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Phase II Study of Intermediate-Dose Cytarabine in Patients with Relapsed or Refractory Ewing Sarcoma: A Report from the Children's Oncology Group

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Abstract

Background—Patients with relapsed or refractory Ewing sarcoma have a poor outcome with conventional therapies. Cytarabine decreases EWS/FLI1 protein levels in Ewing sarcoma cells and has demonstrated preclinical activity against Ewing sarcoma *in vitro* and *in vivo*. The purpose of this phase II clinical trial was to estimate the response rate of intermediate-dose cytarabine in patients with relapsed or refractory Ewing sarcoma.

Procedure—Patients with a histologic diagnosis of Ewing sarcoma were eligible if they were ≤ 30 years of age, had relapsed or refractory measurable disease, and met standard organ function requirements. Patients received cytarabine 500 mg/m²/dose intravenously over 2 hours every 12 hours for 10 doses with cycles repeated every 21 days. Response was assessed according to RECIST criteria.

Results—Ten patients (median age 20 years; 7 males) were treated. Only 5 patients had documented *EWS/FLI1* translocated tumors. No objective responses were seen. One patient had stable disease for 5 cycles before developing progressive disease. All patients evaluable for hematologic toxicity developed grade 4 neutropenia and thrombocytopenia during protocol therapy. Patients were not able to receive therapy according to the planned 21-day cycles, with a median interval of 26.5 days.

Conclusions—Cytarabine at the dose and schedule utilized in this trial resulted in hematologic toxicity that limited delivery of this therapy. This regimen also had minimal activity in this patient population.

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Keywords

Ewing sarcoma; cytarabine; EWS/FLI1

Introduction

Ewing sarcoma is the second most common type of primary bone cancer seen in children and young adults [1]. The tumor is characterized by reciprocal chromosomal translocations in approximately 95% of cases. The most common translocation, t(11;22)(q24;q12), creates a fusion protein involving the amino-terminal domain of EWS and the carboxy-terminal domain of FLI1 [2,3]. The EWS/FLI1 fusion protein serves as an aberrant transcription factor crucial for the pathogenesis of these tumors. Efforts to target these fusion proteins clinically have been hampered by delivery issues for compounds such as antisense RNA and by poor understanding of the downstream effects of EWS/FLI1.

Cytarabine is a nucleoside analogue used mainly for the treatment of hematologic malignancies [4]. Recent preclinical studies have indicated that cytarabine has activity against Ewing sarcoma and that this activity correlates with decreases in *in vitro* EWS/FLI1 levels with cytarabine treatment. Two groups demonstrated that cytarabine inhibits the growth of Ewing sarcoma cells *in vitro* at concentrations in the range of 0.1-0.25 μ M [5,6]. Follow-up studies have shown that cytarabine induces a gene expression signature that is similar to Ewing sarcoma cells treated with RNA interference to knock down *EWS/FLI1* gene expression [6]. Cytarabine also down-regulated EWS/FLI1 protein levels in these experiments [6]. The *in vitro* activity of cytarabine was extended to Ewing sarcoma mouse xenograft studies in which cytarabine significantly decreased tumor growth compared to controls [6].

Patients with relapsed or refractory Ewing sarcoma have a poor outcome, with 5-year overall survival estimates from the time of relapse ranging from 8-23% [7-10]. Given the role of EWS/FLI1 in the growth of these tumors and the preclinical data demonstrating that cytarabine down-regulates EWS/FLI1 protein levels, the Children's Oncology Group initiated this phase II study of cytarabine in this patient population. An intermediate dose of cytarabine was chosen for investigation since this dose was anticipated to produce drug concentrations that far exceed levels active in preclinical studies while avoiding the added toxicity of high-dose cytarabine. The primary aim of the study was to estimate the objective response rate to this regimen.

Methods

Subject Eligibility

Patients ≤ 30 years of age with a histologic diagnosis of Ewing sarcoma or peripheral primitive neuroectodermal tumor were eligible for enrollment at the time of disease progression or disease recurrence. Biopsy at the time of enrollment was not required nor was molecular confirmation of the diagnosis by detection of an *EWS* fusion oncogene. All patients were required to have measurable disease according to the Response Evaluation Criteria in Solid Tumors (RECIST) [11]. Patients were required to have a Karnofsky (> 16 years) or Lansky (≤ 16 years) performance score of $\geq 50\%$ and an anticipated life expectancy of ≥ 8 weeks at study enrollment. Patients were eligible for treatment once they were more than 2 weeks from receipt of chemotherapy, 2 weeks from local radiation therapy, 6 weeks from large field radiation therapy, 6 months from radiation involving $> 50\%$ of the pelvis, and 6 months from autologous stem cell transplantation. All patients were required to have baseline absolute neutrophil count (ANC) $\geq 750/\mu$ L, platelet count $\geq$

75,000/ μ L, hemoglobin $\geq$ 8 gm/dL, total bilirubin $\leq$ 1.5 times the upper limit of normal, alanine aminotransferase (ALT) $\leq$ 2.5 times the upper limit of normal, and normal creatinine based upon age- and sex-based normal values.

Patients or their parents/guardians provided informed consent for study participation using a consent form approved by each treating institution's institutional review board. Assent was obtained as appropriate for patient age.

Treatment

Patients received cytarabine 500 mg/m²/dose intravenously over 2 hours every 12 hours for 10 doses each cycle. Cycles of therapy were scheduled to be repeated every 21 days, although patients were required to have recovered to an ANC $\geq$ 750/ μ L, platelet count $\geq$ 75,000/ μ L, total bilirubin $\leq$ 1.5 times the upper limit of normal, and ALT $\leq$ 2.5 times the upper limit of normal before starting subsequent cycles. Patients meeting toxicity criteria for dose reduction were to have their dose of cytarabine reduced by 25% to 375 mg/m²/dose in subsequent cycles.

Patients received corticosteroid or saline eye drops as conjunctivitis prophylaxis until 24 hours after completing cytarabine. The use of myeloid growth factors was not recommended with the first cycle of therapy, but was recommended in subsequent cycles if patients experienced febrile neutropenia, bacteremia, or prolonged neutropenia. The use of vancomycin in addition to standard institutional empiric therapy was recommended for patients with febrile neutropenia. Other supportive care was administered according to institutional and investigator standard practice. Toxicity was graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events, version 3.0.

No concomitant chemotherapy or biological therapies were allowed. Patients with more than one measurable lesion could receive radiotherapy to localized painful lesions as long as at least one measurable lesion was not treated. In the absence of obvious clinical progression, patients were to receive two cycles of therapy before their first disease evaluation followed by repeat disease evaluations after every third cycle of therapy.

Statistical Considerations

The primary outcome was overall response rate, with the primary outcome measure being best response according to RECIST [11]. Response rates and confidence intervals were constructed according to the method of Chang and O'Brien [12,13]. Evaluable patients with a complete or partial response were categorized as having objective responses. All other evaluable patients were considered non-responders. Patients were considered evaluable if they were on study for at least one cycle of therapy or had progressive disease after receiving at least one dose of cytarabine.

A two-stage phase II design was planned for the study, with 10 evaluable patients in each stage. If no objective responses were obtained in the first stage, the trial was to be terminated due to lack of efficacy. If $\geq$ 6 patients had an objective response, the trial was to be terminated due to demonstrated efficacy. One to 5 objective responses in the first stage was to prompt further evaluation in a second group of 10 patients. If the true response rate was 5%, we would consider cytarabine as not having sufficient activity for further development with a probability of 0.93. If the true response rate was 25%, we would consider cytarabine as having sufficient activity for further development with a probability of 0.88.

Results

Subject Characteristics

The study enrolled 10 patients from May 2007 to February 2008. Since none of the first 10 patients had an objective response (partial or complete response), enrollment was terminated according to the two-stage design. The characteristics of the 10 enrolled patients are shown in Table I. The median age at study entry was 20 years. Seven patients were male. Five patients had tumors known to harbor *EWS/FLI1* fusion oncogenes. One additional patient had a tumor with a translocated *EWS* for which the fusion partner was not reported. In the remaining 4 patients, testing for *EWS* fusion oncogenes had not previously been done or had indeterminate results. Four patients had been previously treated with a different nucleoside analogue, gemcitabine.

Efficacy

No objective responses were observed among the 10 patients treated, for a response rate of 0% (1-sided 95% confidence interval of 0-25.9%). One patient had stable disease. This patient had additional scans obtained beyond those required by the study protocol. These scans obtained during cycles 1-4 confirmed stable disease from the time of study entry for at least 14 weeks before ultimately showing progressive disease at the end of cycle 5. This patient had previously been treated with gemcitabine. A second patient had stable disease after 1 cycle of therapy, but elected to stop protocol therapy because of toxicity. Two patients received 2 cycles of therapy before coming off protocol therapy because of disease progression. Five patients developed progressive disease during the first cycle of therapy. One of these patients developed clinical progression after receiving 5 of 10 planned doses of cytarabine and elected not to receive further therapy. One patient was reported to have progressive disease during the first cycle of therapy, but did not have adequate imaging to fully evaluate response according to RECIST.

Two patients died of progressive disease during the first cycle of therapy. The first patient developed new central nervous system (CNS) metastases that became hemorrhagic and resulted in his death. The second patient developed progression of pleural and pulmonary disease that resulted in his rapid clinical deterioration and death. A possible contribution of cytarabine to this patient's deterioration could not be excluded.

Toxicity

Toxicity data were reported in 16 cycles in 10 patients (Table II). One patient died prior to first hematologic evaluation and is therefore not included in evaluation of hematologic toxicity. The hematologic toxicity of this regimen in this patient population was considerable. All patients evaluable for hematologic toxicity experienced grade 4 neutropenia and grade 4 thrombocytopenia with every cycle of therapy. Ten cycles resulted in infectious complications of neutropenia. In 5 cycles, patients had culture-negative grade 3 febrile neutropenia. In the other 5 cycles, patients had grade 3 infections including: *E. coli* bacteremia; *Klebsiella* bacteremia; polymicrobial bacteremia; cellulitis; and candidal myositis.

This degree of hematologic toxicity compromised delivery of protocol therapy. While no patients required a cytarabine dose reduction due to toxicity, none of the 3 patients who received multiple cycles of therapy received cycles following the planned 21-day interval. The median interval between cycles was 26.5 days (range 23-33 days).

Non-hematologic toxicity was generally mild. One patient developed aspartate transaminase (AST) elevation and 3 patients developed alanine transaminase (ALT) elevation. One patient

developed grade 3 hypoalbuminemia. As expected for this intermediate dose of cytarabine, no patients experienced CNS toxicity. No cases of conjunctivitis were reported, though 1 patient developed grade 2 photophobia. No patients developed grade 3 or 4 nausea, vomiting, or mucositis.

Discussion

These results indicate that cytarabine, given at the dose and schedule utilized, had minimal activity in the treatment of patients with relapsed or refractory Ewing sarcoma. Although one patient had transient disease stabilization with this regimen, the remaining nine patients did not benefit. There are several potential explanations for the observation that the *in vitro* and *in vivo* preclinical evidence of antitumor activity that formed the rationale for this trial did not translate into clinically meaningful activity in patients. One hypothesized mechanism of action of cytarabine against Ewing sarcoma involves down-regulation of the EWS/FLI1 fusion oncoprotein, although direct cytotoxicity can not be excluded. Whether cytarabine has a similar effect on other EWS fusion oncoproteins has not yet been determined. In an effort to establish the generalizability of this therapy to patients with Ewing sarcoma, patients without documented *EWS/FLI1* translocations were eligible to enroll on study. As a result, only 5 of 10 patients had documented *EWS/FLI1* translocations, with a sixth patient with an EWS translocation with unknown fusion partner. This experience argues for the use of eligibility criteria based upon tumor biology for therapies hypothesized to target a specific biologic feature.

Another potential explanation for this negative result may have been inadequate cytarabine concentrations. Given the extensive clinical experience using cytarabine, pharmacokinetic testing was not performed as part of this trial. Instead, an intermediate dose of cytarabine was chosen for evaluation in part based upon the reported plasma and cellular pharmacokinetics of this drug. Based on previous reports in adults and children, a peak plasma cytarabine concentration of approximately 6 micromolar was anticipated at the conclusion of the 2-hour infusion [4,14-16]. This anticipated concentration compares favorably with the concentrations effective against Ewing sarcoma cells *in vitro*. In addition, studies in leukemia cells have indicated that this concentration results in optimal intracellular accumulation of the active form of cytarabine, ara-CTP [15]. While cytarabine has a short plasma half-life, the half-life of intracellular ara-CTP in leukemia cells is several hours [15]. No preclinical data describe the transport or intracellular half-life of cytarabine and metabolites in Ewing sarcoma cells. Cytarabine delivery into solid tumors may be different from cytarabine delivery to circulating leukemia cells. Conditions which should have resulted in prolonged intracellular ara-CTP levels in leukemia cells may have not had similar effects in Ewing sarcoma cells. Moreover, the mechanism of action in Ewing sarcoma cells may not depend upon ara-CTP at all. If the mechanism of action in Ewing sarcoma cells depends instead upon intracellular cytarabine, then a continuous dosing schedule may have been more effective.

Because of the known hematologic toxicity of cytarabine, patients were only scheduled to receive 5 days of therapy per cycle. In addition, none of the cycles were administered at the prescribed 21-day interval, which may have further diminished the efficacy of this regimen for the five patients who did not show progressive disease in the first cycle of therapy. As a result, tumor cells were not exposed to drug for a minimum of 16 days per cycle allowing for the possibility of growth of resistant tumor cells during this interval. The prominent hematologic toxicity of cytarabine monotherapy in this heavily pre-treated patient population exceeded the toxicity reported in a previous study of high-dose cytarabine in children with refractory solid tumors [17]. This degree of toxicity would preclude evaluations of dosing schedules utilizing additional cytarabine doses or shorter dosing

intervals. While a combination approach may decrease the likelihood of tumor resistance, hematologic toxicity in this patient population may be an obstacle to this approach as well.

Cytarabine demonstrated prominent activity in preclinical studies of Ewing sarcoma. In this clinical trial, no responses were observed and possibly minimal antitumor activity, in the form of stable disease for more than three months, was seen in one patient. Given the minimal activity and prominent toxicity observed, further evaluation of this regimen is not indicated. Instead, future efforts should first be directed at studying the mechanism of action of cytarabine in Ewing sarcoma cells. Since gemcitabine appears to have some activity in patients with Ewing sarcoma [18,19], further studies should also investigate whether this nucleoside analogue also modulates EWS/FLI1. These studies may provide insight that allows for the design of a subsequent clinical study using a different dosing schedule perhaps in combination with other active agents in this disease. In addition, future evaluations of cytarabine and other agents hypothesized to target the EWS/FLI1 oncoprotein as their mechanism of activity should be restricted to patients with known EWS/FLI1-containing tumors.

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Table I

Baseline characteristics of the 10 patients treated on study.

Median Age at Study Entry (range)	20 years (8 – 27 years)
Male : Female	7 : 3
Median Time to First Progression (range)	87 weeks (4 - 441 weeks)
Type of <i>EWS</i> Fusion Oncogene	
<i>EWS/FLI1</i>	5
<i>EWS</i> fusion present, type unknown	1
None detected	2
Not assessed	2
Prior Gemcitabine Therapy	
Yes	4
No	6

Table II

Number of episodes of grade 3 and 4 toxicity of intermediate-dose cytarabine in 16 cycles in patients with relapsed or refractory Ewing sarcoma.

Toxicity	Grade 3 n (%)	Grade 4 n (%)
Hematologic Toxicity*		
Anemia	3 / 15 (20%)	1 / 15 (6%)
Neutropenia		15 / 15 (100%)
Febrile neutropenia	5 / 15 (33%)	
Infection with neutropenia	5 / 15 (33%)	
Thrombocytopenia		15 / 15 (100%)
Non-Hematologic Toxicity		
Alanine transaminase	3 / 16 (19%)	
Aspartate transaminase	1 / 16 (6%)	
Hypoalbuminemia	1 / 16 (6%)	
Chest pain	1 / 16 (6%)	

* Data for hematologic toxicity reflect 15 cycles since one patient died of progressive disease before first hematologic evaluation.