Single Ascending Dose Tolerability, Pharmacokinetic–Pharmacodynamic Study of Dihydropyrimidine Dehydrogenase Inhibitor Ro 09-4889

S. Eralp Bellibas, Indra Patel, Emmanuel Chamorey, Bettyna Brivet, Ernest D. Bush, Catherine Kircher, Stephane Nave, Ludger Banken, Nicole Renée, and Gérard Milano

1Department of Clinical Pharmacology, Hoffmann-La Roche Inc., Nutley, New Jersey, and 2Centre Antoine Lacassagne, Oncopharmacology Laboratory, Nice, France

ABSTRACT

Purpose: Ro 09-4889 was designed to enhance the anticancer efficacy of capecitabine (Xeloda) by generating a dihydropyrimidine dehydrogenase inhibitor (DPDi) 5-vinyluracil (5-VU) preferentially in tumor tissues. This study assessed the tolerance to Ro 09-4889 treatment, and related pharmacokinetic and pharmacodynamic data such as inhibition of DPD activity in peripheral blood mononuclear cells (PBMCs) and plasma uracil levels.

Experimental design: This was a single-center, double-blind, placebo-controlled, single-dose escalation study in 64 healthy male volunteers at 1-, 5-, 20-, 50-, 75-, 100-, and 200-mg oral dose of Ro 09-4889. Also, food effect was assessed separately in a group dosed with 20 mg of the compound.

Results: No serious adverse effects or significant laboratory and electrocardiogram abnormalities were observed during the study. Ro 09-4889 has a short elimination half-life (t1/2) of 0.5 h, followed by metabolites 5′-deoxy-5-vinyluridine (5′-DVUR), 5′-deoxy-5-vinylcytidine (5′-DVCR), and 5-VU with t1/2 of 1.3, 1.2, and 2 h, respectively. The major metabolite excreted in urine was 5-DVCR (45% of dose). The inhibition of PBMC DPD activity and the increase in plasma uracil were related to Ro 09-4889 dose. DPD inhibition versus dose and uracil AUC (area under the curve) versus dose were modeled using the Emax model with a baseline effect. The model-predicted ED50 value was 100 mg.

Conclusion: Single oral doses of Ro 09-4889 ranging from 1 to 200 mg were well tolerated. On the basis of these findings, a 10-to-30-mg dose range of Ro 09-4889 combined with capecitabine could be appropriate for further evaluation in cancer patients.

INTRODUCTION

The use of fluoropyrimidines in the therapy of solid tumors is now expanding through the clinical development of new oral formulations (1). Orally administered agents have several potential advantages over parenteral formulations, mainly because they are more convenient for patients and generate no morbidity inherent in the use of indwelling catheters and infusion pumps (2). Most so-called oral fluoropyrimidines include a dihydropyrimidine dehydrogenase (DPD) inhibitor (3). DPD is a ubiquitous enzyme that, at two levels at least, produces a marked variability in the pharmacological behavior of fluoropyrimidines. The first level concerns pharmacokinetics, because DPD controls the first step in 5-fluorouracil (5-FU) liver catabolism and DPD-deficient patients have been shown to be prone to develop more or less severe 5-FU-related toxicity (4). The second level relates to pharmacodynamics, because 5-FU catabolism at the target level itself has been identified, from both experimental (5) and clinical (6) data, as a factor of resistance to the 5-FU antitumor response. Hence, pharmacological DPD inhibition is of clinical relevance because it optimizes 5-FU-based therapy by attenuating unpredictable 5-FU pharmacokinetic variability and suppressing a major factor of tumor resistance to 5-FU.

Capecitabine (Xeloda) was rationally synthesized to be efficiently absorbed from the gastrointestinal tract as a prodrug and to be converted to 5-FU through the action of thymidine phosphorylase (TP) and preferentially in tumors by exploiting the high activity of TP in malignant tissue (7). The compound Ro 09-4889 was designed to optimize the anticancer efficacy of capecitabine by generating a DPD inhibitor, 5′-vinyluracil (5′-VU), preferentially in tumors (Fig. 1). Hence, Ro 09-4889 follows an identical pathway of enzymatic activation to capecitabine. It is converted by esterases abundant in human liver to 5′-deoxy-5-vinylcytidine (5′-DVCR), then to 5′-deoxy-5-vinyluridine (5′-DVUR) by cytidine deaminase, and then to 5-vinyluracil (5-VU), the active metabolite, by TP (Fig. 2). 5-VU inhibits DPD by both mechanism-based inhibition and classically defined competitive inhibition, thereby blocking the catabolism of 5-FU (data in file). Therefore, by combining Ro 09-4889 with capecitabine, it can be anticipated that a higher exposure of 5-FU in tumors could be achieved, thus optimizing efficacy without a detrimental increase in toxicity. This study investigated the tolerability, safety, pharmacokinetics, and pharmacodynamics of a single oral intake of Ro 09-4889 in healthy volunteers. As regards the pharmacodynamics, the impact of drug administration on peripheral blood mononuclear cell (PBMC) DPD activity was taken into consideration as well as the plasma levels of uracil, a natural substrate of DPD. The
information obtained from this study will be useful in optimizing study designs (dose range, dosage frequency, and duration of dosing) for subsequent Phase I safety/efficacy studies in cancer subjects.

MATERIALS AND METHODS

Overall Study Design

This was a Phase 1, single-center, double-blind, placebo-controlled, single ascending dose study. Sixty-four male volunteers were allocated among the seven different dosage groups (1, 5, 20, 50, 75, 100, and 200 mg) in which study medication was administered within 15 min of eating a 94-calorie standard breakfast and one overnight fasted group (20 mg). Within each dosage group, two subjects were randomly assigned to receive placebo. Subjects were admitted to the investigational site the evening before study drug administration and baseline assessments were performed.

The type of adverse event, date of onset, date of resolution, treatment for the adverse event, and outcome were based on information volunteered by or elicited from the subject, or from observations made by the investigator or staff. The investigator evaluated the intensity of each adverse event, related to the dose tested, and whether or not the event was serious. An adverse event was considered to be serious if it clearly presented, or could be expected to present, a threat to the well-being of the subject. Investigators were required to report serious adverse events to the sponsor within 1 working day.

Pharmacokinetic Parameters

Plasma and urine concentrations of Ro 09-4889 and its three metabolites (5'-DVUR, 5'-DVCR, and 5-VU) were measured at the time points indicated in Table 1.

The plasma pharmacokinetic parameters were estimated using WinNonlin Professional (Version 3.0, Pharsight Corporation, Mountain View, CA) based on model-independent techniques according to the Roche internal “Guideline for calculation and analysis of noncompartmental pharmacokinetic parameters” issued in February 1998.

The cumulative amount of Ro 09-4889 and its three metabolites excreted in urine was calculated at 6, 12, 24, 36, and 48 h postdose.

The method for quantifying of Ro 09-4889, 5'-DVUR, 5'-DVCR, and 5-VU in human plasma was validated, from 1 to 1000 ng/ml for Ro 09-4889, 5'-DVUR, and 5'-DVCR, and from 2 to 1000 ng/ml for 5-VU. The analysis procedure is briefly summarized as follows: 200 μl of acidified sodium fluoride/potassium oxalate human plasma samples were fortified with internal standards. The samples were extracted by solid-phase-extraction with Oasis cartridges from Waters Corporation. The reconstituted extract solution was injected into a triple quadrupole liquid chromatography/tandem mass spectrometry (MS/MS) system for separation and detection by positive and negative elec-
High-performance liquid chromatography separation was performed on a 100 × 2 mm 5 μm Phenomenex Columbus C18 column with water and methanol containing ammonium acetate as mobile phase. Detection was conducted in selective reaction monitoring mode on the MS/MS instrument.

The method used to quantify Ro 09-4889, 5'-DVUR, 5'-DVCR, and 5-VU in human urine was validated, from 2 to 1000 ng/ml for Ro 09-4889, 5'-DVUR, and 5-VU and from 5 to 1000 ng/ml for 5'-DVCR. The analysis procedure is briefly summarized as follows: 200 μl urine samples were fortified with internal standards. The samples were extracted by solid-phase-extraction with Oasis HLB cartridges from Waters Corporation. The reconstituted extract solution was injected into a triple quadrupole liquid chromatography/MS/MS system for separation and detection by positive and negative electrospray ionization. High-performance liquid chromatography separation and detection were performed using the identical method as for the plasma samples.

### Assay Performance

Performance of the assay during sample analysis was determined from the results of the quality control samples and back-calculated concentrations of calibration standards. Precision for the human plasma standards and quality control samples (as measured by the percentage coefficient of variation) ranged from 5.4 to 9.3% for Ro 09-4889, from 3.9 to 10.2% for 5'-DVUR, from 4.4 to 10.4% for 5'-DVCR, and from 5.2 to 9.4% for 5-VU. Accuracy for the human plasma standards and quality control samples (as measured by the percentage relative error) ranged from −3.0 to 6.4% for Ro 09-4889, from −6.8 to 6.0% for 5'-DVUR, from −4.4 to 3.6% for 5'-DVCR, and from −4.5 to 3.8% for 5-VU.

### Table 1: Schedule of study assessments

<table>
<thead>
<tr>
<th>Nominal study day of assessment*</th>
<th>−21 to −1 screening</th>
<th>1 (0 h)</th>
<th>2 (24 h)</th>
<th>3 (48 h)</th>
<th>8 (168 h)</th>
<th>11 (240 h)</th>
<th>15 ± 2 days follow-up (336 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Informed consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical examination</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medical history</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vital signs&lt;sup&gt;a&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECG&lt;sup&gt;c&lt;/sup&gt;</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dosing</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pharmacokinetic measurements&lt;sup&gt;d&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DPD activity in PBMCs&lt;sup&gt;e&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Plasma uracil level&lt;sup&gt;f&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine uracil/creatinine and drug levels&lt;sup&gt;g&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory safety tests&lt;sup&gt;g&lt;/sup&gt;</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drugs of abuse&lt;sup&gt;h&lt;/sup&gt;</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adverse events</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Continuously throughout the study</td>
</tr>
</tbody>
</table>

<sup>a</sup> Starting scheduled hour of the day is in parentheses.
<sup>b</sup> Predose and at 1, 2, 4, 8, and 48 h postdose and on day 15.
<sup>c</sup> ECG, electrocardiogram; DPD, dihydropyrimidine dehydrogenase; PBMC, peripheral blood mononuclear cell.
<sup>d</sup> Predose and at 1, 2, 3, 4, 5, 6, 8, 12, 24 and 48 h postdose.
<sup>e</sup> Predose and at 1, 2, 4, 8, 24, 48 h and on day 8 (168 h), day 11 (240 h) and day 15 (336 h).
<sup>f</sup> Predose and at 1, 2, 4, 8, 12, 16, 24, 36, and 48 h postdose and follow-up day 15.
<sup>g</sup> Time 0 to 6 h, 6 to 12 h, 12 to 24 h, 24 to 36 h and 36 to 48 h postdose, some additional spot samples were collected at day 8, day 11, and day 15.
<sup>h</sup> Screening, predose, 48 h postdose, and follow-up day 15.
<sup>i</sup> At randomization.
<sup>j</sup> Predose only.

### Table 2: Summary of adverse events

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Treatment (%)</th>
<th>Placebo (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>13 (27)</td>
<td>4 (25)</td>
</tr>
<tr>
<td>Abdominal pain/cramp or epigastralgia</td>
<td>4 (8)</td>
<td>0</td>
</tr>
<tr>
<td>Back pain</td>
<td>2 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Toothache</td>
<td>2 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Perineal pain</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Vein pain</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Nausea</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>1 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Asthenia</td>
<td>1 (2)</td>
<td>1 (6)</td>
</tr>
</tbody>
</table>
PK-PD Study of DPD Inhibitor Ro 09-4889

E/

- deoxy-5-vinylcytidine; 5"/H11032
- DVCR, 5
- VU, 5-vinyluracil.
- DVUR, 5
- /H11032
- calculation; 5
- error) ranged from −5.0 to 2.8% for Ro 09-4889, from −5.8 to 2.0% for 5"-DVUR, from −8.7 to 6.0% for 5"-DVCR, and from −3.0 to 4.0% for 5-VU.

PD Parameters

PD parameters were DPD inhibition in PBMCs, plasma uracil levels, and urine uracil levels.

Predicted pharmacodynamic (PD) parameters were modeled based on an E\textsubscript{max} model with a baseline effect E\textsubscript{0} using WinNonlin Professional (Version 3.0, Pharsight Corporation, Mountain View, CA).

DPD Activity in PBMCs. DPD activity in PBMCs was measured at the time points indicated in Table 1. DPD activity was measured using a radio-enzymatic method, described previously (8). The following PD parameters were determined: lowest level (minimal) of DPD enzyme activity (E\textsubscript{min}), DPD E\textsubscript{max} (%) is the minimal DPD activity expressed as the percentage of the individual baseline value; time of minimal DPD activity (T\textsubscript{min}); and DPD activity recovery time (T\textsubscript{rec} = time to 90% recovery) is the time DPD takes to recover to 90% of the baseline activity.

Plasma Uracil Levels. Plasma uracil levels were measured at the time points indicated in Table 1 and the following PD parameters were determined from the plasma uracil concentration-time data: maximum plasma uracil concentration (C\textsubscript{max}) and area under the plasma uracil concentration-time curve from zero to 48 h (AUC\textsubscript{0-48 h}).

The method for quantifying uracil in human plasma was validated, from 10 to 3000 ng/ml for uracil (and 5 to 3000 ng/ml for 5-FU). The analysis procedure is briefly summarized as follows: 200 \mu l of acidified sodium fluoride/potassium oxalate human plasma samples were fortified with internal standards. Acetonitrile was added to precipitate the proteins. Supernatant reconstituted in water. The reconstituted solution was injected into a triple quadrupole liquid chromatography/MS/MS system for separation and detection by negative electrospray ionization. High-performance liquid chromatography separation was performed on a 150 × 2 mm 5 \mu m Zorbax Bonus-RP column with water and methanol containing ammonium acetate as mobile phase. Detection was conducted in selective reaction monitoring mode on the MS/MS instrument.

Urine Uracil Levels. Urine uracil and creatinine levels were measured at the time points indicated in Table 1. The cumulative amount of uracil excreted at 6, 12, 24, 36, and 48 h postdose was calculated.
ness and reconstituted in water. The reconstituted solution was injected to triple quadrupole liquid chromatography/MS/MS system for separation and detection by negative electrospray ionization. High-performance liquid chromatography separation and detection were performed as for the plasma samples.

**Statistical Analyses**

All pharmacokinetic and PD parameters were subjected to descriptive analysis including arithmetic means, SDs, geometric means, coefficients of variation, and ranges.

Pharmacokinetic-PD relationships were explored by examining the behavior of plasma and urine uracil level and DPD inhibition in lymphocytes as a function of plasma exposure of Ro 09-4889 and/or 5-VU.

Predicted AUC as a function of Ro 09-4889 dose were modeled with sigmoid $E_{\text{max}}$ model with a baseline effect using the following:

$$E = E_0 + (E_{\text{max}} - E_0) \cdot \frac{C^\gamma}{C^\gamma + EC_{50}}$$

where $E_{\text{max}}$ is the maximal effect, $E_0$ is the baseline effect, $EC_{50}$ is the drug concentration producing 50% of $E_{\text{max}}$, and the exponential constant $\gamma$ controls the slope of the sigmoid-shape curve.

**RESULTS**

**Safety Data**

The overall incidence of adverse events was similar among dosage groups. The most frequent adverse event was headache, which was reported by 25% of placebo-treated subjects and 27% of Ro 09-4889-treated subjects. No adverse event demonstrated a dose relationship. A summary of adverse events by dosage group is presented in Table 2. Adverse events in each dosage group were mild or moderate in severity. No significant biological and electrocardiogram abnormalities were reported.

**PK Data**

Mean data and pharmacokinetic (PK) profiles for Ro 09-4889 and three metabolites obtained in the 75-mg, 100-mg, and 200-mg groups are presented in Table 3 and Fig. 3. The main circulating species was 5'-DVUR followed by 5'-DVCR and 5-VU.

### Table 4  Effect of food on the pharmacokinetic (PK) parameters of Ro 09-4889 and its metabolites (mean ± SD)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Food</th>
<th>$C_{\text{max}}$ (ng/ml)</th>
<th>$t_{\text{max}}$ (h)</th>
<th>$AUC_{0-\infty}$ (ng/h/ml)</th>
<th>$t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ro 09-4889</td>
<td>Fed</td>
<td>5.07 ± 3.18</td>
<td>1.17 ± 0.41</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Fasted</td>
<td>1.92 ± 0.86</td>
<td>1.00 ± 0.00</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5'-DVUR</td>
<td>Fed</td>
<td>85.10 ± 23.16</td>
<td>2.17 ± 0.98</td>
<td>305.15 ± 77.86</td>
<td>1.38 ± 0.26</td>
</tr>
<tr>
<td></td>
<td>Fasted</td>
<td>111.78 ± 33.75</td>
<td>1.17 ± 0.41</td>
<td>250.88 ± 46.72</td>
<td>1.10 ± 0.13</td>
</tr>
<tr>
<td>5'-DVCR</td>
<td>Fed</td>
<td>74.91 ± 20.38</td>
<td>1.33 ± 0.52</td>
<td>227.70 ± 24.33</td>
<td>1.23 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>Fasted</td>
<td>55.55 ± 15.61</td>
<td>1.00 ± 0.00</td>
<td>102.11 ± 23.67</td>
<td>1.07 ± 0.12</td>
</tr>
<tr>
<td>5-VU</td>
<td>Fed</td>
<td>8.94 ± 3.07</td>
<td>3.00 ± 1.10</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Fasted</td>
<td>13.32 ± 5.67</td>
<td>1.83 ± 1.17</td>
<td>50.13 ± 19.30</td>
<td>1.76 ± 0.52</td>
</tr>
</tbody>
</table>

$C_{\text{max}}$, mean maximum plasma uracil concentration; $t_{\text{max}}$, time of $C_{\text{max}}$; $AUC_{0-\infty}$, Area under the curve from zero and extrapolated to the infinity; $t_{1/2}$, elimination half-life; NA, not applicable, either because of levels below the quantification limit or insufficient data points to make a PK calculation; 5'-DVUR, 5'-deoxy-5-vinyluridine; 5'-DVCR, 5'-deoxy-5-vinylcytidine; 5'-VU, 5-vinyluracil.
Cmax for Ro 09-4889 increased dose-dependently ranging from 1.51 to 42.9 ng/ml with tmax ranging from 1.2 to 2.0 h. Ro 09-4889 and the three metabolites were quickly eliminated. Mean values of t1/2 for Ro 09-4889, 5’-DVUR, 5’-DVCR, and 5-VU were 0.5 h, 1.2 h, 1.3 h, and 2 h, respectively.

For the final metabolite with pharmacological efficacy, 5-VU, food caused a 32% decrease in Cmax with values of 13.3 ± 5.67 ng/ml and 8.94 ± 3.07 ng/ml in fasted and fed groups, respectively (Table 4). The intersubject variability in the PK parameters was similar in the 20-mg dosage group in fed and fasted subjects.

The major metabolite excreted in urine was 5’-DVCR, which represented almost one-half of the administered dose (Table 5). Recovery ratios (percentage of Ro 09-4889 dose recovered in urine) in the 200-mg dosage group at 48 h for Ro 09-4889, 5’-DVUR, 5’-DVCR, and 5-VU were 0.34, 2.39, 45.4, and 1.86%, respectively. These ratios were consistent with those seen in other dosage groups.

**PD Data**

**DPD Inhibition in PBMCs.** DPD inhibition in PBMCs by time and dosage group is shown in Fig. 4A. A sustained DPD inhibitory effect was not seen in either the 1-mg or 5-mg dosage groups. The 20-mg dosage group had a 70% decrease in basal DPD activity at 4 h postdose; DPD activity returned to 50 and 70% of the basal level at 24 and 48 h postdose, respectively. The 50-, 75-, and 100-mg dosage groups decreased basal DPD activity 80–90% reaching a minimum DPD activity at 7 h postdose.

Mean Emin, tmin, and t90R are summarized in Table 6. With the exception of the 50-mg and 100-mg dosage groups, the time when DPD recovered to 90% of the baseline activity (T90R) was dose dependent and ranged from 35 to 300 h.

**Uracil Levels.** Mean plasma uracil levels to 48 h postdose are summarized by dosage group in Fig. 4B. Both the mean maximum plasma uracil concentrations (Cmax) and the AUC48 h values were dose dependent ranging from 15 to 300 ng/ml and 676 to 4833 ng·h/ml (Table 6). Tmax was 8 h for all dosage groups. Mean urine uracil levels to 48 h postdose are summarized by dosage group in Fig. 4C. Mean urine concentration of uracil was dose dependent. DPD inhibition in PBMCs showed a nonlinear correlation with plasma uracil exposure with an R2 value of 0.82 as shown in Fig. 5.

**PK/PD Modeling Results.** Observed and predicted DPD Emax values by Ro 09-4889 dose are shown in Fig. 6A. The 20-mg dosage group is marked with an arrow because 20 mg was believed to be a threshold value for nonsystemic DPD inhibition.

---

**Table 5** Mean urinary recovery ratios of Ro 09-4889 and its metabolites (%)

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Ro 09-4889</th>
<th>5’-DVUR*</th>
<th>5’-DVCR</th>
<th>5-VU</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.55 ± 0.24</td>
<td>2.23 ± 0.89</td>
<td>48.22 ± 8.63</td>
<td>0.00</td>
</tr>
<tr>
<td>5</td>
<td>0.77 ± 0.52</td>
<td>0.98 ± 0.60</td>
<td>48.22 ± 4.18</td>
<td>0.20 ± 0.05</td>
</tr>
<tr>
<td>20</td>
<td>0.61 ± 0.48</td>
<td>1.94 ± 0.80</td>
<td>42.02 ± 4.98</td>
<td>0.55 ± 0.14</td>
</tr>
<tr>
<td>50</td>
<td>0.67 ± 0.22</td>
<td>1.42 ± 0.47</td>
<td>45.17 ± 5.47</td>
<td>0.82 ± 0.29</td>
</tr>
<tr>
<td>75</td>
<td>0.51 ± 0.19</td>
<td>1.61 ± 1.04</td>
<td>37.61 ± 7.99</td>
<td>1.19 ± 0.43</td>
</tr>
<tr>
<td>100</td>
<td>0.46 ± 0.15</td>
<td>3.56 ± 2.00</td>
<td>47.37 ± 6.54</td>
<td>1.45 ± 0.33</td>
</tr>
<tr>
<td>200</td>
<td>0.34 ± 0.29</td>
<td>2.39 ± 0.90</td>
<td>45.40 ± 4.74</td>
<td>1.86 ± 0.79</td>
</tr>
</tbody>
</table>

*: 5’-DVUR, 5’-deoxy-5-vinyluridine; 5’-DVCR, 5’-deoxy-5-vinylcytidine; 5-VU, 5-vinyluracil.
inhibition. Ro 09-4889 at a dosage level higher than 20 mg may result in losing the tumor-selective action of capecitabine because of increased 5-FU in both tumor tissues and plasma. Consequently, a 20-mg dose of Ro 09-4889 was considered to be the tumor-selective DPD inhibitor dose.

Observed and predicted plasma uracil AUC from baseline to 48 h postdose are shown in Fig. 6B. The predicted Ro 09-4889 dose is 100 mg for ED$_{50}$.

**DISCUSSION**

The combined use of a DPD inhibitor with an oral fluoropyrimidine offers several potential pharmacological advantages mainly on account of the less unpredictable variability in 5-FU pharmacokinetics and the elimination of a cellular factor of tumoral resistance. For instance, it has been shown that, after the administration of the DPD inhibitor eniluracil in association with oral 5-FU, the clearance of 5-FU was essentially predictable on the basis of the individual value of the estimated creatinine clearance (9). On the other hand, previous experimental data have shown that 5-FU-resistant tumoral cells exhibiting high DPD activity recovered drug sensitivity after the application of a specific DPD inhibitor (10). Interestingly, the combined use of the DPD inhibitor prodrug Ro 09-4889 and the 5-FU prodrug, capecitabine, could offer additional potential benefits. This is explained by the fact that both the 5-FU production from capecitabine and the delivery of the DPD inhibitor 5-VU are strictly dependent on TP activity, which is known to be overexpressed in malignant tissue (11, 12). Therefore, it is reasonable to anticipate that, because of the higher TP activity in tumors, a more intense exposure of 5-FU in malignant cells will be achieved by combining Ro 09-4889 and capecitabine (continuous production from deoxy-5-fluorouridine and less degradation by DPD), thereby optimizing the efficacy without a detrimental increase in the drug-related toxicity. This tumoral selectivity conferred by capecitabine is illustrated by a recent clinical study by Schüller et al. (13) in which colorectal cancer patients received capecitabine 2.5 g/m$^2$/day for 5–7 days before surgical resection of the primary tumor and for hepatic metastases. 5-FU concentrations in primary tumors were on average 3.2-fold higher than in adjacent healthy tissue and, importantly, 21-fold higher than in plasma.

The presence of 5-FU can impact on DPD activity (14, 15). Thus, in theory, after the administration of a drug combination with a DPD inhibitor and a fluoropyrimidine, it can be difficult to distinguish between the PD effects attributable to DPD inhibition and those resulting from 5-FU itself. It was thus judged preferable to perform a separate Phase I study of the DPD inhibitor prodrug Ro 09-4889 to examine the specific pharmacological consequences on DPD activity and on other relevant biochemical variables. The present study indicates that single oral doses of Ro 09-4889 varying from 1 mg to 200 mg were safe and well tolerated by healthy male volunteers. No serious adverse events or premature withdrawals because of adverse events were reported. The overall incidence of minor adverse events was similar in placebo-treated and Ro-4889-treated subjects, and no adverse events showed a Ro 09-4889 dose relationship. In addition, no clinically significant laboratory, vital sign, or electrocardiogram abnormalities were reported.

From the PK part of the study, it appears that the circulating levels of 5-VU (final metabolite and DPD inhibitor) were particularly low. This suggests that a systemic DPD inhibition is improbable and DPD inhibition through 5-VU will preferentially occur in tissues expressing TP. It was found that food caused a 32% decrease in the C$_{max}$ value of 5-VU. Thus, food is likely to raise a problem for the combination of Ro 09-4889 with capecitabine because the latter is recommended to be taken with food (16).

![Fig. 5 Dihydropyrimidine dehydrogenase (DPD) E$_{min}$ levels versus plasma uracil AUC$_{48h}$ values. E$_{min}$, lowest level (minimal) of DPD enzyme activity; AUC$_{48h}$, area under the curve from time zero to 48 h.](clincancerres.aacrjournals.org)
The impact of Ro 09-4889 on DPD activity was examined in PBMCs, although the liver is the main site for 5-FU clearance. Nevertheless, a previous study has shown the existence of a satisfactory correlation between DPD activity in PBMCs and normal hepatic tissue of identical subjects (17). However, the relevance of liver DPD inhibition is limited in the case of a capecitabine–Ro 09-4889 combination because capecitabine generates relatively low 5-FU circulating concentrations (18). The inhibition of DPD activity and the increase in plasma uracil were related to Ro 09-4889 dosage. Plasma uracil thus appears to be a reliable surrogate marker of pharmacological DPD inhibition. Similar conclusions concerning uracil levels and DPD inhibition were reported in recent studies (9, 19). One can question the need to achieve a complete inhibition of DPD activity. Significant DPD activity is present in intestinal mucosa and hematological cells (20). It is thus conceivable that the presence of DPD in these 5-FU-targeted tissues plays a detoxifying role and may limit the cytotoxic impact of 5-FU. Consequently, complete DPD inhibition could be detrimental to the drug therapeutic index. This view is strengthened by recent experimental data showing that the transduction of DPD gene attenuates 5-FU cytotoxicity on murine hematopoietic progenitor cells (21). Bearing this in mind, one can recommend a Ro 09-4889 dose of 20 mg because, in the present study, it has been shown to lead to DPD $E_{\text{min}}$ levels $\approx 80\%$ while producing a minimal increase in plasma uracil levels. More specific and sophisticated pharmacological studies are needed to check whether a 20-mg dose is also sufficient to significantly decrease DPD activity in tumor tissue. At the dose of 20 mg of Ro 09-4889, the average time needed for DPD to recover 90% of the baseline activity was close to 90 h (Table 6). Because of the profound and prolonged inhibition of DPD activity induced by eniluracil, and given the fact that Grem et al. (9) highlighted the occurrence of fatal toxicities (notified by CTEP) in patients rechallenged with 5-FU 4 and 5 weeks after eniluracil, a washout period of 2 months was recommended for eniluracil. The present data argue in favor of a much shorter and more clinically manageable 1-week wash-out period when using a Ro 09-4889 dose in the proposed 10–30 mg range. We previously reported on a population study showing that DPD activity was a mean 15% lower in women than in men (22). To limit the factors of variability, only male subjects were recruited for participation in this study. However, this slight difference in DPD activity does not suggest the need for different Ro 09-4889 dosages in women. The pharmacological and clinical observations reported in the present study may be useful for future development of Ro 09-4889 given in combination with capecitabine. Hattori et al. (23) recently reported encouraging preliminary preclinical data on human lung carcinoma xenografts with more than additive growth inhibition resulting from the combination capecitabine–Ro 09-4889. Because the activation pathways for Ro 09-4889 are the same as those for capecitabine, it is anticipated that forthcoming clinical investigations with the drug combination...
will have to explore the possibility of PK interactions between the two drugs.

REFERENCES

Single Ascending Dose Tolerability, Pharmacokinetic–Pharmacodynamic Study of Dihydropyrimidine Dehydrogenase Inhibitor Ro 09-4889

S. Eralp Bellibas, Indra Patel, Emmanuel Chamorey, et al.


Updated version  Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/10/7/2327

Cited articles  This article cites 20 articles, 11 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/10/7/2327.full.html#ref-list-1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.