Phase I Trial of 6-Hour Infusion of Glufosfamide, a New Alkylating Agent With Potentially Enhanced Selectivity for Tumors That Overexpress Transmembrane Glucose Transporters: A Study of the European Organization for Research and Treatment of Cancer Early Clinical Studies Group

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Purpose: **To determine the maximum-tolerated dose (MTD), the principal toxicities, and the pharmacokinetics of 6-hour infusion of glufosfamide (beta-D-glucosylisophosphoramide mustard; D-19575), a novel alkylating agent with the potential to target the glucose transporter system.**

Patients and Methods: **Twenty-one patients (10 women and 11 men; median age, 56 years) with refractory solid tumors were treated with doses ranging from 800 to 6,000 mg/m^2. Glufosfamide was administered every 3 weeks as a two-step (fast/slow) intravenous infusion over a 6-hour period. All patients underwent pharmacokinetic sampling at the first course.**

Results: **The MTD was 6,000 mg/m^2. At this dose, two of six patients developed a reversible, dose-limiting renal tubular acidosis and a slight increase in serum creatinine the week after the second and third courses of treatment, respectively, whereas three of six patients experienced short-lived grade 4 neutropenia/leukopenia. Other side effects were generally mild. Pharmacokinetics indicated linearity of area under the time–versus-concentration curve against dose over the dose range studied and a short elimination half-life. There was clear evidence of antitumor activity, with a long-lasting complete response of an advanced pancreatic adenocarcinoma and minor tumor shrinkage of two refractory colon carcinomas and one heavily pretreated breast cancer.**

Conclusion: **The principal toxicity of 6-hour infusion of glufosfamide is reversible renal tubular acidosis, the MTD is 6,000 mg/m^2, and the recommended phase II dose is 4,500 mg/m^2. Close monitoring of serum potassium and creatinine levels is suggested for patients receiving glufosfamide for early detection of possible renal toxicity. Evidence of antitumor activity in resistant carcinomas warrants further clinical exploration of glufosfamide in phase II studies.**

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GLUFOSFAMIDE IS A new alkylating agent in which isophosphoramide mustard, the alkylating metabolite of ifosfamide, is linked to beta-D-glucose (β-D-Glu-IPM). In vitro data suggest that cellular uptake of glufosfamide is mediated by the transmembrane transport system of glucose. Initial studies demonstrated a direct cytotoxicity that was reducible by the inhibitors of transmembrane glucose transporters, phlorizin and phloretin.[1] Recently it was found that glufosfamide is conveyed into tumor cells by SAAT1, a low-affinity sodium/glucose cotransporter.[2] Other glucose transporter proteins may also play a role in intracellular translocation of this compound. This targeting mechanism, together with the accelerated metabolic rate and increased glucose consumption of tumor cells, suggests potentially enhanced tumor selectivity for glufosfamide and introduces a novel concept for drug targeting.

Another interesting characteristic of glufosfamide is the lack of release of the urothelium irritant acrolein because of the absence of the oxazophosphorine ring in its structure. Oxazophosphorines are metabolized in the liver by cytochrome P450 to open-ring aldose forms that decompose to acrolein and the alkylating metabolites.[3] Glufosfamide does not require metabolic activation in the liver. It is transported intact into cells, where the active moiety isophosphoramide mustard is thought to be released by either spontaneous hydrolysis or hydrolysis catalyzed by intracellular glucosidases.[4]

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Trial results were presented at the Tenth National Cancer Institute–European Organization for Research and Treatment of Cancer Symposium on new drugs in cancer therapy, Amsterdam, the Netherlands, June 16-18, 1998, and at the Twenty-Third Congress of the European Society for Medical Oncology, Athens, Greece, November 6-10, 1998 (Ann Oncol 9:128, 1998 [suppl 4]).

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Preclinical pharmacokinetic studies of glufosfamide demonstrated rapid renal clearance, high tissue distribution, and low protein binding.[5] Toxicity studies in rodents showed that glufosfamide was more toxic when given orally, apparently because of a pronounced first-pass effect and increased production of toxic metabolites. The lethal dose for 10% of mice was 533 mg/kg for single oral dosing and 1,795 mg/kg for a single intravenous (IV) infusion. The IV-infusion lethal dose for 50% of rats and mice was 1.7 and 3.8 times higher, respectively, compared with oral administration. Nevertheless, the acute and subacute toxicity profiles were similar for both routes of administration. The major toxicity targets identified histologically were the bone marrow, the kidney, the skin, and the genital tract, and the observed alterations were minor and reversible.[6]

The New Drug Development and Coordinating Committee of the European Organization for Research and Treatment of Cancer selected glufosfamide for clinical evaluation because of its interesting pharmacologic properties and its possible selectivity for tumors that express the glucose transporters. This phase I trial of a biphasic, rapid/slow 6-hour infusion of glufosfamide was initiated in June 1997. The schedule was based on a simulation model that was developed by using pharmacokinetic data from an ongoing phase I trial of a 1-hour IV glufosfamide infusion. It aimed to rapidly achieve steady-state concentrations and expose tumor cells to the study drug over a moderately prolonged time, with the intent to optimize selective uptake by the glucose transport system.

PATIENTS AND METHODS

Study Design and Objectives

This was an open-label, uncontrolled, dose-finding and pharmacokinetic phase I trial with between-patient dose escalation. Two European Organization for Research and Treatment of Cancer Early Clinical Studies Group centers collaborated in this study: the Department of Medical Oncology, University of Ioannina, School of Medicine, Ioannina, Greece, and the Cancer Research Campaign Centre for Cancer Therapeutics, The Institute of Cancer Research, Belmont, Sutton, United Kingdom. Study monitoring was done by the New Drug Development Office (NDDO) Oncology, Amsterdam, the Netherlands.

Primary objectives were as follows: (a) to determine the maximum-tolerated dose (MTD) and characterize the toxic effects of glufosfamide in humans when administered as a 6-hour infusion, with one quarter of the total dose given as a rapid half-hour infusion followed by the remaining three quarters of the total dose given as a 5½-hour infusion; (b) to propose a safe dose for phase II evaluation; and (c) to study the pharmacokinetics at the different dose levels. A secondary objective was to collect evidence of antitumor activity with this agent and schedule.

Chemistry and Formulation

Glufosfamide is a β-D-Glucopyranosyl-[N,N′-bis[(2-chloroethyl)]phosphoric acid diamide (International Union of Pure and Applied Chemistry [IUPAC] name, β-D-Glu-IPM). It was supplied by ASTA Medica AG (D-19575; Frankfurt, Germany) in vials containing 500 mg of glufosfamide as a lyophilisate to be reconstituted with 25 mL of 0.9% sodium chloride solution. Figure 1 shows the structural formula of β-D-Glu-IPM.

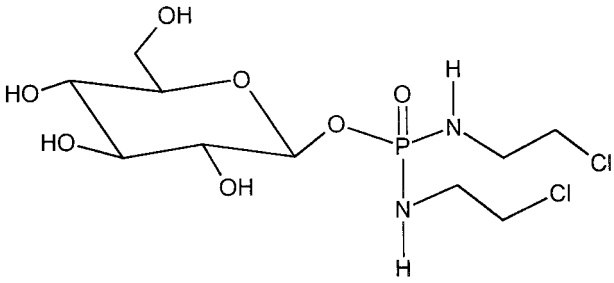

Fig 1. Chemical structure of β-D-Glc-IPM (D-19575).

Administration and Dose Escalation

An extended two-step, 6-hour IV infusion was used in this study: one quarter of the dose was administered in 30 minutes, and the remaining three quarters were given over the next 5½ hours. The starting dose level was 800 mg/m^2, based on data from another, parallel-running phase I study of short infusion (principal investigator: coauthor A.H). Prophylactic antiemetic treatment was allowed for subsequent courses only if nausea and vomiting occurred during the first course.

A minimum of three assessable patients (receiving at least one course of treatment) were entered at nontoxic dose levels. In case of ≥ grade 2 nonhematologic toxicity, except alopecia or inadequately treated nausea and vomiting (no prophylactic antiemetic medication was allowed during the first course, per protocol), or ≥ grade 3 hematologic toxicity, at least three more patients (for a total of six patients) were treated. At a given dose level, at least 2 weeks passed between the entry of the first and the next two patients. Doses were escalated depending on toxicity and the clinical judgment of the investigators. If no toxicity was observed, doses could be escalated by 100%. A "modified Fibonacci" scheme was to be followed if toxicity occurred. Decisions on dose escalation were made by teleconference of the investigators, the Early Clinical Studies Group chairman, and ASTA Medica, with the coordination of NDDO Oncology. Any pharmacokinetic or clinical data available during the course of the study were considered in making a final judgment.

Patients

Twenty-one patients (10 women and 11 men; median age, 56 years; age range, 31 to 70 years) with refractory cancers were enrolled during a 15-month period (from June 1997 to September 1998) and were treated with doses ranging from 800 to 6,000 mg/m^2. All patients had a histologically or cytologically confirmed diagnosis of a solid tumor not amenable to established forms of treatment, gave written informed consent, and fulfilled the established inclusion criteria for this phase I study. The inclusion criteria were as follows: (a) age ≥ 18 years; (b) performance status ≤ 2 on the World Health Organization scale; (c) life expectancy ≥ 3 months; (d) no prior chemotherapy, immunotherapy, or radiotherapy for at least 4 weeks before entry onto the study, and no prior nitrosourea, mitomycin, high-dose carboplatin, or extensive radiotherapy for 6 weeks; (e) WBC count ≥ 3.0×10^9/L and platelet count ≥ 100×10^9/L; (f) adequate hepatic function, characterized by

a bilirubin level less than 25 μmol/L (1.5 mg/dL) and transaminase (AST and ALT) levels ≤ 2.5 times the upper normal limit (unless related to liver metastases, in which case < five times the upper normal limit was allowed); and (g) adequate renal function, defined by creatinine levels ≤ 140 μmol/L (1.6 mg/dL). Exclusion criteria were (a) concomitant treatment with other investigational agents, (b) breast-feeding, pregnancy, or inadequate contraception in female patients, (c) any noncompensated or uncontrolled nonmalignant condition, (d) history of alcoholism, drug addiction, or psychotic disorders, and (e) brain involvement or leptomeningeal disease or prior irradiation to the brain.

Safety and Efficacy Assessment

All patients were seen clinically. Hematology, biochemistry, and urinalysis tests were performed at baseline and weekly thereafter until 4 weeks after the last administration of glufosfamide. If treatment was discontinued due to adverse events, or if possible treatment-related adverse events occurred at the end of treatment, toxicity was assessed until resolution of the abnormality at intervals not exceeding 4 weeks. Toxicity was graded according to the National Cancer Institute of Canada Clinical Trials Group expanded common toxicity criteria (CTC) (revised December 21, 1994). Any pre-existing symptom or laboratory abnormality was taken into consideration. If the symptom worsened by at least one grade during the study, it was characterized as grade 9. If no CTC grading was applicable, a "severity" grade was given (mild, moderate, severe, or life-threatening). Adverse events were recorded throughout the period of the clinical trial and until 4 weeks after the last treatment administration. Any abnormal outcomes were documented as adverse events and characterized by their relationship to the study drug. The MTD was defined as the highest dose that produced grade 3 or higher dose-limiting, nonhematologic toxicity or grade 4 hematologic toxicity in at least two of six patients.

Although efficacy evaluation was not an end point in this phase I study, tumors were assessed for response every two cycles using World Health Organization criteria. Patients were assessable for antitumor activity if disease measurements were recorded for at least 6 weeks after the first dose of therapy. A complete response was defined as the disappearance of all known disease, and partial response was defined as an at least 50% decrease of the sum of the products of the largest perpendicular diameters of all measurable bidimensional lesions or the sum of largest diameters of all unidimensional lesions. Objective responses had to be confirmed with a second assessment not less than 4 weeks apart.

Pharmacokinetics

Sampling procedure. Blood and urine samples for pharmacokinetic analysis were collected from all patients at the first cycle. Five-milliliter blood samples were collected in heparinized 10-mL tubes (NH_4 Heparin Monovette; Sarstedt AG, Nümbrecht, Germany) via an indwelling cannula or by venipuncture of veins from the arm not used for infusion. Blood was taken at 0, 0.5, 1, 3, 6, 6.25, 6.5, 7, 8, 10, 12, 14, 16, 20, 24, and 36 hours after the start of infusion. An additional sample was taken at 48 hours from a number of patients in the 3,200-mg/m^2 and 6,000-mg/m^2 dose groups. Before the second and every following administration of glufosfamide, only predose blood samples were taken. Plasma was separated by centrifugation at 4°C and 3,400 rpm for 10 minutes and was stored at −20°C. To determine the amount of glufosfamide in the urine, three separate urine samples were collected at 0 to 4, 4 to 16, and 16 to 24 hours after treatment. Each micturition was collected completely, transferred into a container filled with 100 mL of 0.01M ammonium acetate buffer (pH 7.4), and immediately put into a refrigerator (4 to 8°C) until the end of each sampling interval, ie, 4, 16, and 24 hours. A 10-mL aliquot of each urine collection was stored at −20°C. Both plasma and urine samples were kept frozen in a horizontal position to allow thawing control and were shipped on dry ice to ASTA Medica for bioanalysis.

Bioanalysis. The analytic work was performed in the Department of Biologic Research Biochemistry, ASTA Medica, and at Analytico Research B.V. (formerly BCO; Breda, the Netherlands). Two methods, A/1 and A/2 (data on file, Knebel and Winkler, 1999), were used at ASTA Medica, and method B/2 was used at Analytico (data on file, Bender, 1998). Plasma was assayed with methods A/1, A/2, and B/2; urine was assayed with method B/2 only.

Samples were pretreated by ultrafiltration (A/1 and A/2) or by solvent protein precipitation (B/2), followed by liquid chromatography (LC) and tandem mass spectrometry (MS/MS) detection with a TurboIon Spray Interface (Applied Biosystems, Foster City, CA). D-24144 (=[^{13}C]D-19575) was used as internal standard. The high-performance LC–MS/MS system was a Finnigan TSQ 7000 (Thermo-Quest Corp, San Jose, CA) or an API 3/300 (PE Sciex, Concord, Ontario, Canada). Frozen plasma and urine samples were thawed at ambient temperature, "vortex"-mixed, and centrifuged for 10 minutes. Plasma/urine samples with expected concentrations above the calibration range were diluted with a 0.009% aqueous sodium acetate solution. For calibration, blank plasma and blank urine samples were spiked daily with glufosfamide at concentrations of 0.05, 0.07, 0.1, 0.3, 0.7, 1, 3, 5, 7, and 10 μg/mL for plasma and 0.2, 0.5, 1, 2, 5, 10, 25, 50, 75, and 100 μg/mL for urine. The chromatograms were evaluated with the internal standard method using peak-area ratios for calculation purposes. Calibration curves were evaluated with linear regression weighted by $1/x$, $1/x^2$, $1/x^{1.5}$, or $1/y^2$, depending on the LC-MS/MS system used and the respective software (data on file, Knebel and Winkler, 1999). All calculations were done using the Quan Guide (Thermo Quest Corporation, San Jose, CA) or MacQuan (PE Sciex) calculation program. Quality control samples at three concentrations (0.15, 1, and 9 μg/mL for plasma and 0.6, 10, and 90 μg/mL for urine) or four concentrations (0.05, 0.5, 0.7, and 5 μg/mL for plasma) were analyzed along with every analytic series (batch). If more than two of six quality control samples or all quality control samples at one concentration level differed by more than ± 15% (plasma) and ± 20% (urine) from the theoretical value within a batch, results were rejected and the test samples reanalyzed.

Pharmacokinetic analysis. All pharmacokinetic evaluations were based on the real blood sampling times as documented on the respective case report forms (CRFs). Noncompartmental pharmacokinetic calculations were performed with validated Excel-based software (FUNCALC; ASTA Medica, 1997). Arithmetic mean, coefficient of variation, median, lower quartile (25% quartile), upper quartile (75% quartile), minimum, and maximum, as well as geometric mean with corresponding coefficient of variation$_{ln}$ and 95% confidence interval$_{ln}$, were calculated for elimination half-life, volume of distribution at steady state, volume of distribution during terminal phase, total plasma clearance, renal clearance, and urinary excretion over all dose groups (normalization was to 1.73 m^2 body surface). For the pharmacokinetic parameters area under the time–versus-concentration curve (AUC) and average concentration during infusion from time point 0 hours to 6 hours ($C_{av,0\text{-}6}$), the arithmetic mean was calculated for each dose group. For calculation of arithmetic mean, values below the limit of quantification were set to zero.

Dose linearity of the average concentration from 0 to 6 hours and AUC was investigated for the 800- to 6,000-mg dose range. Calcula-

tions were performed with the individual values of each dose group. Renal clearance was calculated as the ratio of amount excreted in urine to total plasma AUC. As an approximation, the cumulative amount of glufosfamide excreted in urine over a 48-hour period was used for the numerator.

Ethical considerations. Independent ethical committees at the two centers involved approved the study protocol and the patient informed consent form. Informed written consent was obtained from each patient before enrollment. The trial was conducted in accordance with the laws and guidelines current at the time: the national drug laws, the principles of the Declaration of Helsinki, and the European Note for Guidance for Good Clinical Practice. The protocol informed consent, CRFs, study conduct, and study reports were subject to an internal review.

Monitoring. The study was monitored by NDDO Oncology. Investigators entered the information required by the protocol onto CRFs developed by NDDO Oncology for the study. The CRFs were then forwarded to the data management unit of NDDO Oncology. All data from the CRFs were entered into REMOTE ACES database, version 1.0. An independent visual data check was done in order to ensure the quality of data entry. Monitors visited the investigators regularly to verify the CRFs for correctness and completeness, obtaining corrections at the centers where necessary. The following items were regularly checked against source data: all patient eligibility criteria, baseline physical examination results, prior treatment, drug administration, adverse events, laboratory outcomes, efficacy data, and off-study data. Data quality assurance was done by complete data check. On completion of the clean-up procedures, the database was locked.

RESULTS

Table 1 summarizes the patients' characteristics. Twenty-one patients (10 women and 11 men; median age, 56 years) with a variety of refractory or pretreated solid tumors were enrolled onto this study. Two patients were chemotherapy-naïve; the others had received prior chemotherapy. Five dose levels (800, 1,600, 3,200, 6,000, and 4,500 mg/m^2) were explored. Three patients were entered at dose levels I, II, and III, and six patients were entered at dose levels IV and V (Table 2).

All patients received at least one course of treatment with glufosfamide, and a total of 62 courses were administered. The median number of courses given per patient was two (range, one to eight). Courses were repeated every 3 weeks, and only in four cases was treatment delayed for logistic reasons. One patient with a serum bilirubin concentration of 2.2 mg/dL on the day of the first course was considered eligible because at registration, 3 days before treatment, she had a normal bilirubin level. Two patients discontinued the trial because of adverse events.

Safety

The dose-limiting toxicity of glufosfamide administered as a two-step, 6-hour IV infusion in patients with advanced solid malignancies was renal tubular acidosis (Table 3). This consisted of metabolic acidosis, hypokalemia, hypophosphatemia, phosphaturia, renal glucosuria, polyuria, a high urinary beta-2-microglobulin level, and a mild, short-lived increase in serum creatinine. Dose-limiting renal toxicity occurred in two of six patients treated at the highest dose level of 6,000 mg/m^2, which was defined as the MTD for this schedule of glufosfamide. Accrual continued at the 4,500-mg/m^2 dose level, which was well tolerated. At this dose level, only one of six patients developed grade 2 hypokalemia, grade 2 hypophosphatemia, and a mild increase in serum creatinine. This was a bladder cancer patient who had previously been treated with cisplatin.

At the time of study initiation, no CTC grade was available for inorganic phosphorus and metabolic acidosis. These abnormalities were graded by severity, according to the investigator's opinion. For a clearer picture of the intensity of these events, the nadirs of the inorganic phos-

Table 1. Patient Demographics

Patients	
No. entered	21
Male/Female	11/10
Age, years	
Average	56
Range	31-70
Performance status	
Median	1
Range	0-2
Prior therapy	
Radiotherapy only	0
Chemotherapy only	10
Radio- and chemotherapy	9
None	2
Primary tumor sites	
Colorectal	7
Lung	3
Breast	2
Unknown	1
Bladder	1
Hypopharynx	1
Kidney	1
Melanoma	1
Sarcoma	1
Pancreas	1
Stomach	1
Urachus	1

Table 2. Dose Levels and Patient Cohorts

Dose Level	Dose (mg/m^2)	No. of Patients	No. of Courses (N = 62)
I	800	3	7
II	1,600	3	6
III	3,200	3	10
IV	6,000	6	18
V	4,500	6	21

Table 3. Dose-Limiting Toxicities

Dose (mg/m^2)	Total No. of Patients	Worst Grade per Patient														
		Hypophosphatemia					Hypokalemia					Metabolic Acidosis				
		Severity					CTC Grade					Severity				
		1	2	3	4	9*	1	2	3	4	9*	1	2	3	4	9*
800	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1,600	3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3,200	3	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4,500	6	—	1	—	—	—	2	1	—	—	—	—	1	—	—	—
6,000	6	—	2	—	—	—	—	—	2	—	—	1	1	1	—	—

*Grade 9 indicated pre-existing toxicity that worsened at least one grade while on study.

phorus and plasma pH were reassessed according to the revised National Cancer Institute CTC, version 2.0 (Table 4).

The adverse events that occurred at the lower dose levels and hematologic toxicity as summarized in Table 5 were generally mild. A short-lived grade 4 neutropenia was seen in two and a grade 4 leukopenia was seen in one out of six patients treated at 6,000 mg/m^2, but these toxicities were not complicated by infections. Other reported toxicities were mild nausea, vomiting, fatigue, proteinuria, glucosuria, alopecia, and taste disturbances (dysgeusia).

Dose-Limiting Toxicity: Case Illustration

Patient no. 11. Patient no. 11, a 70-year-old woman with breast cancer metastatic to the liver, was treated with glufosfamide 6,000 mg/m^2 as a third-line chemotherapy. During course 2, she developed grade 3 hypokalemia, moderate hypophosphatemia, a grade 1 increase in creatinine, and metabolic acidosis (grade 3 according to National Cancer Institute CTC version 2.0). Hypophosphatemia and hypokalemia became evident on day 8 after the second dose of the drug and worsened 1 week later. The patient was admitted to the hospital with the diagnosis of renal tubular acidosis and was treated with IV supplementation of potassium bicarbonate and phosphate. The study drug was considered to be the most likely cause of this toxic event. Furthermore, the patient developed grade 3 leukopenia and grade 2 neutropenia. The increase in serum creatinine and the metabolic acidosis resolved on days 11 and 12, respectively. Hypokalemia resolved on day 25. Hypophosphatemia improved but never resolved completely. The patient was taken off treatment and died 2 months later of malignant disease.

Patient no. 14. Patient no. 14, a 61-year-old man with pretreated advanced colon cancer, was enrolled at the 6,000-mg/m^2 dose level. He received a total of three courses of glufosfamide and had significant palliation of his symptoms, a decrease in his tumor markers, and significant shrinkage of the tumor mass that did not qualify as an objective response. A week after his third course of treatment with glufosfamide (on day 8), he was admitted to the Department of Nephrology with grade 3 metabolic acidosis, grade 3 hypokalemia, a grade 2 increase in serum creatinine, grade 1 hypophosphatemia, and grade 1 proteinuria. The study drug was considered to be the cause of this toxic event. The patient was placed on IV potassium and phosphate supplementation. While hospitalized he also developed febrile neutropenia that was successfully

Table 4. Inorganic Phosphorus and Plasma pH Graded According to Standard and the New National Cancer Institute CTC, Version 2.0

Dose Level (mg/m^2)	Assessable Patients	Worst Grade per Patient									
		Severity					New CTC				
		1	2	3	4	9*	1	2	3	4	9*
Hypophosphatemia											
800	3	—	—	—	—	—	—	—	—	—	—
1,600	3	—	—	—	—	—	1	—	—	—	—
3,200	3	1	—	—	—	—	—	2	—	—	—
4,500	6	—	1	—	—	—	—	1	1	—	1†
6,000	6	—	2	—	—	—	—	—	1	1	—
pH											
4,500	3	—	1	—	—	—	—	—	1	—	—
6,000	3	1	1	1	—	—	1	—	2	—	—

*Grade 9 indicates pre-existing toxicity that worsened at least one grade while on study.

†Grade 2 hypophosphatemia at baseline, which worsened to an actual CTC version 2.0 grade 3.

Table 5. Hematologic Toxicity

Dose (mg/m^2)	Total No. of Patients	Worst CTC Grade per Patient														
		Anemia					Leukopenia					Neutropenia				
		1	2	3	4	9*	1	2	3	4	9*	1	2	3	4	9*
800	3	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—
1,600	3	—	1	—	—	—	1	—	—	—	—	—	—	—	—	—
3,200	3	2	1	—	—	—	1	—	—	—	—	—	—	—	—	—
4,500	6	—	1	—	—	3	1	1	2	—	—	2	1	1	—	—
6,000	6	1	—	—	—	3	1	1	2	1	—	—	1	1	2	—

*Grade 9 indicates pre-existing toxicity that worsened at least one grade while on study.

treated with antibiotics. The neutropenia resolved after 2 days, and the fever lasted only 6 hours. The hypokalemia and increased serum creatinine lasted for 2 weeks and resolved on day 22. The proteinuria and hypophosphatemia were resolved 2 months later. The patient died 8 months later of tumor progression.

Efficacy

All included patients had assessable or measurable disease at the start of treatment, but one patient was not considered assessable for response because she had received only one course of treatment. Eight patients had progressive disease and were taken off study after two courses of treatment. Ten patients had stable disease as best overall response, but in two refractory colon carcinoma cases, minor tumor shrinkage on a computed tomography scan and a fall in tumor markers were noticed. Objective responses were seen in two cases: one heavily pretreated breast cancer patient had a partial response after course 4 that failed to be confirmed after course 6, and one chemotherapy-naïve pancreatic cancer patient had a complete tumor response. The complete responder was a 70-year-old man who was treated at the 4,500-mg/m^2 dose level. He tolerated treatment very well and received uneventfully a total of eight courses. The target lesion in this patient was the tumor mass at the pancreas (baseline measurement, 6.5 × 4.7 cm). A partial response was documented after course 2 and confirmed after courses 4 and 6. The tumor disappeared completely after course 8, and a computed tomography scan 4 weeks later confirmed this complete response. Biopsy tissue from this patient was reviewed by external pathologists at the request of ASTA Medica, and the diagnosis was confirmed. The patient has remained in complete remission for 24+ months. The carcinoembryonic antigen tumormarker had been normalized during course 3, and the CA 19-9 marker was normalized during course 5 (Figs 2 and 3).

Pharmacokinetics

Bioanalytics performed reliably, with coefficients of determination (r^2) of the calibration lines ranging from 0.993 to 0.999 for all three analytic methods used. The individual

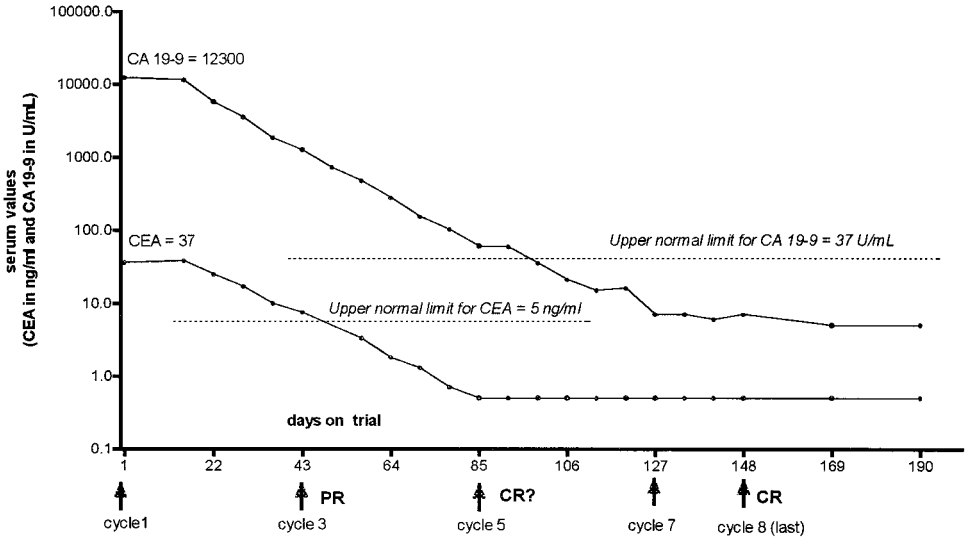

Fig 2. Serial measurements of serum tumor markers in the responder patient with pancreatic cancer. This patient received a total of eight cycles at 4,500 mg/m^2 with no significant toxicity.

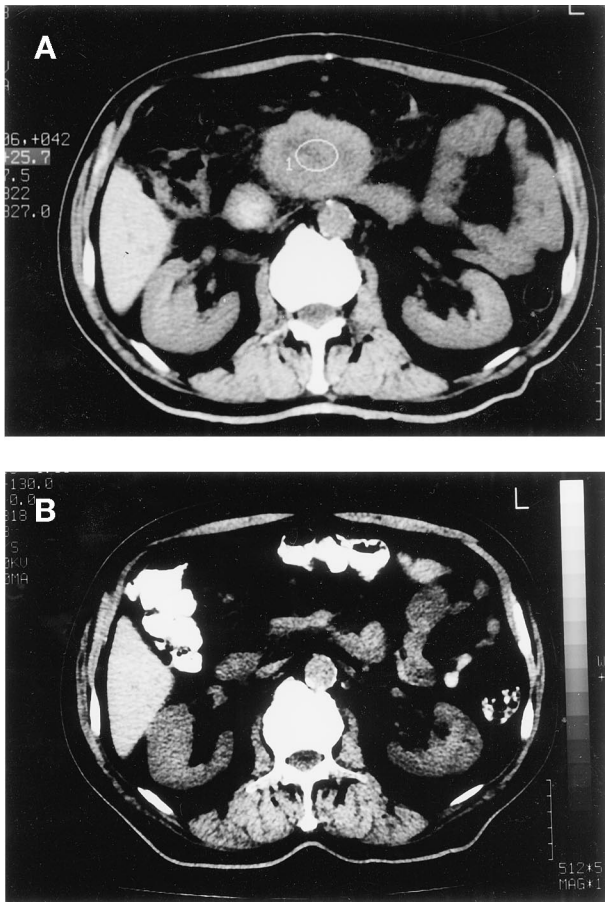

Fig 3. Computed tomography scans of a pancreatic cancer patient (A) before treatment and (B) in complete remission 20 months later.

plasma concentration–time courses of glufosfamide from 18 patients in five different dose groups (800, 1,600, 3,200, 4,500, and 6,000 mg/m^2) were combined. The mean concentration-time profiles are shown in Fig 4.

The relationship between dose and AUC was linear over the dose range studied (Fig 5). The dose-independent pharmacokinetic parameters are summarized in Table 6. The median terminal plasma half-life was 2.3 hours, and the amount excreted intact in the urine amounted to 34.2%. Pharmacokinetic data for the two patients who developed renal toxicity did not differ essentially from the pharmacokinetic data of the others who were treated at the same dose level, although the AUCs obtained by these patients were clearly on the high side. (Table 7).

DISCUSSION

Enhanced antitumor selectivity constitutes a priority in the development of new anticancer agents. Chemical modification of established antitumor drugs may be one way to optimize their pharmacology and possibly improve anticancer selectivity. Glufosfamide is a new alkylating cytotoxic agent developed from ifosfamide that has the potential to target tumor cells by functioning as a substrate for the plasma membrane glucose transport system. Rapidly proliferating and energy-consuming cancer cells are known to overexpress certain glucose transport proteins.[7,8] Initial work done by Pohl et al[1] suggested an active transmembrane transport mechanism for glufosfamide, and recently, Veyhl et al[2] identified a low-affinity sodium/glucose cotransporter that translocates this compound actively across the plasma membrane. This is the SAAT1 molecule, a member of the Na+/glucose gene family of cotransporters that was initially isolated from a pig renal cell line but was also found expressed in various human carcinomas and tumor cell lines.[2,9,10] According to Veyhl et al, uptake of glufosfamide by SAAT1 was phlorizin-inhibitable, stereospecific, and substrate-dependent with a higher affinity and a smaller maximal velocity in comparison to D-glucose. In the same work, the high-affinity SGLT1 transporter was not found to participate in the mechanism of cellular uptake of glufosfamide; the facilitative glucose transporters were not investigated.

Two major observations came out of this study: glufosfamide administered by 6-hour infusion in humans can cause dose-limiting renal tubular toxicity and can produce interesting antitumor activity in refractory tumors. Oxazophosphorine cytotoxic drugs are DNA-alkylating agents that need cytochrome P450 activation in the liver to become therapeutically active. Ifosfamide, a widely used oxazophosphorine, is a potentially nephrotoxic and urotoxic drug. Renal tubules and the bladder constitute primary targets for potentially toxic ifosfamide metabolites undergoing renal excretion, such as chloracetaldehyde, a major metabolite of side-chain oxidation, and acrolein, a metabolite of the oxazophosphorine ring.[11,12] In glufosfamide, the therapeutically active isophosphoramide mustard is coupled to D-glucose via a beta-glycosidic linkage, constituting a hydrophilic conjugate with the potential to release the active drug intracellularly by hydrolysis. Therefore, on theoretical grounds, this drug does not require metabolic activation, and in animal studies it was only found to form metabolites to a limited extent when given intravenously.[5] In our study, hemorrhagic cystitis was not recorded and myelotoxicity was mild at clinically effective dose levels. Nevertheless, renal tubular toxicity was the dose-limiting event for glufosfamide given on this schedule. The observed nephrotoxicity resembled more or less the extensively studied but incompletely understood renal toxicity of ifosfamide, for which proximal tubular damage has been characterized as a primary toxic event.[12,13] One can only hypothesize that

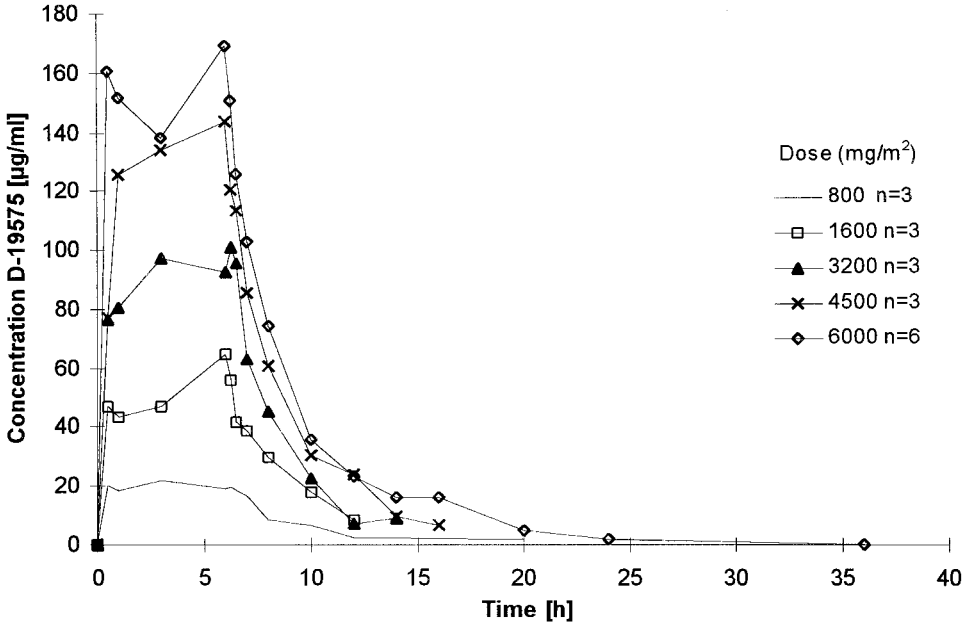

Fig 4. Time course of the arithmetic mean plasma concentrations after administration of different doses of glufosfamide.

tubular damage by both drugs might be related to a common metabolite or to the highly reactive isophosphoramide mustard itself. The first hypothesis is supported by the low urine excretion of intact drug in this study, which suggests a substantial metabolism of glufosfamide in humans. Regarding a possible direct involvement of isophosphoramide mustard in tubular damage, this may have been facilitated through active uptake of glufosfamide by the sodium/glucose cotransporters that are activated at the cell membranes of the proximal tubule for reabsorption of filtered glucose.[14,15]

In this study, our aim was to expose tumor cells over a prolonged time to this rapidly eliminated agent to better exploit the mechanism of the cellular uptake of glufosfamide, given that transportation through the glucose transporters shows saturable substrate dependence.[2] Thus, a biphasic, fast/slow infusion was selected in order to achieve steady-state plasma levels of the drug rapidly and sustain

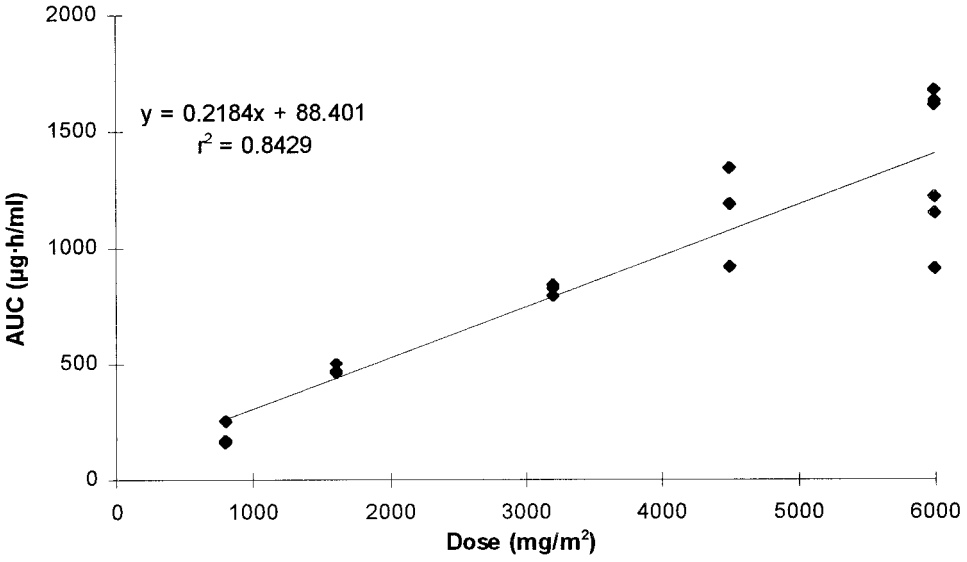

Fig 5. Dose linearity AUC after 6-hour infusion of different doses of glufosfamide (800, 1,600, 3,200, 4,500, and 6,000 mg/m^2).

Table 6. Summarized Pharmacokinetic Parameters Over the Whole Dose Range (800 to 6,000 mg/m²)

	$t_{1/2}$ (hour) (n = 18)	$V_{z,norm}$ (1.73 m^2) (L) (n = 18)	$V_{ss,norm}$ (1.73 m^2) (L) (n = 18)	Total Plasma CL_{norm} (1.73 m^2) (mL/min) (n = 18)	$CL_{ren,norm}$ (1.73 m^2) (mL/min) (n = 9)	Urinary Excretion (%) (n = 9)
Median	2.29	20.87	35.96	109.85	41.39	25.62
Minimum	1.18	11.86	30.90	92.18	20.31	20.62
Maximum	14.60	183.88	83.86	190.85	122.03	67.95
$Mean_{geo}$	2.65	26.88	39.59	117.41	41.76	34.20
95% CI_{ln}	1.95-3.59	19.70-36.67	34.96-44.84	106.11-129.91	26.41-66.02	22.93-51.01

Abbreviations: $t_{1/2}$, elimination half-life; $V_{z,norm}$, volume of distribution during terminal phase; $V_{ss,norm}$, volume of distribution at steady state; CL_{norm}, clearance; $CL_{ren,norm}$, renal clearance; $mean_{geo}$, geometric mean; 95% CI_{ln}, 95% confidence interval, logarithm natural.

them over a relatively prolonged period. Pharmacokinetic analysis showed that the pharmacokinetic simulation was successful. Obtained data confirmed the accuracy of the computer simulation in that the two-step infusion schedule produced the desired profile of rapidly achieved and sustained plasma concentrations for more than 6 hours. Renal excretion of the intact drug was moderate, although data from rodents showed primarily renal excretion of unchanged drug when administered as a single agent.[5] In our study, renal excretion was half of that observed in rats after single bolus administration, indicating a possible difference in metabolism between species or influence of duration of administration on metabolic rate.

Although by design this study was not directed to allow estimation of efficacy, there was strong evidence of antitumor activity in refractory solid tumors. Accelerated rates of glucose transport and increased glucolysis are characteristic features of malignant transformed cells, mediated by overexpression of glucose transporters.[16,17] Of the two major families of transmembrane glucose transporters,[18] ie, the facilitative glucose transporters (GLUT1-5) and the sodium-dependent glucose transporters (SGLT1-2), cancer research has focused mostly on the facilitative glucose transporters.[19] Three types of the family of facilitative glucose transporters (GLUT1, GLUT2, and GLUT3) have been found to be elevated in most cancer tissues studied.[8] Pancreatic carcinoma was theoretically a particularly attractive candidate for glufosfamide therapy because of intense GLUT1 expression,[20] and this was evidenced by an impressive, longstanding complete response in an advanced pancreas carcinoma case that occurred at a subtoxic dosage. Indications of antitumor activity were also seen in two metastatic colon cancers and one breast carcinoma, all heavily pretreated. These tumor types have also been shown to overexpress transmembrane glucose transporters.[21-23]

In conclusion, the principal and dose-limiting toxicity of glufosfamide given as a biphasic, rapid/slow, 6-hour infusion every 3 weeks was reversible renal tubular acidosis. The MTD with this schedule was 6,000 mg/m², and the recommended phase II dose was 4,500 mg/m². Myelotoxicity was seen but was generally mild. Glufosfamide caused renal toxicity, consisting of metabolic acidosis, a transient increase in serum creatinine, hypophosphatemia, hypokalemia, hyperphosphaturia, glucosuria, and an increase in urine beta-2-microglobulin, and this was dose-dependent. The remarkable antitumor activity seen in chemotherapy-resistant carcinomas in this trial warrants further clinical exploration of glufosfamide, despite the renal toxicity. Phase II studies should be conducted under close monitoring of renal function. Checks on serum potassium, creatinine, and, if indicated, blood pH, 8 to 15 days after treatment are strongly advised in patients receiving glufosfamide for early detection of evolving renal tubular damage.

Table 7. Individual Plasma Pharmacokinetic Data From Patients Treated at the MTD

	Individual PK Data of Glufosfamide at 6,000 mg/m²				
Patient No.	$t_{1/2}$ (hour)	$AUC_{(0-t)}$ ($\mu g \cdot h/mL$)	AUC ($\mu g \cdot h/mL$)	Cl_{norm} (1.73 m^2) (mL/min)	$C_{av,0-6}$ ($\mu g/mL$)
10	1.40	1,052.6	1,058.0	163.5	126.6
11*	3.68	1,605.7	1,608.1	107.6	133.9
12	1.82	1,401.6	1,433.0	120.7	164.1
13	3.78	1,663.4	1,664.2	104.0	169.9
14*	3.77	1,621.1	1,621.8	106.7	178.5
15	1.60	1,207.3	1,215.1	142.4	158.4

Abbreviations: PK, pharmacokinetic; $C_{av,0-6}$, average concentration during infusion from time point 0 hours to 6 hours.
*Patients no. 11 and 14 had renal toxicity.

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