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Stereoselective Metabolism and Pharmacokinetics of Tegafur

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ABSTRACT: *Objectives*. UFT is composed of racemic tegafur (FT), a prodrug of 5-fluorouracil (5-FU), and uracil in a fixed molar combination (1:4). FT contains a chiral center and has two stereoisomers, *R*-FT and *S*-FT. The objectives of this study were to assess the stereoselectivity in the metabolism of FT to 5-FU *in vitro* and to determine stereoselective differences in the disposition of FT *in vivo*.

Methodology. R-FT, *S*-FT, and racemic FT were incubated with pooled human liver microsomes and S-9 fraction for a period of up to 30 min for *in vitro* studies. For pharmacokinetics, plasma samples were obtained from fasted cancer patients over a period of 24 h after oral administration of 200 mg UFT. Samples from *in vitro* studies and patient plasma samples were analyzed for FT using a validated achiral and a chiral assay, and for 5-FU using a validated GC/MS assay.

Results. *R*-FT was metabolized at a rate 5.6-times faster than *S*-FT by human liver microsomes. Similarly, stereoselective metabolism of *R*-FT was also seen in the S-9 incubations. In cancer patients, the peak plasma concentrations (C_{max}) and the time to reach C_{max} (T_{max}) were similar for the two isomers after the administration of UFT suggesting no apparent differences in their absorption kinetics. However, the area under the curve from zero to infinity [AUC(INF)] and the terminal elimination half-life (T-HALF) values for *R*-FT were about 4.6- and 4.4-fold lower compared to *S*-FT, respectively, suggesting the preferential elimination of *R*-FT. The T-HALF of racemic FT (8.3 h) was comparable to the T-HALF of *S*-FT (10.3 h) which indicated that the kinetics of the racemate are governed by *S*-FT. The active cytotoxic moiety, 5-FU, exhibited formation rate limited kinetics from *R*-FT because the T-HALF of 5-FU (3.4 h) was similar to that of *R*-FT (2.4 h).

Conclusions. The *R*-isomer of FT is preferentially metabolized to 5-FU compared to the *S*-isomer *in vitro*. The distinct kinetic profiles of the stereoisomers of FT following the administration of UFT is apparently due to the stereoselective disposition of the *R*-isomer relative to the *S*-isomer. These data suggest that the *R*-isomer of FT is worthy of further preclinical and clinical evaluation for safety, efficacy, and pharmacokinetics. Copyright © 2001 John Wiley & Sons, Ltd.

Key words: stereoisomers; stereoselective; pharmacokinetics; tegafur

Introduction

UFT is composed of a fixed molar ratio (1:4) of uracil and tegafur (also referred to as ftorafur; FT). FT, 1-(2-tetrahydrofuranyl)-5-fluorouracil, is a prodrug of the anticancer agent 5-fluorouracil (5-FU) [1]. Uracil is a competitive and reversible inhibitor of dihydropyrimidine dehydrogenase, a rate-limiting enzyme responsible for the catabolism of 5-FU [2]. UFT is commercially available in Japan since 1984 and has been recently approved for marketing in the European Union countries. Over the past several years, UFT has been extensively shown to be clinically safe and effective in the treatment of solid tumors [3]. In recently completed pivotal phase III studies, UFT in combination with leucovorin, has been shown to have a superior safety profile while maintaining equivalent survival rate to intravenous 5-FU/

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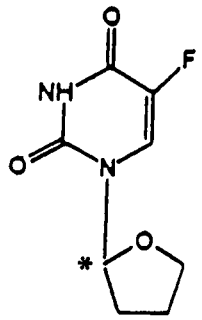

Ftorafur

* denotes the position of the chiral center in FT

Figure 1. Structure of tegafur. The asterisk denotes the position of the chiral center

leucovorin in the first-line treatment of colorectal cancer [4].

While 5-FU does not have a chiral center, FT contains one asymmetric carbon at the 2′ position in the tetrahydrofuran ring (Figure 1) and has two stereoisomers, *R*-FT and *S*-FT (for this paper, the stereoisomers have been prefixed with *R*- and *S*-notations while racemic FT has been identified as 'FT' or 'racemic FT'). UFT contains racemic FT. Horwitz *et al.* have reported the biological activity of the stereoisomers of FT using a clonogenic assay, cell multiplication assay, and ^{14}C-deoxyuridine DNA incorporation assay [5]. Based on these *in vitro* assays, the authors concluded that there was no marked difference in the cytotoxic activity of *R*-FT, *S*-FT, and FT [5]. These data were corroborated by Yasumoto *et al.* by evaluating the *in vivo* antitumor activity of the stereoisomers against AH-130 carcinoma and Yoshida sarcoma tumor in rats [6]. *R*-FT, *S*-FT, and FT inhibited the growth of AH-130 tumor by 62, 72, and 51%, respectively, and that of the Yoshida sarcoma tumor by 34, 41, and 39%, respectively, suggesting their equivalent activity [6].

Since enantiomers of drugs can exhibit stereoselective disposition, the objective of the current investigation was to determine if there are stereoselective differences in the metabolism and pharmacokinetics of FT in humans. The metabolism was evaluated using *in vitro* incubation experiments, and the pharmacokinetics of the stereoisomers were evaluated as part of the clinical study that assessed the effect of food on the oral bioavailability of UFT.

Materials and Methods

Chemicals

R-FT and *S*-FT were obtained from Taiho Pharmaceutical Co., Tokyo, Japan. Racemic FT was purchased from Sigma Chemical Co., St. Louis, MO. All other chemicals were laboratory grade and were obtained from commercial sources. For the clinical pharmacokinetic study, UFT capsules (100 mg, based on racemic FT) and leucovorin tablets (15 mg) were supplied by Taiho Pharmaceutical Co., Tokyo, Japan and Immunex, Seattle, WA, respectively.

In vitro metabolism

R-FT, *S*-FT, and racemic FT were incubated with pooled human liver microsomes and *S*-9 fractions (In-Vitro Technologies, Baltimore, MD) in a shaking water bath at 37°C. The incubation medium was Tris-buffer (pH 7.4) with glucose-6-phosphate (10 mM), NADP (1 mM), $MgCl_2$ (5 mM), and glucose-6-phosphate dehydrogenase (1 IU/ml). The total volume of the incubation medium was 10 ml. The concentration of *R*- and *S*-FT in the final incubation mixture was 5 μg/ml (25 μM), while that for FT was 10 μg/ml (i.e. 5 μg/ml for each enantiomer). The final concentrations of the microsomal and S-9 protein were 0.5 and 3.0 mg/ml, respectively. Immediately after the addition of co-factors (0 min), and at 2, 5, 15, 30, and 60 min, triplicate aliquots of 0.1 and 0.25 ml were withdrawn and mixed with an equal volume of ice-cold methanol, placed briefly over ice, and then stored at −20°C. The 0.1 ml aliquots were assayed for concentrations of 5-FU and the 0.25 ml aliquots were assayed for concentrations of *R*- and *S*-FT. To serve as negative control, racemic FT was incubated with heat-deactivated (5 min at 80°C) microsomes and S-9 fractions.

Pharmacokinetics in cancer patients

The pharmacokinetics of the stereoisomers of FT were assessed as part of a clinical study designed

to evaluate the effect of food on the oral bioavailability of UFT and leucovorin, the results from which were published separately [7]. This was an open label, single dose, two-period, two-treatment, crossover study in 25 patients with histologically confirmed advanced solid malignancy refractory to standard therapy. Patients were randomly assigned to receive UFT plus leucovorin following an overnight fast or 5 min after completion of a high-fat meal with a washout period between treatment of 3 days. The fasted patients were given a regular breakfast 2 h after drug administration. For each treatment, UFT was administered orally at a fixed dose of 200 mg [2 × 100 mg capsules (dose represented in terms of racemic FT)] and leucovorin was given orally at a dose of 30 mg (2 × 15 mg tablets). The study drugs were administered with 5 ounces of room temperature tap water. Patients were allowed to drink water as required during the predose and postdose fasting periods. Blood samples (about 8 ml) were collected after each treatment at predose and at 15 and 30 min, and 1, 1.5, 2, 3, 5, 8, and 24 h after dose in EDTA tubes and kept in chipped ice. Within 1 h of collection, blood samples were centrifuged at 5°C at 1000*g* for 15 min and the resulting plasma was stored at or below −20°C. Out of the 25 patients enrolled in this study, 22–24 patients were evaluable for pharmacokinetics. Since a significant negative food effect was seen in this clinical study [7], for the purpose of assessing the stereoselectivity in the kinetics of the *R*- and the *S*-isomer of FT, plasma samples obtained under fasting conditions in the study were utilized. The protocol was approved by the Institutional Review Boards of the University of Arizona Cancer Center, Tucson, AZ, USA; Ottawa Regional Cancer Center, Ottawa, Canada; and MD Anderson Cancer Center, Houston, TX, USA. Written informed consent was obtained from all patients prior to the initiation of study-related procedures.

Sample analyses

Concentrations of FT in the *in vitro* incubation mixtures and in the plasma samples obtained after dosing UFT under fasting conditions in the clinical study were determined using achiral- and chiral-validated HPLC assays with ultraviolet detection; these assays involved minor modifications of a previously published method [8]. Notable differences for the achiral assay was that the mobile phase, methylene chloride–hexane–ethanol (80:20:1.2) was used instead of ethylene chloride–ethanol (24:1) [8]. The retention time of racemic FT was approximately 10.2 min and the absorbance was measured at 254 nm. For the chiral assay, resolution of *R*- and *S*-FT was achieved using a polysaccharide HPLC column (Chiralpak®, 25 cm × 4.6 mm; Diacel Corp., Exton, PA) and the mobile phase was 40% isopropanol in hexane and 0.1% trifluoroacetic acid. The retention times of *R*- and *S*-FT were 10.0 and 12.5 min, respectively, and the absorbance was measured at 272 nm. Neither stereoisomers underwent chiral conversion during the conditions of storage of study samples. A validated gas chromatographic–mass spectrometric assay was used to quantitate 5-FU. This assay was based on a previously published method for 5-FU [9]. Prior to analysis, interference from FT was eliminated by passing the samples through two C_{18} solid-phase extraction columns; FT was retained on the columns and the eluate from the second column was used for the analysis of 5-FU.

The standard curves for *R*-FT, *S*-FT, racemic FT, and 5-FU were linear ($R^2 \geq 0.997$) over the concentration range of 25–10 000, 50–10 000, 50–20 000, and 1–500 ng/ml, respectively. Based on the analyses of quality control samples, included in each analytical run, the accuracy for all assays were greater than 91% and the inter- and intra-run precision was within 17%. The standard curve and quality control sample data indicated that the assay methods used were precise, accurate, and the analytes were stable in plasma.

Pharmacokinetic analyses

The plasma concentration–time data for tegafur and its two isomers were analyzed by a non-compartmental method [10]. The peak plasma concentration, C_{max}, and the time to reach peak concentration, T_{max}, were recorded directly from experimental observations. The area under the plasma concentration–time curve from time zero to T, AUC(0-T), where T is the time of last measurable concentration, was calculated by the

trapezoidal method. Using no weighting factor, the slope of the terminal phase of the plasma profile, K, was determined by the log-linear regression of at least three data points which yielded a minimum mean square error. The absolute value of K was used to estimate the terminal half-life (T-HALF) by the formula T-HALF = $\ln 2/K$. The area under the plasma concentration–time curve from zero to infinity, AUC(INF), was determined by summing the areas from time zero to the time of last measured concentration, calculated by using the combined log-linear trapezoidal rule and the extrapolated area. The extrapolated area was determined by dividing the final concentration by the slope of the terminal log-linear phase.

Results

In vitro metabolism

In both the human microsomal and S-9 incubations, *R*-FT and *S*-FT were not detected when the incubation substrates were *S*-FT and *R*-FT, respectively, which indicates that interconversion of the two isomers (i. e. racemization) did not occur under the incubation conditions. Over the incubation period of 60 min, concentrations of *R*-FT and *S*-FT did not decrease substantially with time, suggesting that only a small fraction of tegafur was metabolized. Therefore, the metabolism of tegafur was assessed by evaluating the amount of 5-FU formed in the incubation mixtures. Figures 2 and 3 depict the amount of 5-FU formed in the microsomal and S-9 incubations, respectively. In the microsomal incubations, markedly higher amount of 5-FU was formed from *R*-FT relative to *S*-FT at all incubation time points evaluated (Figure 2). The amount of 5-FU produced in the racemic FT incubations was slightly higher than that produced with incubation of *R*-FT. At the last microsomal incubation time interval of 60 min, the amount of 5-FU formed from *R*-FT, *S*-FT, and racemic FT was 380, 68, and 426 ng/mg of protein, respectively. The amount of 5-FU generated upon incubation of *R*-FT, *S*-FT, and racemic FT with human liver S-9 fractions was markedly less compared to that generated in the

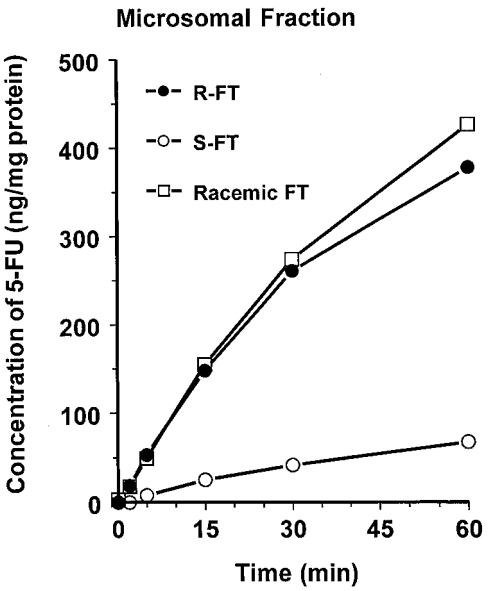

Figure 2. Mean concentration of 5-FU formed after the incubation of *R*-FT, *S*-FT and racemic FT with human liver microsomes for various time intervals

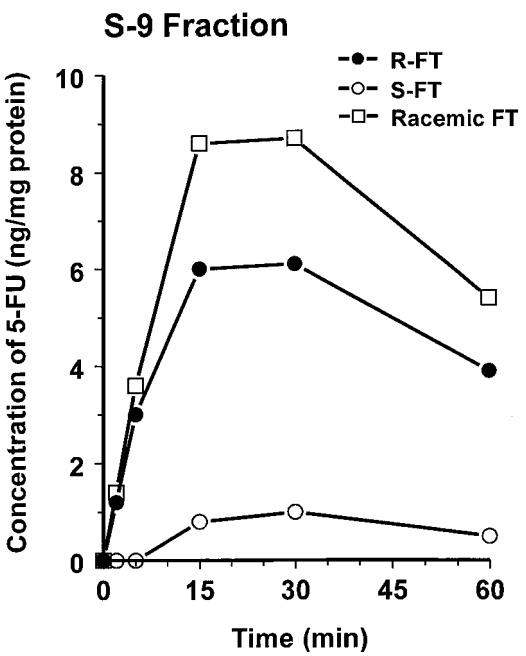

Figure 3. Mean concentration of 5-FU formed after the incubation of *R*-FT, *S*-FT and racemic FT with human liver S-9 fractions for various time intervals

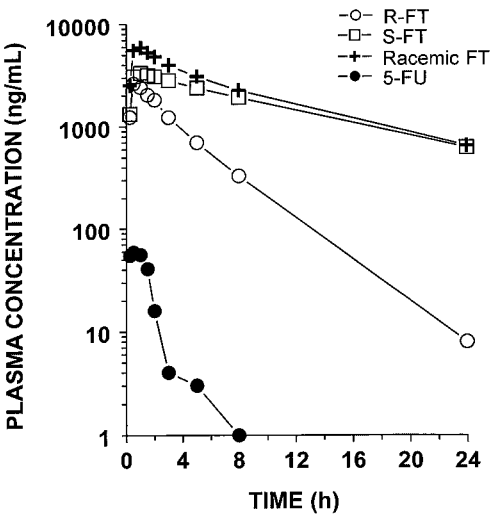

Figure 4. Mean plasma concentration–time profiles for *R*-FT, *S*-FT, racemic FT and 5-FU in cancer patients after oral administration of UF

microsomal incubations (Figure 3). Similar to the results obtained from the microsomal incubations, the amount of 5-FU formed in the S-9 incubations was consistently higher for *R*-FT compared to *S*-FT.

Pharmacokinetics in cancer patients

The plasma concentrations of FT determined using the achiral assay and the chiral assay were in excellent agreement. The mean plasma concentration–time profiles for *R*-FT, *S*-FT, racemate FT, and 5-FU are shown in Figure 4, and the pharmacokinetic parameters are given in Table 1. Following the oral administration of UFT in cancer patients, the mean C_{max} and median T_{max} values for the two isomers were reasonably similar. The T_{max} for both isomers occurred within 1 h. The mean AUC(INF) and T-HALF values were about 4.6- and 4.4-fold higher, respectively, for *S*-FT relative to *R*-FT. Consistent with the plasma concentrations of FT determined using the chiral and achiral assay, the C_{max} and AUC(INF) values of *R*-FT and *S*-FT combined were in excellent agreement with the parameter values for the racemate.

Discussion

There appears to be a striking similarity in the metabolism of FT between several animal species (mouse, rat, rabbit, dog, and monkey) and in humans [1,11–13]. The conversion of FT to active cytotoxic species, the phosphorylated fluoronucleotides, proceeds via the formation of 5-FU, with liver being the primary site of its metabolism. The pathways for the metabolism of FT to 5-FU involves the C-5′ oxidation by the microsomal enzymes and C-2′ hydrolysis by the cytosolic enzymes [14,15]. It should be noted that the identity of the actual enzymes responsible for the metabolism of FT are presently unknown. The C-3′ and C-4′ oxidation products, (the hydroxy-FT metabolites), and a dehydro-FT metabolite (undefined location of the double bond in the

Table 1. Mean (S.D.) pharmacokinetic parameters of *R*-FT, *S*-FT, racemic FT, and 5-FU in cancer patients after oral administration of UFT along with leucovorin

Analyte	Pharmacokinetic parameters[a]			
	C_{max} (ng/mL)	T_{max}[b] (h)	AUC (INF) (h ng/ml)	T-HALF (h)
R-FT	2957 (921)	0.5 (0.25, 2.0)	10759 (6137)	2.4 (1.1)
S-FT	3788 (811)	1.0 (0.5, 3.0)	49324 (15523)	10.3 (3.1)
Racemic FT	6623 (1598)	1.0 (0.5, 2.0)	57622 (20092)	8.3 (2.8)
5-FU	115 (116)	0.5 (0.25, 2.0)	126 (96)	3.4 (2.0)

[a] $n = 22$–24 patients.
[b] Median (minimum, maximum) value.

tetrahydrofuran ring) have also been reported as urinary excretory products in rats, rabbits, and humans but are only minor metabolic components [16]. Moreover, these metabolites are non-cytotoxic, and only the C-4′ hydroxy-FT metabolite is converted to 5-FU to the extent of 5% in humans by thymidine phosphorylase [17]. The excretion of unchanged FT in urine plays only a minor role in the elimination of FT [13]. Hence, metabolism to 5-FU appears to be the predominant route for the clearance of FT.

The *in vitro* metabolism data in humans suggest that *R*-FT is metabolized to 5-FU at an approximately 5-fold faster rate than *S*-FT. This trend was consistent in the microsomal and the S-9 (cytosolic) liver fraction, suggesting that the C-5′ oxidative pathway mediated by the microsomal enzymes and at the C-2′ hydrolytic pathway mediated by the cytosolic enzymes appear to be stereoselective in humans. The cytosolic incubations yielded much lower levels of 5-FU compared to the microsomal incubations. It has been shown by Chaudhuari *et al*. that 5-FU undergoes rapid metabolism via cytosolic enzymes [18], and is presumably the cause of low levels of 5-FU in our incubations with the S-9 fraction. Similarly, incubation with mouse liver fractions have also been reported to yield lower levels of 5-FU by cytosolic enzymes compared to microsomal enzymes [19]. Therefore, the differences in the formation of 5-FU in the human cytosolic versus microsomal fractions is not indicative of the relative importance of the two above-mentioned pathways for the conversion of FT to 5-FU. In microsomal incubations, the formation of 5-FU appears to increase linearly with time at least over a 30 min incubation as shown in Figure 2, and the curves exhibit a trend towards a plateau after incubation for 60 min. It is likely that incubations beyond 60 min may result in further formation of 5-FU by both isomers. Nonetheless, these *in vitro* studies clearly indicate stereoselectivity in the conversion of FT to 5-FU, favoring the *R*-isomer for metabolism.

Au and Sadée have reported that the mouse microsomal enzymes are capable of metabolizing the *R*- and *S*-isomer of FT to a similar extent, thus suggesting that stereoselective cleavage may not occur in the C-5′ position of FT in the mouse [19]. However, these authors have also shown that the cleavage at the C-2′ position to form γ-butyrolactone (a by-product during the formation of 5-FU) by non-microsomal soluble enzymes in mouse and rabbit liver homogenates was greater for *R*-FT compared to S-FT [19]. Overall, these *in vitro* metabolism data suggest that *R*-FT may be more readily converted to 5-FU than *S*-FT; but across species there may be some differences in the rate and extent of the stereoselective metabolism of FT.

The pharmacokinetics of the stereoisomers of FT in cancer patients following administration of racemic FT (as UFT) suggest that there are no apparent differences in the absorption kinetics of the isomers. However, the R-isomer is rapidly eliminated compared to the *S*-isomer in cancer patients after the administration of UFT. This stereoselective disposition of FT isomers in patients may be primarily related to the preferential metabolism of *R*-FT over *S*-FT as seen in the *in vitro* experiments. The elimination of racemic FT in the terminal-phase parallels the terminal elimination phase of *S*-FT, suggesting that the kinetics of the racemate appear to be primarily governed by the kinetics of the *S*-isomer. It also appears from the present investigation that the kinetics of 5-FU are governed by its rate of formation from *R*-FT since the half-life of 5-FU (3.4 h) was in reasonable agreement with the half-life of *R*-FT (2.4 h). Furthermore, since the decline in plasma concentrations of 5-FU parallel *R*-FT rather than *S*-FT, it appears that 5-FU is predominantly formed from *R*-FT rather than from *S*-FT.

Given the longer half-life of the *S*-isomer compared to the *R*-isomer, the potential for accumulation of the *S*-isomer upon repeated administration of UFT in humans would be similar to the racemate. In clinical pharmacological studies, pharmacokinetics of FT were delineated using an achiral assay after the administration of therapeutic doses of 300 mg/m^2/day of UFT (with 75 mg/day of leucovorin) given as three divided doses daily for 28 days. The steady-state exposure on day 28 were similar to the exposure obtained after a single dose on day 1 indicating that significant accumulation of the racemate, and by inference the accumulation of the *S*-isomer does not occur after therapeutic

doses of UFT [20]. Similarly, the steady-state exposure of 5-FU were similar to those obtained after a single dose. Hence, the conclusion that 5-FU is predominantly formed from the *R*-FT rather than *S*-FT from this single-dose study would also be applicable at steady state as there is no substantial accumulation of FT or 5-FU after clinically relevant doses of UFT.

It should be noted that both isomers of FT *per se* are inactive as anticancer agents without their conversion to 5-FU [5] and the anticancer activity of orally administered UFT is due to its conversion to 5-FU [1]. The plasma kinetics of 5-FU are formation-rate limited since 5-FU is further metabolized quite rapidly by the enzyme dihydropyrimidine dehydrogenase. This conversion is inhibited by uracil which is a competitive and reversible inhibitor of the enzyme. However, the half-life of uracil after the administration of UFT is only about 3 h [7]. It can be argued that *S*-FT, which has a longer half-life than *R*-FT, may offer the possibility for the sustained formation of 5-FU. However, considering the minor contribution of 5-FU generated from *S*-FT, as evidenced in the *in vitro* studies, and the short half-life of uracil after the administration of UFT, it appears that 5-FU formed from S-FT would be rapidly metabolized by dihydropyrimidine dehydrogenase. This is evident by the fact that plasma levels of 5-FU remain quantifiable for only about 6–8 h duration after the administration of UFT [7]. Hence, it appears that *R*-FT may be a better candidate than *S*-FT for further evaluation in combination with an inhibitor of dihydropyrimidine dehydrogenase.

Although 5-FU is predominantly formed from the *R*-isomer, at the present time, it is unclear if administration of *R*-FT may be substantially better in activity and toxicity than administration of the racemate (as UFT) in cancer patients, especially since activity is related to the formation of 5-FU. However, data obtained from this study suggest that the *R*-isomer of FT is worthy of further preclinical and clinical evaluation for safety, efficacy, and pharmacokinetics. Finally, it should be noted that oral UFT (containing FT as a racemate) plus leucovorin has been shown to be equivalent in activity and significantly lower in toxicity compared to intravenous 5-FU plus leucovorin in pivotal Phase III studies [4].

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