Phase I and Pharmacokinetic Study of Tasidotin Hydrochloride (ILX651), a Third-Generation Dolastatin-15 Analogue, Administered Weekly for 3 Weeks Every 28 Days in Patients with Advanced Solid Tumors

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Abstract

Purpose: To determine the safety, tolerability, and pharmacokinetics and to seek preliminary evidence of anticancer activity of tasidotin (ILX651), a novel dolastatin analogue, when administered as a 30-minute i.v. infusion weekly for 3 weeks every 4 weeks.

Experimental Design: Thirty patients with advanced solid malignancies were treated with 82 courses at six dose levels ranging from 7.8 to 62.2 mg/m² weekly, initially according to an accelerated dose-escalation scheme, which evolved into a Fibonacci scheme as a relevant degree of toxicity was observed. Plasma and urine were sampled to characterize the pharmacokinetic behavior of tasidotin.

Results: A high incidence of neutropenia complicated by fever (one patient), or precluding treatment on day 15 (three patients), was the principal toxicity of tasidotin, at doses above 46.8 mg/m². At all dose levels, nonhematologic toxicities were generally mild to moderate and manageable. Grade 3 toxicities included diarrhea and vomiting (one patient each). Drug-induced neurosensory symptoms were mild and there was no evidence of cardiovascular toxicity, which has been previously associated with other dolastatins. Tasidotin pharmacokinetics were mildly nonlinear, whereas metabolite kinetics were linear. A patient with non-small cell lung carcinoma experienced a minor response, and a patient with hepatocellular carcinoma had stable disease lasting 11 months.

Conclusions: The recommended dose for phase II studies of tasidotin administered on this schedule is 46.8 mg/m². The mild myelosuppression and manageable nonhematologic toxicities at the recommended dose, the evidence of antitumor activity, and the unique mechanistic aspects of tasidotin warrant further disease-directed evaluations on this and alternative schedules.

The dolastatins, a unique class of antimicrotubule agents from both structural and mechanistic perspectives, were initially isolated from the Indian Ocean sea hare Dolabella auricularia (1). These polypeptide compounds, which contain unique amino acids, such as dolavaline, dolaisoleucine, dolaproline, and N-methyl-valine, block tubulin assembly and mitosis by noncompetitively binding to tubulin at the peptide site of the Vinca alkaloid binding domain (2, 3). Tasidotin hydrochloride (ILX651; N,N-dimethyl-L-valyl-L-valyl-N-methyl-L-valyl-L-prolyl-L-proline-tert-butylamide hydrochloride; Genzyme Corporation, Cambridge, MA; Fig. 1) is a third-generation synthetic, water-soluble dolastatin pentapeptide with superior metabolic stability and oral bioavailability compared with the prior generation compound, cemadotin. Tasidotin is synthesized using the cemadotin backbone, with substitution of the COOH-terminal benzylamide group by tert-butylamide (4). Tasidotin induces dose-dependent inhibition of tubulin polymerization in vitro, with IC₅₀ values ranging from 10 to 20 µM/L (5). Tasidotin has also been shown to have unique effects on microtubules (3). At low concentrations (25-40 µM/L), tasidotin seems to induce a prolonged lag phase in microtubule assembly, which is followed by the standard rate of microtubule assembly. At higher concentrations (>50 µM/L), tasidotin inhibits the extent of microtubule assembly and induces a long lag time, which is not a feature of other antimicrotubule agents. In essence, tasidotin slows down the rates of both microtubule nucleation and elongation (3). These perturbations of microtubule dynamics result in the development of abnormal spindles and chromosome distribution in...
mitotic cells, leading to a prominent block in mitosis (G2-M phase) and ultimately cell death (4).

Tasidotin showed broad and potent antitumor activity in vitro, with submicromolar IC50 values in a wide variety of cancer types, including breast, ovarian, prostate, and colon carcinomas, and melanoma (4, 6). Tasidotin has also shown prominent activity against various murine tumors and human tumor xenografts, including P388 leukemia, and LX-1 lung, CX-1 colon, and PC-3 prostate carcinomas (4). Complete tumor regression was also noted in MX-1 human breast carcinoma and LOX melanoma murine xenograft models (4, 6). Toxicology studies done in rats and dogs revealed that rapidly proliferating tissues, including testes, bone marrow, skin, intestinal epithelium, and lymphoid tissues, were most susceptible to treatment with tasidotin, but toxicity was largely reversible (4). Tasidotin caused slight or no significant changes in cardiovascular variables at myelosuppressive doses in these preclinical studies. An oral formulation of tasidotin has shown acceptable bioavailability in dogs (7).

The biochemical features of tasidotin, as well as its encouraging preclinical antitumor activity, provided a rationale for clinical evaluations. This phase I and pharmacokinetic study was designed to evaluate the feasibility of administering tasidotin as a 30-minute i.v. infusion weekly for 3 weeks every 4 weeks in patients with advanced solid malignancies based on the protracted nature of the binding and retention of antimicrotubule agents in peripheral tissues and tumors, as well as the high therapeutic indices of other agents with similar mechanisms when administered on similar schedules (8–11).

The principal objectives of this study were as follows: (a) characterize the toxicities of tasidotin; (b) determine the maximum tolerated dose (MTD) and the recommended dose for phase II studies of tasidotin on this schedule; (c) characterize the pharmacokinetic profile of the compound; and (d) obtain preliminary evidence of antitumor activity.

Patients and Methods

Eligibility. Patients with metastatic or inoperable solid malignancies for whom standard therapeutic options did not exist were candidates for this study. Eligibility criteria also included age ≥18 years; an Eastern Cooperative Oncology Group performance status ≤2; adequate hematopoietic [absolute neutrophil count (ANC) ≥1500/μL, hemoglobin ≥9 g/dL, platelets ≥100,000/μL], hepatic [total bilirubin <2 mg/dL and transaminases twice or less than the upper limit of normal (less than five times if secondary to liver metastases)], and renal (serum creatinine ≤1.5 mg/dL or calculated creatinine clearance ≥60 mL/min) functions; recovery from all acute toxicities of prior treatment; no active brain metastases or carcinomatous meningitis; and no serious cardiovascular condition including uncontrolled congestive heart failure, angina, history of myocardial infarction within 2 months of enrollment, or cardiac functional capacity class III or IV as defined by the New York Heart Association Classification. Patients gave written informed consent according to federal and institutional guidelines before treatment.

Dosage and drug administration. The starting dose of tasidotin was 7.8 mg/m² administered as an i.v. infusion over 30 minutes at a rate of 100 mL/h. Dose-level increments were formulated according to an accelerated dose-escalation schedule until grade 2 drug-related toxicity was observed in a first course, at which time a more conservative modified Fibonacci scheme was used. At the initial accelerated dose escalation, three patients were to be entered at the starting dose. If no drug-related grade 2 toxicity was observed in the first cycle of treatment, the dose was to be doubled in each new patient. The occurrence of a drug-related grade 2 adverse event during the first course required the expansion of the cohort to three patients, and the subsequent use of the modified Fibonacci scheme, which required treatment of at least three to six patients at each dose level. If no dose-limiting toxicity (DLT) occurred after the initial patient had reached day 22 of the first course, two additional patients were entered at that same dose level. If no DLT was noted after the third patient had reached day 22 of the first treatment course, then a single subject patient was entered on the next dose level. At any dose level, if one of the first three patients experienced a DLT, then up to six patients were to be treated at that dose level. If two of the initial three patients or two of six patients, whichever occurs first, experienced DLT during the first course, then the MTD was exceeded and up to a total of 12 patients were to be treated at the next lower dose level. No intrapatient dose escalation was permitted. Dose reduction by one level was permitted for patients who developed DLT. If marked differences in the incidence and severity of toxicities were noted between the dose level that exceeded MTD and the previous dose level, an intermediate dose level could be explored after consultation between the investigator and the medical monitor.

All toxicities were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0. DLT was defined as any of the following: any grade 3 or 4 drug-related nonhematologic toxicity except grade 3 or 4 nausea/vomiting or diarrhea in the absence of optimal preventative and supportive measures; grade 4 neutropenia lasting longer than 5 days; grade 3 or 4 neutropenia associated with fever (≥38.5°C); platelet count <25,000/μL; treatment delay >7 days due to any unresolved toxicity in patients who experienced any grade 3 drug-related nonhematologic toxicity; or missing one or more scheduled dose in the first course due to toxicity. The MTD was to be determined separately for minimally pretreated and heavily pretreated patients if heavily pretreated patients were more prone to DLT. Heavily pretreated patients were defined a priori as those who had received more than six courses of an alkylating agent-containing regimen (except low-dose cisplatin), more than four courses of carboplatin-containing chemotherapy regimens, more than two courses of nitrosoureas or mitomycin-containing regimen, irradiation to ≥25% of bone marrow reserve, or high-dose chemotherapy requiring hematopoietic stem cell reinfusion. Patients with widespread bone metastases, as determined by the principal investigator, were included in the heavily pretreated cohort. Patients not meeting one of these criteria were considered as minimally pretreated.

Tasidotin was supplied by Genzyme as a sterile injectable formulation containing the active compound as a base in aqueous buffered solution. Each 5-mL vial contained 3 mL of tasidotin at a concentration of 10 mg/mL. The final solution was reconstituted with normal saline (NaCl 0.9%).

Pretreatment and follow-up studies. Histories that included performance status and concurrent medications, physical examinations, and

Fig. 1. Structure of tasidotin hydrochloride and tasidotin-C-carboxylate.
routine laboratory evaluations were done pretreatment and weekly, and at the end of the study. Routine laboratory evaluations included complete blood cell counts, chemistries, coagulation tests, and urine analysis. An electrocardiogram was done pretreatment and before each course, and at the end of the study. Complete blood cell counts and chemistries were assessed every other day if the ANC was <750/μL, platelets were <50,000/μL, or if any grade 3 to 4 chemistry toxicities were observed, respectively. Radiologic studies for disease assessment were conducted pretreatment, after every other course until disease progression, and at the end of the study. Patients were able to continue treatment in the absence of progressive disease, which was defined as a ≥25% increase in the size of any lesion, reappearance of any lesion, or appearance of any new lesion. A complete response was scored if there was disappearance of all disease on two measurements separated by at least 4 weeks, and a partial response required at least 50% reduction in the sum of the product of the bidimensional measurements of all documented lesions separated by at least 4 weeks.

Plasma and urine sampling and assay. Five-milliliter blood samples were collected during course 1 from a vein contralateral to the infusion in prechilled EDTA tubes immediately before tasidotin administration and at 0.25, 0.5, 0.75, 1, 1.5, 3, 5, 8 and 24, 48, and 72 hours after the initiation of the infusion on days 1 and 15. Blood samples were centrifuged for 5 minutes at 4°C at 1,000 × g. Plasma was removed, separated into two aliquots, and transferred to 5 mL polystyrene cryotubes and stored at −20°C, with care to minimize the exposure to UV light. Samples were shipped to MicroConstants, Inc. (San Diego, CA), on dry ice and stored at −20°C until analysis. Samples were analyzed using the methods reported in Lewiston (12) and Cunningham (13).

Also analyzed was the carboxylate metabolite of tasidotin, which had a linear range of 1 to 500 ng/mL. At the lower limit of quantification of the metabolite, the accuracy of the standards was −0.5% with a coefficient of variation of 6.5%. The accuracy of the quality control samples ranged from −0.5% to 1.0% with a coefficient of variation of <10%.

A single urine sample was collected before dose administration on days 1 and 15 of course 1. Urine was then collected in 24-hour aliquots during days 1 through 3 and 15 through 17 of the first course beginning after dose administration on days 1 and 15, respectively. Two 20-mL aliquots were frozen at −20°C, and then shipped to MicroConstants for analysis. Urine tasidotin concentrations were analyzed at Micro-Constants using a validated liquid chromatography-tandem mass spectrometry assay having a linear range of 0.1 to 50 μg/mL (12, 13). The accuracy of the method at the limit of quantification (0.1 μg/mL) was −0.2% from the theoretical value. The coefficient of variation was <10.11% with no quality control sample concentration exceeding ±4.0% from theoretical values.

Pharmacokinetic and pharmacodynamic analyses. Tasidotin and tasidotin-C-carboxylate pharmacokinetic variables were estimated using noncompartmental methods with actual sample times on days 1 and 15 (14). Metabolite ratios were calculated using the ratio of metabolite to parent area under the curve from 0 to infinity [AUC(0-∞)]. All variables were calculated using WinNonlin Professional, version 4.0 (Pharsight Corp., Mountain View, CA), and SAS for Windows, version 8.02 (SAS Institute, Cary, NC). Dose proportionality was assessed using a linear mixed-effects power model with the following dependent variables: In(C0/C), with caretominizetheexposuretoUV

Results

General

Thirty patients were treated with 82 total courses of tasidotin across six dose levels. Tables 1 and 2 detail the pertinent demographics of the patients and the dose-escalation scheme, respectively. The median number of courses administered per patient was two (range 1-11). Thirty patients were fully evaluable for safety. Twenty-seven patients (90%) had been treated previously with chemotherapy. According to the definitions established prospectively, 14 and 9 patients were considered minimally pretreated and heavily pretreated, respectively. Nine patients required dose reduction due to hematologic toxicity and 19 courses involving 12 patients were delayed due to neutropenia (14 courses), scheduling conflict (2 courses), intercurrent adverse events (3 courses), and non-compliance (1 course). One patient treated at 62.2 mg/m² died on study due to progressive disease.

The first patient treated at the 7.8 mg/m² dose level experienced abdominal pain that was initially considered to be possibly related to tasidotin and resulted in the uneventful treatment of two additional patients. It was later determined that the progressive growth of retroperitoneal metastases was responsible for the patient's abdominal pain, and therefore the accelerated dose-escalation scheme was resumed. After no drug-related toxicity was noted in a single patient treated at the 15.6 mg/m² dose level, three patients were treated at 31.2 mg/m² because the first subject experienced a transient grade 2 peripheral sensory neuropathy. Although there were no

| Table 1. Patient characteristics |
| Characteristic | No. patients |
|----------------|-------------|
| Total patients (evaluable) | 31 (30)** |
| Gender, male/female | 19/12 |
| Median number of courses/patient (range) | 2 (1-11) |
| Age, median (range), y | 57 (34-83) |
| Ethnicity | |
| Caucasian | 24 |
| Hispanic | 4 |
| Black | 2 |
| Other | 1 |
| Median performance status (ECOG) | |
| 0 | 9 |
| 1 | 16 |
| 2 | 5 |
| 3 | 1 |
| Prior biotherapy/chemotherapy regimens | |
| <2 | 2 |
| 2-5 | 23 |
| >5 | 6 |
| Tumor type | |
| Colorectal | 13 |
| Melanoma | 4 |
| Renal | 4 |
| Non–small cell lung | 2 |
| Breast | 2 |
| Other (esophageal carcinoma, head and neck squamous cell carcinoma, hepatocellular, mesothelioma, ovarian, prostate) | 1 each |

Abbreviation: ECOG, Eastern Cooperative Oncology Group.
*One patient was not treated due to uncontrolled tumor pain and Eastern Cooperative Oncology Group performance status of 3.
further toxic events noted at this dose level, the dose-escalation scheme reverted to the modified Fibonacci method. DLT was not observed in three patients treated at the next dose level, 46.8 mg/m², but two of three minimally pretreated subjects experienced unacceptable hematologic toxicity following treatment at the 62.2 mg/m² dose level. Due to the discrepancy in tolerance between the 46.8 and 62.2 mg/m² dose levels, an intermediate tasidotin dose of 54.5 mg/m² was next evaluated. At 54.5 mg/m², two of the first six patients, a group that included three minimally pretreated and heavily pretreated patients each, experienced dose-limiting hematologic toxicity. Because both subjects were heavily pretreated patients, the dose-escalation scheme diverged for heavily pretreated and minimally pretreated patients. Six additional heavily pretreated patients were treated with tasidotin at the 46.8 mg/m² dose level, with only one patient experiencing grade 4 neutropenia that precluded treatment on day 15. However, dose-limiting hematologic toxicity occurred in three of eight total minimally pretreated patients treated at the 54 mg/m² dose level, and therefore this dose exceeded the MTD. The 46.8 mg/m² dose level, at which one of six heavily pretreated and none of three minimally pretreated patients experienced DLT, was determined to be the MTD and recommended dose for phase II studies involving both heavily pretreated and minimally pretreated patients.

**Toxicity**

**Hematologic toxicity.** Neutropenia was the principal toxicity of tasidotin and the only DLT in this study. The ANC nadir typically occurred between days 13 and 20, which precluded treatment on day 15 of the first course in 8 (27%) of 30 patients. Of these eight individuals, three and five patients experienced grade 3 and 4 neutropenia, respectively. Only one patient experienced a grade 4 neutropenia with fever. The median day to nadir was 21 days, and the median number of days of grade 4 neutropenia was 5 days (range 3 to 8). The median number of days to recover from any neutropenia was 8 days. There was no evidence of cumulative myelosuppression. The distribution of the grades of neutropenia, as well as the hematologic DLTs, as a function of the dose level is listed in Table 3.

Severe neutropenia was initially noted at the 62.2 mg/m² dose level, with two of the three patients enrolled experiencing grade 3 or 4 uncomplicated neutropenia, respectively, which

### Table 2. Dose-escalation scheme, patients entered, and dose-limiting toxicities

| Dose level (mg/m²) | Prior therapy | Total dose per course (mg/m²) | New patients at dose level (courses) | Patients with dose reduction (courses) | Total patients (courses) | Omission of weekly therapy due to neutropenia | New patients with DLT | No. patients | Day omitted | Grade 3 ANC | Grade 4 ANC | Grade 4 > 5d fever | Grade 3-4 + fever | Grade 4 PLTs (range), µL* | PLTs grade 1-2 |
|-------------------|---------------|-------------------------------|-------------------------------------|---------------------------------------|-------------------------|---------------------------------------------|-----------------------|--------------|-------------|--------------|-------------|-----------------|-----------------|----------------|----------------|
| 7.8               | —             | 23.4                          | 3 (4)                               | 0                                     | 3 (4)                   | 0                                           | 0                     | 0            | 0           | 0            | 0           | 0/3             |                 | April 19, 2006   |               |
| 15.6              | —             | 46.8                          | 1 (2)                               | 0                                     | 1 (2)                   | 0                                           | 0                     | 0            | 0           | 0            | 0           | 0/1             |                 | April 19, 2006   |               |
| 31.2              | —             | 93.6                          | 3 (4)                               | 1 (1)                                 | 4 (5)                   | 0                                           | 0                     | 0            | 0           | 0            | 0           | 0/3             |                 | April 19, 2006   |               |
| 46.8              | MP            | 140.4                         | 3 (8)                               | 5 (18)                                | 8 (26)                  | 0                                           | 0                     | 0            | 0           | 0            | 0           | 0/3             |                 | April 19, 2006   |               |
| 46.8              | HP            | 140.4                         | 3 (7)                               | 6 (15)                                | 9 (22)                  | 1                                           | 15                    | 0            | 1           | 2            | 0           | 0/3             |                 | April 19, 2006   |               |
| 54.5              | MP            | 163.5                         | 8 (13)                              | 0                                     | 8 (13)                  | 3                                           | 15                    | 1            | 2           | 0            | 0           | 0/3             |                 | April 19, 2006   |               |
| 54.5              | HP            | 163.5                         | 3 (4)                               | 0                                     | 3 (4)                   | 2                                           | 15                    | 1            | 0           | 1*           | 2           | 2/3             |                 | April 19, 2006   |               |
| 62.2              | MP            | 186.6                         | 3 (6)                               | 0                                     | 3 (6)                   | 2                                           | 15                    | 1            | 1           | 2            | 0           | 0/3             |                 | April 19, 2006   |               |
| NOTE: Three patients received two of three cycles due to neutropenia. Abbreviations: MP, minimally pretreated; HP, heavily pretreated. *Febrile neutropenia. | | | | | | | | | | | | | | | | | |
precluded treatment on day 15. At the 54.5 mg/m² dose level, two heavily pretreated and three minimally pretreated patients, including the aforementioned patient with neutropenia complicated by fever, experienced grade 3 or 4 neutropenia on day 15 of the first course. At the recommended phase II dose, 46.8 mg/m², only one of the nine patients experienced a grade 4 neutropenia on day 15. Overall, severe (grade 3 or 4) neutropenia occurred in 8 (10%) of 82 courses. Eight patients (27%) experienced grade 3 lymphopenia at doses ranging from 46.8 to 62.2 mg/m². There were no significant effects of tasidotin on platelets or RBC. Anemia related to tasidotin was predominately mild (one patient) or moderate (four patients) in severity, and there was no evidence of cumulative toxicity. Three patients required transfusion of RBC.

Nonhematologic toxicities. The principal nonhematologic toxicities of tasidotin included fatigue [11 patients (37%)], diarrhea [nine patients (30%)], nausea [nine patients (30%)], anorexia [eight patients (27%)], vomiting [five patients (17%)], and abdominal pain [five patients (17%)], as shown in Table 4. These events were generally mild to moderate, brief, and managed successfully with prochlorperazine and loperamide in the case of nausea/vomiting and diarrhea, respectively. Myalgia, lower extremity edema, neurosensory effects, and alopecia were uncommon. Neuropathic manifestations were typically mild in severity. Only one individual who received prior treatment with oxaliplatin experienced grade 2 neuropathy, which was self-limiting.

An 82-year-old male with multifocal, unresectable hepatocellular carcinoma and a 30-year history of ischemic heart disease developed chest pain on the 11th day of his first course of tasidotin at the 54.5 mg/m² dose level. Neither elevation in cardiac enzymes nor electrocardiographic abnormalities indicative of myocardial injury were apparent. The patient received 10 additional courses of tasidotin, without recurrence of the chest pain. However, a minimal pleural effusion and a slightly decreased left ventricular ejection fraction of 40% were shown at that time. Because there had not been a left ventricular ejection fraction assessment before treatment, the precise etiology of these findings was indeterminable. However, a cardiac evaluation done 3 months after discontinuation of tasidotin showed

| Table 4. Drug-related nonhematologic toxicity by patient/course |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Adverse event** | 7.8 (3 Pts/4 courses) | 15.6 (1 Pt/2 courses) | 31.2 (4 Pts/5 courses) | 46.8 (16 Pts/48 courses) | 54.5 (11 Pts/17 courses) | 62.2 (3 Pts/6 courses) | **Total (30 Pts/82)** |

| Gr. 1-2 | Gr. 3-4 | Gr. 1-2 | Gr. 3-4 | Gr. 1-2 | Gr. 3-4 | Gr. 1-2 | Gr. 3-4 | Gr. 1-2 | Gr. 3-4 | Gr. 1-2 | Gr. 3-4 | Gr. 1-2 | Gr. 3-4 | All |
|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|------|
| Abdominal pain | — | — | — | — | — | — | — | — | — | 4/5 | — | 1/2 | — | 5/7 | 5/7 |
| Alopecia | — | — | — | — | — | — | 1/1 | — | — | 2/2 | — | 1/1 | — | 4/4 | 4/4 |
| Anorexia | 1/1 | — | 1/1 | — | 1/1 | — | 2/3 | — | 2/2 | — | 1/1 | — | 8/9 | 8/9 |
| Constipation | — | — | — | — | — | — | 1/2 | — | — | 2/2 | — | 1/1 | — | 2/4* | 2/4* |
| Diarrhea | 1/1 | — | — | — | — | — | 1/1 | 3/6 | — | 3/3 | — | 1/4 | — | 8/14 | 1/9 |
| Fatigue | 1/1 | — | 1/1 | — | 2/2 | — | 4/10 | — | 3/3 | — | — | — | 11/17 | — | 11/17 |
| Myalgia | — | — | — | — | — | — | 1/1 | — | — | 1/1 | — | 2/2 | — | 2/2 |
| Nausea | 2/3 | — | — | — | — | — | 2/3 | — | — | 2/4 | — | 1/2 | — | 9/19 | 9/19* |
| Neuropathy (sensory) | — | — | — | — | — | — | 1/1 | — | — | 2/2 | — | — | — | 3/3 | 3/3 |
| Peripheral edema | — | — | — | — | — | — | 1/1 | — | — | — | — | — | 1/1 | — | 1/1 |
| Rash | — | — | — | — | — | — | — | — | — | 3/3 | — | — | 1/1 | — | 1/1 |
| Vomiting | 2/2 | — | — | — | — | — | 3/3 | — | — | — | — | — | 1/1 | — | 5/5 | 1/1 |

Abbreviations: Pt, patient; Gr., grade.
*Patients are only counted once, but patient experienced adverse event in two different dose levels.
a left ventricular ejection fraction of 51%. No other potential cardiac or thromboembolic events were observed.

**Antineoplastic activity.** A 59-year-old female with non–small cell lung carcinoma that had metastasized to the left adrenal gland, pleura, and pericardium experienced progressive reduction in tumor size to a maximum of 47%, as well as improvement in hemoptysis and other disease-related respiratory symptoms. This heavily pretreated patient whose disease had previously progressed through several chemotherapy regimens that included carboplatin, paclitaxel, docetaxel, vinorelbine, gemcitabine, and gefitinib, was treated initially at the 54.5 mg/m² dose level for two courses and 46.8 mg/m² thereafter due to hematologic toxicity at the higher dose. She presented with progressive disease as manifested by brain metastases and received 11 courses at the 54.5 and 46.8 mg/m² dose levels.

**Pharmacokinetic and pharmacodynamic studies.** Plasma and urine sampling for pharmacologic studies was done on all patients. Representative plasma concentration-time profiles of tasidotin and its principal metabolite tasidotin-C-carboxylate are shown in Fig. 2. Tasidotin concentrations appeared to decline in a biphasic manner. A third, nonquantifiable phase was observed in several patients. Because of this inconsistent phase, effective half-life values, instead of the terminal half-life values, were calculated. Tasidotin-C-carboxylate concentrations were initially lower than parent concentrations, but, whereas parent concentrations rapidly declined, metabolite concentrations declined more slowly. Furthermore, the metabolite concentrations seemed to decline monophasically compared with the biphasic elimination profile of the parent drug. Also, tasidotin and tasidotin-C-carboxylate pharmacokinetics appeared stationary with no evidence of time dependency. Mean ILX651 pharmacokinetic variables as function of tasidotin dose level are listed in Table 5. Tasidotin Cmax values were not dose proportional. As depicted in Fig. 3, they were instead time invariant. A 2-fold increase in dose resulted in a 2.4-fold increase in AUC(0-24) for metabolite. "None" indicates that no values were available for analysis.

### Table 5. Summary of tasidotin pharmacokinetic variables

| Dose level (mg/m²) | No. patients* | Cmax (ng/mL), median (range) | AUC(0-24) (ng h/mL), median (range) | CL (L/h/m²), median (range) | Vdss (L/m²), median (range) | Half-life (min), median (range) |
|-------------------|---------------|-----------------------------|------------------------------------|-----------------------------|-----------------------------|-----------------------------|
| Tasidotin         |               |                            |                                    |                             |                             |                             |
| 7.8               | 3             | 480 (412-528)              | 333 (261-384)                      | 23.7 (20.3-29.9)            | 8.4 (7.7-9.6)               | 23 (22-23)                  |
| 15.6              | 1             | 869 (779-959)              | 727 (626-827)                      | 21.9 (18.9-24.9)           | 10.9 (9.3-12.6)            | 33 (26-40)                  |
| 31.2              | 3             | 1,382 (986-1,734)         | 1,178 (830-1,410)                 | 26.6 (22.1-37.6)            | 14.0 (12.4-21.8)           | 36 (28-41)                  |
| 46.8              | 9             | 3,619 (1,565-5,459)       | 2,797 (1,370-4,049)               | 16.7 (11.6-34.2)            | 9.9 (5.3-22.7)             | 37 (32-92)                  |
| 54.5              | 10            | 3,415 (1,924-5,994)       | 3,589 (1,526-5,198)               | 15.2 (10.5-35.7)            | 11.8 (6.7-21.7)            | 38 (31-61)                  |
| 62.2              | 3             | 3,510 (3,187-4,192)       | 4,170 (3,584-4,849)               | 15.2 (12.8-17.4)            | 13.8 (12.4-17.4)           | 45 (38-49)                  |

### Table 5. Summary of tasidotin pharmacokinetic variables

| Dose level (mg/m²) | No. patients* | Cmax (ng/mL), median (range) | AUC(0-24) (ng h/mL), median (range) | Tmax (h), median (range) | Half-life (h), median (range) | Metabolite ratio, median (range) |
|-------------------|---------------|-----------------------------|------------------------------------|--------------------------|-----------------------------|--------------------------------|
| Tasidotin-C-carboxylate |               |                            |                                    |                          |                             |                                 |
| 7.8               | 3             | 12.0 (9.6-12.9)             | 167 (128-204)                      | 5.0 (3.0-7.9)            | 6.6 (n = 1)                 | 0.53 (n = 1)                  |
| 15.6              | 1             | 30.8 (28.8-32.8)           | 441                               | 5.0 (5.0-5.1)            | None                       | None                           |
| 31.2              | 2             | 62.6 (36.7-71.1)           | 904 (551-989)                     | 5.0 (5.0-5.0)            | 8.6 (6.8-8.6)              | 0.68 (0.67-0.69)             |
| 46.8              | 8             | 97.5 (43.7-128.2)          | 1,308 (591-1,885)                 | 5.0 (3.0-5.1)            | 7.2 (6.7-8.2)              | 0.45 (0.38-0.94)             |
| 54.5              | 11            | 86.3 (28.1-137.1)          | 1,271 (502-2,075)                 | 5.0 (3.0-8.1)            | 8.6 (6.6-13.5)             | 0.51 (0.34-0.82)             |
| 62.2              | 3             | 110.9 (62.5-166.8)         | 1,787 (961-2,278)                 | 6.5 (5.0-8.0)            | 8.2 (6.8-9.7)              | 0.70 (0.58-0.83)             |

Abbreviation: CL, clearance.

*Number of patients having evaluable pharmacokinetics based on AUC(0-24) for parent or AUC(0-24) for metabolite. "None" indicates that no values were available for analysis.
tasidotin was affected neither by dose nor by day of administration. The least-square mean metabolite ratio was 0.59 and contributed about one third to the total circulating AUC of exposure (parent and metabolite).

Pharmacodynamic relationships, specifically between estimated tasidotin \( \text{AUC}_{0-\infty} \), \( C_{\text{max}} \) and cumulative dose in the first course and the principal toxicities, were sought. Scatter plots representing percentage decrements of ANC as a function...
of tasidotin and tasidotin-C-carboxylate dose, AUC, and $C_{\text{max}}$ are depicted in Fig. 4. All measures of exposure were significant predictors of neutropenia, but percentage decrements in ANC were most strongly related to tasidotin-C-carboxylate AUC$_{(0-24)}$ (Spearman’s $r = -0.792$, $P < 0.0001$) and tasidotin-C-carboxylate $C_{\text{max}}$ (Spearman’s $r = 0.776$, $P < 0.0001$). None of the metrics of exposure were significant predictors of nausea or vomiting.

**Discussion**

The dolastatin class of antimicrotubule agents are considered strategic candidates for clinical development in malignant diseases due to their unique effects on tubulin and range of antitumor activity, as well as their favorable pharmacologic and toxicologic profiles in early clinical studies (1–6). The clinical development of two members of this class, dolastatin-10 and cemadotin, was halted prematurely because of lack of clinical activity at tolerable doses, in spite of an excellent in vitro profile, and unacceptably high rates of cardiovascular toxicity (16–21). Therefore, dolastatins with improved therapeutic indices have been sought. Tasidotin showed favorable pharmacologic properties and therapeutic window in preclinical studies with significantly lower cardiovascular toxicity in animal models, all of which supported the rationale for clinical development.

Neutropenia was the principal DLT of tasidotin in the current study. The duration of neutropenia was typically brief and complications were rare. However, the nadir typically occurred around day 14, which precluded drug administration on day 15 of the first course in an unacceptably high proportion of patients at the higher doses and limited dose escalation on this schedule. The administration of tasidotin appeared feasible on the “3 of 4 weeks” schedule at the recommended dose. It is possible that a “2 of 3 weeks” schedule could provide a better dose intensity; however, this was not explored in the current study. No severe effects on platelets or RBC occurred. Furthermore, nonhematologic effects, particularly nausea, vomiting, and diarrhea, were generally mild to moderate and manageable. Unlike cemadotin, only mild elevations of hepatic enzymes occurred in patients treated with tasidotin. An important distinction from cemadotin, as predicted from preclinical studies, was the lack of drug-related cardiovascular toxicity. Similarly, neither cardiovascular nor hepatic toxicities were shown in this study compared with two other phase I studies evaluating alternate schedules of administration (22, 23). The toxicity profile of tasidotin, particularly its principal reversible effects on neutrophils, compares favorably with those of other clinically relevant agents that interfere with microtubule dynamics (24, 25). However, an apparent difference in the toxicologic profiles of the dolastatin analogues, including tasidotin, cemadotin, and dolastatin-10, is the lack of significant neurotoxicity, compared with the taxanes, *Vinca* alkaloids, and epothilones (24, 25). Nevertheless, caution must be exercised in making such comparisons because only three patients in the present study received more than four courses of treatment and the cumulative neurotoxic effects of tasidotin will require further elucidation in subsequent disease-directed trials. The precise reasons for the relative lack of neurotoxicity are unclear but it may represent a predilection of the dolastatins to affect highly dynamic microtubules, such as those constituting the mitotic spindle in contrast to the relatively adynamic microtubules of nonproliferating neurons (26). The water solubility of tasidotin is another relative advantage, which enables a formulation without toxic solubilizers such as polyoxyethylated castor oil (Cremophor EL) that are associated with hypersensitivity phenomena and require premedication and protracted administration schedules. Finally, an oral formulation of tasidotin is under consideration and could represent another significant pharmacologic advantage of this compound (7).

Tasidotin showed mild nonlinearity in its pharmacokinetics, but the metabolite showed linearity. These results are consistent with other phase I studies. The role of the metabolite in the activity of tasidotin is unclear. In vitro, the metabolite has shown anticancer activity, but this did not translate in mouse xenograft models. Theoretically, the metabolite contributed about a third of the total exposure and the metabolite had the highest correlation with neutropenia compared with other exposure measures. Still, the metabolite, which is a carboxylic acid, may be too polar to cross cell membranes and what is actually being seen in the analysis of the neutropenia data may be an indirect measure of tasidotin activity in the tissues. Further studies are needed to examine the role of the metabolite in the activity of tasidotin.

In this study, one patient with non–small cell lung cancer who had failed treatment with other antimicrotubule agents, including paclitaxel, docetaxel, and vinorelbine, experienced a 47% reduction of tumor burden. Interestingly, recent data show that tasidotin has a profound inhibitory effect on polymerization of $\alpha$III tubulin isotype, and showed impressive cytotoxicity against $\beta$-tubulin mutant cell lines that overexpress $\beta$III isotype (27). As aberrant expression of $\beta$III tubulin is associated with paclitaxel and epothilone resistance (27–29), tasidotin may be active against $\beta$III tubulin–overexpressing tumors refractory to other antimicrotubule agents.

In conclusion, the present study shows that the administration of tasidotin as a 30-minute i.v. infusion weekly for 3 weeks every 4 weeks is feasible and well tolerated at the recommended phase II dose of 46.8 mg/m$^2$. The improved pharmacologic and toxicity profiles of tasidotin compared with other dolastatins and the preliminary evidence of antitumor activity, along with a potentially original mechanism of action support disease-directed evaluations of the compound, which are ongoing.

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