A Phase I Study of the Dolastatin-15 Analogue Tasidotin (ILX651) Administered Intravenously Daily for 5 Consecutive Days Every 3 Weeks in Patients with Advanced Solid Tumors

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Abstract

Purpose: To determine the maximum tolerated dose, dose-limiting toxicity, and pharmacokinetics of the dolastatin-15 analogue, tasidotin (ILX651), when administered i.v. daily for 5 days every 3 weeks.

Experimental Design: Thirty-six patients with advanced solid tumors received a total of 114 courses through eight dose levels ranging from 2.3 to 36.3 mg/m^2. Pharmacokinetic samples were collected in cycle 1.

Results: Neutropenia was the principal dose-limiting toxicity at 36.3 mg/m^2/d along with grade 3 ileus and elevated aspartate amino transaminase/alanine amino transaminase (n = 1). At the maximum tolerated dose, 27.3 mg/m^2, 4 of 14 patients experienced dose-limiting grade 4 neutropenia. The other principal toxicities consisted of mild-to-moderate elevated transaminases, alopecia, fatigue, and nausea. One patient with melanoma metastatic to liver and bone treated at 15.4 mg/m^2/d experienced a complete response and received 20 courses of tasidotin. Two other patients with melanoma had mixed responses of cutaneous metastases at 27.3 mg/m^2/d associated with either stable or progressive visceral disease. In addition, nine patients had stable disease. There was no accumulation of tasidotin following repeated daily dosing. Tasidotin decayed from plasma in a biphasic fashion with a half-life of <45 minutes in most cases.

Conclusion: The maximum tolerated dose and recommended phase II dose for tasidotin when administered on this schedule was 27.3 mg/m^2/d. The favorable toxicity profile of tasidotin compared with other antitubulin agents (particularly the lack of severe cumulative neuropathy, peripheral edema, and fatigue), the observed antitumor activity of tasidotin, and its novel mechanism of action support further disease-directed evaluations of this agent on this 5-day schedule every 3 weeks.

Tasidotin (*N*,*N*-dimethyl-L-valyl-L-valyl-*N*-methyl-L-valyl-L-prolyl-L-proline-*tert*-butylamide hydrochloride; Genzyme Corp., San Antonio, TX) is a third-generation dolastatin-15 analogue that is metabolically stable through its resistance to hydrolysis by prolyl oligopeptidases. The dolastatins are a group of peptides with unusual amino acids that were isolated from the Indian Ocean sea hare *Dolabella auricularia* (1–4). The preclinical antitumor potential of the dolastatins initially garnered much enthusiasm (5–8). Cemadotin, a dolastatin-15 pentapeptide, at concentrations as low as 10 to 100 nmol/L, suppressed the rate of tubulin growth more than shortening, a process that differentiated dolastatin-15 analogues from other tubulin-acting agents like *Vinca* alkaloids (which suppress the rate of growth and shortening simultaneously) and taxanes (which preferentially suppress the rate of tubulin growth; ref. 9). However, clinical trial results with cemadotin were disappointing due to rapid metabolic conversion and dose-limiting cardiovascular toxicity, including hypertension, angina, and myocardial infarction (10–13).

Tasidotin induces cell cycle arrest in the G_2 and M phases and inhibits tubulin polymerization *in vitro* similar to cemadotin and the *Vinca* alkaloids. Whereas tasidotin tubulin-binding studies have not been done, evaluation of other dolastatin analogues suggests a binding site distinct from the *Vinca* alkaloids, taxanes, and colchicine (14). The effects of tasidotin on microtubule assembly were examined *in vitro* and compared with those of podophyllotoxin and vinblastine. Tasidotin is unique because at low concentrations, 25 to 40 μmol/L, a prolonged lag phase in microtubule assembly is induced followed by assembly to normal levels (15). At concentrations ≥50 μmol/L, tasidotin inhibits the extent of microtubule assembly.

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Tasidotin is cytotoxic against a broad range of murine and human solid tumors with IC_{50} values ranging from 0.02 to 0.7 μmol/L. Tasidotin was curative in human MX-1 breast, PC-3 prostate, and LOX melanoma xenografts. In addition, significant growth delays were noted in LX-1 non–small cell lung, CX-1 and HT-29 colon carcinoma, and P388 murine leukemia models. In the MX-1 model, tasidotin induced tumor growth delays ranging from 20 to >90 days compared with 15 days for paclitaxel (16). Schedule dependency was observed with tasidotin; repetitive dosing was required to achieve optimal antitumor activity.

Animal toxicology studies revealed effects predominantly on rapidly proliferating tissues, including hematopoietic, lymphoid, and gastrointestinal tissues. Reported cardiovascular events in rats consisted of an insignificant and reversible increase in heart rate. No relevant changes in blood pressure were noted in either rats or beagle dogs. In mongrel dogs, slight, dose-dependent decreases in cardiac output and increases in coronary, femoral, and total peripheral vascular resistance were observed, although these changes were 10-fold less compared with cemadotin.[4]

The impressive preclinical activity, favorable pharmacologic properties, and novel mechanism of action of tasidotin provide the rationale for evaluation in patients with solid tumors refractory to standard treatment. A 5-day every 3 weeks schedule was chosen based on preclinical schedule dependency data. The principal objectives of this study were to determine the maximum tolerated dose of tasidotin when administered according to this schedule, determine the toxicities, characterize the pharmacokinetic behavior, seek preliminary evidence of antitumor activity, and recommend a phase II dose for tasidotin.

Materials 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 variables: age ≥18 years; Eastern Cooperative Oncology Group performance status ≤2; life expectancy ≥12 weeks; no chemotherapy, radiation therapy, or major surgical procedures within previous 4 weeks (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/dL], hepatic [total bilirubin <2.0 mg/dL, aspartate amino transaminase (AST) and alanine amino transaminase (ALT) levels ≤2 × upper limits of normal or <5 × upper limits 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 according to the method of Cockcroft and Gault 17) functions; prior radiotherapy to ≤25% of bone marrow reserves (no whole pelvic irradiation was allowed); no active infection or other coexisting medical problems of sufficient severity to limit study compliance; no prior stem cell or bone marrow transplantation; and no clinically apparent central nervous system metastases. All patients gave written informed consent in accordance with federal and institutional guidelines before treatment.

Dosage and drug administration. The starting dose of tasidotin (2.3 mg/m^2/d) was 1/10th of the maximum tolerated dose in rat toxicology studies. A modified Fibonacci scheme guided tasidotin dose escalation in successive cohorts from an initial dose of 2.3 to 4.6, 7.7, 11.6, 15.4, 20.5, 27.3, and 36.3 mg/m^2. Tasidotin was administered over 30 minutes i.v. daily for 5 consecutive days every 3 weeks. Dose reduction to the previous dose level was permitted for patients experiencing dose-limiting toxicity. The maximal number of dose reductions per patient was two. Up to three new patients were treated at each escalated dose level. No intrapatient dose escalation was permitted. If one of three new patients at any dose level experienced dose-limiting toxicity, then up to a total of six new patients were treated at that dose level. The maximum tolerated dose, or recommended phase II dose, was defined as the highest dose level that induced dose-limiting toxicity in <2 of six new patients. Dose-limiting toxicity was defined during course 1 as: ANC <500/μL; platelet count <25,000/μL; any drug-related ≥grade 3 nonhematologic toxicity except suboptimally treated nausea/vomiting or diarrhea; ≥ grade 3 drug-related vomiting or diarrhea despite optimal treatment; and treatment delays >7 days due to unresolved toxicity in patients with ≥ grade 3 thrombocytopenia, grade 4 neutropenia, or any grade 2 drug-related nonhematologic toxicity. Patients were re-treated provided eligibility requirements were maintained and any nonhematologic toxicity had resolved to ≤grade 1. A re-treatment delay of 2 weeks 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).

Tasidotin (Genzyme) was supplied in 5 mL vials containing 30 mg of tasidotin, which was diluted with 50 mL of 0.9% NaCl and infused over 30 minutes. Prophylactic use of antiemetics and hematopoietic growth factor support were not permitted during course 1.

Pretreatment and follow-up examinations. Histories, physical examinations, performance status assessments, and routine laboratory studies (complete blood counts with differential, biochemistries, clotting times, and urinalysis) were done pretreatment and weekly. A chest X-ray and electrocardiogram were done at baseline. Electrocardiograms were repeated before each course of tasidotin. 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 bidimensional measurements of all measurable disease.

Plasma sampling and assay. Five-milliliter blood samples were collected during course 1 from a vein contralateral to the infusion in prechilled, EDTA (nonseparator) tubes immediately before tasidotin administration and at 0.25, 0.5, 0.58, 0.75, 1, 1.5, 3, 5, 8, and 24 hours after infusion on days 1 and 5, and 48 and 72 hours after day 5. Later amendments removed the day 5 24-, 48-, and 72-hour samples. Samples were centrifuged for 5 minutes at 4°C at 1,000 × *g*; plasma was removed into polypropylene cryotubes and stored at −20°C. Samples were shipped to MicroConstants, Inc. (San Diego, CA) on dry ice and stored at −20°C until analysis.

Plasma tasidotin and ILX651-*C*-carboxylate concentrations were analyzed using a liquid chromatography-mass spectrometry assay having a linear range of 1 to 500 ng/mL for analytes; the coefficient of variation was <12.8% for tasidotin and <9.1% for ILX651-*C*-carboxylate (18).

Pharmacokinetic analysis of tasidotin and ILX651-C-carboxylate. Pharmacokinetic variable estimates for tasidotin were done using noncompartmental methods (19) with WinNonlin Professional (version 4.0, Pharsight Corp., Mountain View, CA). Dose proportionality was assessed using a linear mixed effects power model, the method recommended by the Statisticians in the Pharmaceutical Industry/Pharmaceutics UK Joint Working Party (20). Under the power model

[4] S. Hergenröder, unpublished data.

for dose proportionality, in a linear pharmacokinetic system, area under the curve (AUC) and C_{max} are proportional to dose. Hence, mathematically,

$$\text{AUC or } C_{max} = \beta_0(\text{dose})^{\beta_1} \quad \text{(A)}$$

where β_1 equals 1. Eq. A can be fit using nonlinear regression but is more typically analyzed after natural log (ln) transformation on both sides, which leads to the linear model

$$\ln(\text{AUC}) \text{ or } \ln(C_{max}) = \ln(\beta_0) + \beta_1 \times \ln(\text{dose}) \quad \text{(B)}$$

Dose can be either total dose or dose per square meter. Because of the repeated measures nature of the data, a linear mixed effects power model was used where patients were treated as random effects, whereas day and dose were treated as fixed effects. If day was not statistically significant at the 0.05 level, the factor was removed and the reduced model was refit. Dose proportionality was declared if the 90% confidence interval associated with β_1 contained the value 1.0. All pharmacokinetic statistical analyses were done using SAS (version 8, SAS Institute, Cary, NC).

Pharmacodynamic analysis. Two pharmacodynamic analyses were conducted. The first analysis used ordered logistic regression to model the association between the highest National Cancer Institute grade neutropenia severity across all courses of treatment against measures of exposure. The following measures of exposure were examined: daily total dose, daily dose per square meter, average tasidotin AUC(0−∞), average tasidotin C_{max}, average metabolite AUC(0-8), and average metabolite C_{max}. For nausea and vomiting, patient gender was also included as a covariate. Patients who did not experience either of these events were coded as 0. Significance was declared if $P < 0.05$. The second analysis involved analyzing the maximal percentage change in ANC as a function of measures of exposure. A sigmoid E_{max} model was fit to the data using the Marquardt algorithm. If the value of E_{max} was not significantly different from 100% based on the 95% confidence interval, then E_{max} was set equal to 100% and the model was refit.

Results

General. Thirty-six patients, whose characteristics are depicted in Table 1, received 114 courses of tasidotin (median 2; range 1-20) through eight dose levels. Two patients did not complete the first course of treatment. One patient was removed from the study after the first tasidotin infusion at 2.3 mg/m^2 due to rapidly progressive disease. A second patient who received 2 days of tasidotin at 15.4 mg/m^2 was hospitalized with deep venous thrombosis and was not retreated. Eight patients were dose reduced. Six patients treated at 27.3 mg/m^2 were dose reduced to 20.5 mg/m^2 after two (n = 4), three (n = 1), and six (n = 1) courses of tasidotin. One patient was subsequently dose reduced to 15.4 mg/m^2 after course 3. In addition, both patients treated at 36.3 mg/m^2 were dose reduced to 27.3 mg/m^2 for course 2, with one patient requiring a further dose reduction to 20.5 mg/m^2 after course 3.

Four patients were treated at the first dose level, 2.3 mg/m^2, due to the previously discussed patient with rapidly progressive disease. In addition, four patients were treated at the 15.4 mg/m^2 dose level due to deep venous thrombosis in one patient. Dose escalation proceeded to 20.5 mg/m^2, where grade 3 elevated transaminases was observed in one of three patients. At baseline, this patient had a grade 2 elevated transaminase, which progressed to grade 3 on course 1, day 8, after which the patient was taken off study due to progressive disease. One of the first three patients treated at 27.3 mg/m^2 had dose-limiting neutropenia; however, no further events were seen in the next three patients treated at this dose level. At the next higher dose level, 36.3 mg/m^2, dose-limiting neutropenia was observed in the first two patients. One of these events was associated with grade 3 ileus, dehydration, hyponatremia, and elevated transaminases. Three additional patients were treated at 27.3 mg/m^2 without further dose-limiting toxicity. Having confirmed the maximum tolerated dose at the 27.3 mg/m^2 dose level, a total of 14 patients were treated to further assess the safety profile of tasidotin. Four of 14 patients treated at the maximum tolerated dose experienced dose-limiting toxicity during cycle 1, specifically grade 4 neutropenia.

During this study, only one patient was hospitalized with tasidotin-related toxicity consisting of neutropenia, ileus, hyponatremia, and elevated transaminases as previously described. Hospitalizations unrelated to tasidotin included shortness of breath, increased pain, hypoxia due to pulmonary metastasis, and deep venous thrombosis. One patient died during the study due to disease progression.

Hematologic toxicity. Neutropenia was the principal dose-limiting toxicity of tasidotin. Hematologic toxicities as functions

Table 1. Patient characteristics

Characteristic	No. patients
No. patients (evaluable)	36 (36)
Median number of courses/patient (range)	2 (1-20)
Sex (male/female)	21/15
Median age (range)	58.5 (28-81)
Ethnicity	
Caucasian	29
Hispanic	5
African-American	1
Asian	1
Performance status (ECOG)	
0	13
1	20
2	3
Prior biotherapy/chemotherapy regimens	
≤2	18
>2 <5	15
≥5	3
Prior radiotherapy	17
Primary tumor types	
Melanoma	9
Renal	5
Breast	4
NSCLC	4
Unknown primary	3
Adrenal	2
Colorectal	2
Other (bladder, fibrosarcoma, gastric, pancreatic, prostate, pseudomyxoma peritonei, SCLC)	One each

Abbreviations: NSCLC, non – small cell lung cancer; SCLC, small cell lung cancer; ECOG, Eastern Cooperative Oncology Group.

Table 2. Hematologic toxicity of tasidotin

Tasidotin dose level (mg/m^2)	No. patients evaluable (new)	No. evaluable courses	Neutropenia grade, all courses (course 1)				Thrombocytopenia grade, all courses (course 1)	
			1	2	3	4	1	2-4
2.3	4 (4)	11	0	0	0	0	0	0
4.6	3 (3)	7	2 (1)	0	0	0	0	0
7.7	3 (3)	8	0	0	0	0	0	0
11.6	3 (3)	5	0	0	0	0	0	0
15.4	5 (4)	26	9 (0)	4 (0)	0	0	2 (1)	0
20.5	10 (3)	19	3 (0)	4 (0)	4 (1)	1 (0)	0	0
27.3	16 (14)	36	4 (0)	5 (3)	15 (6)	8 (4)	0	0
36.3	2 (2)	2	0	0	0	2 (2)	1 (1)	0

of the total numbers of patients and courses of tasidotin are shown in Table 2. Dose-limiting neutropenia occurred in course 1 in the first two patients treated at 36.3 mg/m^2. The incidence of grade 4 neutropenia was tolerable at 27.3 mg/m^2 with four episodes during first courses. ANC depression typically occurred on days 14 to 22, with a median time to nadir of 17 days (range, 7-29 days) with recovery usually within 5 days. Treatment delays due to an ANC <1,500/μL at the maximum tolerated dose were required in only two (4%) courses. The only episode of grade 4 neutropenia complicated by fever occurred in a patient treated at 36 mg/m^2. There was no evidence of cumulative neutropenia with repetitive dosing, although this assessment is limited due to the short duration of treatment for most patients.

The incidence of anemia at the maximum tolerated dose was 43%, with all events grade 1 to 2 except one uncomplicated grade 3 event. Thrombocytopenia was only grade 1 and was observed in only two patients. Whereas not included in the determination of dose-limiting toxicity and maximum tolerated dose, seven patients (19%) experienced grade 3 lymphopenia at doses ranging from 2.3 to 27.3 mg/m^2.

Nonhematologic toxicity. The nonhematologic toxicities associated with administration of tasidotin were generally mild to moderate in severity (Table 3). At the maximum tolerated

Table 3. Nonhematologic toxicity

Drug-related adverse events by all patients* (*n* = 36)

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4	Total, *n* (%)
AST/ALT increased†	9	2	3	0	14 (38.8)
Alopecia	4	2	0	0	6 (16.7)
Fatigue/fatigue aggravated	3	2	0	0	6 (16.7)
Nausea	2	1	0	0	3 (8.3)
Diarrhea	0	2	0	0	2 (5.6)
Pain in jaw/face	0	2	0	0	2 (5.6)
Vomiting	0	2	0	0	3 (8.3)
Dyspepsia	2	0	0	0	2 (5.6)
Headache	1	1	0	0	2 (5.6)
Ileus	0	0	1	0	1 (2.8)
Pyrexia	0	0	1	0	1 (2.8)
Anorexia	0	1	0	0	1 (2.8)
Arthralgia	0	1	0	0	1 (2.8)
Dyspnea exertional	0	1	0	0	1 (2.8)
Gastroesophageal reflux disease	0	1	0	0	1 (2.8)
Pain	0	1	0	0	1 (2.8)
Rash, macular	0	1	0	0	1 (2.8)
Sore throat	0	1	0	0	1 (2.8)
Abdominal pain	1	0	0	0	1 (2.8)
Hypoesthesia	1	0	0	0	1 (2.8)
Lethargy	1	0	0	0	1 (2.8)
Myalgia	1	0	0	0	1 (2.8)

*Only one occurrence at the highest grade is counted for each patient.
†AST/ALT shifts are changes in laboratory values that are presumed to be drug related.

dose, the overall incidence of elevated transaminases, alopecia, and fatigue was 21% each. Overall, three patients experienced grade 3 elevated transaminases. A 57-year-old female with metastatic breast cancer treated at 20.5 mg/m^2 experienced worsening of baseline grade 2 AST elevation to grade 3 with grade 3 hyponatremia and grade 2 hyperbilirubinemia, all of which were judged to be disease-related. The patient was not retreated. A second patient, a 78-year-old female with metastatic melanoma treated at 36.3 mg/m^2, experienced grade 3 ALT elevation on course 1, day 10, with grade 4 neutropenia, grade 3 ileus, grade 3 dehydration, and grade 3 hyponatremia. The grade 3 ALT and subsequent grade 1 hyperbilirubinemia were transient, resolving within 4 days, and assessed as drug related. The third patient, a 59-year-old female with colorectal cancer, was treated at 11.6 mg/m^2 and experienced worsening of a baseline grade 2 AST elevation to grade 3 on course 1, day 12, and a subsequent grade 2 hyperbilirubinemia reflected progressive disease and led to her removal from the study. Only one additional episode of hyperbilirubinemia reached grade 2 magnitude, at 2.3 mg/m^2, and was not associated with elevation of other hepatic variables.

Hypertension was observed in two patients and consisted of transient grade 1 diastolic hypertension. Other drug-related adverse events that were infrequently noted include grade 1 to 2 nausea, diarrhea, fatigue, dyspepsia, headache, facial or jaw pain, and vomiting.

Pharmacokinetic studies. Pharmacokinetic samples were obtained from all patients. Tasidotin plasma concentrations increased with increasing dose (Fig. 1). In estimating AUC, in all cases, <5% of the total area was extrapolated, whereas in most cases <0.5% was extrapolated. In the analysis of tasidotin dose proportionality, β_1 was estimated to be 1.19 (90% confidence interval, 1.07-1.32) and 0.99 (90% confidence interval, 0.86-1.12) for AUC and C_{max}, respectively. Whereas a 2-fold increase in dose resulted in a 2-fold increase in C_{max}, a 2-fold increase in dose resulted in a 2.3-fold increase in AUC. Hence, C_{max} was dose proportional whereas AUC was not (Fig. 2).

Because AUC was not dose proportional, neither was systemic clearance (P = 0.0228). Tasidotin clearance decreased with increasing dose, indicating nonlinear pharmacokinetics, and ranged from 11.4 L/h/m^2 (27.3 mg/m^2) to 96.0 L/h/m^2 (2.3 mg/m^2; Table 4). Between-subject variability for clearance was moderate at 38% with an interoccasion variability of 14%. Only 10% of the dose was excreted unchanged in the urine, indicating that renal clearance was a minor route of drug elimination. The least-squares mean renal clearance was 4.1 L/h and was independent of dose and sampling day. Between-subject variability for renal clearance was moderate at 40%. Volume of distribution at steady-state (Vd_{ss}) was dependent on dose (P = 0.0112) with a least-squares mean of 8.1 L/m^2. Vd_{ss} ranged from 2.9 to 25.0 L/m^2. Between-subject variability and

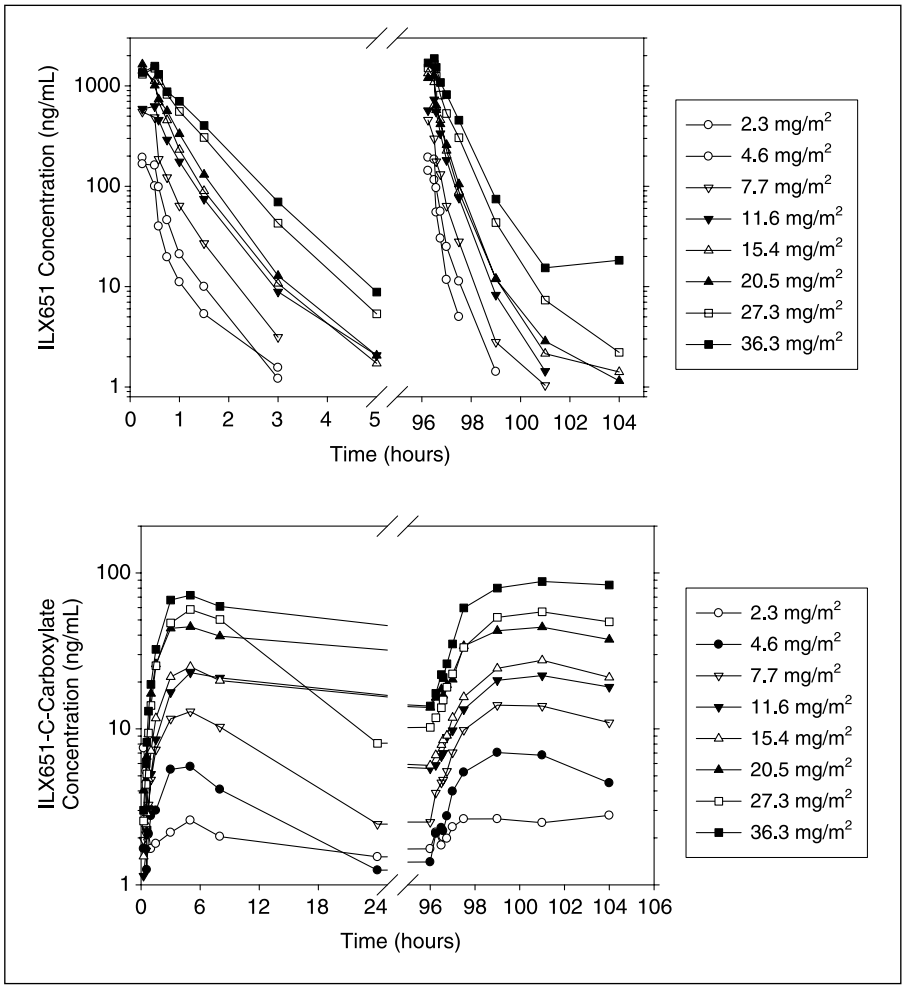

Fig. 1. Scatterplot of mean tasidotin concentration (ng/mL) versus time (hours) on days 1 and 5 (*top*) and mean concentration-time (ng/mL) plot for ILX651-*C*-carboxylate (*bottom*) by dose.

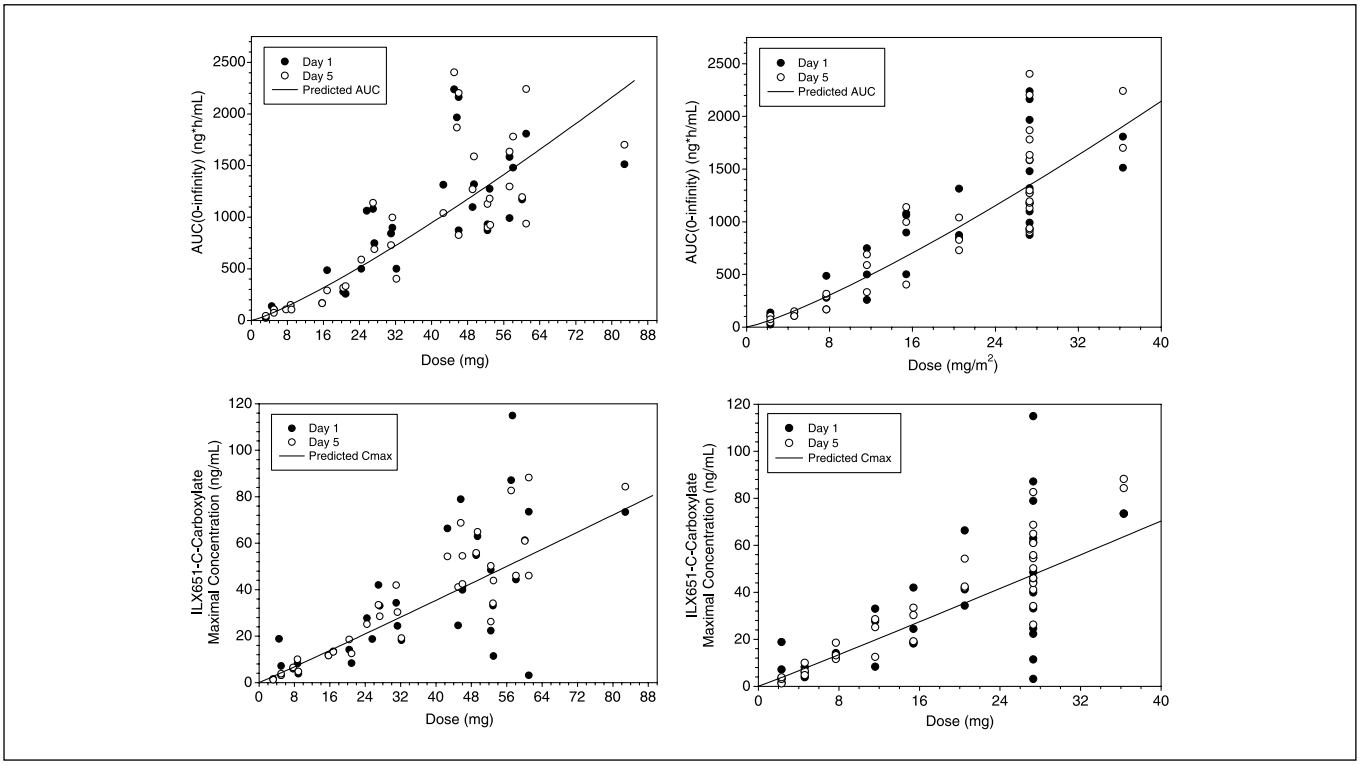

Fig. 2. Scatter plots of tasidotin AUC (*top plots*) and ILX651-*C*-carboxylate C_{max} (*bottom plots*) against total dose and dose per square meter. Solid line, predicted fit based on the power model using the appropriate independent variable.

interoccasion variability for Vd_{ss} were similar to clearance at 44% and 19%, respectively.

Tasidotin exhibited time-invariant, biphasic kinetics with no significant differences observed between days 1 and 5. Tasidotin effective half-life ranged from 0.15 (4.6 mg/m^2) to 1.22 hours (27.3 mg/m^2) and was dose dependent (P < 0.0001). In most instances, effective half-life is the same as the terminal half-life, but for a drug that exhibits multicompartment kinetics, the two may not be equal. There are instances, e.g., vancomycin and gentamicin, where one phase of the concentration-time profile contributes much more to the AUC than the other slower phase such that drug accumulation is not dependent on the terminal half-life but rather on the effective half-life (21). For tasidotin, by 8 hours or so postdose, the time in which the terminal half-life begins to appear (which was not apparent in all subjects), tasidotin concentrations had already declined by >90%. Hence, tasidotin accumulation, which did not occur to any significant extent, was dependent on the faster, effective half-life and not the terminal elimination half-life. The between-subject variability for effective half-life was <10%.

The mean concentration-time profiles by dose for the metabolite of tasidotin, ILX651-*C*-carboxylate, are presented in Fig. 1. Maximal ILX651-*C*-carboxylate concentrations were ~10% of tasidotin C_{max}. Plasma concentrations increased as dose increased and peaked ~5 hours after administration. Maximal ILX651-*C*-carboxylate concentrations displayed dose proportionality over the entire dose range studied (Fig. 2). β_1 for ILX651-*C*-carboxylate C_{max} was estimated at 1.03 (90% confidence interval, 0.86-1.20). Hence, a 2-fold increase in dose will result in a 2-fold increase in the C_{max} of the ILX651-*C*-carboxylate. Accurate assessment of the half-life of ILX651-*C*-carboxylate was not possible but was estimated at 10 hours.

Table 4. Noncompartmental pharmacokinetic variables of tasidotin

Dose level (mg/m^2)	No. patients	C_{max} (ng/mL), median (range)	AUC (ng • h/mL), median (range)	Clearance (L/h/m^2), median (range)	Vd_{ss} (L/m^2), median (range)	Effective half-life (h), median (range)
2.3	4	143 (58-334)	90 (24-137)	26.5 (16.8-96.0)	7.2 (2.9-23.0)	0.28 (0.16-0.59)
4.6	3	200 (129-263)	123 (104-150)	38.1 (30.7-44.1)	11.4 (7.6-20.5)	0.37 (0.15-0.42)
7.7	3	543 (200-898)	284 (167-486)	27.2 (15.9-46.2)	8.9 (5.2-25.1)	0.45 (0.33-0.47)
11.6	3	701 (423-897)	544 (257-748)	21.5 (15.5-45.1)	11.2 (9.1-16.4)	0.43 (0.29-0.66)
15.4	4	1,592 (562-2,141)	997 (403-1,139)	15.5 (13.5-38.3)	6.7 (4.5-17.0)	0.48 (0.25-0.61)
20.5	3	1,179 (979-2,879)	857 (729-1,313)	23.9 (15.6-28.1)	12.5 (5.4-16.4)	0.49 (0.40-0.67)
27.3	14	1,628 (985-2,778)	1,286 (874-2,403)	21.2 (11.4-31.3)	13.6 (7.4-22.7)	0.56 (0.42-1.22)
36.3	2	1,823 (1,526-1,886)	1,754 (1,512-2,241)	20.7 (16.2-24.0)	17.0 (13.0-17.9)	0.63 (0.54-0.67)

The accumulation of ILX651-*C*-carboxylate on the 5-day administration schedule was minimal.

Pharmacodynamic analysis. Dose (P = 0.0009), dose per square meter (P = 0.0125), tasidotin AUC(0-∞) (P = 0.0007), tasidotin C_{max} (P = 0.0075), metabolite AUC(0-8) (P = 0.0005), and metabolite C_{max} (P = 0.0007) were significant predictors of neutropenia with total dose being the most predictive. Interestingly, metabolite AVC(0-8) was a better predictor of neutropenia compared with parent AUC(0-∞) and metabolite C_{max} was a better predictor than parent C_{max}. As any exposure metric increased, so did the probability of neutropenia. The dose of ILX651 in which 50% and 80% of the patients would be expected to experience a grade 3 or higher neutropenic event was estimated at ~47 mg (~19 mg/m^2) and 57 mg (~32 mg/m^2), respectively, indicating that the exposure-response curve was fairly steep. None of the exposure measures were predictive of nausea and vomiting, although females were approximately five to seven times more likely to experience nausea and vomiting than males (P < 0.1).

All measures of exposure were also predictive of maximal percentage reduction in ANC and could be fit to a sigmoid E_{max} model, except for dose per square meter, which did not minimize successfully but was nevertheless correlated with the dependent variable (P = 0.0201). For all exposure measures, the 95% confidence interval for E_{max} contained the value 100%, indicating that complete reduction of ANC was observed in some patients. Hence, E_{max} was fixed to 100% and the models were refit. Based on mean square error, the best predictor of maximal percentage reduction in ANC was total dose administered (R^2 = 0.75), followed by tasidotin AVC(0-∞) and metabolite AUC(0-8), both of which were equally predictive (R^2 = 0.72). The best fit model using total dose as the predictor variable showed that the baseline maximal percentage reduction in ANC was 23.8 ± 5.1%, the total dose that 50% suppression of ANC was 40.9 ± 2.9 mg (~22 mg/m^2) and the shape variable was 5.7 ± 1.7 (Fig. 3).

Antitumor activity. Evidence of antineoplastic activity was observed in three patients. A 61-year-old male with melanoma metastatic to liver and bone previously treated with adjuvant biochemotherapy was treated at 15.4 mg/m^2 (Fig. 4). He experienced a partial response after two courses and a complete response by the end of course 12. After 20 courses of therapy, this patient came off-study with an ongoing complete response (data not shown). Two other patients with metastatic melanoma experienced mixed responses of >50% reduction of cutaneous nodules. Visceral disease remained stable in one patient, but progressed in the other. In addition, nine patients had stable disease. Those patients who had a best response of stable disease or better remained on the study twice as long (median 95 days) as those patients with progressive disease (median 40 days).

Discussion

The present study shows that tasidotin, a synthetic analogue of the natural marine product dolastatin-15, when administered every day for 5 days once every 3 weeks, has objective antitumor activity in patients with advanced solid tumors and has improved pharmacologic properties that translate into an improved safety profile compared with previous dolastatins. Neutropenia was the principal dose-limiting toxicity of tasidotin on this administration schedule. Ileus, elevated transaminases, and hyponatremia were also dose-limiting. In contrast to previous dolastatin analogues, cardiovascular toxicity was not observed on this 5-day tasidotin schedule. Initial studies with cemadotin revealed severe hypertension on three different administration schedules (9–12). In addition, on the single and weekly cemadotin administration schedules, hypertension was associated with myocardial infarction. Whereas myocardial infarction was not observed on this

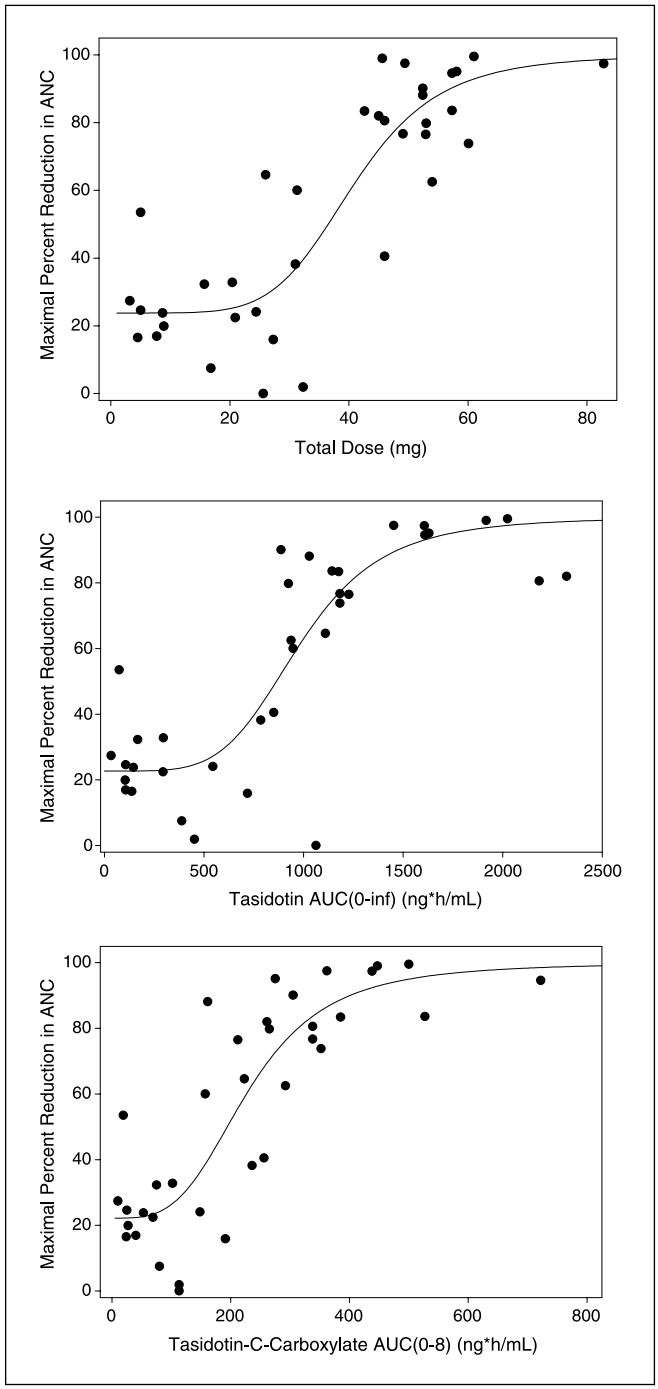

Fig. 3. Scatter plot of maximal percentage reduction in ANC against total dose (*top*), tasidotin AUC (*middle*), and metabolite AUC(0-8) (*bottom*). Solid line, model predicted fit to the data using a sigmoid E_{max} model.

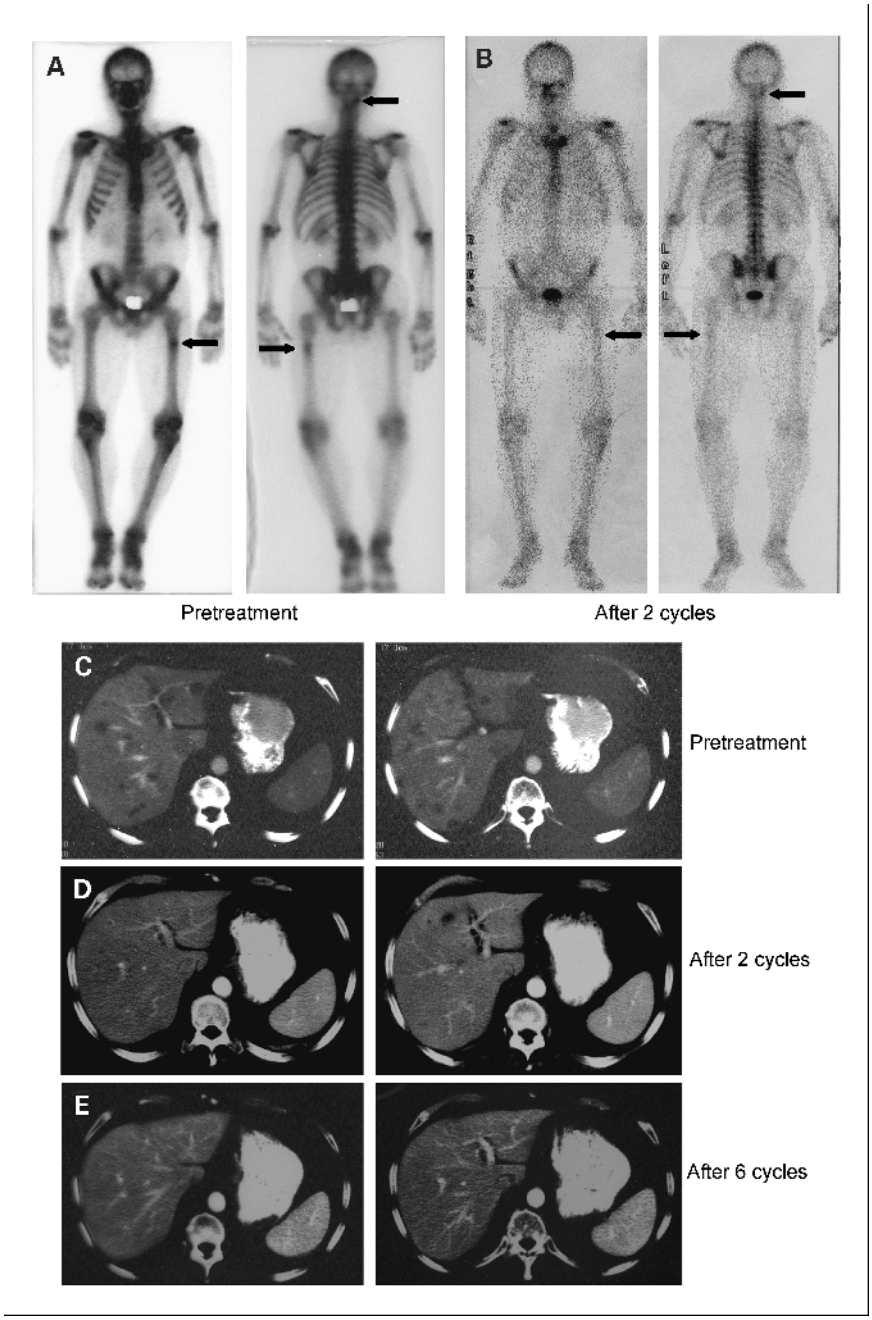

Fig. 4. *A,* radionuclide bone scan and computed tomography images for a patient with metastatic melanoma demonstrating bone metastases in the femur and cervical spine at baseline (*arrows*). *B,* improvement in the bone metastases after two cycles of tasidotin; the bone scan was normalized after 12 cycles of treatment (not shown). *C,* liver metastases at baseline. *D,* dramatic improvement in liver metastases after two cycles of tasidotin. *E,* complete resolution of liver metastases after six cycles of tasidotin.

tasidotin schedule, one patient who received tasidotin at a dose above the maximum tolerated dose on an alternate schedule (days 1, 3, and 5 every 3 weeks) experienced a myocardial infarction on course 3, day 12. In contrast to other dolastatin analogues, such as the dolastatin-10 analogue TZT-1027, tasidotin neurotoxicity was mild and not dose limiting (22). TZT-1027 administered over 1 hour every 3 weeks induced a dose-limiting neurotoxicity syndrome in two of three patients treated above the maximum tolerated dose (2.7 mg/m^2) consisting of mandibular cramps, paresthesias, insomnia, agitation, limb pain, and possibly abdominal pain and constipation. In the current tasidotin study, mild jaw pain, ileus, and hypoesthesia were infrequent (1-2 episodes each), but may have been manifestations of tasidotin-induced neurotoxicity similar to that observed with the *Vinca* alkaloids and TZT-1027. Otani et al. (23) speculate that TZT-1027 treatment-related pain, including abdominal pain and constipation, may be due to tumor-selective hemorrhage that was observed in animal models. The potential for tasidotin to induce similar effects has not been evaluated to date. All three patients with dose-limiting TZT-1027 neurotoxicity had previously received oxaliplatin, suggesting an oxaliplatin neurotoxicity recall phenomenon because peripheral neurotoxicity is the most frequent dose-limiting toxicity of oxaliplatin (24). In the current tasidotin study, only one patient had been treated previously with oxaliplatin and this patient did not experience any neurologic symptoms. It is notable that tasidotin does not cause the typical peripheral neurotoxicity characteristic of other antimicrotubular agents, such as the *Vinca* alkaloids, taxanes, and epothilones. This

lack of peripheral sensory neuropathy, which distinguishes dolastatins from taxanes, was initially noted with cemadotin (9–12) and has now been confirmed with tasidotin and TZT-1027 (22). In addition, the tasidotin toxicity profile is notable for lack of severe peripheral edema, arthralgia, myalgia, fatigue, and nail changes.

In comparison with the weekly or days 1, 3, and 5 every 3 weeks administration schedules, the schedule evaluated in this study maximizes tasidotin dose intensity (25). However, dose intensity in the current study may not be optimized as the definition for dose-limiting toxicity for neutropenia did not specify duration of neutropenia as is typical in many phase I studies. Thus, patients who are minimally pretreated may tolerate a higher dose of tasidotin than the maximum tolerated dose recommended for this 5-day schedule. In addition, a prolonged complete response was observed in a patient with metastatic melanoma previously treated with adjuvant biochemotherapy. Antitumor activity in previously treated metastatic melanoma was also seen in two patients who experienced improvement in cutaneous melanoma metastases, albeit with stable visceral disease in one patient and overt progression of disease in the other, indicating that tasidotin merits further evaluation in patients with this disease.

The pharmacokinetic behavior of tasidotin on the 5-day schedule is consistent with that reported for the days 1, 3, and 5 and weekly administration schedules (26–28). Lack of dose proportionality was observed with tasidotin AUC, but was observed with tasidotin C_{max} and ILX651-*C*-carboxylate C_{max}. Although not definitive, this phenomenon is probably related to saturation of metabolic clearance pathways, particularly metabolism by peptidases, because tasidotin clearance increased with increasing dose, a hallmark of Michaelis-Menten kinetics. Although there was statistical evidence of nonlinear pharmacokinetics, the deviation from linearity was mild and can be considered clinically irrelevant. More impressive is the low interoccasion variability for tasidotin clearance and Vd_{ss}, 14% and 19%, respectively, indicating that subjects will have similar concentration-time profiles with repeated drug administration.

The *C*-carboxylate metabolite of tasidotin is also the metabolite of cemadotin (*n*-desbenzylamino-cemadotin). This metabolite also prevents microtubule assembly in microtubule polymerization assays, but exhibits 8-fold less cytotoxicity than cemadotin *in vitro* (9). Tasidotin was rapidly cleared from plasma with an effective half-life of <45 minutes in most cases, whereas ILX651-*C*-carboxylate concentrations peaked at 5 hours and had a half-life of ~10 hours. However, due to the limited sampling scheme, the reported ILX651-*C*-carboxylate half-life is at best an estimate. The longer half-life of the metabolite compared with the parent indicates that ILX651-*C*-carboxylate clearance was elimination rate limited in its removal from the blood. Interestingly, tasidotin concentrations decreased to negligible levels whereas metabolite concentrations were still increasing. The most likely explanation for this observation is that tasidotin metabolism occurs at a site distal from the sampling site, such as tumor or liver, and the metabolite then slowly distributes back to the venous circulation.

Statistical analysis showed a relationship between tasidotin exposure and biological activity, namely neutropenia, as all measures of exposure were significant predictors of grade of neutropenia. The maximum tolerated dose defined in this study was 27.3 mg/m^2, with the next higher dose being 36.3 mg/m^2. Logistic modeling predicted that 80% of patients would experience grade 3 or 4 neutropenia at 32 mg/m^2, which was consistent with the observations in this study. Metabolite measures of exposure seemed to be better predictors of grade of neutropenia than parent concentrations, indicating that the metabolite has equal, if not better, activity than the parent. None of the exposure measures were predictive of grade of nausea or vomiting, but there were few counts in each category and no grade 3 or 4 events were observed, which may limit the validity of the these results. Modeling of maximal percentage reduction in ANC showed that 50% suppression of ANC could be achieved with ~22 mg/m^2 and that the reduction of ANC was fairly steep because the slope variable was >1.

In conclusion, tasidotin administered on this 5-day schedule was active at tolerable doses and allowed administration of tasidotin at >10-fold higher doses than its predecessor, cemadotin. Despite a higher C_{max} for tasidotin than cemadotin, tasidotin proved considerably safer than cemadotin with no observed cardiotoxicities. Pharmacodynamic analysis indicated that tasidotin exhibits biological activity with increasing exposure leading to a reduction in ANC and increased probability of neutropenia. Because of the antitumor activity and favorable toxicity profile observed in this study, further evaluation in phase II disease-specific studies in advanced melanoma, non–small cell lung cancer, and prostate cancer were initiated.

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