CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20-896/S-006

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Date: September 12, 2000
From: John K. Leighton, Ph.D., DABT JKL 9/12/00
Acting Pharm/Tox Team Leader
To: Files for NDA 20-896
Re: Approvability for Pharmacology and Toxicology
Xeloda™

Xeloda, or Capecitabine, is supplied as film coated tablets. Capecitabine is actually a pro-drug, with the active metabolite being 5-fluorouracil. Xeloda is currently approved for the treatment of patients with metastatic breast cancer resistant to both paclitaxel and an anthracycline-containing chemotherapy regimen. In this supplemental application, Hoffmann-La Roche seeks approval of Xeloda for the first-line treatment of patients with metastatic colorectal carcinoma. Dr. McGuinn reviewed several studies for this supplemental NDA that are relevant to the package insert. These include:

- Excretion of metabolic products of Capecitabine into milk in mice,
- Inhibition of Cytochrome P450s by Capecitabine and its metabolites
- 2-year carcinogenicity study of Capecitabine in mice.

The most important finding in Dr. McGuinn's review was the inadequacy of the mouse carcinogenicity study to conclusively demonstrate that Capecitabine is not carcinogenic in this species. This conclusion differs from the sponsor's conclusion. The rationale for Dr. McGuinn's conclusion is that the doses used in this study were too low relative to the clinical dose to full assess carcinogenic potential. Dr. McGuinn notes that there is a published report indicating that the active metabolite of Capecitabine, 5-fluorouracil, is carcinogenic in mice. Carcinogenicity studies are not necessary to support the approval of Xeloda in this patient population, and additional studies are not necessary at this time.

A labeling review was provided by Dr. McGuinn and I agree with the requested changes.

Recommendations: The pharmacology and toxicology data supports approval of this NDA. There are no outstanding issues.

Original NDA
cc: Div file
HFD-150
DMcGuinn
MPelosi
AMartin

Pelosi

SEP 8 2000

Division of Oncology Drug Products, HFD-150
Review and Evaluation of Pharmacology and Toxicology Data
NDA Review, review #2 - (page 21 is missing)

NDA: 20-896 **Type:** SE1, BZ, SNC **Serial #:** 006
Submission: Supplemental NDA Received September 20, 1999
Completed

Key Words: Capecitabine, Xeloda, colorectal, colon, cancer, and prodrug.

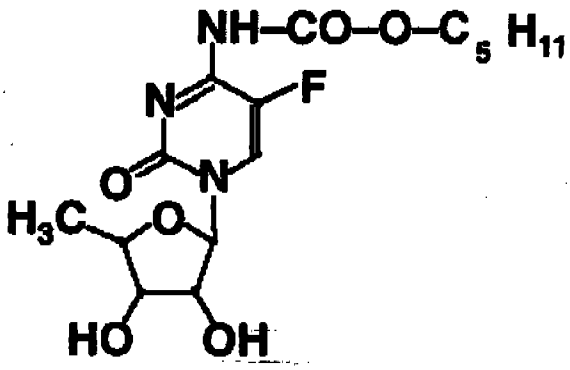
Information to be conveyed to the sponsor: YES

Reviewer: W. David McGuinn, Jr., Ph. D., D.A.B.T.

Sponsor: Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, NJ 07110-1199

Drug Name:
Code name: Ro 09-1978
Generic Name: Capecitabine
Trade Name: XELODA
Chemical Name: N^4-Pentyloxycarbonyl-5'-deoxy-5-fluorocytidine
FW: 359.35

Structure:



Class cytotoxin, 5-Fluorouracil pro-drug
Indications: The first-line treatment of patients with metastatic colorectal carcinoma.

Related drugs: 5-FU, Furtulon

Related INDs: ( ) Hoffman-La Roche Inc.
( ) discontinued)

Related NDAs: None

Route of Administration: PO

Formulation: film–coated tablets containing 150 mg Capecitabine or 500 mg Capecitabine. The inactive ingredients are: anhydrous lactose, croscarmellose sodium, hydroxypropyl methylcellulose, microcrystalline cellulose, magnesium stearate and purified water. The peach or light peach film coating contains hydroxypropyl methylcellulose, talc, titanium dioxide, and synthetic yellow and red iron oxides.

Dose: 2500 mg/m^2 daily for two weeks followed by a one-week rest, 3-week cycles

Previous Clinical Studies.

In a phase I study with XELODA, the MTD dose as a single agent in the treatment of patients with solid tumors was 3000 mg/m^2 /dX14 q21d. The dose–limiting toxicities were diarrhea and leukopenia.

The sponsor did a large phase II multicenter trial with 162 patients with advanced or metastatic breast cancer. This heavily pretreated patient population was refractory to previous paclitaxel therapy. Most patients were also resistant (41%) or had failed (26%) previous anthracycline therapy and 82% had been exposed to 5–FU. XELODA was administered at a dose of 2510 mg/m^2/d X14 q21d. The sponsor claims a median survival was 384 days. Again, the major toxicities were diarrhea and leukopenia. Other studies in other diseases are ongoing. The Food and Drug Administration has approved XELODA for the treatment of locally advanced or metastatic breast cancer after failure of paclitaxel and an anthracycline–containing chemotherapy regimen.

In the study that supports this application, 603 patients were treated with XELODA at a daily dose of 2500 mg/m^2 for two weeks followed by 1-week rest. The control arm received 5-FU, 425-mg/m^2 IV bolus, and leucovorin, 20 mg/m^2, on days one through five every 28 days. The control arm for this trial is no longer the standard of care.

Table of contents:

Studies Not Reviewed:

The sponsor submitted many studies in this NDA. I have reviewed only those relevant to the sponsor's new label claims. For a complete list of all the studies in this submission, see Volume 1, page 13, NDA 20-896.

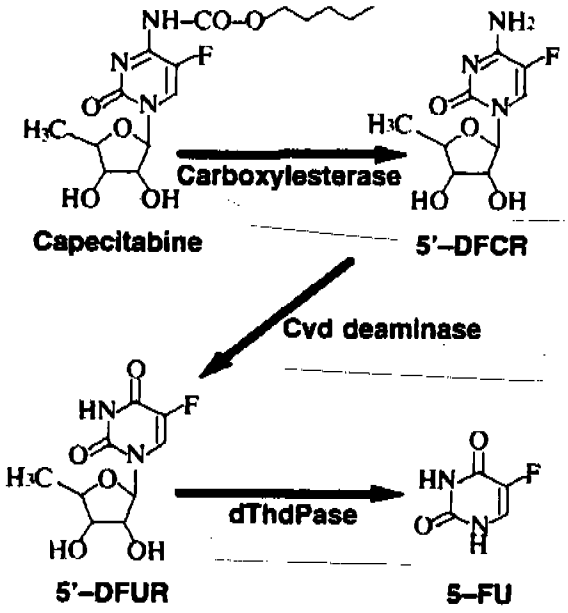
Rationale

In 1982, A. Kono *et al.* (1982, *Chem. Pharmacol. Bull.* **31**:175-178) showed that some animal tumors contained higher levels of pyrimidine nucleoside phosphorylase (PyNPase) than did normal tissue. PyNPase is a reversible enzyme normally responsible for adding ribose-1-phosphate to uracil to form uridine. In the reverse direction the enzyme can hydrolyze 5-deoxy-ribose from 5'-deoxy-5-fluorouridine (5'-DFUR) to form 5-FU. If PyNPase is over-expressed in a tumor, this reaction would allow higher concentrations of 5-FU to form in tumor than in normal tissue. This increased expression might increase the efficacy of this treatment, and consequently the therapeutic index, over that of standard 5-FU treatment. Japan has approved the oral prodrug, 5'-DFUR or FURTULON, for use in cancer chemotherapy. This drug's dose-limiting toxicity is diarrhea, caused by the presence of high concentrations of PyNPase in the intestinal tract. Thus, much of the drug never reaches the tumor and causes significant local toxicity.

To overcome this absorption problem Roche developed _____ This drug is not a substrate for gastric PyNPase, so it is absorbed past the gastric lumen without the loss of 5-deoxy-ribose. In the liver, acylamidase (acylamide amidohydrolase, EC 3.5.1.) cleaves _____ group to form 5'-deoxy-5-fluorocytidine (5'-dFCR). Cytidine deaminase then hydrolyzes 5'-dFCR to 5'-DFUR (also 5'-FUDR below) in tissues and tumor throughout the body. Some tumor tissues may express higher concentrations of cytidine deaminase than most normal tissue.

Roche abandoned efforts to develop _____ for chemotherapy when they found it was a poor substrate for acylamidase in humans. Subsequently, Roche developed Ro 09-1978 by replacing _____ with a N^4-pentyloxycarbonyl group. Roche claims that the activity of acylamidase is 50 times greater for this compound than for _____ This increase in activity shifts the kinetics of the metabolic pathway and increases the available

concentrations of 5'-DFUR. Roche hopes to market this compound as an oral 'tumor specific' chemotherapeutic agent. The graph below shows this catalytic scheme.



Glossary of Abbreviations:

5'–DFCR	5'–Deoxy–5–fluorocytidine
5'–DFUR	5'–Deoxy–5–fluorouridine (doxifluridine, FURTULON)
DPD	Dihydropyrimidine dehydrogenase
dThdPase	Thymidine phosphorylase, same as Pyrimidine nucleoside phosphorylase
FBAL	α–Fluoro–β–alanine
FdUMP	2'–Deoxy–5–fluorouridine mono–phosphate
FUH_2	Dihydrofluorouracil
FUPA	α–Fluoro–β–ureidopropionate
FUTP	Fluorouridine tri–phosphate
5–FU	5–Fluorouracil
FUdR	2'–Deoxy–5–fluorouridine
LC–MS/MS	Liquid chromatography–tandem mass spectrometry with ion–spray interface
PyNPase	Pyrimidine nucleoside phosphorylase
TS	Thymidylate synthase
UrdPase	Uridine phosphorylase

I have excerpted portions of this review directly from the Sponsor's submission.

Review:

physical characteristics:

pK_a = 8.8
partition coefficient (octanol/ pH 7.4 Buffer) = 4.5

Reproductive Toxicology

3125 H Onodera, 1998, Transfer of the fetus and milk (*sic*) in mice after single oral administration of ^{14}C- Ro 09-1978. RR J-146751. Volume 21 page 1.

Animal	Female BDF1 _____ (34.1 to 39.9 g at time of dosing) Group 1 – gestation, mated at 9 weeks, 16 day of pregnancy Group 2 – lactating, mated at 9 weeks, 9th or 10th day after delivery
Drug	^{14}C-Ro 09-1978/602 (Lot 12352B9g Mo) labeled at position 6 of fluorouracil moiety, 2.47 MBq/mg, 96% radio chemical purity R0 09-1978 unlabeled (Lot 24289-74-A)
Dose	198 mg/kg (594 mg/m^2), 6.25 MBq/kg
Vehicle	citrate buffer (40 mM, pH 6) plus 5% gum Arabic, 19.8 mg/mL
Route	PO gavage
Schedule	Single dose
Sample times	Group 1 – 30 min, 2, and 24 hours post dosing Tissues – plasma, blood, brain, heart, lung, liver, kidney, adrenal gland, uterus, ovary, placenta, mammary gland, amniotic fluid and fetus. Group 2 – milk 30 min, 1, 2, 4, 7, 24, and 48 hr post dosing Group 2 – plasma, 15, 30 min, 1, 2, 4, 7, 24 and 48 hours post dosing
Analysis	_____

Y. Karasawa did this study at _____ in Japan. I did not find a GLP statement in the report.

Results:

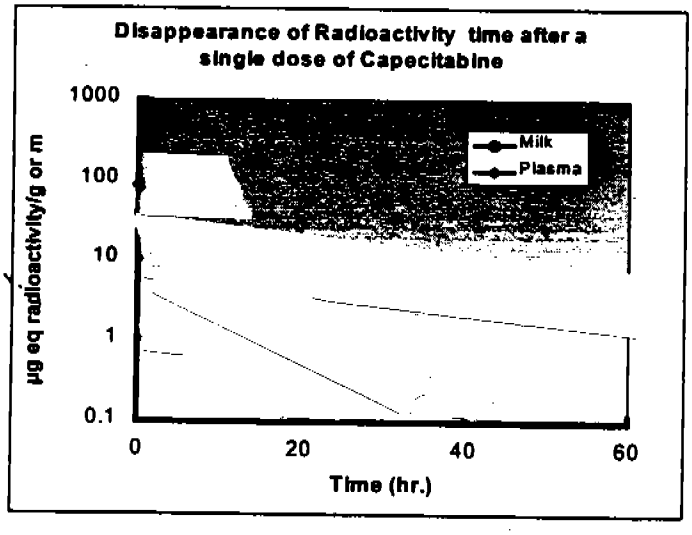
The following table shows the results of the experiment with Group 1, mice during gestation. The researchers reported the results as µg eq. of Ro 09-1978/g or mL but because of the method of

analysis, these figures must be µg eq. total radioactivity.

	Radioactivity Concentration		
	µg eq of total radioactivity/g or mL		
Maternal	30 min	2 hr	24 hr
Plasma	**139.9**	**46.3**	**0.6**
Blood	129.2	39.8	0.4
Brain	9.2	10.2	0.3
Heart	119.1	32.7	0.6
Lung	114	37.4	0.9
Liver	**296.2**	**255.4**	**4.6**
Kidney	**333.1**	**326.4**	**2.5**
Adrenal gland	121.6	42.9	ND
Uterus	73.2	37.4	1.6
Ovary	118.4	33.1	1.6
Placenta	66.4	54.9	2.1
Mammary gland	40.1	17.5	1.3
Amniotic fluid	9.2	18.4	2.1
Fetus	**53.1**	**62**	**1.8**

The table shows that the drug appears in vascular tissues at approximately the concentration in plasma except in liver, kidney and brain. In liver and kidney, the total radioactivity is higher than in plasma by more than a factor of two, suggesting metabolism in these tissues. The radioactivity clears from plasma more quickly than from these metabolic tissues, suggesting that membranes or liposomes retain these metabolic products. At 30 minutes, the concentration in fetus is lower than in plasma, but at 2 and 24 hours the concentration is greater. This probably results from accumulation of metabolic products in the developing metabolic organs of the fetus. The radioactivity in the brain suggests that the radioactivity does not cross the Blood Brain Barrier.

The following table shows that the concentration of total radioactivity is higher in milk than in plasma after a little more than two hours.



The metabolic products of Capecitabine are not particularly lipophilic so I can only conjecture on the mechanism of this concentration effect. Unfortunately, the researchers did not analyze breast tissue. Nevertheless, the sponsors warning in the label that Capecitabine is secreted in to mouse milk is justified. Lastly, the following table shows that the elimination half-life from milk is longer than that from plasma and that the AUC is greater.

Parameter	Unit	Milk	Plasma
C_{max}	µg eq./mL	37.6	107.7
T_{max}	Hr.	0.5	0.58
$t_{1/2}\alpha$	Hr.	0.7	0.8
$t_{1/2}\beta$	Hr.	15	12
AUC	µg eq*hr./mL	396	256

Toxicokinetics and Pharmacokinetics.

3126 – H. Onodera. Whole body phosphor imaging in the male and female mouse following single oral administration of ^{14}C Ro 09-1978. Study RR J-146750. Volume 21, page 48.

Animal	male and female B6D2F1 mice
Drug	^{14}C Ro 09-1978, Batch 12352120 Mo, specific activity 2.47 µCi/mg
Dose	198 mg/kg
Route	PO gavage
Schedule	single dose
Vehicle	40 mM citrate buffer (pH 6.0) containing 5% gum arabic
Time points	0.5, 2, 6, 24 hours
Analysis	
GLP	Yes

At 30 minutes the highest concentrations of radioactivity were in the GI tract and the urine. Kidney and liver also contained high concentrations. The drug was distributed broadly at lower concentrations throughout the body consistent with a uniform plasma concentration. Concentrations lower than that of systemic distribution occurred in the bone, eye, brain, and spinal cord.

At two hours, the highest concentrations were seen in the kidney medulla, urinary bladder and GI. Lower concentrations were seen in the kidney cortex, liver and testis. The drug had cleared from other tissues.

At six hours the distribution was the same as at 2 hours but at much lower concentrations. At 24 hours only the urinary bladder contained drug. These observations are consistent with the pharmacokinetics of the drug and with tissue distribution studies.

3127 J. Racha, *In vitro* cytochrome P450 inhibition studies with Ro 09-1978 and its metabolites (5'-DFCR, 5-FU and FBAL) using human liver microsomes: Evaluation of clinical drug interaction potential. Study RR N-181302. Volume 21, page 107.

Tissue	Pooled human liver microsomes
Drug	Ro 09-1978, 5'DFCR, 5-FU, and FBAL in separate experiments
Analysis	
P450 isoenzymes	1A2, 2A6, 2C9, 2C19, 2D6, 2E1, 3A4

Ro 09-1978 or one of its metabolites was mixed with microsomes in the presence of test substrate. After an appropriate incubation time, the reaction was stopped and the test solution was analyzed for reaction product. The amount of product formed in the presence of Ro 09-1978 or one of its metabolites was compared with the amount formed in the absence of the drug or metabolite. 5-FU (100μM) decreased 1A2 by about 30 %. Ro 09-1978 decreased 1A2 activity by about 25% and 3A4 activity by about 22%. All other drugs inhibited the activity of these enzymes less than 20%. Of all the compounds tested, 5-FU was the most potent and broad spectrum inhibitor. The study shows that Ro 09-1978 has little potential for interaction with drugs metabolized by these P450 isoenzymes. This study supports the sponsor's label claim.

3128 H. Onodera. Twenty four-month oral (feed admix) carcinogenicity study of Ro 09-1978/000 in mice – toxicokinetics study. Study RR J-146817. Volume 21, page 121.

For the experimental details of this study, see the carcinogenicity study below. Investigators determined the concentrations of compounds in urine by ^{19}F-NMR on day 4, month 6 and month 12 (24-hour collection).

		Day 4		Month 6		Month 12		
Analyte	Dose mg/kg	Actual Dose	Amount Excreted µmole	Actual Dose	Amount Excreted µmole	Actual Dose	Amount Excreted µmole	accumulation ratio (dose corrected **) d4 to m12
Capecitabine	30	28.7	0.13	32.5	0.17	36.8	0.19	1.1
	60	58.4	0.23	59	0.23	70.7	0.3	1.1
	90	87.2	0.34	97.6	0.38	114.5	0.55	1.2
5'-DFCR	30	28.7	0.19	32.5	0.2	36.8	0.35	1.4
	60	58.4	0.32	59	0.46	70.7	0.63	1.6
	90	87.2	0.57	97.6	0.79	114.5	1.16	1.5
5'-DFUR	30	28.7	0.43	32.5	0.58	36.8	0.69	1.3
	60	58.4	0.71	59	1.11	70.7	1.15	1.3
	90	87.2	1.09	97.6	1.65	114.5	1.84	1.3
FUPA	30	28.7	0.24	32.5	0.25	36.8	0.37	1.2
	60	58.4	0.41	59	0.59	70.7	0.67	1.3
	90	87.2	0.65	97.6	0.9	114.5	0.9	1.1
FBAL	30	28.7	0.12	32.5	0.18	36.8	0.39	2.5
	60	58.4	0.21	59	0.46	70.7	0.55	2.2
	90	87.2	0.4	97.6	0.74	114.5	0.93	1.8
Sum of metabolites	30	28.7	1.11	32.5	1.38	36.8	1.99	1.4
	60	58.4	1.88	59	2.85	70.7	3.3	1.4
	90	87.2	3.05	97.6	4.46	114.5	5.38	1.3

**I calculated the accumulation ratio as:

$$(\text{Amount excreted}_{\text{month12}}/\text{Actual dose}_{\text{month12}}) \div (\text{Amount excreted}_{\text{day 4}}/\text{Actual dose}_{\text{day4}})$$

The concentrations of 5-FU and 5-FUH$_2$ were below the detection limit of the method. Between 49 and 69% of an administered dose was collected in the urine across the study. The results show that the

excretion of Capecitabine and all its metabolites, except FBAL, remained approximately constant through the first year of the experiment. FBAL excretion increased about two-fold. I have no other information to determine the significance of this increase. Males and females eliminated Capecitabine similarly except that female mice produced somewhat less 5'DFCR. The following table shows the percent of the total moles excreted as each major metabolite and the parent compound. Assuming no significant changes in metabolism occurred with repeated dosing, this table establishes the excretion profile for Capecitabine and its metabolites in mice.

Analyte	Dose mg/kg	Day 4 % of total moles excreted	Month 6 % of total moles excreted	Month 12 % of total moles excreted	average	St Dev
Capecitabine	30	11.7%	12.3%	9.5%	11.2%	1.5%
	60	12.2%	8.1%	9.1%	9.8%	2.2%
	90	11.1%	8.5%	10.2%	10.0%	1.3%
average					**10.3%**	**1.6%**
5'-DFCR	30	17.1%	14.5%	17.6%	16.4%	1.7%
	60	17.0%	16.1%	19.1%	17.4%	1.5%
	90	18.7%	17.7%	21.6%	19.3%	2.0%
average					**17.7%**	**2.0%**
5'-DFUR	30	38.7%	42.0%	34.7%	38.5%	3.7%
	60	37.8%	38.9%	34.8%	37.2%	2.1%
	90	35.7%	37.0%	34.2%	35.6%	1.4%
average					**37.1%**	**2.6%**
FUPA	30	21.6%	18.1%	18.6%	19.4%	1.9%
	60	21.8%	20.7%	20.3%	20.9%	0.8%
	90	21.3%	20.2%	16.7%	19.4%	2.4%
average					**19.9%**	**1.7%**
FBAL	30	10.8%	13.0%	19.6%	14.5%	4.6%
	60	11.2%	16.1%	16.7%	14.7%	3.0%
	90	13.1%	16.6%	17.3%	15.7%	2.2%
average**					**14.9%**	**3.0%**

** The numbers in the rows marked average are the average for all nine measurements for that metabolite. Likewise, the standard deviation is that of all nine measurements. The averages under the column 'average' are that for the measurements at a particular dose. Likewise, the standard deviation is for the measurements at a particular dose.

Humans excrete about 57% of an administered oral dose into the urine as FBAL and only about 3% as the parent compound. Thus, the excretion profile in humans is significantly different from that in mice. This is but one more piece of information that confirms that mice are a poor model for Capecitabine toxicity.

Carcinogenicity:

2503 A. Kawashima. Twenty four-month oral (feed admix) carcinogenicity study of Ro 09-1978/000 in mice. RR J-146816. 1998. Volume 11, page 1, through Volume 13.

Animals	male and female BDF1 mice (~5 weeks old at start of study)
Drug	Capecitabine
Lot	24289-190 (purity 99.5%)
Doses	0 (control 1), 0 (control 2), 30, 60, 90 mg/kg/day 0, 0, 90, 180, 270 mg/m^2/day
N	50 per sex per dose group 3 males and 3 females were assigned to satellite groups at each dose level for urine sampling
Route	PO, feed admix, *ad libitum*, housed individually "Ro 09-198/000 was admixed to the powdered feed, NIH open formula, with a feed mixing machine [redacted] The admixed food was prepared weekly. The drug concentrations in the feed were calculated based on the body weights and food intake of the previous week for each group of both sexes."
Observations	
Clinical Signs	Daily
Body Wt.	Weekly for 12 weeks and monthly thereafter
Food Cons	Weekly
Palpation	monthly for three months, once every two weeks for the second three months and weekly thereafter.
Hematology	at termination
Gross Pathology	at termination at 24 months
Histopathology	Investigators at [redacted] only examined tissues from all animals in the Control 1, the high dose group and animals dying before scheduled necropsy. They did not examine the tissues of low and mid-dose animals. See table for tissues examined.
Statistical Methods	Fisher's exact test and Peto's method to assess tumor incidence, α = 5% one-tailed.

Researchers at [redacted] Japan, did this study for the sponsor. [redacted] of Japan did the histopathology. The study director, A. Kawashima initiated the study on September 18, 1995 and signed the GLP statement October 30, 1998. The study included a QA report. The investigators based their dose selection on a 13-week PO study in mice submitted to NDA 20-896, submission 000, October 31, 1997. [N. Shishido *et al.*, Thirteen week oral (feed admix) toxicity study of Ro 09-1978/000 in mice (Pilot study for carcinogenicity study) GCR J-146'497]. In this study, the low dose, 90 mg/kg caused slight anemia and a slight increase in extramedullary hematopoiesis in the spleen. This study was irrelevant to the initial NDA and I have yet to review it. The sponsor did not request a CAC consultation on the dose.

Results:

Mortality: The following table summarizes total mortality for males and females in each dose group.

Dose Group	Cont. 1	Cont. 2	30 mg/kg	60 mg/kg	90 mg/kg
Males	7 (14%)	4 (8%)	6 (12%)	7 (14%)	3 (6%)
Females	14 (28%)	14 (28%)	16 (32%)	15 (30%)	8 (16%)

In males, there were no statistically significant differences among the dose groups. In females, the low mortality in the high dose group was different from both controls and both lower dose groups, but the difference did not reach statistical significance. Most of the deaths occurred after the 75th week.

Clinical Signs — No differences between controls and treated animals

Body weight — No toxicologically significant differences between controls and treated animals. Any statistical differences were sporadic and not related to dosing. Consistent with *ad labium* feeding, the weights of males increased to about 45 g and that of females to about 30 g. The total tumor incidence reflects these high average weights.

Food Cons. — No toxicologically significant differences between controls and treated animals

Dose Validation: The following table shows the calculated mean compound-intake values in mg/kg.

Dose Group	Cont. 1	Cont. 2	30 mg/kg	60 mg/kg	90 mg/kg
			mg/kg	mg/kg	mg/kg
Males	0	0	31.2	62.7	93.1
Females	0	0	30.2	62.2	93.0

Palpation — No differences between controls and treated animals

Hematology — The following table shows the hematological parameters that were statistically significantly ($p < 0.01$) different from Control 1 in the mid and hid dose males at the end of the study.

		Control 1	Mid dose	% difference	High dose	% difference
RBC	10E6/µl	9.42			8.26	-12.3
HCT	%	48.1			44.5	-7.5
Hb	g/dl	14.8			13.1	-11.5
MCV	fl	51.1	52.3	2.3	54.2	6.1
MCH	pg	15.1	15.4	2.0	15.9	5.3

The following table shows hematological changes seen in females at the end of the experiment ($p < 0.05$).

		Control 1	Low dose	% difference	Mid dose	% difference	High dose	% difference
Plt	10E3/µl	1138					1298	14.1
RBC	10E6/µl	9.3			8.75	-5.9	8.4	-9.7
MCV	fl	51.2	52.9	3.3	53.8	5.1	54.7	6.8
MCH	pg	15.2	15.7	3.3	16	5.3	16.3	7.2

I have shown these changes in detail because these toxicities are the ones on which the sponsor bases the claim that the doses were adequately high. None of these toxicities demonstrated a statistically significant dose response. In this case, MCH is a function of MCV. The increases in both are probably up-regulation as a compensation for the decreases in RBC. The decreases in RBC are probably the only toxicologically significant changes in hematological parameters. This decrease is probably a direct result of decreased RBC formation.

Organ Weights — decreases in absolute weight of thymus (~17%) and testes (~5%) in HD males. Small but statistically significant decrease in HD female heart weight and left kidney weights were probably incidental.

Gross Pathology — Major findings included nodular lesions in the Harderian gland, liver, lung, lymph nodes, spleen, pituitary gland, and uterus, and focal lesions in the pituitary (predominantly in females). The incidences of these findings did not demonstrate any dose-related toxicity. Indeed, dosing "tended to suppress nodular lesions and enlargement in the liver, lymphatic tissues and uterus in females."

Presumptive Cause of Death

Most animals that died on study demonstrated pathology consistent with death due to a neoplastic process. None of these causes of death showed or suggested a dose effect. Lethal tumors included malignant lymphoma (4 males, 17 females), histocytic sarcoma (5 males, 27 females), blood vessel tumors (6 males, 7 females) and liver tumors (2 males). Lesions killing but a single animal included lung, intestinal, pituitary, thyroid, Harderian gland, neural and ovarian tumors. Non-tumor causes of death included amyloidosis (1 male), renal lesions (4 females), UTI (1 female), arteritis (2 males and 2 females), Ovarian lesions (1 female), convulsion (1 male), and unclear (3 males and 4 females).

Tumor Incidence:

The following table shows the incidence of major tumors (5% or more in the HD group) by tissue.

	Dose Group, Males					Dose Group, Females				
	Control 1	Control 2	30 mg/kg	60 mg/kg	90 mg/kg	Control 1	Control 2	30 mg/kg	60 mg/kg	90 mg/kg
Number examined	50	4	6	7	50	50	14	16	15	50
Hemolymphoreticular tumors										
Malignant Lymphoma	4	0	1	1	3	6	5	5	4	6
Histocytic sarcoma	4	1	1	3	2	16	4	6	8	6**
Harderian gland tumors										
Adenoma	1	1	0	0	4	3	0	2	0	4
Adenocarcinoma	0	0	0	0	0	1	0	0	0	0
Liver tumors										
Hepatocellular adenoma	10	0	2	0	10	1	0	2	0	1
Hepatocellular carcinoma	3	0	0	1	3	2	0	0	0	1
Hemangioma	1	0	0	0	3	4	1	1	0	3
Hemangiosarcoma	2	1	0	3	1	2	0	0	0	2
Lung tumors										
Bronchiolo-alveolar adenoma	11	2	0	0	4**	3	0	1	0	0
Bronchiolo-alveolar carcinoma	3	0	0	0	2	0	0	0	1	2
Ovarian tumors						n=49		n=15		
Cystadenoma	-	-	-	-	-	1	1	0	0	3
Pituitary tumors						n=49	n=12			n=48
Anterior adenoma	0	0	0	0	0	5	0	0	0	4
Blood vessel tumors										
Hemangioma	6	0	0	0	4	5	1	1	2	3
Hemangiosarcoma	3	2	0	4	3	5	2	2	0	2

**$p < 0.05$ significant difference from Control-1, The sponsor did not analyze the data of groups other than the 90 mg/kg group statistically.

There were two statistically significant differences in the incidence of specific tumors. In males, there was a significant decrease in bronchiolo-alveolar adenoma (11 in control, 4 in HD group), and in females, there was a decrease in histocytic sarcoma (16 in control, 6 in HD group).

The following table shows the total incidences of tumors.

	Dose Group, Males					Dose Group, Females				
	Control 1	Control 2	30 mg/kg	60 mg/kg	90 mg/kg	Control 1	Control 2	30 mg/kg	60 mg/kg	90 mg/kg
Number examined	50	4	6	7	50	50	14	16	15	50
Number benigh tumors	37	4	3	0	30	25	2	6	2	15
Number malignant tumors	28	6	2	13	20	34	13	14	13	22
Number tumors	65	10	5	13	50	59	15	20	15	37
Number benign tumor bearers	23	2	2	0	22	16	2	6	2	14
Number malignant tumor bearers	18	3	2	7	11	30	10	12	13	20
Number multipal tumor bearers	18	2	1	3	12	13	2	5	2	7
Number tumor bearers	33	3	4	7	30	38	11	13	13	28

In HD males and females, the numbers of tumors and tumor bearers were less than in controls in all cases. Since only animals dying during the study were examined microscopically, no dose relationship could be determined. There was no evidence to suggest a difference in the time course of tumor formation in treated animals.

As expected in an old mouse population, non-tumor microscopic pathology was considerable. Male HD mice showed greater angiectasis and sinus hemorrhage in the lymph nodes than did controls, but this was the only difference seen in proliferative tissues including spleen, stomach, intestine and thymus. These are the tissues where one would expect direct 5-FU toxicity. There were no other toxicologically significant differences in non-tumor microscopic pathology.

Comments and conclusions:

This study failed to demonstrate any carcinogenesis associated with Capecitabine dosing. The statistician's report concurs that there is no significant difference in the incidence of tumors between control and treated animals. Indeed, the study failed to demonstrated any significant toxicity associated with Capecitabine other than a ~10% decrease RBC and a concomitant decrease in Hct in males and females. The dose given to the high dose group is 90 mg/kg or 270 mg/m^2/day. This dose is only 10.8% the recommended clinical dose of 2500 mg/m^2/day for two-weeks. The investigators based their choice of doses on a 13-week oral feeding study in mice (see above). In this study, the group receiving 90 mg/kg suffered "slight anemia and a slight increase in extra-hematopoiesis in the spleen, however no drug-related change in body weight was observed." This dose may have been too low for this carcinogenicity study, but twice this dose, 180 mg/kg, in the 13-week study caused unacceptable weight loss, anemia, increased spleen weight, and regressive changes in proliferative tissues.

Both the rat and the mouse are unacceptable models for toxicity tests for this prodrug. The spectrum of metabolites they each generate is the same as humans, but the plasma concentrations of these metabolites are considerably different. The mouse is more sensitive to Capecitabine than humans

and the rat is extremely insensitive due to high folate reserves. The only relevant species is the monkey. The sponsor did 26-week and 52-week studies in the monkey and reported no signs of carcinogenicity. The monkeys in these studies suffered anemia, but leukopenia and damage to the gastrointestinal tract, spleen, bone marrow and lymph nodes were dose limiting. The spectrum of toxicities in monkeys is similar to that in humans. The spectrum of toxicities seen in mice in the present study is not.

A. Cavaliere *et al.* (*Tumori* 1990 Apr 30;76(2):179-81) have reported that a dose of 5-FU of 30 mg/kg once a week IP in BALB/C mice induced a significant increase in lung tumors in both sexes (males, p less than 0.05; females, p less than 0.01) and tumors of the lymphoreticular system in female mice (p less than 0.001). These results suggest that 5-fluorouracil is carcinogenic in mice. If the current study had been positive, it would have been difficult to determine whether the ultimate carcinogen was Capecitabine or 5-FU or both.

I seriously doubt the sponsor can ever adequately assess the carcinogenic potential of this compound in either the rat or mouse. Nevertheless, Capecitabine is a 5-FU prodrug. Patients receiving cumulative doses of 0.24-1.0 g of fluorouracil parenterally have shown an increase in numerical and structural chromosome aberrations in peripheral blood lymphocytes. 5-FU is probably carcinogenic to humans, so again any differences between carcinogenicity associated with Capecitabine and that of 5-FU would be very difficult to discern. Despite this serious potential toxicity, 5-FU is routinely used as an adjuvant treatment for non-metastatic colorectal cancer after surgical resection. Thus, the results of this study are of little clinical importance.

Summary

The pharmacology of Capecitabine is interesting because Roche intentionally designed this drug to overcome significant problems associated with oral 5-FU treatment. The absorption of 5-FU across the gastric lumen is variable and DPD in the plasma rapidly degrades the compound. Adding ribose-1-phosphate to 5-FU (Furtulon) increases absorption, but this greatly increases GI toxicity. This increased toxicity is due to large concentrations of PyNPase in the human GI. Adding an _____ _____ to Furtulon protects the compound, increases absorption and provides good oral bioavailability, but humans have limited ability to remove this moiety. Finally, by replacing the _____ _____ with a N^4-pentyloxycarbonyl group, Roche found a compound that crossed the human GI and can be readily cleaved by three enzyme steps to generate 5-FU systemically. The pharmacology section of the original NDA describes an impressive body of generally good scientific investigation that led to the clinical development of Capecitabine.

The k_m of PyNPase in human lung cancer tissue was 0.24 mM for thymidine, the endogenous substrate, and 1.7 mM for 5'-DFUR. The physical significance of these numbers is questionable because humans express at least two PyNPase enzymes. The k_m of cytidine deaminase from human leukemic granulocytes for cytidine was 11 μM. This activity is not rate limiting in humans. The total cytidine deaminase activity was lower in leukemic cells than in normal granulocytes. Expression increases with differentiation.

In *in vitro* efficacy studies of Capecitabine, 5'-DFCR, 5'-DFUR and 5-FU in the same tumor cell lines, Capecitabine was relatively non-toxic. In most cases, the IC_{50} of the parent drug was greater than 1000 μM. This suggests that most tumor cells do not express significant carboxylesterase activity. Likewise, concentrations of 5'-DFCR less than 90 μM were toxic to only Scabber cells. Again, this suggests that most tumor cells do not express significant cytidine deaminase activity. Most of this activity is in the circulation or the liver. Only 5'-DFUR and 5-FU killed most tumor cells effectively at concentrations below 100 μM. Nevertheless, in all but two cell lines, the IC_{50}s of 5'-DFUR were at least ten times higher than those of 5-FU. This suggests that Capecitabine *in vitro* is relatively non-toxic, but also that 5-FU itself is more effective than any of the metabolites at the cellular level in tumor. In most cases, the expression of PyNPase is not sufficient to make 5'-DFCR as effective as 5-FU.

In humans, the great majority of carboxylesterase activity is in the liver. Tumor and normal tissue express about the same activity. Human liver, kidney, stomach and lower GI tissue express the largest amounts of cytidine deaminase activity, but the blood also contains considerable activity. In human tumors, the expression of this activity is more variable than in normal tissue. Some individual tumors, within a large sampling of colon, ovarian, and cervical tumors, appear to express more of this activity than normal tissue. PyNPase is widely expressed in normal tissue, but the largest activities are found in the liver. Many tumors from many different tissues express more activity of this enzyme than adjacent normal tissue, but the variability is large. This means that some tumors express much more PyNPase than normal tissue and some express much less. Without specific testing, an oncologist could not know *a priori* whether a tumor expressed excess PyNPase activity. Thus, there is insufficient scientific evidence to support the sponsor's claim that Capecitabine therapy is 'tumor specific'.

In the mouse, carboxylesterase activities and PyNPase activities are greatest in small intestine, most cytidine deaminase activity is in the kidney. In the rat, most of the carboxylesterase activity is in the small intestine and liver, and most PyNPase is in the lung and small intestine. The rat expresses relatively low cytidine deaminase activities. In monkeys, most carboxylesterase activity is in the liver. Most major organs in the monkey express significant cytidine deaminase and PyNPase activity. Though the carboxylesterase activity is six times higher in human liver than in monkey liver, the distribution of this activity and of that of the other two enzymes is similar to that of humans in all tissues. Again, this justifies the use of monkeys in the development of Capecitabine. The tissue distributions suggest that the limiting toxicities will be in the gastrointestinal system in monkeys and in the liver in humans. These predictions turn out to be true for both species.

Human liver expresses two carboxylesterase activities but human colon expresses only one, called isoenzyme B. The other isoform, isoenzyme A, cleaves N^4-alkoxycarbonyl-5'-DFCR compounds, such as Capecitabine. Isoenzyme B does not. Thus, Capecitabine crosses the human GI relatively intact and is then cleaved to 5'-DFCR in the liver. The substrate specificity and distribution of carboxylesterase enzymes in the monkey is similar to those of human. Those in mouse are not. Thus, the monkey is an appropriate model for the preclinical development of Capecitabine.

PyNPase in humans is homologous with platelet-derived endothelial cell growth factor (rPD-ECGF). rPD-ECGF has thymidine phosphorylase activity. These results imply that PyNPase activity may be important in tumor angiogenesis. Cytokines such as TNFα, IL-1α and IFNγ can increase the

expression of PyNPase in various tumor cell lines. This increase in expressio n increases the toxicity of 5'-DFUR in tumor cell lines.

Repeated dosing with Capecitabine does not induce carboxylesterase or cytidine deaminase activity in the colon or liver of the monkey. In safety pharmacology studies, Capecitabine caused little toxicity other than that associated with anticipatable 5-FU toxicity.

Rats express little cytidine deaminase. Consequently, they develop high plasma concentrations of 5'-DFCR and low steady state concentrations of 5'-DFUR and 5-FU when given oral Ro 09-1978. The rat toxicity data cannot be considered predictive for humans. In contrast, monkeys develop comparably high plasma concentrations of 5'-DFUR and parent compound. Thus, the monkey is probably the most predictive species for the human response to Ro 09-1978.

No mice died in single dose gavage toxicity studies, so the rodent LD_{50} of Capecitabine remains unknown. The only clinical symptom observed in these single-dose studies was hypoactivity. This hypoactivity persisted for about 1 hr in mice receiving 3000 mg/m^2 and for from 2 to 4 hr in mice receiving 6000 mg/m^2. These single doses in mice caused no gross pathology. Similarly, in rats, doses to 12000 mg/m^2 caused only decreased activity. This symptom was more frequent in males than in females.

Similarly, Capecitabine caused no clinical symptoms in rats dosed daily for 4 weeks by gavage to doses of 3231 mg/m^2. Rats dosed for 26 weeks at doses to 2154 mg/m^2 (gavage) suffered minor changes in hematological parameters, including increased MCV in females and MCH in males. Serum protein was decreased in males suggesting mild hepatic toxicity. At 3231 mg/m^2 these same symptoms increased in severity. This dose caused slight degeneration of rectal cells in males, but killed no rats.

Monkeys dosed with 2154 mg/m^2 daily for 28 days suffered diarrhea and slight weight loss. Thymus weight and WBC decreased. This dose caused some microscopic damage in the small intestine and in lymphatic and hematopoietic organs. A dose of 4308 mg/m^2 daily for 28 days rendered two male monkeys moribund on days 20 and 27. This dose is about one-third higher than the approved clinical dose. This dose increased the severity of the symptoms seen at the lower dose and caused and a decrease in spleen weight, an increase in adrenal weight. The moribund monkeys were emaciated and had low WBC. The dose limiting toxicities in monkeys are degeneration of the gastrointestinal system and myelosuppression. A longer dosing schedule, 26 weeks, with 1728 mg/m^2 caused a decrease in red cell parameters, RBC, Hct, Hbg. This high dose rendered one female monkey moribund on day 57. This monkey was emaciated. These signs and symptoms were consistent with 5-FU toxicity. Nevertheless, the monkey does not predict the hepatotoxicity seen in humans, probably because they express less carboxylesterase activity in the liver.

In reproductive function tests in the mouse (Segment I), Capecitabine caused a dose dependent and severe decrease in female fertility. The percentage of fertile females decreased from 83 in controls to 13 in mice given 2280 mg/m^2/d. This decrease was associated with continuous diestrus. This high dose also decreased the weight of testes and epididymides in mated males. Capecitabine caused a dose dependent, severe, decrease in the total number of corpora lutea, number of live fetuses, the percentage of live fetuses relative to implantation and early deaths. The impairment of fertility in female dams appeared reversible.

In a study of toxicity during organogenesis in the mouse (Segment II), doses as high as 2373 mg/m^2/d caused only a decrease in body weight gain in the dams. Nevertheless, it caused a 100% decrease in the number of corpora lutea in the high dose group. This decrease was dose dependent. All fetuses in the high dose group died early in pregnancy and again this increase in fetal death was dose dependent. Fetal external anomalies in the low and mid dose group included cleft palate, anophthalmia, microphthalmia, oligodactyly, polydactyly, syndactyly and kinky tail. The total incidence of abnormalities increased with dose. Visceral abnormalities included the ones mentioned above plus esophagectopy and dilation of the renal pelvis. Skeletal abnormalities included cleft palate, fusion of cervical vertebra, abnormal shape of cervical vertebra, fusion of thoracic vertebra, wavy rib and fusion of metacarpus. A decrease in ossification in the caudal vertebra was seen in the LD and MD groups. Twenty-four of 65 LD fetuses had a rudimentary 14th rib.

In a study of exposure during late pregnancy and lactation (Segment III) in mice, Capecitabine at less than half the proposed clinical dose on a mg/m^2 basis (1200 mg/m^2) caused little toxicity to the dams. The doses in this study caused no differences the number of live neonates, implantations, lactation indices, external abnormalities or reproductive function in the F1 generation. The highest dose did cause a slight decrease in F1 female body weight and some neurological parameters (increased rearing and walking) may have been affected. High dose F1 mice showed some damage to reproductive organs though there was no decrease in reproductive function.

In the monkey, doses of 1080 and 2160 mg/m^2/d were embryo lethal during organogenesis (Segment II). The high dose decreased fetal ovary weight significantly (to 30% of control) and there was evidence that other organs may have been smaller as a function of dose (brain, thymus, lung, spleen, and kidney). Capecitabine at doses less than the proposed clinical dose on a mg/m^2 basis is fetotoxic and embryolethal. The parent drug and its metabolites are excreted into milk in considerable concentrations.

Capecitabine did not cause mutations in the Ames assay with or without S9 activation. Likewise it did not cause mutations in V79 Chinese hamster lung cells (HPRT assay) with or without metabolic activation. At the highest dose tested, it did cause an approximately five-fold increase in chromosome aberrations (excluding gaps) in human peripheral blood lymphocytes exposed for 24 hours *in vitro* without S9. With a 48 hour exposure, the number of aberrations (excluding gaps) increased approximately 23-fold. This clastogenicity was not evident with metabolic activation, probably because of the short exposure times. In the mouse micronucleus test, Capecitabine caused a two-fold increase in micronuclei. This increase did not reach the level of statistical significance. 5-FU is positive in the mouse micronucleus test. A two-year carcinogenicity study in the mouse with a high dose of 270 mg/m^2/day did not demonstrate any increase in tumors. The doses in this study were too low and the study provides little useful information. The concentration of the enzymes that metabolize Capecitabine in the mouse is considerably different from that of humans. These differences possibly protect them from much of Capecitabine or 5-FU's potential genotoxicity. It is unlikely that the sponsor could design a study in rodents that would provide adequate exposure to demonstrate carcinogenicity. Nevertheless, clinical doses of Capecitabine are probably clastogenic in humans.

Capecitabine is rapidly absorbed across the GI in the monkey. In most studies, apparent T_{max} was between 30 min and one hour. Oral bioavailability of Capecitabine in solution is at least 81% in the monkey. The three different clinical formulations provide roughly the same bioavailability as measured

by urinary excretion. Maalox did not significantly affect the absorption of an oral dose in rats. Studies in monkeys suggest that food may slow Capecitabine absorption.

Capecitabine and its metabolites distribute widely throughout the body. In the mouse, the largest concentrations are found in the stomach and bladder at 0.5 and 2 hours. High concentrations are also found in the liver and kidney. Concentrations in the brain are low, implying that Capecitabine does not cross the blood brain barrier. The concentration in most other major tissues uniform. Relatively high concentrations are found in the intestines at 6 hours despite rapid absorption. This information and plasma concentration curves suggest that Capecitabine and 5'-DFUR may recirculate within the enterohepatic system.

Most of a dose of Capecitabine is eliminated in the urine. Little is eliminated in the feces. Studies in monkeys suggest that food may slow Capecitabine absorption. Mice also eliminate most of a dose in the urine. The major metabolites of Capecitabine were FBAL in monkey urine, 5'–DFCR in rat urine and 5'–DFUR in mouse urine.

In multiple dose studies in the monkey, the AUC values for Capecitabine and its metabolites 5'-DFCR and 5'-DFUR increased with dose, but in some cases this increase was greater than linear at the higher doses. In rats the AUC values for Capecitabine, 5'-DFCR and 5'-DFUR increased linearly over the range of 179.5 to 538.5 mg/kg. Repeated dosing did not affect the AUC values in the monkey. There were no consistent gender differences in monkeys. In rats the AUC values for Capecitabine and 5'-DFUR were similar in males and females, but the AUC values for 5'-DFUR were 40 to 120% higher in females than in males. Capecitabine dosing did not induce or inhibit any cytochrome P450 tested.

Recommendation

Based on the pharmacology and toxicology data presented in this NDA, I have no objection to the approval of Capecitabine for this indication.

Discussed with the Medical Officer:

The doses in the mouse carcinogenicity study are too low to demonstrate conclusively that Capecitabine is not carcinogenic in that species. The study does not support the sponsor's label claim.

At least one patient has died from Capecitabine overdose. Should this patient's death and the lethal dose be described in the drug label?

Labeling Comments and Changes:

Under the section Drug-Drug interactions, the text currently reads:

DRAFT

It should be changed to read:

DRAFT

Under the section ***Carcinogenesis, Mutagenesis and Impairment of Fertility***, the first sentence describing the results of the two-year mouse carcinogenicity currently reads:

DRAFT

This statement should be eliminated or it should be changed to read:

DRAFT

Under the section ***Nursing Women***, the text currently reads:

DRAFT

It should be changed to read:

The section Overdosage should be changed to _____
_____ The sentence describing

/S/

W. David McGuinn, Jr., Ph.D., D.A.B.T.

cc: Original NDA
HFD-150 Division file
/WD McGuinn
/J Leighton
/A Martin
/M Pelosi

/S/
9/8/00

September 7, 2000

Division of Oncology Drug Products, HFD-150
Review and Evaluation of Pharmacology and Toxicology Data
NDA Review, review # 1

NDA: 20-896 **Type:** SE1, BZ, SNC **Serial #:** 006
Submission: Supplemental NDA Received September 20, 1999
Completed

Key Words: Capecitabine, Xeloda, colorectal, colon, cancer, and prodrug.

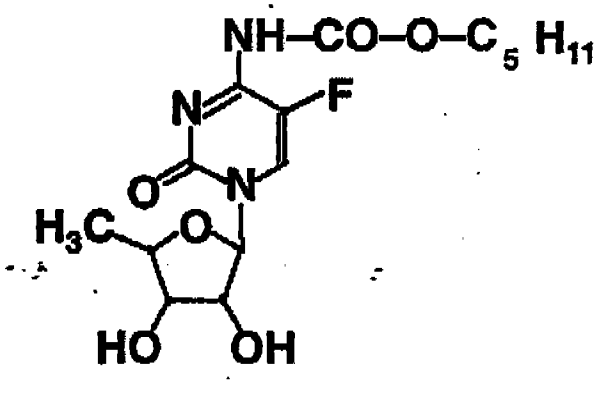
Information to be conveyed to the sponsor: YES

Reviewer: W. David McGuinn, Jr., Ph. D., D.A.B.T.

Sponsor: Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, NJ 07110-1199

Drug Name:
Code name: Ro 09-1978
Generic Name: Capecitabine
Trade Name: XELODA
Chemical Name: N^4-Pentyloxycarbonyl-5'-deoxy-5-fluorocytidine
FW: 359.35

Structure:



Class cytotoxin, 5-Fluorouracil pro-drug
Indications: The first-line treatment of patients with metastatic colorectal carcinoma.

Related drugs: 5-FU, Furtulon, _____

Related INDs: _____ Hoffman-La Roche Inc.
_____ (discontinued)

Related NDAs: None

Route of Administration: PO

Formulation: film–coated tablets containing 150 mg Capecitabine or 500 mg Capecitabine. The inactive ingredients are: anhydrous lactose, croscarmellose sodium, hydroxypropyl methylcellulose, microcrystalline cellulose, magnesium stearate and purified water. The peach or light peach film coating contains hydroxypropyl methylcellulose, talc, titanium dioxide, and synthetic yellow and red iron oxides.

Dose: 2500 mg/m^2 daily for two weeks followed by a one-week rest, 3-week cycles

Previous Clinical Studies.

In a phase I study with XELODA, the MTD dose as a single agent in the treatment of patients with solid tumors was 3000 mg/m^2 /dX14 q21d. The dose–limiting toxicities were diarrhea and leukopenia.

The sponsor did a large phase II multicenter trial with 162 patients with advanced or metastatic breast cancer. This heavily pretreated patient population was refractory to previous paclitaxel therapy. Most patients were also resistant (41%) or had failed (26%) previous anthracycline therapy and 82% had been exposed to 5–FU. XELODA was administered at a dose of 2510 mg/m^2/d X14 q21d. The sponsor claims a median survival was 384 days. Again, the major toxicities were diarrhea and leukopenia. Other studies in other diseases are ongoing. The Food and Drug Administration has approved XELODA for the treatment of locally advanced or metastatic breast cancer after failure of paclitaxel and an anthracycline–containing chemotherapy regimen.

In the study that supports this application, 603 patients were treated with XELODA at a daily dose of 2500 mg/m^2 for two weeks followed by 1-week rest. The control arm received 5-FU, 425-mg/m^2 IV bolus, and leucovorin, 20 mg/m^2, on days one through five every 28 days. The control arm for this trial is no longer the standard of care.

Table of contents:

Studies Not Reviewed:

The sponsor submitted many studies in this NDA. I have reviewed only those relevant to the sponsor's new label claims. For a complete list of all the studies in this submission, see Volume 1, page 13, NDA 20-896.

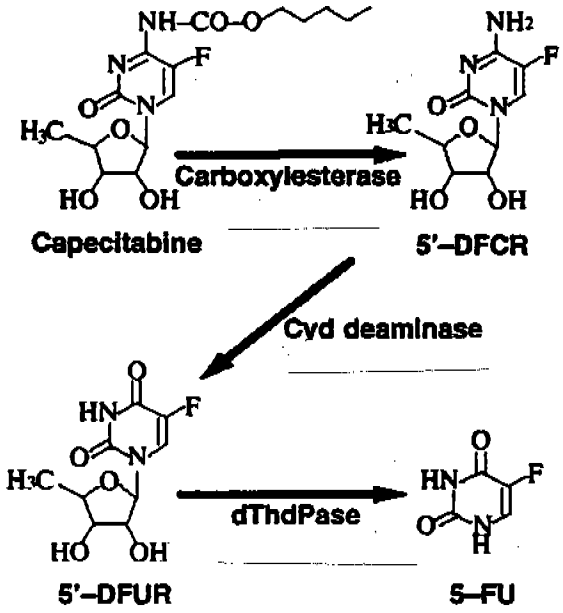
Rationale

In 1982, A. Kono *et al.* (1982, *Chem. Pharmacol. Bull.* 31:175-178) showed that some animal tumors contained higher levels of pyrimidine nucleoside phosphorylase (PyNPase) than did normal tissue. PyNPase is a reversible enzyme normally responsible for adding ribose-1-phosphate to uracil to form uridine. In the reverse direction the enzyme can hydrolyze 5-deoxy-ribose from 5'-deoxy-5-fluorouridine (5'-DFUR) to form 5-FU. If PyNPase is over-expressed in a tumor, this reaction would allow higher concentrations of 5-FU to form in tumor than in normal tissue. This increased expression might increase the efficacy of this treatment, and consequently the therapeutic index, over that of standard 5-FU treatment. Japan has approved the oral prodrug, 5'-DFUR or FURTULON, for use in cancer chemotherapy. This drug's dose-limiting toxicity is diarrhea, caused by the presence of high concentrations of PyNPase in the intestinal tract. Thus, much of the drug never reaches the tumor and causes significant local toxicity.

To overcome this absorption problem Roche developed _____ _____ This drug is not a substrate for gastric PyNPase, so it is absorbed past the gastric lumen without the loss of 5-deoxy-ribose. In the liver, acylamidase (acylamide amidohydrolase, EC 3.5.1.) cleaves _____ to form 5'-deoxy-5-fluorocytidine (5'-dFCR). Cytidine deaminase then hydrolyzes 5'-dFCR to 5'-DFUR (also 5'-FUDR below) in tissues and tumor throughout the body. Some tumor tissues may express higher concentrations of cytidine deaminase than most normal tissue.

Roche abandoned efforts to develop _____ for chemotherapy when they found it was a poor substrate for acylamidase in humans. Subsequently, Roche developed Ro 09-1978 by replacing _____ with a N^4-pentyloxycarbonyl group. Roche claims that the activity of acylamidase is 50 times greater for this compound than for _____ This increase in activity shifts the kinetics of the metabolic pathway and increases the available

concentrations of 5'-DFUR. Roche hopes to market this compound as an oral 'tumor specific' chemotherapeutic agent. The graph below shows this catalytic scheme.



Glossary of Abbreviations:

5'–DFCR	5'–Deoxy–5–fluorocytidine
5'–DFUR	5'–Deoxy–5–fluorouridine (doxifluridine, FURTULON)
DPD	Dihydropyrimidine dehydrogenase
dThdPase	Thymidine phosphorylase, same as Pyrimidine nucleoside phosphorylase
FBAL	α–Fluoro–β–alanine
FdUMP	2'–Deoxy–5–fluorouridine mono–phosphate
FUH_2	Dihydrofluorouracil
FUPA	α–Fluoro–β–ureidopropionate
FUTP	Fluorouridine tri–phosphate
5–FU	5–Fluorouracil
FUdR	2'–Deoxy–5–fluorouridine
LC–MS/MS	Liquid chromatography–tandem mass spectrometry with ion–spray interface
PyNPase	Pyrimidine nucleoside phosphorylase
TS	Thymidylate synthase
UrdPase	Uridine phosphorylase

I have excerpted portions of this review directly from the Sponsor's submission.

Review:

physical characteristics:

$pK_a = 8.8$
partition coefficient (octanol/ pH 7.4 Buffer) = 4.5

Reproductive Toxicology

3125 H Onodera, 1998, Transfer of the fetus and milk (*sic*) in mice after single oral administration of ^{14}C- Ro 09-1978. RR J-146751. Volume 21 page 1.

Animal	Female BDF1 [redacted] (34.1 to 39.9 g at time of dosing) Group 1 – gestation, mated at 9 weeks, 16 day of pregnancy Group 2 – lactating, mated at 9 weeks, 9th or 10th day after delivery
Drug	^{14}C-Ro 09-1978/602 (Lot 12352B9g Mo) labeled at position 6 of fluorouracil moiety, 2.47 MBq/mg, 96% radio chemical purity R0 09-1978 unlabeled (Lot 24289-74-A)
Dose	198 mg/kg (594 mg/m^2), 6.25 MBq/kg
Vehicle	citrate buffer (40 mM, pH 6) plus 5% gum Arabic, 19.8 mg/mL
Route	PO gavage
Schedule	Single dose
Sample times	Group 1 – 30 min, 2, and 24 hours post dosing Tissues – plasma, blood, brain, heart, lung, liver, kidney, adrenal gland, uterus, ovary, placenta, mammary gland, amniotic fluid and fetus. Group 2 – milk 30 min, 1, 2, 4, 7, 24, and 48 hr post dosing Group 2 – plasma, 15, 30 min, 1, 2, 4, 7, 24 and 48 hours post dosing
Analysis	[redacted]

Y. Karasawa did this study at [redacted] in Japan. I did not find a GLP statement in the report.

Results:

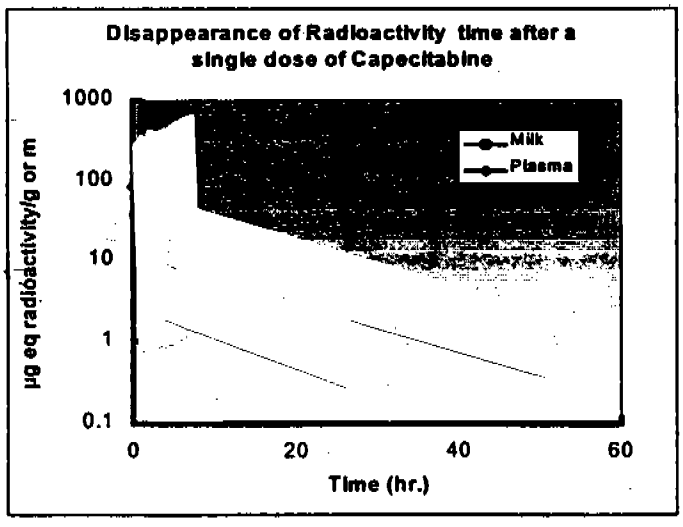
The following table shows the results of the experiment with Group 1, mice during gestation. The researchers reported the results as µg eq. of Ro 09-1978/g or mL but because of the method of

analysis, these figures must be µg eq. total radioactivity.

	Radioactivity Concentration		
	µg eq of total radioactivity/g or mL		
Maternal	30 min	2 hr	24 hr
Plasma	139.9	46.3	0.6
Blood	129.2	39.8	0.4
Brain	9.2	10.2	0.3
Heart	119.1	32.7	0.6
Lung	114	37.4	0.9
Liver	296.2	255.4	4.6
Kidney	333.1	326.4	2.5
Adrenal gland	121.6	42.9	ND
Uterus	73.2	37.4	1.6
Ovary	118.4	33.1	1.6
Placenta	66.4	54.9	2.1
Mammary gland	40.1	17.5	1.3
Amniotic fluid	9.2	18.4	2.1
Fetus	53.1	62	1.8

The table shows that the drug appears in vascular tissues at approximately the concentration in plasma except in liver, kidney and brain. In liver and kidney, the total radioactivity is higher than in plasma by more than a factor of two, suggesting metabolism in these tissues. The radioactivity clears from plasma more quickly than from these metabolic tissues, suggesting that membranes or liposomes retain these metabolic products. At 30 minutes, the concentration in fetus is lower than in plasma, but at 2 and 24 hours the concentration is greater. This probably results from accumulation of metabolic products in the developing metabolic organs of the fetus. The radioactivity in the brain suggests that the radioactivity does not cross the Blood Brain Barrier.

The following table shows that the concentration of total radioactivity is higher in milk than in plasma after a little more than two hours.



The metabolic products of Capecitabine are not particularly lipophilic so I can only conjecture on the mechanism of this concentration effect. Unfortunately, the researchers did not analyze breast tissue. Nevertheless, the sponsors warning in the label that Capecitabine is secreted in to mouse milk is justified. Lastly, the following table shows that the elimination half-life from milk is longer than that from plasma and that the AUC is greater.

Parameter	Unit	Milk	Plasma
C_{max}	µg eq./mL	37.6	107.7
T_{max}	Hr.	0.5	0.58
$t_{1/2}\alpha$	Hr.	0.7	0.8
$t_{1/2}\beta$	Hr.	15	12
AUC	µg eq*hr./mL	396	256

Toxicokinetics and Pharmacokinetics.

3126 – H. Onodera. Whole body phosphor imaging in the male and female mouse following single oral administration of ^{14}C Ro 09-1978. Study RR J-146750. Volume 21, page 48.

Animal	male and female B6D2F1 mice
Drug	^{14}C Ro 09-1978, Batch 12352120 Mo, specific activity 2.47 µCi/mg
Dose	198 mg/kg
Route	PO gavage
Schedule	single dose
Vehicle	40 mM citrate buffer (pH 6.0) containing 5% gum arabic
Time points	0.5, 2, 6, 24 hours
Analysis	
GLP	Yes

At 30 minutes the highest concentrations of radioactivity were in the GI tract and the urine. Kidney and liver also contained high concentrations. The drug was distributed broadly at lower concentrations throughout the body consistent with a uniform plasma concentration. Concentrations lower than that of systemic distribution occurred in the bone, eye, brain, and spinal cord.

At two hours, the highest concentrations were seen in the kidney medulla, urinary bladder and GI. Lower concentrations were seen in the kidney cortex, liver and testis. The drug had cleared from other tissues.

At six hours the distribution was the same as at 2 hours but at much lower concentrations. At 24 hours only the urinary bladder contained drug. These observations are consistent with the pharmacokinetics of the drug and with tissue distribution studies.

3127 J. Racha, *In vitro* cytochrome P450 inhibition studies with Ro 09-1978 and its metabolites (5'-DFCR, 5-FU and FBAL) using human liver microsomes: Evaluation of clinical drug interaction potential. Study RR N-181302. Volume 21, page 107.

Tissue	Pooled human liver microsomes
Drug	Ro 09-1978, 5'DFCR, 5-FU, and FBAL in separate experiments
Analysis	
P450 isoenzymes	1A2, 2A6, 2C9, 2C19, 2D6, 2E1, 3A4

Ro 09-1978 or one of its metabolites was mixed with microsomes in the presence of test substrate. After an appropriate incubation time, the reaction was stopped and the test solution was analyzed for reaction product. The amount of product formed in the presence of Ro 09-1978 or one of its metabolites was compared with the amount formed in the absence of the drug or metabolite. 5-FU (100μM) decreased 1A2 by about 30 %. Ro 09-1978 decreased 1A2 activity by about 25% and 3A4 activity by about 22%. All other drugs inhibited the activity of these enzymes less than 20%. Of all the compounds tested, 5-FU was the most potent and broad spectrum inhibitor. The study shows that Ro 09-1978 has little potential for interaction with drugs metabolized by these P450 isoenzymes. This study supports the sponsor's label claim.

APPEARS THIS WAY
ON ORIGINAL

3128 H. Onodera. Twenty four-month oral (feed admix) carcinogenicity study of Ro 09-1978/000 in mice – toxicokinetics study. Study RR J-146817. Volume 21, page 121.

For the experimental details of this study, see the carcinogenicity study below. Investigators determined the concentrations of compounds in urine by ^{19}F-NMR on day 4, month 6 and month 12 (24-hour collection).

		Day 4		Month 6		Month 12		
Analyte	Dose mg/kg	Actual Dose	Amount Excreted µmole	Actual Dose	Amount Excreted µmole	Actual Dose	Amount Excreted µmole	accumulation ratio (dose corrected **) d4 to m12
Capecitabine	30	28.7	0.13	32.5	0.17	36.8	0.19	1.1
	60	58.4	0.23	59	0.23	70.7	0.3	1.1
	90	87.2	0.34	97.6	0.38	114.5	0.55	1.2
5'-DFCR	30	28.7	0.19	32.5	0.2	36.8	0.35	1.4
	60	58.4	0.32	59	0.46	70.7	0.63	1.6
	90	87.2	0.57	97.6	0.79	114.5	1.16	1.5
5'-DFUR	30	28.7	0.43	32.5	0.58	36.8	0.69	1.3
	60	58.4	0.71	59	1.11	70.7	1.15	1.3
	90	87.2	1.09	97.6	1.65	114.5	1.84	1.3
FUPA	30	28.7	0.24	32.5	0.25	36.8	0.37	1.2
	60	58.4	0.41	59	0.59	70.7	0.67	1.3
	90	87.2	0.65	97.6	0.9	114.5	0.9	1.1
FBAL	30	28.7	0.12	32.5	0.18	36.8	0.39	2.5
	60	58.4	0.21	59	0.46	70.7	0.55	2.2
	90	87.2	0.4	97.6	0.74	114.5	0.93	1.8
Sum of metabolites	30	28.7	1.11	32.5	1.38	36.8	1.99	1.4
	60	58.4	1.88	59	2.85	70.7	3.3	1.4
	90	87.2	3.05	97.6	4.46	114.5	5.38	1.3

**I calculated the accumulation ratio as:

$$(\text{Amount excreted}_{\text{month12}}/\text{Actual dose}_{\text{month12}}) \div (\text{Amount excreted}_{\text{day 4}}/\text{Actual dose}_{\text{day4}})$$

The concentrations of 5-FU and 5-FUH$_2$ were below the detection limit of the method. Between 49 and 69% of an administered dose was collected in the urine across the study. The results show that the

excretion of Capecitabine and all its metabolites, except FBAL, remained approximately constant through the first year of the experiment. FBAL excretion increased about two-fold. I have no other information to determine the significance of this increase. Males and females eliminated Capecitabine similarly except that female mice produced somewhat less 5'DFCR. The following table shows the percent of the total moles excreted as each major metabolite and the parent compound. Assuming no significant changes in metabolism occurred with repeated dosing, this table establishes the excretion profile for Capecitabine and its metabolites in mice.

Analyte	Dose mg/kg	Day 4 % of total moles excreted	Month 6 % of total moles excreted	Month 12 % of total moles excreted	average	St Dev
Capecitabine	30	11.7%	12.3%	9.5%	11.2%	1.5%
	60	12.2%	8.1%	9.1%	9.8%	2.2%
	90	11.1%	8.5%	10.2%	10.0%	1.3%
average					**10.3%**	**1.6%**
5'-DFCR	30	17.1%	14.5%	17.6%	16.4%	1.7%
	60	17.0%	16.1%	19.1%	17.4%	1.5%
	90	18.7%	17.7%	21.6%	19.3%	2.0%
average					**17.7%**	**2.0%**
5'-DFUR	30	38.7%	42.0%	34.7%	38.5%	3.7%
	60	37.8%	38.9%	34.8%	37.2%	2.1%
	90	35.7%	37.0%	34.2%	35.6%	1.4%
average					**37.1%**	**2.6%**
FUPA	30	21.6%	18.1%	18.6%	19.4%	1.9%
	60	21.8%	20.7%	20.3%	20.9%	0.8%
	90	21.3%	20.2%	16.7%	19.4%	2.4%
average					**19.9%**	**1.7%**
FBAL	30	10.8%	13.0%	19.6%	14.5%	4.6%
	60	11.2%	16.1%	16.7%	14.7%	3.0%
	90	13.1%	16.6%	17.3%	15.7%	2.2%
average**					**14.9%**	**3.0%**

** The numbers in the rows marked average are the average for all nine measurements for that metabolite. Likewise, the standard deviation is that of all nine measurements. The averages under the column 'average' are that for the measurements at a particular dose. Likewise, the standard deviation is for the measurements at a particular dose.

Humans excrete about 57% of an administered oral dose into the urine as FBAL and only about 3% as the parent compound. Thus, the excretion profile in humans is significantly different from that in mice. This is but one more piece of information that confirms that mice are a poor model for Capecitabine toxicity.

Carcinogenicity:

2503 A. Kawashima. Twenty four-month oral (feed admix) carcinogenicity study of Ro 09-1978/000 in mice. RR J-146816. 1998. Volume 11, page 1, through Volume 13.

Animals	male and female BDF1 mice (~5 weeks old at start of study)
Drug	Capecitabine
Lot	24289-190 (purity 99.5%)
Doses	0 (control 1), 0 (control 2), 30, 60, 90 mg/kg/day 0, 0, 90, 180, 270 mg/m^2/day
N	50 per sex per dose group 3 males and 3 females were assigned to satellite groups at each dose level for urine sampling
Route	PO, feed admix, *ad libitum*, housed individually "Ro 09-198/000 was admixed to the powdered feed, NIH open formula, with a feed mixing machine( )The admixed food was prepared weekly. The drug concentrations in the feed were calculated based on the body weights and food intake of the previous week for each group of both sexes."
Observations	
Clinical Signs	Daily
Body Wt.	Weekly for 12 weeks and monthly thereafter
Food Cons	Weekly
Palpation	monthly for three months, once every two weeks for the second three months and weekly thereafter.
Hematology	at termination
Gross Pathology	at termination at 24 months
Histopathology	Investigators at( ) only examined tissues from all animals in the Control 1, the high dose group and animals dying before scheduled necropsy. They did not examine the tissues of low and mid-dose animals. See table for tissues examined.
Statistical Methods	Fisher's exact test and Peto's method to assess tumor incidence, $\alpha = 5\%$ one-tailed.

Researchers at( ) Japan, did this study for the sponsor. ( ) Japan did the histopathology. The study director, A. Kawashima initiated the study on September 18, 1995 and signed the GLP statement October 30, 1998. The study included a QA report. The investigators based their dose selection on a 13-week PO study in mice submitted to NDA 20-896, submission 000, October 31, 1997. [N. Shishido *et al.*, Thirteen week oral (feed admix) toxicity study of Ro 09-1978/000 in mice (Pilot study for carcinogenicity study) GCR J-146'497]. In this study, the low dose, 90 mg/kg caused slight anemia and a slight increase in extramedullary hematopoiesis in the spleen. This study was irrelevant to the initial NDA and I have yet to review it. The sponsor did not request a CAC consultation on the dose.

Results:

Mortality: The following table summarizes total mortality for males and females in each dose group.

Dose Group	Cont. 1	Cont. 2	30 mg/kg	60 mg/kg	90 mg/kg
Males	7 (14%)	4 (8%)	6 (12%)	7 (14%)	3 (6%)
Females	14 (28%)	14 (28%)	16 (32%)	15 (30%)	8 (16%)

In males, there were no statistically significant differences among the dose groups. In females, the low mortality in the high dose group was different from both controls and both lower dose groups, but the difference did not reach statistical significance. Most of the deaths occurred after the 75th week.

Clinical Signs — No differences between controls and treated animals

Body weight — No toxicologically significant differences between controls and treated animals. Any statistical differences were sporadic and not related to dosing. Consistent with *ad labium* feeding, the weights of males increased to about 45 g and that of females to about 30 g. The total tumor incidence reflects these high average weights.

Food Cons. — No toxicologically significant differences between controls and treated animals

Dose Validation: The following table shows the calculated mean compound-intake values in mg/kg.

Dose Group	Cont. 1	Cont. 2	30 mg/kg	60 mg/kg	90 mg/kg
			mg/kg	mg/kg	mg/kg
Males	0	0	31.2	62.7	93.1
Females	0	0	30.2	62.2	93.0

Palpation — No differences between controls and treated animals

Hematology — The following table shows the hematological parameters that were statistically significantly ($p < 0.01$) different from Control 1 in the mid and hid dose males at the end of the study.

		Control 1	Mid dose	% difference	High dose	% difference
RBC	10E6/µl	9.42			8.26	-12.3
HCT	%	48.1			44.5	-7.5
Hb	g/dl	14.8			13.1	-11.5
MCV	fl	51.1	52.3	2.3	54.2	6.1
MCH	pg	15.1	15.4	2.0	15.9	5.3

The following table shows hematological changes seen in females at the end of the experiment ($p < 0.05$).

		Control 1	Low dose	% difference	Mid dose	% difference	High dose	% difference
Plt	10E3/µl	1138					1298	14.1
RBC	10E6/µl	9.3			8.75	-5.9	8.4	-9.7
MCV	fl	51.2	52.9	3.3	53.8	5.1	54.7	6.8
MCH	pg	15.2	15.7	3.3	16	5.3	16.3	7.2

I have shown these changes in detail because these toxicities are the ones on which the sponsor bases the claim that the doses were adequately high. None of these toxicities demonstrated a statistically significant dose response. In this case, MCH is a function of MCV. The increases in both are probably up-regulation as a compensation for the decreases in RBC. The decreases in RBC are probably the only toxicologically significant changes in hematological parameters. This decrease is probably a direct result of decreased RBC formation.

Organ Weights — decreases in absolute weight of thymus (~17%) and testes (~5%) in HD males. Small but statistically significant decrease in HD female heart weight and left kidney weights were probably incidental.

Gross Pathology — Major findings included nodular lesions in the Harderian gland, liver, lung, lymph nodes, spleen, pituitary gland, and uterus, and focal lesions in the pituitary (predominantly in females). The incidences of these findings did not demonstrate any dose-related toxicity. Indeed, dosing "tended to suppress nodular lesions and enlargement in the liver, lymphatic tissues and uterus in females."

Presumptive Cause of Death

Most animals that died on study demonstrated pathology consistent with death due to a neoplastic process. None of these causes of death showed or suggested a dose effect. Lethal tumors included malignant lymphoma (4 males, 17 females), histocytic sarcoma (5 males, 27 females), blood vessel tumors (6 males, 7 females) and liver tumors (2 males). Lesions killing but a single animal included lung, intestinal, pituitary, thyroid, Harderian gland, neural and ovarian tumors. Non-tumor causes of death included amyloidosis (1 male), renal lesions (4 females), UTI (1 female), arteritis (2 males and 2 females), Ovarian lesions (1 female), convulsion (1 male), and unclear (3 males and 4 females).

Tumor Incidence:

The following table shows the incidence of major tumors (5% or more in the HD group) by tissue.

	Dose Group, Males					Dose Group, Females				
	Control 1	Control 2	30 mg/kg	60 mg/kg	90 mg/kg	Control 1	Control 2	30 mg/kg	60 mg/kg	90 mg/kg
Number examined	50	4	6	7	50	50	14	16	15	50
Hemolymphoreticular tumors										
Malignant Lymphoma	4	0	1	1	3	6	5	5	4	6
Histocytic sarcoma	4	1	1	3	2	16	4	6	8	6**
Harderian gland tumors										
Adenoma	1	1	0	0	4	3	0	2	0	4
Adenocarcinoma	0	0	0	0	0	1	0	0	0	0
Liver tumors										
Hepatocellular adenoma	10	0	2	0	10	1	0	2	0	1
Hepatocellular carcinoma	3	0	0	1	3	2	0	0	0	1
Hemangioma	1	0	0	0	3	4	1	1	0	3
Hemangiosarcoma	2	1	0	3	1	2	0	0	0	2
Lung tumors										
Bronchiolo-alveolar adenoma	11	2	0	0	4**	3	0	1	0	0
Bronchiolo-alveolar carcinoma	3	0	0	0	2	0	0	0	1	2
Ovarian tumors						n=49		n=15		
Cystadenoma	-	-	-	-	-	1	1	0	0	3
Pituitary tumors						n=49	n=12			n=48
Anterior adenoma	0	0	0	0	0	5	0	0	0	4
Blood vessel tumors										
Hemangioma	6	0	0	0	4	5	1	1	2	3
Hemangiosarcoma	3	2	0	4	3	5	2	2	0	2

**p< 0.05 significant difference from Control-1, The sponsor did not analyze the data of groups other than the 90 mg/kg group statistically.

There were two statistically significant differences in the incidence of specific tumors. In males, there was a significant decrease in bronchiolo-alveolar adenoma (11 in control, 4 in HD group), and in females, there was a decrease in histocytic sarcoma (16 in control, 6 in HD group).

The following table shows the total incidences of tumors.

	Dose Group, Males					Dose Group, Females				
	Control 1	Control 2	30 mg/kg	60 mg/kg	90 mg/kg	Control 1	Control 2	30 mg/kg	60 mg/kg	90 mg/kg
Number examined	50	4	6	7	50	50	14	16	15	50
Number benigh tumors	37	4	3	0	30	25	2	6	2	15
Number malignant tumors	28	6	2	13	20	34	13	14	13	22
Number tumors	65	10	5	13	50	59	15	20	15	37
Number benign tumor bearers	23	2	2	0	22	16	2	6	2	14
Number malignant tumor bearers	18	3	2	7	11	30	10	12	13	20
Number multipal tumor bearers	18	2	1	3	12	13	2	5	2	7
Number tumor bearers	33	3	4	7	30	38	11	13	13	28

In HD males and females, the numbers of tumors and tumor bearers were less than in controls in all cases. Since only animals dying during the study were examined microscopically, no dose relationship could be determined. There was no evidence to suggest a difference in the time course of tumor formation in treated animals.

As expected in an old mouse population, non-tumor microscopic pathology was considerable. Male HD mice showed greater angiectasis and sinus hemorrhage in the lymph nodes than did controls, but this was the only difference seen in proliferative tissues including spleen, stomach, intestine and thymus. These are the tissues where one would expect direct 5-FU toxicity. There were no other toxicologically significant differences in non-tumor microscopic pathology.

Comments and conclusions:

This study failed to demonstrate any carcinogenesis associated with Capecitabine dosing. The statistician's report concurs that there is no significant difference in the incidence of tumors between control and treated animals. Indeed, the study failed to demonstrated any significant toxicity associated with Capecitabine other than a ~10% decrease RBC and a concomitant decrease in Hct in males and females. The dose given to the high dose group is 90 mg/kg or 270 mg/m^2/day. This dose is only 10.8% the recommended clinical dose of 2500 mg/m^2/day for two-weeks. The investigators based their choice of doses on a 13-week oral feeding study in mice (see above). In this study, the group receiving 90 mg/kg suffered "slight anemia and a slight increase in extra-hematopoiesis in the spleen, however no drug-related change in body weight was observed." This dose may have been too low for this carcinogenicity study, but twice this dose, 180 mg/kg, in the 13-week study caused unacceptable weight loss, anemia, increased spleen weight, and regressive changes in proliferative tissues.

Both the rat and the mouse are unacceptable models for toxicity tests for this prodrug. The spectrum of metabolites they each generate is the same as humans, but the plasma concentrations of these metabolites are considerably different. The mouse is more sensitive to Capecitabine than humans

and the rat is extremely insensitive due to high folate reserves. The only relevant species is the monkey. The sponsor did 26-week and 52-week studies in the monkey and reported no signs of carcinogenicity. The monkeys in these studies suffered anemia, but leukopenia and damage to the gastrointestinal tract, spleen, bone marrow and lymph nodes were dose limiting. The spectrum of toxicities in monkeys is similar to that in humans. The spectrum of toxicities seen in mice in the present study is not.

A. Cavaliere *et al.* (*Tumori* 1990 Apr 30;76(2):179-81) have reported that a dose of 5-FU of 30 mg/kg once a week IP in BALB/C mice induced a significant increase in lung tumors in both sexes (males, p less than 0.05; females, p less than 0.01) and tumors of the lymphoreticular system in female mice (p less than 0.001). These results suggest that 5-fluorouracil is carcinogenic in mice. If the current study had been positive, it would have been difficult to determine whether the ultimate carcinogen was Capecitabine or 5-FU or both.

I seriously doubt the sponsor can ever adequately assess the carcinogenic potential of this compound in either the rat or mouse. Nevertheless, Capecitabine is a 5-FU prodrug. Patients receiving cumulative doses of 0.24-1.0 g of fluorouracil parenterally have shown an increase in numerical and structural chromosome aberrations in peripheral blood lymphocytes. 5-FU is probably carcinogenic to humans, so again any differences between carcinogenicity associated with Capecitabine and that of 5-FU would be very difficult to discern. Despite this serious potential toxicity, 5-FU is routinely used as an adjuvant treatment for non-metastatic colorectal cancer after surgical resection. Thus, the results of this study are of little clinical importance.

Summary

The pharmacology of Capecitabine is interesting because Roche intentionally designed this drug to overcome significant problems associated with oral 5-FU treatment. The absorption of 5-FU across the gastric lumen is variable and DPD in the plasma rapidly degrades the compound. Adding ribose-1-phosphate to 5-FU (Furtulon) increases absorption, but this greatly increases GI toxicity. This increased toxicity is due to large concentrations of PyNPase in the human GI. Adding an _____ to Furtulon protects the compound, increases absorption and provides good oral bioavailability, but humans have limited ability to remove this moiety. Finally, by replacing _____ with a N^4-pentyloxycarbonyl group, Roche found a compound that crossed the human GI and can be readily cleaved by three enzyme steps to generate 5-FU systemically. The pharmacology section of the original NDA describes an impressive body of generally good scientific investigation that led to the clinical development of Capecitabine.

The k_m of PyNPase in human lung cancer tissue was 0.24 mM for thymidine, the endogenous substrate, and 1.7 mM for 5'-DFUR. The physical significance of these numbers is questionable because humans express at least two PyNPase enzymes. The k_m of cytidine deaminase from human leukemic granulocytes for cytidine was 11 μM. This activity is not rate limiting in humans. The total cytidine deaminase activity was lower in leukemic cells than in normal granulocytes. Expression increases with differentiation.

In *in vitro* efficacy studies of Capecitabine, 5'-DFCR, 5'-DFUR and 5-FU in the same tumor cell lines, Capecitabine was relatively non-toxic. In most cases, the IC_{50} of the parent drug was greater than 1000 μM. This suggests that most tumor cells do not express significant carboxylesterase activity. Likewise, concentrations of 5'-DFCR less than 90 μM were toxic to only Scabber cells. Again, this suggests that most tumor cells do not express significant cytidine deaminase activity. Most of this activity is in the circulation or the liver. Only 5'-DFUR and 5-FU killed most tumor cells effectively at concentrations below 100 μM. Nevertheless, in all but two cell lines, the IC_{50}s of 5'-DFUR were at least ten times higher than those of 5-FU. This suggests that Capecitabine *in vitro* is relatively non-toxic, but also that 5-FU itself is more effective than any of the metabolites at the cellular level in tumor. In most cases, the expression of PyNPase is not sufficient to make 5'-DFCR as effective as 5-FU.

In humans, the great majority of carboxylesterase activity is in the liver. Tumor and normal tissue express about the same activity. Human liver, kidney, stomach and lower GI tissue express the largest amounts of cytidine deaminase activity, but the blood also contains considerable activity. In human tumors, the expression of this activity is more variable than in normal tissue. Some individual tumors, within a large sampling of colon, ovarian, and cervical tumors, appear to express more of this activity than normal tissue. PyNPase is widely expressed in normal tissue, but the largest activities are found in the liver. Many tumors from many different tissues express more activity of this enzyme than adjacent normal tissue, but the variability is large. This means that some tumors express much more PyNPase than normal tissue and some express much less. Without specific testing, an oncologist could not know *a priori* whether a tumor expressed excess PyNPase activity. Thus, there is insufficient scientific evidence to support the sponsor's claim that Capecitabine therapy is 'tumor specific'.

In the mouse, carboxylesterase activities and PyNPase activities are greatest in small intestine, most cytidine deaminase activity is in the kidney. In the rat, most of the carboxylesterase activity is in the small intestine and liver, and most PyNPase is in the lung and small intestine. The rat expresses relatively low cytidine deaminase activities. In monkeys, most carboxylesterase activity is in the liver. Most major organs in the monkey express significant cytidine deaminase and PyNPase activity. Though the carboxylesterase activity is six times higher in human liver than in monkey liver, the distribution of this activity and of that of the other two enzymes is similar to that of humans in all tissues. Again, this justifies the use of monkeys in the development of Capecitabine. The tissue distributions suggest that the limiting toxicities will be in the gastrointestinal system in monkeys and in the liver in humans. These predictions turn out to be true for both species.

Human liver expresses two carboxylesterase activities but human colon expresses only one, called isoenzyme B. The other isoform, isoenzyme A, cleaves N^4-alkoxycarbonyl-5'-DFCR compounds, such as Capecitabine. Isoenzyme B does not. Thus, Capecitabine crosses the human GI relatively intact and is then cleaved to 5'-DFCR in the liver. The substrate specificity and distribution of carboxylesterase enzymes in the monkey is similar to those of human. Those in mouse are not. Thus, the monkey is an appropriate model for the preclinical development of Capecitabine.

PyNPase in humans is homologous with platelet-derived endothelial cell growth factor (rPD-ECGF). rPD-ECGF has thymidine phosphorylase activity. These results imply that PyNPase activity may be important in tumor angiogenesis. Cytokines such as TNFα, IL-1α and IFNγ can increase the

expression of PyNPase in various tumor cell lines. This increase in expression increases the toxicity of 5'-DFUR in tumor cell lines.

Repeated dosing with Capecitabine does not induce carboxylesterase or cytidine deaminase activity in the colon or liver of the monkey. In safety pharmacology studies, Capecitabine caused little toxicity other than that associated with anticipatable 5-FU toxicity.

Rats express little cytidine deaminase. Consequently, they develop high plasma concentrations of 5'-DFCR and low steady state concentrations of 5'-DFUR and 5-FU when given oral Ro 09-1978. The rat toxicity data cannot be considered predictive for humans. In contrast, monkeys develop comparably high plasma concentrations of 5'-DFUR and parent compound. Thus, the monkey is probably the most predictive species for the human response to Ro 09-1978.

No mice died in single dose gavage toxicity studies, so the rodent LD_{50} of Capecitabine remains unknown. The only clinical symptom observed in these single-dose studies was hypoactivity. This hypoactivity persisted for about 1 hr in mice receiving 3000 mg/m^2 and for from 2 to 4 hr in mice receiving 6000 mg/m^2. These single doses in mice caused no gross pathology. Similarly, in rats, doses to 12000 mg/m^2 caused only decreased activity. This symptom was more frequent in males than in females.

Similarly, Capecitabine caused no clinical symptoms in rats dosed daily for 4 weeks by gavage to doses of 3231 mg/m^2. Rats dosed for 26 weeks at doses to 2154 mg/m^2 (gavage) suffered minor changes in hematological parameters, including increased MCV in females and MCH in males. Serum protein was decreased in males suggesting mild hepatic toxicity. At 3231 mg/m^2 these same symptoms increased in severity. This dose caused slight degeneration of rectal cells in males, but killed no rats.

Monkeys dosed with 2154 mg/m^2 daily for 28 days suffered diarrhea and slight weight loss. Thymus weight and WBC decreased. This dose caused some microscopic damage in the small intestine and in lymphatic and hematopoietic organs. A dose of 4308 mg/m^2 daily for 28 days rendered two male monkeys moribund on days 20 and 27. This dose is about one-third higher than the approved clinical dose. This dose increased the severity of the symptoms seen at the lower dose and caused and a decrease in spleen weight, an increase in adrenal weight. The moribund monkeys were emaciated and had low WBC. The dose limiting toxicities in monkeys are degeneration of the gastrointestinal system and myelosuppression. A longer dosing schedule, 26 weeks, with 1728 mg/m^2 caused a decrease in red cell parameters, RBC, Hct, Hbg. This high dose rendered one female monkey moribund on day 57. This monkey was emaciated. These signs and symptoms were consistent with 5-FU toxicity. Nevertheless, the monkey does not predict the hepatotoxicity seen in humans, probably because they express less carboxylesterase activity in the liver.

In reproductive function tests in the mouse (Segment I), Capecitabine caused a dose dependent and severe decrease in female fertility. The percentage of fertile females decreased from 83 in controls to 13 in mice given 2280 mg/m^2/d. This decrease was associated with continuous diestrus. This high dose also decreased the weight of testes and epididymides in mated males. Capecitabine caused a dose dependent, severe, decrease in the total number of corpora lutea, number of live fetuses, the percentage of live fetuses relative to implantation and early deaths. The impairment of fertility in female dams appeared reversible.

In a study of toxicity during organogenesis in the mouse (Segment II), doses as high as 2373 mg/m^2/d caused only a decrease in body weight gain in the dams. Nevertheless, it caused a 100% decrease in the number of corpora lutea in the high dose group. This decrease was dose dependent. All fetuses in the high dose group died early in pregnancy and again this increase in fetal death was dose dependent. Fetal external anomalies in the low and mid dose group included cleft palate, anophthalmia, microphthalmia, oligodactyly, polydactyly, syndactyly and kinky tail. The total incidence of abnormalities increased with dose. Visceral abnormalities included the ones mentioned above plus esophagectopy and dilation of the renal pelvis. Skeletal abnormalities included cleft palate, fusion of cervical vertebra, abnormal shape of cervical vertebra, fusion of thoracic vertebra, wavy rib and fusion of metacarpus. A decrease in ossification in the caudal vertebra was seen in the LD and MD groups. Twenty-four of 65 LD fetuses had a rudimentary 14th rib.

In a study of exposure during late pregnancy and lactation (Segment III) in mice, Capecitabine at less than half the proposed clinical dose on a mg/m^2 basis (1200 mg/m^2) caused little toxicity to the dams. The doses in this study caused no differences the number of live neonates, implantations, lactation indices, external abnormalities or reproductive function in the F1 generation. The highest dose did cause a slight decrease in F1 female body weight and some neurological parameters (increased rearing and walking) may have been affected. High dose F1 mice showed some damage to reproductive organs though there was no decrease in reproductive function.

In the monkey, doses of 1080 and 2160 mg/m^2/d were embryo lethal during organogenesis (Segment II). The high dose decreased fetal ovary weight significantly (to 30% of control) and there was evidence that other organs may have been smaller as a function of dose (brain, thymus, lung, spleen, and kidney). Capecitabine at doses less than the proposed clinical dose on a mg/m^2 basis is fetotoxic and embryolethal. The parent drug and its metabolites are excreted into milk in considerable concentrations.

Capecitabine did not cause mutations in the Ames assay with or without S9 activation. Likewise it did not cause mutations in V79 Chinese hamster lung cells (HPRT assay) with or without metabolic activation. At the highest dose tested, it did cause an approximately five-fold increase in chromosome aberrations (excluding gaps) in human peripheral blood lymphocytes exposed for 24 hours *in vitro* without S9. With a 48 hour exposure, the number of aberrations (excluding gaps) increased approximately 23-fold. This clastogenicity was not evident with metabolic activation, probably because of the short exposure times. In the mouse micronucleus test, Capecitabine caused a two-fold increase in micronuclei. This increase did not reach the level of statistical significance. 5-FU is positive in the mouse micronucleus test. A two-year carcinogenicity study in the mouse with a high dose of 270 mg/m^2/day did not demonstrate any increase in tumors. The doses in this study were too low and the study provides little useful information. The concentration of the enzymes that metabolize Capecitabine in the mouse is considerably different from that of humans. These differences possibly protect them from much of Capecitabine or 5-FU's potential genotoxicity. It is unlikely that the sponsor could design a study in rodents that would provide adequate exposure to demonstrate carcinogenicity. Nevertheless, clinical doses of Capecitabine are probably clastogenic in humans.

Capecitabine is rapidly absorbed across the GI in the monkey. In most studies, apparent T_{max} was between 30 min and one hour. Oral bioavailability of Capecitabine in solution is at least 81% in the monkey. The three different clinical formulations provide roughly the same bioavailability as measured

by urinary excretion. Maalox did not significantly affect the absorption of an oral dose in rats. Studies in monkeys suggest that food may slow Capecitabine absorption.

Capecitabine and its metabolites distribute widely throughout the body. In the mouse, the largest concentrations are found in the stomach and bladder at 0.5 and 2 hours. High concentrations are also found in the liver and kidney. Concentrations in the brain are low, implying that Capecitabine does not cross the blood brain barrier. The concentration in most other major tissues uniform. Relatively high concentrations are found in the intestines at 6 hours despite rapid absorption. This information and plasma concentration curves suggest that Capecitabine and 5'-DFUR may recirculate within the enterohepatic system.

Most of a dose of Capecitabine is eliminated in the urine. Little is eliminated in the feces. Studies in monkeys suggest that food may slow Capecitabine absorption. Mice also eliminate most of a dose in the urine. The major metabolites of Capecitabine were FBAL in monkey urine, 5'–DFCR in rat urine and 5'–DFUR in mouse urine.

In multiple dose studies in the monkey, the AUC values for Capecitabine and its metabolites 5'-DFCR and 5'-DFUR increased with dose, but in some cases this increase was greater than linear at the higher doses. In rats the AUC values for Capecitabine, 5'-DFCR and 5'-DFUR increased linearly over the range of 179.5 to 538.5 mg/kg. Repeated dosing did not affect the AUC values in the monkey. There were no consistent gender differences in monkeys. In rats the AUC values for Capecitabine and 5'-DFUR were similar in males and females, but the AUC values for 5'-DFUR were 40 to 120% higher in females than in males. Capecitabine dosing did not induce or inhibit any cytochrome P450 tested.

Recommendation

Based on the pharmacology and toxicology data presented in this NDA, I have no objection to the approval of Capecitabine for this indication.

Discussed with the Medical Officer:

The doses in the mouse carcinogenicity study are too low to demonstrate conclusively that Capecitabine is not carcinogenic in that species. The study does not support the sponsor's label claim.

At least one patient has died from Capecitabine overdose. Should this patient's death and the lethal dose be described in the drug label?

Labeling Comments and Changes:

Under the section Drug-Drug interactions, the text currently reads:

DRAFT LABELING

It should be changed to read:

Draft

Under the section ***Carcinogenesis, Mutagenesis and Impairment of Fertility***, the first sentence describing the results of the two-year mouse carcinogenicity currently reads:

Draft

This statement should be eliminated or it should be changed to read:

Draft

Under the section ***Nursing Women***, the text currently reads:

Draft

It should be changed to read:

The section Overdosage should be changed to . The sentence describing

/S/

W. David McGuinn, Jr.; Ph.D., D.A.B.T.

September 7, 2000

cc: Original NDA
HFD-150 Division file
/WD McGuinn
/J Leighton
/A Martin
/M Pelosi

/S/
9/8/00

APPEARS THIS WAY
ON ORIGINAL

Histopathology Table

Study animal	J-146'257 Cynomolgus monkey	J-146'171 Rat	J-146'258 Rats	J'146'413 Cynomolgus monkey
adrenals	X	X	X	X
aorta	X	X	X	X
bone marrow				
femoral	X	X	X	X
sternum				
brain				
cerebrum	X	X	X	X
cerebellum	X	X	X	X
brain stem	X	X	X	X
spinal cord	X			X
bronchus	X			X
epididymides	X	X	X	X
esophagus	X	X	X	X
eyeball	X	X	X	X
femur	X			X
gall bladder	X			X
heart	X	X	X	X
harderian gland		X	X	
kidneys	X	X	X	X
lacrimal gland	X			X
large intestine				
cecum	X			
colon	X	X	X	X
rectum	X	X	X	X
liver	X	X	X	X
lung	X	X	X	X
lymph node				
submandibular	X			
mesenteric	X	X	X	X
mammary gland	X	X	X	X
muscle	X	X	X	X
ovaries	X	X	X	X
pancreas	X	X	X	X
parathyroid	X		X	X
pituitary	X	X	X	X
prostate	X	X	X	X

salivary glands		X	X	X
sciatic nerve	X	X	X	X
seminal vesicle	X	X	X	X
skin	X	X	X	X
small intestine				
duodenum	X	X	X	X
jejunum	X	X	X	X
ileum	X	X	X	X
spinal cord	X	X	X	X
spleen	X	X	X	X
sternum	X	X	X	X
stomach		X	X	X
fundus	X			
pylorus	X			
testes	X	X	X	X
thymus	X	X	X	X
thyroid	X	X	X	X
tongue	X	X	X	X
tonsil	X			X
trachea	X		X	X
urinary bladder	X	X	X	X
uterus	X	X	X	X
vagina	X	X	X	X
gross lesions	X	X	X	X