Cancer Chemother Pharmacol (2002) 49: 425–428
DOI 10.1007/s00280-002-0429-3

CLINICAL TRIAL REPORT

**Jacqueline Ming Liu · Li Tzong Chen · Yee Chao
Anna FY Li · Chew Wen Wu · Tai-Shun Liu
Her Shiong Shiah · Jang Yang Chang · Jong Dong Chen
Hsiao Wei Wu · Wei Chun Lin · Chieh Lan
Jacqueline Whang-Peng**

Phase II and pharmacokinetic study of GL331 in previously treated Chinese gastric cancer patients

Received: 10 October 2001 / Accepted: 9 January 2002 / Published online: 8 March 2002

Abstract *Purpose:* A phase II and pharmacokinetic study was designed to assess the efficacy and toxicity profile of an epidophyllotoxin analogue, GL331, in previously treated Chinese gastric cancer patients, with concurrent pharmacokinetic evaluation of the drug's metabolism. *Material and methods:* GL331 was given at 200 mg/m^2 as a daily 3-h infusion for 5 days every 4 weeks. *Results:* Enrolled in the study were 15 patients. One patient died from neutropenic sepsis before evaluation, one patient did not receive the full dose for reasons unrelated to GL331, nine patients had progressive disease with a median survival of 80 days, and five had stable disease with a median survival of 240 days. Grade 3 and 4 myelosuppression occurred in 10 of the 15 patients, with one death from neutropenic sepsis. This patient's peak GL331 concentration was 16.8 μg/ml, which was high compared to the mean peak drug concentration of 6 ± 4.1 μg/ml. The mean systemic GL331 clearance was 12.1 ± 7.2 l/h per m^2, much lower than 23.3 ± 8.2 l/h per m^2 found in the phase I trial. Topoisomerase IIα was determined by immunohistochemistry and overexpression was detected in 3 of 11 specimens. *Conclusions:* GL331 was ineffective at this dose and schedule in this group of patients in spite of adequate blood levels of the drug.

Keywords GL331 · Chinese · Refractory · Gastric cancer · Topoisomerase IIα

J.M. Liu · L.T. Chen · H.S. Shiah · J.Y. Chang · H.W. Wu
W.C. Lin · C. Lan · J. Whang-Peng (✉)
Division of Cancer Research,
National Health Research Institutes,
Veterans General Hospital-Taipei,
Shipai Road, Taipei, Taiwan
E-mail: jmliu@vghtpe.gov.tw
Tel.: +886-2-28757224
Fax: +886-2-28716467

Y. Chao
Cancer Center,
Veterans General Hospital-Taipei, Taipei, Taiwan

A.F. Li
Pathology Department,
Veterans General Hospital-Taipei, Taipei, Taiwan

C.W. Wu
Surgery Department,
Veterans General Hospital-Taipei, Taipei, Taiwan

T.-S. Liu
Genelabs Biotechnology, HsinChu, Taiwan

J.D. Chen
Division of Biotechnology and Pharmaceutical Research,
National Health Research Institutes,
Veterans General Hospital-Taipei,
Shipai Road, Taipei, Taiwan

Introduction

Gastric cancer ranks as the sixth most common cancer in Taiwan with an incidence of 18.7/100,000, and 3093 new cases in 1996 were reported [6]. These figures are high compared to the USA incidence rate of 6.7/1000,000 in the same year [15]. The high local occurrence of gastric cancer has been attributed mainly to cigarette smoking [12]. The overall 5-year survival for gastric cancer is only 5–15% because many patients present at a late stage [2]. Therefore it is important to continually explore new improved therapeutic agents for this cancer.

The most commonly administered single agent, 5-fluorouracil (5-FU), has been reported to have a 21% overall response rate. Single-agent etoposide has resulted in response rates ranging from 10% to 20% [18]. Chemotherapy regimens utilized for gastric cancer often include the following combinations: 5-FU/high-dose methotrexate/doxorubicin, etoposide/doxorubicin/cisplatin, etoposide/5-FU/leucovorin, epirubicin/cisplatin/5-FU. Response rates with these regimens range from 25% to 40%, with a median survival of 6–8 months in patients with metastatic disease [11]. In Japan, 5-FU- and cisplatin-based combination chemotherapy is preferred over regimens which incorporate an anthracycline [17]. In Taiwan, a regimen based on weekly 24-h 5-FU infusion is usually given up-front with responses ranging

from 40% to 80% and median survival of 8–11 months [4, 5, 13]. Administration of 5-FU by infusion is preferred since thymidylate synthase inhibition by 5-FU is significantly greater in patients given 5-FU by continuous infusion than by bolus injection [19].

With infusion 5-FU as a backbone, the challenge lies in the selection of efficacious agents to be given in combination. Cisplatin [16], the taxanes [11], and camptothecins [3, 11] have all shown promise. New developments in topoisomerase inhibitors include oral etoposide [1], but a more efficacious epidophyllotoxin would be better. GL331 is a new etoposide analogue whose principle mechanism of action is through inhibition of topoisomerase IIα (T2α) [8]. Overexpression of T2α is usually predictive of etoposide responsiveness [20]. GL331 has the added advantage over etoposide of having a higher affinity for transport into the cancer cell, and thus the potential to circumvent drug resistance attributed to *mdr*1 gene overexpression [9, 10]. A phase II and pharmacokinetic (PK) study was initiated to ascertain the efficacy and toxicity of GL331 in previously treated Chinese patients, with concomitant determination of the initial tumor T2α content.

Patients and methods

This study was approved by the Taipei Veterans' General Hospital Ethics Committee and the Department of Health of the Republic of China.

Patients

All patients provided informed consent to the protocol treatment. Eligibility criteria included: proof of gastric cancer pathology; at least one bidimensionally measurable lesion; progression after first-line chemotherapy with a minimum 4-week interval from any prior treatment; Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2 [14]; normal liver function (bilirubin ≤ 1.5 mg/dl) and renal function (creatinine ≤ 2 mg/dl); adequate absolute neutrophil count (ANC) ($\geq 1500/mm^3$) and platelet count ($> 100{,}000/mm^3$). Assessment of response was according to ECOG criteria, and evaluation of toxicity was according to the common toxicity criteria [14].

Follow-up studies

Full blood counts were obtained weekly during the first course to monitor nadir white cell count and ANC, and blood chemistry study was done prior to each course of GL331. Tumor marker studies, coagulation profiles, and imaging studies for measurement of tumor status were performed every 3–4 weeks during the first two courses in order to detect any rapidly regressive or progressive disease, and then after every two courses.

Drug administration

GL331 has a molecular formula of $C_{27}H_{24}N_2O_9$. It is formulated in a vehicle consisting of 8% Tween-80 dissolved in a mixture of 62% polyethylene glycol 300 and 30% absolute ethanol [8, 10]. The maximum tolerated dose determined in the phase I trial was 300 mg/m^2 as a 3-h infusion for five consecutive days every 3–4 weeks [8]. Our starting dose was therefore set at 200 mg/m^2 given in 2000 ml normal saline (using non-PVC containers and tubing) in the same schedule, and if tolerable, with rapid escalation in 20% fractions every subsequent cycle. Premedication was not advised unless the patient vomited, and subsequent prescription of oral metoclopramide was considered sufficient. A 20% dose reduction would be instituted for a nadir ANC $\leq 500/mm^3$ or platelet count $\leq 50{,}000/mm^3$, and a 20% dose escalation would be instituted if the nadir ANC was $\geq 1000/mm^3$ and platelet count was $\geq 100{,}000/mm^3$. Treatment was to be stopped if progressive disease occurred, but continued for stable or responsive disease. Overall survival was counted from the day of initiation of GL331, and progression-free survival was counted from the day of treatment until disease progression. Survival was analyzed according to the Kaplan-Meier method.

Pharmacokinetics

A PK study was performed during the first course of treatment. High-performance liquid chromatography (HPLC) was performed using a Hewlett Packard 1100 system with elution peaks at 394 nm. Blood samples were drawn via an indwelling venous catheter, and 3-ml aliquots were spun down at 800 rpm for 4 min and the plasma stored at –80°C until analysis. The zero time-point was completion of the 3-h GL331 infusion on the 5th day of treatment. Blood samples for the PK study were taken at –48, –24, 0, 1, 2, 3, 4, 6, 8, 12, 48, 60, 84 and 96 h, a total of 16 sampling points. Systemic clearance, volume of distribution, and parent drug elimination half life were evaluated using WinNonlin software based on a two-compartment model.

Immunohistochemistry

Immunohistochemical evaluation of 11 available specimens was carried out using a T2α antibody (gift from Dr J.L. Huang, Academia Sinica, Taiwan). Briefly, 4–5 μm thick paraffin sections were cut, fixed, deparaffinized and stained with the primary T2α antibody. A LSAB-2 kit (DAKO, Carpinteria, California) was used for detection. Overexpression was considered to be present if the nucleus of the cancer cells showed red staining.

Statistics

Statistical analysis was based on a projected response rate of 20%. Accepting a 4% rejection error (α), a minimum of 14 patients would be needed for the study. Thus, for a true 20% response rate, the chance of observing 14 consecutive failures would be less than 5% [7].

Results

A total of 15 previously treated gastric cancer patients were enrolled in this study (Table 1). All had adenocarcinoma of the stomach. Pathology was diagnosed by endoscopic biopsy in 2 patients, and 13 others underwent laparotomy. One patient had inoperable disease and all the others received partial or total gastric resection.

The first patient developed renal insufficiency with decreased urine output on the 3rd day of treatment for reasons unrelated to GL331. GL331 was stopped in this patient but he was still evaluated for response and toxicity on an intention to treat basis. A total of 35 courses were delivered, with one to six courses per patient Ten patients completed at least two courses, four patients

Table 1. Patient demographics ($n = 15$)

Age (years)	
Mean	60.5
Range	38–77
Male/female	12/3 (80%/20%)
Performance status (ECOG)	
1	3 (20%)
2	12 (80%)
Local recurrence	2 (13.3%)
Metastatic site	
Lung	4 (26.6%)
Bone	2 (13.3%)
Skin	1 (6.7%)
Liver	6 (40%)
Omentum	4 (26.6%)
Retroperitoneum/nodes	5 (33.3%)
Other	5 (33.3%)
Previous therapy	
Surgery (laparotomy)	12 (80%)
Chemotherapy	15 (100%)
Radiotherapy	5 (33.3%)

received only one cycle, and patient L01 received only 40% of the first cycle. All patients received 200 mg/m^2 as a starting dose. In subsequent cycles, four patients required a dose reduction to 160 mg/m^2, two patients were escalated to 240 mg/m^2, and four continued at the same dose.

Response

There were no objective responses. Nine patients had progressive disease, and five had stable disease, including patient L01 who died from progressive disease 31 days after starting GL331. Patient L07 did not reach the evaluation time. The median survival for all patients was 90 days, for those with stable disease 240 days, and for those with progressive disease only 80 days.

Toxicity

Toxicities were moderate (see Table 2). Ten patients had grade 3 or 4 neutropenia, with three developing neutropenic fever requiring hospitalization, and one dying of septic shock. Nine patients had grade 3 or 4 anemia, often exacerbated by chronic bleeding from the remnant/recurrent gastric cancer. Nausea and vomiting was mild. Mucositis was uncommon. Many patients complained of asthenia. Three patients lost over 10% of their on-study weight. Anorexia was noted starting on day 3 or 4 of drug administration.

Pharmacokinetics

PK study was performed in patients L02–L15. Patient L07 had grade 4 leukopenia and thrombocytopenia, and a very high peak GL331 blood level of 16.8 μg/ml. Five other patients experiencing grade 4 neutropenia had peak levels of 1.6, 5.2, 4.8, 8.6 and 9 μg/ml. The mean peak parent drug concentration was 6 ± 4.1 μg/ml, the mean systemic clearance was 12.1 ± 7.2 l/h per m^2, the volume of distribution was 66.9 l/m^2, and the median elimination half-life (t1/2β) of the parent drug was 7.3 h (2.2–13.9 h). In the initial phase I study, in which GL331 was given at 162–375 mg/m^2 per day over 2–3 h daily for five consecutive days, the mean peak parent drug concentration was 4.7 ± 1.8 μg/ml, the mean systemic clearance was 23.3 ± 8.2 l/h per m^2, the volume of distribution was 57.8 l/m^2, and the median elimination half-life of the parent drug was 1.75 h (1.6–16.9 h) [8].

Overexpression of T2α was detected in 3 of 11 specimens. The mean survival of these patients was 763 days, whereas it was only 523 days in those without T2α overexpression.

Table 2. Toxicity profile according to the common toxicity criteria for 15 patients (values are percent of patients)

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Leucopenia	13.3	6.7	13.3	26.7	40
Anemia	6.7	0	33.3	53.3	6.7
Anorexia	26.7	20	40	13.3	0
Asthenia	53.3	26.7	13.3	0	6.7
Nausea	53.3	26.7	20	0	0
Vomiting	80	6.7	13.3	0	0
Thrombocytopenia	60	26.7	0	6.7	6.7
Stomatitis	67.7	20	13.3	0	0
Fever	80	0	0	13.3	6.7
Infection	80	0	0	13.3	6.7

Discussion

GL331 given at a dose of 200 mg/m^2 as a short daily infusion for five consecutive days every 3 weeks in previously treated gastric cancer patients resulted in no objective responses. In addition, ten patients experienced grade 3 or 4 neutropenia, with one death from septic shock. After patient L07 had died, close questioning of his children revealed that he had been taking Chinese herbal medication in spite of adamant denials previously, so the high serum GL331 level of 16.8 μg/ml may have been a consequence of uncertain drug interactions, or of a direct contribution to the concentration of GL331 drug from herbal components, since it was much higher than the peak GL331 concentration of 4.7 ± 1.8 μg/ml found in the phase I study. Of the minor toxicities, asthenia was not serious but was a frequent complaint, and adversely affected quality of life.

Comparison of the PK data from the phase I study and the present study revealed that Chinese patients clear GL331 more slowly than Caucasian patients (12.1 ± 7.2 vs 23.3 ± 8.2 l/h per m^2) [8], so that the t1/2 of the parent drug was 7.3 h in this study, but only 1.75 h in the initial study [8]. However, the HPLC analyses for the two trials were performed in different laboratories. Further PK studies may be needed to confirm this apparent ethnic difference in drug metabolism, and also to

ascertain whether this can be extrapolated to other epidophyllotoxins.

Even though GL331 was administered at an adequate dose to our patients as determined from the PK study, it caused major toxicity and resulted in no objective responses. It was therefore pertinent to ascertain the presence of the main drug target, T2α. T2α was overexpressed in 3 of 11 specimens. Two of these patients had stable disease and one progressed after two cycles of treatment. These three patients enjoyed a longer survival than patients without T2α overexpression, but the numbers were too small to draw a meaningful conclusion. The drawback of the specimen sampling is that the assay was performed on the initial tumor since sampling tumor after relapse was technically challenging.

GL331 was ineffective at this dose and in this schedule in refractory gastric cancer patients in spite of attaining adequate drug levels. The PK data from this group of Chinese patients hint at ethnic differences in the metabolism of epidophyllotoxins which require further studies for confirmation.

Acknowledgements This study was supported by grants from the National Health Research Institutes of Taiwan and the Chen Shuyi Cancer Foundation.

References

1. Ajani JA, Takiuchi H (1999) Recent developments in oral chemotherapy options for gastric carcinoma. Drugs 58 [Suppl 3]:85
2. Alexander HR, Kelsen DG, Tepper JC (1997) Cancer of the stomach. In: DeVita VT Jr, Hellman S, Rosenberg SA (eds) Cancer: principles and practice of oncology, 5th edn. Lippincott, Philadelphia, p 1021
3. Bleiberg H (1999) CPT-11 in gastrointestinal cancer. Eur J Cancer 35:371
4. Cheng AL, Yeh KH, Lin JT, Hsu C, Liu MY (1998) Cisplatin, etoposide and weekly high dose 5-FU and leucovorin infusion (PE-HDFL) – a very effective regimen with good patient compliance for advanced gastric cancer. Anticancer Res 18(2B):1267
5. Chi KH, Chao Y, Chan WK, Lo SS, Chen SY, Yen SH, Chen KY, Wu CW, Lee SD, Lui WY (1998) Weekly etoposide, epirubicin, cisplatin, 5-FU and leucovorin: an effective chemotherapy in advanced gastric cancer. Br J Cancer 77:1984
6. Department of Health of the Republic of China (1996) Health statistics. http://www.doh.gov.tw/lane/statist/85/890301-l.htm
7. Gehan EA (1961) The determination of the number of patients required in a preliminary and follow up trial of a new chemotherapeutic agent. J Chron Dis 13:346
8. Genelabs Technologies (1997) GL331 clinical study. GL93-01 final report. Genelabs Technologies, Redwood City
9. Huang TS, Shu CH, Shih YL, Huang HC, Su YC, Chao Y, Yang WK, Whang-Peng J (1996) Protein tyrosine phosphatase activities are involved in apoptotic cancer cell death induced by GL331, a new homolog of etoposide. Cancer Lett 110:77
10. Huang TS, Lee CC, Chao Y, Shu CH, Chen LT, Chen LL, Chen MH, Yuan CC, Whang-Peng J (1999) A novel podophyllotoxin-derived compound GL331 is more potent than its congener VP-16 in killing refractory cancer cells. Pharm Res 16:997
11. Kohne CH, Wils JA, Wilke HJ (2000) Developments in the treatment of gastric cancer in Europe. Oncology 14 [Suppl 14]:22
12. Lee HH, Wu HY, Chuang YC, Chang AS, Chao HH, Chen KY, Chen HK, Lai GM, Huang HH, Chen CJ (1990) Epidemiologic characteristics and multiple risk factors of stomach cancer in Taiwan. Anticancer Res 10:875
13. Lin YC, Liu HE, Wang CH, Wang HM, Yang TS, Liau CT, Chen JS (1999) Clinical benefit and response in patients with gastric cancer to weekly 24 hour infusion of high dose 5-fluorouracil (5-FU) and leucovorin. Anticancer Res 19(6C):5615
14. Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP (1982) Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649
15. Ries LAG, Eisner MP, Kosary CL, Hankey BF, Miller BA, Clegg L, Edwards BK (eds) (2000) SEER cancer statistics review, 1973–1997. National Cancer Institute, Bethesda. http://seer.cancer.gov/Publications/CSR1973_1997/
16. Roth A, Kolaric K, Zupanc D, Oresic V, Ebling Z (1999) High doses of 5-FU and epirubicin with or without cisplatin in advanced gastric cancer: a randomized study. Tumori 85:234
17. Sasaki T, Maeda Y, Kobayashi T, Okamoto R, Omuro Y, Takada N (2000) Standard chemotherapy for gastrointestinal malignancies based on evidence. Gan To Kagaku Ryoho 27:166
18. Schipper DL, Wagener DJ (1996) Chemotherapy of gastric cancer. Anticancer Drugs 7:137
19. Tsujinaka T, Kido Y, Shiozaki H, Iijima S, Homma T, Sakaue M, Mori T (1992) Schedule dependent inhibition of thymidylate synthase by 5-fluorouracil in gastric cancer. Cancer 70:2761
20. Zhou Z, Zwelling LA, Kawakami Y, An T, Kobayashi K, Herzog C, Kleinerman ES (1999) Adenovirus mediated human topoisomerase II alpha gene transfer increases the sensitivity of etoposide resistant human breast cancer cells. Cancer Res 59:4618