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A Phase 1 Trial of TPI 287 as a Single Agent and in Combination With Temozolomide in Patients With Refractory or Recurrent Neuroblastoma or Medulloblastoma

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Background. The primary aim of this Phase I study was to determine the maximum tolerated dose (MTD) of TPI 287 and the safety and tolerability of TPI 287 alone and in combination with temozolomide (TMZ) in pediatric patients with refractory or recurrent neuroblastoma or medulloblastoma. The secondary aims were to evaluate the pharmacokinetics of TPI 287 and the treatment responses. ***Procedure.*** Eighteen patients were enrolled to a phase I dose escalation trial of weekly intravenous infusion of TPI 287 for two 28-day cycles with toxicity monitoring to determine the MTD, followed by two cycles of TPI 287 in combination with TMZ. Samples were collected to determine the pharmacokinetic parameters C_{max}, $AUC_{0\text{-}24}$, $t_{1/2}$, CL, and V_d on day 1 of cycles 1 (TPI 287 alone) and 3 (TPI 287 + TMZ) following TPI 287 infusion. Treatment response was evaluated by radiographic (CT or MRI) and radionuclide (MIBG) imaging for neuroblastoma. ***Results.*** We determined the MTD of TPI 287 alone and in combination with temozolomide to be 125 mg/m^2. The non-dose-limiting toxicities at this dose were mainly anorexia and pain. The dose-limiting toxicities (DLTs) of two patients at 135 mg/m^2 were grade 3 hemorrhagic cystitis and grade 3 sensory neuropathy. ***Conclusions.*** Overall, TPI 287 was well tolerated by pediatric patients with refractory and relapsed neuroblastoma and medulloblastoma at a dose of 125 mg/m^2 IV on days 1, 8, and 15 of a 28 day cycle. Pediatr Blood Cancer 2016;63:39–46.

Key words: brain tumors; medulloblastoma; neuroblastoma; pediatric hematology/oncology; phase I clinical trials; TPI 287

INTRODUCTION

Neuroblastoma is the most common pediatric extracranial solid tumor and accounts for 7–10% of childhood cancers.[1,2] Although the prognosis for infants with neuroblastoma is generally good, currently only 30% of children diagnosed after 12–15 months of age survive despite aggressive multimodal therapies.[3,4] High-dose chemotherapy (HDC) followed by hematopoietic stem cell transplantation (HSCT) and maintenance therapy with retinoic acid and anti-GD2 antibody improves survival in children presenting with metastatic NB, but the 5-year event-free survival remains below 60%.[5,6] Medulloblastoma is the most common malignant brain tumor in children and accounts for 16% of all brain tumors in children 0–14 years old and 6% in adolescents 15–19 years old.[7] Current therapies for children with disseminated disease are associated with severe long-term toxicities, and lead to cure in only a minority of cases.[8] Thus, the development of new therapies—especially ones with more favorable toxicity profiles—would represent a significant improvement in the treatment of this disease. Consequently, the evaluation of new drugs is strongly needed in these diseases.

TPI 287 is a novel anti-microtubule agent of the taxane family developed by Tapestry Pharmaceuticals, Inc. (now Cortice Biosciences, Inc.). It is synthetically manufactured from naturally occurring taxanes extracted from yew starting material. The synthesis involves modifications of the side chain to make the drug more lipophilic, and modification of the baccatin ring structure in order to circumvent Multi-Drug Resistant (MDR) pathways and allow for binding to mutant tubulin. TPI 287 has been found to be potent against several neuroblastoma and medulloblastoma cell lines in vitro (unpublished results, Cortice Biosciences). In neuroblastoma this was also demonstrable in transplanted xenografts, showing greater activity than paclitaxel, docetaxel, or nab-paclitaxel (unpublished data, Cortice Biosciences). In vitro studies have indicated that TMZ is effective in tumor cell lines resistant to other agents.[9] A preclinical study reported activity of TMZ in four xenograft models of human neuroblastoma.[10] TPI

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Abbreviations: ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; AUC0-24, area under the curve between 0–24 hr; BUN, blood urea nitrogen; CBC, complete blood count; CL, clearance; C_{max}, maximum concentration; CNS, central nervous system; CT, computed tomography; DLT, dose limiting toxicity; EKG, electrocardiogram; HDC, high dose chemotherapy; HRPC, hormone refractory prostate cancer; HSCT, hematopoietic stem cell transplantation; LDH, lactate dehydrogenase; MB, medulloblastoma; MDR, multiple drug resistant; MIBG, 123I-metaiodobenzylguanidine; MRI, magnetic resonance imaging; MTD, maximum tolerate dose; NB, neuroblastoma; NCI-CTCAE v3.0, National Cancer Institute—Common Terminology Criteria for Adverse Events v3.0; PK, pharmacokinetics; PNS, peripheral nervous system; RECIST, response evaluation criteria in solid tumors; SGPT, serum glutamic pyruvate transaminase; t1/2, biologic half-life; TMZ, temozolomide; ULN, upper limit of normal; V_d, apparent volume of drug distribution

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287 was also shown to have increased efficacy when TPI 287 is combined with TMZ in neuroblastoma cell lines (unpublished data, Sholler Lab).

In vitro, TPI 287 was shown to have comparable cytotoxicity to paclitaxel in several MDR- cell lines, but was 5–3,900-fold more active than several comparator compounds in MDR+ cell lines.[11] In MCF-7-AR breast cancer cells, which display MDR, TPI 287 was 20 times more active than paclitaxel. Similar findings were observed in MDR+ cells derived from colorectal, breast, and prostate cancers.[12] TPI 287 was also evaluated in a variety of xenograft models. In an orthotopic xenograft using U251 cells implanted in the brains of nude mice, treatment with either TPI 287, TMZ, or combinations were compared to control animals, evaluating median survival (10 animals per group) as well as animals whose survival extended beyond 110 days. Significant synergy and improvement in long-term survival was seen with the combination of TMZ plus TPI 287.[13] Salient features of TPI 287 include a structure similar to the taxanes, microtubule stabilization in vitro, and significant brain permeability. In animal studies, brain distribution was determined after a single injection into mice and rats. Multiple-fold elevation was observed in brain as compared with plasma in both species, in terms of C_{max}, AUC, and other pharmacologic parameters. Using an experimental model of brain metastasis of triple-negative breast cancer, a 55% reduction in the formation of large metastases using three intravenous injections of 18 mg/kg TPI 287 was reported.[12]

TPI 287 has been investigated in Phase I and II trials in adult cancer populations. A Phase I trial of 29 eligible patients established a MTD of 125 mg/m^2, although some patients demonstrated a 57% reduction in one lesion as assessed by Response Evaluation Criteria in Solid Tumor (RECIST) at a dose of 85 mg/m^2. The drug was well tolerated in this population (unpublished data, Cortice Biosciences). The most frequent adverse events included anemia, nausea, diarrhea, and neuropathy. Dose limiting toxicities observed at a dose of 185 mg/m^2 included grade 3 neuropathy, neutropenia, and elevated AST. A second Phase I trial of 21 eligible patients investigated a dose up to 160 mg/m^2 administered every 21 days. Dose limiting toxicities were similar to the previously discussed study and included grade 3 neuropathy and grade 4 neutropenia. The most common adverse events were also similar with the most frequent (in four or more patients) being anemia, neuropathy, neutropenia, fatigue, diarrhea, nausea, vomiting, asthenia, abdominal pain, and myalgia. A potential side effect of all microtubule-stabilizing drugs is peripheral neuropathy. Although neurotoxicity is the principal toxic effect observed clinically with microtubule stabilizing drugs, how and why this happens is not well understood,[12] although it is thought to be due, at least in part, to the inhibition of axonal microtubules that are necessary for axonal transport in neurons.[14] Other than the obvious explanation that neural cells have large amounts of tubulin, studies addressing this phenomenon have been hampered by the lack of suitable in vitro or animal models. TPI 287 was administered on Days 1, 8, and 15 at a dose of 127 mg/m^2/dose in a phase I adult trial for patients with advanced cancer and found to be well tolerated.[15] This dose schedule has been further evaluated in a number of phase II trials in combination with temozolomide for patients with melanoma.

TMZ has been evaluated in pediatric populations with solid tumors[16,17] as well as with CNS tumors[18,19] and has shown some activity against recurrent medulloblastoma. TMZ is rapidly and completely absorbed after oral administration.[20] A multicenter, Phase 2 evaluation of an oral, daily schedule of TMZ (200 mg/m^2/d $\times$ 5 days every 28 days) was undertaken in children with refractory or relapsed high-risk neuroblastoma. Twenty-five patients were enrolled, and response was observed in five patients with a median duration of 6 months. The authors concluded that TMZ shows activity in heavily pretreated patients with neuroblastoma, and deserves further evaluation in combination with another drug.[21]

The Neuroblastoma and Medulloblastoma Translational Research Consortium (NMTRC) performed a pediatric phase I trial specifically designed to test the safety, clinical toxicity, maximum tolerated dose (MTD), and pharmacokinetics of TPI 287 alone and in combination with temozolomide for the treatment of patients with recurrent or refractory neuroblastoma or medulloblastoma. The secondary objectives of the study were to evaluate pharmacokinetics and treatment response. This paper will describe the toxicity profile, dose limiting toxicities (DLTs), MTD, pharmacokinetics, and treatment responses of patients with recurrent/refractory neuroblastoma or medulloblastoma when treated with TPI 287 alone and in combination with TMZ.

METHODS

Eligibility

Patients >12 months of age and diagnosed before the age of 21 with histologically proven neuroblastoma and confirmation of refractory or recurrent disease or medulloblastoma with histologic confirmation at diagnosis or at the time of recurrence/progression were eligible for this study. Residual abnormal tissue at a primary or metastatic site must have measured more than 1 cm in any dimension by standardized imaging (CT or MRI) for measurable disease or for patients with only skeletal disease neuroblastoma, there must have been at least two persisting skeletal foci on ^{123}meta-iodobenzylguanidine (MIBG) follow-up scans. Patients without bone marrow metastases were required to have an ANC >750/μl and platelet count >50,000/μl. Patients had to demonstrate adequate liver function as defined by total bilirubin ≤1.5× upper limit of normal (ULN) for age and SGPT (ALT) <10× upper limit of normal for age and a bilirubin <1.5 mg/dl ULN for age and no other significant organ toxicity defined as >Grade 2 by National Cancer Institute Common Toxicity Criteria for Adverse Events version 3 (NCI-CTCAE v3.0). This trial was approved by the Western Institutional Review Board as well as by local institutional review boards at each enrolling site. Informed consent from the patient or their guardian(s) and assent, as appropriate, were obtained prior to study entry.

Study Design

TPI 287 was supplied as a sterile, parenteral solution to be diluted prior to administration, consisting of 10/mg/ml TPI 287 in a 50:50 v/v mixture of Cremophor® EL-P and dehydrated alcohol, USP by Archer Biosciences. The drug was administered intravenously (IV) at the assigned dose once per week for 3 weeks. Due to potential hypersensitivity reactions due to Cremophor® EL-P, all patients were pretreated with a corticosteroid, an antihistamine, and a H_2 blocker. TPI 287 was dosed in a 3 + 3 study design with dosing beginning at 90 mg/m^2 IV with dosing escalated by 20–25 mg/m^2 per cohort until a maximum

tolerated dose (MTD) was determined. Patients received TPI 287 as a single agent on days 1, 8, and 15 of each 28 day cycle in cycles 1 and 2, followed by the same schedule of TPI 287 in combination with TMZ (100 mg/m^2/d orally on days 1–5) starting in cycle 3. Patients were allowed to move directly to Cycle 3 if they experienced a progression during cycles 1 or 2.

Pharmacokinetic Studies

Pharmacokinetic (PK) analysis for TPI 287 and its analytes TPI 511 and TPI 513 was done on all patients at the MTD level on day 1 of the first and third cycles. Blood samples (2 ml) were collected prior to beginning infusion (Time 0), at the end of infusion (Time 1) as well as 0.25, 0.5, 1, 2, 4, and 6 hr post-time 0 collection. The blood samples were processed to plasma and the concentration of TPI 287 was assayed by a validated liquid chromatography/tandem mass spectrometry (LC/MS-MS) method. A standard calibration curve was performed by using internal standards and varying concentrations of TPI 287 and metabolites to blank human plasma and processed similar to clinical samples. PK parameters ($AUC_{0\text{-}7}$, $AUC_{0\text{-}\infty}$, Cl, V_{ss}, $t_{1/2}$, C_{max}, and t_{max}) were determined as appropriate using non-compartmental procedures (R version 2.15.1 PK package and SAS v 9.2 software). Individual subject measurements, as well as summary statistics (mean, standard deviation, and range) were reported.

Toxicity Monitoring

Safety analysis was conducted on all patients who received at least one dose of study drug, and included the frequency of all adverse events and laboratory abnormalities as well as frequency of dose interruptions, dose reductions, and treatment discontinuation. Weekly monitoring for treatment related toxicities included a physical exam, CBC, AST/ALT, LDH, Bilirubin, electrolytes, BUN, Creatinine, albumin, total protein, and neurological questionnaire. Vital signs, including temperature, pulse rate, and blood pressure (sitting) were performed pre-dose and at the end of infusion. Patients without bone marrow metastases were required to have adequate bone marrow function as defined by ANC >750/μl and platelets >50,000/μl before starting chemotherapy. At the beginning of Cycles 1 and 3 an EKG was also performed. Clinical and laboratory adverse events were graded according to the NCI-CTCAE v3.

Definition of DLT and MTD

The MTD of single agent therapy was defined as the dose level below that in which there were DLTs in two out of six patients who completed at least one 28-day cycle of therapy at that dose level. Patients that went off therapy for reasons other than a DLT before completing one full cycle were replaced in order to complete the cohort of three patients. DLT was defined as any of the following events that were at least possibly attributable to TPI 287: Grade 5 toxicity that occurred within 30 days of taking study drug, Grade 3 neurotoxicity, Grade 4 neutropenia or thrombocytopenia that persisted for ≥7 days, Grade 3 thrombocytopenia with bleeding, Grade 3 elevation of transaminases that persisted for ≥7 days, Grade 2 cardiotoxicity, or any other Grade 3 non-hematologic toxicity, excluding alopecia or inadequately treated nausea, vomiting, or diarrhea. TPI 287 was discontinued and patients removed from study if toxicity persisted up to 21 days.

Criteria for Assessment of Response

This study used RECIST measurements from the NCI modified for pediatrics as well as MIBG or PET and bone marrow response. Tumor assessments/imaging studies were obtained at baseline >7 days from prior therapy and <21 days from the start of study therapy. These were repeated at the end of the first cycle (optional), second cycle, and again after every other cycle. All neuroblastoma patients enrolled were of the INRG high risk group.[22] Definitions of responses were consistent with INRC guidelines.[3]

Statistical Methods

Descriptive statistics were examined. Mean and median times to progression were estimated.

RESULTS

Patients

Eighteen patients with refractory or recurrent neuroblastoma or medulloblastoma were enrolled in this study between March 2009 and June 2011. One patient (125 mg/m^2) was excluded from analysis due to initial diagnosis at >21 years of age. The patient characteristics are shown in Table I. Every patient had previously received standard therapy and had relapsed or was refractory to therapy. The median age was 7 years old, with a range of 4–19 years old. Patients were enrolled at one of four escalating doses. Three patients were enrolled at 90 mg/m^2, six patients at 110 mg/m^2, two patients at135 mg/m^2, and seven patients at 125 mg/m^2. Eighteen patients received at least one dose of TPI 287 as a single agent and were evaluable for safety (16 with neuroblastoma and two with medulloblastoma). Thirteen evaluable patients (12 neuroblastoma and one medulloblastoma) completed ≥1 cycle comprising the TPI 287 single agent efficacy population whereas eight patients (seven neuroblastoma and one medulloblastoma) completed ≥3 cycles comprising the TPI 287 and temozolomide combination treatment efficacy population. Of the eight patients who received the additional combination therapy, one patient completed 1 cycle, four patients completed 2 cycles (including one medulloblastoma patient), two patients completed 10 cycles, and one patient completed 23 cycles of TPI 287 and temozolomide combination treatment.

TABLE I. Patient Characteristics

Patient Characteristics	Number
Patients enrolled	18
Male	12
Female	6
Median age (y) (range)	7.5 (4–25)
Race	
Caucasian	12
Hispanic	4
Other	2
Diagnosis	
Neuroblastoma	16
Medulloblastoma	2

Toxicities

Taxanes, including paclitaxel and docetaxel, have been studied in phase I and II pediatric trials. The principal dose limiting toxicity observed was neutropenia. As in adults, hypersensitivity was observed and patients required pre-medication. Additional toxicities included hematologic, neurologic, and dermatologic.[23,24] TPI 287-related non-dose-limiting toxicities observed during cycles 1 and 2 are shown in Table II. Anorexia (n = 2), AST elevation (n = 2), pain (n = 4), and vomiting (n = 2) were the most common toxicities. Anorexia was only observed at the 125 mg/m^2 dose. AST elevation was more severe at the dose of 125 mg/m^2 compared to that of 90 mg/m^2, but was not observed at 110 or 135 mg/m^2. Pain was not observed at the 110 mg/m^2 dose. It was observed as grade 2 at 90, 125, and 135 mg/m^2, as well as grade 3 at 125 mg/m^2. It is particularly challenging to determine the etiology of pain in pediatric patients, whether from drug-induced neuropathy or progressive disease; therefore, pain without clear other etiology is always reported as related to treatment. Vomiting was observed as grade 2 at both 110 and 125 mg/m^2 doses, but was absent in other dose cohorts.

The toxicities observed in all cycles of TPI 287 combination therapy are shown in Table III. Anemia (n = 3) was the most common toxicity noted across all dose levels for TPI 287. It was observed as a grade 2 on one occasion in each dose level. The most common toxicities at 125 mg/m^2 included one observation each for gastritis (grade 2), anemia (grade 2), leukopenia (grade 2), and sensory neuropathy (grade 3).

One unexpected toxicity that occurred above the MTD was hemorrhagic cystitis. The DLT occurred 13 days after the last dose of TPI 287. The patient was evaluated for infectious causes; culture and BK virus testing were negative. The DSMB determined that it was most likely due to a viral infection, but because the relationship to TPI 287 could not be ruled out they deemed the event to be possibly related and recommended reducing the TPI 287 dose by 10 mg/m^2. The patient did not recover from the SAE within the protocol-required timeline and was withdrawn from the study.

No cardiotoxicity was noted on 5 min ECGs done throughout the study. Three DLTs occurred during the safety phase of this trial. One DLT, grade 3 seizure, occurred in the 110 mg/m^2 dose cohort which was determined to be due to extensive metastatic medulloblastoma disease. Three additional patients were enrolled (n = 6 for this cohort) and no other DLTs occurred. Two DLTs occurred in the 135 mg/m^2 cohort. These included a grade 2 hemorrhagic cystitis and a grade 3 sensory neuropathy. Following these DLTs the DSMB

TABLE II. Toxicities Observed During the First Two Cycles With TPI 287 Alone

	No. subjects											
	Dose 90 mg/m^2 (n = 3)			Dose 110 mg/m^2 (n = 6)			Dose 125 mg/m^2 (n = 6)			Dose 135 mg/m^2 (n = 2)		
Toxicity	Grade 2	3	4	2	3	4	2	3	4	2	3	4
Abdominal distention							1					
Albumin							1					
ALT		1										
ANC									1[a]			
Anorexia							1	1				
AST	1							1				
Constipation				1								
Dehydration				1								
Diarrhea							1[a]					
Drug Reaction					1							
Fatigue								1				
GGT							1					
Hemoglobin							1					
Hemorrhagic cystitis											1[b]	
Hyperbilirubinemia									1			
Hypokalemia							1					
INR								1				
Leukocytes								1[a]				
Lip sore				1								
Liver dysfunction							1					
Neuropathic pain				1								
Neuropathy sensory							1				1[b]	
Neutrophil	1											
Pain	1						1	1		1		
Peripheral neuropathy							1					
Seizure					1[a,b]							
SIADH				1								
Vomiting				1			1					

Number listed represents frequency of occurrence in the population followed by percentage in parenthesis. ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; INR, international normalized ratio. [a]Medulloblastoma subject; [b]Dose limiting toxicities.

TABLE III. Toxicities Observed During Co-Administration of TPI 287 and Temozolamide

	No. Subjects								
	Dose 90 mg/m^2 (n = 2)			Dose 110 mg/m2 (n = 3)			Dose 125 mg/m^2 (n = 3)		
	Grade 2	3	4	2	3	4	2	3	4
ALT	1								
Anorexia				1					
Fever					1				
Gastritis							1		
Hemoglobin	1			1			1		
Leukocytes							1[a]		
Nausea				1					
Neuropathic pain	1								
Neuropathy sensory								1	
Neutrophil	1								
Stress fracture	1								
Vomiting				1					

ALT indicates alanine aminotransferase. There were no subjects from the 135 mg/m^2 dose that received therapy combined with temozolmide.
[a]Medulloblastoma subject.

made the decision to move to a lower dose, 125 mg/m^2. Seven patients were enrolled in the 125 mg/m^2 dose and no additional DLTs were observed. Thus, the MTD of TPI 287 recommended for Phase II evaluation is 125 mg/m^2.

Pharmacokinetics

Pharmacokinetic sampling was achieved on six out of six patients treated at the MTD of 125 mg/m^2 of TPI 287 and the pharmacokinetic parameters that were derived using non-compartmental procedures are listed in Table IV. Pharmacokinetic analysis was done on day 1 of cycle 1 (TPI 287 alone) and of cycle 3 (TPI 287 with temozolomide). Consistently, the C_{max} was captured immediately following completion of the TPI 287 infusion with steady decline at all other time points. This was consistent between both cycle 1 and cycle 3 for all patients. Due to the small sample size, high inter-patient variability was seen in the range of data; however data appear to be more consistent during cycle 1 as compared to cycle 3. On day 1 of cycle 1, PK analysis showed mean peak serum levels of 3.21 μg/ml at 1 hr post completion of the TPI 287 infusion. The same trend was also observed on day 1 of cycle 3 with mean peak serum levels 3.44 μg/ml at 1 hr post TPI 287 infusion as well. The $AUC_{0\text{-}7}$ was similar on each of the days measured, with means of 5.10 μg/ml-hr and 5.47 μg/ml-hr on day 1 of cycles 1 and 3, respectively. It should also be noted that two subjects did not have samples collected at the last time point (6 hr post-time 0) on day 1 of cycle 3.

Response

Twelve patients with neuroblastoma and one patient with medulloblastoma were evaluable for efficacy following TPI 287 treatment alone. According to RECIST criteria, five patients (all with neuroblastoma) displayed stable disease, and eight had progressive disease. Of the eight patients (seven with neuroblastoma and one with medulloblastoma) that were evaluable for efficacy with TPI 287 and temozolomide, one showed a partial response (neuroblastoma), three had stable disease (neuroblastoma), and four had progressive disease (three neuroblastoma and one medulloblastoma) (Table V).

One patient (neuroblastoma) was non-evaluable for disease response to therapy. This patient experienced a DLT (peripheral neuropathy) after one dose of TPI 287 alone and was withdrawn from the study. The mean progression free survival (PFS) for all 12

TABLE IV. Pharmacokinetics of TPI 287 and Metabolites TPI 511 and TPI 513

				Mean ± SE						
Analyte	Treatment	N	Dose (mg/m^2)	$AUC^a_{0\text{–}7}$ (ng/ml-hr)	$AUC_{0\text{–}\infty}$ (ng/ml-hr)	Cl (L/hr/m^2)	Vss (L/m^2)	$t_{1/2}$ (hr)	C_{max} (ng/ml)	t_{max} (hr)
TPI 287	C1D1	5[b]	125	5,055.40 ± 327.68	6,097.05 ± 571.74	21 ± 2	80 ± 18	2.70 ± 0.77	3,208.00 ± 223.79	1
TPI 287	C3D1	4[c]	125	5,474.90 ± 684.96[a]	6,857.78 ± 1652.43	18 ± 4	78 ± 34	2.98 ± 1.85	3,435.00 ± 872.61	1
TPI 511	C1D1	5[b]	125	282.46 ± 28.81	353.10 ± 56.35	354 ± 56	1548 ± 419	3.03 ± 1.15	126.38 ± 25.41	1
TPI 511	C3D1	4[c]	125	201.55 ± 29.25[a]	226.16 ± 33.40	553 ± 82	1817 ± 458	2.28 ± 0.55	89.83 ± 29.09	1
TPI 513	C1D1	5[b]	125	522.22 ± 77.52	562.79 ± 78.67	222 ± 31	569 ± 143	1.78 ± 0.34	299.40 ± 92.96	1
TPI 513	C3D1	4[c]	125	377.31 ± 45.76[a]	418.10 ± 69.41	299 ± 50	866 ± 259	2.01 ± 0.76	215.25 ± 55.31	1

[a]Two subjects (108, 302) with no data at 7 hr time point for C3D1 treatment; [b]One subject (801) with no data at 1 hr timept. for C1D1 treatment, excluded from analysis; [c]Two subjects (202, 801) with no data for C3D1 treatment.

TABLE V. Study Subject Responses to Treatment With TPI 287 Alone and in Combination With Temozolomide

Dose (mg/m^2)	N	Partial response	Stable disease	Progressive disease
TPI 287 alone				
90	3	0	2	1
110	5	0	1	4
125	4	0	1	3[a]
Total	12	0	4	8
TPI-287 in combination with temozolomide				
90	2	0	1	1
110	3	0	1	2
125	3	1	1	1[a]
Total	8	1	3	4

[a]Includes 1 medulloblastoma patient.

patients with neuroblastoma completing at least one cycle of TPI 287 was 6.2 months (median 2.1 months). Two patients with neuroblastoma on this study were removed from treatment at parental request with no progression of disease. PFS for these patients were both censored at 10.7 months. Another patient was removed due to unacceptable toxicity (grade 3 sensory neuropathy and grade 2 gastritis) with stable disease. PFS for this patient was censored at 4.0 months. One patient experienced a partial response and remained on therapy for 21.8 months before moving on to other treatment with a final PFS for this patient of 36.4 months. One patient with medulloblastoma was non-evaluable for response to combination therapy due to unacceptable toxicity (grade 3 seizure) after one dose of TPI 287 and was withdrawn from the study. The second patient with medulloblastoma had PFS of 1.7 months. Individual subject details can be found in Table VI including assigned cohort, disease at status entry, number of previous relapses, responses, and reason for off-therapy.

DISCUSSION

TPI 287 is a novel anti-microtubule agent of the taxane family with the intent to circumvent MDR-based resistance and allow for binding to mutant tubulin as well. TPI 287 has a very high preclinical potency against several neuroblastoma cell lines and xenograft models. Although TPI 287 has been previously studied in adult cancer populations, amassing a substantial amount of safety data, this is the first study in a pediatric population.

This study determined the MTD of TPI 287 in children to be 125 mg/m^2 given IV on days 1, 8, and 15 of a 28 day cycle. This is the same as the MTD determined for adult (communication Cortice Biosciences). The DLTs of two patients at 135 mg/m^2 were Grade 3 sensory neuropathy and Grade 3 hemorrhagic cystitis. DLT in adults were similar, including neuropathy and neutropenia.

When TPI 287 was administered alone at the MTD, the most common toxicities in our pediatric population were anorexia and pain, whereas in adults they were fatigue and nausea. The most common toxicities when TPI 287 was given at the MTD in combination with TMZ were gastritis, changes in hemoglobin and leukocyte counts, and sensory neuropathy.

Side effects in adults included anemia, diarrhea, vomiting, and abdominal pain. Based on these previous data, many of the gastrointestinal and neurological toxicities observed in this study were expected, including nausea, vomiting, diarrhea, abdominal pain, neuropathy, and neutropenia.

TABLE VI. Patient Details Including Cohort, Disease at Status Entry, Number of Previous Relapses, Responses, and Reason for Off-Therapy

Patient #	Cohort dose mg/m^2	Diagnosis	Relapsed/ refractory	Disease status at entry	Number of previous relapses	Response at end of TPI 287 alone	Response at end of combination	Best response	Number of cycles completed	Reason for off-therapy
1	90	NB	Relapsed	Progressing	5	PD	NE	PD	1	Progression
2	90	NB	Relapsed	Progressing	3	SD	PD	SD	4	Progression
3	90	NB	Refractory	Progressing	refractory	SD	SD	SD	12	Completed
4	110	NB	Refractory	Stable[b]	refractory	SD	SD	SD	12	Completed
5	110	NB	Relapsed	Stable[b]	2	PD	PD	PD	3	Progression
6	110	MB	Relapsed	Progressing	1	NE	NE	NE	<1	Seizure
7	110	NB	Refractory	Progressing	2	PD	NE	PD	<1	Progression
8	110	NB	Relapsed	Progressing	3	PD	PD	PD	4	Progression
9	110	NB	Relapsed	Progressing	2	PD	NE	PD	1	Progression
10	135	NB	Relapsed	Progressing	2	NE	NE	NE	<1	DLT-neuropathy
11	135	NB	Relapsed	Progressing	3	NE	NE	NE	1	DLT-hemorrhagic cystitis
12	125	MB	Relapsed	Progressing	4	PD	PD	PD	4	Progression
13	125	NB	Relapsed	Progressing	5	SD	SD	SD	4	Parental withdrawal to go on new therapy
14	125	NB	Relapsed	Stable[b]	3	PD	NE	PD	2	Progression
15	125	NB	Relapsed	Progressing	1	SD	PR	PR	23	Completed
16[a]	125	NB	Relapsed	Progressing	refractory	PD	NE	PD	2	Progression
17	125	NB	Relapsed	Stable[b]	3	PD	NE	PD	1	Progression
18	125	NB	Refractory	Progressing	refractory	NE	NE	NE	1	Elevated bilirubin

[a]Pt 16 greater than 21 years of age at diagnosis censored; [b]Subjects stable with persistent metastatic neuroblastoma.

The toxicities observed must be considered in the light of the solvents used in the preparation of TPI 287. Both cremaphor and alcohol have toxicities similar to those noted in the study. Cremaphor EL (CrEL) is a formulation vehicle used for various poorly water-soluble drugs, including paclitaxel (Taxol). In contrast to earlier reports, CrEL is not an inert vehicle, but exerts a range of biological effects, some of which may have important clinical implications. Its use has been associated with severe hypersensitivity reactions (potentially severe), hyperlipidemia, aggregation of erythrocytes, and peripheral neuropathy.[25,26] Hypersensitivity reaction prophylaxis is recommended and was used in all patients on this study. Peripheral neuropathy is also a known toxicity of cremaphor and was seen as a toxicity related to TPI 287 in this study. In addition, ethanol serves as a solvent and microbial preservative in oral liquid medications and is the second most commonly used solvent in liquid formulations, following water, to dissolve water-insoluble ingredients.[27,28] There is limited information on the physiologic effects of ethanol in pediatric populations except signs of acute ethanol exposure include hypoglycemia, acidosis, tachycardia, hypothermia, hyper-responsiveness, and disorders of consciousness.[27,28] Patients on this study did not appear to have ethanol-related toxicities.

Pharmacokinetics of TPI 287 at the MTD dose of 125 mg/m^2 showed that although the C_{max} was similar between pediatric (3.2 μg/ml) and adults studies at a dose of 126 mg/m^2 (3.4 μ/ml), the AUC of 5.10 μg/ml-hr was less than that in adults, with an AUC of 6.1 μg/ml-hr (communication, Cortice Biosciences). This is likely due to the lengthened half-life in adults of 9.26 hr, whereas in our patients this was only 2.7 hr, suggesting faster clearance of the medication in children. This would suggest that dosing of TPI 287 may require more frequent dosing in pediatrics and should be addressed in future studies.

Although this was a Phase I study and was not powered to evaluate treatment response, our initial results show that TPI 287 may have efficacy against relapsed/recurrent neuroblastoma and medulloblastoma. Of the 11 patients with neuroblastoma and one patient with medulloblastoma who completed one cycle of treatment with TPI 287 alone, five out of 11 patients with neuroblastoma demonstrated stable disease. In the seven patients with neuroblastoma that continued on TPI 287 combination treatment with temozolomide, three had stable disease and one showed a partial response. It should be noted that this response may be due to temozolomide alone as this is a known active agent in neuroblastoma. In patients with medulloblastoma, one had progressive disease and one withdrew early before scans were done. A recent review of six Phase I and two Phase II studies in pediatric taxanes use showed similar results with 5% response and 15% stabilization of disease.[24] This article identifies preclinical studies that suggest the optimum schedule of administration of taxanes may involve metronomic dosing due to anti-angiogenic properties of taxanes on endothelial cells.[24,29]

In conclusion, TPI 287 alone and in combination with TMZ was well tolerated in pediatric and young adult patients with relapsed/refractory neuroblastoma and medulloblastoma. Generally, toxicities associated with TPI 287 at the MTD were mild and/or controllable with medication. Stabilization of disease in this cohort of extensively treated patients suggested potential efficacy in neuroblastoma as well and should be tested at an earlier stage of disease. Given these results, 125 mg/m^2 TPI 287 is currently being assessed for its anti-tumor efficacy in a Phase I/II trial assessing a new formulation. Novel dosing regimens should be examined as well in future studies.

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REFERENCES

1. Society AC. Cancer facts and figures. Atlanta, GA: American Cancer Society; 2008.
2. Bernstein ML,Leclerc JM, Bunin G, Brisson L, Robison L, Shuster J, Byrne T, Gregory D, Hill G, Dougherty G, Scriver C, Lemieux B, Tuchman M, Woods WG. A population-based study of neuroblastoma incidence, survival, and mortality in North America. J Clin Oncol 1992;10:323–329.
3. Brodeur GM, Pritchard J, Berthold F, Carlsen NL, Castel V, Castelberry RP, De Bernardi B, Evans AE, Favrot M, Hedborg F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. J Clin Oncol 1993;11:1466–1477.
4. Park JR, Eggert A, Caron H. Neuroblastoma: Biology, prognosis, and treatment. Pediatr Clin North Am 2008;55:97–120.
5. Hartmann O, Valteau-Couanet D, Vassal G, Lapierre V, Brugieres L, Delgado R, Couanet D, Lumbroso J, Benhamou E. Prognostic factors in metastatic neuroblastoma in patients over 1 year of age treated with high-dose chemotherapy and stem cell transplantation: A multivariate analysis in 218 patients treated in a single institution. Bone Marrow Transplant 1999;23:789–795.
6. Matthay KK, Villablanca JG, Seeger RC, Stram DO, Harris RE, Ramsay NK, Swift P, Shimada H, Black CT, Brodeur GM, Gerbing RB, Reynolds CP. Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid. Children's Cancer Group. N Eng J Med 1999;341:1165–1173.
7. (CBTRUS) CBTRotUS. 2007–2008 Primary Brain Tumors of the United States. 2008:2000–2004 (Years Data Collected).
8. Partap S, Fisher PG. Update on new treatments and developments in childhood brain tumors. Curr Opin Pediatr 2007;19:670–674.
9. Raymond E, Izbicka E, Soda H, Gerson SL, Dugan M, Von Hoff DD. Activity of temozolomide against human tumor colony-forming units. Clin Cancer Res 1997;3:1769–1774.
10. Middlemas DS, Stewart CF, Kirstein MN, Poquette C, Friedman HS, Houghton PJ, Brent TP. Biochemical correlates of temozolomide sensitivity in pediatric solid tumor xenograft models. Clin Cancer Res 2000;6:998–1007.
11. Emerson DL, Bell C, Jones ME, Schiemann B, Tapolsky G. Oral bioavailabity and antitumor activity of TPI 287 a new taxane analog with greater activity than paclitaxel against tumor cells with demonstrated mutant tubulin. AACR Meeting Abstracts 2006;2006:116-b-117.
12. Fitzgerald DP, Emerson DL, Qian Y, Anwar T, Liewehr DJ, Steinberg SM, Silberman S, Palmieri D, Steeg PS. TPI-287, a new taxane family member, reduces the brain metastatic colonization of breast cancer cells. Mol Cancer Ther 2012;11:1959–1967.
13. Emerson D, Jones M, Bell C, Brown E. TPI 287 crosses the blood brain barrier and contributes to antitumor activity in the U251 glioblastoma intracranial tumor model in nude mice. AACR Meeting Abstracts 2007:1441.
14. Huff LM, Sackett DL, Poruchynsky MS, Fojo T. Microtubule-disrupting chemotherapeutics result in enhanced proteasome-mediated degradation and disappearance of tubulin in neural cells. Cancer Res 2010;70:5870–5879.
15. Modiano MR, Plezia P, Basche M, Cohn AL, Baram Y, Tapolsky G, Yancik S, Pugliese L, Silberman S. A phase I study of TPI 287, a novel taxane, administered every 21 days in patients (pts) with advanced cancer. ASCO Meeting Abstracts 2008;26:13510.
16. Estlin EJ, Lashford L, Ablett S, Price L, Gowing R, Gholkar A, Kohler J, Lewis IJ, Morland B, Pinkerton CR, Stevens MC, Mott M, Stevens R, Newell DR, Walker D, Dicks-Mireaux C, McDowell H, Reidenberg P, Statkevich P, Marco A, Batra V, Dugan M, Pearson AD. Phase I study of temozolomide in paediatric patients with advanced cancer. United Kingdom Children's Cancer Study Group. Br J Cancer 1998;78:652–661.
17. Nicholson HS, Krailo M, Ames MM, Seibel NL, Reid JM, Liu-Mares W, Vezina LG, Ettinger AG, Reaman GH. Phase I study of temozolomide in children and adolescents with recurrent solid tumors: A report from the Children's Cancer Group. J Clin Oncol 1998;16:3037–3043.
18. Baruchel S, Diezi M, Hargrave D, Stempak D, Gammon J, Moghrabi A, Coppes MJ, Fernandez CV, Bouffet E. Safety and pharmacokinetics of temozolomide using a dose-escalation, metronomic schedule in recurrent paediatric brain tumours. Eur J Cancer 2006;42:2335–2342.
19. Broniscer A, Gururangan S, MacDonald TJ, Goldman S, Packer RJ, Stewart CF, Wallace D, Danks MK, Friedman HS, Poussaint TY, Kun LE, Boyett JM, Gajjar A. Phase I trial of single-dose temozolomide and continuous administration of o6-benzylguanine in children with brain tumors: A pediatric brain tumor consortium report. Clin Cancer Res 2007;13:6712–6718.
20. Newlands ES, Stevens MF, Wedge SR, Wheelhouse RT, Brock C. Temozolomide: A review of its discovery, chemical properties, pre-clinical development and clinical trials. Cancer Treat Rev 1997;23:35–61.
21. Rubie H, Chisholm J, Defachelles AS, Morland B, Munzer C, Valteau-Couanet D, Mosseri V, Bergeron C, Weston C, Coze C, Auvrignon A, Djafari L, Hobson R, Baunin C, Dickinson F, Brisse H, McHugh K, Biassoni L, Giammarile F, Vassal G, Societe Francaisedes Cancers de lE, United Kingdom Children Cancer Study Group-New Agents Group S. Phase II study of temozolomide in relapsed or refractory high-risk neuroblastoma: A joint Societe Francaise des Cancers de l'Enfant and

United Kingdom Children Cancer Study Group-New Agents Group Study. J Clin Oncol 2006;24:5259–5264.
22. Cohn SL, Pearson AD, London WB, Monclair T, Ambros PF, Brodeur GM, Faldum A, Hero B, Iehara T, Machin D, Mosseri V, Simon T, Garaventa A, Castel V, Matthay KK, Force IT. The International Neuroblastoma Risk Group (INRG) classification system: An INRG Task Force report. J Clin Oncol 2009;27:289–297.
23. Seibel NL, Reaman GH. New microtubular agents in pediatric oncology. Invest New Drugs 1996;14:49–54.
24. Andre N, Meille C. Taxanes in paediatric oncology: And now? Cancer Treat Rev 2006;32:65–73.
25. Irizarry L, Luu TH, McKoy JM, Samaras AT, Fishe MJ, Carias EE, Raisch DW, Calhoun EA, Bennett CL. Cremophor EL-containing paclitaxel induced anaphylaxis: A call to action. Community Oncol 2009;6:132–134.
26. Gelderblom H, Verweij J, Nooter K, Sparreboom A. Cremophor EL. The drawbacks and advantages of vehicle selection for drug formulation. Eur J Cancer 2001;37:1590–1598.
27. Smolinske SC. Handbook of food, drug, and cosmetic excipients. Boca Raton: CRC Press; 1992. p. 439.
28. Marek E, Kraft WK. Ethanol pharmacokinetics in neonates and infants. Curr Ther Res Clin Exp 2014;76:90–97.
29. Pasquier E, Honore S, Pourroy B, Jordan MA, Lehmann M, Briand C, Braguer D. Antiangiogenic concentrations of paclitaxel induce an increase in microtubule dynamics in endothelial cells but not in cancer cells. Cancer Res 2005;65:2433–2440.
30. Saulnier Sholler GL, Bergendahl G, Lenox S, Zage PE, Roberts W, Kraveka JM, Eslin D, Kaplan J, Higgins T, Ferguson W. A phase I trial of TPI-287 as a single agent and its combination with temozolomide in relapsed neuroblastoma or medulloblastoma. ASCO Meeting Abstracts 2010;28:TPS329.
31. Saulnier Sholler GL, Eslin D, Roberts WD, Kaplan J, Bergendahl G, Ashikaga T, Higgins T, Lenox S, Silberman S, Ferguson W. Phase I trial of TPI 287 as a single agent and in combination with temozolomide (TMZ) in patients with refractory or recurrent neuroblastoma (NB) or medulloblastoma (MB). ASCO Meeting Abstracts 2011;29:9554.