin level at the time of scheduled measurement was >15 ng/mL, the dose was held for one day and then reduced by 1 mg and a repeat rapamycin level was drawn 3 days after the dose adjustment had been made. If that level was then nontoxic, the patient continued at that dose. If the level was still >15 mg/mL, drug was held until it returned to therapeutic range with serial level checks.

Responses were defined according to the International Working Group criteria for AML as stable disease, partial response (PR), complete response (CR), and CR with incomplete count recovery (CRi) [21].

2.2. Definition of dose-limiting toxicity

Adverse events were graded per the NCI Common Toxicity Criteria version 3.0 (http://ctep.cancer.gov). Dose-limiting toxicity (DLT) was defined with cycle 1 of therapy. Drug-related non-hematologic toxicity of grade 3 or 4 was considered a DLT with the exception of infections, fatigue, weight loss, stomatitis, nausea and emesis, and electrolyte imbalances, all anticipated in relapsed AML patients irrespective of treatment. For DLT, if the toxicity occurred in 2 or more patients at a single-dose level, that dose was deemed the maximum tolerated dose (MTD). The Data Safety Monitoring Board of the James P. Wilmot Cancer Center of the University of Rochester ensured that no unexpected adverse events occurred at each dose level before enrollment on the next dose level. The last person on the prior cohort had to be followed for at least 30 days from the start of therapy before enrollment on the next dose could commence.

2.3. Cytogenetics, molecular markers, and ancillary studies

Routine banding cytogenetics and flow cytometry were performed as part of routine clinical analysis. Rapamycin levels were assessed through the clinical laboratories on days 9, 16, and 25 (therapeutic range 5–15 ng/mL). Triglyceride and cholesterol levels were checked on day 1 of each cycle.