Phase I and Pharmacokinetic Study of the Dolastatin-15 Analogue Tasidotin (ILX651) Administered Intravenously on Days 1, 3, and 5 Every 3 Weeks in Patients with Advanced Solid Tumors

Casey Cunningham,[1] Leonard J. Appleman,[2] Miora Kirvan-Visovatti,[2] David P. Ryan,[3] Eileen Regan,[3] Svetislava Vukelja,[1] Peter L. Bonate,[4] Francis Ruvuna,[4] Robert J. Fram,[4] Antti Jekunen,[4] Steve Weitman,[4] Lisa A. Hammond,[4] and Joseph P. Eder, Jr.[2]

Abstract

Purpose: To determine the maximum tolerated dose (MTD), dose-limiting toxicity (DLT), and pharmacokinetics of tasidotin (ILX651), a dolastatin-15 analogue, when administered on days 1, 3, and 5 every 3 weeks in patients with advanced solid tumors.

Patients and Methods: Thirty-two patients were treated with 92 courses of tasidotin through seven dose levels determined by a modified Fibonacci scheme ranging from 3.9 to 45.7 mg/m^2. Pharmacokinetic samples were collected during the first course.

Results: Neutropenia was the principal DLT at the 45.7 mg/m^2/d dose level. In addition, one patient also experienced grade 3 neutropenia complicated with grade 3 esophageal candidiasis and grade 3 dehydration. Only 1 of 11 patients treated at the MTD, 34.4 mg/m^2, experienced dose-limiting neutropenia. Other common, drug-related toxicities included mild to moderate fatigue, anemia, nausea, anorexia, emesis, alopecia, and diarrhea. The best observed antitumor response consisted of stable disease and was noted in 10 patients (31%); the median duration on study for those patients with stable disease was 99.5 days compared with 37.5 days for those patients with progressive disease. Tasidotin plasma concentrations declined biphasically with an effective half-life of ≤55 minutes, and ~11% was excreted unchanged in the urine.

Conclusion: The recommended dose for phase II studies and the MTD when tasidotin is administered on days 1, 3, and 5 every 3 weeks is 34.4 mg/m^2. The favorable toxicity profile of tasidotin compared with other antitubulin agents, including other dolastatin analogues, and its novel mechanism of action support further disease-directed evaluation of this agent.

Tubulin is a well-established target for anticancer agents. Although available antitubulin agents, including the taxanes and *Vinca* alkaloids, are highly effective in cancer therapy, their clinical usefulness is limited by intrinsic or acquired resistance and systemic toxicity (1). Thus, it is important to develop new agents that target the tubulin/microtubule system with efficacy against resistant tumors and an improved side effect profile.

The dolastatins, a group of peptides isolated from the Indian Ocean sea hare *Dolabella auricularia* (2–5), bind to tubulin subunits and inhibit tubulin-dependent GTP hydrolysis *in vitro* (6). *In vivo*, these actions inhibit the assembly of new microtubules, induce the depolymerization of existing microtubules, and inhibit cell cycle progression in mitosis (7, 8). However, initial clinical evaluation of dolastatin-10 and cemadotin, a synthetic analogue of dolastatin-15, showed disappointing results (9–11), possibly due, in part, to poor cellular uptake. In addition, cemadotin is rapidly converted to a metabolite with dose-limiting cardiovascular toxicities, including hypertension, angina, and myocardial infarction, that limited its therapeutic efficacy (12–15).

To address these problems, a new generation of dolastatins, represented by tasidotin (Genzyme Corp., San Antonio, TX), has been created that offer several advantages over most other antitubulins. Tasidotin is a pentapeptide (*N*,*N*-dimethyl-L-valyl-L-valyl-*N*-methyl-L-valyl-L-prolyl-L-proline-*tert*-butylamide hydrochloride; Fig. 1), chemically modified to improve the pharmacologic properties of cemadotin resulting in a more metabolically stable compound. Tasidotin induces a prolonged lag phase in microtubule assembly at concentrations of 25 to 40 μmol/L (16-26 μg/mL) followed by recovery with microtubule assembly returning to normal levels (16). These effects are in contrast to those seen with other antitubulins, such as podophyllotoxin and vinblastine (17–21), and produce cell cycle arrest in the G_2 and M cell cycle phases. At concentrations ≥50 μmol/L (32 μg/mL), tasidotin inhibits the extent of microtubule assembly, which is also an atypical finding for antitubulins. Finally, in addition to its potentially enhanced therapeutic window, tasidotin is orally bioavailable (22).

Authors' Affiliations: [1]Tyler Cancer Center, Tyler, Texas; [2]Dana-Farber Cancer Institute, [3]Massachusetts General Hospital, Boston, Massachusetts; and [4]Genzyme Corp., San Antonio, Texas

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Requests for reprints: Peter L. Bonate, Genzyme Corp., 4545 Horizon Hill Boulevard, San Antonio, TX 78229. Phone: 210-949-6662; Fax: 210-949-6219; E-mail: peter.bonate@genzyme.com.

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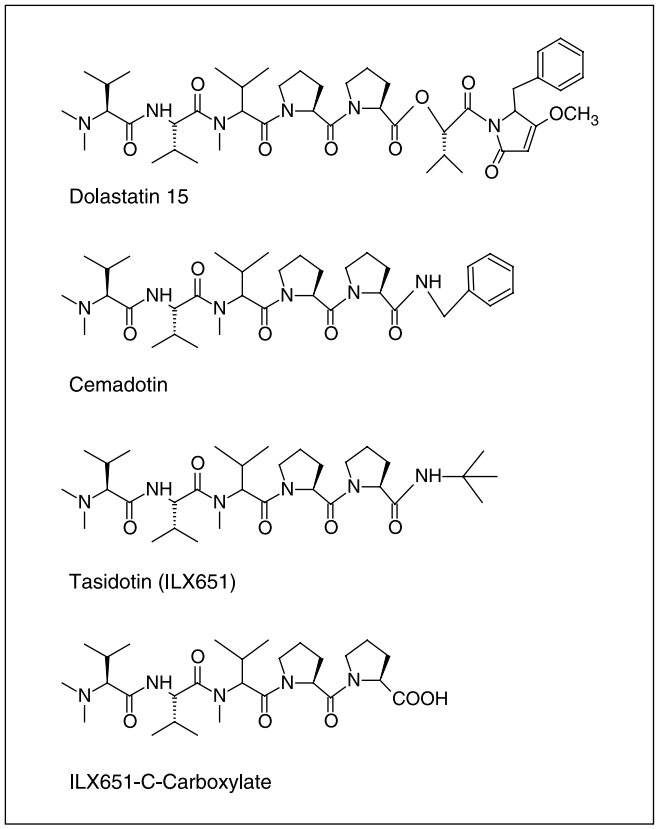

Fig. 1. Structures of dolastatin-15, cemadotin, Tasidotin, and ILX651-C-carboxylate.

The cytotoxic potential of tasidotin was evaluated in the National Cancer Institute tumor cell line screen (including melanoma, non–small cell lung cancer, prostate, breast, colon, central nervous system, and leukemia cell lines); GI_{50} values ranged from <10 nmol/L (6 ng/mL) to >1 mmol/L (0.6 mg/mL; ref. 23). Complete responses were documented in early-stage and late-stage breast carcinoma (MX-1), melanoma (LOX), and prostate (PC-3) cancer mouse models, and survival time was significantly increased in the P388 murine leukemia model. In the MX-1 model, tasidotin induced tumor growth delays ranging from 20 to >90 days compared with 15 days for paclitaxel (23). Of note, repetitive dosing schedules of tasidotin showed superior activity compared with single dosing schedules (24).

Toxicology studies in rats and dogs showed that tasidotin principally affects the bone marrow, gonads, lymphoid tissues, and kidney. Administration of tasidotin i.v. in dogs resulted in emesis, diarrhea, and neutropenia. Neutropenia was maximal at days 6 to 13, and recovery occurred during days 15 to 29. Mesangioproliferative glomerulopathy was only observed in rats treated at the highest dose of tasidotin (30 mg/kg i.v. × 5 days). When administered to mongrel dogs, tasidotin induced dose-dependent decreases in cardiac output and increases in coronary, femoral, and total peripheral vascular resistance that were 10-fold less than cemadotin (24). After both i.v. and oral administration of tasidotin in dogs, the plasma concentrations showed a linear increase and a half-life of ~2.3 hours (22). The mean bioavailability was 23%. Studies with [^{14}C]tasidotin using a single i.v. dose of tasidotin in rats revealed that 55% to 60.5% of drug was recovered in the feces, whereas 22.8% to 30.6% was excreted renally (internal data).

Given its novel mechanism of action, impressive preclinical antineoplastic activity, favorable preclinical pharmacologic properties, and reduction in toxicities over previous dolastatins, a phase I trial of tasidotin given i.v. to patients with advanced solid tumors refractory to standard treatment was conducted. To mimic the repetitive dosing schedules associated with superior antitumor activity in the preclinical studies, tasidotin was administered i.v. over 30 minutes on days 1, 3, and 5 every 3 weeks. The principal objectives of this study were to determine the maximum tolerated dose (MTD) of tasidotin administered i.v. on this schedule, determine the toxicities of tasidotin, characterize the pharmacokinetic behavior of tasidotin, seek preliminary evidence of antitumor activity, and recommend a dose of tasidotin for phase II studies.

Patients and Methods

Eligibility. Patients with histologically documented, advanced solid tumors refractory to conventional therapy, or for whom no effective therapy existed, were candidates. Eligibility criteria also required the following: age, ≥18 years; Eastern Cooperative Oncology Group performance status, ≤2; life expectancy, ≥12 weeks; no prior chemotherapy, radiation therapy, or major surgical procedures within 4 weeks of study entry (6 weeks for mitomycin C or nitrosourea); adequate hematopoietic [absolute neutrophil count (ANC), ≥1,500/μL; platelet count, ≥100,000/μL; and hemoglobin, ≥9.0 g/d], hepatic (total bilirubin, <2.0 mg/dL; aspartate amino transaminase and alanine amino transaminase levels, ≤2 × upper limit of normal or <5 × upper limit of normal for patients with liver metastases), metabolic (calcium within institutional limits of normal), and renal (serum creatinine, ≤1.5 mg/dL or creatinine clearance, ≥60 mL/min; ref. 25) variables; prior radiotherapy to <25% of bone marrow reserves; no active infection or coexisting medical problems of sufficient severity to limit compliance with the study; no apparent central nervous system metastases; no prior stem cell or bone marrow transplantation; and no cardiac functional capacity of class III or IV, using the New York Heart Association Classification. All patients gave written informed consent in accordance with federal and local institutional guidelines before treatment.

Dosage and drug administration. The starting dose of tasidotin was 3.9 mg/m^2/d (one tenth of the MTD in rat studies ref. 24) administered i.v. over 30 minutes on days 1, 3, and 5 of each 21-day treatment course. Successive cohorts of patients were dose escalated per a modified Fibonacci scheme from the initial dose of 3.9 to 7.8, 13.0, 19.5, 25.9, 34.4, and 45.7 mg/m^2. Dose reduction to the previous dose level was permitted for patients experiencing dose-limiting toxicity (DLT); however, a maximum of two dose reductions per patient was permitted. The MTD, or recommended phase II dose, was defined as the highest dose level that induced DLT in less than two of six new patients treated in the first course. At least three new patients were treated at each escalated dose level, except at the MTD where the cohort was expanded to 11 patients to gain greater safety data at that dose level. DLT was defined during the first course for determination of MTD as follows: ANC, <500/μL; platelet count, <25,000/μL; grade ≥3 drug-related nonhematologic toxicity except nausea/vomiting or diarrhea that was not optimally treated with antiemetics or antidiarrheal regimens, respectively; grade ≥3 drug-related vomiting or diarrhea despite optimal treatment; and treatment delays of >7 days due to unresolved toxicity in patients with platelet count < 25,000/μL, ANC < 500/μL, or grade 2 drug-related nonhematologic toxicity. Patients were retreated at 3-week intervals provided the eligibility requirements for hematologic, hepatic, and renal variables were maintained and nonhematologic toxicity had resolved to grade ≤1. A delay of 2 weeks in dosing of new cycles was permitted; if delayed >2 weeks, patients

were removed from the study. Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria (version 2.0; ref. 26). Tasidotin (Genzyme) was supplied in 5-mL vials containing 30 mg of tasidotin, which was diluted with 50 mL of 0.9% NaCl.

Pretreatment and follow-up studies. Histories, physical examinations, performance status assessments, and routine laboratory studies were done pretreatment and weekly. A chest X-ray, electrocardiogram, and urinalysis were done at baseline. Electrocardiograms were repeated before each course. Tumor measurements were done pretreatment and after every other course according to the modified WHO criteria. A complete response required disappearance of all active disease on two measurements separated by ≥4 weeks. A partial response required ≥50% reduction in the sum product of the bidimensional measurements of all documented lesions separated by ≥4 weeks. Progressive disease was defined as ≥25% increase in the sum of products of the bidimensional measurements of measurable disease.

Plasma sampling, urine sampling, and bioanalytic assay. Blood samples (5 mL) for pharmacokinetic analyses using EDTA as the anticoagulant were collected from the arm contralateral from the infusion arm during the first course immediately before tasidotin administration and at 0.25, 0.5, 0.58, 0.75, 1, 1.5, 3, 5, 8, 24, 48, and 72 hours (day 5 only) after initiation of the infusion on days 1 and 5. The 48-hour sample was a trough concentration collected before administration of the second dose in the cycle. Preliminary pharmacokinetic data from the first nine patients indicated that the half-life of tasidotin was less than an hour; thus, the pharmacokinetic sampling scheme was modified to remove the 24-, 48-, and 72-hour samples. Urine samples were collected in 24-hour collections for the first 2 days (0-24 and 24-48 hours) after the first dose and for 3 days after the third dose (0-24, 24-48, and 48-72 hours) in cycle 1.

Plasma tasidotin concentrations were analyzed at MicroConstants, Inc. (San Diego, CA) using a Good Laboratory Practices–validated liquid chromatography tandem mass spectrometry assay having a linear range of 1 to 500 ng/mL. The internal standard used was β-casomorphin (1-4) amide. Briefly, 100-μL plasma was precipitated with acetonitrile, evaporated, and then reconstituted with mobile phase. Samples were analyzed using a XDB-phenyl (150 × 2.1 mm, 5 μm, Agilent Technologies, Wilmington, DE) column at 45°C and a flow rate of 0.3 mL/min. Samples were eluted using gradient elution with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Initial conditions were 85% A/15% B. The mobile phase was changed to 15% A/85% B at 3 minutes, changed back to the original conditions at 6 minutes, and had a total run time of 9 minutes. Tasidotin and the internal standard had a retention time of 4.7 and 4.4 minutes, respectively. Samples were detected using a tandem quadrupole mass spectrometer (Quattro LC, Waters Corp., Milford, MA) that monitored mass transitions m/z 607.4 → 340.10 for tasidotin and m/z 522.3 → 408.3 for the internal standard. Samples about the upper limit of quantification of the assay (500 ng/mL) were diluted into the range of the standard curve. The accuracy and precision of 10× diluted quality control samples were ≤13.1% from their theoretical value with a 5.6% coefficient of variation, respectively. Samples were stable for at least 180 days when stored at −20°C and were stable for at least three freeze-thaw cycles. The accuracy of the lower limit of quantification (1 ng/mL) was within 3.0% of the theoretical value. The coefficient of variation for the method was <12.3% and did not deviate from theoretical values by >2.8%. Tasidotin-C-carboxylate concentrations were not monitored in this study. Further details of the method can be found in Lewiston et al. (27).

Urine tasidotin concentrations were analyzed at MicroConstants using a Good Laboratory Practices–validated liquid chromatography tandem mass spectrometry assay having a linear range of 0.1 to 50 μg/mL. The internal standard was substance-P with mass transitions monitored at m/z 613.10 → 596.10. Briefly, urine samples were diluted with mobile phase and injected on-column after centrifugation. Samples were analyzed using a Zorbax SB-Phenyl (150 × 2.1 mm, 5 μm, Agilent Technologies) column at 45°C and a flow rate of 0.3 mL/min. The mobile phase proportions were changed to 70:30:0.1 (v/v/v) water/acetonitrile/formic acid. The run time was 5 minutes with tasidotin eluting at 2.8 minutes and substance-P eluting at 3.6 minutes. Urine samples were stable for at least 170 days at −20°C and were stable for at least three freeze-thaw cycles. The accuracy of the method at the lower limit of quantification (0.1 μg/mL) was −1.1% from the theoretical value. The coefficient of variation for the urine method was <7.6% and did not deviate from theoretical values by >1.0%.

Pharmacokinetic analysis of tasidotin in plasma and urine. Pharmacokinetic variable estimates for tasidotin were done using noncompartmental methods (28) with WinNonlin Professional (version 4.0, Pharsight Corp., Mountain View, CA). Concentrations below the lower limit of quantification after the first dosing event were set equal to missing, whereas all predose concentrations were set equal to 0. Area under the curve (AUC) was calculated using the linear trapezoidal rule. Estimation of the elimination rate constant was based on at least three data points in the terminal phase with a coefficient of determination of at least 0.95. AUC from 0 time to extrapolated to infinity ($AUC_{0-\infty}$) was calculated provided the percent area extrapolated was <20%; otherwise, the value was set to missing. Renal clearance was assessed as the ratio of the amount of tasidotin excreted in urine in a 24-hour period to the AUC over a 24-hour period.

Pharmacokinetic data were summarized using descriptive statistics. Individual concentration values below the limit of quantification after the start of the first dosing event were set equal to 0 for graphical purposes. Dose proportionality was assessed using a linear mixed effects power model (29). Natural log-transformed (ln) $AUC_{0-\infty}$ and maximal concentrations (C_{max}) were used as the dependent variables; ln transformation was used to stabilize the variance of the residuals. Patients were treated as random effects, whereas Day and ln(Dose) were treated as continuous fixed effects. If Day was not statistically significant at the 0.05 level, the factor was removed and the reduced model refit. Dose proportionality was declared if the 90% confidence interval associated with the slope for ln(Dose) contained the value 1.0. Repeated measures ANOVA was also done on the pharmacokinetic variables, clearance, volume of distribution at steady state, half-life, and renal clearance, using Dose and Day as a continuous fixed effects. Patients were treated as random effects. Fixed effects that were not significant at the 0.05 level were removed from the model, and the reduced model fitted. All pharmacokinetic statistical analyses were done using SAS for Windows (version 8.0, SAS Institute, Cary, NC).

Results

General. Thirty-two patients, whose characteristics are depicted in Table 1, received 92 courses of tasidotin (median, 2; range, 1-14) through seven dose levels (Table 2). An additional patient was enrolled on this study but taken off study before receiving tasidotin due to the presence of brain metastases. All 32 patients were assessable for toxicity in the first course. One patient treated at 7.8 mg/m^2 had a treatment interruption on day 3 of course 1 due to an episode of grade 1 sinus tachycardia, subsequently judged unrelated to study drug. An additional three patients missed the following tasidotin infusions at the following dose levels: course 2, day 3 due to anemia (25.9 mg/m^2); course 2, days 3 and 5 due to rapidly progressive disease (34.4 mg/m^2); and course 3, day 5 due to pyrexia (45.7 mg/m^2). Three patients were dose reduced from 45.7 to 34.4 mg/m^2 after one (n = 1) and three (n = 2) courses. One patient subsequently required further dose reduction to 25.9 mg/m^2 after course 6.

Three patients were treated at each of the first two dose levels (3.9 and 7.8 mg/m^2) without incidence. Four patients were treated at the next dose level, 13.0 mg/m^2, as one patient did

not complete the full 3 weeks of evaluation due to rapidly progressive disease. Three patients each were then treated at 19.5, 25.9, and 34.4 mg/m^2 dose levels without DLT. The first episodes of dose-limiting grade 4 neutropenia were observed at 45.7 mg/m^2 and were seen in three of five patients. In addition, a fourth patient treated at this dose level experienced grade 3 neutropenia complicated by grade 3 esophageal candidiasis and grade 3 dehydration. Further patient accrual proceeded at 34.4 mg/m^2 to allow evaluation of 11 patients. At this dose level, only one patient experienced a DLT during the first course (grade 4 neutropenia), confirming that this was indeed the MTD.

Two patients were hospitalized for drug-related toxicity as follows: course 1, day 9 due to grade 3 esophageal candidiasis, grade 3 neutropenia, grade 3 dehydration, and grade 3 odynophagia; and course 3, day 12 due to a myocardial infarction. Other reasons for hospitalizations during the study that were unrelated to tasidotin include dyspnea, urosepsis, colostomy stoma bleed, dehydration, constipation, atrial fibrillation/bigeminy, mental status changes due to brain metastases, brain infarct in a patient with progressive brain metastases receiving radiation therapy, and abdominal pain/obstruction due to progressive abdominal metastases. One patient died during course 4 due to rapidly progressive disease.

Hematologic toxicity. The rates of relevant hematologic toxicities as functions of the total numbers of patients and courses of tasidotin are shown in Table 3. Decrements in ANCs typically occurred on days 14 to 22. The median time to ANC nadir was 14 days (range, 6-29 days) with recovery typically occurring within 5 days. Treatment delays due to an ANC <1500/μL occurred in only 7 of 92 (7.6%) courses. There was no evidence of cumulative neutropenia with repetitive dosing, although there is limited ability to assess this due to the short duration of treatment for most patients.

Anemia and thrombocytopenia were both relatively mild. Anemia was only grade 1 to 2 in magnitude and observed, as a change from baseline, in 44% of patients. Thrombocytopenia was only grade 1 in intensity and was observed in only two patients. Although not included in the determination of MTD and DLT, 10 of 32 patients (31%) experienced grade 3 lymphopenia; three of these patients had grade 3 lymphopenia at baseline.

Nonhematologic toxicity. The drug-related nonhematologic toxicities associated with administration of tasidotin were generally mild to moderate in severity, as shown in Table 4. Fatigue, nausea, alopecia, and transaminitis were the most common nonhematologic drug-related toxicities observed. Four patients experienced neuropathy. One patient treated at 25.9 mg/m^2 experienced grade 2 sensory neuropathy assessed as drug related during course 3 that lasted 25 days and required the patient to be removed from study. Two patients treated at 45.7 mg/m^2 experienced grade 1 unilateral hand numbness; one developed on day 1 of course 3 and resolved 4 weeks after study exit; and the other occurred on day 15 of course 2. This patient subsequently developed brain metastases after course 5 and was removed from study; thus, the duration of the mild hand numbness is unknown. Finally, a patient treated at 13 mg/m^2 experienced grade 2 peripheral motor neuropathy on day 1 of course 1 that was assessed as not drug related, because the patient had rapidly progressive disease and expired off study 32 days after the first dose of tasidotin.

Two patients experienced grade ≥3 cardiac-related events. A 58-year-old female with pancreatic cancer and hypertension, initially treated at 45.7 mg/m^2, subsequently dose reduced to 34.4 mg/m^2 due to neutropenia, experienced a myocardial infarction on course 3 day 12. Although it was assessed as tasidotin related, it did not occur during or immediately after infusion as seen with previous dolastatin analogues. This patient recovered without further sequelae but was not redosed with tasidotin. A second patient, an 81-year-old male with colorectal cancer and a history of bigeminy, stroke, and hypertension treated at 13.0 mg/m^2, experienced grade 3 atrial fibrillation and bigeminy on day 27 of course 2 that were assessed as not related to tasidotin. In addition, transient elevations of diastolic readings compatible with grade 1 hypertension were observed in two patients.

Gastrointestinal adverse events were predominantly grade 1 to 2 and included nausea, vomiting, diarrhea, transaminitis, constipation, and abdominal pain. Nausea and vomiting occurred predominantly peritreatment, were controlled with prochlorperazine, and only occasionally required use of a $5HT_3$ antagonist. One episode of severe ileus occurred in an 80-year-old female with squamous cell carcinoma of the tonsil with lung metastases treated at 45.7 mg/m^2 who was hospitalized on course 1 day 10 due to grade 3 bowel obstruction. Radiographic studies revealed ileus without evidence of an obstructing mass. After oral intake resumed this patient was taken off study and not retreated.

Table 1. Patient characteristics

Characteristic	No. patients
No. patients (evaluable)	33 (32)*
Median no. courses/patient (range)	2.0 (1-14)
Sex (male/female)	12/20
Median age (range)	59 (37-83)
Ethnicity	
Caucasian	31
Asian	1
Performance status (ECOG)	
0	4
1	23
2	5
Prior chemotherapy regimens	
≤2	6
>2 to <5	15
≥5	11
Prior radiation therapy	15
Primary tumor types	
Colorectal	14
Lung	5
Pancreatic	3
Breast	2
Ovarian	2
Other (adrenal, melanoma, renal, tonsil, unknown primary, uterine leiomyosarcoma)	1 each

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
*An additional patient was enrolled on this study but taken off the study before receiving tasidotin due to the presence of brain metastases.

Table 2. Dose escalation scheme and rate of DLTs in course 1

Tasidotin dose level	Tasidotin dose (mg/m^2/d)	Total dose (mg/m^2)	No. patients (new)	No. evaluable courses	ANC <500/μL	Platelets <25,000/μL	Grade 3-4 nonhematologic toxicity	No. of dose reductions course 1 (all courses)	New patients with any DLT/no. new patients
1	3.9	11.7	3 (3)	6	0	0	0	0 (0)	0/3
2	7.8	23.4	3 (3)	5	0	0	0	0 (0)	0/3
3	13.0	39.0	4 (4)	6	0	0	0	0 (0)	0/4
4	19.5	58.5	3 (3)	7	0	0	0	0 (0)	0/3
5	25.9	77.7	4 (3)	11	0	0	0	0 (0)	0/3
6	34.4	103.2	14 (11)	46	1	0	0	0 (1)	1/11
7	45.7	137.1	5 (5)	9	3	0	1*	1 (3)	4/5

*One patient with grade 3 oral candidiasis, grade 3 dehydration, and grade 3 neutropenia.

One severe respiratory event occurred in a 38-year-old male with non–small cell lung cancer treated at 34.4 mg/m^2 who was admitted on course 4 day 12 with grade 4 dyspnea, grade 4 neutropenia, a pulmonary cavitary mass with air fluid levels, and bacteremia and expired 2 days later. Other adverse events that were less frequently noted include grade 1 to 2 infections, hyperbilirubinemia, myalgias, arthralgias, and jaw pain.

Pharmacokinetics. Pharmacokinetic samples were obtained in 31 patients at all dose levels. One patient dosed at the MTD had no pharmacokinetic samples collected because the site was unable to obtain peripheral blood access for pharmacokinetic samples. Table 5 summarizes the pharmacokinetic variables across doses. Tasidotin plasma concentrations increased with increasing dose (Fig. 2), and rapidly declined biphasically to <1% maximal concentrations within about 8 hours after dosing. The presence of a third γ phase, which appeared very late in the profile, was seen in some patients but could not be quantified because most samples were below the lower limit of quantification by 8 hours after dose, although in some patients, concentrations were quantifiable; thus, longer sampling may have helped in characterizing this phase. Nevertheless, given the current sampling scheme, >97.5% of the total AUC was observable (<2.4% of the total AUC was extrapolated in all cases with the average being 0.26% extrapolated); thus, the contribution this γ phase makes to the total AUC seemed negligible. In the dose proportionality analysis, the slope associated with ln(Dose) was estimated to be 1.19 for $AUC_{0-\infty}$ (90% confidence interval, 1.04-1.33) and 1.27 for C_{max} (90% confidence interval, 1.07-1.46). Hence, neither $AUC_{0-\infty}$ nor C_{max} exhibited dose proportionality over the range of doses studied (Fig. 3). However, the nonlinearity was mild and not clinically relevant as a 2-fold increase in dose resulted in a 2.3-fold increase in $AUC_{0-\infty}$ and a 2.4-fold increase in C_{max}, ~20% higher than would be expected assuming linear pharmacokinetics.

Because $AUC_{0-\infty}$ was not dose proportional, neither was systemic clearance (P = 0.0203; Fig. 4). Clearance decreased as C_{max} increased and ranged from 8.6 L/h/m^2 (45.7 mg/m^2) to 43.2 L/h/m^2 (25.9 mg/m^2). Between-subject variability for clearance was moderate at 30%. Renal clearance was a minor route of tasidotin elimination, as only 11% of the dose was excreted unchanged in the urine. The least-squares mean renal clearance for tasidotin was 4.5 L/h and was independent of dose and day of sampling. Between-subject variability in renal clearance was large, ~86%. Volume of distribution at steady state (V_{dss}) was independent of dose with a least-squares mean of 13.2 L/m^2 and between-subject variability of 37%. V_{dss} ranged from 5.0 to 35.4 L/m^2 (after removal of a single outlier). Tasidotin seemed to exhibit biphasic kinetics with an effective half-life ranging from 21 minutes (34.4 mg/m^2) to 55 minutes (45.7 mg/m^2). The effective half-life was dose dependent (P < 0.0001) with a small between-subject variability of ~12%. As expected with such a short effective half-life, no accumulation of tasidotin was observed on day 5

Table 3. Hematologic toxicities of tasidotin on a days 1, 3, and 5 every 3-week schedule

Tasidotin dose level (mg/m^2)	No. new patients (evaluable)	No. evaluable courses	Neutropenia grade all courses (course 1)				Thrombocytopenia grade all courses (course 1)	
			1	2	3	4	1	2-4
3.9	3 (3)	6	0	0	0	0	0	0
7.8	3 (3)	5	0	0	0	0	1	0
13.0	4 (4)	6	0	0	0	0	0	0
19.5	3 (3)	7	0	0	0	0	0	0
25.9	3 (4)	11	1 (0)	0	0	0	0	0
34.4	11 (14)	46	8 (1)	16 (3)	10 (2)	4 (1)	2 (0)	0
45.7	5 (5)	9	0	0	3 (2)	6 (3)	1 (0)	0

Table 4. Nonhematologic toxicity

Drug-related adverse events by all patients* (*N* = 32)					
Adverse event	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Total, *n* (%)**
AST/ALT increased †	13	3	0	0	17 (50.0)
Fatigue/fatigue aggravated	4	6	0	0	10 (31.2)
Nausea	7	3	0	0	10 (31.2)
Alopecia	6	3	0	0	9 (28.1)
Vomiting	4	1	0	0	5 (15.6)
Diarrhea	3	1	0	0	4 (12.5)
Anorexia	2	2	0	0	4 (12.5)
Pyrexia	2	0	0	0	2 (6.3)
Dermatitis	2	0	0	0	2 (6.3)
Pain in limb	2	0	0	0	2 (6.3)
Dizziness	2	0	0	0	2 (6.3)
Constipation	1	0	0	0	1 (3.1)
Edema	1	0	0	0	1 (3.1)
Headache	1	0	0	0	1 (3.1)
Oral candidiasis	0	0	1	0	1 (3.1)
Erythema	0	1	0	0	1 (3.1)
Pruritus	1	0	0	0	1 (3.1)
Peripheral neuropathy	0	1	0	0	1 (3.1)
Weight decreased	0	1	0	0	1 (3.1)
Menstruation, irregular	0	0	1	0	1 (3.1)
Myocardial infarction	0	0	0	1	1 (3.1)
Pain in jaw	1	0	0	0	1 (3.1)

Abbreviations: AST, aspartate amino transaminase; ALT, alanine amino transaminase.
*Only one occurrence at the highest grade is counted for each patient.
†Grade 1 and 2 AST/ALT shifts in laboratory values are presumed to be drug related.

compared with day 1. Tasidotin showed stationary pharmacokinetics with no significant pharmacokinetic differences observed between days 1 and 5.

Antitumor activity. The best antitumor response was stable disease that was observed in 10 patients. Four patients had stable disease for more than four courses: breast cancer (n = 1) for five courses, undifferentiated small cell carcinoma of unknown primary (n = 1) for six courses, ovarian cancer (n = 1) for eight courses, and non–small cell lung cancer (n = 1) for 14 courses. Overall, the median duration on study for patients with stable disease was 99.5 days compared with 37.5 days for patients with progressive disease.

Discussion

The medicinal use of the shell-less mollusk *D. auricularia* dates back to Roman times when extracts from this marine animal were used to poison the emperor Claudius and his stepson (30).

Table 5. Noncompartmental pharmacokinetic variables of tasidotin after i.v. administration of tasidotin on days 1, 3, and 5 every 3 weeks

Dose level (mg/m^2)	No. patients	C_{max} (ng/mL), median (range)	$AUC_{0-\infty}$ (ng · h/mL), median (range)	CL ($L/h/m^2$), median (range)	V_{dss} (L/m^2), median (range)	Half-life (min), median (range)
3.9	3	228 (15-261)	165 (158-173)	23.6 (22.6-24.7)	9.8 (9.4-14.1)	26 (23-36)
7.8	3	390 (237-411)	249 (205-431)	31.4 (18.1-38.1)	14.2 (11.6-35.4)	26 (24-28)
13.0	4	816 (599-1,554)	593 (394-990)	21.9 (13.1-33.0)	11.9 (5.0-20.5)	36 (25-42)
19.5	3	941 (702-1,140)	751 (574-865)	26.4 (22.6-34.0)	16.3 (13.8-21.9)	36 (35-37)
25.9	3	1,177 (766-1,494)	889 (600-1,512)	31.1 (17.1-43.2)	20.1 (13.1-29.3)	33 (26-50)
34.4	10	2,722 (1,415-4,538)	1,676 (1,332-3,828)	20.5 (9.0-25.8)	11.3 (5.7-19.4)	37 (21-45)
45.7	5	2,414 (1,611-4,811)	2,523 (1,524-5,344)	18.1 (8.6-30.0)	15.2 (7.7-24.8)	36 (33-55)

Abbreviation: CL, clearance.

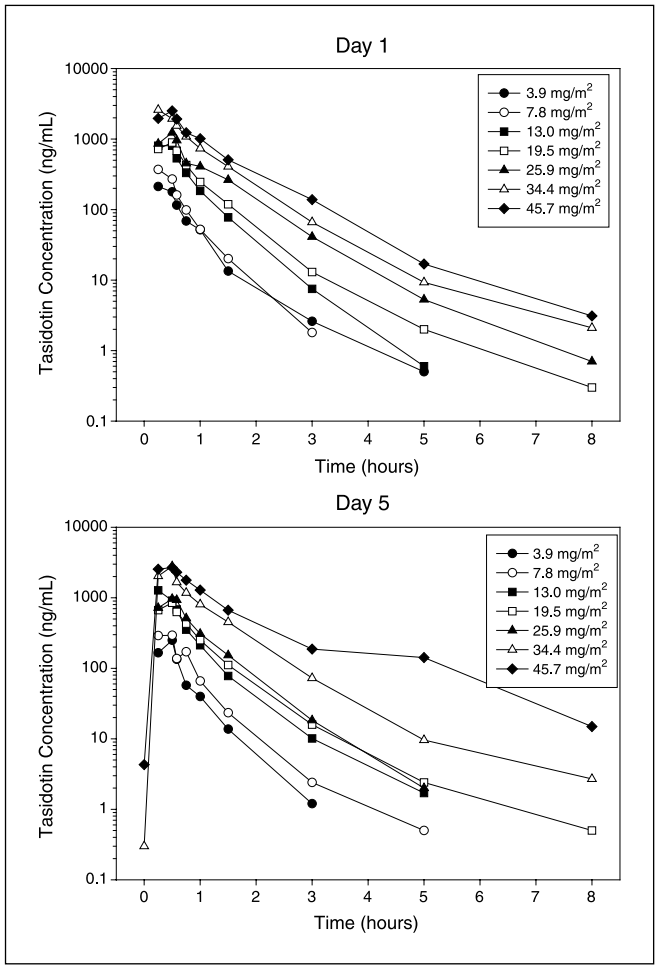

Fig. 2. Mean plasma tasidotin concentration-time profile (days 1 and 5) of patients treated with tasidotin by dose level.

However, not until two decades ago was the antitumor potential of the dolastatin peptides discovered (31). The first dolastatin analogue to overcome synthesis and solubility challenges was cemadotin. Initial studies showed that this agent binds to tubulin and strongly suppresses microtubule dynamics in a unique manner by suppressing the rate of growth of microtubules more than the rate of shortening thus enhancing the frequency of rescue with little effect on the frequency of catastrophe (32). However, cemadotin is a weak agonist for the angiotensin II type 1 receptor and produced a dose-dependent increase in diastolic blood pressure preclinically (33). Unfortunately, this translated into dose-limiting cardiovascular toxicities in the clinic, including myocardial infarction, peripheral edema, and hypertension (9–12), all of which were associated with the magnitude of peak blood levels of the parent drug or its metabolites (34). Further modification of dolastatin-15 led to the synthesis of tasidotin, which has now been evaluated on three different administration schedules in the phase I setting. The current study was designed to evaluate the feasibility of administering tasidotin as a 30-minute i.v. infusion on days 1, 3, and 5 every 3 weeks.

Tasidotin was well tolerated on this administration schedule, with neutropenia as the principal DLT. In contrast to previous clinical studies with dolastatins, the incidence of cardiovascular toxicity was diminished (9–12) with only one myocardial infarction and no severe hypertensive episodes observed. However, no myocardial infarction was observed on the phase I tasidotin trial using a daily for 5 days every 3-week administration schedule (35). Furthermore, in contrast to other dolastatin analogues, such as TZT-1027, neurotoxicity associated with tasidotin administration was mild and not dose limiting (36). However, mild jaw pain, ileus, and hypoesthesia were infrequently seen and may have been manifestations of tasidotin-induced neurotoxicity similar to that observed with TZT-1027 and the *Vinca* alkaloids. Overall, however, the results of this study suggest that efforts to chemically modify cemadotin to improve its toxicity profile have been successful. This conclusion is also confirmed in the trial of tasidotin administered on a daily × 5 schedule (37).

Although higher individual day dosing of tasidotin was feasible on this day 1, 3, and 5 schedule (34.4 mg/m^2 at the MTD) compared with the daily for 5-day schedule (27.3 mg/m^2 at the MTD), the dose intensity was actually higher on the 5-day schedule compared with the day 1, 3, and 5 schedule, 45.5 and 34.4 mg/m^2, respectively. In addition, although this is a phase I study with the primary purpose to determine the MTD and not

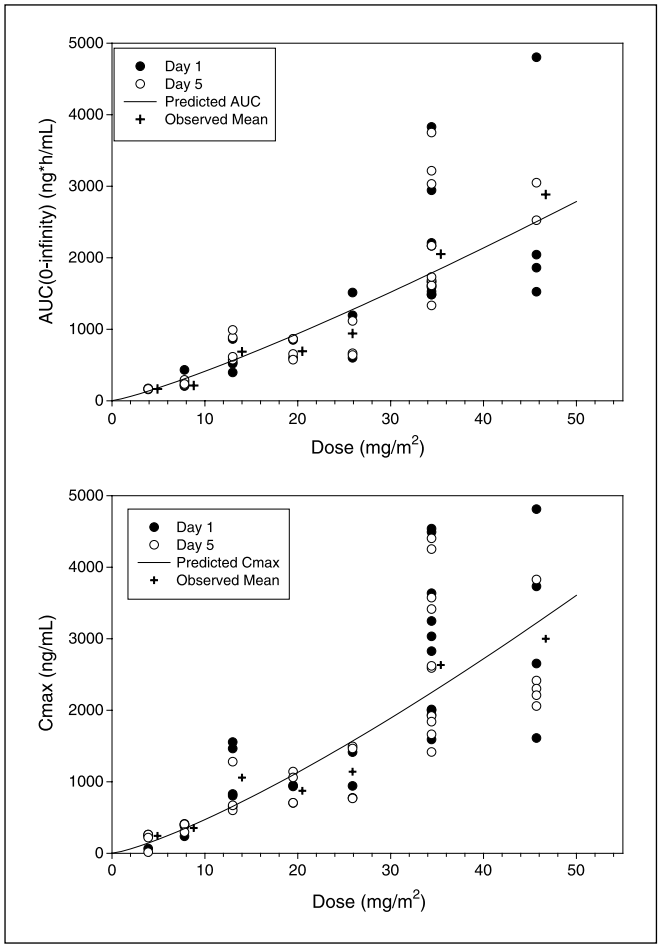

Fig. 3. Scatter plots showing the distributions of total tasidotin $AUC_{0-\infty}$ values (*top*) and C_{max} values (*bottom*) as a function of tasidotin dose stratified by day of administration. Solid line is the predicted value under the reduced linear mixed effects power model: $AUC_{0-\infty} = \exp[3.28 + 1.19 \times \ln(\text{Dose})]$ and $C_{max} = \exp[3.24 + 1.27 \times \ln(\text{Dose})]$. Plus signs are the observed means with dose jittered by +1 mg/m^2 for clarity.

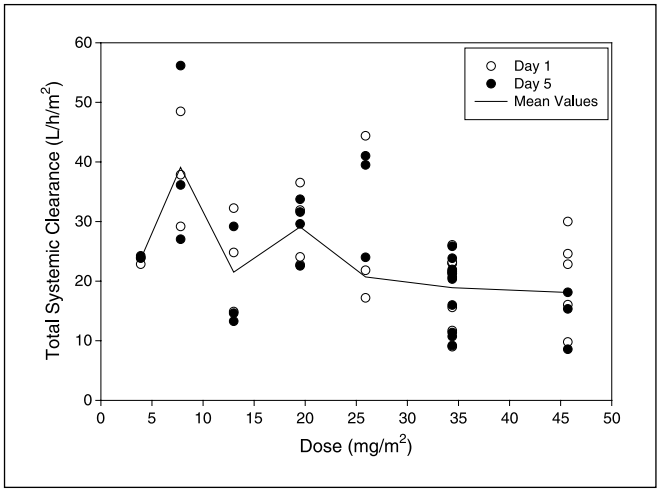

Fig. 4. Scatter plot of tasidotin systemic clearance against dose stratified by day of administration. The solid line connects the mean values pooled across days.

response, the fact that there was a complete response and two mixed responses in melanoma on the 5-day schedule suggests that improvement in dose intensity may translate into improved antitumor activity. However, it is important to recognize that tasidotin dose intensity may have been higher on the 5-day schedule due to the less heavily pretreated status of the patients (37).

The pharmacokinetic profile of tasidotin on this day 1, 3, and 5 treatment schedule is consistent with that observed on the daily for 5 days and weekly tasidotin schedules (38). Tasidotin exposure increased disproportionately to dose but the nonlinearity was mild and clinically not relevant. The degree of nonlinearity did not seem large as doubling the dose would increase exposure only 20% higher than expected under linear pharmacokinetics. Because systemic clearance decreased with increasing maximal concentrations, the source of nonlinearity was suggested to be Michaelis-Menten elimination kinetics. Volume of distribution was independent of dose, indicating that tasidotin distributes predominantly to the extracellular fluid compartment. These two components, high systemic clearance relative to hepatic plasma flow and small volume of distribution, contribute to a short half-life. In some patients, a third γ phase was observed in the concentration-time profile but could not be quantified. Even in the presence of nonlinear pharmacokinetics, the half-life of tasidotin was <1 hour across all doses resulting in lack of drug accumulation on this every other day dosing schedule. Due to the pharmacokinetic characteristics of tasidotin on this schedule, drug accumulation was not expected to occur with a daily 5-day dosing schedule, which was confirmed clinically (35).

Of note, the pharmacokinetics of tasidotin were analyzed by a Good Laboratory Practices–validated mass spectral assay, whereas cemadotin was analyzed by RIA and had severe cross-reactivity problems with metabolites in the sample (34). Thus, any measurement of pharmacokinetic variables of cemadotin actually reflects a conglomerate of parent and metabolite concentrations. In contrast, due to the high selectivity afforded by mass spectroscopy, the assay used herein is able to distinguish parent compound from metabolite. In all likelihood, the pharmacokinetics of tasidotin are not likely to be substantially different from cemadotin, and the reported half-life of cemadotin of 13.2 hours (11) actually reflects that of the carboxylate-metabolite.

In conclusion, tasidotin administered on days 1, 3, and 5 every 3 weeks achieves plasma concentrations higher than that required for cytotoxic activity *in vitro* and preliminarily seems active with prolonged stable disease being observed at tolerable doses. This alternate day administration schedule allows dosing of tasidotin at >10-fold higher doses than was possible with its predecessor, cemadotin. However, due to the lack of an objective response and lesser dose intensity on the current schedule, in contrast to the complete response in melanoma observed on the daily for five-consecutive-days schedule, the latter tasidotin schedule is currently being evaluated in disease-directed phase II studies in melanoma, non–small cell lung cancer, and prostate cancer.

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