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A Phase I Trial of Imetelstat in Children with Refractory or Recurrent Solid Tumors: A Children's Oncology Group Phase I Consortium Study (ADVL1112)

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Abstract

Purpose: Imetelstat is a covalently-lipidated 13-mer thiophosphoramidate oligonucleotide that acts as a potent specific inhibitor of telomerase. It binds with high affinity to the template region of the RNA component of human telomerase (hTERC) and is a competitive inhibitor of telomerase enzymatic activity. The purpose of this study was to determine the recommended phase II dose of imetelstat in children with recurrent or refractory solid tumors.

Experimental Design: Imetelstat was administered intravenously more than two hours on days 1 and 8, every 21 days. Dose levels of 225, 285, and 360 mg/m^2 were evaluated, using the rolling-six design. Imetelstat pharmacokinetic and correlative biology studies were also performed during the first cycle.

Results: Twenty subjects were enrolled (median age, 14 years; range, 3–21). Seventeen were evaluable for toxicity. The most common toxicities were neutropenia, thrombocytopenia, and lymphopenia, with dose-limiting myelosuppression in 2 of 6 patients at 360 mg/m^2. Pharmacokinetics is dose dependent with a lower clearance at the highest dose level. Telomerase inhibition was observed in peripheral blood mononuclear cells at 285 and 360 mg/m^2. Two confirmed partial responses, osteosarcoma ($n = 1$) and Ewing sarcoma ($n = 1$), were observed.

Conclusions: The recommended phase II dose of imetelstat given on days 1 and 8 of a 21-day cycle is 285 mg/m^2. *Clin Cancer Res; 19(23); 6578–84. ©2013 AACR.*

Introduction

Telomeres, specialized structures found at the end of chromosomes, are involved in the replication and stability of the chromosome. Telomeres consist of tandem repeats of the DNA sequence TTAGGG and associated proteins. During the process of cell division, most human cells undergo telomere shortening because they lose some of these tandem repeats, approximately 50 to 200 base pairs (bp) per cell division (1, 2). When telomeres become critically short, cells either become senescent or undergo apoptosis.

The enzyme telomerase plays an important role in the formation, maintenance, and renovation of telomeres. Telomerase, or telomere terminal transferase, is a ribonucleoprotein that catalyzes the *de novo* synthesis and elongation of telomeric repeats at chromosomal ends by using an RNA segment within the RNA subunit as a template (3–5). Telomerase consists of at least two essential components, the RNA template (hTERC) and the catalytic subunit (hTERT).

Cancer development is accompanied by the preservation of telomere length, which in most cases results from the reactivation of telomerase (6, 7). This reactivation is believed to be critical for tumor progression because it enables cancer cells to maintain their telomere length and avoid apoptosis (8). Approximately 90% of biopsies from a range of human cancers have been found to express telomerase activity (7, 9, 10), including a wide variety of pediatric tumors such as hepatoblastoma, Ewing sarcoma, rhabdomyosarcoma, and osteosarcoma (11). Furthermore, correlations between tumor stage and telomerase activity have been observed, with early-stage tumors having less telomerase activity than late-stage tumors (10, 12–14). On the basis of the high level of telomerase expression common to most cancers and their relatively shorter telomeres compared with their normal tissue counterparts, along with a low expectation of major toxicities occurring in normal tissues, telomerase is a rational target for the treatment of cancer with potentially broad applicability.

Imetelstat has demonstrated broad activity *in vitro* and *in vivo* against a variety of tumor types. Inhibition of xenograft

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Translational Relevance

Telomeres, specialized structures found at the end of chromosomes, are involved in the replication and stability of the chromosome. When telomeres become critically short, cells either become senescent or undergo apoptosis. The enzyme telomerase plays an important role in the formation, maintenance, and renovation of telomeres. Telomerase activation is believed to be critical in cancer progression because it enables tumor cells to maintain their telomere length and avoid apoptosis. Inhibition of telomerase is an attractive new target for cancer therapy. Imetelstat is a covalently-lipidated 13-mer thiophosphoramidate oligonucleotide that acts as a potent specific inhibitor of telomerase and is a first in class agent. This study reports the first trial of imetelstat in patients with pediatric cancer. Twenty subjects were enrolled in this phase I study. Telomerase inhibition was demonstrated in 5 of 6 patients at or exceeding the phase II recommended dose and two confirmed partial responses were observed. Further development of this novel agent for pediatric cancer therapy should be considered.

tumor growth and metastases in rodents at plasma exposures that overlap with plasma exposures attained in patients participating in imetelstat clinical trials have been demonstrated in breast cancer, myeloma, small-cell lung cancer, and non–small cell lung cancer models (15–17). Evidence also suggests that telomerase inhibition is a potential candidate for targeted therapy in pediatric brain tumors. In malignant gliomas, telomerase is positive in 10% to 100% of anaplastic astrocytomas and in 26% to 100% of glioblastoma multiforme (GBM; ref. 18). In 76% of primary medulloblastomas and other primitive neuroectodermal brain tumors have upregulated hTERT mRNA expression compared with normal human cerebellum (19). Imetelstat has also been observed to cross the blood–brain barrier in an orthotopic GBM mouse model. Tumor cells isolated from the orthotopic tumors following systemic administration of imetelstat showed approximately 70% inhibition of telomerase activity (20).

Imetelstat was administered as a single agent in three phase I studies in adults (advanced solid tumors, multiple myeloma, and chronic lymphoproliferative diseases) with the most activity observed in essential thrombocythemia and multiple myeloma (21). The recommended phase II dose and schedule for further testing of imetelstat as a single agent in adults is 9.4 mg/kg on days 1 and 8 of a 21-day cycle (22). In the solid tumor patient study hematologic toxicity was dose limiting; cytopenias were unacceptable at 11.7 mg/kg. However, at 9.4 mg/kg, 12 patients were treated without first cycle dose-limiting toxicity (DLT; ref. 22).

We report the results of a phase I trial of imetelstat in children with recurrent or refractory solid tumors. The primary objectives of this trial were to determine the maximum tolerated dose (MTD) and/or recommended phase II dose, define and describe the toxicities, and characterize the pharmacokinetics of imetelstat. We also assessed the biologic activity by analyzing peripheral blood mononuclear cell (PBMC) extracts for telomerase activity, hTERT, hTERC expression, and telomere length before and after imetelstat therapy.

Materials and Methods

Patient eligibility

Patients older than 12 months and younger than 22 years with measurable or evaluable recurrent or refractory solid tumors were eligible for enrollment. Histologic verification of malignancy from either the time of original diagnosis or relapse was required. Other eligibility criteria included Lansky or Karnofsky score $\geq$60; recovery from the acute toxic effects of prior therapy; $\geq$6 months since total body irradiation, craniospinal or hemi-pelvic radiation; and $\geq$3 months since a stem cell transplant; adequate bone marrow function for patients with solid tumors (peripheral absolute neutrophil count $\geq$1,000/μL, platelets $\geq$100,000/μL transfusion independent), hemoglobin $\geq$8.0 g/dL; adequate renal function (age-adjusted normal serum creatinine or a glomerular filtration rate $\geq$70 mL/min/1.73 m^2); adequate liver function (total bilirubin $\leq$ 1.5 $\times$ institutional upper limit of normal for age, ALT $\leq$ 110 U/L and albumin $\geq$2 g/dL); and adequate coagulation (PTT $\leq$ 1.2 $\times$ upper limit of normal). Patients with solid tumors with known bone marrow metastatic disease were eligible but were not evaluable for hematologic toxicity. Patients were excluded if they were pregnant or lactating or if they had uncontrolled infections.

This trial was approved by the Institutional Review Board of each participating institution. Written informed consent and assent, as appropriate, were obtained in accordance with federal and institutional guidelines.

Drug administration

Imetelstat was supplied by Geron Corporation as a white to pale yellow sterile lyophilized powder. The drug was reconstituted with 0.9% sodium chloride for injection to yield a reconstituted drug concentration of 33.33 mg/mL. An appropriate amount of reconstituted drug was then added to a sufficient volume of 0.9% sodium chloride for injection to achieve a final imetelstat concentration of 1 mg/mL for the 225 mg/m^2 and 285 mg/m^2 dose levels and to a final concentration of 1.3 mg/mL for the 360 mg/m^2 dose level. Drug was administered more than 2 hours.

The starting dose of imetelstat was 225 mg/m^2 (equivalent to approximately 80% of the adult recommended dose of 9.4 mg/kg) with dose escalations in approximately 30% increments to 285 mg/m^2 and 360 mg/m^2. De-escalation of imetelstat to 165 mg/m^2 was planned if DLT was observed at the starting dose level.

Study design

Briefly, up to 6 patients were enrolled concurrently at the starting dose. Enrollment to subsequent dose levels was determined by the number of enrolled patients, the number

Table 1. Characteristics for eligible patients ($n = 20$)

Characteristic	Number (%)
Age, y	
Median	14.9
Range	3.4–21.9
Sex	
Male	13 (65)
Female	7 (35)
Race	
White	14 (70)
Asian	1 (5)
American Indian or Alaska Native	0 (0)
Black or African American	1 (5)
Unknown	4 (20)
Ethnicity	
Non-Hispanic	15 (75)
Hispanic	4 (20)
Unknown	1 (5)
Diagnoses	
Ewing sarcoma	6 (30)
Neuroblastoma	6 (30)
Hepatocellular carcinoma, fibrolamellar	2 (10)
Other[a]	6 (30)
Prior therapy	
Chemotherapy regimens	
Median	3
Range	0–8
Number of patients with prior radiotherapy	11

[a]Includes one patient each with: Wilm tumor, adrenal cortical carcinoma, embryonal rhabdomyosarcoma, osteosarcoma, alveolar soft part sarcoma, and Hodgkin lymphoma.

with DLT, and the number at risk for DLT using the rolling-six design (23).

Toxicity was graded according to the Common Terminology Criteria for Adverse Events version 4.0 (http://ctep.cancer.gov). Hematologic DLT was defined as grade 4 neutropenia for more than 7 days; grade 4 thrombocytopenia on 2 separate days, or requiring a platelet transfusion on 2 separate days within a 7-day period; or myelosuppression that caused a delay of more than 14 days between treatment cycles. Nonhematologic DLT was defined as grade 3 or 4 nonhematologic toxicity attributable to the investigational drug with the exclusion of grade 3 nausea and vomiting of less than 3 days duration; grade 3 transaminase elevation that returned to $\leq$grade 1 or baseline before the time for the next treatment cycle; grade 3 fever or infection; or grade 3 electrolyte abnormalities responsive to oral supplementation. Nonhematologic DLTs included any nonhematologic toxicity that caused a delay of 14 or more days between treatment cycles.

Tumor response was reported using the Response Evaluation Criteria in Solid Tumors (24).

Study evaluations

Patient history, physical examination, and laboratory studies were obtained before treatment and then weekly throughout the first cycle of therapy and before subsequent courses thereafter. Complete blood counts (CBCs) were obtained at least twice weekly during the first cycle and weekly thereafter. Disease evaluations were obtained at baseline, at the end of cycle 1, and after every other cycle $\times$ 2 and then after every third cycle.

Pharmacokinetic studies

Sample collection. Blood samples (3 mL for patients > 10 kg and 2 mL for patients $\leq$ 10 kg) for imetelstat pharmacokinetic studies were placed in EDTA tubes. Samples were collected at the end of infusion and at 0.5, 1, 2, 4, 6 to 8, and 24 ($\pm$2) hours after the infusion following the first imetelstat dose. A 48-hour sample was also obtained in consenting patients. Serum was separated by centrifugation at 1,200 to 1,500 $\times$ *g* relative centrifugal force (RCF) for a minimum of 10 minutes, transferred into cryogenic tubes a polypropylene tube, and stored at -70°C until analysis.

Sample analysis. Plasma imetelstat concentrations were measured using a validated hybridization-ELISA assay. The lower limit of quantitation for imetelstat in human plasma was 367 ng/mL, and the assay dynamic range was between 367 ng/mL and 2448 ng/mL. The interday accuracy was 97.9% to 104.3% with a coefficient of variation of 5.2% to 9.8%. Pharmacokinetic parameters for imetelstat were calculated using noncompartmental analysis with WinNonlin Enterprise, version 5.2 (Pharsight Corporation).

Table 2. Summary of dose-limiting toxicities

Dose level	Number of patients entered	Number of patients evaluable	Number of patients with DLT	Type of DLT (*n*)
225 mg/m^2	7	5	0	
285 mg/m^2	7	6	1	Platelet count decreased (1)
360 mg/m^2	6	6	2	Neutrophil count decreased (1), Platelet count decreased (2)

Table 3. Toxicities (grade 3 or greater) observed in evaluable patients and attributed at least possibly related to the drug

	Maximum grade per patient			
	(Cycle 1, total 17 courses)		(Cycles 2–8, total 17 courses)	
Toxicity type	Grade 3	Grade 4	Grade 3	Grade 4
Anemia	1	0	2	0
Lymphopenia	1	0	1	0
Neutropenia	0	0	0	1
Thrombocytopenia	0	0	2	3
Leukopenia	0	0	1	0
Catheter related infection	0	0	1	0

Biologic assays

Analysis of telomerase activity in PMBCs. PBMCs were isolated from patients' whole blood by density gradient centrifugation using Ficoll-Paque PLUS (GE Healthcare Life Sciences) according to the manufacturer's instructions. Briefly, room temperature 1X PBS without Ca^{2+}, Mg^{2+} (Hyclone laboratories Inc.) was added to the whole blood to a total volume of 15 mL. This mixture was slowly added to a 50 mL conical tube containing 13 mL of Ficoll-Paque PLUS and centrifuged at 400 × *g* for 30 minutes at room temperature. After centrifugation, the PBMC containing layer was collected and washed three times with three volumes of room temperature 1X Hank's balanced salt solution (Mediatech) and centrifuged a second time at 365 × *g* for 10 minutes at 20°C. The cells were resuspended in 3 mL cold PBS and the cell count and viability were determined. Cells were then pelleted at 3,200 × *g*, at 4°C for 1 minute, and cell pellets were flash-frozen in dry ice and immediately stored at −80°C until assays were performed. PBMCs were collected before treatment; 6 to 8 hours, and 24 (±2 hours) hours after the first imetelstat dose; before the imetelstat dose on day 8; and before the imetelstat dose on day 1 of cycles 2 and 3. PBMC extracts were analyzed for telomerase activity before and following therapy with imetelstat. Telomerase enzyme activity was assessed using the TRAPeze Telomerase Detection and TRAPeze XL Telomerase Detection Kits (Millipore). Cell extracts were prepared according to protocols provided by the manufacturer and 400 ng of total protein was assayed for telomerase activity.

Results

Twenty patients were enrolled on study between June 2011 and April 2012. Three patients were not fully evaluable for toxicity because they did not complete cycle 1. One of the 3 patients was removed for progressive disease. One withdrew before starting treatment and 1 withdrew (patient preference) before day 8, drug administration. Patients received a median of one cycle of therapy (range, 1–8). Patient characteristics are summarized in Table 1.

Toxicity

Table 2 summarizes the observed DLTs. At the third dose level (360 mg/m^2), 1 patient experienced thrombocytopenia leading to a delay in treatment of more than 14 days and the second patient experienced both neutropenia and thrombocytopenia leading to a delay in therapy of more than 14 days.

Table 3 summarizes adverse events grade 3 or greater at least possibly attributable to imetelstat in the 17 evaluable patients.

Responses

Two partial responses were observed in the 16 patients who were evaluable for response. One partial response was observed at the 225 mg/m^2 dose level in a patient with metastatic osteosarcoma to the lung. Unfortunately, the patient had prolonged thrombocytopenia following his third course and had to be removed from protocol therapy. The second partial response was observed at the 285 mg/m^2 dose level in a patient with a paraspinal Ewing sarcoma. This

Table 4. Summary of imetelstat pharmacokinetic parameters

Dose level					
(mg/m^2)	No. of patients	C_{max} (μg/mL)	Half-life (h)	AUC (μg/mL·h)	Cl (mL/h/m^2)
225	7	63.7 ± 18.7	3.7 ± 1.8	307 ± 154	855 ± 298
285	6	96.7 ± 31.4	8.0 ± 5.3	614 ± 248	792 ± 602
360	5	164 ± 41.3	4.4 ± 1.5	1240 ± 395	318 ± 117

patient received 8 cycles of therapy before eventually having progressive disease.

Pharmacokinetics

Results of day 1, cycle 1 pharmacokinetic studies are shown in Table 4. Pharmacokinetic analyses were completed for 18 patients. The mean half-life for imetelstat was 5.3 ± 3.7 hours. Imetelstat clearance was dose-dependent. A 3-fold increase in C_{max} and a 4-fold increase in AUC were observed when the dose was escalated from 225 mg/m^2 to 360 mg/m^2.

Telomerase activity in PBMCs following imetelstat treatment

The effect of imetelstat on telomerase activity in PBMC extracts was assessed using the telomeric repeat amplification protocol (TRAP). PBMCs were collected before the treatment; 6 to 8 hours, and 24 (±2 hours) hours after the first imetelstat dose; before the imetelstat dose on day 8; and before the imetelstat dose on day 1 of cycle 2. Samples from 6 patients, including 3 at the recommended phase II dose (285 mg/m^2), could be analyzed. Although baseline and posttreatment samples were submitted from 17 patients, only 6 patients had samples that could be evaluated for telomerase inhibition. There did not seem to be any issues with assay quality. For the samples analyzed the internal controls functioned appropriately and problems seemed to be a result of poor sample quality related to shipping and handling. If telomerase activity could not be assessed at baseline, the patient was considered inevaluable for assessment of telomerase inhibition.

Telomerase inhibition in PBMCs was observed in 5 of 6 patients including all 3 that were evaluable at the recommended phase II dose. In one of these patients telomerase inhibition was sustained through day 8. In the other 2 patients, telomerase activity returned by the second dose of drug on day 8 (for one inhibition was sustained for 6–8 hours and for the other inhibition lasted through 24 hours). At the 360 mg/m^2 level, 2 of 3 patients had inhibition of telomerase activity in PBMCs. For both patients, telomerase activity returned by the second dose of drug on day 8. Fig. 1 shows the telomerase inhibition results for patient 12, the patient with Ewing sarcoma who had a partial response at 285 mg/m^2.

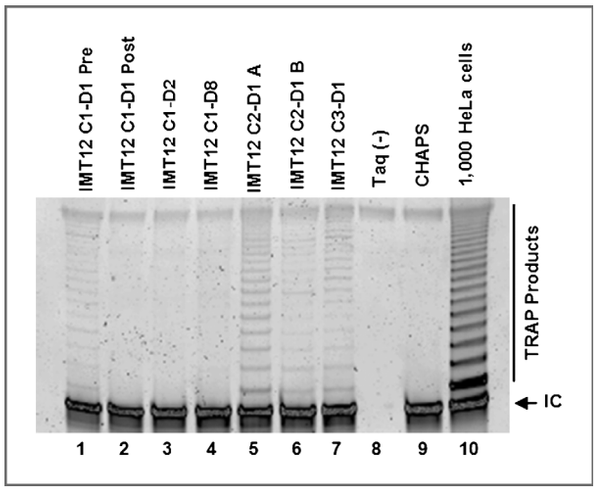

Figure 1. PBMC telomerase activity in a representative patient as assessed by the TRAP. Lane 1 shows presence of telomerase activity predose characterized by 6-bp telomeric repeat ladder TRAP products, but no activity at day 2 or day 8 of cycle 1, lanes 3 and 4. Lane 2 represents telomerase activity 6 to 8 hours after the first dose. Telomerase activity is again observed on the predose sample for cycle 2; lane 5 and 6, indicating that telomerase inhibition is sustained through day 8. A and B in lanes 5 and 6 indicate blood samples from two different vials drawn at the same time point. This patient with paraspinal Ewing sarcoma had a partial response to therapy before having progressive disease after cycle 8 of therapy. Lane 7 represents telomerase activity predose at day 1 of cycle 3. IC is the PCR internal control; lane 8, Taq polymerase negative control; lane 9, telomerase activity negative control (lysis buffer only, CHAPS); lane 10, telomerase activity positive control (from 1,000 HeLa cells).

Table 5 shows imetelstat pharmacokinetics (peak concentration and AUC) for the patients in whom telomerase inhibition could be assessed. As shown in the table, duration of telomerase inhibition did not clearly correlate with either imetelstat concentration or imetelstat AUC. However, the data are limited and insufficient to definitively evaluate the association between pharmacokinetics and duration of telomerase inhibition.

Table 5. Relationship between pharmacokinetics and telomerase inhibition

Patient ID	Dose level (mg/m^2)	C_{max} (μg/mL)	AUC (μg/mL·h)	Duration of PBMC telomerase inhibition[a]
10	285	141	816	8 hours
11	285	113	906	24 hours
12	285	115	770	8 days
15	360	199	1,508	24 hours
16	360	207	1,308	24 hours
18	360	169	1,696	No inhibition observed

[a]PBMCs were collected at the following time points: (i) before the first dose of drug on day 1, (ii) 6 to 8 hours after the first dose, (iii) 24 (±2) hours after the first dose, (iv) before the imetelstat dose on day 8, and (v) before the imetelstat dose on day 1 of cycles 2 and 3. Duration of inhibition is determined as the last time point at which telomerase activity was absent.

Discussion

This study examined the toxicity and tolerable dose of imetelstat in pediatric patients with refractory or recurrent solid tumors. The MTD of imetelstat was 285 mg/m^2 intravenously more than 2 hours on days 1 and 8 of a 21-day cycle. The dose-limiting toxicities observed at 360 mg/m^2 were myelosuppression leading to delay in therapy. In general, the hematologic and nonhematologic toxicities on this study were minor. No nonhematologic toxicities grade 3 or greater were observed on any of the courses of therapy delivered.

Drug disposition of imetelstat was investigated on day 1 of cycle 1. Pharmacokinetic analyses were completed for 18 patients. The mean half-life for imetelstat was 5.3 ± 3.7 hours. Consistent with the adult pharmacokinetic data, imetelstat clearance in pediatric patients was dose-dependent. A 3-fold increase in C_{max} and a 4-fold increase in AUC were observed when the dose was escalated from 225 mg/m^2 to 360 mg/m^2. The C_{max} (96.7 ± 31.4 μg/mL) and AUC (614 ± 248 μg/mL·h) at the recommended phase II dose (285 mg/m^2) were less than have been reported for adults. In studies in adult patients with solid tumors at doses of 7.5 to 9.4 mg/kg (approximately 225–282 mg/m^2), the C_{max} and AUC were 136 to 190 μg/mL and 1,028 to 1,036 μg/mL·h, respectively (22).

Correlative biology studies show that imetelstat decreased telomerase activity in PMBCs. Telomerase inhibition was observed for 5 patients including all 3 at the at the recommended phase II dose (285 mg/m^2) who had evaluable samples. In 1 of these patients telomerase inhibition was sustained through day 8. In the other 2 patients, telomerase activity returned by the second dose of drug on day 8. At the 360 mg/m^2 dose level telomerase inhibition was observed in 2 of 3 patients and activity returned by day 8 in both patients. There was no clear predictor of the duration of telomerase inhibition. In summary, 20 children with recurrent or refractory solid tumors were enrolled in the phase I trial of imetelstat, and we recommend a phase II dose of 285 mg/m^2 on days 1 and 8 of a 21-day cycle. Imetelstat pharmacokinetics was dose dependent and telomerase inhibition was observed at the recommended phase II dose. Two partial responses were also observed. Overall the drug was well tolerated and the preliminary response data suggest further development of imetelstat as both a single agent or in combination studies for children. A phase II single-agent study in pediatrics is planned.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: P.A. Thompson, R. Drissi, M. Fouladi, S.M. Blaney
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): P.A. Thompson, E. Panditharatna, A.M. Ingle, C.H. Ahern, J.M. Reid, T. Lin, S.M. Blaney
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