CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20-896

CHEMISTRY REVIEW(S)

Division of Oncologic Drug Products
Review of Chemistry, Manufacturing and Controls

NDA #: 20-896 **CHEM. REVIEW#:** 1 **REVIEW DATE:** Feb. 27,1997

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	Oct. 28, 1997	Oct. 31,1997	Nov. 4, 1997

NAME AND ADDRESS OF APPLICANT: Hoffmann-La Roche Inc.
340 Kingsland St.
Nutley, NJ 07110-1199

DRUG PRODUCT NAMES:

Proprietary:	XELODA Tablets
Nonproprietary/USAN:	Capecitabine
Code Name/#:	CAS 154361-50-9
Chem. Type/Ther. Class	1-P

PHARMACOL. CATEGORY/INDICATION:

DOSAGE FORM/STRENGTHS: 150 and 500 mg/tablets

ROUTE OF ADMINISTRATION: Oral

MANUFACTURER:

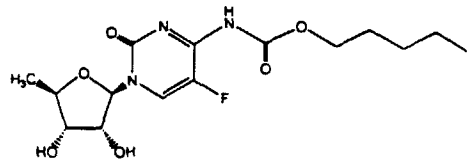
A. Drug Substance
Roche Carolina Inc.
6173 E. Old Marion Highway
Florence, SC 29506-9330

B. Drug Product
Hoffmann-La Roche Inc.
340 Kingsland St.
Nutley, NJ 07110-1199

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR WEIGHT:

5'-Deoxy-5-fluoro-N-[4-pentyloxycarbonyl]-cytidine

$C_{15}H_{22}FN_3O_6$, MW = 359.35



SUPPORTING DOCUMENTS:

DMF

DMF

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IND

CONSULTS:

Consult	Status	Comments
EER	Pending	Submitted on 12/10/97
Methods Validation	Pending	Will be submitted after the deficiencies have been addressed by the applicant
Trademark	Pending	Submitted on 12/12/97
Statistics	Pending	Submitted on 12/12/97
Biopharmaceuticals	Pending	Submitted on 12/12/97

Environmental Assessment.	Categorical exclusion	Acceptable

REMARK/COMMENTS:
Drug Substance: There are several deficiencies relating to the drug substance. Most of the deficiencies are not serious and should be relatively simple for the applicant to address.

Drug Product: There are several deficiencies relating to the drug product. Most of the deficiencies are not serious and should be relatively simple for the applicant to address. The most concerned deficiencies for the drug product are the specifications.

CONCLUSIONS AND RECOMMENDATIONS:
The NDA is approvable with respect to CMC issue if the deficiencies are addressed satisfactorily and the several consults and the EER are outstanding.

/S/ 2/27/98
Chengyi Liang, Ph.D. Review Chemist

/S/ 3/27/98
Liang Zhou, Ph.D.
Acting Chemistry Team Leader

CC:

Orig. NDA 20-896
HFD-150 Division File
HFD-150/CLiang
HFD-150/LZhou
HFD-150/MPelosi

Division of Oncologic Drug Products

Review of Chemistry, Manufacturing and Controls

NDA #: 20-896 **CHEM. REVIEW#:** 2 **REVIEW DATE:** 3/28/98

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	10/28/1997	10/31/1998	11/4/1997
BC	3/12/1998	3/13/1998	3/17/1998
BC	3/16/1998	3/18/1998	3/23/1998
BC	3/18/1998	3/19/1998	3/24/1998

NAME AND ADDRESS OF APPLICANT: Hoffmann-La Roche Inc.
340 Kingsland St.
Nutley, NJ 07110-1199

DRUG PRODUCT NAMES:

Proprietary:	XELODA Tablets
Nonproprietary/USAN:	Capecitabine
Code Name/#:	CAS 154361-50-9
Chem. Type/Ther. Class	1-P

PHARMACOL. CATEGORY/INDICATION:

DOSAGE FORM/STRENGTHS: 150 and 500 mg/tablets

ROUTE OF ADMINISTRATION: Oral

MANUFACTURER:

A. Drug Substance
Roche Carolina Inc.
6173 E. Old Marion Highway
Florence, SC 29506-9330

B. Drug Product
Hoffmann-La Roche Inc.
340 Kingsland St.
Nutley, NJ 07110-1199

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR WEIGHT:

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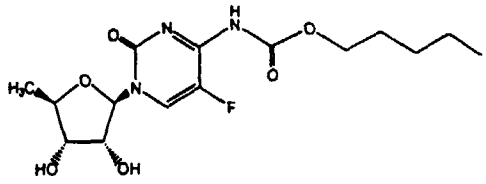

SUPPORTING DOCUMENTS: IND

Statistics	Received dated 3/18/98. The proposed expiration dating period is acceptable based on the stability data.	
Biopharmaceuticals	Acceptable	
Environmental Assessment	Categorical exclusion	Acceptable

REMARK/COMMENTS:
The amendments (BC) dated 3/12/98 and 3/16/98 provided for the batch records and the information about process change during the manufacture of DP tablets. The amendment BC dated 3/18/98 responded to the deficiencies which were found in the original NDA review. The details see review notes enclosed.

CONCLUSIONS AND RECOMMENDATIONS:
Adequate information is provided to address remained deficiencies for the drug substance and drug product. Approval is recommended for this NDA with respect to CMC issues. Pending EER. However, the project manager should communicate with the applicant as follows:

1. Provide a commitment

2. The applicant is recommended to include the code for each test method in the specifications of The example can be seen in original NDA, (Vol.6, pp 19-20) for

3. Provide a phase IV commitment

4. Please provide a copy of revised bottle labels.

/S/ 4/2/98
Chengyi Liang, Ph.D., Review Chemist

/S/ 4/2/98
Liang Zhou, Ph.D.
Chemistry Team Leader

Orig. NDA 20-896
HFD-150/Division File
HFD-150LZhou
HFD-150CYLiang
HFD-150/MPelosi
HFD-810/CHoiberg/JSimmons

Division of Oncologic Drug Products

Review of Chemistry, Manufacturing and Controls

NDA #: 20-896 **CHEM. REVIEW#:** 3 **REVIEW DATE:** 4/14/98

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	10/28/1997	10/31/1998	11/4/1997
BC	3/12/1998	3/13/1998	3/17/1998
BC	3/16/1998	3/18/1998	3/23/1998
BC	3/18/1998	3/19/1998	3/24/1998
BC	4/9/1998	4/10/1998	4/13/1998

NAME AND ADDRESS OF APPLICANT: Hoffmann-La Roche Inc.
340 Kingsland St.
Nutley, NJ 07110-1199

DRUG PRODUCT NAMES:

Proprietary:	XELODA Tablets
Nonproprietary/USAN:	Capecitabine
Code Name/#:	CAS 154361-50-9
Chem. Type/Ther. Class	1-P

PHARMACOL. CATEGORY/INDICATION:

DOSAGE FORM/STRENGTHS: 150 and 500 mg/tablets

ROUTE OF ADMINISTRATION: Oral

MANUFACTURER:

A. Drug Substance
Roche Carolina Inc.
6173 E. Old Marion Highway
Florence, SC 29506-9330

B. Drug Product
Hoffmann-La Roche Inc.
340 Kingsland St.
Nutley, NJ 07110-1199

CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR WEIGHT:

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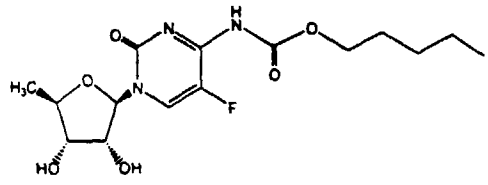

SUPPORTING DOCUMENTS: IND

DMF

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DMF

DMF

DMF

CONSULTS:

Consult	**Status**	**Comments**
EER	Submitted on 12/10/97, approved at 1/27/98	
Methods Validation	Pending	Will be submitted after the deficiencies have been addressed by the applicant

Trademark	The Committee has found (3/1/98) that the proposed name "Xeloda" is acceptable.	
Statistics	Received dated 3/18/98. The proposed expiration dating period is acceptable based on the stability data.	
Biopharmaceuticals	Acceptable	
Environmental Assessment	Categorical exclusion	Acceptable

REMARK/COMMENTS:
The amendment (BC) dated 4/9/98 was submitted to respond the questions raised in the review of previous amendments (BC) dated 3/12, 3/16 and 3/18.

FDA Request #1:
Provide a commitment that the drug product tablets manufactured with revised process procedure will be put on stability studies in order to demonstrate that the process change does not impact the stability of the drug product during the shelf life.

Response:
Stability Conunitment: Three batches of each dosage strength of Xeloda (capecitabine) Tablets manufactured using the improved manufacturing process will be placed on stability, and the data will be included in the annual progress report.

Comment: Acceptable.

FDA Request #2:
The applicant is recommended to include the code for each test method in the specifications of the drug product. The example can be seen in original NDA (Vol. 6, pp. 19-20) for drug substance.

Response:
As stated in the March 18, 1998 amendment, Sponsor Reply #4, page 3, we do not use a numbering system for test methods because the test methods and specifications used at Hoffmann-La Roche Inc. (Roche) are contained in a single document. The specification example referred to in this request (original ADA, Vol. 6, pp. 19-20) belongs to Roche Carolina Inc. (RCI), where the test methods exist as separately numbered documents. As you requested, Roche has numbered the test methods to correspond to the specifications within a single document.

Comment:
The firm has agreed to develop method codes for DP.

FDA Request #3:
Provide a phase IV commitment

Response:

Comment:
In the applicant's response to the Agency's comment #3, this Phase IV commitment is not acceptable.

The manufacturer should be able to produce the drug substance within the tightened specifications. Alternatively, the applicant ought to demonstrate these high amount of residual solvents (e.g. not individual residual solvent) in the drug substance or drug product would not cause any adverse effect in the clinical studies. Given a special consideration of the drug product used as a third line therapy in the treatment of patients with locally advanced or metastatic breast cancer, we agree with the applicant's commitment to justify this specification at within one year of the approval of the drug product. However, the applicant should address these issues in a prior-approval supplement rather than submitting in an NDA Annual Report.

FDA Request #4:
Please provide a copy of revised bottle labels

Sponsor Reply #4:
Revised labeling is included in Attachment 2.

Comment: Acceptable.

CONCLUSIONS AND RECOMMENDATIONS:
Adequate information is provided to address remained deficiencies for the drug substance and drug product. Approval is recommended for this NDA with respect to CMC issues pending EER.

/S/ 4/14/98

Chengyi Liang, Ph.D., Review Chemist

/S/ 4/15/98

Liang Zhou, Ph.D.
Chemistry Team Leader

Orig. NDA 20-896
HFD-150/Division File
HFD-150LZhou
HFD-150CLiang
HFD-150/MPelosi
HFD-810/CHoiberg/JSimmons

Division of Oncologic Drug Products

Review of Chemistry, Manufacturing and Controls

NDA #: 20-896 **CHEM. REVIEW#:** 4 **REVIEW DATE:** 4/23/98

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	10/28/1997	10/31/1998	11/4/1997
BC	3/12/1998	3/13/1998	3/17/1998
BC	3/16/1998	3/18/1998	3/23/1998
BC	3/18/1998	3/19/1998	3/24/1998
BC	4/9/1998	4/10/1998	4/13/1998
BC	4/15/1998	4/16/1998	4/23/1998

NAME AND ADDRESS OF APPLICANT: Hoffmann-La Roche Inc.
340 Kingsland St.
Nutley, NJ 07110-1199

DRUG PRODUCT NAMES:

Proprietary:	XELODA Tablets
Nonproprietary/USAN:	Capecitabine
Code Name/#:	CAS 154361-50-9
Chem. Type/Ther. Class	1-P

PHARMACOL. CATEGORY/INDICATION:

DOSAGE FORM/STRENGTHS: 150 and 500 mg/tablets

ROUTE OF ADMINISTRATION: Oral

MANUFACTURER:

A. Drug Substance
Roche Carolina Inc.
6173 E. Old Marion Highway
Florence, SC 29506-9330

B. Drug Product
Hoffmann-La Roche Inc.
340 Kingsland St.
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CHEMICAL NAME, STRUCTURAL FORMULA, MOLECULAR WEIGHT:

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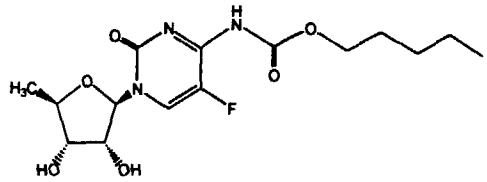

SUPPORTING DOCUMENTS: IND

DMF

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CONSULTS:

Consult	**Status**	**Comments**
EER	AC for	Approved at 1/27/98
	AC for	Approved at 4/17/98
Methods Validation	Pending	Will be submitted

Trademark	The Committee has found (3/1/98) that the proposed name "Xeloda" is acceptable.	
Statistics	Received dated 3/18/98. The proposed expiration dating period is acceptable based on the stability data.	
Biopharmaceuticals	Acceptable	
Environmental Assessment	Categorical exclusion	Acceptable

REMARK/COMMENTS:
The amendment (BC) dated 4/16/98 was submitted to respond the questions raised in the review of previous amendments (BCs) dated 4/14. EER is attached to this review note (AC recommendation from Office of Compliance).

CONCLUSIONS AND RECOMMENDATIONS:
Adequate information is provided to address remained deficiency for the drug substance and drug product. Approval is recommended for this NDA with respect to CMC issues.

/S/

Chengyi Liang, Ph.D., Review Chemist

/S/ 4/23/98

Liang Zhou, Ph.D.
Chemistry Team Leader

Orig. NDA 20-896
HFD-150/Division File
HFD-150LZhou
HFD-150CLiang
HFD-150/MPelosi
HFD-810/CHoiberg/JSimmons

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:NDA 20-896

PHARMACOLOGY REVIEW(S)

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

NDA 20-896 **Submission:** # 000 **Type:** NDA
Original NDA Received October 31, 1997
Completed April 17, 1998
Revised April 20, 1998

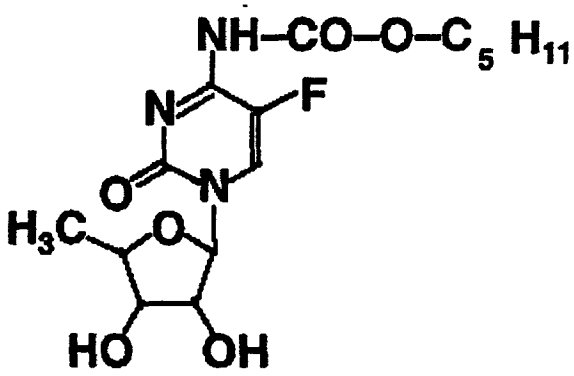
Sponsor: Hoffmann-La Roche Inc.
340 Kingsland Street
Nutley, NJ 07110-1199

Information to be conveyed to the sponsor: YES

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

Drug Name: Ro 09-1978, Capecitabine
Chemical Name: N^4-Pentyloxycarbonyl-5'-deoxy-5-fluorocytidine
FW: 359.35

Structure:



Class cytotoxin, 5-Fluorouracil pro-drug

Indications: The treatment of patients with locally advanced or metastatic breast cancer after failure of paclitaxel and an anthracycline–containing chemotherapy regimen

Related drugs 5-FU, Furtulon, NeoFurtulon

Related INDs

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.

Breast Carcinoma: 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.

Studies Reviewed:

Studies Not Reviewed:

The sponsor submitted many studies in this NDA. I have reviewed only the most relevant. For a complete list of all the studies in this submission, see Volume 1, page 13, NDA 20-896.

Rationale

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 NeoFURTULON (N^4-trimethoxybenzoyl-5'-deoxy-5-fluorocytidine). 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 the N^4-trimethoxybenzoyl 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 NeoFURTULON for chemotherapy when they found it was a poor substrate for acylamidase in humans. Subsequently, Roche developed Ro 09-1978 by replacing the N^4-trimethoxybenzoyl group of NeoFURTULON with a N^4-pentyloxycarbonyl group. Roche claims that the activity of acylamidase is 50 times greater for this compound than for NeoFURTULON. 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.

Glossary of Abbreviations:

ACNU	Nimustine
ACU	6–Amino–5–chlorouracil
AUC	Area under the concentration–time curve
CDDP	Cisplatin
Cmax	Maximum concentration
CPA	Cyclophosphamide
Cyd	Cytidine
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
DXR	Doxorubicin
ED_{50}	Dose at which tumor growth is inhibited by 50%
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

hr	Human recombinant
IC_{50}	Dose at which cell growth is inhibited by 50%
IFN	Interferon
LC–MS/MS	Liquid chromatography–tandem mass spectrometry with ion–spray interface
IL	Interleukin
MEM	Modified Eagle's Medium
MMC	Mitomycin C
MTT	3–[4,5–Dimethythiazole–2–yl]–2,5–diphenyltetrazolium bromide
MoAb	Monoclonal antibody
MTX	Methotrexate
PD–ECGF	Platelet derived endothelial cell growth factor
PyNPase	Pyrimidine nucleoside phosphorylase
THU	Tetrahydrouridine
TNF	Tumor necrosis factor
TS	Thymidylate synthase
U	Unit
UFT	Fixed combination of tegafur and uracil (1:4)
UrdPase	Uridine phosphorylase
UTP	Uridine tri–phosphate

I have excerpted portions of this review directly from the Sponsors submission.

Review:

physical characteristics:

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

Pharmacology:

1101) H. Ishitsuka *et al.* (1980) Role of uridine phosphorylase for antitumor activity of 5'-deoxy-5-fluorouridine. *Gann (Jpn J Cancer Res.)*. 71:112-123. Volume 17, 1-12.

This paper simply confirms the original work of Cook et al. (1979, J. Med. Chem. 22:1330) where 5'-deoxy-5-fluorouridine (5'-DFUR) was first described. 5'-DFUR is hydrolyzed to 5-FU by uridine phosphorylase (pyrimidine nucleotide phosphorylase, PyNPase). Different tumors express different concentrations of this enzyme. Ishitsuka *et al.* confirmed that 5'-DFUR inhibited the growth of Sarcoma-180 tumor cells implanted in mice in a dose proportional manner whether administered i.v. or i.p. This cytostatic effect correlated with a high concentration of PyNPase within these tumor cells. Likewise, mice implanted with the P388 leukemia cell line survived longer than controls when given 5'-DFUR. Survival was dose proportional. 5'-DFUR was not cytotoxic to a similar leukemia cell line resistant to 5-FU. Uridine concentration > 10 mg/ml reversed the cytostatic effect of 5'-DFUR in sarcoma 180 cells in culture. The statistical methods used to interpret the data in this paper are inappropriate.

1102) A. Kono *et al.* (1983) Activation of 5'-deoxy-5-fluorouridine by thymidine phosphorylase in human tumors. *Chem. Pharm. Bull.* 31:175-178. Vol 17, 13-16.

Kono *et al.* assessed the activity of PyNPase in cytosolic extracts of human tumor tissues, normal tissues from corresponding organs taken at surgery and tumor tissue from mice and guinea pig. They determined enzyme activity using thymidine, uridine and 5'-DFUR as substrates. This manuscript has many deficiencies. The procedure for extraction of product is not validated. The chromatographic determination of products is inadequately described, validated and calibrated. The assay volume is unnecessarily small. Small assay volumes frequently lead to inaccuracies. The maximum concentration of 5'-DFUR used is only 5X greater than k_m, thus it is uncertain that the enzyme was saturated with substrate and that Michaelis-Menten approximations were appropriate. (Thymidine $k_m = 0.24$ mM, 5'-DFUR $k_m = 1.7$ mM). The investigators did not specify the pH and temperature optima of the reaction.

The enzyme activity in human tumor, normal tissue and animal tumor under standard conditions was isolated as the 105,000Xg supernatant. Yet, when the researchers partially purified the enzyme, they

fractionated the 7000Xg supernatant with ammonium sulfate. No fractional recoveries are given. Thus, one cannot be sure what percent of the enzyme was recovered in the 105,000Xg supernatants and how this varies with different tissues.

In this research the human tumor tissue contained from 5 to 10 times more PyNPase activity than corresponding normal tissue. Activity decreased with substrate roughly, thymidine 20X > 5'-DFUR 4X > uridine in human tumor and in normal tissue. In animal tumors, this activity relationship was significantly different, uridine 4X > thymidine ~ 5'-DFUR. 1-(2'-Deoxy-b-D-glucopyranosyl) thymine (GPT) sharply inhibited PyNPase activity in mouse tumors for all three substrates. Nevertheless, GPT inhibited only uridine phosphorylase activity in human tumor extracts. GPT did not inhibit 5'-DFUR phosphorylase activity and thymidine phosphorylase activity increased. Thus, the human tissue extract probably contained more than one PyNPase enzyme. A study I have reviewed below (1111) shows this to be the case. So, the mouse is a poor model for the pharmacodynamics of this compound. The deficiencies cited above render this work qualitative at best.

1103) Miwa *et al.* (1986) Enzymatic cleavage of various fluorinated pyrimidine nucleosides to 5-FU and their anti-proliferative activities in human and murine tumor cells. Chem. Pharm. Bull. 34:4225-4232. Vol 17, 17-24.

This paper determines the hydrolysis of numerous synthetic 5-FU derivatives by several tissues including mouse sarcoma and human stomach tumors. This study does not include R0-09-1978, only earlier derivatives.

1104) W. Bollag and H. R. Hartmann (1980) Tumor inhibitory effects of a new fluorouracil derivative: 5'-deoxy-5-fluorouridine. Eur. J. Cancer, 1990; 16:427-432. Vol. 17, 25-30.

Bollag and Hartmann showed that 5'-DFUR was cytostatic against Crocker sarcoma S180, Lewis lung carcinoma and squamous cell carcinoma of the skin in mice. This cytostatic effect was dose proportional. 5'-DFUR was less effective against leukemia L1210 and B-16 melanoma. This finding suggests that PyNPase may not be over-expressed in these tumors. The cytostatic effect correlated with increased mouse survival (approximately 10 fold over 5-FU as measured by the authors calculated therapeutic index) over the observation period. 5'-DFUR was myelosuppressive only at doses 24 times higher than myelosuppressive doses of 5-FU (in mg/kg or about 10 times higher in mmol/kg).

1105) Y Ninomiya *et al.* (1990) Comparative anti-tumor activity and intestinal toxicity of 5'-deoxy-5-fluorouridine and its pro-drug trimethoxybenzoyl-5'-deoxy-5-fluorocytidine. Jpn J Cancer Res. 81:188-195. Vol 17, 31-38.

NeoFURTULON or Ro 09-1390 is the N^4-Trimethoxybenzoyl analog of Ro 09-1978. Hoffmann-La Roche synthesized NeoFURTULON as a prodrug of FURTULON (5'-DFUR) in an attempt to circumvent the GI toxicity caused when 5'-DFUR is given p.o. Japan has approved NeoFURTULON for cancer chemotherapy.

The mice used in the study bore implanted Lewis lung carcinoma. When the investigators gave mice 1 mmol/kg 5'-DFUR p.o., they found that intestinal tissue converted most of the dose to 5-FU in less than an hour. Concentrations were as high as 12 μmol/g of intestinal tissue. 5'-DFUR was almost undetectable in blood at 15 min, but concentrations of 5-FU were nearly 400 nmol/ml. Concentrations of 5'-DFUR < 70 nmol/g did accumulate in tumor tissue. This 5'-DFUR was converted to 5-FU, concentration < 50 nmol/g. By contrast when the mice received 1 mmol/kg NeoFURTULON p.o., concentrations of 5-FU and 5'-DFUR were < 400 nmol/g of intestinal tissue. 5-FU was undetectable in blood at 15 minutes. Total pyrimidine metabolite concentrations in tumor tissue were > 400 nmol/g of tissue. NeoFURTULON caused tumor shrinkage over 20 days similar to that caused by 5'-DFUR. NeoFURTULON caused less weight loss and was less toxic to intestinal tissue than 5'-DFUR. Intestinal tissue contains low concentrations of acylamidase, one of the two enzyme necessary to convert NeoFURTULON to 5'-DFUR. Thus, this study showed that significant concentrations of PyNPase exists in the intestine of mice. The work shows that NeoFURTULON passes through the intestine with much less conversion to 5-FU than 5'-DFUR, thus more is available to accumulate in tumor.

1106) Camiener GW, Smith CG. Studies of the enzymatic deamination of cytosine arabinoside – 1. Enzyme distribution and species specificity. Biochem. Pharm., 1965; 14: 1405–1416. Vol 17, 39–50.

Pyrimidine nucleotide deaminase activity in human tissues rapidly converts cytidine to uridine and cytosine arabinoside (CA) to uracil arabinoside (UA). This activity limits the therapeutic usefulness of cytosine arabinoside. Camiener and Smith determined the distribution and species specificity of this activity. The following table shows that the deaminase activity is greater in human liver homogenates than in human kidney homogenates. In separate paper chromatography and UV spectrophotometry experiments, these investigators demonstrated that the product of these deamination reactions were indeed uridine and UA depending on the starting material.

Deamination of Cytosine Arabinoside by Homogenates of Human Tissue

Reaction	Human Tissue Homogenate	[CA] in reaction mixture (mg/ml)	Apparent [CA] remaining after incubation (mg/ml)
1	none	1000	>900
2	liver	none	<60
3	liver	1000	<60
4	kidney	none	<60
5	kidney	1000	460

They also showed that the amount of product formed increased linearly with time and with crude enzyme concentration. UA and uracil, the products of this deamination activity, inhibited the activity.

Camiener and Smith then examined the distribution of this activity in man and eleven species of animals. The following table shows that only Rhesus monkeys have similar activities in the liver and kidneys that are similar to man. Nevertheless, these animals also have significant activities in heart and striated muscle that humans lack.

TABLE 2. DISTRIBUTION OF PYRIMIDINE NUCLEOSIDE DEAMINASE ACTIVITY IN VARIOUS ANIMAL TISSUES*

Species	Liver	Kidney	Heart	Striated muscle
Postnatal man†	5+‡	3+	1+	—
Prenatal man	—	2+§	—	—
Rhesus monkey	5+	4+	4+	4+
Squirrel monkey	4+	—	—	—
Rabbit (New Zealand white)	2+	—	—	—
Rat (Wistar)	—	—	—	—
Dog (beagle)	1+	—	—	—
Guinea pig	—	5+	—	—
Mouse (Swiss)	—	5+	—	—
Frog (*R. pipiens*)	1+	not run	—	—
Pigeon (White King)	>3+	not run	—	—
Cat‡	2+	—	—	—
Pig‡	—	5+	—	—

* The incubation mixtures were prepared in 12-ml centrifuge tubes in an ice bath; they contained 0·2 ml of 1·25 M glycylglycine buffer at pH 8·0, 0·1 ml of either CA or CR prepared in KR buffer, and 0·2 ml of a 25% centrifuged homogenate of the indicated tissue. The amount of substrate used in the reaction mixtures was varied in the manner described in the text. The tubes were incubated for 1 hr at 37°, and their contents were assayed spectrophotometrically at 290 mμ, as described in Methods.

† Eight months to 75 years of age.

‡ The symbols refer to the average number of μμmoles of UA formed from CA under the assay conditions described above: —, <50; 1+, 50–100; 2+, 100–200; 3+, 200–400; 4+, 400–800; 5+, >800. The omission from the table of the data obtained with CR is discussed in the text.

§ These data were derived from the tissues of only one specimen. All other data were obtained from two or more animals.

1107. G. Giusti *et al.* (1970), Deoxycytidylate deaminase and deoxycitidine deaminase in normal and neoplastic human tissue. *Enzym. Biol. Clin.*, 11:375-383. Vol.17, page 51.

In this brief article, Guisti et al. measured the activity of deoxycytidylate deaminase and deoxycytidine deaminase in a variety of human normal and tumor tissues. They used two different methods to determine this activity, a spectrophotometric method that measured the change in optical density between 280 and 267 nm as the reaction progressed and a colorimetric method that measured the ammonia cleaved from the either of the two substrates.

Unfortunately, the comparison between normal and tumor tissues is not systematic. Worse, the authors give no rationale for using one assay on a particular tissue and the other assay on another tissue. The spectrophotometric assay appears to give consistently higher activities than the colorimetric assay, but the authors provide no comparison or standardization.

As tissue progresses from normal to benign tumor to malignant tumor the ratio of deoxycytidine deaminase activity to deoxycytidylate deaminase activity consistently decreases (from 0.99 in normal skin to 0.17 in epidermal carcinoma). The results imply that progression to malignancy in some tumors increases the expression of deoxycytidylate deaminase activity or decreases expression of deoxycytidine deaminase activity or both. It is obvious from the results that at least two different enzymes are responsible for these activities.

1108. B. A. Chabner *et al.* (1974) Purification and properties of cytidine deaminase from normal and leukemic granulocytes. *J. Clin. Invest.*, 53:922-931, Vol 17. p 60.

This is one of the best and most comprehensive classical enzymology papers I have seen lately. This is not surprising considering the source. The authors determined cytidine deaminase by two methods, the first using radioactive substrate, Dowex-50 H^+ separation of product and reactant and scintillation counting, and second using a coupled enzyme assay that measured ammonia production. In an early purification step they selectively eliminated the deoxycytidylate deaminase activity by heat inactivation.

They first demonstrated that the cytidine deaminase activity was lower in leukemia cells than in normal WBCs as the following table shows.

Group	Age	Activity in units/mg protein $X10^{-3}$
AML	33	0.19 ± 0.17
CML	35	1.40 ± 0.70
Normal	29	3.52 ± 1.86

where one unite = 1 nmol of substrate deaminated per hour at 37 C.

The authors then purified the enzyme activity by salt precipitation and size exclusion from the initial specific activity of 3.52 units/mg protein $X10^{-3}$ in normal WBCs to a final solution with an activity of 2530 units/mg protein $X10^{-3}$ (700 fold). V_{max} did not change during the purification procedure for a variety of substrates implying that the procedure involved a single activity. The purified enzyme tolerated a broad pH range. Activity was maximal at pH 5 and declined little to pH 9. The authors found no dialyzable cofactors and estimated the molecular weight of the enzyme as 51kD by size exclusion. The isoelectric point was 4.8. With the purified enzyme they determined the Michaelis constant for 10 similar substrates as follows:

Substrate	Km (μM)
Cytidine	11
Deoxycytidine	26
Ara-C	88
5-AzaC	430
5-Fluorocytidine	30

The purified enzyme did not deaminate CMP, dCMP, CTP, dCTP and Cytosine

Tetrahydrouridine inhibited the activity at very low concentrations. The k_i by Linweaver-Burke analysis was 54 nM with cytidine as the substrate and 55 nM with deoxycytidine as the substrate. The inhibition was competitive.

The authors separated normal mature granulocytes from normal immature granulocytes of bone marrow aspirates by Ficoll density gradient and determined the enzyme activity in both population. The relative activity (mature/immature) ranged from 3.55 to 14.2 implying that expression increases with

granulocyte maturity. They measured a similar ratio, 13.8, when they compared the activity in mature and immature CML cells from peripheral blood.

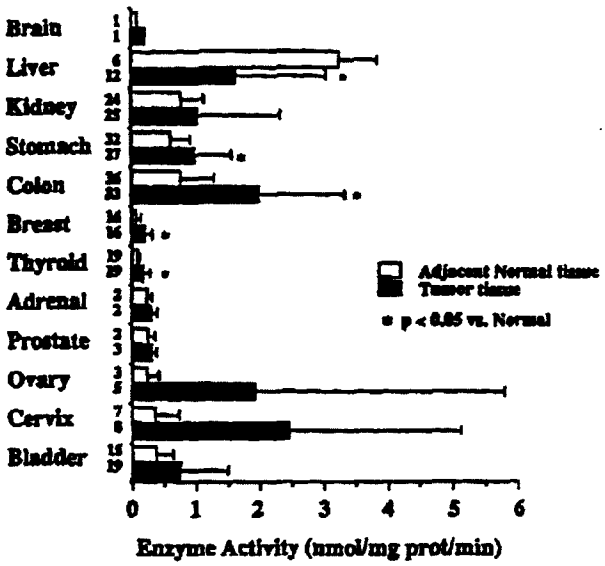
I can venture comparison with limited confidence, but this enzyme activity appears to be the same as the deoxycytidine activity described in reference 1107. That the activity decreases with progression to malignancy here is consistent with the results of that paper.

1109) K. Mori *et al.* (1994) Activity of cytidine deaminase and pyrimidine nucleoside phosphorylase in various human tumor tissues and their adjacent normal tissues. Roche Report J-146210, January 24. Vol 17, 70-81.

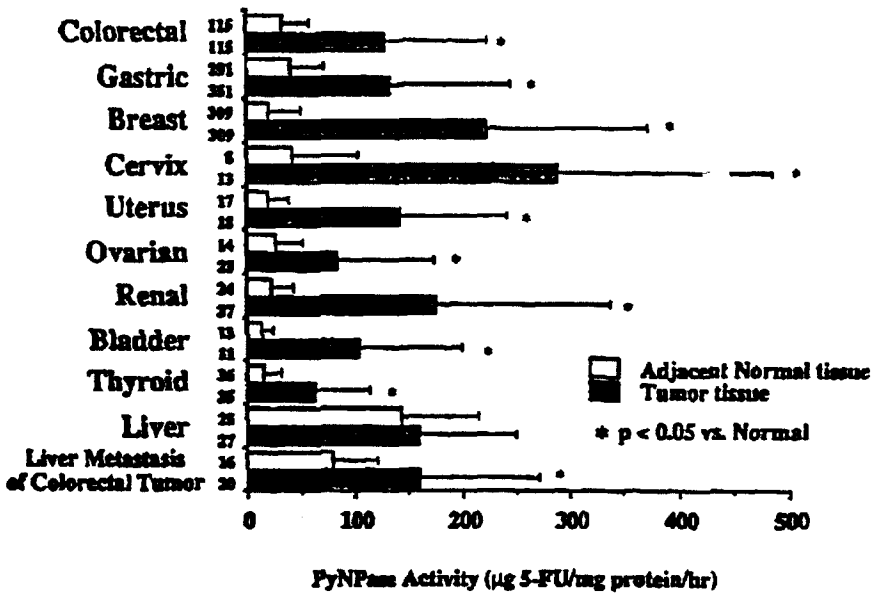
Mori *et al.* determined cytidine deaminase activity and PyNPase activity in a 9 tumor tissues and the corresponding normal tissue from an impressive number of patients, n = 5 to 33. These researchers used 5'DFCR as the substrate for the enzyme. They isolated the enzyme activity as the 105,000Xg supernatant, and determined the products of the reaction by HPLC. Again, the researchers did not show adequate standardization for the analysis.

Colorectal, gastric, breast, and thyroid tumor tissue contained significantly more cytidine deaminase activity than normal tissue. Normal liver contained more activity than tumor tissue as the following graph shows. This is significant, since it implies a large first pass effect and that the concentrations of 5'-dFUDR, and hence 5-FU, will be high in the liver. Other tissues showed no statistically significant difference, but this appears to be because of large variance in all tumor tissues. This is also significant, since it implies that whatever increased expression a tumor may have it is unpredictable.

Cytidine Deaminase



Similarly these researcher examined PyNPase activity in 11 tissues using 5'-DFUR as substrate. The results of these assays are shown in the following graph.



The activity of PyNPase was higher in tumor tissue in all tissues but liver, but again the results showed great variability. These results suggest that Ro 09-1978 might only be effective in colorectal, gastric, breast and thyroid cancers. More importantly, the high activities for both enzymes in normal liver suggest that Ro 09-1978 may cause significant 5-FU related hepatotoxicity in humans. Hepatotoxicity is seen clinically.

1110. M. Miwa *et al.* (1997) Design of a new oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumors by enzymes concentrated in human liver and cancer tissues. Report J-146'658. Vol. 17, page 82.

Miwa *et al.* studied the relative efficacy of capecitabine and its metabolites *in vitro* in a number of tumor cell lines. The following table shows the IC_{50} values for these cell lines.

		IC_{50} (μM)			
	Culture Period	5-FU	5'-DFUR	5'-DFCR	Capecitabine
Colo205	5	3.1	127	>1000	>1000
HCT116	4	3.7	39	803	>1000
DLD-1	4	7.6	190	>1000	>1000
MCF-7	4	13	91	>1000	>1000
AR-75-1	7	0.25	0.36	207	ND
MKN45	4	3.3	38	174	994
MKN28	5	2.9	65	>1000	>1000
SIHA	4	7.5	67	92	578
HT-3	4	21	84	>1000	>1000
Scabber	3	0.72	3.7	9.3	97
T24	3	4.3	90	>1000	ND

In vitro capecitabine shows little activity against these cell types. Only the Scabber cells have carboxylesterase activity necessary for cleaving the capecitabine side chain. AR-75-1, MKN45, SIHA and Scabber cells appear to have significant cytidine deaminase activity. These results suggest that capecitabine itself is relatively non-toxic in the absence of these two enzyme activities. More importantly, the results suggest that most of these tumor cell lines have little PyNPase or cytidine deaminase activity. In all the models the activity of 5-FU 1.4 to 41 times higher than the activity of 5'-DFUR. This suggests that 5-FU produced systemically may be more important in the mechanism than 5-FU produced within the tumor.

These researchers then compared the *in vivo* efficacy of capecitabine and its active metabolites in two *in vivo* tumor models, HCT116 Human Colon Carcinoma and MX-1 Human Mammary Carcinoma

implanted in BALB/c nu/nu mice. The following table shows the results for the HCT116 HCC experiments.

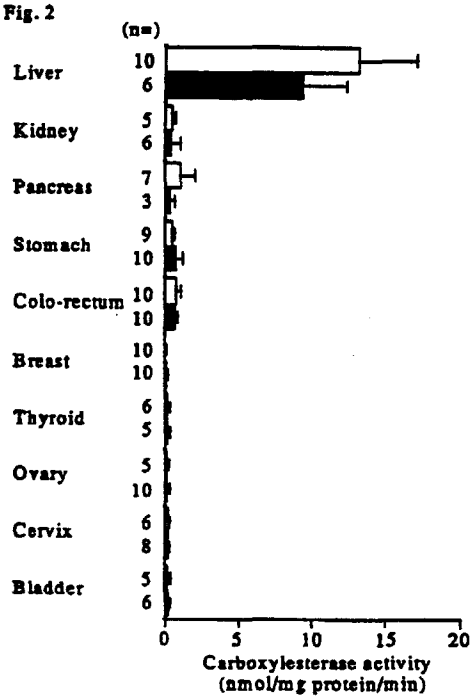
		Antitumor effect		toxicity		
Drug	Dose	Tumor vol. Change	%Growth Inhibition	Survival on d34	Body wt. change	Occult blood
	mmol/kg	mm3			grams	
Vehicle		1700		12/12	1.5	
5'-FUra	0.067	1663	2	6/6	1.2	
	0.1	1562	8	6/6	0.9	
	0.15	1365	20	6/6	0.3	
	0.225	681	60	4/6	-3.7	±
UFT	0.044	1794	-6	6/6	0	
	0.067	1312	23	6/6	1.3	
	0.1	1027	40	6/6	0.4	±
	0.15			0/6		(++)
5'-dFUrd	0.2	1165	31	6/6	0.3	
	0.3	1280	25	6/6	1.5	
	0.44	909	47	6/6	-0.9	±
	0.67	361	79	6/6	-3.2	+
	1	71	96	2/6	-6.4	+
	1.5			0/6		(+++)
Capecitabine	0.2	956	44	5/5	0	
	0.3	964	43	6/6	0	
	0.44	653	62	6/6	-0.8	
	0.67	529	69	6/6	-1	
	1	298	82	6/6	-1.4	
	1.5	-44	103	6/6	-3.1	±
	2.25	-173	110	1/6	-4.8	±

In these experiments Capecitabine had the best therapeutic index of all the compounds tested, 4.5 in the HCT116 model and 3.4 in the MX-1 model. The authors calculated the therapeutic index as the toxic (lethal) dose over the ED_{50}.

In their final series of experiments the authors measured the activity of the three enzymes involved in the conversion of capecitabine to 5-FU, carboxylesterase, cytidine deaminase, and PyNPase (or dThdPase), in human tumor tissue and in normal tissue resected from the tumor margins. Unfortunately the authors did not include the actual measured numbers for these experiments. Instead they presented the data as graphs. In each of these graphs the "closed" or solid bar demonstrates the

activity in normal tissue, the open bar that of tumor tissue. I have included each of these graphs below because they are important to the sponsor's claim that capecitabine toxicity occurs selectively in tumor *in vivo*. In the results and discussion the authors say "Carboxylesterase is concentrated in the liver; Cyd deaminase is located in the liver and various types of tumor tissues, while dThPase is concentrated in tumor tissue." Figure 2 demonstrates that carboxylesterase activity is much higher in the liver than in other tissues, and the concentration of this activity in adjacent tumor tissue is greater than that of normal tissue. Nevertheless, the difference is not significant.

Fig. 2



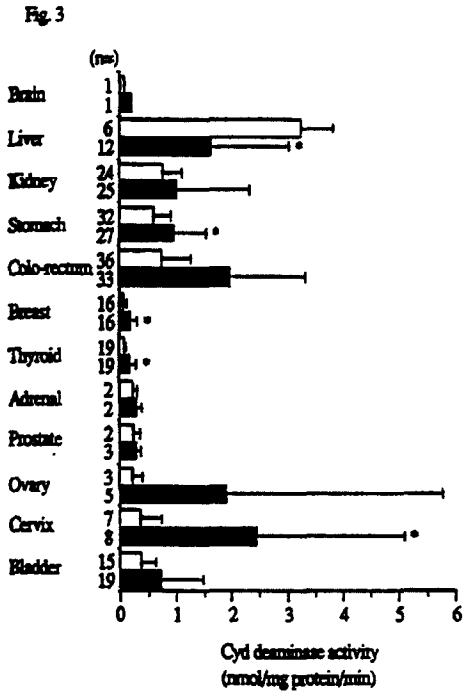
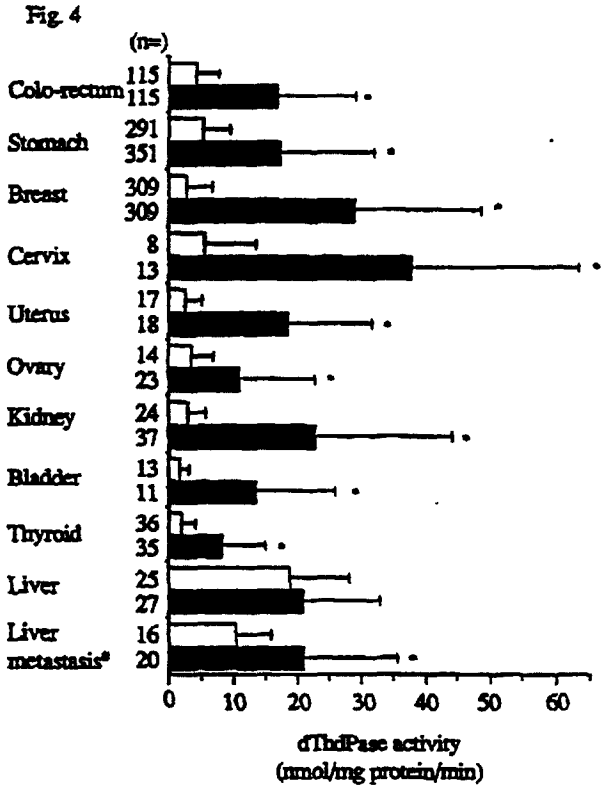


Figure 3 shows that cytidine deaminase activity is widely distributed and that, except in liver, the activity is higher in normal tissue. Figure 4 shows that in all cases dThdPase activity is higher in the normal tissue. The sponsor should clarify the difference between their statement and the presentation of their results. The results of these experiments are central to several claims the sponsor makes in the proposed labeling. Nevertheless, the variability within the data is so large that even if the graphs are mislabeled and the bars representing normal and tumor tissue are reversed, the data does not convincingly support the sponsor's claim. This study might have been more informative if the investigators had correlated normal versus tumor tissue expression for individuals patients.

Tumor from a biopsy sample contains numerous kinds of tissue in addition to tumor cells, including stroma, immune cells, endothelial cells from the vasculature. These cells, stimulated to proliferate by the tumor, might actually express the high dThdPase activities leading to these results. The total activity in the sample represents the mean expression of all the tissues in the sample. For numerous reasons, the *in vitro* activity many not accurately represent the actual intracellular activity.



1111) M Miwa *et al.* (1994) A liver specific acylamidase isozyme activates the 5-fluorouracil oral pro-drugs, N^4-alkoxycarbonyl-5'-deoxy-5-fluorocytidines. Roche Report J-146211, February 21. Vol 17, 106-130.

This is Roche's pivotal *in vitro* research to identify Ro-09-1978 as the best 5-FU pro-drug. Miwa *et al.* purified acylamidase from human liver, human colon, and various organs of Cynomolgus monkey and mouse. Acylamidase (carboxylesterase) hydrolyzes the substituted carboxyl group from N^4-(substituted carboxyl)-5'-dFCyd derivatives. The investigators determined the hydrolysis of many N^4-(substituted carboxyl)-5'-dFCyd derivatives by HPLC after *in vitro* incubation. They isolated acylamidase activity from human liver by the following sequence: acid precipitation, 80% ammonium sulfate precipitation, Blue Sepharose CL-6B with KCl gradient, DEAE with KCl gradient, DEAE with KCl gradient, and hydroxylapatite with sodium phosphate gradient. The enzyme isolation from human colon eliminated the acid precipitation and the Blue Sepharose, but added a size exclusion step. These purifications were inefficient. In most cases purification improved the specific activity less than 50 fold with losses of 80 to 95% of total activity.

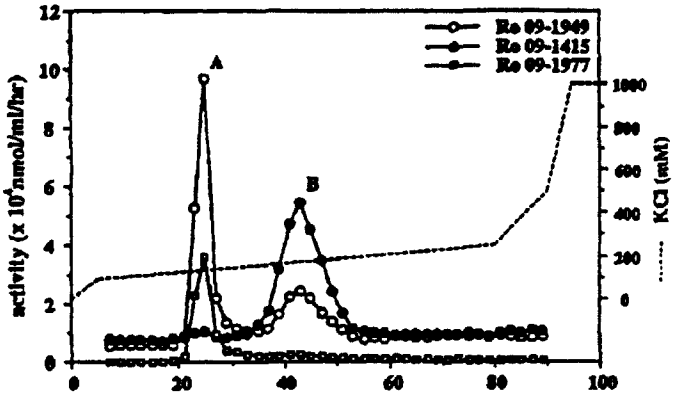
The researchers found two distinct enzyme activities, isozyme A and B. Liver contained both these isozymes, but colon mucosa contained only isozyme B. These distinct activities were identified by

differences in their substrate specificity. Isozyme A hydrolyzes both Ro 09-1949 (an N^4-acyl-5'-dFCyd) and Ro 09-1977 (an N^4-alkoxycarbonyl-5'-dFCyd). Isozyme B hydrolyzes 09-1415 (an N^4-acyl-5'-dFCyd) selectively. Thus, Ro 09-1977 identifies isozyme A and Ro 09-1415 identifies isozyme B. Isozyme A had a formula weight of approximately 60Kd. Isozyme B is at least twice as large.

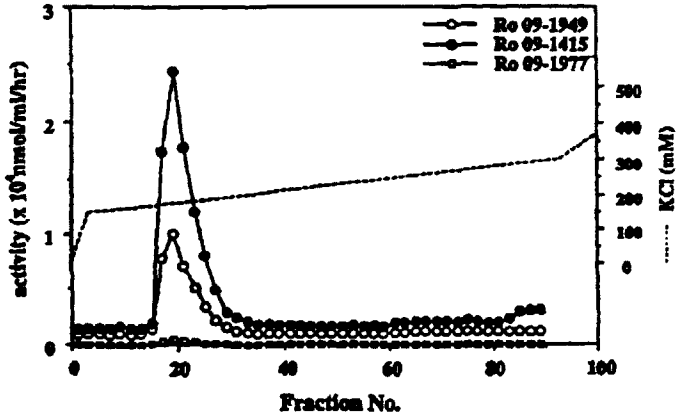
The following graphs show the elution profiles of these activities from liver and colon. Note that the fractional position in the two chromatograms do not exactly correlate. The peak in the chromatogram from colon mucosa is isozyme B.

Fig. 2 DEAE TOYOPEARL 650S Column Chromatography of Acylamidases from Human Liver and Colon Mucosa

2a) Liver

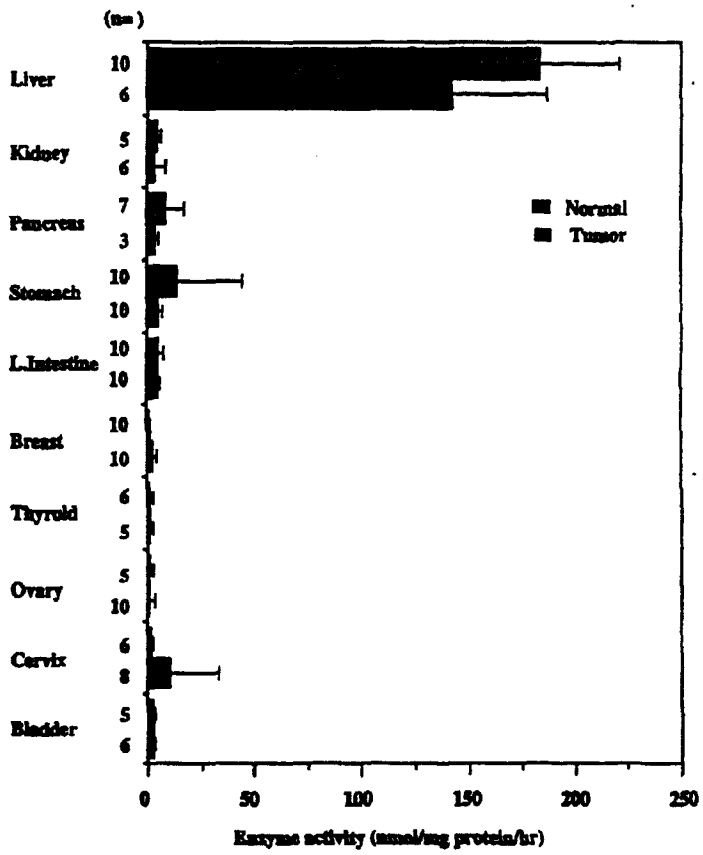


2b) Colon Mucosa



The two different enzymes had different tissue distributions. The highest isozyme B activities are expressed in the liver. Nevertheless, the researchers found activities one third those of liver in normal kidney. They also found considerable activities in extra-hepatic tissues such as pancreas, stomach and intestine. Liver tumor tissue contained isozyme B activities equal to normal liver, and extra-hepatic tumor tissue frequently contained isozyme B activity as high as one fourth that of normal liver. In contrast, little isozyme A activity was found outside the liver in either normal or tumor tissue. The following bar graphs show these results.

Fig. 5 Tissue Distribution of Acylamidase Isozyme A in Human Normal and Tumor Tissues



The researchers compared the activities of crude liver and intestinal extracts from monkey and mouse for hydrolysis of 22 different substrates. Both liver and intestine of the mouse contained varying activities for the 22 compounds. Nevertheless, only monkey liver contained activity, monkey intestinal extracts did not hydrolyze any of the substrates. The following table shows the activities of these tissues in humans, monkeys and mice for the hydrolysis of Ro 09-1978.

	Ro 09-1978 activity in crude extracts		
	liver	intestine	stomach
	nmol/mg/h	nmol/mg/h	nmol/mg/h
Human	190	7	12
monkey	29	0	
mouse	32	100	

Roche chose Ro 09-1978 from among the 22 synthetic derivatives because the ratio of hydrolysis in the liver to that in the intestine was greatest. Thus more drug should survive intestinal absorption intact. These results also suggest that mouse is a poor model for this therapy. Miwa et al. state in their discussion that they could not find an enzyme in mouse liver with a substrate specificity corresponding to that of human acylamidase A. Though the monkey has much lower activities in the liver than humans, like humans the intestinal activity is lower still. The substrate specificity of the monkey enzyme is similar to that of human acylamidase A. The relatively high activity in human liver suggest that acylamidase activity may not be rate limiting in the human as it is with the pro-drug Ro 09-1390. This assumption is central to the sponsor's hypothesis. 5'-DFCR will accumulate much faster in human liver than in monkey liver. Hepatotoxicity may be dose limiting in humans as suggested by the limited previous human experience.

1115) T Ishikawa *et al.* (1993) Antitumor efficacy of N^4-alkoxycarbonyl substituted 5'-deoxy-5-fluorocytidines in mice correlated with their pharmacokinetic parameters. Report J-146'209, Vol. 17, page 141.

In this brief paper the authors again demonstrated that capecitabine (Ro 09-1978) had the best therapeutic index of nine N^4-alkoxycarbonyl substituted compounds tested in an *in vivo* BALB/c mouse model bearing CXF280 Human colon carcinoma implants.

In a second set of experiments the investigators gave mice baring CXF280 tumors 2 mmol/kg of each of the different N4-alkoxycarbonyl substituted compound PO. At different time points they killed the mice and collected plasma and tumor tissue. They then determined the concentration of 5-FU in the plasma by HPLC and in tumor tissues by GC-MS. They did not specify how may mice were killed at each time point or how many time points they studied, so I cannot assess the quality of the data or confirm the results. Of the compounds tested Ro 09-1978 and Ro 09--2132 had the lowest ED_{50} and the highest AUC in plasma and tumor tissue. These results evidently helped the sponsor choose capecitabine for development from among these competing drugs.

1117) M Arasaki *et al.* (1992) N4-Oxycarbonyl substituted 5'-deoxy-5-fluorocytidines. European patent application 92121538.0, December18.

This is a patent application. It contains little information that the sponsor does not present elsewhere. It defines every compound conceivable that could ever have ambitions of being enzymatically transmogrified to 5'-FU.

1119) M. Miwa *et al.* 1997 No induction of carbolylesterase and cytidine deaminase activities by capecitabine in monkeys, Report -146'547, Vol 18, p 44.

Miwa *et al.* surgically resected liver tissue from monkeys one day after completing treatment with capecitabine (control and 0.50 mmol/kg/d, PO daily for 28 days, study J-146'259 below). They homogenized the tissue and isolated the 700g supernatant. They then dialyzed the supernatant. They determined the carbolylesterase and cytidine deaminase activity of this crude preparation to determine whether dosing induced the expression of these activities. They used standardized HPLC and UV assays for the carbolylesterase and cytidine deaminase activity respectively. The authors do not mention removing colon tissue in the materials and methods section, but they present results for colon tissue in the tables and graphs. I assume they prepared the enzyme from colon as they did from liver. The following table shows the results of these experiments.

	Liver				Colon			
	control		0.5		control		0.5	
	mmol/kg/k	SD	mmol/kg/k	SD	mmol/kg/k	SD	mmol/kg/k	SD
Carboxylesterase nmol/mg prot/hr	11.7	1	14.2	2.2	4.5	4.1	2.6	0.4
Cytidine deaminase nmol/mg prot/hr	1.1	0.1	1.2	0.3	7.4	1.1	6	1.6

These results show that daily dosing does not significantly increase these enzyme activities in the colon or liver of monkeys.

1120. M Ura *et al.* 1997. No large differences between Japanese and Caucasians in the levels of enzymes that metabolize capecitabine to 5-FU. Report J-146'672. Vol 18. p 52.

Ura *et al.* obtained human breast tumor tissue from Japanese patients and European patients. They obtained normal human liver tissue from Japanese cancer patients (biopsy) and from an American tissue bank (autopsy). They analyzed the dThdPase expression by ELISA and activity by HPLC. For the ELISA dThdPase assay, they homogenized the tissue and prepared a crude enzyme extract as the 8000g supernatant. For the HPLC dThdPase assay they homogenized the tissues and prepared a 105,000g

supernatant that they subsequently dialyzed. They also used this 105,000g supernatant for the cytidine deaminase assay. For the carboxylesterase assay, they homogenized the tissues and prepared a 700g supernatant that they subsequently dialyzed. They determined carboxylesterase and cytidine deaminase activity by HPLC. They did not present HPLC or ELISA standardization data or the raw results of these experiments.

From the ELISA experiments, the dThdPase activity in breast tumor tissue showed a Poisson distribution. There is considerable variability in the expression of this enzyme. The following table shows the parameters of this distribution.

	n	Lower limit units/mg	upper limit units/mg	Mean units/mg	SD	Median units/mg
Japanese	945	0.7	1437	134	127	99.9
European	261	1.2	843	138	117	105

The following table shows the results of the enzyme activity assays in Japanese and Americans.

	American				Japanese	
	Caucasian		African			
n	17	SD	3	SD	12	SD
Carboxylesterase nmol/mg protein/hr	48.4	7.1	52.4	6.5	68.3	14.7
Cytidine deaminase nmol/mg protein/hr	2.46	0.49	1.92	0.46	2.66	1.15
dThdPase µg 5-FU/mg prot/hr	94.8	25.4	80	12.7	86.9	45.5

Note that the carboxylesterase is significantly higher in Japanese than in Americans ($p<0.05$ to Caucasians, Bonferroni/Dunn test), but this difference is less than the SD for the Japanese samples. It is probably not toxicology significant. Unfortunately, the investigators did not correlate dThdPase activity with expression. Such a correlation might have strengthened their contention that this activity is higher in tumor in humans.

1121) M Miwa *et al.* (1994) Tissue distribution patterns of acylamidase, cytidine deaminase and pyrimidine nucleoside phosphorylase activity in the human and experimental animals. Roche Report J-146259, April 18. Vol 18, 62-74.

Miwa *et al.* determined the tissue distribution of acylamidase, cytidine deaminase and pyrimidine nucleoside phosphorylase. They prepared acylamidase as a crude 700Xg supernatant

dialyzed against buffer. Enzyme activity was determined by the hydrolysis of Ro 09-1978 measured by HPLC. They prepared cytidine deaminase and pyrimidine nucleoside phosphorlase as crude 105,000Xg supernatants dialyzed against buffer. Cytidine deaminase activity was determined by the deamination of 5'-DFCR to 5'-DFUR measured by HPLC. The researchers determined Pyrimidine nucleoside phosphorylase activity by the hydrolysis of 5'-DFUR to 5-FU measured by HPLC. They determined enzyme activities in tissues from mouse, rat, monkey and human. The following charts show these results.

Fig. 3 Tissue Distribution of Acylamidase, Cytidine Deaminase, and Pyrimidine Nucleoside Phosphorylase Activity in Rats

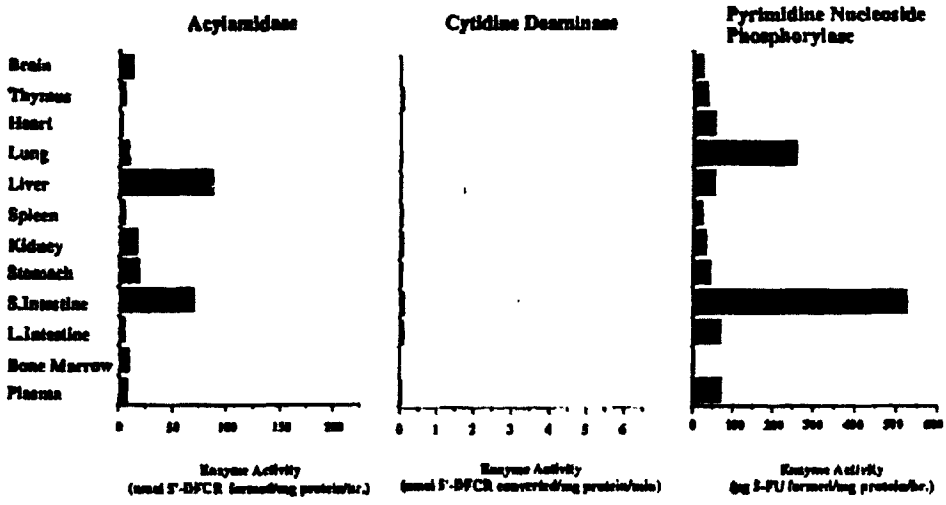


Fig. 2 Tissue Distribution of Acylamidase, Cytidine Deaminase, and Pyrimidine Nucleoside Phosphorylase Activity in Mice

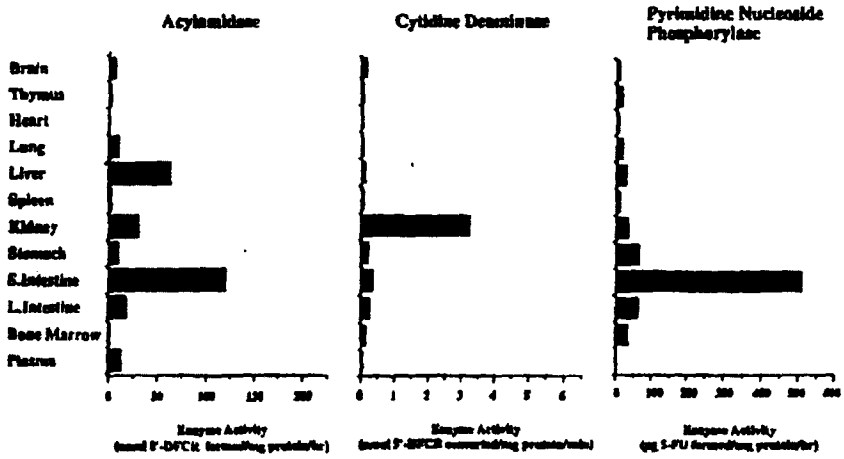


Fig. 4 Tissue Distribution of Acylamidase, Cytidine Deaminase, and Pyrimidine Nucleoside Phosphorylase Activity in Monkeys

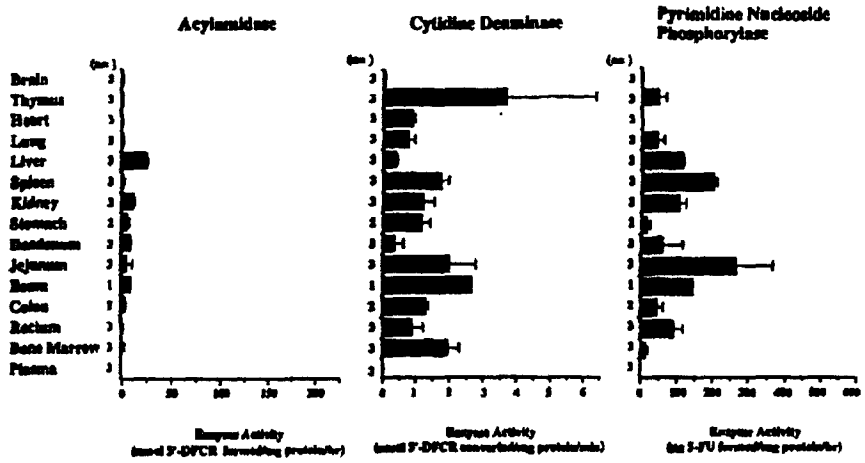
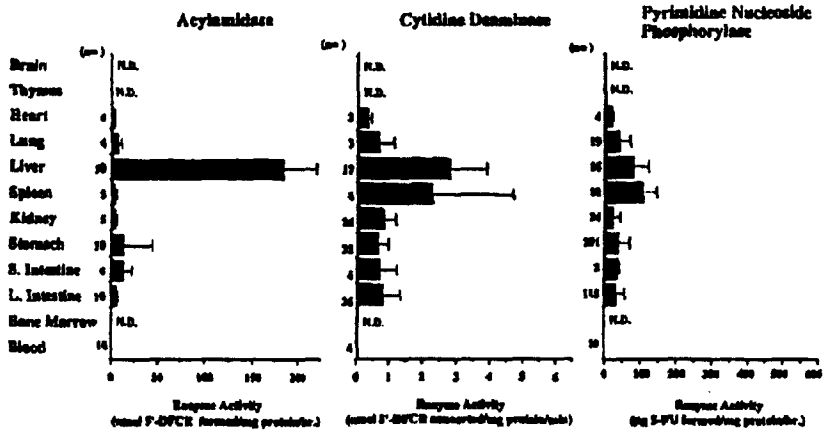


Fig. 1 Tissue Distribution of Acylamidase, Cytidine Deaminase, and Pyrimidine Nucleoside Phosphorylase Activity in Humans



N. D. ; not done

Mouse acylamidase substrate specificity is significantly different from that of human acylamidase. Additionally, most cytidine deaminase activity is found in the kidney instead of the blood, liver and spleen as in humans. Mouse small intestine contain the highest concentrations of PyNPase, whereas highest concentrations are found again in human liver and spleen. These results suggest that much of the 5'-DFUR would be eliminated by the mouse kidney and that the mouse should be relatively insensitive to Ro 09-1978 toxicity. Dose limiting toxicity should be seen in the intestine. The toxicology studies below are consistent with this observation

For the rat, the researchers found the highest concentrations of acylamidase in liver and small intestine. None of the rat tissues studied by Miwa et al. contained significant concentrations of cytidine deaminase. Lung and small intestine contained the highest concentrations of PyNPase. The rat is very insensitive to Ro 09-1978 toxicity.

Monkey liver contained the highest acylamidase activities, but again these activities were at least 6 fold lower than those found in human liver. Cytidine deaminase was broadly distributed. Spleen, liver, bone marrow and gastrointestinal tissues contained the highest PyNPase activities. These results predict that myelosuppression and GI degeneration should be dose limiting in the monkey. These toxicities are the first observed in sub-chronic studies.

Human liver contains the highest acylamidase activities of any tissue and species studied. Cytidine deaminase is also found in high concentrations in liver but the enzyme is broadly distributed. The profile for PyNPase is similar to that of cytidine deaminase. Again, cytidine deaminase activity is probably rate limiting in humans, not acylamidase activity as seen with Ro 09-1978. Thus dose limiting toxicities should be seen in those tissues with the highest cytidine deaminase activities. This predicts that unlike the other species hepatotoxicity may be dose limiting in human.

1122. T Sumizawa *et al.* (1993) Thymidine phosphorylase activity associated with platelet-derived endothelial cell growth factor. J. Biochem. (Tokyo), 114:9-14, Vol. 18, p 75-80.

Sumizawa *et al.* purified dThdPase from human placenta. The isolated a cDNA clone for dThdPase. They then determined sequence homology between the 120 amino acids of human dThdPase and the sequence of platelet-derived endothelial cell growth factor (PD-ECGF) in humans. The 120 residues of dThdPase aligned identically with residues 125-244 of PD-ECGF. They also showed that rPD-ECGF has a thymidine phosphorylase specific activity similar to that of purified human dThdPase. Since PD-ECGF is angiogenic, these results imply that dThdPase expression may be important in tumor angiogenesis.

1123. F. Ishikawa *et al.* (1989) Identification of angiogenic activity and the cloning and expression of platelet-derived endothelial cell growth factor, *Nature*, 338:557-562, Vol 18 page 81-86.

This paper is an essential reference in the previous paper. The authors purified PD-ECGF to homogeneity from human platelets. The protein has a molecular weight of ~45kD, does not bind to heparin and does not stimulate the proliferation of fibroblasts. The authors determined the composition of PD-ECGF by N-terminal sequencing and by analysis of fragments. Using Southern blots they established that there is only one copy of the PD-ECGF gene in the human genome. They determined that partially purified PD-ECGF is angiogenic *in vivo* in chick chorioallantoic membranes. Finally, using microscopic analysis, they established that tumors transfected with PD-ECGF cDNA had a higher density of blood vessels than non-transfected tumors.

1124. A Moghaddam *et al.* (1995) Thymidine phosphorylase is angiogenic and promotes tumor growth. *Proc. Natl. Acad. Sci.*, 92:998-1002. vol. 18, p 87-91.

Moghaddam *et al.* implanted circular polyether sponge discs SC in male Wistar rats. They injected the sponges with vehicle, dThdPase, antibodies to dThdPase, and dThdPase plus antibodies. They determined blood flow through the discs by measuring the clearance of ^{133}Xe containing saline from the sponge. They claim that the clearance of ^{133}Xe containing saline from the sponge was significantly increased over that of control with a $p < 0.001$, but they do not describe the method of statistical analysis. If they used Students-t test, as I suspect, the significance is overestimated. Nevertheless, an ANOVA would still probably show that the difference was significant. Small doses of dThdPase, 0.1 or 1 pmole, did not cause an increase in flow, but 100 pmole did not significantly increase the flow over that caused by 10 pmol. They then showed that dThdPase containing pellets implanted around a freeze injured skin graft hastened the increased in blood flow. The following graphs show the results of this work. Note that at least in the case of the sponge implants, the increase in flow is more rapid than control, but that the final flow appears to be converging by day 14.

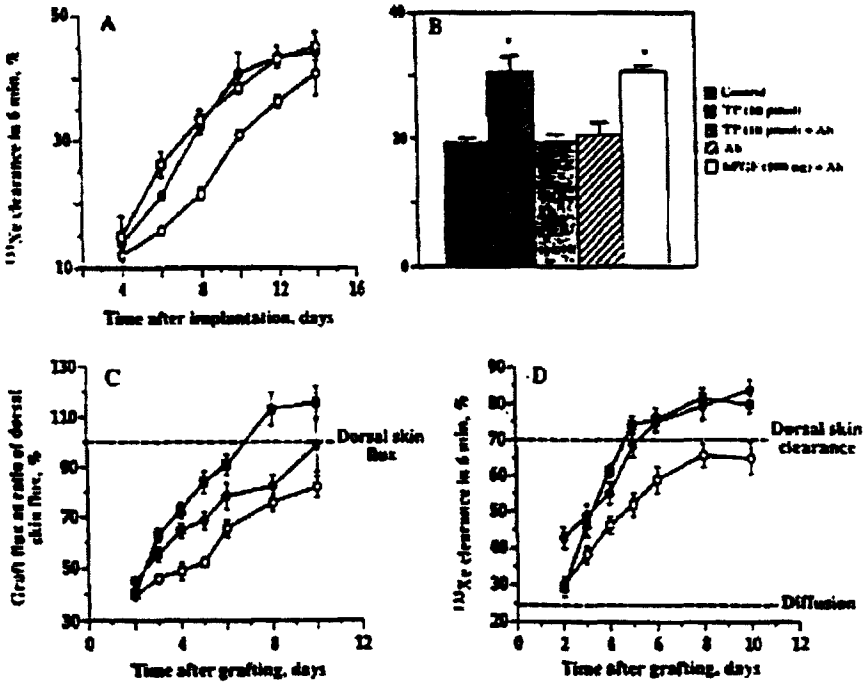


FIG. 1. Angiogenic activity of TP in the rat sponge and freeze-injured skin graft models. (*A*) Dose–response curves of the angiogenic activity of recombinant human TP (○, PBS control; ●, 10 pmol of TP per day; □, 100 pmol of TP per day) in the rat sponge model ($n = 4$, mean ± SEM). A daily dose of 0.1 or 1 pmol of TP gave no significant stimulation above controls. (*B*) Blocking of TP-induced sponge angiogenesis by polyclonal rabbit anti-TP antibodies (Ab). Polyclonal anti-TP antibodies failed to block basic fibroblast growth factor (bFGF)-induced angiogenesis ($n = 4$, mean ± SEM). (*C* and *D*) Angiogenic activity of human recombinant TP in the freeze-injured skin graft model. Graft blood flow was measured by laser Doppler flowmetry and expressed as a percentage of that determined for the uninjured skin of the dorsal flank (*C*) and by monitoring the rate of ^{133}Xe clearance and expressed as the percentage of clearance over 6 min (*D*). ○, Control; ●, TP containing pellets; ■, non-freeze-injured graft. Data are the mean ± SEM ($n = 5$ to 38). Some animals were sacrificed for histology on days when measurements were made; thus, the number of data points fell as the experiment progressed.

The investigators then constructed dThdPase mutants by site directed mutagenasis. These mutants were catalyticly inactive or had 1.3% the activity of the wild type enzyme. None of these mutants increase the clearance of ^{133}Xe containing saline in the sponge implant model.

The following graphs show that dThdPase caused migration of bovine aortic endothelial cells in a modified Boyden microchemotaxis chamber.

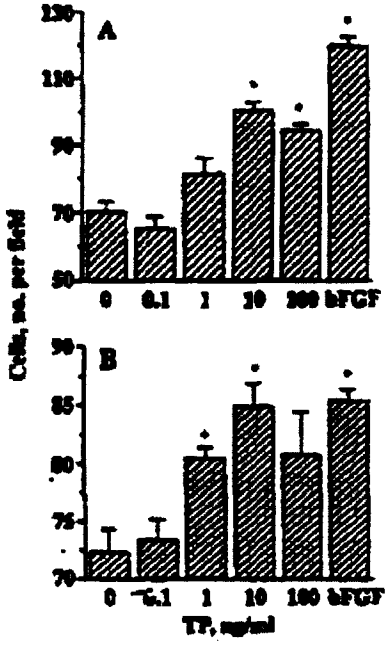


FIG. 4. Migration of bovine aortic endothelial cells in a modified Boyden chamber. (*A*) In DMEM supplemented with 1% fetal calf serum. (*B*) In DMEM supplemented with 0.1% bovine serum albumin and 100 nM thymidine. Data are the mean ± SEM ($n = 4$). *, $P < 0.001$; +, $P < 0.02$. Each experiment was repeated three times with similar results. bFGF, basic fibroblast growth factor.

Finally the investigators transfected the dThdPase gene, under control of a cytomegalovirus promoter, into MCF-7 breast carcinoma cells. Isolated clones of these cells were then grafted into oopherectomized BALB/c nu/nu mice. The following figures show that over-expression of dThdPase did not effect the growth of these tumor cells *in vitro*, but it significantly increased the growth rate of tumors *in vivo*.

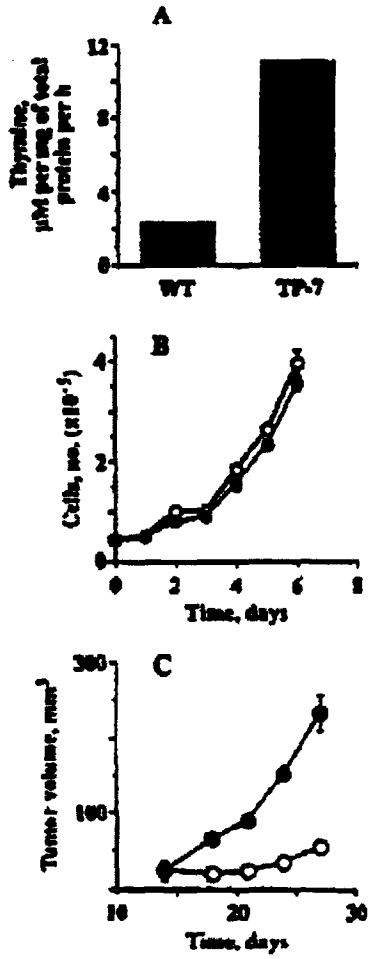


FIG. 6. Effect of overexpression of TP on MCF-7 breast carcinoma growth. (*A*) TP activity of WT cells (transfected with vector alone) and TP-transfected (TP-7) cells. (*B*) Growth of WT (○) and TP-7 (●) cells in 1% fetal calf serum *in vitro*. (*C*) Growth of WT (○) and TP-7 (●) cells when xenografted into BALB/c *nu/nu* mice (n = 10; average ± SD). Xenografts were performed twice with three overexpressing clones. All overexpressing TP clones gave statistically significant faster growing tumors than did WT cells. Representative data for TP-7 are shown.

1125. M Toi *et al.* (1995) Expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in human breast cancer. Int. J. Cancer, 64:79-82. Vol. 18 p 92-95.

These investigators determined the expression of PD-ECGF (dThdPase) in human breast cancer tissues using anti-PD-ECGF monoclonal antibodies and immunohistochemical staining. They found that of 100 invasive ductal carcinoma samples, only 39 were PD-ECGF positive. They saw enzyme

expression mainly in the cytoplasm of the tumor cells. Expression was associated with microvessel density ($p < 0.05$), but was not associated with menopausal status, tumor size, axillary lymph-node metastases, hormone receptor status, epidermal growth factor receptor, erb-B-2 protein expression or p53 expression.

This paper is a good example of the variability of expression of dThdPase. These results suggest that tumors with rapid neovascularization may be more susceptible to capecitabine, but not all tumors show rapid neovascularization. This argues against the sponsors claim that capecitabine is tumor specific.

1126. T Ishikawa *et al.* (1997) Tumor selective conversion of capecitabine to 5-FU in human cancer xenografts. Study # J-146'549. Vol. 18, page 96.

Ishikawa et al. implanted Human colon cancer HCT116, COLO205, CXF280 and WiDr cells SC in BALB/c nu/nu mice. The WiDr cells line is resistant to 5-FU, the other two lines are susceptible. The investigators then treated the mice with single doses of capecitabine (PO, 1.5 mmol/kg), 5'dFUrd (PO, 0.75 mmol/kg) or 5-FU (IP, 0.15 mmol/kg). They collected blood, thigh muscle, and tumor tissue at 0.25, 0.5, 1, 2, 4, 7 and 24 hours from three mice at each time point. The determined the concentration of 5-FU and 5'dFUrd by liquid chromatography plus ion-spray ionization tandem mass spectrometry with isotopically labeled internal standards. They did not provide validation for this method or raw data in this report. In separate experiments these investigators also looked at inhibition of tumor growth by these three fluoropyrimidines. They gave tumor bearing mice injections daily X7/wk or daily X5/wk for three to four weeks. The doses they chose are MTDs determined by body weight change and lethality.

In the pharmacokinetic analysis the C_{max} and T_{max} values are apparent maxima, due to the limited sampling. The investigators assumed a single exponential model for the $t_{1/2}$ values and calculated the AUC by the linear trapezoidal rule. The kinetic parameters must be considered approximations due to the limited sampling and the modeling method, but this is acceptable considering the scope of these experiments.

The following table shows that capecitabine and 5'dFUrd (PO) were more effective than 5-FU (IP) in the HCT116, COLO205, CXF280 tumor models. The WiDr tumors were refractory to treatment.

Drug	Route	mmol/kg/wk	HCT116				CXF280				COLO205				WiDr			
			qdX7/wk		qdX5/wk		qdX7/wk		qdX5/wk		qdX7/wk		qdX5/wk		qdX7/wk		qdX5/wk	
			mm^3	%	mm^3	%	mm^3	%	mm^3	%	mm^3	%	mm^3	%	mm^3	%	mm^3	%
Vehicle	PO		1373		1188		1395		913		1413		1259		1373		887	
capecitabine	PO	10.5	191	86	-9	101	-25	102	36	96	320	77	495	61	745	46	740	17
5'dFUrd	PO	5.25	464	66	327	72	98	93	211	77	467	67	586	53	881	36	806	9
5-FU	IP	1.05	1153	16	ND		228	84	ND		862	39	ND		907	34	714	20
5-FU	PO	1.05	1056	23	728	39	ND		393	57	ND		813	35	927	33	ND	

The following table shows the toxicokinetic parameters of 5-FU in the three sampled tissues. Capecitabine administration appears to generate the highest 5-FU concentrations in tumors and the

greatest differences between AUCs in tumor and that in plasma or muscle (ratio tumor/plasma and tumor/muscle). This increase even occurs in the refractory tumor, WiDr, but the numbers vary considerably.

Toxicokinetic parameters of 5-FU in Tumor, Plasma and Muscle

		Capecitabine				5'dFUrd				5-FU			
xenograft	tissue	C_{max} µg/ml tissue	T_{max} µg/ml tissue	AUC_t µg*hr/ g tissue	$t_{1/2}$ hr	C_{max} µg/ml tissue	T_{max} µg/ml tissue	AUC_t µg*hr/ g tissue	$t_{1/2}$ hr	C_{max} µg/ml tissue	T_{max} µg/ml tissue	AUC_t µg*hr/ g tissue	$t_{1/2}$ hr
HCT116	Tumor	14.2	0.5	39.4	4.5	5.3	0.5	9.19	4.1	1.13	0.25	1.11	2.3
	Plasma	0.2	0.5	0.31	1	0.39	0.25	0.44	2.1	1.96	0.25	0.61	1.6
	Muscle	0.45	0.5	1.82	4.3	0.55	0.25	1.63	3.8	2.6	0.25	3.05	6.6
	$Ratio_{(tumor/plasma)}$			**127**				20.56				**1.8**	
	$Ratio_{(tumor/muscle}$			**21.6**				5.6				**0.4**	
CSF280	Tumor	7.62	1	37.6	5.5	3.11	0.5	10.2	7.4	1.5	0.25	1.7	12.4
	Plasma	0.12	0.5	0.18	1	0.24	0.5	0.3	4	2.13	0.25	0.62	0.3
	$Ratio_{(tumor/plasma)}$			**209**				34				**2.7**	
COLO205	Tumor	8.04	0.5	27.3	17.4	3.57	0.5	7.62	10.2	2.56	0.25	1.68	15.1
	Plasma	0.24	0.5	0.24	0.54	0.23	0.25	0.35	1.7	3.06	0.25	0.88	0.4
	$Ratio_{(tumor/plasma)}$			**114**				21.8				**1.9**	
WiDr	Tumor	2.45	1	8.16	5.5	1.27	0.25	29	5.2	0.58	0.25	1.48	7.8
	Plasma	0.13	0.5	0.22	1.5	0.25	0.25	0.27	3.2	2.21	0.25	0.66	0.68
	$Ratio_{(tumor/plasma)}$			**37.1**				10.7				**2.2**	

1129) T Ishikawa *et al.* (1993) Comparative antitumor activities of N^4-alkoxycarbonyl-5'-deoxy-5-fluorocytidine derivatives and its related fluoropyrimidines. Roche Report J-146208, December 29. Vol. 18, 118-150.

These experiments compare the ability of Ro 09-1978 and Ro 09-1979 to inhibit the growth of human tumor xenografts in mice relative to other fluorocytidine derivatives. The investigators studied growth inhibition in 12 human tumor models grafted into BALB/c nu/nu mice. This manuscript is very similar to submission 1110 above. It confirms that Ro 09-1978 is a more effective tumor cytotoxin than Ro 09-1979. Interestingly, tumor baring mice given capecitabine were less cachexic than tumor baring mice given vehicle. Capecitabine treated mice had near normal serum glucose and gained weight while vehicle treated mice had abnormally low serum glucose and lost weight.

1130) T Ishikawa *et al.* (1997) The capecitabine metabolites 5'-DFCR and 5'dfur become cytotoxic only after conversion to 5-FU. Roche Report J-146'548. Vol. 18, p 151.

In these experiments Ishikawa *et al.* used a number of human tumor cell lines (colon cancer HCT116, COLO205, DLD-1, breast cancer ZR-75-1 and MCF-7, bladder cancer Scabber and T-24, cervix cancer SIHA and HT-3, gastric cancer MKN45 and MKN28) to determine the cytotoxicity of capecitabine and its metabolites. They measured cytotoxicity as inhibition of cell growth relative to controls and expressed as the IC_{50}. They added THU, an inhibitor of cytidine deaminase, in a series of cultures to prevent the conversion of the 5'dFCyd to 5'-dFUrd. They also added 6-amino-5-chlorouracil (ACU), an inhibitor of dThdPase, in another series to prevent the conversion of 5'-dFUrd to 5-FU.

The study of cytotoxicity in the different cell lines is identical to that presented in submission 1110. The pharmacology section of this NDA contains numerous redundant presentations of data. This practice complicates the review process unnecessarily. Again the investigators did not provide raw data, only graphs, for the cytotoxicity experiments with inhibitors. The following graph shows that 5'-dFCyd, 5'dFUrd and 5'dFUrd plus THU cause roughly equivalent cytotoxicity in SIHA cells. This is consistent with the hypothesis that 5'-dFCyd and 5'dFUrd are converted to 5-FU in the cells. The authors calculated that the IC_{50} of 5-FU alone was 7.5 μM in SIHA cells. They also calculated the values of 67 μM for 5'-dFUrd and 92 μM for 5'-dFCyd. The value for 5'dFUrd does not appear to agree with the following graph. I cannot explain the difference. The addition of THU decreases the cytotoxicity of 5'-dFCyd by at least a factor of 10.

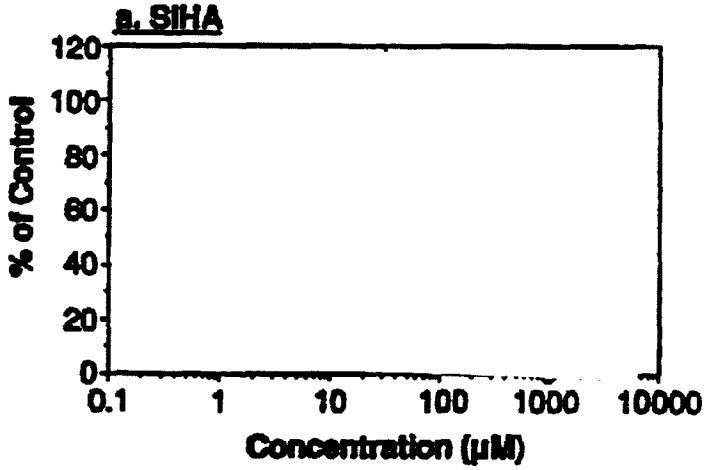


Fig. 1 Antiproliferative activity of 5'-dFCyd and 5'-dFUrd with a Cyd deaminase inhibitor, THU, against two human cancer cell lines. A single-cell suspension (1 x 10^4 cells/well) of human cervix cancer SIHA (a) and bladder cancer Scaber (b) was added to the serially diluted 5'-dFCyd and 5'-dFUrd with or without 200 μM THU in the wells of a 96-well plate. The cells were then cultured at 37°C for 8 (SIHA) or 5 (Scaber) days

Likewise the following graph shows that ACU decreases the cytotoxicity of 5'-dFUrd by approximately 50 fold. These experiments suggest that the intermediate metabolites of capecitabine, 5'-dFCyd and 5'-dFUrd are less toxic than 5-FU.

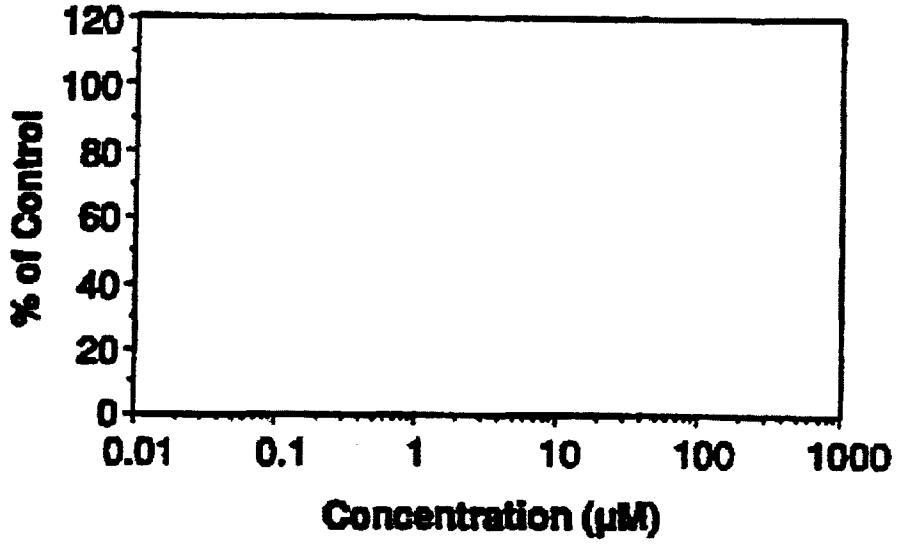


Fig. 2 Antiproliferative activity of 5'-dFUrd with a dThdPase inhibitor, ACU, against a human cancer cell line. A single-cell suspension (1 x 10^3 cells/well) of human breast cancer ZR-75-1 was added to the serially diluted 5'-dFUrd with or without 100 µM ACU in the wells of a 96-well plate. The cells were then cultured at 37°C for 8 days

1131. T Ishikawa, *et al.* (1997). Effects of the 5-FU catabolites, FUH2, FUPA, and FBAL on the anti-proliferative activity of 5-FU in human cancer cells. Roche Report J-146'716. Vol. 18, p 166.

In the introduction to this series of experiments Ishikawa et al. state that they had observed that capecitabine dosing produced higher serum concentrations of FBAL than did 5-FU dosing in clinical and preclinical experiments. They did not identify these experiments. 5-FU is hydrogenated to dihydrofluorouracil (FUH2 by dihydropyrimidine dehydrogenase) which in turn is oxidized to α-fluoro-β-ureidopropionate (FUPA) and ultimately to α-fluoro-β-alanine (FBAL) which is excreted in the urine. The investigators did the current studies to determine whether the catabolites of capecitabine affect the anti-proliferative activity of 5-FU in human colon HT29 and breast AR-75-1 cancer cell lines. As one would expect the metabolites of 5-FU are much less toxic than 5-FU (~100 fold by IC_{50}). High concentrations of the catabolites (10 fold greater than that of 5-FU in a fixed ratio) did not diminish the cytotoxicity of 5-FU as measured by the IC_{50}. Thus, there is no product inhibition of 5-FU toxicity in

the breakdown scheme of 5-FU. This is interesting, but it does not explain why capecitabine causes higher serum concentrations of FBAL than comparable doses of 5-FU. I suspect the reason is in the route. Large PO doses of capecitabine will be rapidly converted to 5-FU and ultimately to FBAL by a large first pass effect in the liver. IV doses of 5-FU are rapidly distributed throughout the body. Though dehydrogenation to FUH2 is also rapid, conversion to subsequent metabolites is probably relatively slow.

1146) H Eda *et al.* (1993) Cytokines induce thymidine phosphorylase expression in tumor cells and make them more susceptible to 5'-deoxy-5-fluorouridine. Cancer Chemother. Pharmacol. 32:333-338. Vol 19, 66-71.

This paper is a confirmation of the results of the next paper using the same cytokines and three human tumor cell lines *in vitro* (COLO201, MKN45, WiDr). These cytokines did not increase the cytotoxicity of 5'-DFUR or 5-FU against normal fibroblast WI38 cells. The authors show that the cytokines increase the activity of thymidine phosphorylase activity and thymidine phosphorylase mRNA expression (Northern Blot). The Northern probe was 17mer cDNA sequence based on the structure of human thymidine phosphorylase. From this research it is again unclear how many individual enzyme activities contribute to total pyrimidine nucleotide phosphorylase (PyNPase) activity. Expression of this activity is complex and varies from tissue to tissue, from tumor to tumor, and from species to species, thus compromising any prediction of toxicity from animal studies of Ro 09-1978.

1147) H. T. Tevaearai *et al.* (1992) Interactions of interferon-α2a with 5'-deoxy-5-fluorouridine in colorectal cancer cells *in vitro*. Eur. J Cancer. 28:368-372. Vol. 19, 72-76.

This appears to be the original paper in this series of three on similar subjects. IFNα decreased the IC_{50} of 5'-DFUR by approximately 50% at the highest concentrations used in cultures of 5 tumor lines (WiDr, GT-29, 513, SW-480 and Co-115). IFNα caused a similar decrease in IC_{50} of 5-FU in only one cell line, SW-480. The decrease in IC_{50} of 5'-DFUR correlated with an approximate doubling of PyNPase activity in the sensitive cell lines. Thus, IFNα induces PyNPase activity in some tumors.

1152) T Ishikawa *et al.* (1994) Selective inhibition of spontaneous pulmonary metastasis of Lewis lung carcinoma by 5'-deoxy-5-fluorouridine. Roche Report J-146'207, February 2. Vol. 4, p 131.

TNFα, IL-1α and IFNγ increase the expression of PyNPase in Lewis Lung Carcinoma (LLC) cell cultures. As shown in papers reviewed above this increase in PyNPase expression increases the antiproliferative effect of 5'-DFUR. These factors also increase the expression of type IV collagenase, a factor that enhances the metastatic ability of a cancer. In the current paper 5'-DFUR inhibited LLC metastasis from the s.c. inoculation site to the lung of mice. 5'-DFUR reduced the number of tumor nodules in the lung at doses 17 to 46 times lower than doses necessary to prevent primary tumor growth.

1153) Y Tanaka et al. **(1990) Anticachetic activity of 5'-deoxy-5-fluorouridine in a murine tumor cachexia model, colon 26 adenocarcinoma. Cancer Res.** 50:4528-4532. **Vol. 19, 175-179.**

Colon 26 adenocarcinoma causes cachexia in mice. When mice with large tumor burdens were given 5'-DFUR (p.o. d 22 to d 29 post tumor implant) decreases in carcass weight, adipose tissue weight and serum glucose reversed proportional to dose. 5'-DFUR also restored diminished hepatic catalase and cytochrome P-450 activity in tumor bearing mice after 7 days of treatment. Cyclophosphamide, nimustine and 2'deoxy-5-fluorouridine were less effective at reversing this induced cachexia. 5-FU, tegafur, mitomycin, cis-platin, and doxorubicin did not reverse this experimental cachexia. 5'-DFUR reversed cachexia at concentrations that did not slow tumor growth. The authors claim that this shows that the anti-cachectic effect of 5'-DFUR is independent of it anti-tumor effect. Perhaps the authors overstate this conclusion pending further evidence.

1154) Y Fukase *et al.* (1994) Dose dependent anti-tumor activity of Ro 09-1978. Roche Report J-146'260, April 7.

This study determined that a dosing schedule of Ro 09-1978 p.o., 5d/week for 9 weeks caused only non-lethal toxicity at a dose of 26.25 mmol/kg/3 wk. The test animal was athymic mice with human colon cancer HCT116 xenografts. This dose was lethal to 3/6 and 4/6 mice at two other schedules, daily administration for 9 weeks or daily for two weeks then one week off for 9 weeks, respectively. The investigators reported only death as toxic end-point. All three schedules inhibited tumor growth by 88% or more on day 55 compared to controls. Ro 09-1978 was more effective than comparable doses of 5-FU.

1155) Y Satoh et al. **(1994) General pharmacology studies on Ro 09-1978, the NeoFurtulon successor, in laboratory animals. Roche Report J146262, April 26. Vol 19, 192-220.**

These investigators gave capecitabine to rats, mice, guinea pigs and beagle dogs to doses of 300 mg/kg PO or 30 mg/kg IV in a standard battery of pharmacology safety tests. These doses produced no severe adverse effects in behavior tests, the CNS, respiratory system, cardiovascular system, or GI *in vivo* or *in vitro*. At the highest doses (1000 mg/kg) capecitabine cause some hypomobility in mice and bradycardia in rats. Such high doses also caused some changes in the cardiohemodynamic parameters in anesthetized dogs, the excretion of sodium and potassium in anesthetized dogs and in the potassium ratio in rats.

9) H Eda *et al.* (1993) Cytokines induce uridine phosphorylase in mouse colon 26 carcinoma cells and make the cells more susceptible to 5'-deoxy-5-fluorouridine. Gann (Jpn J Cancer Res) 84:341-347. **Vol. 4, p 91.**

Eda *et al.* showed that tumor necrosis factor α (TNFα), interleukin-1a (IL-1α), and interferon γ (IFNγ) increase the cytotoxicity of 5'-DFUR and 5-FU *in vitro* (mouse colon 26 carcinoma cells). Cytotoxicity increases as much as 12.4 fold for 5'-DFUR and 2.7 fold for 5-FU when a mixture of these

cytokines are added to tumor cell cultures. The authors demonstrated that this increase in cytotoxicity is due primarily to induction of tumor cell expression of uridine phosphorylase activity.

Pharmacology 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 a N^4-trimethoxybenzoyl group (NeoFurtulon) 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 N^4-trimethoxybenzoyl group 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 this submission describes an impressive body of generally good scientific investigation that lead to the clinical development of capecitabine.

The sponsor provided but a minimum of information about the enzymology of this reaction sequence. Kono *et al.* (1102) determined that 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. These numbers are poorly determined and human tissue contains at least two PyNPase enzymes. Nevertheless, this large value for thymidine and the seven fold increase for 5'-DFUR might have suggested early on that this therapy might generate equivocal clinical results.

In a more thorough enzymology paper Chabner *et al.* (108) determined that the k_m of cytidine deaminase from human leukemic granulocytes for cytidine was 11 μM. This activity is not rate limiting in humans. This enzyme should quickly convert most 5'-DFCR to 5'-DFUR in the blood stream. Interestingly, total activity in units/mg protein was lower in leukemic cells than in normal granulocytes. Expression increased with differentiation.

As one would expect with this class of compounds, the sponsor has provided an impressive body of work describing the ability of various fluoropyrimidine derivatives to inhibit tumor growth *in vivo* and *in vitro*. The most interesting of these is the *in vitro* study by Miwa *et al.* (1110) where the investigators compared the *in vitro* efficacy of capecitabine, 5'-DFCR, 5'-DFUR and 5-FU in the same tumor cell lines. Capecitabine was not toxic to this broad spectrum of tumor cells. In most cases the IC_{50} was greater than 1000 μM. This suggests that most tumor cell lines 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, the expression of PyNPase is not sufficient to make 5'-DFCR as effective as 5-FU.

In this large and pivotal work, Miwa *et al.* also present three graphs that compare activities of the three enzymes in the metabolic sequence. These graphs show activity in tumor tissue from major organs and in adjacent normal tissue in humans. Only the liver expresses significant carboxylesterase activity and the difference between tumor and normal tissue is not significant. The figure legend for the graph of cytidine deaminase and PyNPase activity says that closed bars represent normal tissue and open bars represent tumor tissue. Reading the graphs this way, cytidine deaminase activity is lower in normal liver than in liver tumor. It is higher in all other normal tissues than in tumor. Yet in the results the authors say "Except in hepatoma, this activity in tumors was also higher than that of all corresponding normal tissues." Likewise, in the graph of PyNPase activity the graph shows high activity in normal tissue and the text claims that the activity is higher in tumor. The figure legends may be incorrect. In an earlier manuscript on which Dr. Miwa is also an author, K. Mori *et al.* (1109) present exactly the same graphs of cytidine deaminase and PyNPase activity. In this submission, the authors labeled tumor activities as closed bars and normal tissue as open bars. The labeling in this manuscript supports the possibility that the graphs in submission 1110 are mislabeled. Nevertheless, this redundant presentation of data slows the review process.

Regardless of the labeling of the graphs in these two manuscripts, the results do not support the sponsors claim that the metabolism of 5'-DFUR to 5-FU is 'tumor specific'. Though the differences in activities between tumor and normal tissue reach significance (however it is labeled) the variability is very large. This implies that some tumors express large amounts of the enzymes and that others do not. The paper by Tori *et al.* (1125) is also a good example of the variability of expression of dThdPase. They found that of 100 invasive ductal carcinoma samples, only 39 were dThdPase positive Their results suggest that tumors with rapid neovascularization may be more susceptible to capecitabine, but not all tumors show rapid neovascularization. This also argues against the sponsors claim that capecitabine is tumor specific.

The sponsor, nor a practicing oncologist, cannot predict *a priori* which tumors will have increased enzyme expression and which will not. Thus, it is inappropriate to imply that this therapy is tumor specific. Other papers argue for the 'tumor specificity' of capecitabine, but they are similarly unconvincing.

The paper by Miwa *et al.* (1111) is the most elegant and important of all the manuscripts by the Roche group. The authors isolated carboxylesterase activity from human liver and colon. They established that human liver expresses two carboxylesterase activities and that human colon expresses only one, 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. This paper also established that the substrate specificity and distribution of carboxylesterase enzymes in the monkey are similar to those of human. Those in mouse are not. This paper justifies the use of the monkey as a model for the preclinical development of capecitabine.

Miwa *et al.* (1119) established that repeated dosing (28 days) with capecitabine did not induce carboxylesterase or cytidine deaminase activity in the colon or liver of the monkey. These investigators

are to be commended for acquiring this important information from animals used in a toxicology experiment. Ura *et al.* (1120) showed that breast cancer tissues from European American, African American and Japanese patients express about the same cytidine deaminase and PyNPase activity. Assays showed that tissue from Japanese patients expressed more carboxylesterase activity than tissue from European American, but a test for enzyme expression by ELISA showed no difference. In all cases the expression showed great variability, again to the detriment of the sponsor's claim of tumor specificity.

Miwa *et al.* (1121) determined the enzyme activity for the three enzymes that transform capecitabine to 5-FU in major organ tissues of mouse, rat, monkey and human. In the mouse, they found that most of the carboxylesterase activity and PyNPase activity were in small intestine, most cytidine deaminase activity was in the kidney. In the rat, they found most of the carboxylesterase activity in the small intestine and liver, most PyNPase in the lung and small intestine, and very low cytidine deaminase activity throughout. In monkeys, they found most of the carboxylesterase activity in the liver and widely distributed cytidine deaminase and PyNPase activity. Though the carboxylesterase activity was six times higher in human liver than in monkey liver, the distribution of this activity and of the other two was similar to that of humans in all tissues. Again this study 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.

Sumizawa *et al.* (1122) established that the PyNPase in humans is homologous with platelet-derived endothelial cell growth factor. They also established that rPD-ECGF has thymidine phosphorylase activity. These results imply that PyNPase activity may be important in tumor angiogenesis. Moghaddam *et al.* (1124) then established that PyNPase is angiogenic.

In a series papers the sponsor established that 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 cells *in vitro*.

In safety pharmacology studies, capecitabine caused little toxicity other than that associated with anticipatable 5-FU toxicity.

Toxicology

2101) A. Kawashima and I. Horii, 1993, Oral single dose toxicity study of Ro 09-1978 in mice Report J-146'169, Volume 20, page 1.

Species	male and female BDF1 mice, 5/sex/dose group
Drug	Ro 09-1978, Lot KM-016,
Doses	vehicle control, 3000 and 6000 mg/m^2 (given as 0, 1000, and 2000 mg/kg)
Vehicle	40 mM Na^+-Citrate buffer pH 6 plus 5% gum Arabic solution v/v.
Route	PO, gavage
Schedule	single dose
Observations	
Clinical Signs	daily
Body Weight	before dosing, d1, 2, 3, 7, 10 and 14
Food Cons.	before dosing, d1, 2, 3, 7, 10 and 14
Gross Path	at necropsy on day 14

The GLP statement was signed by A. Kawashima. He and Dr. Horii did the study at

Mortality	None
Clinical Signs	Almost all animals in both dose groups showed decreased activity from 15 min to 1 hr after dosing. This included bradypnea in some animals. These signs disappeared ~4 hr after dosing in HD males and ~2 hr after dosing in HD females. The LD animals returned to normal within ~1 hr.
Body Wt.	No toxicologically significant changes
Food Consumption	Slight decrease in LD and HD males on day 1.
Gross Path	No drug related changes.

Single dose oral capecitabine causes little toxicity in mice at doses to 6000 mg/m^2.

2102) A. Kawashima and I. Horii, 1993, Oral single dose toxicity study of Ro 09-1978 in rats Report J-146'168, Volume 20, page 27.

Species	male and female SD-Slc Rats, 5/sex/dose group
Drug	Ro 09-1978, Lot LS-1,
Doses	vehicle control, 6000 and 12000 mg/m^2 (given as 0, 1000, and 2000 mg/kg)
Vehicle	40 mM Na^+-Citrate buffer pH 6 plus 5% gum Arabic solution v/v.
Route	PO, gavage
Schedule	single dose
Observations	
Clinical Signs	daily

Body Weight	before dosing, d1, 2, 3, 7, 10 and 14
Food Cons.	before dosing, d1, 2, 3, 7, 10 and 14
Gross Path	at necropsy on day 14

The GLP statement was signed by A. Kawashima. He and Dr. Horii did the study at

Mortality	None
Clinical Signs	Almost all HD rats showed decreased activity from 15 min to 1 hr after dosing. These signs disappeared within 4 hr. Only one LD female showed decreased activity.
Body Wt.	No toxicologically significant changes
Food Consumption	No toxicologically significant changes
Gross Path	No drug related changes.

Capecitabine appears even less toxic to rats than to mice.

2103) A. Kawashima and I. Horii, 1993, Oral pyramiding (sic) dose toxicity study of Ro 09-1978 in male monkey, Research Report J-146'170, Vol 23, page 51.

Species	Cynomolgus Monkey, two males
Drug	Ro 09-1978, Lot LS-1
Doses	6000, 12000, 24000 mg/m^2 (given as 500, 1000 and 2000 mg/kg)
Note	This is not a single dose study, the same two monkeys were given these doses at 3 day intervals, day -6, day -3 and day 0 in the order 500, 1000 and 2000 mg/kg.
Vehicle	40 mM Na^+-Citrate buffer pH 6 plus 5% gum Arabic solution v/v.
Route	PO, NG tube, 2.0 ml/kg volume
Schedule	escalating dose every 3 days
Observations	
Clinical Signs	daily
Body Weight	daily from day -6 to day 3, d7, d10, d14
Food Cons.	daily
Clinical Chem.	before dosing, wk 4, wk 8, wk 13, wk 17 recovery
Necropsy	none

The GLP statement was signed by H. Sameshima, D.V.M. He and Dr. Horii did the study at

Mortality	None
Clinical signs	LD day -6, emesis containing drug for ~10 min post dosing MD day -3, white foamy emesis at 1.5 or 6 hr.

	HD day 0, emesis containing drug for ~15 min. Salivation.
	one male diarrhea before the HD and on days 1 through 5.
	one male diarrhea on day -5, 6 hr after the MD, and from days 2 through 8.
Body weight	No changes (no control)
Food consumption	No changes (no control)

This is an unusual study. It provides little useful information about capecitabine in monkeys. Like rodents, they are fairly tolerant to a few high doses. These doses at this schedule did not overwhelm the monkey's ability to recover though the doses were clearly toxic. The investigators did not attempt to quantify how much drug was lost due to vomiting. Nevertheless, these doses are very high compared to the proposed clinical dose.

2203) A Kawashima and I Horii, 1993. Four-week oral toxicity study of Ro 09-1978 in rats. Report J-146'171. Volume 21 page 1.

Animal	Sprague-Dawley (DS-SLC) rats, 5 per dose level per sex
Drug	Ro-09-1978, Lots LS-1 and KM-016
Doses	0, 1077, 2154 and 3231 mg/m^2/day (given as 0, 179.5, 359 and 538.5 mg/kg/day, or 0, 0.5, 1.0 or 1.5 mmol/kg/day)
Route	PO, 1.0 ml/100g dose volume
Schedule	once daily for 28 days
Vehicle	40 mM citrate buffer, pH 6, in 5% gum Arabic solution.

Observations:

Clinical signs	daily
Body weight	twice weekly
Food cons.	twice weekly
Hematology	necropsy
Clin Chem.	necropsy
Urinalysis	"before final dosing"
Necropsy	at four weeks, with histopathology and organ weights

The investigators did this study at the
Mr. Kawashima signed a GLP statement.

Results:

Mortality	all rats survived to necropsy
Clinical signs	no drug related signs or symptoms reported
Body weight	HD males were about 8% less than controls. No change in other groups.
Food cons.	Decrease in HD males, no change in other groups.
Hematology	no toxicologically significant differences
Clinical Chem	no toxicologically significant differences.
Urinalysis	no toxicologically significant differences.

Organ Wt.	no toxicologically significant differences.
Gross Path	no toxicologically significant differences.
Histopathology	Slight degeneration of the rectal crypt cells in 3 HD males and one HD female No other drug related changes

Despite the fact that the HD appRochees doses given to patients on a mg/m^2 basis, capecitabine caused very little toxicity in these rats. Rats are obviously very tolerant of capecitabine.

2204) A Kawashima and I Horii, 1994. Twenty six-week oral toxicity study of Ro 09-1978 in rats. Report J-146'258. Volume 21 page 61.

Animal	Sprague-Dawley (DS-SLC) rats, 20 per dose level per sex
Drug	Ro-09-1978, KM-021
Doses	0, 1077, 2154 and 3231 mg/m^2/day (given as 0, 179.5, 359 and 538.5 mg/kg/day, or 0, 0.5, 1.0 or 1.5 mmol/kg/day)
Route	PO, Gavage, 1.0 ml/100 g dose volume
Schedule	once daily for 26 weeks
Vehicle	40 mM citrate buffer, pH 6, in 5% gum Arabic solution.

Observations:

Clinical signs	daily
Body weight	twice weekly
Food cons.	twice weekly
Hematology	necropsy
Clin Chem.	necropsy
Ophth Exam	before necropsy on 6 animals per dose group per sex.
Urinalysis	"before final dosing"
Necropsy	at four weeks, with histopathology and organ weights

The investigators did this study at the
Mr. Kawashima signed a GLP statement.

Results:

Mortality	all rats survived to necropsy
Clinical signs	no line listing but investigators report "no drug related changes", they also say that some animals had "loose passage, salivation just after dosing."
Body weight	The following table shows in body weight in each group as a percent of control.

Dose mg/kg	Control	179.5	359	538.5
male	100%	95%	90%	87%
female	100%	107%	99%	95%

Food cons.	Decrease in MD and HD males, no change in other groups.
Hematology	The following table shows the significant changes in parameters in males as percent of control.

Dose	MCV	MCH	RBC	WBC	Basophil ratio
Control	100%	100%	100%	100%	100%
LD	103%	101%	99%	86%	89%
MD	103%	103%	98%	88%	82%
HD	104%	103%	97%	89%	79%

	Changes in HCT, MCV, MCH, MCHC in females reached statistical significance but were less than 3% in magnitude
Clinical Chem.	Total protein decreased in males with dose but the decrease was <5% other differences were not toxicologically significant
Urinalysis	Urinary protein and specific gravity were increased in males. These increases were not severe but were dose dependent ($p < 0.01$ for protein). This increase may have been secondary to decreased volume (dose dependent). Total protein was also increased in females without significant changes in volume.
Ophth exam	no toxicologically significant differences.
Organ Wt.	dose related decrease in absolute heart (7% in HD) and liver (13% in HD) wt. increases in relative brain, kidney, and testes weight. no toxicologically significant changes in females.
Gross Path	no dose related changes
Histopathology	Drug related degeneration of crypt cells (3 HD males) and dilation of glandular lumen (2 HD males) of the rectum.

This study appears well done and well reported. Again the high dose was minimally toxic even on the long schedule. The most obvious toxicity is weight loss which is more profound in males than females. The increases in relative organ weight in brain, kidney and testes are probably secondary to this body weight loss. Nevertheless, the significant decreases in absolute heart and liver weight are cause for concern. These toxicities are consistent with the liver toxicity observed clinically. I am curious why this toxicity does not manifest microscopically. This suggests general atrophy. The increase in urinary protein and the decrease in serum protein suggest kidney toxicity at the glomerulus. The changes in hematological parameters suggest hematopoietic damage. Unfortunately the investigators did not do a marrow smear. This experiment would have been more informative if the low dose group had been eliminated in favor of a 2 mmol/kg dose group.

2206) A. Kawashima and I. Horii, 1995, 13 week oral toxicity study in monkeys, Research Report J-146'413, Vol 23, page 1.

Species	Cynomolgus Monkey, 4/sex/dose group one monkey/sex/dose group was allowed to recover for four weeks.
Drug	Ro 09-1978, Lot 24289-74-A, Purity 99.9%
Doses	vehicle control, 648, 1296, 2580 mg/m^2 (given as 0, 54, 108, and 215 mg/kg/d, or 0, 0.15, 0.3, 0.6 mmol/kg/d) for day 0 through 31. The investigators did not dose the

	HD group on days 32 through 34. They reduced the high dose to 1944 mg/m^2/d (162 mg/kg/d, or 0.45 mmol/kg/d). The high initial HD was too toxic.
Vehicle	40 mM Na^+-Citrate buffer pH 6 plus 5% gum Arabic solution v/v.
Route	PO, NG tube, 2.0 ml/kg volume
Schedule	daily
Observations	
Clinical Signs	daily
Body Weight	before dosing, twice weekly and at necropsy
Food Cons.	daily
Ophth. Exam	before dosing and before necropsy
Liver function	wk 12, phenolsulfonphthalein and indocyanine clearance
Renal function	wk 12, creatinine clearance
Urinalysis	before dosing, wk 4, wk 8, wk 13, wk 17 recovery
Hematology	before dosing, wk 4, wk 8, wk 13, wk 17 recovery
Clinical Chem.	before dosing, wk 4, wk 8, wk 13, wk 17 recovery
Bone Marrow	at necropsy
Gross Path	at necropsy
Histopathology	at necropsy, light microscopy of major organs, electron-microscopy of liver and kidneys

The GLP statement was signed by A. Kawashima. He and the other investigators did the study at

Results

Mortality	One HD male found dead on d34
	One HD female moribund on d45
Clinical Signs	anorexic d12, diarrhea d18 to death, d24 emesis, staggering gate, d27 onward inactive and in poor condition.
	anorexic d22 till death, diarrhea d33 till death, hypothermia d42 onward, staggered gate day 43.
	Other HD animals developed anorexia and diarrhea before the dose was decreased, they recovered during the cessation of dosing and remained in nominal condition through the experiment.
	One MD female developed loose stool day 73 to 79.
Body Weight	lost ~25% of their initial body weight before they died
	These decreases accounted for most of the changes in the means for the HD group. The LD and MD males gained weight rapidly in the first week of recovery to 10 to 20% above controls. They maintained this greater weight through recovery. HD animals gained only slightly.
Food Cons.	Body weight decreased with food consumption for
	LD and MD males did not increase their consumption during recovery, indeed it was decreased in wk13 in all groups
	All females including controls ate ~ half-normal at the cessation of dosing, controls,

LD and HD recovered rapidly but MD continued to eat less.

Ophth. Exam No toxicologically significant changes

Liver function No toxicologically significant changes

Renal function No toxicologically significant changes

Urinalysis

Hematology had low RBC, HCT at wk4 and at death, low WBC at death, high MCV and Lymphocyte ratio (LYMP) at death.
had low WBC wk4 and at death, LYMP an bone marrow count at death, high Plt and NEUT at death

The following table shows hematological parameters that changed significantly from control as a function of dose. Most of these changes are >10%.

In males

	Dose	Control	LD	MD	HD	% of control in HD
	day					
WBC 10^3/µl	28	17.4	14.4	15.5	7.7	44%
	56	17.1	14.2	17.4	5.4	32%
RBC 10^6/µl	28	7.1	6.7	6.9	5.8	82%
	91	7.1	6.4	6.4	5.8	82%
HCT %	28	48.7	47.6	47.9	37.9	78%
Hb g/dl	28	13.5	13.5	13.68	11.2	83%
	91	13.3	13.2	12.8	11.6	87%
EOSI %	28	4.4	3.5	1.6	1.3	30%
	91	4	4.25	1.4	1.23	31%
BASO %	28	0.7	0.5	0.4	0.2	29%
MONO %	56	4.1	3.7	6	10.6	259%
	91	5.2	3.93	7	10.7	206%
Pro. Time Sec	91	12.4	12.4	11.5	11.7	94%

In Females

	Dose day	Control	LD	MD	HD	
WBC $10^3/\mu l$	28	13.2	11.6	13	7.3	55%
RBC $10^6/\mu l$	91	6.6	6.3	5.6	5.3	80%
HCT %	28	44.2	44.8	43	38	86%
	91	42.1	39.9	35.6	36.6	87%
MCH pg	56	18.6	19	19.5	21.2	114%
	91	18.4	18.7	19.5	20.9	114%
MCHC g/dl	56	28.9	29.1	30.4	30.2	104%
	91	28.4	29.7	30.6	30.3	107%
MONO %	56	3.3	4.8	5.3	7.4	224%
	91	4	4.7	6.5	10.5	263%

Clinical Chem. No toxicologically significant changes except in the two dogs that died. These changes are consistent with the whole body failure.

Organ Wt. In the HD animals that died - low absolute and relative thymus or spleen. Other changes consistent with weight loss.

In surviving HD animals - Low absolute and relative thymus and low spleen in one male. Low absolute and relative thymus in one female.

Gross Path In the animals that died - small thymus and spleen. decreased adipose, enlarged adrenals.

In the surviving MD and HD animals - one male small thymus and spleen, one female small thymus in each group.

Histopathology In the animals that died - Atrophy of mucosa, enlargement of nuclei of epithelium and crypt cells, dilation of glandular lumina in GI, atrophy of lymphatic organs, Hypertrophy of cortical cells in the adrenals. Cellular damage was wide-spread and ranged from slight to severe.

In surviving MD and HD animals - Regressive changes in spleen, lymph nodes and thymus, tonsil in HD only.

2207) I. Horii and H. Sameshima, 1994, Twenty six week oral toxicity study of Ro 09-1978 in Cynomolgus monkeys. Report J-146'257, Volume 24 page 1.

Species Cynomolgus Monkey, 3/sex/dose group

Drug Ro 09-1978, Lot KM-21, Purity 99.6%

Doses vehicle control, 216, 648, 1728 mg/m^2 (given as 0, 18, 54, and 144 mg/kg)

Vehicle	40 mM Na^+-Citrate buffer pH 6 plus 5% gum Arabic solution v/v.
Route	PO via catheter (sic)
Schedule	daily
Observations	
Clinical Signs	thrice daily, before and after dosing and 2-3 hr after dosing
Body Weight	before dosing, weekly and at necropsy
Ophth. Exam	before dosing, wk 13, wk 26, with ketamine anesthesia.
Fecal Exam	before dosing, wk 4, wk 13, wk 26 guaiac method
Urinalysis	before dosing, wk 4, wk 13, wk 26 metabolic cages.
Hematology	before dosing, wk 4, wk 13, wk 26
Clinical Chem.	before dosing, wk 4, wk 13, wk 26
Bone Marrow	at necropsy
Gross Path	at necropsy
Histopathology	at necropsy, light microscopy of major organs, electron-microscopy of liver and kidneys

The GLP statement was signed by H. Sameshima, D.V.M. He and Dr. Horii did the study at

Results

Mortality	One female in the high dose group moribund on day 57. All other monkeys survived to necropsy
Clinical Signs	The moribund female had soft stool d29, diarrhea d31, decreased activity day 33, decreased appetite wk5, emaciation d36, prone d56. One HD male had soft stool d61 reversed by d75
Body Weight	decreased wt wk5, by wk8 ~60% of control
Ophth. Exam	no abnormalities
Fecal Exam	no abnormalities
Urinalysis	no dose related changes
Hematology	In female low RBC (80% of control), Hct (80%), Hgb, WBC (15%) and segmented leukocytes, high lymphocytes by wk4. Other HD females had low lymphocytes wk13 and WBC wk26. Low WBC in two HD males wk 13 and 36 (30 to 40% of controls). Low red cell parameters in one HD male In the MD group, one male had low RBC
Clinical Chem.	Female low cholesterol, glucose, Ca^{++}, Na^+, K^+, Cl^-, high BUN, creatinine and α_1-globulin
Bone Marrow	Decreased nucleated cells in
Gross Path	In female involution of the thymus, enlarged adrenals, aqueous contents in the large intestine
Organ Wt.	decrease in absolute heart, liver and kidney, increase in brain, lung and adrenals
Histopathology	No test related changes in the LD or MD groups.

In female degeneration of the mucosal epithelium in ileum, cecum, colon and rectum. Fibroplasia of the submucosa in the duodenum, jejunum, ileum, cecum, colon and rectum. Brown pigment in the macrophages in the submucosa of the cecum. Numerous GI changes. Atrophy of the thymus and lymphoid follicles of the spleen. Lymphocyte depletion of the mesenteric lymph node. Decrease in cellularity of the bone marrow with low remodeling activity and spongy bone. Hypoplasia of the squamous epithelium in the skin, mammary gland, tongue, esophagus, and vagina. Atrophy of the hair follicles. Degranulation of the acinar cells of the pancreas.

In surviving HD animals, atrophy of the lymphoid follicles of the spleen in one male. Atrophy of the thymus in two males and one females. Brown pigmentation of the Kupffer cells in one male and one female.

Capecitabine toxicity clearly killed HD female The pathology associated with her death is that often seen with 5-FU. The organ system most affected was the GI. The decrease in absolute heart, liver, and kidney weight and the increase in brain, lung and adrenals is consistent with wasting. The changes in serum chemistry were determined too close to death to have much meaning. The myelosuppression seen in this animal and others is consistent with fluoropyrimidine toxicity. No other monkeys suffered such profound toxicity at this dose. This makes me wonder if monkeys have a polymorphism for DPD similar to humans or if this death was associated with more effective metabolism of capecitabine.

2208) A. Bryson, (1996), a 52 week oral toxicity and toxicokinetic study in the Cynomolgus monkey, Report W-142581, Vol. 25, page 1.

The sponsor also studied the effects of capecitabine given to monkeys for 52 weeks at doses of 432, 862 and 1296 mg/m^2/d (given as 36, 72, and 108 mg/kg/d), study 2208). This was a GLP study done in the United Kingdom, but was similar to the studies I have described above. No monkeys died prior to necropsy in this study. The toxicities Dr. Bryson described in this study are similar to but less in severity than those described in the shorter term studies. This is consistent with the lower dose levels of this long term study. Dr. Bryson did not report any drug related tumors.

Toxicology Summary

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 the monkeys develop comparably high plasma concentrations of 5'-DFUR and parent compound. Roche considers the monkey to be the most predictive species for the human response to Ro 09-1978. Nevertheless, none of the data submitted establishes comparable expression of these enzymes in monkey and man. The study by Miwa

et al. (1121 above) shows that acylamidase activity in monkey liver is 6 fold less than in humans. The investigators in the sub-chronic monkey studies missed an excellent opportunity to determine these enzyme expressions and activities in control and induced monkeys at the end of the study.

No mice died in either the acute of sub-chronic studies, so the investigators could not estimate an LD_{50}. The only clinical symptom observed in the acute study was hypoactivity from 15 min after dosing. This hypoactivity persisted for about 1 hr in mice receiving 1000 mg/kg and for from 2 to 4 hr in mice receiving 2000 mg/kg. All dosed mice gained weight similar to controls and no unusual pathologies were seen at necropsy. Similarly, no clinical signs were observed in mice given Ro 09-1978 daily for 4 weeks by gavage. No mice died and body weight gain was unaffected. Similarly, an LD_{50} could not be determined in rats. The following tables summarize the most toxicologically significant changes in some of the studies of Ro 09-1978 in rat and monkey. The most notable toxicity is myelosuppression.

Rat

Dose mg/m²	Dose mg/kg	Mortality	Clinical Signs	Weight Change	Necropsy	hematology and Clinical Chemistry	Histopathology, Day 14
Acute Studies				Oral gavage, suspension in 40 mM Citrate buffer, 5% gum Arabic			
		Controls not shown, no changes					
6000	1000	0/5/sex	↓ activity < 30 min	None	No Changes	NA	NA
12000	2000	0/5/sex	↓ activity < 1 hr	None	No Changes	NA	NA
Sub-Chronic							
daily X 28				Oral gavage, suspension in 40 mM Citrate buffer, 5% gum Arabic			
		Controls not shown, no changes					
1,077	179.5	0/5/sex	None	None	None	None	None
2,154	359	0/5/sex	None	None	None	None	None
3,231	538.5	0/5/sex	None	↓ male	None	None	Slight degeneration rectal crypt cells, 3 male, 1 female
26 week				Oral gavage, suspension in 40 mM Citrate buffer, 5% gum Arabic			
		Controls not shown, no changes					
1,077	179.5	0/20/sex	None	None	None	None	None
2,154	359	0/20/sex	None	↓ male	None	↓ protein male, ⇑ MCV female, ⇑ MCH male	None
3231	538.5	0/20/sex	None	↓ male	None	↓ protein male, ↓ RBC male, ⇑ MCV female, ⇑ MCH both	Slight degeneration rectal cells, male

Monkey

Sub-Chronic			Oral suspension in 40 mM citrate buffer, 5% gum Arabic				Controls not shown, no changes	
	daily X 28							
	Dose mg/m²	Dose mg/kg	Mortality	Clinical Signs	Weight Change	Necropsy	hematology and Clinical Chemistry	Histopathology
	430.8	35.9	0/3/sex	None	None	None	None	negligible
	2154	179.5	0/3/sex	diarrhea	↓ slight	↓ thymus wt	↓ WBC,	slight damage, intestine, lymphatic and hematopoietic organs
	4308	359	2 of 3 male only	diarrhea all monkeys after day 8	↓	↓ thymus wt, ↓ spleen wt, ⇑ adrenal wt.	↓ WBC, 2 moribund male ⇑ BUN, glucose, creatinine, PO_4	severe intestine, moderate lymphatic & hematopoietic organs
			males moribund on day 20 and 27	emaciation, hypothermia, bradypnea, prone				
	26 week		Oral suspension in 40 mM citrate buffer, 5% gum Arabic				Controls not shown, no changes	
	Dose mg/m²	Dose mg/kg	Mortality	Clinical Signs	Weight Change	Necropsy	hematology and Clinical Chemistry	Histopathology
	216	18	0/3/sex	None	None	None	None	None
	648	54	0/3/sex	None	None	None	None	None
	1,728	144	1 female of 3/sex	Soft stool in 1 male	None	None	↓ WBC, RBC, Hct, Hbg	thymic atrophy in 3, lymphoid depletion in spleen in 1.
			female moribund on day 57	emaciation, hypothermia, bradypnea, prone, d 57	↓ from week 5	enlarged adrenals, involuted thymus, low thymus wt	↓ WBC, RBC, Hct, Hbg, ⇑ BUN, ↓ creatinine, glu, Na, Cl	degeneration intestine, spleen, thymus, bone marrow, esophagus

Reproductive Toxicology

2301) S. Takizawa and I. Horii. 1995. Reproduction segment I study of Ro 09-1978 in mice, Report J-146'381. Volume 27 page 1.

Animal	24 male and 24 female BDF-1 mice per dose group
Drug	Ro 09-1978, Lot 20867-147
Doses	0, 570, 1140, and 2280 mg/m2/day (given as 0, 190, 380, and 760 mg/kg/d)
Route	PO by "intubation"
Schedule	daily, males for 28 days before mating and through mating females for 14 days before mating and through gestation day 6.
Vehicle	not specified
Observations:	
Clinical signs	daily
Body weight	twice weekly, pregnant females on gestation day 0-6, 9, 12, 15, 18
Food cons.	twice weekly
Necropsy	gestation day 18 for females
Histopathology	Uteri and ovaries of non-pregnant females male reproductive organs

Fourteen females dosed with 760 mg/kg/d did not mate successfully. Drug administration was discontinued in these animals and they were allowed to mate with treated males.

The investigators did this study at the
Mr. Takizawa signed a GLP statement.

Results:

Parents

Mortality	10 animals were killed by gavage error during the experiment
Clinical signs	Several HD males and females suffered emaciation or decreased activity
Body Wt	Dose related diminished food consumption and body weight gain in females and males < 5% during mating but to 30% in females by gestation day 18, some of the decrease is probably due to decreased number of fetuses
Fertility	The following table shows female fertility decreased with dose. This decrease was statistically significant and severe.

Dose Group	Control	Low	Medium	High
% fertile males	83		92	
% fertile females	83		71	

Many high dose females had disordered estrus. Most appeared to be in continuous diestrus.

Organ Wt Decrease in testes and epididymides in HD males

Histopathology

Infertile HD males had decreases in spermatocytes and spermatids, degeneration of spermatocytes and spermatids in the testes, and giant cell formation in the seminiferous tubules. They also had degenerated spermatogenic cells and decreased numbers of sperm in the epididymides. Similar but less severe changes occurred in fertile HD males.

Some infertile MD and HD females had changes that indicated failed pregnancy, corpus luteum graviditatis (3/6 and 5/7), decidual changes of the uterine endometrium (1/6 and 3/7) and stratified columnar epithelium of the vagina (1/6 MD). One MD female had uterine atrophy.

Fetuses

The following table demonstrates the embryolethality of capecitabine.

Dose Group	Control	Low	Medium	High
n	17		15	
total corpora lutea	184		164	
no corpora lutea per liter (mean)	10.8		10.9	
total implantation	166		147	
% implantation relative to corpora lutea (mean)	89.6		98.2	
total live fetuses	155		26	
% live fetuses relative to implantation	91.5		19	
total early death	9		121	
% early death relative to implantation	7		81	
Body Weight grams				
male mean	1.2	1.0	1.0	
female mean	1.2	1.0	0.8	

Then investigators stopped dosing the fourteen females mice in the HD group that did not achieve pregnancy. They re-mated these mice, seven with HD treated males and seven with control males. Fertility in these treated groups was 83% (5/6, one death due to gavage error) and 100% (7/7). The one female that failed to mate after recovery had uterine atrophy. The investigators documented no other toxicologically significant changes in the recovered dams or their offspring. This implies that the effects of capecitabine on fertility are reversible in mice. Nevertheless, capecitabine is clearly embryolethal when given during mating and implantation. It was more toxic to male fetuses than to female; the number of dead male fetuses in the MD group was higher than the number of female (9 live males compared to 17 live females).

2302) M. Hayashi *et al.* 1994, Reproduction segment II study of Ro 09-1978/000 in mice. Report J-146'446. Volume 27, page 56.

Animal	~20 female BDF-1 mice per dose group
Drug	Ro 09-1978, Lot 20867-147
Doses	0, 594, 1185, and 2373 mg/m^2/day (given as 0, 198,395, 791 mg/kg/d)
Route	PO by gavage
Schedule	daily, days 6 to 15 of gestation.
Vehicle	40 mM citrate buffer, pH 6, plus 5% gum Arabic solution

Observations:

Clinical signs	daily
Body weight	gestation day 0-5, 17
Food cons.	gestation day 0-5, 17
Necropsy	gestation day 17

Half the fetuses from each group were fixed for skeletal examination, the remaining fetuses were fixed for visceral examination.

The investigators did this study at the
M. Hayashi signed a GLP statement.

Results:

Mortality	one HD female died by gavage error on gestation d6
Clinical signs	no toxicologically significant changes
Body Wt.	Dose dependent decrease in body weight gain, ~100% in MD and HD. Parallel decrease in food consumption.

Litter Data

Dose Group	Control	LD	MD	HD
number of Dams	24		20	
Corpora lutea				
total	242		12	
mean number per litter	10.1		12	
Implantation				
Total	210		209	
mean	8.8		10.5	
mean % relative to corpora lutea	86		100	
Live Fetuses				
male	92		3	
female	105		2	
mean number per litter	8.2		0.3	
mean % of implantations	93		2	
Early Death				
Total	11		203	
mean % to implantations	6		98	
% litters with early death	41.7		100	
Late death				
Total	2		1	
mean % to implantations	1		0.4	
% litters with early death	8		5	
Body weight of live fetuses				
mean male	0.9	0.8	0.8	
mean female	0.9	0.8	0.8	

Fetal abnormalities

External anomalies in the low dose group included cleft palate, anophthalmia, microphthalmia, oligodactyly, polydactyly, syndactyly and kinky tail. One of five live fetuses in the MD group had oligodactyly. The total incidence of abnormalities increased with dose; 2/197, 11/108, 1/5 for the control, LD and MD groups. No fetuses survived in the HD group. The authors did not report the total number of litters in each group that had abnormalities.

Visceral abnormalities included the ones mentioned above plus esophagectopy and dilation of the renal pelvis. Again in the fetuses examined, the number of abnormalities increased with dose; 1/89, 17/43 and 0/2 for the control, LD and MD groups.

Skeletal abnormalities included cleft palate, fusion of cervical vertebra, abnormal shape of cervical vertebra, fusion of thoracic vertebra, wavy rib and fusion of metacarpus. Though none of these changes reached statistical significance, the total incidence of abnormalities increased with dose; 3/108, 10/65 and 1/3 for control, LD and MD groups. 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. This difference was statistically significant.

2303) S. Takizawa, *et al.* (1995) Supplementary embryo-fetal development study of Ro 09-1978/000 in mice. Report J-146'449. Volume 27, page 94.

2304) S. Takizawa, *et al.* (1996) Ro 09-1978/000 Supplementary Segment II: Oral study for effects on embryo-fetal development in mice. Report J-146'575. Volume 27, page 131.

The sponsor studied the Segment II toxicity of capecitabine in mice in two other studies at lower doses; 0, 25, 50, and 100 mg/kg/d in study J-146'449, and 0, 50, 100, and 200 mg/kg/d in study J'-146'575. These studies were essentially identical to this one, so I review them here only in summary. Again these doses caused no deaths among the dams. At the higher two doses, 100 and 200 mg/kg, capecitabine again caused a slight decrease in maternal weight gain (50% in the 200 mg/kg/d group). The 200 mg/kg dose prolonged pregnancy about a day (~5%) in the 200 mg/kg/d group. Again the higher doses decreased viability and body weight of the pups. These doses caused the same retardation of ossification and increase in incidence of a 14th rib described above. The other anomalies described above were seen. The authors estimate that 50 mg/kg/d (150 mg/m^2/d) is a no effect dose for the pups. This dose is nearly 20 fold lower than the recommended daily clinical dose.

2305) S. Takizawa and I. Horii. A preliminary study for effects on embryo-fetal development of Ro 09-1978 via oral administration in Cynomolgus monkeys. Report J-146'607. Vol. 28, page 1.

Animal	Cynomolgus monkey (Macaca fascicularis), two females per dose group. The monkeys were housed with males from the same breeder for three days on d12,d13 and d14 of their menstrual cycle. Pregnancy was determined by ultrasound with ketamine anesthesia 18 days after mating.
Drug	Ro 09-1978, Lot 24289-175, 99.5% purity.
Dose	1080 and 2160 mg/m^2/d (given as 90 and 180 mg/kg/d or 0.25 and 0.5 mmol/kg/day)
Route	PO, gastric tube, 2 ml/kg
Schedule	daily on days 20 to 50 of gestation (organogenesis)
Vehicle	40 mM citrate buffer, pH 6, plus 5% gum Arabic solution.
Observations	
Clinical signs	Twice daily
Pregnancy	fetal heart beat and growth by ultrasound under ketamine on days 25,30, 40, 50, 60, 70, 80, and 90 of gestation.
Body weight	days 0, 20, 27, 34, 41, 51, 60, 70, 80, 90, and 100 of gestation.
Food consumption	days 18, 20, 23, 27, 30, 34, 37, 41, 44, 48, 51, 58, 61, 70, 80, 90, 99
Plasma Drug	before dosing, and 1, 3, 7, and 24 hr after dosing on days 20 and 50 see study number J 146'607.
Cesarean Section	at fetal death or on days 100 to 102 of gestation.
Fetuses	viability, sex, body weight, external observations, morphological parameters, visceral observations, skeletal observations.

Takizawa and Horii did this study at Dr. S. Oneda signed a statement affirming that the study was done in accordance with ICH guidelines.

Adult females	
Mortality	None
Abortion	two in HD group, embryonic death on day 40 and spontaneous abortion between day 40 and 50 one in LD group day 50, embryonic death.
Body weight	no abnormalities.
Food consumption	decrease in dams that suffered embryonic death.
Fetuses	
Survival	Only one LD fetus survived to term
Body weight	No abnormalities
Visceral Obs.	No abnormalities
Skeletal Obs.	No abnormalities

This study established that 90 and 180 mg/kg/day were embryo-lethal. The investigators used these results to set the doses for the following study.

2306) S. Takizawa and I. Horii. A study for effects on embryo-fetal development of Ro 09-1978 via oral administration in Cynomolgus monkeys. Report J-146'626. Vol. 28, page 35.

Animal	Cynomolgus monkey (Macaca fascicularis), five females per dose group. The monkeys were housed with males from the same breeder for three days on d12,d13 and d14 of their menstrual cycle. Pregnancy was determined by ultrasound with ketamine anesthesia 18 days after mating.
Drug	Ro 09-1978, Lot 24289-175, 99.5% purity.
Dose	vehicle control, 270, 540 and 1080 mg/m^2/d (given as 22.5, 45 and 90 mg/kg/d or 0.0625, 0.125 and 0.25 mmol/kg/day)
Route	PO, gastric tube, 2 ml/kg
Schedule	daily on days 20 to 50 of gestation (organogenesis)
Vehicle	40 mM citrate buffer, pH 6, plus 5% gum Arabic solution.
Observations	
Clinical signs	Twice daily
Pregnancy	fetal heart beat and growth by ultrasound under ketamine on days 25,30, 40, 50, 60, 70, 80, and 90 of gestation.
Body weight	days 0, 20, 27, 34, 41, 51, 60, 70, 80, 90, and 100 of gestation.
Food consumption	days 18, 20, 23, 27, 30, 34, 37, 41, 44, 48, 51, 58, 61, 70, 80, 90, 99
Plasma Drugbefore	dosing, and 1, 3, 7, and 24 hr after dosing on days 20 and 50 see study number J 146'638.
Cesarean Section	at fetal death or on days 100 to 102 of gestation.

Fetuses	viability, sex, body weight, external observations, morphological parameters, visceral observations, skeletal observations.

Takizawa and Horii did this study at Dr. S. Oneda signed the GLP statement.

Results:

Pregnant females

Mortality	None
Abortion	one in HD group between day 30 and 40 one in LD group day 30, embryonic cardiac arrest.
Body weight	no abnormalities.
Food consumption	no abnormalities

Fetuses

Survival	With the exception of the two abortions described above, all other fetuses survived to term (18 total including control)
Body weight	no differences
Visceral Obs.	Low ovary weights in two of two HD females (~30% of control)
Skeletal Obs.	Bilateral absence of the 12^{th} rib in 1 fetus of the MD group.

The abortion in the HD group is probably dose related as is the decrease in ovary weights. The authors state that they analyzed visceral findings in the fetuses by Chi-square. I am not sure if this includes organ weights. An ANOVA or some other test for a dose response would be appropriate for these findings. The weights of some fetal organs other than ovaries appear to trend lower with dose. These organs include brain, thymus, lung, spleen, and kidney.

The other abortion and the skeletal abnormality are possibly incidental, because there is no clear dose effect. The doses used in this study are considerably lower than the dose used clinically. The choice of such low doses is reasonable considering the number of abortions in the dose range-finding study above. This profound toxicity probably precludes the observation of teratogenicity.

2307) S. Takizawa, N Jukatsu and I Horii. Segment III: Oral study for effects on pre- and postnatal development in the mouse. Report J-146'606. Vol. 28, page 77.

Animal	male and female BDF1 mice.
Drug	Ro 09-1978, Lot 20867-147.
Dose	vehicle control, 300, 600, 1200 mg/m^2/d (given as 100, 200, and 400 mg/kg/d, 22, 20, 22 and 23 females respectively in these dose groups)
Route	PO, gavage, 10 ml/kg
Schedule	daily from day 15 of gestation to day 20 of lactation
Vehicle	40 mM citrate buffer, pH 6, plus 5% gum Arabic solution.
Observations	

Clinical signs	daily
Body weight	days 0, 7, 14 to 18 of gestation and days 0, 4, 7, 14 and 21 of lactation
Food consumption	days 0, 7, 14 to 18 of gestation and days 0, 4, 7, 14 and 21 of lactation
Fetuses	after culling to approximately four males and four females per litter. viability, sex, body weight (weekly), external observations, lactation parameters, weaning parameters, visceral and skeletal examinations, emotional and learning tests, mating

Takizawa and Horii did this study at Dr. S. Oneda signed the GLP statement.

Effects on dams

Mortality	No drug related deaths, one gavage error
Clinical Signs	No toxicologically significant differences
Body Wt.	No toxicologically significant differences
Food Con.	No toxicologically significant differences

F1

Live neonates	No drug related differences
Implantations	No toxicologically significant differences
Lactation	No toxicologically significant differences
External Abnormalities	No toxicologically significant differences
Body Weight	Slight decrease in HD females
Neurological	Some suggestion of increased rearing and walking with dose
Reproductive Function	No toxicologically significant differences
Histopathology	Some suggestion of degenerative changes in reproductive organs of infertile male and female F1 animals but no clear dose response.
Cesarean section	Statistically significant decrease in number of corpora lutea and implantations.
Necropsy	No dose related differences

Capecitabine given during late gestation and lactation caused only minor toxicity. Nevertheless, these doses are considerably less than the proposed clinical dose.

Reproductive Toxicity Summary

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. In live fetuses, body weight decreased relative to control. This difference did not reach significance, but this is probably because no fetuses survived in the high dose group. The impairment of fertility in female dams was 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. The decrease in live fetal body weight did not reach significance, again probably because no fetuses survived in the high dose group. 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. Though none of these changes reached statistical significance, the total incidence of abnormalities increased with dose. 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. This difference was statistically significant.

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, 1 of 2 fetuses and 2 of 2 fetuses at the respective doses). In a subsequent Segment II study, doses from 270 to 1080 mg/m^2/d caused little maternal toxicity. Again one high dose fetus died between day 30 and 40. One low dose fetus had no heart beat on day 30. In the 18 surviving fetuses, one mid dose animal was missing the 12th ribs bilaterally. These last two changes may have been incidental. 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 that less than the proposed clinical dose on a mg/m2 basis is fetotoxic and embryolethal.

Mutagenicity and Genotoxicity

1) Mutagenicity evaluation of Ro 09-1978 in the Ames test, Study No 70M97. S Albertini. Originally submitted to IND

Bacteria	S. typhimurium
Drug	Ro 09-1978
Concentrations	µg/plate dose selection study µg/plate main experiment
Batch	Lot. 27732-120-2, purity 94.8%
Batch	960022, purity 100.1%
Solvent	DMSO
S9 induction	phenobarbital and β-naphthoflavone

Strain	Mutant Allele	other Markers	Plasmid	Positive Control	Mutation Detected
TA1535	hisG46	rfa, ΔuvrB	-	Sodium Azide	base pair substitution
TA97	hisD6610	rfa, ΔuvrB	pKM101	ICR 191	frameshift
TA98	hisD3052	rfa, ΔuvrB	pKM101	2-nitrofluorene	frameshift
TA100	hisG46	rfa, ΔuvrB	pKM101	Sodium Azide	base pair substitution
TA102	hisG428 (pAQ1)	rfa	pKM101, pAQ1	Mitomycin C	responds to oxidizing & crosslinking compounds

In this standard Ames mutation assay, Albertini showed that R0 09-1978 did not cause an increase in forward mutations in any of the test strains with or without S9 activation. He also tested purified R0 09-1978 at the highest concentration and showed that it did not cause forward mutations with or without S9. Concentrations above 100 µg/plate were toxic. All positive controls were positive.

2402) E. Gocke. Evaluation of the mutagenic potential of the anticancer agent Ro 09-1978/000 with the Ames test. Report B-161'190, Volume 29, page 17.

Bacteria	S. typhimurium
Drug	Ro 09-1978
Concentrations	µg/plate
Batch	3061 B1799, purity 99.7%
Solvent	DMSO
S9 induction	phenobarbital and β-naphthoflavone
Animal	Albino male rats for induction.

Strain	Mutant Allele	other Markers	Plasmid	Positive Control	Mutation Detected
TA1535	hisG46	rfa, DuvrB	-	Sodium Azide	base pair substitution
TA1537		rfa, DuvrB	-	ICR 191	frameshift
TA97	hisD6610	rfa, DuvrB	pKM101	ICR 191	frameshift
TA98	hisD3052	rfa, DuvrB	pKM101	2-nitrofluorene	frameshift
TA100	hisG46	rfa, DuvrB	pKM101	Sodium Azide	base pair substitution
TA102	hisG428 (pAQ1)	rfa	pKM101, pAQ1	Mitomycin C	responds to oxidizing & crosslinking compounds
E. Coli Wp2 uvrA	auxotrophic for tryptophan	uvrA		4-nitro-quinoline-oxide	

2-aminoanthracene was used with all strains with and without S9 to demonstrate metabolic activation.

Dr. Gocke did this study at He included and signed a GLP statement.

In this standard Ames mutation assay, Gocke showed that R0 09-1978 did not cause an increase in forward mutations in any of the test strains with or without S9 activation. Concentrations above 100 µg/plate were toxic. All positive controls were positive and in the range of historical results. Ro 09-1978 did not precipitate appreciably from the DMSO solutions even at the highest concentrations. Cytotoxicity varied with test strain and with S9 activation. TA100 survived doses to 5000 µg/plate without S9, but in the presence of S9 doses above 50 µg/plate were toxic to TA97. This is a curious but unexplained difference that implies the presence of a toxic metabolite.

2403) W. Muster. 1994, Gene mutation test in cultured mammalian cells with the NeoFurtulon successor Ro 09-1978 (V79/HPRT Test) Report B-163'202. Volume 29, page 54.

Cells	V79-4 Chinese hamster lung cells, fibroblast-like morphology.
Drug	Ro 09-1978/000, Batch 30601B1799, 99.7% pure by HPLC
Positive controls	Ethylmethanesulfonate (EMS) and 9,10-Dimethyl-1,2-benz(a)anthracene (DBMA)
Selective Medium	HAM F10-10 + 10 µg/ml 6-thioguanine
S9	Fü-albino rats, induced with phenobarbital and β-naphtoflavone

Dr. Muster did this study at He signed and included a GLP statement.

Dr. Muster exposed cells to capecitabine for 16 hours in the absence of S9 activation at concentrations between 100 and 5000 µg/ml. A concentration of 4000 µg/ml reduced cell viability to between 42 and 51%. The highest concentration, 5000 µg/ml, killed all the cells. In the presence of S9 activation, cells were exposed for 5 hours over the same concentration range. 5000 µg/ml reduced viability to between 50 and 92%.

The number of mutants was determined on day 7. In the absence of S9 activation, the positive control, EMS, was strongly positive (~20 fold). The number of HPRT-mutant among cells exposed to capecitabine was similar to controls. In the presence of S9 activation, again capecitabine did not cause an increase in mutations and the positive control was strongly positive.

2404) B Miller. 1994, Chromosome analysis in human peripheral blood lymphocytes treated *in vitro* with the anti-neoplastic agent Ro 09-1978/000 in the presence and in absence of a metabolic activation system. Report B-161'115. Volume 29, page 86.

Cells	Human peripheral blood lymphocytes from three donors
Drug	Ro 09-1978/000, Batch 30601B1799, 99.7% pure by HPLC
Positive controls	Bleomycin sulfate, cyclophosphamide, colcemid.
S9	Fü-albino rats, induced with phenobarbital and β-naphtoflavone

Dr. Miller did this study at He signed and included a GLP statement.

Dr. Miller pre-incubated the cells for 48 hours and then exposed them to capecitabine or controls for 3, 24 or 48 hours. He examined the cells at 21 or 44 hours after treatment. In the absence of S9 activation, concentrations of 250 µg/ml were cytotoxic. In the presence of S9 activation, concentration as high as 5000 µg/ml did not kill the cells.

In the presence of S9, capecitabine did not cause an increase in chromosomal aberrations. Nevertheless, in the absence of S9 and after long exposure, capecitabine induced a significant number of aberrations. The following table summarizes these results.

Test substance	Dose	Average generation time	Mitotic index	Cells with structural aberrations		Polyploid cells
				excluding gaps	including gaps	
	µg/ml	h	%	%	%	%
Treatment for 24 hours without S9						
Negative control	0	12.7	11.5			
Solvent control	0	13.1	11.6	1.0	3.0	1.5
R0 09-1978	50	13.9	7.7	3.0	6.0	1.0
	250	13.0	6.1	1.0	8.0	1.0
	500	13.6	4.1	5.5*	8.5*	1.0
Positive control						0.0
Bleomycin	5		3.8	54**	68**	12**
Colcemide	0.06	22.6				
Treatment for 48 hours without S9						
Negative control	0	16.6				
Solvent control	0	17.9	6.4	0.5	1.0	2.0
R0 09-1978	250	17.8	1.8	7.0 **	16.5 **	0.0
	500	17.7	2.1	11.5 **	13.0 **	1.0
Positive control						
Colcemide	0.6	37.5				20.0 **

* Statistically different from control with 5% confidence
** Statistically different from control with 1% confidence

S9 microsomal preparations are toxic to cells after exposures longer than three hours. The short exposure time of the experiments with S9 may account for the absence of chromosomal damage in these experiments. 5-FU can cause chromosomal abnormalities. Human lymphocytes are probably capable of converting capecitabine to 5-FU during the longer exposure times.

2405) B. Miller, 1994. Micronucleus test in mouse bone marrow after oral administration of the antineoplastic Ro 09-1978/000. Report B-161'114. Volume 29, page 129.

Animal	male and female Fullinsdorm Moro Albino mice
Drug	Ro 09-1978/000, Batch 30601B1799, 99.7% pure by HPLC
Route	PO
Doses	500, 1000 and 2000 mg/kg in the 24 hour experiment 2000 mg/kg in the 48 hour experiment
Positive controls	Procarbazine HCl

Dr. Miller did this study at He signed and included a GLP statement.

Dr. Miller gave doses capecitabine to mice and killed them 24 hours after treatment to examine bone marrow. These mice showed no increase in micronuclei in polychromatic erythrocytes. The positive control was strongly positive (13 fold increase).

When mice were dosed with 2000 mg/kg and killed at 48 hours, the median % of PCE with micronuclei increased from 0.12% in controls to 0.25%. Though two fold, this increase was not statistically significant. Dr. Miller concluded that capecitabine was not clastogenic in the mouse micronucleus test.

These results are not surprising. Mice have an acylamidase in the intestine that catalyzes the conversion of capecitabine to 5'-DFCR before it reaches the liver. Thus metabolism to 5-FU and ultimately its less toxic metabolites is faster in the mouse (see Pharmacokinetics section). According to the labeling, 5-FU does cause an increase in micronuclei. The 5-FU label does not specify the route of administration, but it is probably IV.

Mutagenicity Summary

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 assay) with or without metabolic activation. At the highest dose tested, it did cause a ~5 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 ~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. 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. *In vivo*, clinical doses of capecitabine are probably clastogenic.

Toxicokinetics and Pharmacokinetics.

3101) H Onodera and I Horii. 1997. Excretion of Radioactivity in the Cynomolgus monkey following single oral administration of [^{14}C]-capecitabine. Report J-146'650, Volume 32, page 1.

Animal: Cynomolgus monkeys 4 male, 2.8-3 kg
Drug: ^{14}C-Ro 09-1978/602 (Lot 12352M95.2 No)
Dose: 648 mg/m^2 (54 mg/kg) 5 ml/kg
40 µCi/monkey
Route: PO
Schedule: single dose
Vehicle 40 mM citrate, pH 6.0, in 5% Gum Arabic solution
Assay: Solid samples were oxidized before counting.

did this study for D J. Hall signed the GLP statement.

These investigators made no attempt to identify parent compound or active metabolites in this experiment. The results show only total radioactivity. The following table shows the results of this experiment. The numbers in the table are percent of total administered dose. The results show that 70 or more percent of the administered dose is excreted in the urine. The unaccounted radioactivity is probably explained by uncollected urine in the cages. The difficulty the investigators encountered accounting for all of the dose is not unusual with monkeys and is not unacceptable.

Time interval hr	Urine	sd	Feces	sd	Cage Wash	sd	Cage debris	sd	Total	sd
0-4	46	13	NA		NS		NS		NC	
0-8	63	15	NA		NS		NS		NC	
0-12	64	15	0.08		NS		NS		64	15
0-24	69	14	0.8	0.5	2.2	1.1	0.2	0.3	72	13
0-48	71	12	1.6	0.6	2.7	1.4	0.3	0.3	75	11
0-168	73	11	2.1	0.7	7.2	4.4	0.5	0.4	82	8

NA Not applicable
NC Not calculated
NS No Sample

3102) H Tahara *et al.* , Single dose oral (16.4 mg/kg) pharmacokinetics and effect of food studies of Ro 09-1978 in the Cynomolgus monkey. Report J-146'239, Volume 32, page 48.

Animal: Cynomolgus monkeys, 5 male, 3.7 to 6.1 kg
Drug: Ro 09-1978/000 (Batch 920921 (92-38)/USNEB1 (93-51)- unlabeled)
Dose: 197 mg/m^2 (16.4 mg/kg)
Route: PO, solution
Schedule: single dose
Vehicle: 40 mM citrate, pH 6.0, in 5% Gum Arabic solution

Study Design: In Study 92-38, monkeys were fasted overnight, fed 100 g of primate chow 7 hr post dosing.
In Study 93-51, monkeys were fasted overnight and then fed 30 g of primate chow 30 min before dosing and 70 g of chow 7 hr post dosing. These are two separate studies and not a direct cross over.

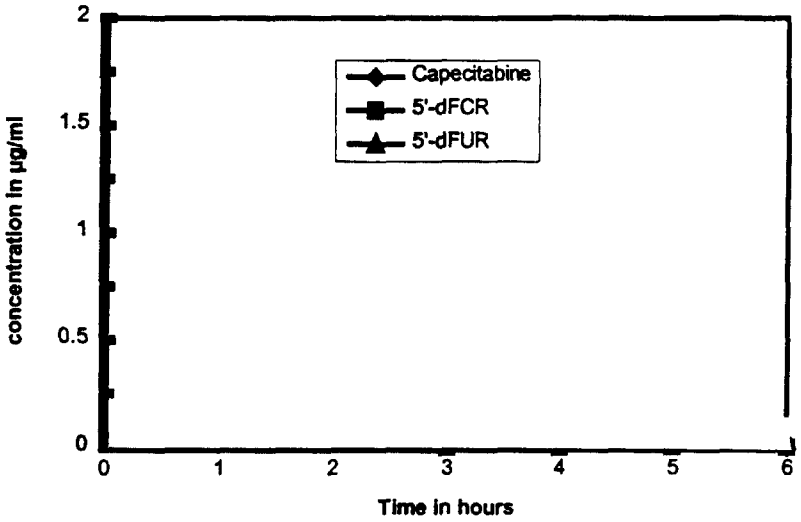
Assay: for Ro 09-1978, 5'-dFCR, and 5'-dFUR
detection limits 0.10, 0.05 and 0.05 µg/ml respectively
^{19}F-NMR for urinalysis of metabolites.

Calculation: Apparent T_{max} and C_{max}, $AUC_{0\text{-}6hr}$; The investigators could not calculate $t_{1/2}$ because of the drug concentration dropped below the detection limit at the later time points.

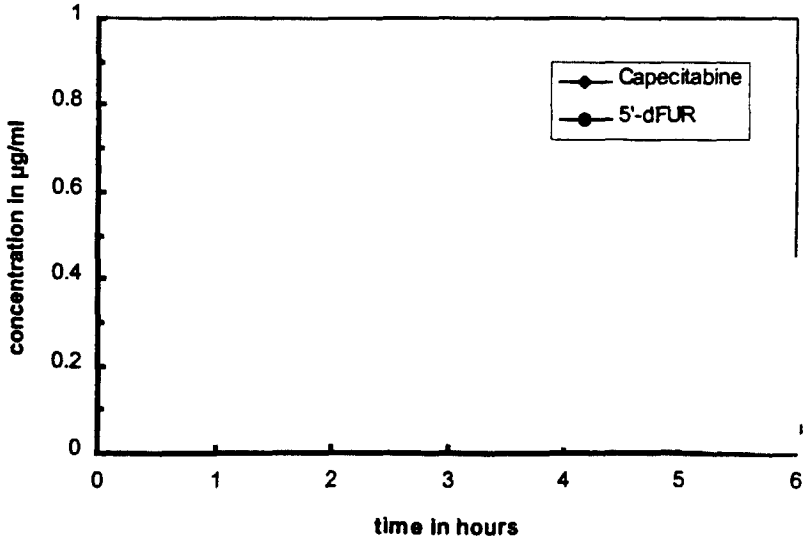
Blood Collection 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, and 6 hours after dosing.

did this study for This was not a GLP study.

Elimination of Capecitabine and Metabolites from Plasma after a single PO dose in fasted monkeys



Plasma concentration of Capecitabine and metabolites after a single PO dose in fed monkeys



Fasted monkeys appear to absorb capecitabine rapidly. They also appear to eliminate the parent compound rapidly. Capecitabine concentration approaches the detection limit within three hours after dosing. The graph suggests that both capecitabine and 5'-DFUR may recirculate through the liver and GI. The number of time points does not allow me to postulate enterohepatic recirculation with complete confidence, but the consistency of the second and even third peak (fasted monkey) in the plasma concentration curve is very strong evidence. The hydrophobicity of capecitabine would account for its recirculation. The similarity of 5'-DFUR to endogenous prymidines might account for its conservation. 5'-DFCR concentrations in the plasma remain near the detection limit. This suggests that cytidine deaminase activity is considerably faster than carboxylesterase activity in the monkey. The concentrations of 5'-DFUR are higher that those of capecitabine at all points past approximately 30 minutes. This suggests that PyNPase activity is rate limiting in the monkey.

The second graph showing the plasma concentrations in fed monkeys suggests that food delays capecitabine absorption. The C_{max} for the parent compound is delayed by at least 30 min, it is lower in magnitude by a factor of two and the peak is broader by a factor of two. The 5'-DFCR peak is not shifted though it is slightly lower in magnitude. This strongly suggests that hydrolysis by carboxylesterase is unrelated to absorption. It also supports the conclusion that much of this activity is hepatic. This is a useful and well-done study.

3106) H Onodera *et al.* (1997) Effect of Maalox on the absorption of Ro 09-1978 in rats. Report J-146'640. Volume 32, page 163.

Animal	Rat, Sprague-Dawley, 6 male
Drug	^{14}C-Ro 09-1978 Lot 9347.151 Hug (2.72 MBq/mg) label in the 6th position of the fluorouracil moiety Ro 09-19787 Batch 20867-147
Dose	900 mg/m^2 (given as 150 mg/kg).
Vehicle	40 mM citrate buffer, pH 6.0, 5% gum Arabic.
Route	PO, gavage
Schedule	Single dose
Drug	Maalox, 3 rats with 3 without.
Dose	1 ml/rat
Sample times	0.25, 0.5, 1, 2, 3, 4, 5, 6, 7 and 24 hours post dosing, IV tail vein
Analysis	

did these studies at . I did not find a GLP statement.

Results.

		Control Ro 09-1978	sd	Maalox Ro 09-1978 + Maalox	sd
Tmax	hr	0.33	0.14	0.5	0
Cmax	µg eq/ml	153	6	134	27
AUC0-4	µg eq*hr/ml	236	10	230	13
AUC0-24	µg eq*hr/ml	292	9	294	17

		Control Ro 09-1978	*sd*	*Maalox Ro 09-1978 + Maalox*	*sd*
Urine	0-4 hr	84.9	1.4	71.1	17.7
Urine	0-24 h	92.9	2	74.9	1
Feces	0-24 h	2.9	1.2	2.4	0.1
Total	0-24 h	95.9	0.8	97.4	0.8

This experiment appears well done and the sampling times are adequate. The experiment demonstrates that Maalox dose not affect the absorption of Capecitabine from the gastrointestinal tract of the rat.

3107) H Onodera *et al.* (1996) Pharmacokinetic studies of Ro 09-1978 in male and female mice: tissue distribution and excretion after a single oral administration of ^{14}C-Ro 09-1978. J-146'487. Volume 32, page 182.

Animal	male and female BDF1 mice
Drug	^{14}C-Ro 09-1978 Lot 9347.151 Hug (2.72 MBq/mg) label in the 6th position of the fluorouracil moiety Ro 09-19787 Batch 20867-147
Dose	594 mg/m^2 (given as 198 mg/kg)
Route	PO
Schedule	Single dose
Observation	Radioactivity in blood tissues and excreta concentration of Capecitabine and its metabolites in blood, small intestine, liver and kidneys.
Necropsy	0.5, 2, 6, and 24 hours, four males per group 0.5 and 24 hours, four females per group

did these studies at . I did not find a GLP statement.

The following table shows the major results of these experiments in male mice. At six hours most of the dose is in the stomach, kidneys, liver, bladder and spleen. The drug does not appear to

accumulate in tissues other than those involved in its elimination. At 0.5 hours the amount of drug in brain is significantly lower than the amount in plasma or other tissues, implying that capecitabine does not cross the blood brain barrier. At 6 hours, the percentage of the dose in the brain is about the same as the percentage in other major tissues such as heart and lung. This implies that these tissues have already cleared most of their burden of capecitabine.

Table 1 Tissue concentrations and distribution of radioactivity after a single oral administration of ^{14}C-Ro09-1978 to male mice at a dose of 198 mg/kg

	0.5 h		2 h		6 h		24 h	
	µg eq/g tissue	% of dose	µg eq/g tissue	% of dose	µg eq/g tissue	% of dose	µg eq/g tissue	% of dose
BLOOD	74.53 ± 7.66	1.86 ± 0.18	30.25 ± 0.78	0.79 ± 0.09	3.02 ± 1.22	0.08 ± 0.03	0.22 ± 0.06	0.01 ± 0.00
PLASMA	83.96 ± 8.01		31.76 ± 3.02		3.63 ± 1.74		0.26 ± 0.05	
BRAIN	2.05 ± 2.22	0.07 ± 0.02	2.72 ± 2.63	0.09 ± 0.02	1.39 ± 0.26	0.04 ± 0.01	0.27 ± 0.03	0.00 ± 0.00
THYMUS	45.01 ± 4.55	0.06 ± 0.01	21.82 ± 2.56	0.07 ± 0.01	2.30 ± 0.19	0.00 ± 0.00	0.60 ± 0.14	0.00 ± 0.00
LIVER	194.34 ± 35.34	4.50 ± 0.38	169.40 ± 12.54	3.62 ± 0.35	10.29 ± 6.92	0.47 ± 0.17	1.91 ± 0.43	0.07 ± 0.02
KIDNEY	208.51 ± 30.44	1.67 ± 0.28	127.69 ± 29.33	1.05 ± 0.30	19.70 ± 5.95	0.15 ± 0.05	1.14 ± 0.15	0.01 ± 0.00
HEART	98.06 ± 10.80	0.17 ± 0.02	21.96 ± 1.80	0.06 ± 0.01	2.66 ± 1.11	0.01 ± 0.01	0.35 ± 0.11	0.00 ± 0.00
LUNG	94.07 ± 6.42	0.13 ± 0.01	22.50 ± 2.79	0.06 ± 0.01	2.52 ± 1.02	0.01 ± 0.01	0.42 ± 0.14	0.00 ± 0.00
STOMACH	4751.82 ± 455.50	46.12 ± 5.69	7912.26 ± 483.13	14.53 ± 5.89	138.85 ± 211.94	2.13 ± 2.93	1.15 ± 0.18	0.01 ± 0.00
SMALL INTESTINE	908.33 ± 28.03	1.94 ± 0.55	27.41 ± 5.71	0.49 ± 0.13	4.47 ± 2.57	0.09 ± 0.05	0.64 ± 0.32	0.01 ± 0.00
SMALL INTESTINE CONTENTS		1.52 ± 0.58		1.81 ± 0.21		0.18 ± 0.14		0.02 ± 0.01
LARGE INTESTINE	79.27 ± 6.15	0.35 ± 0.05	85.73 ± 20.15	0.86 ± 0.17	97.18 ± 42.30	1.14 ± 0.45	1.19 ± 0.13	0.02 ± 0.00
SPLEEN	53.82 ± 7.35	0.07 ± 0.01	22.34 ± 1.73	0.03 ± 0.00	4.23 ± 3.09	0.00 ± 0.01	0.30 ± 0.19	0.00 ± 0.00
PANCREAS	64.68 ± 10.87	0.26 ± 0.12	20.40 ± 2.22	0.05 ± 0.01	4.96 ± 3.72	0.02 ± 0.02	0.37 ± 0.03	0.00 ± 0.00
PITUITARY GLAND	18.63 *		35.38 *		7.32 *		N.D.(2.87)*	
ADRENAL GLAND	19.52 *		19.38 *		2.90 *		N.D.(2.69)	
EYE BALL	21.70 ± 2.62	0.02 ± 0.00	14.55 ± 3.35	0.01 ± 0.00	3.01 ± 0.47	0.00 ± 0.00	0.38 ± 0.07	0.00 ± 0.00
CARCASS**	58.87 ± 4.15	21.67 ± 1.86	23.94 ± 3.56	8.13 ± 1.86	5.00 ± 2.38	1.96 ± 0.87	0.32 ± 0.08	0.11 ± 0.02
LYMPH NODE	35.05 ± 11.41		18.20 ± 2.37		4.78 ± 4.31		0.64 ± 0.15	
BROWN FAT PAD	20.68 ± 0.40		8.92 ± 2.37		1.57 ± 0.59		0.98 ± 0.35	
WHITE FAT PAD	3.54 ± 1.67		3.02 ± 0.37		2.89 ± 1.56		0.32 ± 0.24	
SKIN	20.35 ± 0.83		10.07 ± 1.41		1.75 ± 0.40		0.40 ± 0.13	
MUSCLE	48.91 ± 6.07		17.49 ± 1.73		3.49 ± 3.36		0.18 ± 0.05	
BONE	29.31 ± 7.17		11.63 ± 1.74		2.88 ± 0.90		0.36 ± 0.08	
BLADDER	2760.52 ± 2176.49	0.95 ± 0.69	715.02 ± 709.61	0.28 ± 0.28	276.35 ± 302.98	0.10 ± 0.10	0.63 ± 0.26	0.00 ± 0.00
TESTIS	23.72 ± 5.89	0.09 ± 0.02	28.72 ± 1.40	0.08 ± 0.01	3.17 ± 2.90	0.01 ± 0.01	0.41 ± 0.13	0.00 ± 0.00
URINE		8.95 ± 2.14		45.37 ± 4.50		85.92 ± 2.18		93.73 ± 2.66
FECES		0.01 ± 0.01		0.02 ± 0.01		1.45 ± 1.51		3.23 ± 1.73
TOTAL		92.52 ± 7.26		98.31 ± 1.22		93.80 ± 2.73		97.22 ± 1.48

* Pooled sample
** A whole animal after extraction of the organs and whole organ of tissues
N.D. Not detected the value in the parenthesis is the detection limit
Each value represents the mean ± S.D. of 4 mice

The following table shows that the results in female mice are similar to those in males.

Table 2 Tissue concentrations and distribution of radioactivity after a single oral administration of ^{14}C-Ro09-1978 to female mice at a dose of 198 mg/kg

Tissue Name	0.5 h		24 h	
	μg eq./g tissue	% of dose	μg eq./g tissue	% of dose
BLOOD	137.04 ± 13.00	4.15 ± 0.45	0.18 ± 0.04	0.00 ± 0.00
PLASMA	131.34 ± 11.09		0.22 ± 0.04	
BRAIN	9.39 ± 0.63	0.13 ± 0.01	0.19 ± 0.04	0.00 ± 0.00
THYMUS	118.04 ± 28.61	0.07 ± 0.14	0.58 ± 0.05	0.00 ± 0.00
LIVER	346.31 ± 61.78	6.65 ± 1.31	2.07 ± 0.28	0.06 ± 0.00
KIDNEY	352.72 ± 78.24	2.17 ± 0.33	1.08 ± 0.20	0.01 ± 0.00
HEART	94.07 ± 11.36	0.22 ± 0.02	0.30 ± 0.03	0.00 ± 0.00
LUNG	81.13 ± 12.81	0.24 ± 0.04	0.32 ± 0.03	0.00 ± 0.00
STOMACH	3930.75 ± 421.32	22.96 ± 4.52	0.95 ± 0.52	0.01 ± 0.00
SMALL INTESTINE	118.37 ± 37.35	2.40 ± 0.83	0.74 ± 0.13	0.01 ± 0.00
SMALL INTESTINE CONTENTS		4.45 ± 1.53		0.01 ± 0.00
LARGE INTESTINE	137.78 ± 6.50	1.50 ± 0.14	1.89 ± 0.11	0.02 ± 0.00
SPLEEN	85.35 ± 11.57	0.12 ± 0.02	0.48 ± 0.03	0.00 ± 0.00
PANCREAS	85.26 ± 30.09	0.11 ± 0.07	0.28 ± 0.08	0.00 ± 0.00
PITUITARY GLAND	30.47 *		N.D.(2.87)*	
ADRENAL GLAND	38.81 *		0.36 *	
EYE BALL	31.42 ± 5.06	0.09 ± 0.01	0.28 ± 0.10	0.00 ± 0.00
CARCASS**	92.87 ± 8.85	28.77 ± 2.69	0.36 ± 0.11	0.13 ± 0.04
LYMPH NODE	63.08 ± 14.04		0.50 ± 0.10	
BROWN FAT PAD	35.04 ± 12.37		0.80 ± 0.10	
WHITE FAT PAD	7.85 ± 2.04		N.D.(0.19)	
SKIN	47.25 ± 4.75		0.39 ± 0.31	
MUSCLE	25.16 ± 3.96		0.15 ± 0.02	
BONE	33.94 ± 1.71		0.32 ± 0.05	
BLADDER	145.00 ± 104.96	0.85 ± 0.94	N.D.(0.55)	
UTERUS	42.57 ± 11.36	0.07 ± 0.03	0.44 ± 0.07	0.00 ± 0.00
OVARY	99.00 *		N.D.(0.64)*	
URINE		14.27 ± 8.78		99.83 ± 0.62
FECES		0.85 ± 0.64		2.95 ± 1.06
TOTAL		94.27 ± 5.80		103.85 ± 0.40

* Pooled sample
** A whole animal after subtraction of the organs and whole or part of dosage
N.D. Not detected (the value in the parenthesis is the detection limit)
Each value represents the mean ± S.D. of 4 mice

Table 5 Tissue concentrations of intact drug and its metabolites after a single oral administration of ^{14}C-Ro 09-1978 to male mice at a dose of 198 mg/kg

		BLOOD			SMALL INTESTINE		
		0.5 h	2 h	6 h	0.5 h	2 h	6 h
Ro 09-1978	μg/g tissue	15.00 ± 3.61	1.73 ± 0.96	N.D.	46.87 ± 35.64	2.17 ± 0.94	N.D.
	% of ^{14}C-radioactivity	20.21 ± 5.22	3.67 ± 3.90	N.D.	40.73 ± 22.54	8.27 ± 4.14	N.D.
5'-DFCR	μg/g tissue	29.92 ± 3.12	10.42 ± 0.12	0.73 ± 0.56	16.72 ± 1.96	3.79 ± 0.76	0.64 ± 0.68
	% of ^{14}C-radioactivity	38.89 ± 1.84	30.08 ± 1.60	31.42 ± 13.92	25.25 ± 7.92	20.53 ± 3.72	17.84 ± 8.80
5'-DFUR	μg/g tissue	9.84 ± 1.84	3.02 ± 0.14	0.30 ± 0.16	3.24 ± 0.68	1.01 ± 0.80	N.D.
	% of ^{14}C-radioactivity	19.23 ± 2.76	14.44 ± 0.44	13.78 ± 2.20	4.72 ± 1.14	5.23 ± 3.42	N.D.
5-FU	μg/g tissue	N.D.	N.D.	N.D.	2.15 ± 0.32	0.32 ± 0.12	N.D.
	% of ^{14}C-radioactivity	N.D.	N.D.	N.D.	5.96 ± 1.40	5.30 ± 1.20	N.D.
Total	% of ^{14}C-radioactivity	98.33 ± 3.62	70.19 ± 3.92	45.20 ± 15.76	76.66 ± 12.68	39.33 ± 7.60	17.84 ± 8.80

		LIVER			KIDNEY		
		0.5 h	2 h	6 h	0.5 h	2 h	6 h
Ro 09-1978	μg eq./g tissue	9.15 ± 1.66	4.18 ± 2.12	N.D.	16.35 ± 4.40	2.00 ± 1.36	N.D.
	% of ^{14}C-radioactivity	4.82 ± 1.30	2.69 ± 1.52	N.D.	7.74 ± 1.62	1.53 ± 1.02	N.D.
5'-DFCR	μg eq./g tissue	54.33 ± 5.62	9.97 ± 1.80	0.70 ± 0.70	20.37 ± 2.54	5.60 ± 1.34	0.67 ± 0.78
	% of ^{14}C-radioactivity	25.96 ± 1.78	9.21 ± 2.14	4.62 ± 3.40	14.35 ± 1.70	6.25 ± 1.84	4.22 ± 3.56
5'-DFUR	μg eq./g tissue	2.33 ± 0.14	0.63 ± 0.26	N.D.	73.18 ± 15.60	19.31 ± 4.34	2.23 ± 2.42
	% of ^{14}C-radioactivity	1.78 ± 0.26	0.59 ± 0.28	N.D.	50.50 ± 4.72	21.05 ± 4.22	14.15 ± 11.54
5-FU	μg eq./g tissue	2.74 ± 0.36	0.93 ± 0.12	0.37 ± 0.08	3.55 ± 0.40	1.58 ± 0.42	0.54 ± 0.20
	% of ^{14}C-radioactivity	3.92 ± 0.66	1.60 ± 0.14	6.06 ± 2.06	4.67 ± 0.26	3.18 ± 0.20	7.41 ± 0.58
Total	% of ^{14}C-radioactivity	56.48 ± 2.94	14.08 ± 3.68	10.61 ± 2.12	77.26 ± 6.12	32.02 ± 6.92	23.78 ± 15.34

N.D.: Not detected
Each value represents the mean ± S.D. of 4 mice.

The values in the blood and the liver suggest that the cytidine deaminase step may be rate limiting in the mouse. 5'-DFCR appears to accumulate slightly. Nevertheless, this apparent accumulation results because the kidney eliminates 5'-DFUR rapidly. The concentrations of this metabolite in these organs are relatively high at 0.5 hours and 6 hours. Whatever the reason, the pharmacokinetics of capecitabine elimination appears to be quite different from that of the monkey.

The following table shows the apparent half-lives of capecitabine and its major metabolites in blood, liver, kidney and small intestine. These are only apparent half-lives because the investigators collected data so few time points. The numbers probably do not justify three significant figures. Nevertheless, the values provide a good comparison among the metabolites and the organs.

Table 7 **Apparent mean $t_{1/2}$ of Intact drug and metabolites in tissues/organs after a single oral administration of Ro 09-1978 in male mice at a dose of 198 mg/kg**

	$t_{1/2}$ in tissues (h)			
	Ro 09-1978	5'-DFCR	5'-DFUR	5-FU
Blood	0.481	1.03	1.11	-
Liver	1.33	0.992	0.795	2.06
Kidney	0.495	1.15	1.13	2.12
Small Intestine	0.338	1.23	0.892	0.892

These appear to be well done and comprehensive experiments. They provide considerable information about the elimination of capecitabine in the mouse and imply no here-to-fore unnoticed toxicity.

3108) H Onodera *et al.* (1997). Excretion of Ro 09-1978 and its fluorinated metabolites in urine after a single oral administration of Ro 09-1978 to male mice with ^{19}F-NMR technique. Report J-146'633. Vol. 32. page 232.

Animal	twelve male BDF1 mice
Drug	Ro 09-1978
Dose	30, 90, 270 and 594 mg/m^2 (given as 10, 30, 90 & 198 mg/kg)
Vehicle	40 mM citrate buffer, pH 6.0, 5% gum Arabic
Route	PO
Schedule	Single dose
Sampling	0 to 24 hours in metabolism cages
Analysis	
Necropsy	24 hours

did these studies at I did not find a GLP statement.

For capecitabine, 5'-DFCR, 5'-DFUR, and for the sum of all the fluorinated compounds, the amount excreted in the urine increased linearly with dose between 10 and 198 mg/kg. For FUPA and FBAL the amount of fluorinated compound increased with dose between 30 and 198 mg/kg. The concentrations of these metabolites was below the detection limit at the 10 mg/kg does. In all these cases the linear correlation coefficient was greater than 0.97.

The following table shows the percentage of capecitabine and it's metabolites in mouse urine (0-24 hours collection) after a single oral doses of the parent drug.

Dose	10 mg/kg	sd	30 mg/kg	sd	90 mg/kg	sd	198 mg/kg	sd
Ro 09-1978	14.3	2.1	11.4	1.4	12.8	3.6	11.8	1.2
5'-DFCR	17.7	2.6	23.4	2.3	26.6	3.8	30.9	2.2
5'-DFUR	39.9	5.9	37.5	2.5	29.1	4.8	29.8	2.7
5-FU	ND		ND		ND		0.5	0.1
5-FUH2	ND		ND		ND		ND	
FUPA	ND		12.3	1.2	11.8	0.9	9.8	1.9
FBAL	ND		14	1.6	10.3	2.2	9.7	1.7
Total	71.8	9.5	98.7	6.3	90.5	10.9	92.5	3
< 5-FU	ND		26.4	1.2	22	1.6	20	2.9

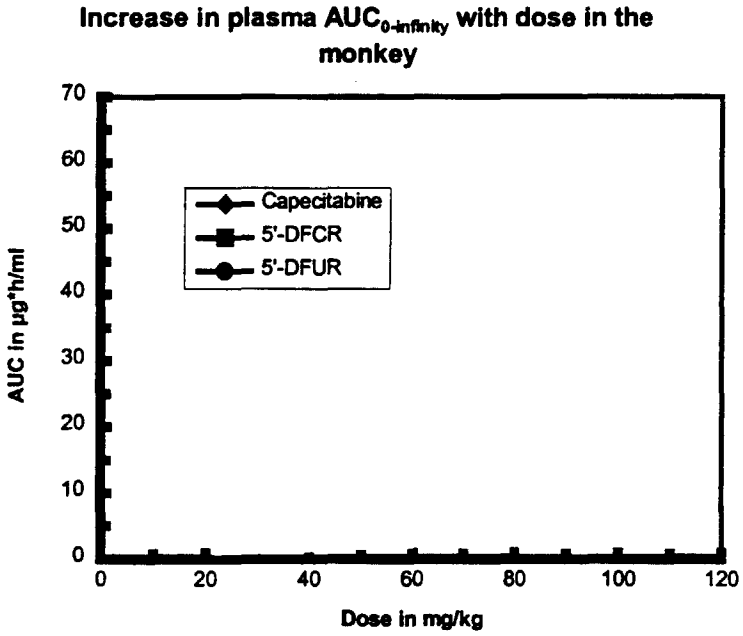
During the first twenty four hours after a single oral dose of capecitabine approximately 12% of the total excreted fluoropyrimidine in the urine is parent compound. About 60% of the total fluorpyrimidine appears as the metabolites in the cleavage pathway to 5-FU, 5'-DFCR and 5'-DFUR. 5-FU appears in the urine only at the highest dose (594 mg/m^2), but this implies that the degradation of capecitabine is saturable in the mouse. This dose is four fold lower than the proposed clinical dose on a mg/m^2 basis. At all doses above 10 mg/kg, only 20 to 25 percent of the total dose appears in the urine as 5-FU or one of its metabolites. The mouse clears much of the dose of capecitabine before it is metabolized to 5-FU.

3109) M. Utoh *et al.* (1997). Plasma level profiles of Ro 09-1978 and its metabolites following oral administration of Ro 09-1978 in monkeys. Report J-146'651. Volume 32, page 254.

Animal	Nine male Cynomolgus monkeys (Macaca fascicularis) 3/dose group
Drug	Ro 09-1978/000, Lot 24289-190
Dose	324, 648, and 1296 mg/m^2 (given as 27, 54, 108 mg/kg)
Route	PO
Schedule	Single dose
Vehicle	40 mM citrate buffer, pH 6.0, 5% gum Arabic
Sample times	0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 24 and 48 hr. 1.3 ml/sample
Analysis	

did these studies at I did not find a GLP statement.

		capecitabine			5'-DFCR			5'-DFUR		
Dose	mg/kg	27	54	108	27	54	108	27	54	108
Cmax	µg/ml	9.2	11.8	44.4	1.8	2.1	4.8	7.2	11.65	23.2
Tmax	hr	0.8	0.8	2	0.8	1.2	2	1	2	2
t1/2	hr	0.31	0.35	0.37	0.38	0.34	0.41	0.36	0.35	0.51
AUC0-inf.	µg*h/ml	7.8	18.7	66.4	1.8	3.1	9.8	8.4	21.68	44.6
NCmax	µg/ml	3.4	2.2	4.1	0.66	0.4	0.44	2.67	2.16	2.15
NAUC$_{0-t}$	µg*h/ml	2.9	2.9	6.1	0.65	0.57	0.73	3.08	3.8	4.72
Cl/F	L/h	4.2	3.1	1.7						



This is one of the better pharmacokinetic studies in the monkey. Nevertheless, many of the concentrations were not quantifiable beyond six hours. There was also considerable variability among the animals. This is not unusual with monkeys. This means that all the parameters are estimates at best. In the graph above, the AUC values for the two metabolites increase with dose. I did not calculate the correlation coefficients since there are so few points available. Still the highest dose is half the proposed clinical dose on a mg/m^2 basis. This limits the usefulness of extrapolation to humans. The graph and the normalized AUC value suggests that the parent compound may be accumulating at the highest dose.

The most interesting result revealed by the graph is that the concentration of 5'-DFCR remains very low compared to the other two drugs. This implies that the hydrolysis of this compound by cytidine deaminase is rapid compared to the other two enzymatic steps. The sharp increase in Capecitabine AUC at the highest dose suggests that carboxylesterase activity may be rate limiting in the monkey. The half-lives of the metabolites are about equal to that of the parent at all dose levels. This also suggests that the carboxylesterase step may be dose limiting.

Curiously, this report includes the toxicokinetic data from a 52 week toxicology study. I believe this study is report number W-142581 above, but I found no specific reference. I will not review this information except to say that the data does not suggest that the clearance of capecitabine changes significantly with long term repeat dosing. This is consistent with the other studies in the monkey.

3112) T. H. Kawashima *et al.* 1994. Four- week oral toxicokinetic studies of Ro 09-1978 in monkey. Report J-146'238, Volume 33, page 1.

The data in this study is combined from three studies of the oral toxicity of capecitabine. The studies are identified as:

Protocol 93-24 (male, 0.1 mmol/kg/d)
Protocol 92-74 (male, 0.5 mmol/kg/d) and
Protocol 93-17 (female, 01 and 0.5 mmole/kg/d)

From this information I have not been able to positively identify the toxicity studies from which this information came. There are two four-week toxicity studies in the submission, study 2205 and 2209. The animal numbers and the results for capecitabine toxicity in these two studies are identical. Kawashima is an author of all these studies. The appearance of a set of data in several studies is not unusual in this submission. This practice would not cause difficulty if the authors of later studies made appropriate reference to the original studies.

Animal:	Cynomolgus monkeys 3/sex/dose group, 3-4 kg
Drug:	Ro 09-1978/000
	batch USNEB for 92-74
	batch KM016 for 93-17 and 93-24
Dose:	430 mg/m^2/d (0.1 mmol/kg/d, 35.9 mg/kg/d)
	2154 mg/m^2/d (0.5 mmol/kg/d, 179.5 mg/kg/d)
	No control
Route:	PO
Schedule:	daily
Duration:	28 days
Vehicle	40 mM citrate, pH 6.0, in 5% Gum Arabic solution

Kawashima *et al.* did these studies at the

Dr. Bruce Micro signed the GLP statement.

Analytical Procedure:

Calculations:

The investigators did not collect sufficient data to allow calculation of elimination rate constants. They report only T_{max} and C_{max} and $AUC_{0\text{-}24hr}$. They calculated AUC by the linear trapezoidal method. I must commend this report as one of the few I have seen to pay attention to significant figures.

Results:

Analyte	Day	Dose mg/kg/d	Dose $mg/m^2/d$	C_{max} µg/ml	$AUCt_1\text{-}t_2$ µg*hr/ml	T_{max} hr	AF	GF
Ro 09-1978	1	35.9	430	4.6	6.8	1.2		0.44
	28	35.9	430	9.8	8.1	0.7	1.2	1.16
	1	179.5	2154	36	58.9	1.1		0.8
	28	179.5	2154	44	68	1.2	1.2	0.98
5'-dFCR	1	35.9	430	0.45	0.97	2		0.15
	28	35.9	430	0.58	0.73	0.8	0.75	0.8
	1	179.5	2154	0.53	1.7	1.5		0.3
	28	179.5	2154	1.39	3.7	1.3	2.2	0.6
5'dFUR	1	35.9	430	3.9	6.2	1.3		0.55
	28	35.9	430	4.5	5.3	0.9	0.85	0.58
	1	179.5	2154	18.7	47.6	1.6		0.53
	28	179.5	2154	24.2	57.6	1.5	1.2	0.67

AF = Accumulation Factor = AUC_{d28}/AUC_{d1}

GF = Gender Factor = AUC_{female}/AUC_{male}

In all cases $T_1\text{-}T_2$ is 0 to 24 hours. Considering that many of the values post 6 hr were below the detection limit the AUC values must be considered estimates.

Considering the inadequate sampling in this study and the large deviation in the data points, I will not attempt to make a quantitative interpretation of this data. C_{max} appears to increase consistently from day 1 to day 28 for Ro 09-1978 and its metabolites. Increases in AUC are not consistent. Considering this and the inadequacies of the AUC calculations differences in the accumulation factor are probably not significant. This implies the rate of absorption increases with repeat dosing but that elimination is unaffected. Decreases in T_{max} are consistent with this implication. The AUC of the metabolites in females is consistantly less than the AUC in males. Females may eliminate the metabolites more rapidly than males. I do not understand the numbers the authors have reported for T_{max}. They say that the numbers were taken from the graphs of the data, so I do not see how the values can be other than 0.5, 1 or 2 hours. Nevertheless, these are the values reported in the summary table.

3113) Y. Tsukamoto *et al.* 1997. Thirteen-week oral toxicity study of R0 09-1978/000 in monkeys (including recovery and toxicokinetic study). Report J-146-637. Submission 137 IT.

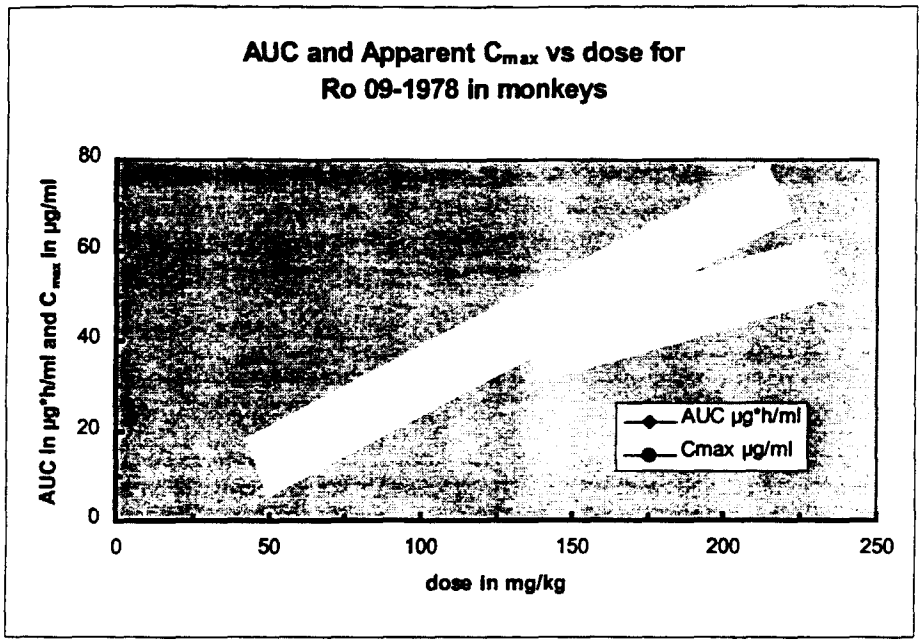
Animals	four male and four female Cynomolgus monkeys (Macaca fascicularis)
Drug	Ro 09-1978
Batch	Lot No. LS-1
Doses	54, 108, 162 or 215 mg/kg/d 215 mg/kg from days 0 to 31, high dose reduced to 162 mg/kg/d days 35 to 90 no control group
Schedule	daily for 13 weeks
Route	PO
Vehicle	40 mM citrate buffer (pH 6) in 5% gum Arabic solution
Analysis	
Sample times	0.5, 1, 2, 4 and 6 hours on days 0, 35, and 84 at doses 54 and 108 mg/kg 0.5, 1, 2, 4 and 6 hours after dosing on days 0 and 31 at 215 mg/kg. 0.5, 1, 2, 4 and 6 hours after dosing on days 35 and 90 at 162 mg/kg

The summary page refers to the study as GLP, but I found no signed statement. I am confident that this toxicokinetic data comes from Toxicology Report J-146'413, but I found no direct reference to this study.

On day 31 (32nd dose) the investigators suspended dosing for two days. They resumed dosing at the lower dose of 162 mg/kg on day 35. They do not give the reason for this dose reduction. Toxicology Report J-146'413 describes the same change in dose schedule and says that it was due to unacceptable toxicity. From the data tables it seems that none of the monkeys died. This is not consistent with the 13wk toxicity study, but the disparity may arise because the investigators used only a subset of the monkeys from that study for this study. The investigators describe no toxicity or clinical symptoms.

The investigators did not calculate half-lives or any kinetic parameters other than apparent C_{max} (which they consistently refer to as C_{max}) and crude $AUC_{0\text{-}6hr}$ because they did not collect blood at enough time points. Neither did they report the volume of the blood samples. They determined the AUC by the trapezoidal method.

The following table shows that AUC and apparent C_{max} for the parent compound, Ro 09-1978, increase with dose. In this graph, I have combined the data from the day 0 and day 35 dosing to show all four doses. The results for the metabolites are similar.



AUC and apparent C_{max} do not change significantly with repeat daily dosing over thirteen weeks. The values are means of the combined data from both males and females. The confidence intervals are somewhat higher than they should be in these tables because the values of C_{max} and AUC for females were consistently 70 to 80% those of males. The confidence intervals reflect this difference. Thus there is a significant difference in the elimination of Ro 09-1978 and its metabolites by male and female monkeys. Females appear to eliminate these compounds more quickly. The investigators did not report the results in molar units making direct comparison of the relative amounts of the metabolites more difficult. Nevertheless, the concentrations of 5'-DFCR are significantly lower than the concentrations of the parent compound or 5'-DFUR. Again this implies that the cytidine deaminase step in the enzymatic cleavage pathway is not rate limiting *in vivo*. The following tables show the major results of this experiment.

C_{max} Ro 09-1978

Dosage	Day 0		Day 31		Day 35		Day 84	
mg/kg	µg/ml	%CV	µg/ml	%CV	µg/ml	%CV	µg/ml	%CV
54	13.8	46			16	57	13.6	51
108	34.8	74			29.9	57	29.9	55
162					39	81	44.6	70
215	51.6	56	47.8	36				

AUC Ro 09-1978

Dosage	Day 0		Day 31		Day 35		Day 84	
mg/kg	µg*h/ml	%CV	µg*h/ml	%CV	µg*h/ml	%CV	µg*h/ml	%CV
54	13	58			13.8	42	11.6	54
108	36.5	71			32.3	48	32.4	60
162					53.4	84	55.1	48
215	71.4	50	60.1	11				

C_{max} 5'-DFCR

Dosage	Day 0		Day 31		Day 35		Day 84	
mg/kg	µg/ml	%CV	µg/ml	%CV	µg/ml	%CV	µg/ml	%CV
54	3.44	37			3.25	39	3.13	32
108	5.74	39			5.29	30	5.38	53
162					8.43	80	7.18	41
215	8.56	47	8.74	32				

AUC 5'-DFCR

Dosage	Day 0		Day 31		Day 35		Day 84	
mg/kg	µg*h/ml	%CV	µg*h/ml	%CV	µg*h/ml	%CV	µg*h/ml	%CV
54	3.33	32			3.27	24	2.42	38
108	7.04	28			6.29	29	4.95	49
162					14.4	85	8.51	38
215	12.7	33	13.3	31				

C_{max} 5'-DFUR

Dosage	Day 0		Day 31		Day 35		Day 84	
mg/kg	µg/ml	%CV	µg/ml	%CV	µg/ml	%CV	µg/ml	%CV
54	11.5	36			11.6	27	12.8	35
108	17.7	36			23	11	21.4	31
162					26.4	63	34.4	33
215	29.1	33	35.6	16				

AUC 5'-DFUR								
Dosage	Day 0		Day 31		Day 35		Day 84	
mg/kg	µg*h/ml	%CV	µg*h/ml	%CV	µg*h/ml	%CV	µg*h/ml	%CV
54	14.6	19			15.1	17	16.1	23
108	29.4	27			37.3	42	33.6	25
162					61.3	62	66	24
215	65.9	27	75.9	23				

This experiment is fraught with problems. It seems poorly planned and poorly presented. Nevertheless, it does yield a very important piece of information, capecitabine metabolism does not appear to change significantly with repeated dosing.

3114) H. Tahara *et al.* 1994. Twenty six-week oral toxicokinetic study of Ro 09-1978 in monkey. Report J-146'240, Volume 33, page 202.

Animal: Cynomolgus monkeys 3/sex/dose group, 2.5 to 3.5 kg
Drug: Ro 09-1978/000, Batch KM021.
Dose: 216 mg/m^2/d (0.05 mmol/kg/d, 18 mg/kg/d)
648 mg/m^2/d (0.15, mmol/kg/d, 54 mg/kg/d)
1728 mg/m^2/d (0.40 mmol/kg/d, 144 mg/kg/d)
No control
Route: PO
Schedule: daily
Duration: 26 weeks
Vehicle: 40 mM citrate, pH 6.0, in 5% Gum Arabic solution

Tahara *et al.* did these studies at . Dr. Bruce Micro signed the GLP statement.

Analytical Procedure:

Calculations:

The investigators did not collect sufficient data to allow calculation of elimination rate constants. They report only T_{max} and C_{max} and $AUC_{0\text{-}6hr}$. They calculated AUC by the linear trapezoidal method.

Kinetic parameters of Ro 09-1978 in the monkey after repeat dosing for 26 weeks.

Dose mg/kg/d	Dose $mg/m^2/d$	Day	Cmax µg/ml	AUC_{t1-t2} µg*hr/ml	T_{max} hr	AF	GF
18	216	1	4.5	4	0.6		0.86
		10	2.4	2.6	0.7	0.65	0.77
		35	2.96	2.5	0.9	0.63	1.3
		77	2.52	2.2	0.6	0.54	1.1
		182	2.36	2.4	0.9	0.61	1.5
54		1	16	13	0.5		1.5
		10	18.1	12.6	0.5	0.97	0.95
		35	21.2	15.6	0.5	1.2	1.45
		77	18.2	14.4	0.8	1.1	0.46
		182	15.7	13.9	0.6	1.1	0.66
144		1	39	49.9	1.3		2.1
		10	36	42.4	0.6	0.85	0.68
		35	38	53.6	0.9	1.1	1.3
		77	25	29.7	1.7	0.6	2.2
		182	38	43.9	0.7	0.9	1.6

AF = Accumulation Factor = AUC_{d28}/AUC_{d1}

GF = Gender Factor = AUC_{female}/AUC_{male}

In all cases T1-T2 is 0 to 6 hours. Considering that many of the values post 6 hr were below the detection limit the AUC values must be considered estimates.

Again I cannot make quantitative interpretations of this information. The kinetic sampling is not adequate and the data is highly variable. In this study C_{max} does not increase with repeat dosing, even when observed at day 35 (closest to day 28, see above). Again AUC does not change with repeat dosing within the variability of the data. AUC increases with dose. Ro 09-1978 does not appear to accumulate with repeat dosing within the variability of the data. Likewise, I can see no consistent patterns in the Gender Factor for capecitabine.

The C_{max} of 5'-DFCR appears to reach a maximum at doses above 54 mg/kg/d (data not shown). This could mean that the carboxylesterase activity is saturable in monkeys. Consistent with this idea, the AUC for 5'-DFCR increases just less than linearly with dose. At these higher doses concentration of 5'-DFCR are 10 to 20 times lower than those of capecitabine or 5'-DFUR, again consistent with the hypothesis that the carboxylesterase step may be rate limiting at high doses. This does not conflict with the possibility that PyNPase may be rate limiting at low doses. The accumulation factor is, but for one point, consistently greater than 1 (range 0.9 to 1.7) for 5'-DFCR implying some accumulation with repeat dosing. Myelosuppression could decrease systemic cytidine deaminase activity with time. The gender factor is highly variable (range).

The C_{max} for 5'-DFUR increases less than linearly with dose (data not shown). This is consistent with acylamidase activity being rate limiting. The accumulation factor may be slightly greater than one (range '). Again the gender factor is variable (range).

3116) A study for effects on embryo-fetal development of Ro 09-1978 via oral administration in Cynomolgus monkeys - Toxicokinetics of Ro 09-1978. Research report number J-146'638, in IT submission #137 of the original IND.

Animals	five female Cynomolgus Monkeys per dose group
Drug	Ro 09-1978
Batch	Lot No not specified
Doses	22.5, 45 and 90 mg/kg/d no control
Schedule	daily on study days 20 to 50 (organogenesis)
Route	PO
Vehicle	40 mM citrate buffer (pH 6) in 5% gum Arabic solution
Analysis	
Sample collection	pre-dosing, 1, 3, 7, and 24 hours after dosing on days 20 and 50

This study is similar to the previous one. Again, I could not find a signed GLP statement. Again, the investigators did not collect samples at enough time points to allow them to calculate half-lives or other kinetic parameters. This study is probably a subset of the toxicology study, Report J-146'626, but the investigators make no reference to this study. Again, the investigators failed to report the amount of blood they took from the monkeys at the sampling times. Thus, I cannot determine if the reason for such sparse sampling is the fear of excessive blood loss. The sample extraction procedure is simple and the method is sensitive, so I do not think inordinately large samples of blood would be necessary. It is difficult to call it a toxicokinetic study, as opposed to a pharmacokinetic study, because again the investigators failed to report any toxicity results. The doses in this study are lower by half than those of the previous study. The investigators state that there were no deviations from protocol that would affect the results, but several data points are missing. They did not include the raw data in the report.

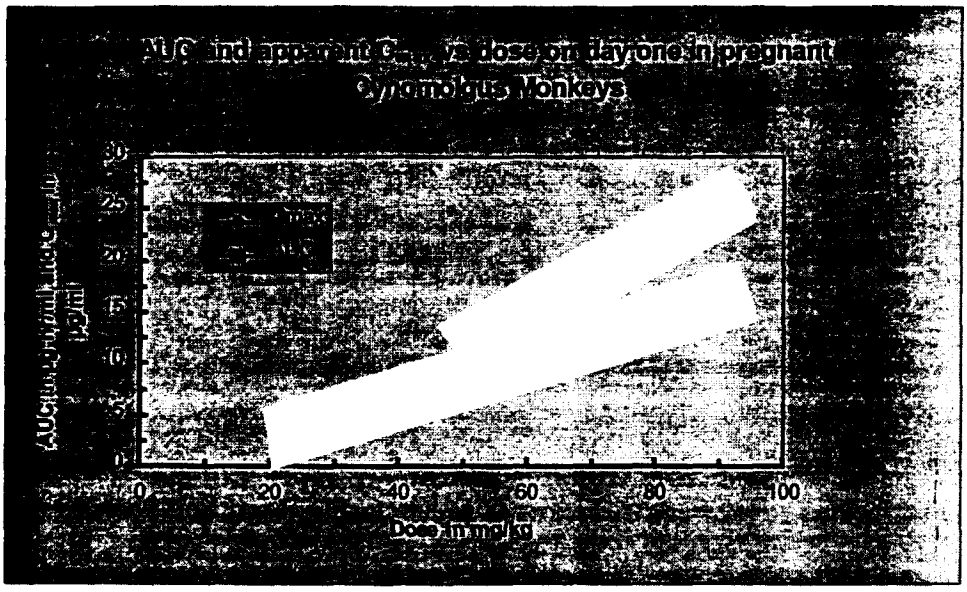
Again the investigators report apparent C_{max} and AUC values. The following tables show the major results of this study.

Ro 09-1978

	Day20					Day 50				
Dosage mg/kg	C_{max} µg/ml	%CV	AUC µg*hr/ml	%CV	AUC/dose	C_{max} µg/ml	%CV	AUC µg*hr/ml	%CV	AUC/dose
22.5	1.05	67	1.7		76	0.73	52	1.3		58
45	2.57	51	4.91	40	109	4.8	87	8.5	62	189
90	10.1	24	18.3	29	203	17.6	56	27	56	300

5'-DFCR

Dosage mg/kg	Day20 C_{max} µg/ml	%CV	AUC µg*hr/ml	%CV	AUC/dose	Day 50 C_{max} µg/ml	%CV	AUC µg*hr/ml	%CV	AUC/dose
22.5	0.76	99	1.4		62	0.58	72	0.9		40
45	1.7	36	2.8	35	62	1.9	82	3.8	54	84
90	3.1	64	5.8	66	64	5.2	43	9.3		103



3119) H Onodera *et al.* (1995). *In vitro* plasma protein binding and red cell partitioning of Ro 09-1978 and 5'-DFCR in human and animals. report J-146'402. Volume 36, page 1.

Test system: Plasma Human Japanese female.
Sprague Dawley Rat, male
BDF1 Mouse, male
Cynomolgus monkey, male

Drug: Ro 09-1978, batch LS-1
^{14}C-Ro 09-1978, Lot 9347.151 Hug
5'-DFCR, Lot 890926

Method

Onodera *et al.* did these studies at . I did

not find a GLP statement.

The following table shows the results of the determinations of the binding of Ro 09-1978 to plasma protein:

Table I Binding of Ro 09-1978 to plasma protein (%)

Species		Concentration of Ro 09-1978 (µg/ml) 0.2	2	20	200
Human	mean	55.1	54.4	53.9	53.2
	± S.E.	0.6	1.2	1.2	0.6
Monkey	mean	71.9	72.8	71.6	60.7
	± S.E.	0.4	0.6	0.5	0.8
Rat	mean	53.3	55.5	54.6	51.8
	± S.E.	0.7	0.6	0.4	1.1
Mouse	mean	50.8	53.1	49.6	49.5
	± S.E.	2.2	1.9	0.3	0.7

(n=3)

Binding to human plasma protein is constant across four orders of magnitude. Binding to Rat and mouse plasma protein is almost identical to that in humans. The binding in monkeys is greater, but considerable drug still remains unbound. The following table shows that 5'-DFCR is poorly bound to plasma. This is not surprising since the hydrophobic side chain of Capecitabine has been cleaved and the compound resembles an endogenous nucleotide. Again binding to monkey plasma is greater than to that of humans.

Table II Binding of 5'-DFCR to plasma protein (%)

Species		Concentration of 5'-DFCR (µg/ml) 0.5	5	50	500
Human	mean	9.9	10.0	8.6	9.4
	± S.E.	0.3	0.9	0.7	0.5
Monkey	mean	19.1	20.7	17.8	11.7
	± S.E.	1.5	1.2	0.8	1.1
Rat	mean	8.6	9.5	9.6	10.1
	± S.E.	1.9	0.7	0.9	0.3
Mouse	mean	19.1	20.1	21.0	15.5
	± S.E.	1.5	1.1	1.0	2.3

(n=3)

In a third experiment the investigators showed that approximately 35% of added capecitabine binds to albumin, 10 to 14% binds to α1-AGP, and another 10 to 12% binds to globulins irrespective of the concentration. Approximately 30% of any given concentration distributes into human RBCs. This is about the same percentage that partitions into monkey RBCs. About 55% of a given dose distributes into rat RBCs and 45% into mouse RBCs irrespective concentration over four orders of magnitude. Under similar conditions 36 to 42% of 5'-DFCR partitions into RBCs irrespective of starting concentration or species.

3122) H Satoh *et al.* (1997). *In vitro* drug interaction studies with Capecitabine (Ro 09-1978) and Furtulon (Ro 21-9738) using human liver microsomes. Volume 36, page 34.

Test System Human Liver Microsomes, Human Liver Bank
Drug Ro 09-1978

The investigators studied the effect of capecitabine on well characterized cytochrome P450 reactions. They reacted substrates with microsomes in the presence of O_2 and NADPH with and without capecitabine. The analyzed for product by detection. The substrates they chose are accepted as at least partially specific for human P450 isoforms. The following table shows the results of these experiments.

Cytochrome P450 isoform	*Substrate*	*Reaction*
1A2	caffeine	3-demethylase
2A6	coumarin	7-hydroxylase
2C9	tolbutamide	methylhydroxylase
2C19	s-mephenytoin	4-hydroxylase
2E1	chlorzoxazone	6-hydroxylase
2D6	+-bufuralol	1-hydroxylase
3A4	Midazolam	1-hydroxylase

Capecitabine had little effect on these microsomal reactions. It did cause some inhibition of several of the reactions, but only at very high, non-physiologic, concentration. This was probably more a result of disruption of the system than inhibition. These results suggest that capecitabine does not inhibit cytochrome P450 and that it is probably not a good P450 substrate.

Pharmacokinetics and Toxicokinetics Summary

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 when 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 is uniform. Relatively high concentrations are found in the intestines at 6 hours despite rapid absorption. Little of the compound or its metabolites is eliminated in the feces. This information and plasma concentration curves suggest that capecitabine and 5'-DFUR may recirculate within the enterohepatic system.

The following table summarizes the excretion of capecitabine in the monkey or the rat. The percentage of dose is measured as total radioactivity or in the case of study 3102 as total fluoride containing compounds (Fluoride NMR).

Species	Number & Sex	Dose mg/kg	Dose mg/m^2	Collection Period hr	Urine % dose	Feces % Dose	Tissue & Carcass % Dose	Total Recovery % Dose	Study
Monkey	4M	54	648	0-4	45.9	NS			3101
				0-8	62.7	NS			
				0-24	71.1	0.8		71.9	
				0-168	80.5	2.1		82.6	
	5M	16.4	196.8	0-24	74.6				3102
	6M	100[d,e]		0-24	76.1				3103
		100[d,f]		0-24	68.3				
		100[d,g]		0-24	66.3				
Rat	9M 3/period	150	900	0-0.5	12.9	NS	80.7	93.6	3104
				0-3	79.7	0.03	16.8	96.5	
				0-24	95	2.5	0.4	97.9	
	3M	150	900	0-7	87	1.2		88.2	3105
				0-24	91	2.8		93.8	
	3M	15 IV [h]	90	0-7	89	0.95		90	3105
				0-24	92.6	1.7		94	
	3M	150	900	0-4	84.9	NS		84.9	3106
				0-24	92.9	2.9		95.9	
	3M	150 with Maalox	900	0-4	71.1	NS		71.1	
				0-24	94.9	2.4		97.4	

NS - No Sample
d - dose in mg
e - clinical formulation - drinking solution
f - clinical formulation - unbuffered tablet
g - clinical formulation - buffered tablet
h - given IV in saline solution

The table shows that most of a dose of capecitabine is eliminated in the urine. Again, little is eliminated in the feces. The percentage of the dose in the rat carcass indicates that radioactive products of capecitabine are almost completely eliminated at 24 hours. Mice also eliminate most of a dose in the urine (not shown).

The major metabolites were: FBAL in monkey urine (ca. 24% of the dose), 5'–DFCR in rat urine (ca. 71%) and 5'–DFCR (ca.18–31%, depending on the dose level) and 5'–DFUR (ca. 40–29%) in mouse urine. The following table shows the urinary excretion of capecitabine and its fluorinated metabolites in rats, mice and monkeys.

Species	Monkey				Rat		Mouse			
Ref.	5-3102-32-67	5-3103-32-100			5-3105-32-144		5-3108-32-240			
No., Sex	5M	6M	6M	6M	3M	3M	3M	3M	3M	3M
Dose (mg/kg)	16.4	100[a,b]	100[a,c]	100[a,d]	150	151 I.V.	10	30	90	198
Drug/ Metabolites	(% Dose)	(% Dose)	(% Dose)	(% Dose)	(% Dose)[e]	(% Dose)[e]	(% Dose)	(% Dose)	(% Dose)	(% Dose)
Intact Drug	4.7	4.0	4.1	4.0	3.9	10.3	14.3	11.4	12.8	11.8
5'-DFCR	14.7	15.1	11.4	15.0	70.9	64.5	17.7	23.4	26.6	30.9
5'-DFUR	6.1	9.0	8.3	7.9	2.0	2.2	39.9	37.5	29.1	29.8
5-FU	2.8	2.5	2.3	2.3			ND[f]	ND	ND	0.5
$5\text{-}FUH_2$	0.9	0.3	0.5	0.7			ND	ND	ND	ND
FUPA	19.9	14.9	11.1	12.8			ND	12.3	11.8	9.8
FBAL	20.4	26.0	27.6	22.1			ND	14.0	10.3	9.7
Co-FBAL	4.0	4.0	3.0	3.0						
F	1.2									
Metabolite[g]					14.1	15.6				
Total	74.6	76.1	68.3	66.3	91.0	92.6	71.8	98.7	90.5	92.5

[a]Dose in mg.
[b]Clinical formulation – drinking solution.
[c]Clinical formulation – unbuffered tablet.
[d]Clinical formulation – buffered tablet.
[e]Data represent the fraction of the dose recovered as 14C-capecitabine derived radioactivity.
[f]Not detected.
[g]Unidentified 14C-capecitabine derived radioactivity.

The following table summarizes plasma protein binding in humans, monkeys, rats and mice.

Compound	Matrix	Concentration	Extent Binding				Sect-Ref.-Vol-Pg
		(µg/ml)	Human (%)	Monkey (%)	Rat (%)	Mouse (%)	
Capecitabine	Plasma	0.2	55.1	71.9	53.3	50.8	5-3119-36-6
		2	54.4	72.8	55.5	53.1	
		20	53.9	71.6	54.6	49.6	
		200	53.2	60.7	51.8	49.5	
	Albumin	0.2	37.1				5-3119-36-7
		2	35.8				
		20	35.8				
		200	34.0				
	α1-AGP	0.2	14.3				5-3119-36-7
		2	14.1				
		20	12.4				
		200	9.6				
	Globulins	0.2	10.1				5-3119-36-7
		2	10.2				
		20	12.8				
		200	12.0				
5'-DFUR	Plasma	0.2	62.6	20.4		0.7	5-3120-36-23
		2	62.4	21.9		<0	
		20	59.9	18.7		<0	
		200	35.7	11.1		<0	
	Albumin	2	46.9			11.8	5-3120-36-23
5'-DFCR	Plasma	0.5	9.9	19.1	8.6	19.1	5-3119-36-6
		5	10.0	20.7	9.5	20.1	
		50	8.6	17.8	9.6	21.0	
		500	9.4	11.7	10.1	15.5	

[a]Data represent the mean of 3 determinations.

The following table summarizes the results of the 4 week and 26 week toxicokinetic studies in the monkey.

Species, Duration Dosing	No., Sex/ Dose	Dosing Day	Daily Dose (mg/kg)	Compound	Cmax (µg/ml)	Tmax (h)	AUC T_1-T_2 (µg·h/ml)	T_1-T_2 (h)	LF*	AF**	Sect–Ref.–Vol–Pg
Monkey 4 weeks	3M, 3F	1	35.9	Intact Drug	4.6	1.2	6.77 (52)	0–24	1.0		5–3112–33–14
				5'–DFCR	0.45	2.0	0.973 (126)	0–24	1.0		
				5'–DFUR	3.9	1.3	6.18 (42)	0–24	1.0		
			179.5	Intact Drug	35.7	1.1	58.9 (55)	0–24	1.7		
				5'–DFCR	0.53	1.5	1.71 (79)	0–24	0.4		
				5'–DFUR	18.7	1.6	47.6 (70)	0–24	1.5		
		28	35.9	Intact Drug	9.8	0.7	8.11 (42)	0–24	1.0	1.2	
				5'–DFCR	0.58	0.8	0.727 (63)	0–24	1.0	0.7	
				5'–DFUR	4.5	0.9	5.27 (40)	0–24	1.0	0.9	
			179.5	Intact Drug	44.0	1.2	68.0 (31)	0–24	1.7	1.2	
				5'–DFCR	1.4	1.3	3.74 (78)	0–24	1.0	2.2	
				5'–DFUR	24.2	1.5	57.6 (31)	0–24	2.2	1.2	
Monkey 13 weeks	4M, 4F	1	54	Intact Drug	13.8	0.7	13.0 (58)	0–6	1.0		5–3113–33–74
				5'–DFCR	3.4	0.7	3.33 (32)	0–6	1.0		
				5'–DFUR	11.5	0.8	14.6 (19)	0–6	1.0		
			108	Intact Drug	34.8	0.8	36.5 (71)	0–6	1.4		
				5'–DFCR	5.7	0.8	7.04 (28)	0–6	1.1		
				5'–DFUR	17.7	1.1	29.4 (27)	0–6	1.0		
			215	Intact Drug	51.6	0.9	71.4 (50)	0–6	1.4		
				5'–DFCR	8.6	0.8	12.7 (33)	0–6	1.0		
				5'–DFUR	29.1	1.5	65.9 (27)	0–6	1.1		

[a]215 mg/kg dose group sampled Day 32
[b]Dose reduced to 593 mg/kg day 37.
[c]Additional 17–week–old mice dosed at 593 mg/kg.
*LF = Linearity Factor = AUC normalized to the lowest dose
**AF = Accumulation Factor = Ratio of AUC after repeated dosing to AUC on Day 1

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 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 cytochrome P450.

The following table compares the pharmacokinetics of capecitabine and its metabolites in monkeys and in human patients.

Species	Number (Sex)	Dose (mg/kg)	Dose (mg/m^2)	Reference
Monkey	3 (M)	54	665	3109
Man	8	37	1255	5111

Parameter	Intact Drug		5'-DFCR		5'-DFUR	
	Monkey	Man	Monkey	Man	Monkey	Man
C_{max} (µg/ml)	12.0 (2.1)	3.9 (1.0)	2.1 (0.8)	1.9 (1.0)	12.0 (1.1)	7.7 (1.0)
T_{max} (h)	0.8	0.5–3	1.2	0.5–4	2.0	0.5–3
AUC_{0-T} (µg·h/ml)	15.9 (1.9)	5.75 (1.0)	3.10 (0.5)	4.43 (1.0)	20.5 (0.9)	16.1 (1.0)
$T_{1/2}$ (h)	0.4	0.5	0.3	1.2	0.4	0.7

Parameter	5-FU		FUH_2		FBAL	
	Monkey	Man	Monkey	Man	Monkey	Man
C_{max} (µg/ml)	0.41 (0.4)	0.66 (1.0)	1.0 (1.2)	0.57 (1.0)	4.6 (0.6)	5.1 (1.0)
T_{max} (h)	2	0.5–4	2.3	1–3	2.6	2–4
AUC_{0-T} (µg·h/ml)	0.819 (0.4)	1.33 (1.0)	2.42 (1.0)	1.60 (1.0)	21.4 (0.6)	24.6 (1.0)
$T_{1/2}$ (h)	0.4	0.7	0.8	1.4	2.2 7.0	3.1[a]

[a]A slower terminal half-life of around 15 h has been found in man [5111]

When Roche submitted the original IND, they attempted to use pharmacokinetic data from the human study to estimate the starting dose. The method they chose relied on many, then dubious, assumptions but their original estimate turned out to be fairly good. Roche determined that patients given the MTD of NeoFURTULON (3000 $mg/m^2/d$) had an average 5'-DFUR AUC equal to 15.6 µg*hr/ml. They assumed this to be the target concentration for 5'-DFUR. A single dose of 306 mg/m^2 of Ro 09-1978 results in an average AUC of 5'-DFUR equal to 2.33 µg*hr/ml in humans. Simple linear extrapolation of this AUC to 15.6 µg*hr/ml estimates a dose of Ro 09-1978 of 2000 $mg/m^2/d$. Roche then allowed a safety factor of four and estimated a safe starting dose of 500 $mg/m^2/d$. The extrapolation is well out side the region where the curve is known and the AUC for capecitabine is not necessarily linear but the extrapolation predicted a dose very close to the eventual MTD.

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 a N^4-trimethoxybenzoyl

group (NeoFurtulon) 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 N^4-trimethoxybenzoyl group 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 this submission describes an impressive body of generally good scientific investigation that lead 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 increased 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} 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 a lot of activity is also found in the blood. 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 larger activities 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 determine *a priori* whither a tumor expressed excess PyNPase activity. Thus, the scientific evidence does not support the sponsors 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 the other two 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 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 the 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 toxicity studies, so the 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 suffered minor changes in hematological parameters, including increased MCV in females and MCH in males. Serum protein was decreased in males. 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 increased the severity of the symptoms 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. The high dose rendered one female monkey

moribund on day 57. Again this monkey was emaciated. The monkey does not predict the hepatotoxicity seen in humans, probably because they express less carboxylesterase activity.

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 that less than the proposed clinical dose on a mg/m^2 basis is fetotoxic and embryolethal.

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 (assay) with or without metabolic activation. At the highest dose tested, it did cause a ~5 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 ~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. 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. *In vivo*, clinical doses of capecitabine are probably clastogenic.

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. The oral bioavailability of capecitabine in solution was at least 81% in the monkey. The three different formulations (solution, buffered and unbuffered tablets) provide roughly the same bioavailability as measured by urinary excretion. A capecitabine dose is almost completely eliminated at 24 hours. Maalox did not significantly affect the absorption of an oral dose in rats. Studies in monkeys suggest that food may slow capecitabine absorption. Mice also eliminate most of a dose in the urine (not shown). The major metabolites of capecitabine were FBAL in monkey urine, 5'–DFCR in rat urine and 5'–DFCR 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 cytochrome P450.

Recommendation

On the basis of 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 pharmacology data does not support the sponsors contention that the activity capecitabine is 'tumor specific.' This claim should not be included in the product label.

Labeling changes:

The following text should replace the corresponding sections in the sponsor's proposed label.

Redacted 1

pages of trade

secret and/or

confidential

commercial

information

Questions and Comments to the sponsor:

1) The text of the report of Miwa *et al.* (study 1110) does not match the data in Figures 2, 3 and 4. Please confirm that the authors misidentified normal and tumor tissues in the legends for these figures. If the text of the legends is correct, please explain the discrepancy between the text and the legends.

2) The results of the studies of the pharmacology of capecitabine do not support your claim that the cleavage of 5'-DFUR to form 5-FU by dThdPase is 'tumor specific'. Though the differences in dThdPase activities between tumor and normal tissue reach significance in some cases the variability is very large (studies 1109 and 1110). This implies that some tumors express large amounts of the enzymes and that others do not.

The paper by Toi *et al.* (1125) shows an example of this variability of expression. These investigators determined the expression of PD-ECGF (dThdPase) in human breast cancer tissues. They found that of 100 invasive ductal carcinoma samples, only 39 were PD-ECGF positive. Expression was associated with microvessel density. This suggests that tumors with rapid neovascularization may be more susceptible to capecitabine, but not all tumors show rapid neovascularization.

The activity of an enzyme in a tissue homogenate does not necessarily predict the intracellular activity. The total activity in a tumor is the mean of all the different cell types. The activity you are measuring may actually be in vascular tissue and not in the tumor cells. This still might result in tumor destruction, but it is not tumor cell specific.

A practicing oncologist, cannot predict *a priori* which tumors will have increased enzyme expression and which will not. Thus, it is inappropriate to imply that this therapy is tumor specific.

/S/

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

Completed April 17, ~~1998~~ April 23 98
Modified April 20, 1998
Modified April 22, 1998
Modified April 23, 1998

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

4/23/98

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