Phase I Study of TLK286 (Glutathione S-Transferase P1-1 Activated Glutathione Analogue) in Advanced Refractory Solid Malignancies


INTRODUCTION

The purpose of this study was to determine the dose-limiting toxicities (DLTs), the maximum tolerated dose, and the pharmacokinetics of the novel glutathione analogue TLK286 administered by i.v. infusion.

Experimental Design: Patients with advanced malignancies received i.v. TLK286 administered as a 30-min constant rate infusion once every 3 weeks in escalating doses from 60 to 1280 mg/m². Patients underwent tumor assessment on day 43 and continued on treatment until disease progression or unacceptable toxicity.

Results: A total of 35 patients were treated with 109 cycles of TLK286. At 1280 mg/m², 3 of 5 patients developed one of two observed dose limiting toxicities (DLTs). The DLTs were: mild pancreatitis (1 of 5) and bladder symptoms (2 of 5) consisting of hematuria, dysuria, and urinary frequency. All of the patients with DLTs continued on TLK286 (2 of 5) consisting of hematuria, dysuria, and urinary frequency. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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4 The abbreviations used are: GST, glutathione S-transferase; NSCLC, non-small cell lung cancer; MAP, mitogen-activated protein; JNK, c-Jun NH₂-terminal kinase; BSA, body surface area; DLT, dose-limiting toxicity; MTD, maximum-tolerated dose; ECOG, Eastern Cooperative Oncology Group; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease; LC/MS/MS, liquid chromatography with automated tandem mass spectrometry; AUC, area under the concentration versus time curve from 0 to infinity; Vss, steady-state volume of distribution; MEK, mitogen-activated protein/extracellular signal-regulated kinase kinase.

Dose-proportional increases in both maximum blood concentrations and area under the blood-concentration-time curve were observed over the dose range of 60–960 mg/m².

Conclusion: TLK286 was well tolerated in this study. TLK286 safety and pharmacokinetics support disease-specific evaluations of TLK286 at doses <1280 mg/m² administered once every three weeks in the treatment of patients with advanced malignancies.

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the MAP kinase MEK4, JNK, and caspase 3, terminating in apoptosis, as evidenced by DNA fragmentation and membrane asymmetry. The current model for the induction of apoptosis by TLK286 through the stress kinase pathway is shown in Fig. 2. The proapoptotic and antitumor activity of TLK286 has been confirmed in vitro against human cancer cell lines and in vivo in a variety of murine tumor models, particularly in those that have elevated levels of GST P1-1 and have increased resistance to other anticancer agents (29, 30).

Preclinical toxicology studies of TLK286 were conducted in rats and dogs. In the rat, the nontoxic single i.v. dose of TLK286 was 400 mg/kg (2400 mg/m² BSA). The principal pathologic findings in rats treated at higher doses were a modest reduction in WBC count and thymic lymphatic atrophy. In the dog, the nontoxic single i.v. dose of TLK286 was 10 mg/kg (200 mg/m²). The dog was the most sensitive species, with hematuria as the DLT.

The decision to pursue the clinical development of TLK286 was based on the novel mechanism of action of the compound and broad spectrum of antitumor activity in preclinical tumor models including cancers exhibiting resistance to standard cytotoxic agents. The principal objectives of this Phase I safety and pharmacokinetic study in patients with advanced refractory solid malignancies were to: (a) determine the toxicities of TLK286 administered once every 3 weeks; (b) determine the MTD; and (c) characterize the pharmacokinetics of TLK286.

PATIENTS AND METHODS

Patient Selection. The Institutional Review Board of the University of California Los Angeles Cancer Center approved the protocol describing treatment and procedures used in this study. Written informed consent was obtained from all of the patients before entering the study. Patients with histologically confirmed advanced solid malignancies and non-Hodgkin’s lymphomas refractory to standard therapy or for whom no effective therapy existed were eligible. Eligibility requirements were: age of ≥18 years; ECOG performance status ≤2; life expectancy of ≥12 weeks; no chemotherapy, radiation therapy, immunotherapy, or investigational agents within 4 weeks (6 weeks for nitrosoureas or mitomycin); adequate hematopoietic function (absolute neutrophil count ≥1,500/mm³, platelet count ≥100,000/mm³, and hemoglobin ≥9.0 g/dl); adequate hepatic function (total bilirubin <2.0 mg/dl, AST and ALT concentrations ≤3.0 × the institutional upper limits of normal, unless in the presence of hepatic metastases, in which case elevations ≤5.0 × upper limits of normal were permitted); and adequate renal function (creatinine <2.0 mg/dl). At least 2 weeks must have elapsed from major surgery, administration of hematopoietic growth factors, or blood transfusion(s).

Exclusion criteria included: pregnancy or lactation; symptomatic brain metastases; carcinomatous meningitis or hydrocephalus; uncontrolled infection; hematuria; concomitant malignancy within the last 5 years other than curatively treated carcinoma in situ of the uterine cervix or basal cell skin cancer; and any coexisting medical problem of sufficient severity to limit full compliance with the study.

Drug Formulation and Administration. TLK286 was supplied by Telik, Inc. in vials containing 265 mg of sterile lyophilized drug product. The drug was reconstituted in Water for Injection, USP, to a concentration of 50 mg/ml followed by dilution of the appropriate dose of TLK286 into 250 ml of 5% Dextrose for Injection, USP. TLK286 was to be administered as a constant rate infusion over 30 min through a peripheral vein once every 3 weeks. A cycle of treatment was defined as 3 weeks.

Dose Escalation, Definition of DLTs, and MTD. The starting dose of TLK286 was 60 mg/m² administered once every 3 weeks, which was equivalent to one-third the nontoxic single dose in the dog, the most sensitive species tested, and one-fourtieth the nontoxic single dose in the rat. Dose escalation proceeded by a modified Fibonacci design with a minimum of 3 patients at each dose level (Table 2). If a DLT was observed in 1 of the first 3 patients, then 3 additional patients were entered at the same dose level. The MTD was defined as the dose at which 2 patients of 3–6 patients treated at that dose level experienced a DLT. A DLT is defined as any drug-related grade 3 or greater nonhematologic toxicity (excluding alopecia, nausea, vomiting, or diarrhea in the absence of optimal medical management) or drug-related grade 4 hematologic toxicity according to the WHO toxicity criteria scale. Intrapatient dose escalation was allowed, providing a subsequent higher dose was declared safe in a minimum of 3 patients.

Duration of Therapy and Dose Modifications. Patients underwent tumor assessments on day 43, and those patients who received clinical benefit (tumor regression or stabilization of disease) and who had experienced no unacceptable toxicities were allowed to continue on TLK286 therapy. Additional disease assessments were performed every 6 weeks. Patients remained on therapy until the development of PD, unacceptable
toxicity, or withdrawal of patient from study. Dose modifications were specified in the protocol as follows. For nonhematologic toxicity of grade 3 or 4 and thought to be TLK286-related, treatment was held until recovery to grade 1 or baseline, and treatment could then be reinstituted at the next lower dose level. For hematologic toxicity, the protocol specified that TLK286 treatment would be withheld until recovery of absolute neutrophil count to ≥1,500/mm³ and platelet count ≥100,000/mm³. For nadir counts of ANC <1,000/mm³ or platelets <50,000/mm³, TLK286 treatment was to be resumed with a 25% dose reduction. Nadir counts of ANC <500/mm³ or platelets <25,000/mm³ required TLK286 treatment to be resumed with a 50% dose reduction.

Pretreatment and Follow-Up Studies. After obtaining written informed consent, and no more than 10 days before treatment initiation, all of the participants underwent a screening evaluation including complete medical history, concomitant medications and prior anticancer therapy, physical examination with vital signs, ECOG performance status, and 12-lead electrocardiogram. A chest X-ray and other imaging studies required to assess extent of disease were performed within 4 weeks of treatment initiation. Pretreatment laboratory evaluation included complete blood count with differential and platelets, serum chemistry profile, urinalysis, and pregnancy test (in women of childbearing potential). Appropriate radiographic assessment of size of both primary and metastatic tumors, and measurement of tumor markers were performed at baseline. Laboratory assessments and physical examination with vital signs, determination of ECOG performance status, recording of concomitant medications, and recording and grading of adverse events were repeated on day 1 (before infusion), and on days 2, 8, 15, and 22 of each cycle.

Tumor responses were assessed by standard response criteria. Tumor size was determined by the product of two perpendicular diameters of marker lesions applied at the widest portion of tumor. All of the measurable lesions were evaluated every two cycles. A CR required the complete disappearance of all of the measurable and evaluable disease without the appearance of new lesions or disease-related symptoms for two measurements at least 4 weeks apart. A PR required a ≥50% decrease from the baseline sum of the bidimensional products of the perpendicular diameters of all measurable lesions without the appearance of new lesions or progression of evaluable disease documented by two measurements at least 4 weeks apart. PD was defined as an increase of ≥25% in the sum of the products of the perpendicular diameters of all measured lesions, the appearance of any new lesion, or the reappearance of any lesion that had disappeared. SD was defined as any measurements that did not meet criteria for CR, PR, or PD.

Pharmacokinetics. To study the pharmacokinetics of TLK286, venous whole blood samples were obtained from an indwelling heparin lock catheter placed in the arm contralateral to the drug infusion for all of the patients during the first cycle of therapy. On day 1 of cycle 1 samples were collected 1 h before infusion; and at 15, 30, 40, and 50 min; and 1, 2, 4, 6, and 24 h after the start of the infusion. Blood samples were drawn into a syringe, and 2 ml of whole blood was immediately added to 9.4 ml of FACN solution (0.05 M formic acid in acetonitrile) in a 15-ml centrifuge tube followed by the addition of 0.6 ml of 60 mM sodium citrate. The tubes were vortexed for 10 s and centrifuged for 5 min at 3500 rpm. The total volume of urine for each
sample was recorded. A 5-ml aliquot of each sample was transferred immediately to a tube containing 15 ml of FACN solution, vortexed, and centrifuged for 5 min at 3500 rpm. An aliquot (12 ml) of the supernatant was transferred into a fresh tube and stored at −70°C until analysis.

Blood and urine concentrations of TLK286 were determined by LC/MS/MS methods. In these methods, TLK286 and an internal standard, TLK48157 (a glutathione analog), were extracted from blood and urine with acetylated acetone. For blood, extracts are first dried, then reconstituted to 100 µl followed by 10 µl injections. For urine, extracts are first dried, then reconstituted to 50 µl followed by 20 µl injections. The aliquot of the extract was analyzed by LC/MS/MS on a Betasil C18 column (100 × 2 mm, 5 µm) using a mobile phase consisting of 50% acetonitrile and 50% water containing 10 mM ammonium formate (pH 4.0) and a flow rate of 0.25 ml/min. The specificity of the assay was evaluated by checking chromatograms for the presence of any interferences from endogenous materials in six different lots of human blood. No interfering peaks were observed in the blank extracts from the six lots. The precision and accuracy of this analytical procedure were evaluated by the analysis of quality control samples at three concentrations, each run in six replicates in each of the three validation runs performed on separate days. A coefficient of variation of 4.9%, 4.3%, and 4.7% was observed at concentrations of 0.03 µg/ml, 0.8 µg/ml, and 1.6 µg/ml, respectively. The assays were carried out at MDS Pharma Laboratories (Sunnyvale, CA).

Individual blood TLK286 concentration data were analyzed by noncompartmental methods (31) to compute the following parameters for TLK286: Cmax, time to maximum observed blood concentrations (Tmax); the magnitude of the slope of the linear regression of the log concentration versus time profile during the terminal phase (Keq); half-life (t1/2), computed as (ln 2)/Keq; AUC, calculated using the linear trapezoidal rule to the last blood level above the quantitation limit of the assay and adding the term CLQCT/Keq, where CLQCT is the blood concentration at the last time point above the quantitation limit (0.01 µg/ml); clearance (Cl), computed as dose/AUC; apparent volume of distribution in the terminal elimination phase (volume), computed as Cl/Keq; Vss, computed as dose × AUMC/AUC2 – T × Dose/2 × AUC, where AUMC is the area under the first moment of the concentration-time curve and T is the duration of infusion; renal clearance (Clr), computed as the amount excreted in urine (A) divided by AUC over the collection interval. Where appropriate, the pharmacokinetic parameters were corrected for body weight as well as BSA. BSA was approximated using the method of DuBois (32): BSA (m²) = 0.20427 × height (m)0.725 × weight (kg)0.425. Actual times were used for analysis of blood and urine concentrations. Concentrations below the quantitation limit for TLK286 were set to zero. Actual doses and times of infusion and sample collection were used to compute the pharmacokinetic parameters.

Statistical Analysis. Blood and urine concentrations, as well as urine amounts and computed parameters were listed and summarized by dose (mean, standard deviation, coefficient of variation, minimum, maximum, and number of observations). Mean and by subject blood concentrations versus time were plotted for each dose on both linear and natural logarithm scales. The data were analyzed using an ANOVA appropriate for a parallel dose ascending design on the dose-adjusted parameters to assess dose proportionality. In addition, the effect of demographic variables such as age (>65 versus <65), gender and race (Caucasian versus non-Caucasian) on the pharmacokinetics of TLK286 were analyzed using ANOVA. Microsoft Excel (33) version 4 was used to calculate the pharmacokinetic parameters. Statistical analyses were done using the SAS procedure, PROC GLM (34).

RESULTS

Patient Demographics. A total of 35 patients were enrolled between January 2000 and December 2000. Characteristics of the 35 patients enrolled in this study are summarized in Table 1. Approximately equal numbers of males and females were enrolled. All of the patients were adults with a median age

### Table 1. Patient characteristics

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<tr>
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<tr>
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<td>≥3</td>
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* Includes leiomyosarcoma (n = 3) and chondrosarcoma, synovial cell sarcoma, and liposarcoma (one each).
** Includes adenocarcinoma (n = 3) and Non-Small cell, not otherwise specified (n = 2).
† Includes one each of endometrial carcinoma, renal cell carcinoma, transitional cell carcinoma of the urinary bladder, adenocarcinoma of the pancreas, adenocarcinoma of unknown primary, medullary carcinoma of the thyroid, adenoid cystic, squamous cell carcinoma of the anus, malignant germ cell, and non-Hodgkin’s lymphoma.
of 54 years (range, 25–74). Protocol eligibility criteria allowed enrollment of patients with ECOG performance status 0–2. At enrollment, 3 of the patients had an ECOG status of 0, 32 had an ECOG status of 1, and none had an ECOG status of 2. This Phase I study enrolled patients with a variety of solid tumors. Four types of malignancies were the most common seen among patients enrolled. These were colorectal, breast, NSCLC, and sarcoma. Ten other types of malignancies were each represented once among enrolled patients. All of the patients had disease metastatic to one or more sites. The most common sites of metastatic disease were lung (66%) and liver (57%). All of the patients had received multiple prior chemotherapy regimens (median, 5; range, 1–8), and 63% had received prior radiation therapy.

Dose Escalation, DLT, and MTD. All of the patients enrolled received treatment with TLK286. Thirty-five patients received a total of 109 cycles of TLK286 at nine dose levels ranging from 60 to 1280 mg/m² BSA once every 3 weeks. The dose escalation scheme, as well as the number of patients and cycles administered as a function of dose level, are reported in Table 2. The median number of cycles administered per patient was two (range, 1–9). At least 3 patients were treated at each dose level. Two cohorts (180 mg/m² and 720 mg/m²) were expanded to 6 patients during dose escalation. The second patient in the 180-mg/m² cohort experienced a grade 3 elevation in AST and a grade 2 elevation in ALT after cycle 2 of TLK286. Grade 3 elevated AST was classified as a DLT. The third DLT was reported for the fifth patient in this cohort. This patient, a 65-year-old woman with NSCLC, developed transient grade 3 hematuria after cycle 1 of TLK286 treatment. The grade 3 elevated AST was classified as a DLT. The AST elevation resolved without treatment within 24 h, and the patient tolerated retreatment well at a reduced dose (540 mg/m²). After 5 additional patients were treated in the 720 mg/m² cohort without additional DLTs, dose escalation was resumed.

The 1280 mg/m² dose cohort was expanded when a DLT was observed. A total of 5 patients were treated at this dose level. DLTs were reported for 3 of the 5 patients, which identified 1280 mg/m² as the MTD. The first DLT was reported in the second patient treated in this cohort. This patient, a 36-year-old female with pancreatic cancer metastatic to liver, developed symptoms of bladder inflammation (grade 3 urinary frequency and urinary incontinence, and grade 2 dysuria, nocturia, and urine discoloration) 1 day after receiving cycle 1 of TLK286 at 1280 mg/m². The symptoms were resolved within 48 h and already subsiding when the urinalysis specimen was obtained. The urinalysis was normal. This adverse event was classified as a possibly drug-related DLT, based on urinary frequency and incontinence, and the 1280 mg/m² cohort was expanded. This patient was subsequently treated at a reduced dose (960 mg/m²) and tolerated TLK286 well without recurrence of the DLT.

The second DLT was reported in the fourth patient treated with 1280 mg/m² of TLK286, and was classified as a drug-related serious adverse event. This patient, a 53-year-old male, had renal cell carcinoma metastatic to bone, lung, and brain. Twenty-four h after infusion of cycle 1 of TLK286 at 1280 mg/m², the patient developed grade 3 nausea and vomiting accompanied by grade 2 abdominal pain. He was hospitalized 2 days later. Laboratory assessments revealed grade 3 hyperbilirubinemia, hyperglycemia, and elevated amylose, and grade 2 elevated lipase. An abdominal computed tomography scan showed pancreatic edema. The patient was diagnosed with grade 3 pancreatitis and responded to supportive care without sequelae. The symptoms of pancreatitis resolved over 4 days. The patient continued to receive TLK286 at a reduced dosage of 960 mg/m² for 9.5 months without recurrence of any DLTs. There were no other TLK286-related serious adverse events at any dose level.

The third DLT was reported for the fifth patient in this cohort. This patient, a 65-year-old woman with NSCLC, developed transient grade 3 hematuria 2 days after receiving the first infusion of TLK286. This was accompanied by grade 2 dysuria and urgency, which resolved to grade 1 in 2 days. This patient received TLK286 at a reduced dosage of 960 mg/m² without a recurrence of the DLT.

On the basis of these findings the MTD of TLK286 administered i.v. once every 3 weeks was established at 1280 mg/m².

Other Toxicity. Most toxicities seen were mild (grade 1–2) and were often consistent with events expected in heavily pretreated patients with advanced solid malignancies. None of the patients died or discontinued study treatment prematurely because of an adverse event considered to be related to TLK286 administration. The DLTs reported for the study were transient and resolved either without therapy or with supportive care. The
DLTs had no sequela and were noncumulative. None of the DLTs occurred during the first treatment cycle. There was no evidence of cumulative toxicity in the study. The most common toxicities during all of the cycles of TLK286 treatment are listed in Tables 3 and 4. There were no treatment-related deaths.

**Hematologic Toxicity.** TLK286 treatment-related hematologic toxicities reported in this study were in all cases mild or moderate (grade 1 or 2 only; Table 3). The reported hematologic toxicities were not clinically significant and did not generally limit TLK286 treatment. Anemia was the most frequently reported of these events (37% of patients). Thrombocytopenia (23%) and neutropenia (11%) were the only other treatment-related hematologic toxicities reported for ≥10% of patients. There was no evidence for cumulative hematologic toxicity. The only treatment-related hematologic adverse events reported for the patients receiving ≥2 cycles of TLK286 treatment were 1 grade 1 anemia in cycle 4 and grade 1 thrombocytopenias in cycle 3.

**Nonhematologic Toxicity.** The most frequent, possibly drug-related nonhematologic toxicities reported were nausea, vomiting, fatigue, transient microscopic hematuria, and hypokalemia (Table 4). These toxicities were generally mild to moderate (grade 1–2) with no grade 4 drug-related toxicities reported. Prophylactic antiemetics were routinely used with doses >720 mg/m². Nausea was well controlled when prophylactic prochlorperazine and dexamethasone were used. Hydroxyl tryptamine type 3 inhibitor antagonists were rarely used. Treatment-related grade 1 hematuria was reported for 10 patients (29%) and transient grade 3 hematuria for 1 patient (3%). Bladder inflammation was an expected adverse event based on the results from preclinical toxicology studies. Five patients were granted exceptions to enroll in the study with microscopic hematuria thought to be because of a variety of underlying conditions (e.g., renal stones and tumor invasion). None of these patients developed clinically significant hematuria after treatment or any exacerbation of their underlying microscopic hematuria. Grade 1 or 2 hypokalemia possibly related to TLK286 treatment was reported for 11 patients (31%), and was treated with oral potassium supplementation in some cases.

A total of 16 cycles of TLK286 at a dose of 960 mg/m² (the dose below the MTD) was administered to 6 patients, 3 each from the 960 mg/m² cohort and the 1280 mg/m² cohort, respectively. Repeated cycles of TLK286 at this dose were well tolerated in this group. Given the overall safety observed in this Phase I study, TLK286 given once every 3 weeks at doses of 960 mg/m² is recommended for disease-specific Phase II studies of TLK286.

**Pharmacokinetic Studies and Analysis.** Pharmacokinetic studies were completed and assessed in all 35 of the patients on cycle 1, days 1 and 2. Blood and urine concentrations of TLK286 were determined by LC/MS/MS methods. The time course for the mean blood concentrations of TLK286 at different dose levels is shown in Fig. 3. Measured blood and urine concentrations were used to calculate the parameters for TLK286 shown in Table 5.

TLK286 exhibited linear pharmacokinetics over the 60–960 mg/m² dose range. As expected, the time to peak concentration in the majority of the subjects occurred at the end of the 30-min infusion. Mean peak blood concentrations (Cmax) of TLK286 increased proportionally to dose from 906 ± 205 to 38.5 ± 16.9 µg/ml over this dose range (P = 0.498; Table 5; Fig. 4). Similarly, total AUC also increased proportionally to dose from 0.630 ± 0.0948 to 23.5 ± 7.25 µg·h/ml over this dose range (P = 0.506; Table 5; Fig. 5). Total body clearance was high and independent of dose when corrected for body weight (range, 17.3 ± 5.29 to 36.2 ± 12.2 ml/min/kg; P = 0.702) or BSA (range, 727 ± 238 to 1558 ± 311 ml/min/m²; P = 0.378).

Greater than proportional increases in the pharmacokinetics of TLK286 were observed at the highest dose studied, 1280 mg/m². For example, a 33% increase in the dose from 960 to 1280 mg/m² resulted in a 2-fold increase in mean Cmax from 28.5 ± 16.9 to 76.4 ± 27.4 µg/ml and mean AUC from 23.5 ± 7.25 to 50.3 ± 20.9 µg·h/ml. However, whereas the clearance corrected for body weight was independent of dose (P = 0.1698) over a dose range up to 1280 mg/m², clearance corrected for BSA demonstrated a dose-dependent decrease (P = 0.034).

Over the entire dose range of 60–1280 mg/m² the volume of distribution in the terminal elimination phase (volume) as well as the Vss approximated total body water, and were independent of dose whether or not corrected for body weight and BSA. TLK286 was rapidly eliminated from the blood with mean half-lives ranging between 0.223 and 0.403 h (approximately 13–24 min) and was independent of dose. The percentage of the administered dose recovered in the urine as TLK286 was highly variable and ranged between a mean of 0.5% and 12.8%.

The pharmacokinetic results were examined in an attempt to identify possible relationships between the measures of drug exposure to TLK286 such as Cmax, AUC, and 24-h urinary excretion of TLK286 and the development of DLTs. There was no evidence for a relationship between a higher maximum concentration of TLK286 in blood (Cmax) or a higher total

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Table 3  Principal hematologic toxicities of TLK286a (n = 35)

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<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
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</table>

a Number of patients with the most frequent hematologic toxicities reported as “possibly-related” or “probably-related” to TLK286.

Table 4  Principal nonhematologic toxicities for TLK286b (n = 35)

<table>
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<tr>
<th>Adverse event</th>
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<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Total</th>
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<td>1</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

a Number of patients with the most frequent hematologic toxicities reported as “possibly-related” or “probably-related” to TLK286.
b Prophylactic antiemetics instituted at dose levels ≥720 mg/m².

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exposure (AUC) and the development of a DLT with urinary symptoms (Table 6). There was no evidence for a relationship between a higher amount of TLK286 excretion in the urine and the development of urinary symptoms as DLT. The single patient who developed pancreatitis after a dose of 1280 mg/m² had a C_max (67.8 µg/ml) and AUC (42.1 µg*h/ml) that was lower than the average C_max (76.4 µg/ml) and AUC (50.3 µg*h/ml) for all of the patients treated at 1280 mg/m². Likewise, there was no apparent effect of age (<65 years versus ≥65 years), gender, or race (Caucasian versus non-Caucasian) on the pharmacokinetic parameters of half-life, V_ss, Cl, or C_max (Ps from ANOVA all ≥0.2). In summary, TLK286 has a mean T_1/2 of 18 min (range, 13–24 min) and a wide volume of distribution. The pharmacokinetics of blood TLK286 is linear and predictable over the dose range recommended for disease-directed Phase II studies. In this study, there was no indication that variations in drug levels from patient to patient were predictive for development of DLTs (Table 6).

Antitumor Effects. Although this was a safety and pharmacokinetic study, 31 of 35 patients were evaluable for objective tumor response. Measurable disease was not required for entry into the study, and 4 patients were ineligible for tumor response because of the absence of measurable disease at baseline. Responses were categorized as CR, PR, or SD. Disease progression was defined by increasing lesion size, worsening evaluable disease, reappearance of any lesions that had disappeared previously, or the appearance of any new lesion or site. Patients were considered evaluable for tumor response if they had received a minimum of two cycles of treatment and underwent day 43 tumor assessment. No objective CRs or PRs (≥50% reduction in tumor area) were observed among the patients with measurable disease. Four patients with measurable disease experienced tumor regressions. These significant patients, 1 with NSCLC, 1 with renal cell carcinoma, 1 with bladder carcinoma and one with colorectal cancer achieved maximum reduction of tumor area after five and six cycles of TLK286 treatment, respectively. In this study, 9 of 31 evaluable patients experienced SD across tumor types: colorectal (2), NSCLC (2), bladder, adenocarcinoma of unknown primary, breast, sarcoma, and renal cell. All of these patients were heavily pretreated with multiple chemotherapy regimens (median, 5; range, 1–8). Median number of cycles of TLK286 treatment in these 9 patients with SD was 6 (range, 4–9). Duration of SD was a median of 4.5 months (range, 3–9.5 months; Table 7).

DISCUSSION

TLK286 is a novel glutathione analog designed to undergo selective activation by GST P1-1, an enzyme that is overexpressed in many human malignancies. Preclinical studies in cell culture and in xenograft models have confirmed the relationship between GST P1-1 levels and TLK286 activity. The IC_{50}s for cytotoxicity for a series of 11 human malignant cell lines treated with TLK286 ranged from 6 to 67 µM (mean, 29 µM; median, 26 µM). Recent results have shown that even brief exposure to TLK286 at concentrations as low as 4 µM TLK286

Fig. 3 Mean blood concentrations (logarithmic scale) of TLK286 versus time (linear scale) after a single 30-min i.v. infusion of TLK286 for dose levels 60–1280 mg/m². The dose level (in mg/m²) is indicated to the right of the figure.

5 F. Meng and J. Keck, unpublished observations.
Table 5  Pharmacokinetic parameters for TLK286 administered once every 3 weeks (Mean ± SD)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Dose Level (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (µg/ml)</td>
<td>0.96 ± 0.205 0.47 ± 0.144 0.20 ± 0.055</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>6.71 ± 4.87 8.17 ± 5.22 8.87 ± 6.17</td>
</tr>
<tr>
<td>$C_{\text{ss}}$ (µg/ml)</td>
<td>4.35 ± 3.88 5.42 ± 3.73 6.71 ± 5.22</td>
</tr>
<tr>
<td>$V_{\text{ss}}$ (L)</td>
<td>27.31 ± 8.06 25.51 ± 7.23 21.43 ± 5.94</td>
</tr>
<tr>
<td>$CL$ (mL/min/kg)</td>
<td>0.07 ± 0.05 0.10 ± 0.07 0.14 ± 0.09</td>
</tr>
<tr>
<td>$CL_{R}$ (mL/min/m²)</td>
<td>0.31 ± 0.27 0.30 ± 0.25 0.38 ± 0.30</td>
</tr>
<tr>
<td>$K_{\text{el}}$ (1/h)</td>
<td>1.13 ± 0.21 1.32 ± 0.35 1.50 ± 0.28</td>
</tr>
<tr>
<td>Half-life (h)</td>
<td>3.46 ± 0.36 3.28 ± 0.31 3.44 ± 0.36</td>
</tr>
<tr>
<td>$D_{\text{LTT}}$ (mg/m²)</td>
<td>60 120 180 240 300 360 420 480 540 600 720 840 960 1200 1280</td>
</tr>
<tr>
<td>$AUC$ (µg*h/ml)</td>
<td>70.4 ± 56.3 80.6 ± 56.3</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (g/ml)</td>
<td>0.205 ± 0.112 0.417 ± 0.144 0.519 ± 0.144</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.02 ± 1.02 1.01 ± 1.01 1.04 ± 1.04</td>
</tr>
<tr>
<td>$AUC$ (µg*h/ml)</td>
<td>6.62 ± 6.62 8.32 ± 8.32 10.77 ± 10.77</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (g/ml)</td>
<td>0.233 ± 0.071 0.379 ± 0.124 0.329 ± 0.107</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.32 ± 0.22 1.43 ± 0.27 1.50 ± 0.28</td>
</tr>
<tr>
<td>$AUC$ (µg*h/ml)</td>
<td>6.80 ± 6.80 8.32 ± 8.32 10.77 ± 10.77</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (g/ml)</td>
<td>0.704 ± 0.304 0.806 ± 0.304 0.97 ± 0.304</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.27 ± 0.27 1.43 ± 0.27 1.50 ± 0.28</td>
</tr>
<tr>
<td>$AUC$ (µg*h/ml)</td>
<td>6.62 ± 6.62 8.32 ± 8.32 10.77 ± 10.77</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (g/ml)</td>
<td>0.205 ± 0.112 0.417 ± 0.144 0.519 ± 0.144</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>1.02 ± 1.02 1.01 ± 1.01 1.04 ± 1.04</td>
</tr>
<tr>
<td>$AUC$ (µg*h/ml)</td>
<td>6.62 ± 6.62 8.32 ± 8.32 10.77 ± 10.77</td>
</tr>
</tbody>
</table>

Fig. 4  TLK286 blood $C_{\text{max}}$ versus dose over the dose range 60–1280 mg/m² after a single 30-min i.v. infusion.

Fig. 5  TLK286 blood $AUC$ versus dose over the dose range 60–1280 mg/m² after a single 30-min i.v. infusion.

Table 6  Selected pharmacokinetic parameters for patients treated at 1280 mg/m² TLK286: lack of relation to development of hematuria, dysuria, or urinary frequency DLT

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Yes (n = 2)</th>
<th>No (n = 3)</th>
<th>Mean</th>
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</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$</td>
<td>µg/ml</td>
<td>70.4</td>
<td>80.6</td>
<td></td>
</tr>
<tr>
<td>AUC</td>
<td>µg*h/ml</td>
<td>41</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>0–24 h urinary excretion</td>
<td>mg</td>
<td>195</td>
<td>366</td>
<td></td>
</tr>
</tbody>
</table>

...can induce apoptosis in cultured human cancer cells. The pathway to apoptosis in TLK286-treated cells is the stress response pathway, involving sequential activation of the MAP kinase MEK4, the MAP kinase JNK, and caspase 3 (28). Activation of this specific pathway to apoptosis may result from the novel characteristics of one or both of the products of TLK286 activation by GST P1-1, a tetrafunctional electrophile, tetrakis (2-chloroethyl) phosphorodiamidate and a vinylsulfone analog of glutathione. TLK286 is a rationally designed chemical entity that exploits a novel target in human malignancies and induces a pathway leading to cell death by apoptosis.
The objectives of this Phase I study were to establish the safety and pharmacokinetics of TLK286 administered once every 3 weeks. During dose escalation of TLK286, DLTs were observed at 1280 mg/m² that established the MTD for i.v. TLK286 administered once every 3 weeks. Two patients developed symptoms consistent with bladder inflammation 2 days after treatment with TLK286, and 1 of the 2 patients developed transient grade 3 hematuria. In both cases, the symptoms resolved and the patients were retreated with TLK286 at a reduced dose (960 mg/m²) without recurrence of bladder symptoms. Preclinical toxicology studies established bladder inflammation previously as the DLT in single-dose and repeat-dose studies in the dog, the most sensitive species tested. Although the nature of the DLTs was not identical for the 2 patients with grade 3 bladder symptoms, the determination of MTD was based on a constellation of bladder-related toxicities observed in 2 patients. Transient asymptomatic microscopic hematuria, possibly related to TLK286, was observed in 10 patients at 6 h after infusion and resolved within 24 h. However, with the exception of the patient experiencing a DLT, hematuria was uniformly grade 1 and not of clinical significance. Five patients had microscopic hematuria because of underlying disease at the time of study entry. None of these patients developed an exacerbation of their baseline hematuria subsequent to TLK286 treatment. Mesna has been used to ameliorate the bladder cystitis caused by the antineoplastic agents ifosfamide and cyclophosphamide. However, the use of mesna with TLK286 is not recommended. The chemistry of TLK286 is distinct from that of cyclophosphamide or ifosfamide where mesna protects against the urinary bladder cystitis caused by the acrolein metabolite of these agents. Acrolein is not a metabolite of TLK286.

The second DLT observed at the MTD was pancreatitis that developed 24 h after cycle 1 TLK286 infusion. The patient presented with symptoms of nausea, vomiting, and abdominal pain. Physical exam, laboratory abnormalities, and computerized tomography were consistent with the diagnosis of mild pancreatitis. The patient recovered with standard supportive care consisting of i.v. fluid hydration and pain medication. Although this episode was classified as possibly related to TLK286 because of its close temporal relationship to the TLK286 infusion, it was the only report of this toxicity. The patient subsequently received TLK286 at 960 mg/m² for 9.5 months without recurrence of this DLT. A definite causal relationship between TLK286 exposure and pancreatitis has not been established.

This study has demonstrated that TLK286 administered as a 30-min constant-rate i.v. infusion once every 3 weeks was well tolerated. There were no TLK286-related deaths, and no TLK286-related grade 4 toxicities were reported. Preclinical toxicology studies established that TLK286 causes less myelosuppression and thrombocytopenia than many standard anticancer agents. The principal possibly TLK286-related hematologic toxicity in the present study was grade 1 or 2 anemia. Only mild thrombocytopenia and neutropenia were reported. These events were not clinically significant and did not limit treatment with TLK286. The present report confirms that myelosuppression was not a clinically significant toxicity for TLK286, even for this group of heavily pretreated cancer patients who would be expected to have limited bone marrow reserve.

Nonhematologic toxicities related to TLK286 were also mild. Antiemetic prophylaxis was not required until the 720-mg/m² dose level cohort. Nausea and vomiting were well controlled when standard antiemetic agents such as prochlorperazine and dexamethasone were used prophylactically. Toxicities common to many anticancer agents such as alopecia, mucositis, and peripheral neuropathy were not reported for TLK286 in this study. In the patients who received repeat cycles of TLK286 therapy, there was no evidence for cumulative toxicity. In addition, TLK286-related toxicities appearing beyond 48 h after infusion were rare.

This study characterized the pharmacokinetics of TLK286 when administered as a constant-rate infusion i.v. over 30 min once every 3 weeks. Data regarding TLK286 blood and urine levels were available for all of the dose levels and for all 35 of the patients for the initial cycle of therapy. TLK286 is rapidly cleared from the blood with a mean half-life of 18 min. The pharmacokinetic parameters for TLK286 in blood were linear and predictable over the broad range of doses from 60–960 mg/m². In higher dose level cohorts, including the dose recommended for Phase II studies, blood levels of TLK286 equivalent to those required for induction of apoptosis in cultured cancer cells were achieved. The mean \( C_{\text{max}} \) in the blood of patients treated at 960 mg/m² (38.5 μg/ml) exceed by 2-fold the median \( IC_{50} \) (21 μg/ml) for TLK286-induced cytotoxicity in the 11 tested cell lines. There was some evidence for nonlinearity in TLK286 pharmacokinetics at the highest dose level tested. A more than proportional increase in mean \( C_{\text{max}} \) and mean AUC was observed between the 960 mg/m² and 1280 mg/m² dose levels. However, the clinical significance of this finding is in

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose cohort (mg/m²)</th>
<th>Primary cancer diagnosis</th>
<th>TLK286 cycles received</th>
<th>Weeks SD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>001–005</td>
<td>120</td>
<td>Adenocarcinoma (unknown primary site)</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>001–006</td>
<td>120</td>
<td>Adenocarcinoma of the colon</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>001–010</td>
<td>180</td>
<td>Bladder carcinoma</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>001–011</td>
<td>180</td>
<td>NSCLC</td>
<td>8</td>
<td>26</td>
</tr>
<tr>
<td>001–012</td>
<td>180</td>
<td>Adenocarcinoma of the breast</td>
<td>6</td>
<td>18</td>
</tr>
<tr>
<td>001–021</td>
<td>540</td>
<td>Sarcoma</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>001–029</td>
<td>720</td>
<td>NSCLC</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>001–040</td>
<td>1280</td>
<td>Adenocarcinoma of the colon</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>001–041</td>
<td>1280</td>
<td>Renal cell carcinoma</td>
<td>9</td>
<td>41</td>
</tr>
</tbody>
</table>

* SD duration is calculated from the date of first treatment with TLK286 until the date of radiographic confirmation of PD.
doubt, as nonlinearity of TLK286 pharmacokinetics was not apparent when doses were corrected for patient body weight. In addition, the only dose for which nonlinearity was observed, 1280 mg/m², is above the recommended dose for disease-directed Phase II studies.

The amount of TLK286 recovered as parent drug in the urine was variable. However, in the 2 patients treated with TLK286 at 1280 mg/m² who subsequently developed the DLT of bladder inflammation, recovery of TLK286 from urine was no higher (mean of 195 mg) than for the 3 patients who did not develop bladder inflammation (mean of 366 mg). Likewise, the development of pancreatitis or bladder inflammation was not associated with higher Cmax s or AUCs than those of patients who did not develop DLTs at this dose level (Table 6).

This Phase I dose-escalation study was designed to determine the safety and pharmacokinetics of TLK286 administered i.v. once every 3 weeks. The patients had received multiple prior chemotherapy regimens with standard and, in some cases, investigational anticancer agents. The results of this study indicate that TLK286, administered on this dose schedule, was well tolerated in this group of heavily pretreated patients with advanced malignancies who would be expected to have limited bone marrow reserve. The most frequently observed side effects of TLK286 are easily managed. DLTs observed were transient, resolved without sequelae, and were noncumulative. The pharmacokinetics of TLK286 are linear and demonstrate that biologically relevant concentrations of TLK286 are achieved in the blood of patients treated at doses below the MTD. The low level of toxicity associated with TLK286 observed in this study and the rather short half-life (~18 min) warrant study of additional dose schedules of TLK286, especially more frequent dosing. Results of a Phase I trial of weekly dosing of TLK286 have been reported recently (35) and suggest that dose intensity can be increased with a weekly dosing schedule.

Duration of study drug treatment during Phase I studies has been identified as a predictor of drug activity in later phase studies of anticancer agents (36). Of 31 evaluable patients, 9 patients continued on TLK286 therapy beyond day 43 tumor assessment (two cycles). Nine patients had SD and remained on TLK286 therapy beyond day 43 tumor assessment (two cycles). Nine patients had SD and remained on TLK286 therapy beyond day 43. Patients continued on TLK286 therapy beyond day 43 tumor assessment (two cycles). Nine patients had SD and remained on TLK286 therapy beyond day 43. Patients continued on TLK286 therapy beyond day 43.

REFERENCES


Phase I Study of TLK286 (Glutathione S-Transferase P1-1 Activated Glutathione Analogue) in Advanced Refractory Solid Malignancies


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