Cancer Chemother Pharmacol (2006) 58: 136–142
DOI 10.1007/s00280-005-0129-x

CLINICAL TRIAL REPORT

Muhammad Wasif Saif · Reid von Borstel

5-Fluorouracil dose escalation enabled with PN401 (triacetyluridine): toxicity reduction and increased antitumor activity in mice

Received: 3 August 2005 / Accepted: 2 September 2005 / Published online: 27 September 2005

Abstract *Purpose*: PN401, an oral prodrug of uridine yields more bioavailable uridine than oral administration of uridine itself. PN401 may therefore be useful for permitting dose escalation of 5-fluorouracil (5-FU) with consequent improvements in antitumor efficacy. *Experimental design*: Female BALB/c mice (Colon 26 adenocarcinoma) were treated with 5-FU with PN401 to define the MTD, and pharmacokinetic analyses were done. A comparison of 5-FU/PN401 was made to 5-FU/eniluracil (EU) and 5-FU/LV. The best timing of the first dose of PN401 relative to 5-FU was evaluated by administering groups of mice PN401 beginning 2, 24, or 48 h after 5-FU dose. *Results*: The MTD of 5-FU was 100 mg/kg/week whereas the MTD of 5-FU + PN401 was 200 mg/kg/week. A complete response (CR) of 80% and partial response (PR) of 20% was observed with 5-FU (200 mg/kg) + PN401, CR of 40% and PR of 60% with 5-FU (175 mg/kg) + PN401, PR of 10% with 5-FU (150 mg/kg) + PN401 while no response with 5-FU (100 mg/kg) + PN401. Analysis of 5-FU pharmacokinetics displayed nonlinearity as a function of administered dose in mice. In the comparison study, the best response was achieved with PN401 when compared to EU and LV. Mice that did not receive PN401 died by day 12, while other groups were alive at day 31. The proportion of mice surviving was highest in the group which received PN401 at 2 h followed by 24 and 48 h. *Conclusions*: There is a threshold 5-FU dose after which the efficacy is dramatically improved—in mice bearing Colon 26 adenocarcinoma, that threshold is a dose of >150 mg/kg/week, and the increased efficacy correlates with about a fourfold increase in the AUC of 5-FU. PN401 used to rescue mice from the lethal toxicity of 5-FU entails that PN401 can be used as an antidote even when used up to 48 h after a 5-FU overdose.

Key words 5-Fluorouracil · PN401 · Fluoropyrimidines · Uridine · DPD

M. W. Saif (✉)
University of Alabama at Birmingham (U.A.B.),
Birmingham, AL, USA
E-mail: wasifyale@yahoo.com
Tel.: +1-203-7371875
Fax: +1-203-7375698

R. von Borstel
Wellstat Therapeutics Corporation,
Gaithersburg, MD, USA

M. W. Saif
Section of Medical Oncology, Yale University School of Medicine,
333 Cedar Street FMP; Room: 116,
06520-8032 New Haven, CT, USA

Introduction

Fluorouracil (5-FU) is one of the most commonly used chemotherapeutic agents and constitutes the mainstay of chemotherapy for most gastrointestinal tumors as well as others [1]. The cytotoxicity of 5-FU involves (1) inhibition of thymidylate synthase, principally via the actions of its metabolite, fluorodeoxyuridine monophosphate (FdUMP) and (2) synthesis of defective RNA as a result of incorporation of a second metabolite, fluorouridine triphosphate (FUTP), into RNA [2]. The principal toxicities of 5-FU include neutropenia, mucositis, diarrhea, and hand–foot syndrome, with the latter two adverse effects predominating when 5-FU is administered as a continuous intravenous (IV) infusion [3]. Like other conventional cytotoxic antineoplastic agents, 5-FU has a relatively narrow therapeutic index, because toxicity often limits the dose of 5-FU that can be administered, limiting its overall therapeutic usefulness.

Because of the association between the incorporation of 5-FU into RNA and dose-limiting toxicities, uridine has previously been examined for potential reduction of host toxicity. Uridine, a naturally occurring pyrimidine nucleoside, augments cellular UTP pools and competes with FUTP for incorporation into the RNA of hematopoietic progenitor and gastrointestinal mucosal cells, thereby attenuating 5-FU toxicity in these normal tissues

[4–8]. In mouse models, administration of uridine following 5-FU selectively reduces toxicity to normal tissues, permitting substantial 5-FU dose escalation and increasing the overall efficacy of 5-FU [4–8]. Preclinical and clinical studies have revealed that sustained uridine concentrations of at least 50 μmol/l are required to confer protection to normal tissues from the toxic effects of 5-FU [6]. Differences in uptake and utilization of uridine between tumor and normal tissues underlie uridine's ability to reduce the toxicities of 5-FU without proportionally reducing antitumor activity [3]. Both hematopoietic and gastrointestinal mucosal progenitors efficiently incorporate exogenous uridine (via the "salvage pathway"), whereas most other tissues, including malignant tumors, favor the de novo pathway of uridine nucleotide biosynthesis, in which free uridine is not an intermediate [3]. Thus, exogenous uridine is more effective in competing with FUTP for incorporation into RNA in normal tissues versus all solid tumors tested to date in murine systems. Although uridine has also been demonstrated to protect against 5-FU toxicity in humans, its low oral bioavailability and the requirement for central venous access for parenteral administration impair clinical utility [9–12].

PN401 (2′,3′,5′-tri-*O*-acetyluridine; Fig. 1; Wellstat Therapeutics Corporation, Gaithersburg, MD, USA), an orally active prodrug of uridine, represents a more effective uridine administration technique. PN401 is efficiently absorbed from the gastrointestinal tract and deacetylated by nonspecific esterases, yielding uridine and acetate. In contrast to oral uridine, PN401 is not a substrate for the catabolic enzyme uridine phosphorylase and does not require the pyrimidine transporter for absorption. Consequently, administration of PN401 results in substantially more bioavailable uridine than does oral administration of uridine itself. Using this PN401 rescue, it has been possible to increase the therapeutic index of 5-FU in BALB/c mice bearing advanced transplants of Colon 26 adenocarcinoma. Further, in the latter tumor system, it has been possible to increase the dose of 5-FU resulting in a significant increase in antitumor activity without increased toxicity.

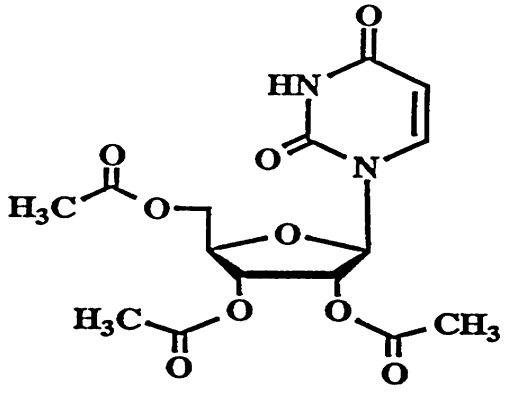

Fig. 1 Structure of PN401 showing 2′,3′,5′-tri-*O*-acetyluridine

Purpose

The primary objectives of the present study were to determine antitumor efficacy of 5-FU dose escalation enabled by delayed administration of PN401, to compare the efficacy of high-dose 5-FU plus PN401 with other 5-FU modulators or regimens and to evaluate the role of PN401 as an antidote to 5-FU overdose.

Experimental design

Murine tumor system

Frozen samples of Colon 26 adenocarcinoma were obtained from the National Cancer Institute (Frederick, MD, USA) and maintained by s.c. serial transplantation in BALB/c mice. For experimentation, 0.3 ml of a 5% tumor brei (in modified Dulbecco's phosphate-buffered saline at pH 7.2) prepared from 4–6 tumors was transplanted s.c. into 8-week-old BALB/c mice. Approximately 7–9 days later, when tumors were palpable, they were measured and the animals were distributed among the experimental groups of ten mice each so that animals carrying approximately equal-sized tumors were represented in each group.

Drug and chemicals

5-FU and leucovorin were obtained from the Sigma Chemical Co. (St Louis, MO, USA). Triacetyluridine and eniluracil were synthesized at Wellstat Therapeutics Corporation. 5-FU was dissolved in 0.85% NaCl solution immediately prior to use and administered by intraperitoneal (i.p.) injection such that the desired dose was contained in 0.1 ml/10 g of mouse weight. PN401 was suspended in 1% aqueous hydroxypropylmethylcellulose at a concentration of 80 mg/ml and administered by gavage.

Tumor measurements

Two axes of the tumor (the longest axis L and the shortest axis W) were measured with the aid of a vernier caliper. Tumor volume was estimated according to the formula:

$$\text{Tumor volume}\,(\text{mm}^3) = L \times W^2 \times \frac{\pi}{6}.$$

Statistical evaluation

Student's t test was used for the statistical evaluation of difference in mean tumor size between groups of treated

nice. Differences between groups with a statistical probability of 0.05 or less were considered significant.

Toxicity measurements

Animal body weights were recorded immediately before and weekly after the initiation of treatment. Weight change was calculated as a percentage of the initial body weight of the animals.

Groups of ten female BALB/c 8-week-old mice bearing subcutaneous Colon 26 adenocarcinoma were treated with weekly 3× i.p. injections of 5-FU alone at MTD (100 mg/kg) or at escalated doses of 5-FU (150, 175, and 200 mg/kg) with delayed administration of PN401. PN401 was administered as 2,000 mg/kg PO starting 2 h after 5-FU dose, q8h ×5. Tumor volumes were measured weekly, with final tumor volumes measured 1 week after the last 5-FU dose. Analysis of 5-FU pharmacokinetics was performed according to the methods described elsewhere [13].

Then a comparison was made to several 5-FU dosing schedules corresponding to common clinical regimens for 5-FU administration. 5-FU is often administered on a daily 5× schedule (repeated every 3–4 weeks) with or without leucovorin (LV). Eniluracil (EU) inhibits 5-FU degradation and thereby enables oral 5-FU administration and furthermore provides plasma 5-FU pharmacokinetics approximating prolonged intravenous infusion. In the following comparative regimens, 5-FU was administered at MTD for BALB/c mice: Regimen A: 5-FU 200 mg/kg i.p. days 1 and 7, PN401 2,000 mg/kg PO starting 2 h after 5-FU dose every 8 h for a total of five doses (following both on 2 and 7 days 5-FU); Regimen B: 5-FU 35 mg/kg i.p. for 5 days; Regimen C: 5-FU 35 mg/kg i.p. + LV 100 mg/kg i.p. for 5 days; Regimen D: 5-FU 2 mg/kg + PO EU 2 mg/kg PO days 1–9. Groups each comprised ten female BALB/c 8-week-old mice bearing subcutaneous Colon 26 adenocarcinoma. Tumor volume was measured on days 7 and 14.

Finally, to investigate the best timing of the first dose of PN401 relative to 5-FU administration as an important determinant of the effectiveness of PN401 in ameliorating the adverse effects of 5-FU, female BALB/c mice (nontumor bearing) received a lethal dose of 5-FU (400 mg/kg i.p.). Groups of mice then received oral PN401 beginning 2, 24, or 48 h after the 5-FU dose. Survival was monitored for 30 days.

Results

The pretreatment mean tumor volume was approximately 200 mm^3. In untreated animals, the tumors grew to a mean size of about 3,400 mm^3. 5-FU at its normal MTD (100 mg/kg) partially inhibited tumor growth, resulting in a final mean tumor volume of about 1,500 mm^3, and no regressions were observed. In the group that received 200 mg/kg/week 5-FU, a dose tolerated only due to toxicity reduction by PN401, durable complete tumor regressions were observed in 8/10 mice and partial regressions (>50% regression) in the remaining two mice. At 175 mg/mg dose of 5-FU with PN401, a CR rate of 40% and a PR rate of 60% was obtained. 5-FU at 150 mg/kg/week + PN401 yielded 10% PR rate and mean tumor volume of 690 mm^3 (Table 1). Significantly, the improved antitumor response of high-dose 5-FU enabled by PN401 was achieved without a toxicity penalty as determined by body weight changes (Table 1). Analysis of 5-FU pharmacokinetics displayed nonlinearity as a function of administered dose in mice. In female CD-1 mice receiving 5-FU by i.p. injection the "area under the curve" (AUC) for plasma 5-FU at a dose of 100 mg/kg is 135 μmol h/ml (mean for five mice). At a dose of 150 mg/kg, the AUC is 197 μmol h/ml and at 200 mg/kg, the AUC is 485 μmol h/ml. Thus, the twofold increase in 5-FU MTD in mice receiving PN401 corresponds to about a fourfold increase in the plasma AUC of 5-FU.

In the comparison study, the tumor volumes on days 7 and 14 are indicated in Table. 2 and 3. 5-FU at 200 mg/kg with delayed oral PN401 induced significant tumor regression (9/10 CR). The other regimens (5-FU daily × 5 + LV and 5-FU PO daily × 9 + EU resulted in the inhibition of tumor growth, but no regressions were observed, even though toxic weight loss was greater in these groups than in mice treated with high-dose 5-FU plus PN401.

In the timing of administration study, after a single lethal dose of 5-FU (400 mg/kg i.p.), mice that did not receive PN401 died by day 12 while most animals in the other three groups were alive at day 31 (Fig. 2). Moreover, the proportion of mice surviving was highest in the group that received PN401 at 2 h followed by 24 and 48 h. A significant improvement in survival was seen even when PN401 was administered 48 h after 5-FU.

About 12 separate variations on the Colon 26 experiments (slightly different dosing regimens, different comparison arms) were done, all corroborating the reported results. Such variations included individual dose as well as the cumulative dose of PN401 administered following 5-FU, timing between 5-FU and the first dose of PN401 ranging from 2 to 24 h, and the number of doses of PN401 required following 5-FU.

Discussion

This study has shown that there is a threshold 5-FU dose after which the efficacy is dramatically improved—in mice, that threshold is a dose of >150 mg/kg/week (i.e. 175 and 200 mg/kg/week), and the increased efficacy correlates with about a fourfold increase in the AUC of 5-FU. This finding is consistent with previous studies including the use of injected uridine [4], uridine

Table 1 Results of the study to determine MTD of 5-FU given alone versus with PN401 and responses (tumor volume)

Treatment (mg/kg)	Tumor vol. (mm^3)	Control (%)	No. dead/no. treated	Regressions		
				PR	CR	Total
Control (no 5-FU)	3,406 ± 263	–	3/10	0/10	0/10	0/10
5-FU 100	1,503 ± 267	44.1	0/10	0/10	0/10	0/10
5-FU 200 + PN401	12 ± 10	0.3	0/10	2/10	8/10	10/10

Tumor: subcutaneous Colon 26 adenocarcinoma, initial size 198 ± 14 mm^3; 5-FU: i.p. injection, weekly 3×; PN401: five oral doses at 8 h intervals, beginning 2 h after 5-FU; evaluation: 7 days after the last of three weekly 5-FU doses. The MTD of 5-FU alone was 100 mg/kg/week whereas the MTD of 5-FU when given with PN401 was 200 mg/kg/week. A complete response (CR) of 80% and partial response (PR) of 20% was observed with 5-FU (200 mg/kg) + PN401 compared to CR of 40% and PR of 60% with 5-FU (175 mg/kg) + PN401, no CR and PR of 10% with 5-FU (150 mg/kg) + PN401 while no responses were seen with 5-FU (100 mg/kg) + PN401*PR* partial response, > 50% decrease in tumor size; *CR* complete response, no tumor detectable

Table 2 Results of comparative regimen study: evaluating responses of 5-FU given alone, with LV, EU, and PN401

Group	Day 7 tumor vol. (mm^3)	Day 14 tumor vol. (mm^3)
Control	1,191 ± 215	1,230 ± 154
5-FU 200 + PN401	13 ± 2	0.5 ± 0.4
5-FU 35×5 d	266 ± 71	633 ± 144
5-FU 35×5 d + LV	159 ± 51	515 ± 122
5-FU 2×9 d + EU	225 ± 47	924 ± 98

Initial tumor size 82 ± 9 mm^3. In the regimen comparison study, tumor volume measured on day 7 and day 14 showed that the best response was achieved with PN401 when compared to EU and LV
LV leucovorin, *EU* ethynyluracil (DPD inhibitor that permits oral 5-FU administration)

diphosphoglucose (UDPG) [14] and PN401 in combination with 5-(benzyloxybenzyl)barbituric acid acyclonucleoside (BBBA), an inhibitor of uridine phosphorylase (UrdPase) [15], indicating that these agents can allow the escalation of 5-FU doses for better chemotherapeutic efficacy. This is the first report that delayed administration of PN401 alone is sufficient for enabling dose escalation of 5-FU to a degree sufficient to induce regressions of the Colon 26 adenocarcinoma.

5-FU is administered in a broad range of dosing regimens and no single dose schedule has ever emerged as clearly superior at maximum tolerated doses [1–3], and the trend towards superiority of one regimen over another may depend on the tumor type. At standard doses, inhibition of thymidylate synthase (TS) apparently predominates as a mechanism of antitumor cytotoxicity, and this inhibition is perhaps best exploited by continuous infusion of 5-FU, although resistance through compensatory overexpression of TS almost inevitably occurs. Acute TS inhibition is maximal at relatively low doses of 5-FU; the problem is to maintain durable inhibition. Unlike TS inhibition, RNA-directed cytotoxicity of 5-FU cannot be saturated at clinically achievable 5-FU concentrations, presenting, in principle, an opportunity for increased activity with dose escalation. However, this mechanism is operative only when concentrations of the 5-FU anabolite FUTP are sufficient to compete with endogenous UTP for incorpora-

Table 3 Comparative 5-FU pharmacokinetics after various clinical dosing regimens

5-FU regimen	5-FU dose (mg/m^2/day)	Dose rate	No. of doses/month	Total dose (mg/m^2/month)	Cl ($l/h/m^2$)	$AUC_{0–24\ h}$ (μmol h)	$AUC_{0–30\ days}$ (μmol h)
Daily × 5	375	1–2 min	5	1,875	42.2	73.5	368
Weekly bolus	500	20 min	4	2,000	65.3	55.9	224
Weekly bolus	500	2 min	4	2,000	42.5	103	412
PN401 weekly bolus	1,400	30 min	3	4,200	24.1	466	1,398
Continuous infusion	146	Continuous	30	4,380	183	7.2	216
120 h infusion	1,000	120 h	5	5,000	156	50.4	252
Weekly 24 h infusion	1,500	24 h	4	6,000	127	96	384
72 h infusion	2,000	72 h	3	6,000	159	156	468

The comparison of Phase I studies [13, 20] studies and various other intravenous schedules of 5-FU [3] have showed disproportionate increases in AUC and corresponding decreases in clearance with increasing 5-FU dose (range 1,200–1,950 mg/m^2). While the dose increases over a standard dose ranged from 2.4 to 3.9-fold, average 5-FU exposures, as measured by AUC, ranged from 5.6 to 18.6-fold [21, 22]. The intrapatient variability in exposure decreased with increased 5-FU dose. With high-dose 5-FU enabled by PN401, clearance is reduced to approximately threefold than that observed with the maximum inhibition of DPD with EU [23]

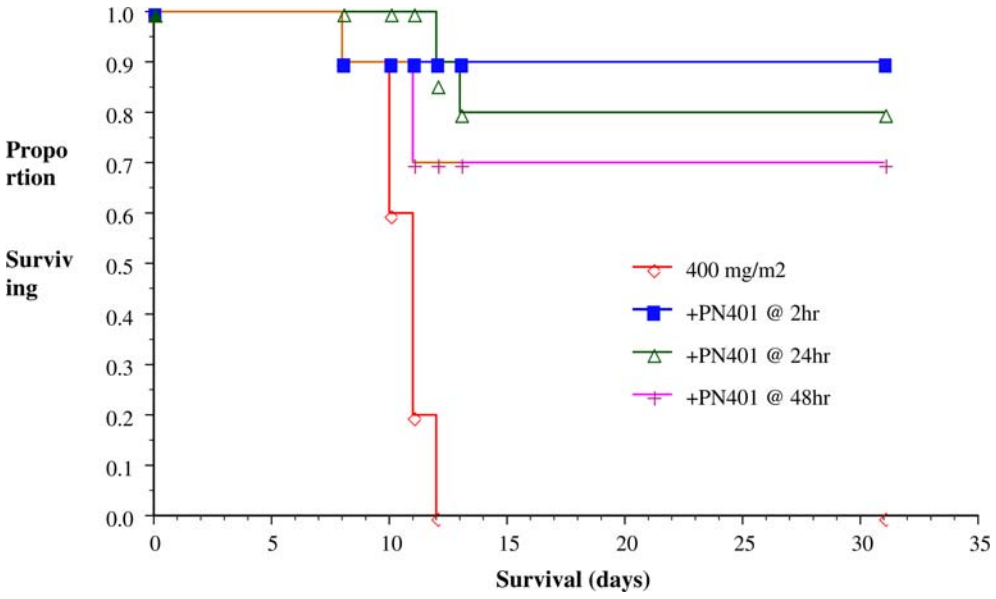

Fig. 2 Survival in mice treated with 400 mg/m^2 5-FU and PN401 and relation to time of dosing. Mice that did not receive PN401 died by day 12, while the other three groups were alive at day 31. Moreover, the proportion of mice surviving was highest in the group which received PN401 at 2 h followed by 24 and 48 h

tion into nuclear RNA, which requires high extracellular concentrations of 5-FU. Thus, bolus administration of 5-FU may be necessary for exploitation of RNA-directed 5-FU toxicity, but dose-limiting 5-FU toxicities intervene. The fact that uridine is an effective antidote for both gastrointestinal and hematologic toxicities due to bolus 5-FU implies that RNA-directed effects account for dose-limiting toxicities in this situation, since delayed uridine does not reverse inhibition of TS by FdUMP [16].

RNA-related toxicities of 5-FU are largely a function of the concentration × time product, equivalent to the AUC for plasma pharmacokinetics [17]. It is noteworthy that although different total amounts of 5-FU are administered in various clinical regimens, the AUC for intact 5-FU (versus total 5-FU plus inactive 5-FU catabolites) during the course of periods of therapy of equivalent duration (i.e., 30 days, to account for different dosing schedules), is quite similar, whether 5-FU is administered by weekly bolus, daily × 5 bolus, 24 or 48 h infusion, or continuous 28 day infusion [3]. This suggests that 5-FU therapy is limited by an ''AUC ceiling'', integrated over a cycle of treatment. An important feature of 5-FU pharmacokinetics is that the clearance rate varies inversely with plasma concentration, due to saturable degradation processes, e.g., by the enzyme dihydropyrimidine dehydrogenase (DPD) [18]. A large fraction of an administered dose of 5-FU is degraded rather than anabolized to cytotoxic fluoropyrimidine nucleotides, especially during prolonged infusion, so that the AUC achieved is perhaps a more meaningful measure of 5-FU exposure than the administered dose. Thus, despite different total doses of 5-FU administered in various regimens, the systemic exposure to intact 5-FU per treatment cycle of equivalent duration is similar, and this may account for the therapeutic equivalence of the various regimens. However, it is tantalizing that the dose response for clinical activity of bolus 5-FU has a very steep slope [19], i.e., a relatively small increase in tumor exposure to intact 5-FU could in principle lead to a substantial increase in clinical efficacy.

Delayed uridine administration after high-dose 5-FU has been demonstrated to improve antitumor efficacy versus 5-FU at MTD in a variety of tumor models, and clinical implementation of this strategy has been attempted. Uridine itself is poorly absorbed, resulting in osmotic diarrhea at doses required to elevate plasma uridine into the therapeutic range, and intravenous administration of uridine is cumbersome due to the large amounts of uridine required, resulting in hyperthermia or phlebitis if the infusion rate and route are not carefully controlled [9–12]. In contrast, PN401 (triacetyluridine) acts as a lipophilic prodrug of uridine that is efficiently absorbed and rapidly deacetylated, yielding more bioavailable circulating uridine than the oral administration of uridine itself. The strong antitumor efficacy of high-dose 5-FU with delayed PN401, with less toxicity (weight loss) than was observed with lower doses of 5-FU without PN401, which only attenuated tumor growth without inducing regressions, indicates that PN401 rescue was relatively selective for normal tissues versus the tumors.

The clinical feasibility of 5-FU dose escalation with PN401 has been demonstrated in phase I studies [13, 20]. These studies have showed disproportionate increases in AUC and corresponding decreases in clearance with increasing 5-FU dose (range 1,200–1,950 mg/m^2) (Table 3; Fig. 3). While the dose increases over a standard dose ranged from 2.4 to 3.9-fold, average 5-FU exposures, as measured by AUC, ranged from 5.6 to 18.6-fold [21, 22]. The intrapatient variability in exposure decreased with increased 5-FU dose. With high-dose 5-FU enabled by PN401, clearance is reduced to approximately threefold than that observed with the maximum inhibition of DPD with EU [23]. In an ongoing phase III clinical study of high-dose 5-FU in

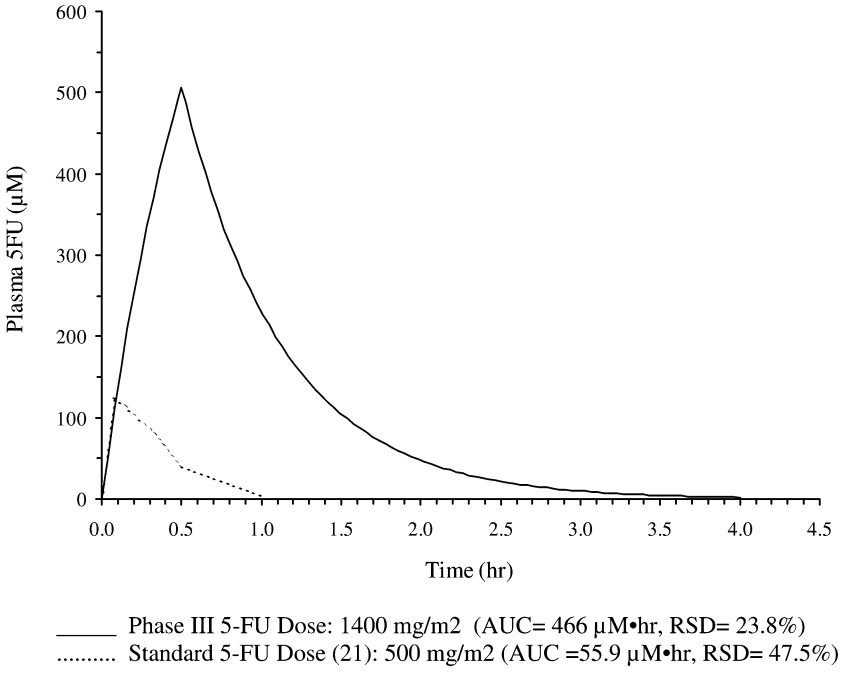

Fig. 3 Plasma 5-FU concentration–time profile: phase III dose versus conventional dose. Comparison of AUC achieved with high-dose 5-FU enabled by delayed oral administration of PN401 versus standard 5-FU bolus dose showed disproportionate increases in AUC when 5-FU was given with PN401

pancreatic cancer, patients receive 1,400 mg/m^2 5-FU per week 3× with 1 week rest, with PN401 6 g administered q8h for eight doses, beginning 8 h after a rapid (30 min) 5-FU infusion. In a phase II clinical study in gastric cancer conducted by SWOG, patients received 5-FU at 1,200 mg/m^2 per week 6× with leucovorin, followed 8 h after 5-FU with PN401 6 g q8h for eight doses. High-dose 5-FU enabled with PN401 is also in principle compatible with combination chemotherapy involving the same spectrum of agents used with standard doses of 5-FU, e.g., oxaliplatin, irinotecan, cisplatin, epirubicin, taxotere.

These experiments also indicated that the timing of the first dose of PN401 may be an important determinant of 5-FU toxicity reduction and the degree of safe 5-FU dose escalation as mice can tolerate higher 5-FU doses if PN401 rescue starts earlier than later. PN401 used to rescue mice from the lethal toxicity of 5-FU not only allows administration of high 5-FU doses to tumors unresponsive to conventional doses of 5-FU but also entails that PN401 can be used as a *antidote* even when used after 48 h in patients who have received an accidental overdose or severe untoward toxicity of 5-FU, in particular RNA-medicated host toxic effects. This issue is particularly important in situations including pump malfunction or ingestion of excessive quantities of 5-FU prodrugs like capecitabine. Moreover, the potential of PN401 in DPD-deficient patients to allow 5-FU treatment also warrants investigation, in particular when they have 5-FU sensitive tumors, which may affect up to about 3% of the population [24].

In summary, the results of this study demonstrate that uridine exposure resulting from PN401 not only allows administration of high 5-FU doses to tumors unresponsive to conventional doses of 5-FU but also entails that PN401 can be used as an antidote even when used up to 48 h after a 5-FU overdose. Because of the nonlinearity of 5-FU pharmacokinetics, the two times increase in the MTD of 5-FU was associated with a fourfold increase in 5-FU exposure (i.e., AUC). Future exploratory studies aiming at evaluating the role of PN401 in DPD-deficient patients as well as clinical trials to further confirm this biochemical modulation with PN401 is warranted.

Acknowledgements We thank James D. O'Neil, Ph.D. (of Wellstat) for the human pK data and the human PK table and Mahmoud el Kouni Ph.D. for the mouse 5-FU pharmacokinetic measurements.

References

1. Allegra CJ, Grem JL (1997) Antimetabolites. In: DeVita VT Jr, Hellman S, Rosenberg SA (eds) Cancer: principles and practice of oncology, 5. Lippincott-Raven, Philadelphia, pp 432–451
2. Sobrero AF, Aschele C, Bertino JR (1997) Fluorouracil in colorectal cancer: a tale of two drugs—implications for biochemical modulation. J Clin Oncol 15:368–381
3. Grem JL (1996) 5-Fluoropyrimidines. In: Chabner BA, Longo DL (eds) Cancer chemotherapy and biotherapy: principles and practice. Lippincott-Raven, Philadelphia, pp 149–211
4. Martin DS, Stolfi RL, Sawyer RC et al (1982) High-dose 5 fluorouracil with delayed "uridine" rescue in mice. Cancer Res 42:3964–3970
5. Klubes P, Cerna I, Meldon MA (1982) Uridine rescue from the lethal toxicity of 5-fluorouracil in mice. Cancer Chemother Pharmacol 8:17–21
6. Martin DS, Stolfi RL, Sawyer RC et al (1989) Use of oral uridine as a substitute for parenteral uridine rescue of 5-fluorouracil therapy, with and without the uridine phosphorylase inhibitor 5-benzylacyclouridine. Cancer Chemother Pharmacol 24:9–14

7. Klubes P, Leyland-Jones B (1989) Enhancement of the antitumor activity of 5-fluorouracil by uridine rescue. Pharmacol Ther 41:289–302
8. Nord LD, Stolfi RL, Martin DS (1992) Biochemical modulation of 5-fluorouracil with leucovorin or delayed uridine rescue: correlation of antitumor activity with dosage and FUra incorporation into RNA. Pharmacology 43:2543–2549
9. Leyva A, van Groeningen CJ, Kraal I et al (1984) Phase I and pharmacokinetic studies of high-dose uridine intended for rescue from 5-fluorouracil toxicity. Cancer Res 44:5928–5933
10. van Groeningen CJ, Peters GJ, Nodal JC et al (1991) Clinical and pharmacologic study of orally administered uridine. J Natl Cancer Inst 83:437–441
11. Seiter K, Kemeny N, Martin D et al (1993) Uridine allows dose escalation of 5-fluorouracil when given with *N*-phosphonacetyl-L-aspartate, methotrexate, and leucovorin. Cancer 71:1875–1881
12. Schwartz GK, Christman K, Saltz L et al (1996) A phase I trial of a modified, intensive FAMTX regimen (high dose 5-fluorouracil + doxorubicin + high dose methotrexate + leucovorin) with oral uridine rescue. Cancer 78:1988–1995
13. Kelsen DP, Martin D, O'Neil J et al (1997) Phase I trial of PN401, an oral prodrug of uridine, to prevent toxicity from fluorouracil in patients with advanced cancer. J Clin Oncol 15:1511–1517
14. Codacci-Pisanelli G, Van der Wilt CL, Smid K, Noordhuis P, Voorn D, Pinedo HM, Peters GJ (2002) High-dose 5-fluorouracil with uridine-diphosphoglucose rescue increases thymidylate synthase inhibition but not 5-fluorouracil incorporation into RNA in murine tumors. Oncology 62(4):363–370
15. Ashour OM, Naguib FN, Panzica RP, Al Safarjalani ON, el Kouni MH (2000) Modulation of 5-fluorouracil host toxicity by 5-(benzyloxybenzyl)barbituric acid acyclonucleoside, a uridine phosphorylase inhibitor, and 2′,3′,5′-tri-*O*-acetyluridine, a prodrug of uridine. Biochem Pharmacol 60(3):427–431
16. Peters GJ, van Dijk J, van Groeningen CJ et al (1988) In vitro biochemical and in vivo biological studies of the uridine rescue of 5-fluorouracil. Br J Cancer 57:259–265
17. Inaba M, Mitsuhashi J, Ozawa S (1990) Kinetic analysis of 5-fluorouracil action against various cancer cells. Jpn J Cancer Res 81:1039–1044
18. Harris BE, Song R, Soong S et al (1990) Relationship between dihydropyrimidine dehydrogenase activity and plasma 5-fluorouracil levels with evidence for circadian variation of enzyme activity and plasma drug levels in cancer patients receiving 5-fluorouracil by protracted continuous infusion. Cancer Res 50:197–201
19. Hryniuk WM, Figueredo A, Goodyear M (1987) Applications of dose intensity to problems in chemotherapy of breast and colorectal cancer. Semin Oncol 14(Suppl 4):3–11
20. Hidalgo M, Villalona-Calero MA, Eckhardt SG, Drengler RL, Rodriguez G, Hammond LA, Diab SG, Weiss G, Garner AM, Campbell E, Davidson K, Louie A, O'Neil JD, von Borstel R, Von Hoff DD, Rowinsky EK (2000) Phase I and pharmacologic study of PN401 and fluorouracil in patients with advanced solid malignancies. J Clin Oncol 18(1):167–177
21. Larsson A, Carlsson G, Gustavsson B et al (1996) Different intravenous administration techniques for 5-fluorouracil: pharmacokinetics and pharmacodynamic effects. Acta Oncol 35:207–212
22. McDermott BJ, van der Berg HW, Murphy RF (1982) Nonlinear pharmacokinetics for the elimination of 5-fluorouracil after intravenous administration in cancer patients. Chemother Pharmacol 9:173–178
23. Baker S (2000) Pharmacology of fluorinated pyrimidines: eniluracil. Invest New Drugs 18(4):373–381
24. Ezzeldin H, Diasio R (2004) Dihydropyrimidine dehydrogenase deficiency, a pharmacogenetic syndrome associated with potentially life-threatening toxicity following 5-fluorouracil administration. Clin Colorectal Cancer 4:181–189