

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

22-273

PHARMACOLOGY REVIEW



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-273
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DATE RECEIVED BY CENTER:	11/19/07
PRODUCT:	Oral fludarabine phosphate
INTENDED CLINICAL POPULATION:	B-cell chronic lymphocytic leukemia
SPONSOR:	Xanthus Pharmaceuticals, Inc.
DOCUMENTS REVIEWED:	Module 4 Nonclinical Study Reports
REVIEW DIVISION:	Division of Oncology Drug Products
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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability:

The non-clinical studies adequately support the use of oral fludarabine phosphate for the treatment of patients with B-cell chronic lymphocytic leukemia (CLL).

B. Recommendation for nonclinical studies: No additional non-clinical studies are required.

C. Recommendations on labeling: A separate review will be conducted.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The intravenous formulation of fludarabine phosphate (NDA 20-038) has been approved in the US since 1991 and is marketed as Fludara® (fludarabine phosphate for injection) for patients with B-cell chronic lymphocytic leukemia (CLL) who have not responded to or whose disease has progressed during treatment with at least one standard alkylating agent containing regimen.

Non-clinical studies were conducted specifically to support the oral formulation of fludarabine phosphate. These include *in vivo* efficacy studies in rodent models, absolute bioavailability studies in rats and dogs using solution and hand-filled capsule formulations, tissue distribution, metabolism and excretion studies and single dose and multiple dose oral toxicity studies in rats and dogs.

Pharmacology:

The *in vivo* antitumor efficacy of intragastric (i.g.), intraperitoneal (i.p.) and intravenous (i.v.) dosing of fludarabine phosphate was studied in *in vivo* syngeneic and CDF1 mice bearing L1210 leukemia models. Antitumor activity of fludarabine phosphate was affected by the schedule and the route of drug administration in *in vivo* tumor models. The antitumor activity increased almost three-fold when the number of treatment was increased. Single doses were less effective than daily treatment for five days or intermittent administration on days 1, 5 and 9. The antitumor efficacy of the drug was comparable following i.v. versus. p.o. administration.

Safety pharmacology:

In safety pharmacology studies, fludarabine phosphate was assessed for its potential effects on CNS, respiratory, gastrointestinal and cardiovascular system. Fludarabine phosphate at i.v doses ≥ 250 mg/kg (750 mg/m²) caused piloerection and hypomotility. Slow, irregular breathing and tremors were observed at doses ≥ 500 mg/kg (1500 mg/m²) in mice. All observations were mild and disappeared within 2 hrs post-dosing. Fludarabine phosphate i.v doses up to 500 mg/kg (1500 mg/m²) did not affect

gastrointestinal transport in mice. In anesthetized rats, fludarabine phosphate reduced respiratory and cardiovascular functions immediately after doses of 100 mg/kg [600 mg/m²(~15X human dose)] intravenously. At this dose (100 mg/kg i.v.), heart rate remained decreased (23% from pre-treatment baseline) after 60 minutes. Effects on heart rate were accompanied by an increase in carotid arterial blood flow. In ECG examinations, slight lowering T-wave was noted about 5 and 10 minutes after 100 mg/kg i.v. dose. At this dose, a slight increase (10 mm Hg) in blood pressure was also noted. Additional cardiovascular effects of fludarabine phosphate were assessed during 4-week oral toxicity study in dogs. Daily dose levels of fludarabine were 0, 4, 16 and 60 mg/kg/day (0, 80, 320 and 1200 mg/m²/day). ECG examinations were conducted during study weeks 1, 3, 4 and 8 (recovery period) pre-dose and 1 hr post-dose. Based on an assessment of the ECG, there were no fludarabine phosphate effects on heart rate or ECG parameters including QRS and QT interval in dogs.

Pharmacokinetics:

Pharmacokinetic studies of fludarabine phosphate (2F-ara-AMP) have been conducted following single and repeated i.v. and oral doses in rats and dogs. Proportional increases of C_{max} and AUC were demonstrated in both routes of administration. 2F-ara-AMP plasma levels declined with a terminal half-life of 2.0-3.5 h in rats and ~1.4-2.5 h in dogs. Similar pharmacokinetics were observed after single and repeated doses. Bioavailability ranged 65.9 to 93.5% in rats and 77.9 to 100.8% in dogs. Tissue distribution studies have shown that fludarabine phosphate distributes primarily to the kidney, liver and intestines. After oral dosing to pregnant rats, total radioactivity in the fetuses was similar to maternal blood concentrations at 1 and 4 h. Concentrations in the placenta were higher than those in the fetus at 1, 4 and 24 h.

Fludarabine phosphate (2F-ara-AMP) is rapidly hydrolyzed to 2F-ara-A, the principal metabolite, in plasma, serum and tissues. Metabolism occurs in the liver but does not appear to involve the P450 enzymes. Metabolic pathways of fludarabine phosphate are similar in animals and man. Following oral administration in man, maximum 2F-ara-A levels were reached at 1-2 h post-dose and were ~20-30% of the corresponding intravenous C_{max} levels. The mean systemic 2F-ara-A availability was in the range of 50-65% following single and repeated doses.

Toxicology:

Toxicological studies of oral fludarabine phosphate were conducted in rats and dogs. In an acute study (Study Report AB98), single intragastric doses of 100 mg/kg (600 mg/m²) of fludarabine phosphate produced no death or toxic effects except transient hypoactivity in male rats at ~1.5 h post-dose. In the second study (Study Report A22092), rats received a single dose of 1500 or 2000 mg/kg. Transient dose-related clinical signs of gastrointestinal (diarrhea, emaciation, discolored feces), and general toxicity (decreased body weight and retarded growth) were observed.

The pivotal 14-day repeat dose toxicity studies were conducted comparing i.v. doses of 20, 75 and 200 mg/kg (120, 450 and 1200 mg/m²) and p.o. doses of 20, 75, 200 and 250 mg/kg (120, 450, 1200 and 1500 mg/m²) in rats over 14 days (Study Report AA95). In a pivotal non-rodent study, toxicology was assessed in dogs which received oral doses of 1.5, 15 and 150 mg/kg (30, 300 and 3000 mg/m²) and an i.v. dose of 150

mg/kg (3000 mg/m²) over 5 days (Study Report AB94). In the rat study, fludarabine phosphate was lethal at doses ≥ 1200 mg/m² independent of route of administration. Mortality was higher after i.v. doses than p.o. doses. Dose-related clinical signs (soft feces/diarrhea, nasal discharge, signs of poor condition, weight loss), hemoconcentration, lymphopenia and leucopenia were observed by both routes at doses ≥ 450 mg/m². Drug-induced lesions included lymphoid depletion in thymus, spleen, lymph nodes, acute inflammatory changes and congestion in the gastrointestinal tract. Additionally, cortical hemorrhage, pulmonary hemorrhage and pulmonary thrombosis were observed in animals administered 1500 mg/m²/day that died preterminally.

In the dog study, toxic effects were observed only at doses ≥ 3000 mg/m²/day by both routes. Clinical signs were limited soft stool, salivation, ocular discharges and lymphopenia. Increased ALP, ALT, AST, BUN, and creatinine values were observed at doses ≥ 150 mg/kg (≥ 3000 mg/m²) which were indicative of liver and kidney damage, however, drug-induced lesions were not seen. Skin lesions were noted in the 150 mg/kg orally dosed group. Drug-induced lesions included mild to moderate inflammation of GI tracts, lymphoid depletion in thymus and mesenteric lymph node, and moderate bone marrow hypocellularity. Route specific differences in toxicological profiles were not observed in dogs treated for 14 days.

4-week oral toxicity studies were conducted (Study Report A22369 and Study Report A22370) in rats and dogs, respectively. Rats were given daily p.o. doses of 10, 25 and 75 mg/kg (60, 150 and 450 mg/m²) for 4 weeks followed by 4-week recovery period. One high dose male died on day 23. Toxic effects were noted mostly in the MD and HD groups. Clinical signs of piloerection were limited to primarily the HD group. Affected organs included the peripheral blood, gastrointestinal tract and lymphatic organs. Increased fibrinogen values were noted in the MD and HD group. Elevated liver enzymes were noted with no signs of histopathologic changes. Most drug-related macroscopic changes resolved, however, testicular changes (tubular atrophy, Sertoli cell vacuolation) noted at the terminal sacrifice were also present at the recovery sacrifice in the HD group.

In the 4-week oral toxicity study (Study Report A22370), beagle dogs received daily oral doses of 4, 16 or 60 mg/kg (80, 320 or 1200 mg/m²) for 4 weeks followed by 4-week recovery period. No mortality or drug-related clinical signs were noted. Slightly decreases in red cell parameters and leukocyte counts and increased fibrinogen values were observed in the MD and HD groups. At MD and HD, increased adrenal weight, decreased thymus weight, lymphocytic depletion, and testicular atrophy were noted. Drug-induced lesions were noted in the thymus and genital tract. Lymphoid depletion of thymus was apparent in all animals at the MD and HD groups. As in the rat study, most drug-related changes were reversible at the end of recovery period, but testicular changes in the MD and HD group at the terminal sacrifice were still present in the recovery animals.

Genetic toxicology:

Fludarabine phosphate was not mutagenic to bacteria (Ames test) or mammalian cells (HGPRT assay in CHO cells) either in the presence or absence of metabolic activation. Fludarabine phosphate was clastogenic *in vitro* to Chinese hamster ovary cells. This was evidenced by chromosomal aberrations in the presence of metabolic

activation and sister chromatid exchanges both with and without metabolic activation). In addition, fludarabine phosphate was clastogenic in an *in vivo* mouse micronucleus assay.

Developmental and reproductive toxicity:

The teratogenic potential of fludarabine phosphate was tested in rats and rabbits. Pregnant rats were given daily i.v. doses of 0, 1, 10 or 30 mg/kg/day (0, 6, 60 or 180 mg/m²/day) and i.v. doses of 0, 1, 5 or 8 mg/kg/day (0, 12, 60 or 96 mg/m²/day) were given to pregnant rabbits on day 6-15 gestation. No drug-related deaths nor toxic effects on maternal and fetal weights were observed but dose-related teratogenic effects were observed at 10 and 30 mg/kg/day in rats by an increased incidence of various skeletal malformations. In the rabbit study, dose-related teratogenic effects (external and skeletal malformations) were observed at 5 and 8 mg/kg/day. These data demonstrate that fludarabine phosphate is teratogenic to rats and rabbits.

B. Pharmacologic activity

Fludarabine phosphate, 2F-ara-AMP, is a synthetic purine nucleotide analog. Fludarabine phosphate is rapidly dephosphorylated to fludarabine (9-β-D-arabino furanosyl-2fluoro adenine, 2F-ara-A) upon reaching the systemic circulation, where 2F-ara-A is transported into cells via the adenosine transport system. Intracellularly, 2F-ara-A is converted to 2F-ara-ATP, the cytotoxic metabolite. The primary action of the drug is inhibition of DNA synthesis and replication, by inhibiting DNA polymerase alpha and ribonucleotide reductase.

C. Nonclinical safety issues relevant to clinical use

Reduction in WBC and lymphocytes were observed in a dose related manner during pivotal repeat oral toxicity studies in rats and dogs. Treatment-related microscopic changes were observed in the lymphoid system, GI tract and genital tract of rats and dogs. Following intravenous doses of fludarabine phosphate in rats, respiratory and cardiovascular functions (heart rate, blood pressure, ECG changes) were reduced. However, oral doses of fludarabine phosphate for 4 weeks did not affect heart rate or cause ECG changes in dogs. Distribution in pregnant rats suggests that oral fludarabine phosphate crosses the blood-brain barrier, placental barrier and may distribute in milk. Fludarabine is a clastogen and a teratogen.

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2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

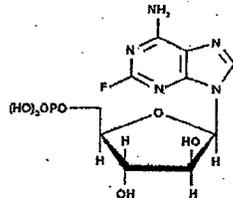
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-273
Review number: 1
Sequence number/date/type of submission: 000/19-Nov-2007/Commercial
Information to sponsor: Yes (x) No ()
Sponsor and/or agent: Xanthus Pharmaceuticals, Inc.
 300 Technology Square
 Cambridge, MA 02139

Manufacturer for drug substance: Bayer Schering Pharma AG
 D-13342 Berlin, Germany

Reviewer name: Doo Y. Lee Ham, Ph. D.
Division name: Division Oncology Drug Products
HFD: DDOP
Review completion date: August 25, 2008

Drug:
Trade name: Oral Fludarabine Phosphate
Generic name: Fludarabine phosphate; 2F-ara-AMP
Code name: NSC-312887; ZK 153851; SHL 573A
Chemical name: IUPAC: 2-Fluoro-9-(5-O-phosphono-β-D-arabinofuranosyl)-9H-purine-6-amine
 CAS: 9H-purine-6-amine, 2-Fluoro-9-(5-O-phosphono-β-arabinofurnosyl)-
 75607-67-9
CAS Registry number: 75607-67-9
Molecular formula: C₁₀H₁₃FN₅O₇P
Molecular weight: 365.2
Structure:



Relevant INDs/NDAs/DMFs:

Xanthus; oral fludarabine phosphate; IND _____ and 78,332
 Berlex; Fludara® (fludarabine phosphate) for injection; NDA 20-038
 Schering DMF Type 2 for fludarabine oral tablets

b(4)

Drug class: Nucleotide metabolic inhibitor/Antimetabolite

Intended population: Patients with B-cell chronic lymphocytic leukemia (CLL)

Clinical formulation: 10 mg Tablets

Fludarabine phosphate film-coated tablets are capsule shaped tablets that are salmon pink in color and marked one side with "LN" in a regular hexagon. Each tablet contains 10 mg fludarabine phosphate. The tablet core consists of microcrystalline cellulose, lactose monohydrate, colloidal silicon dioxide, croscarmellose sodium and magnesium stearate. The film-coat contains hypromellose, talc, titanium dioxide (E171) and ferric oxide pigment (red/E172, yellow/E172).

Route of administration: Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Introduction and Drug History:

Fludara® for injection (intravenous formulation of fludarabine phosphate) was approved in 1991 for the treatment of patients with B-cell chronic lymphocytic leukemia (CLL) who have not responded to or whose disease has progressed during treatment with at least one standard alkylating-containing regimen. The recommended adult dose and schedule is 25 mg/m² administered intravenously over a period of approximately 30 minutes daily for five consecutive days, every 28 days.

The oral formulation of fludarabine phosphate (tablet formulation) was approved in the UK in 2000 as Fludara® Oral. Currently the oral tablet is approved in 75 countries worldwide, but not in the US, where the sponsor is currently seeking approval of oral fludarabine phosphate tablets for patients with B-cell chronic lymphocytes leukemia (CLL). Clinical trials of oral fludarabine phosphate in over 700 patients as first-line and second-line therapy in more than _____ patients indicate that the oral formulation has a similar safety profile compared to IV administered fludarabine phosphate.

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This NDA was submitted pursuant to section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act, for Oral Fludarabine Phosphate.

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Studies reviewed in previous submission:

Toxicology

Study Report AA95: 14-day repeated dose toxicity study of fludara administration intravenously or orally to SD rats

Studies reviewed within this submission:

Pharmacology

Primary Pharmacodynamic Studies

Study Report A348: Summary of anti-tumor activity of fludarabine phosphate, NSC-312887

Study Report AU77: Antitumor efficacy of fludarabine phosphate (i.v.) on 5 consecutive days in SCID mice intraperitoneally inoculated with human leukemic JOK-1 cells

Study Report AV28: Antitumor effects of fludarabine phosphate (oral) in human leukemic SCID mouse

Pharmacokinetics:

Study Report AE99: Dose response linearity and absolute bioavailability study following intravenous and oral administration of Fludara in Sprague-Dawley rats: non-compartmental analysis of Berlex Lab dated 7/29/94

Study Report AF08: Fludarabine phosphate bioavailability studies beagle dogs. ————— P03-01/IRDC study 592-001 dated 8/24/88

Study Report A20177: Quantitative whole body autoradiographic study following intragastric administration of 20 mg/kg ¹⁴C-ZK 153851 to rats

Study Report AL67: Metabolism and excretion of 3H-2F-ara-A in male and female SD rats following a single intravenous and intragastric liquid dose of 20 mg/kg tritiated fludarabine phosphate/kg

Study Report A21917: Metabolic patterns of ¹⁴C-ZK 153851 in plasma and urine from female and male rats after intravenous injection and intragastric administration

Study Report A21908: Formation of ¹⁴CO₂, excretion and mass balance of ¹⁴C-ZK153851 after single intravenous and intragastric administration to male and female rats

Toxicology

Single-dose Toxicity

Study Report AB98: Acute oral toxicity study of fludarabine phosphate tablets in rats

Study Report A22092: Acute toxicity of SHL 573A (ZK 153851) in male and female rats after single i.g. (intragastric) administration

Repeat-dose Toxicity

Study Report A22369: A 4-week systemic toxicity study by repeated oral administration to SD rats with a subsequent recovery period of 4-weeks

Study Report AB94: Pilot multiple dose toxicity study of fludarabine in dogs

Study Report A22370: A 4-week systemic toxicity study by repeated oral administration to Beagle dogs with a subsequent recovery period of 4 weeks

Studies not reviewed within this submission: None

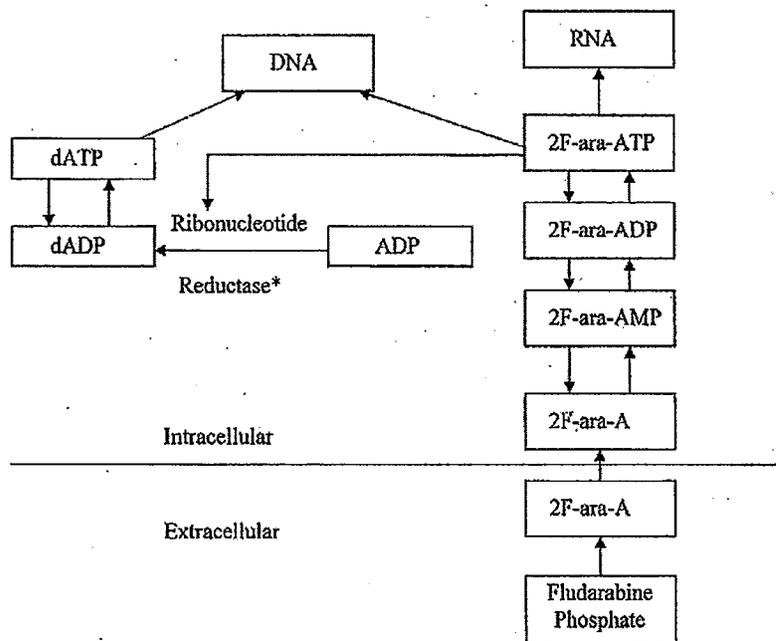
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2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Fludarabine phosphate, 2F-ara-AMP, is a synthetic purine nucleotide analog. Fludarabine phosphate is rapidly dephosphorylated to fludarabine (9-β-D-arabino furanosyl-2fluoro adenine, 2F-ara-A) upon reaching the systemic circulation, where 2F-ara-A is transported into cells via the adenosine transport system. Intracellularly, 2F-ara-A is converted to 2F-ara-ATP, the cytotoxic metabolite. The primary action of the drug is inhibition of DNA synthesis and replication, by inhibiting DNA polymerase alpha and ribonucleotide reductase.

Figure 2.6.2: Metabolism and mechanisms of actions of fludarabine phosphate



Abbreviations: A, adenosine; dA, deoxyadenosine.
 *Ribonucleotide reductase also catalyzes the reductive synthesis of dCDP from CDP.

Figure excerpted from the sponsor

The objective of the primary pharmacodynamic studies was to compare the antitumor activity of intragastric (i.g.), intraperitoneal (i.p.) and intravenous (i.v.) dosing of fludarabine phosphate using a single dose or multiple doses in different tumor models representing various spectrum of cancer indications including human leukemia, the intended indication for fludarabine phosphate.

2.6.2.2 Primary pharmacodynamics

A. Antitumor effects of fludarabine phosphate, NSC-312887, in various tumor models (Study Report A348) Module 4.2.1.1.1.

The study was performed comparing the antitumor efficacy of oral (p.o.), intraperitoneal (i.p.) and intravenous (i.v.) dosing of fludarabine phosphate in CDF1 mice bearing L1210 leukemia. Mice were inoculated with 1×10^5 cells on day 0 (i.p.). Fludarabine phosphate was administered via i.p., p.o., or i.v. 24 hrs after tumor inoculation. The effects of schedule and route of administration on the antitumor activity of fludarabine phosphate were evaluated. The antitumor efficacy was measured in increase in lifespan (ILS) of the animals.

Results:

Table 1. Effect of schedule and route of administration on antitumor activity of Fludarabine phosphate against i.p. implanted L1210 leukemia

Schedule	Route	Dose Range (mg/kg)	Optimal dose (mg/kg)	ILS%	Tumors S/T	Normal S/T
Q1D, Day 1 only	i.p.	1350-266	900	42	0/10	8/8
Q3H, Q1D, Day 1 only	i.p.	250-50	250	98	0/10	8/8
Q1D, Days 1-5	i.p.	600-118	266	122	0/10	8/8
Q4D, Days 1, 5, 9	i.p.	1000-200	670	122	0/10	7/7
Q3H, Q4D, Days 1, 5, 9	i.p.	125-25	125	525	6/10	7/8
Q1D, Day 1 only	p.o.	1600-100	1600	-	0/10	8/8
Q1D, Days 1-5	p.o.	1600-100	1600	75	0/10	6/8
		HNTD	800	50	0/10	7/8
Q1D, Day 1 only	i.v.	1350-266	600	28	0/10	7/8
Q1D, Days 1-5	i.v.	600-118	266	95	0/10	6/8
		HNTD	177	71	0/10	8/8

CDF1 mice were inoculated i.p. with 1×10^5 L1210 leukemia cells on day 0.

Table excerpted from the sponsor

The drug was active following i.p. doses on all treatment schedules and the antitumor activity increased ~3-fold when the number of drug treatments increased. A maximum ILS value of 122% was produced following i.p. dose of 266 mg/kg on days 1-5. An ILS value of 525% was produced following 125 mg/kg i.p. on Q4D when administered every 4 hours on days 1, 5 and 9 schedules. In comparison, the same dose given intravenously on days 1-5 resulted in 95% ILS whereas a dose of 1600 mg/kg, p.o. produced a 75% ILS. Less activity, as measured by increased ILS, was observed when given i.v. or p.o. compared to i.p.

Table 4: Summary of antitumor activity of fludarabine phosphate against a spectrum of murine tumors and human tumor xenografts

Type of Tumors	Treatment schedule	Activity
Murine tumors:		
i.p. B16 melanoma	i.p. Q1D, Days 1-6	-
s.c.CDF mammary tumor	i.p. Q7D, Days 1-29	+
s.c. Colon 38 tumor	i.p. Q7D, Days 2, 9	-
i.p. L1210 leukemia	i.p. Q1D, Days 1-9	++
i.v. Lewis lung	i.p. Q1D, Days 1-9	-
i.p. P388 leukemia	i.p. Q1D, Days 1-9	+
Human tumor xenografts:		
s.r.c*. CX-1 colon tumor	s.c. Q1D, Days 1-10	-
s.r.c.LX-1 lung tumor	s.c. Q1D, Days 1-10	++
s.r.c. MX-1 mammary tumor	s.c. Q1D, Days 1-10	-

* s.r.c.:sub-renal capsule

Table excerpted from the sponsor

As in Table 4, fludarabine phosphate demonstrated significant antitumor activity against the murine i.p. implanted L1210 leukemia and the human LX-1 lung tumor xenografts implanted beneath the renal capsule.

B. Antitumor efficacy of i.v. fludarabine phosphate in SCID mice with human leukemic JOK-1 cells (Study Report AU77) Module 4.2.1.1.2.

A human leukemia SCID mouse model was established by i.p. inoculation of the human B-cell CLL JOK-1 cells (2×10^7 cells) into CD-17/SCID mice. The antitumor activity (increased median survival time, MST) of fludarabine phosphate was studied following once a day injection (300 mg/kg) or twice a day injections at 6 hr intervals (135 mg/kg) into SCID JOK-1 mice on 5 consecutive days from days 1, 11, 16 or 21 after tumor inoculation.

Treatment Schedule

Substances	Schedules	Treatment time	Doses (mg/kg/injection)
Fludarabine phosphate	Q1D x 5	Days 1-5	300
		Days 11-15	
		Days 16-20	
		Days 21-25	
	Q6H x 2 x 5	Days 1-5	135
		Days 11-15	
		Days 16-20	
		Days 21-25	
Control	-	-	-

* Q1D x 5: Once a day on 5 consecutive days

**Q6H x 2 x 5: Twice a day at 6-hr interval on 5 consecutive days

In a separate experiment, mice received a single optimized dose/schedule (135 mg/kg, Q6Hx2/day for 5 days) followed by no-treatment 10 days for one and two cycles. The antitumor efficacy (MST) of fludarabine phosphate for two courses was compared with doxorubicin at 5 mg/kg injected the mice at days 1 and 16 after inoculation. Antitumor efficacy (MST) was assessed by the mean survival time (MST).

Results:

Fludarabine phosphate administered twice a day for 5 consecutive days treatment (Q6Hx2x5D) significantly prolonged the survival time of mice compared with the controls or once a day treatment (Q1Dx5D) in SCID mice (Table 1).

Table 1. Antitumor efficacy of fludarabine phosphate by treatment once or twice a day at different times after tumor inoculation

Schedule	Schedule Days	Doses mg/kg	Mortality Days	MST Days	ILS %
Control			31,32,33,33,36	33.0	-
Q1Dx5D	Days 1-6	300	31,32,32,34,49	32.5	-1.5
	Days 11-15	300	16,31,32,33,38	32.5	-1.5
	Days 16-20	300	23,33,34,35,38	34.5	4.5
	Days 21-25	300	29,33,35,35,36	35.0	6.1
Q6Hx2x5D	Days 1-5	135	33,39,40,49,63	40.5*	22.7
	Days 11-15	135	32,33,34,35,36	34.5	4.5
	Days 16-20	135	32,35,38,38,49	38.0	15.2
	Days 21-25	135	28,35,36,38,50	36.5	10.6

MST-Median survival time,

*-p<0.05 vs.control by Log-Rank test

Table excerpted from the sponsor

As shown in Table 2, the control mice died with a MST of 28.6 days. The mice treated with fludarabine phosphate showed 11.9% ILS by one course and 32.9% ILS by two courses. Both courses significantly prolonged survival time of the mice as compared with the controls (p<0.0001). The antitumor activity of fludarabine phosphate following two courses (32.9%) was the same as that of doxorubicin (33.9% ILS).

Table 2. Antitumor efficacy of fludarabine phosphate by two treatment courses

Drugs	Schedule Days	Doses mg/kg	Mortality Days	MST Days	ILS %
Control			25,25,26,27,27,28 29,29,29,30	28.6	-
Fludarabine phosphate	Q6Hx2, days 1-5 (one course)	135	29,30,31,31,32,32, 32,36,49,63	32.0*	11.9
	Q6Hx2, days 1-5,16-20 (two courses)	135	33,35,35,35,37,38, 40,41,48,57	38.0*	32.9
DOX	Q15D, days 1,16	5	30,32,35,37,38,38 39,41,41,49	38.3*	33.9

*-p<0.0001 vs.control by Log-Rank test

Table excerpted from the sponsor

C. Antitumor efficacy of oral fludarabine phosphate in SCID mouse animal model (Study Report AV28) Module 4.2.1.1.3.

A human leukemia SCID mouse model was established by i.p. inoculation of the human CLL JOK-1 cells into mice. Fludarabine phosphate was given orally at doses of 135, 270, 350 and 1100 mg/kg or intravenously at 135 mg/kg twice a day at 6 hr intervals (n=10). The treatment regimen consisted of two 5 day courses (days 1-5 and 16-20) with 10 non-treatment days. Untreated tumor-bearing SCID mice served as controls. The antitumor efficacy was assessed by MST and ILS.

Results:

Table 1. Antitumor efficacy of oral administration of fludarabine phosphate

Schedule	Routes	Doses Mg/kg	Mortality	MST	ILS
			Days	Days	%
Twice a daily at 6 hr intervals days 1-5,16-20.	p.o.	135	33,33,33,34,34,36,39,44,51,55	36.0	22.4
		270	7,7,8,33,38,38,39,40,40,42	38.3	30.3
		550	4,6,6,6,6,6,7,17,40	6.3	<0
		1100	5,5,5,5,5,6,6,6,6	5.4	<0
Control	i.v.	135	21,33,34,34,35,36,37,38,38,44	36.0	22.4
			28,28,29,29,29,29,30,30,30,33	29.4	-

Table excerpted from the sponsor

A significant increase in ILS (22.4%) was observed after i.v. administration of fludarabine phosphate (135 mg/kg). The median survival time of the control mice was 29.4 days. Fludarabine phosphate administered p.o. at doses of 135 and 270 mg/kg produced 22.4% and 30.3% ILS, respectively, which were comparable to that of the i.v. treatment (22.4% ILS). Body weight decreases were comparable (i.v. vs. p.o.) at 135 mg/kg dose level. However, the p.o. doses of 550 and 1100 mg/kg were toxic to mice with no ILS. Fludarabine phosphate resulted in reduction of body weight (more than 20%), and caused mortality in 8/10 and 10/10 mice treated with 550 and 1100 mg/kg, respectively, by day 10.

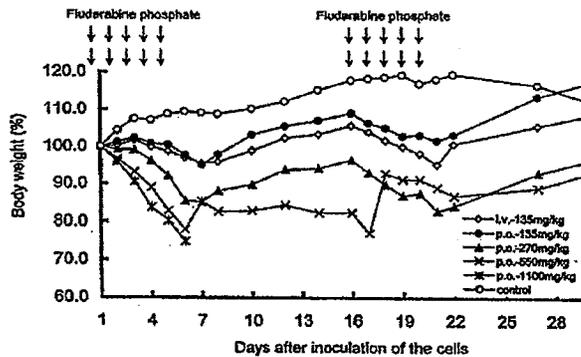


Figure 1 Changes in body weight of the mice orally treated with fludarabine phosphate

Figure excerpted from the sponsor

2.6.2.3 Secondary pharmacodynamics

No studies submitted

2.6.2.4 Safety pharmacology

No studies submitted

2.6.2.5 Pharmacodynamic drug interactions

No studies submitted

2.6.2 PHARMACOLOGY TABULATED SUMMARY

Primary Pharmacodynamics of Fludarabine Phosphate			
Species/ Strain	Study Type	Compound /Dose/ Route/Duration	Noteworthy Findings
Mouse, CDF1 Module 4.2.1.1.1	<i>In vivo</i> syngeneic and xenograft anti-tumor models	2F-ara-AMP i.v.: 118, 177, 266, 400, 600 mg/kg Q1Dx1, Q1Dx5 p.o.: 100, 200, 400, 800, 1600 mg/kg Q1Dx1, Q1Dx5 i.p.: 118, 177, 266, 400, 600 mg/kg Q1Dx1, Q1Dx5	<i>In vivo</i> antitumor activity of fludarabine phosphate against murine L1210 leukemia cells, human LX-1 lung cells and murine CD8F1 mammary carcinoma. Multiple daily i.p. treatments (25-125 mg/dose) increased the number of surviving animals and ILS greater than single large i.p., i.v. or p.o. doses. Oral daily dosing for 5 days significantly increased ILS.
CD-17/ SCID mouse Module 4.2.1.1.2	<i>In vivo</i> B-CLL xenograft anti- tumor models	2F-ara-AMP i.v.: 135 or 300 mg/kg Q1Dx5, Q6Hx2x5, 1-2 cycles	Twice daily i.v. (Q6Hx2) fludarabine phosphate for 5 days increased the MST of SCID mice inoculated with JOK-1 cells. Two courses of treatments (with a 10 day interval between courses) were more efficacious than one course (11.9% ILS vs. 32.9% ILS; p<0.0001).
CD-17/ SCID mouse Module 4.2.1.1.3	<i>In vivo</i> B-CLL xenograft anti- tumor models	2F-ara-AMP i.v. or p.o.: 135, 270, 550, 1100 mg/kg Q6Hx2x5D, 1-2 cycles	Twice daily oral administration (135, 270 mg/kg) or i.v. administration (135 mg/kg) of 2F-ara-AMP for 5 days increased the MST of SCID mice with JOK-1 cells by 22%. Body weight decreases were comparable (i.v. vs. p.o.) at 135 mg/kg. However, twice daily oral doses of 550 and 1100 mg/kg were lethal to 8/10 and 10/10 mice, respectively, by day- 10.

2.6.3 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Pharmacokinetic studies of fludarabine phosphate (2F-ara-AMP) have been conducted following single and repeated i.v. and oral (i.g.) doses in rats and dogs. Dose-linear pharmacokinetics were observed following single and repeated dosing of fludarabine phosphate administration (dose-dependent increase of C_{max} and AUC, constant CL, CLR, T_{1/2}, and V). 2F-ara-A plasma levels declined with a terminal half-life of 2.0-3.5 h in rats and ~1.4-2.5 h in dogs. Similar pharmacokinetics was observed after single and repeated doses. Bioavailability ranged 65.9 to 93.5% in rats and 77.9 to 100.8% in dogs.

Following a single ¹⁴C-fludarabine phosphate oral dose to male and pregnant albino rats, tissue distribution and excretion were studied by QWBA (Quantitative whole body autoradiography). Within 30 min after a dose of 20 mg/kg ¹⁴C-fludarabine phosphate was detected in well-perfused organs. After one hour dosing, peak concentrations were observed in kidney, liver, the GI tract, and spleen. Tissue concentrations declined and were below the detection level in most tissues 7 days post-dose. In pregnant rats, the distribution pattern of total radioactivity was similar to that observed for males. At 1 and 4 h post-dose, total radioactivity in the fetuses was similar to maternal blood concentrations. Concentrations in the placenta were higher than those in the fetus at 1, 4 and 24 hr post-dose. Concentrations in both fetus and placenta decreased with time. Concentrations in mammary gland were similar to those in blood 1 hr post-dose, and may suggest distribution of radiolabeled 2F-ara-A and other metabolites in milk.

After i.v. or oral administration of ³H-2F-ara-AMP/kg in male and female rats, similar metabolic patterns were observed. 2F-ara-AMP was rapidly dephosphorylated to 2F-ara-A, the principal metabolite, in plasma, serum and tissues. Metabolites of ³H-fludarabine were renally (70-80%) and fecally (10-12%) excreted, primarily during the first 24 hr after a single dose. No original prodrug was detected in urine and feces. The metabolites 2F-ara-A and 2F-ara-Hx (the hypoxanthine nucleoside analog) were found in serum or plasma and excreted in the urine of mice, rats, dogs, and patients dosed orally or intravenously with fludarabine phosphate. Metabolic pathways of 2F-ara-AMP are similar in laboratory animals and man.

No additional biotransformation products were identified when 2F-ara-AMP (at concentrations up to 100 uM) was incubated with human CYP isozymes 1A2 or 3A4 or with human liver microsomes.

2.6.4.2 Methods of Analysis

(see under individual study reviews)

2.6.4.3 Absorption

Study Report AE99: Dose response linearity and absolute bioavailability study following intravenous and oral administration of Fludara in Sprague-Dawley rats (Report No. AE99). Module 4.2.2.2.1

Pharmacokinetic studies were conducted with oral fludarabine phosphate (2F-ara-AMP) following single and repeated doses in rats. SD rats (n=10/sex/group) received either 20 or 75 mg 2F-ara-AMP/kg solution as single or daily repeat doses (14 days) via i.v. and i.g. administration. Plasma was sampled on days 1 and 14 up to 24 hr post administration using individual rats at 5 different time points. Urine was collected cumulatively over 24 hr on days 1 and 12. 2F-ara-A plasma and urine levels were analyzed by HPLC with fluorescence detection.

Results:

Figure 2.6.3: Single Dose Pharmacokinetics of 2F-ara-A in Rats Given Single Oral Doses of Fludarabine Phosphate

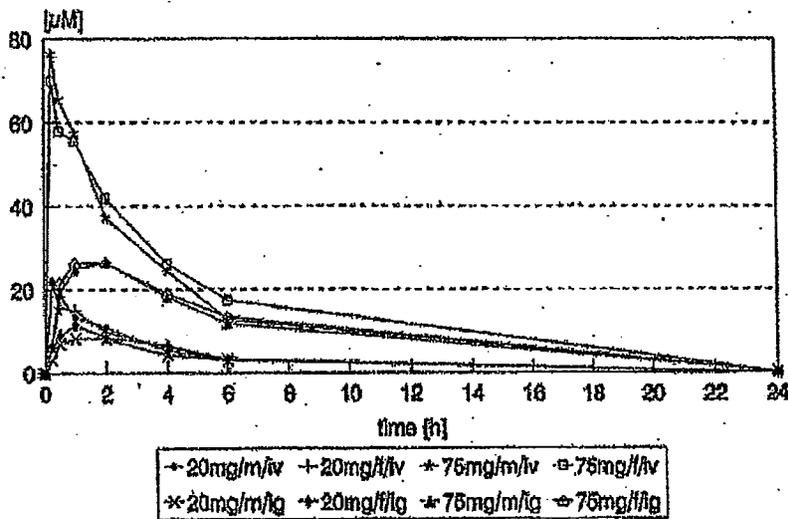


Figure excerpted from sponsor

Mean C_{max} after single oral 20 or 75 mg/kg doses ranged from 8.21 to 26.5 μM. The terminal half-life after oral administration in rats was identical to the terminal half-life after i.v. administration; 2.5-3.5 hr.

Table 1. F-ara-A Pharmacokinetic Parameters following single or multiple daily oral and intravenous administration of 20 and 75 mg/kg Fludarabine Phosphate to SD rats

Parameter	Single dose				Repeated dose			
	20 mg/kg		75 mg/kg		20 mg/kg		75 mg/kg	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
i.v. administration								
C _{max} (uM)	21.9	21.3	76.5	69.9	17.9	13.5	66.6	61.4
T _{max} (h)	0.25	0.25	0.25	0.29	0.50	0.25	0.26	0.50
T _{1/2} (h)	3.40	3.24	2.97	2.92	2.64	3.24	3.58	2.04
AUC(uM-h)	84.2	80.3	323	373	58.7	53.7	208	196
V(L/kg)	3.20	3.19	2.73	2.32	3.56	4.77	5.11	3.08
CL(mL/min/kg)	10.8	11.4	10.6	9.20	15.6	17.0	16.5	17.4
CLR(mL/min/kg)	4.50	4.02	3.26	3.19	6.26	6.33	3.39	4.36
Ae(%)	41.3	35.2	30.7	34.6	40.2	37.2	20.6	24.9
i.g. administration								
C _{max} (uM)	8.21	11.5	26.5	26.5	12.7	9.07	16.0	13.5
T _{max} (h)	1.0	1.0	2.0	2.0	1.0	1.0	2.0	1.0
T _{1/2} (h)	2.68	3.55	3.43	3.21	3.15	3.17	3.08	3.16
AUC(uM-h)	59.9	72.1	226	245	54.9	35.3	138	133
CLR(mL/min/kg)	4.40	3.54	2.36	2.50	4.77	7.03	2.80	3.49
Ae(%)	28.8	27.8	15.5	17.8	28.6	27.2	11.3	13.6
F-plasma(%)	71.3	90.0	70.0	65.9	93.5	65.7	66.3	67.9
F-Urine(%)	69.9	78.8	50.4	51.5	71.4	73.0	54.9	54.4

Note: CLR: renal clearance; Ae: total urinary excretion

Table excerpted from sponsor's submission

After i.v. administration, plasma clearance was ~11 mL/min/kg and the volume of distribution was ~3.2 L/kg, slightly larger than total body water. There was no apparent difference between males and females, and the pharmacokinetics were apparently linear between the 20 and 75 mg/kg doses after both a single dose and 14 daily doses.

After i.g. administration, absorption was rapid (1.0 to 2.0 hrs). C_{max} values after single 20 or 75 mg/kg doses ranged from 8.21 to 26.5 uM. Plasma concentrations after oral doses were similar in both male and female rats on Days 1 and Days 14. The terminal half-life after oral administration in rats was identical to the terminal half-life after i.v. administration, 2.5-3.5 h. Bioavailability ranged from 65.9% to 93.5% following oral administrations of 20 and 75 mg/kg fludarabine phosphate to SD rat (Figure 2.6.3 and Table 1).

Study Report AF08: Fludarabine phosphate bioavailability studies beagle dogs (Report No. A08) Module 4.2.2.2.2

This study was designed as a single dose, cross-over study in 8 beagle dogs (4/sex/dose). Animals received single i.v. and p.o. doses of 86.7 mg/m² and 260 mg 2F-ara-AMP/m². Blood samples were taken up to 24 hr post dose. 2F-ara-A plasma levels were analyzed by HPLC with UV detection and internal standardization.

Results:

Table 1: 2F-ara-A pharmacokinetics after single i.v. and i.g. administration of 86.7 and 260 mg 2F-ara-AMP/m² in beagle dogs (n=4/group)

Parameter	Dog			
	i.v.	i.v.	p.o.	p.o.
Dose, mg/m ²	86.7	260	86.7	260
C _{max} , μM	20.3±1.0	72.2±16.6	15.4±3.8	36.7±10.8
T _{max} , h	0.07	0.07	0.6	1.0
AUC, μM·h/ml	46.2±7.7	164±40.8	46.8±11.9	128±33.1
T _{1/2} , h	1.6-2.3	1.6-2.3	1.5-2.5	1.4-2.2
Cl, ml/min/m ²	89±14	77±23	-	-
F, %	-	-	100.8±15.4	77.9±1.4

Table excerpted from sponsor's submission

Plasma levels and AUC values increased dose-dependently following single oral doses of 86.7 and 260 mg 2F-ara-AMP/m² to beagle dogs. Median peak 2F-ara-A plasma levels were reached at 0.6 h and 1.0 h post dose. Plasma levels declined dose-independently with a mean terminal half-life of about 1.8 h as in Table 1. The oral bioavailability was 100.8 and 77.9% in doses of 86.7 and 260 mg/m², respectively.

The Pharmacokinetics of 2F-ara-A for multiple species was compared with those found for patients given a single 50 mg (ca. 31 mg/m²) dose of 2F-ara-AMP orally and intravenously (Table 2.6.19). Pharmacokinetic data were calculated by validated software such as TOPFIT, NONLIN or CSTRIP.

Table 2.6.19 2F-ara-A Pharmacokinetics in Different Species

Mean Pharmacokinetics of 2F-ara-A After Single Oral and IV Doses of 2F-ara-AMP									
SPECIES Strain/Gender (Source)	Dose	CL _{sp} mL/min	V _{ss} L	T _{1/2} (IV) hr	T _{1/2} (PO) hr	F %	C _{max} (PO) μM	T _{max} (PO) hr	AUC (PO) μM·hr
Mouse BDF ₁ F (Noker et al., 1983)	40 mg/m ²	0.37	0.061	1.9	ND ^b	ND	ND	ND	ND
Rat Sprague Dawley M+F (AE99)	120 mg/m ²	2.75	0.8	3.3	3.1	81	9.9	1.0	66.1
Dog beagle M+F (AF08)	86.7 mg/m ²	44.5	7.65	2.0	1.9	100.8	4.39	0.6	46.6
Monkey rhesus F Dareer et al., 1980)	30 mg/m ²	NR ^a	NR	2.1	ND	ND	ND	ND	ND
Human M+F TB03-1105 (IV) ME95101 (PO tabs.)	50 mg	226	153	21.1	NR (22.5) ^c	56	1.05	1.1	6.17

^aNR, not reported

^bND, not determined

^cT_{1/2} from Day 5 in study ME94204

Table excerpted from sponsor's submission

Systemic plasma clearance of 2F-ara-A in mice, rats, and dogs after single i.v. doses of fludarabine phosphate was low compared to humans. The volume of distribution was also modest in all species ranging from 0.8 L/kg in dogs to 3.2 L/kg in rats. These volumes exceed estimates for total body water in all species and suggest some tissue distribution and binding to intra-vascular and extra-vascular compartments. The terminal half-life in animals was short (~ 2-3 hrs), and different than the apparent half-life in humans (~ 22 hrs). Multiphase elimination was evident for all species. The oral bioavailability of 2F-ara-A in rats and dogs given single oral doses of 2F-ara-AMP was high, 81 and 100.8%, respectively.

2.6.4.4 Distribution

Study Report A20177: Quantitative whole body autoradiographic (QWBA) study following intragastric administration of 20 mg/kg ^{14}C -2F-ara-AMP to rats (Study report A20177) Module 4.2.2.3.1

Six male and 4 pregnant female albino rats received 20 mg ^{14}C -labeled 2F-ara-AMP as single i.g. dose. Male animals were killed at 0.5, 1, 4, 24, 72, 168 hr post-dose and pregnant female animals were killed 1, 4, 24 hr post-dose and frozen. Sagittal sections were cut from embedded carcasses, exposed to an imaging plate and the ^{14}C quantitated by laser-induced luminescence. Tissue concentrations were calculated against standard curves of known amounts of ^{14}C embedded with each section.

Results:

In male rats:

Quantitative whole body autoradiographic studies of male and pregnant albino rats dosed with ^{14}C -labeled 2F-ara-AMP demonstrated that distribution was rapid and widespread to well perfused organs at 30 min post-dose. The peak concentrations of total radioactivity observed 1 hr post-dose in both well and poorly perfused tissues. Highest concentrations of radioactivity were detected in major organs; kidney, liver, spleen and intestines. Relatively low concentrations were in bone, brain, and spinal cord. Tissue concentrations declined with time and were below the limit of quantitation in most tissues 7 days post-dose. Concentrations in brain were low but detectable 30 min post-dose, increased with time up to the 4 hr sample, and were still present 7 days post-dose (Table TT3 below). The highest concentrations 7 days post-dose were found in skin, 3.09 ug-eq./g.

TT3. Concentration of Radioactivity (ug-eq/g) in tissues at 30 min, 1 h, 4 h, 1 d, 3 d, and 7 d After single Intra-gastric Administration of 20 mg ¹⁴C-2F-ara-AMP to Male Albino Rats

Organs	30 min		1 h		4 h		1 d		3 d		7 d	
	Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD
Bone	0.868±0.545		1.14 ±0.190		1.11±0.805		0		0		n.a	
Brain	0.511±n.a.		1.42±0.064		2.74±0.291		0.339±0.064		0.223±n.a.		0.173±n.a.	
Heart (myocard)	9.59±0.731		14.1±n.a.		6.34±0.235		0.465±0.011		0.176±0.022		0	
Kidney cortex	11.6±0.545		17.1±0.847		5.05±1.29		1.75±0.003		0.974±0.085		0.379±n.a.	
Kidney medulla	15.8±11.2		40.7±18.0		55.8±3.78		0.725±n.a.		0.333±n.a.		0.281±0.185	
Large intestine w.	7.49±3.34		7.48±n.a.		16.3±n.a.		n.a.		0.923±n.a.		0.595±n.a.	
Large intestine c.	0.752-0.047		0.916±n.a.		61.6±n.a.		n.a.		0.410±0.045		0	
Liver	43.6±2.96		37.5±4.46		17.3±0.857		1.68±0.118		0.732±0.079		0.173±0.014	
Lung	4.17±0.176		7.21±1.14		3.49±0.211		0.292±n.a.		0.463±0.006		0	
Lymph node	9.71±n.a.		26.7±n.a.		28.9±3.47		1.11±0.261		n.a.		n.a.	
Nose mucosa	2.65±n.a.		173±n.a.		0.119±0.549		n.a.		n.a.		n.a.	
Esophagus	3.32±38.6		n.a.		n.a.		1.09±n.a.		n.a.		n.a.	
Pancreas	6.92±0.108		10.2±1.26		7.39±0.413		1.20±0.044		0.711±n.a.		0	
Skeletal muscle	3.48±0.677		6.69±0.038		4.79±0.901		0.425±0.001		0.260±0.015		0	
Skin	2.97±0.389		6.78±0.442		4.89±0.179		2.20±n.a.		1.45±n.a.		3.09±n.a.	
Small intestine content	361-179		37.3-31.4		45.0-15.4		22.6-0.248		0.669-0.214		n.a.	
Spinal cord	1.26±1.35		1.59±n.a.		2.65±n.a.		0.199±n.a.		0.180±0.034		0	
Spleen	±		±		±		±		±		±	
Stomach content	965-517		333-287		75.0-18.7		0		0		0	
Stomach gl. p.	74.4-31.4		14.5-13.3		7.88±0.023		0.759-0.662		0.410-0.377		0	
Testicles	1.44±93.1		4.23±0.276		3.37±0.194		0.420±0.042		0.264±0.0108		0.212±0.015	
Tongue	8.22±n.a.		17.3±n.a.		10.3±0.973		0.555±n.a.		0.282±n.a.		n.a.	
Thymus	5.69±0.351		11.8±n.a.		6.44±0.737		0.697±0.064		0.441±0.051		n.a.	
Vitreous body	1.33±0.857		3.09±0.157		0.947±n.a.		0.212±n.a.		0		0	
LLOQ	0.333-0.294		1.20-0.397		0.643-0.459		0.204-0.104		0.238-0.091		0.152-0.137	

* due to the broad range of radiolabel concentrations (inhomogenous distribution) in the organs of gastrointestinal tract concentration ranges were given instead of mean and standard deviation.

n.a.=not available; w=wall; c.=content; gl.p.=glandular part; LLOQ= lower limit of quantitation; values below LLOQ were set to 0.

In pregnant rats

After oral doses of ¹⁴C-2F-ara-AMP to pregnant rats, the distribution pattern of total radioactivity (¹⁴C) was similar to that observed for males. ¹⁴C concentrations in the fetus were similar to maternal blood concentrations 1 and 4 hr post-dose and were still present 24 hr post-dose. These data demonstrate that 2F-ara-A or metabolites cross the placental barrier and in fetus. Placenta maternal concentrations were greater than concentrations in the fetus at all times sampled. Concentrations in amniotic fluid were low, and below the limit of quantitation 4 hr post-dose. Concentrations in mammary gland were similar to those in blood 1 hr post-dose, and may suggest distribution of radiolabeled 2F-ara-A and other metabolites in milk.

Organs	key	1 h		4 h		1 d (24 h)	
		Mean	SD	Mean	SD	Mean	SD
Vena cava	vc	8.18±n.a		4.71±n.a.		0.250±n.a.	
Amniotic fluid	af	0.425 ± n.a.		0		0	
Fetus	fe	6.26 ± 0.480		4.42 ± 0.061		0.342 ± 0.038	
Mammary gland	mg	8.08 ± 1.20		3.29 ± 1.43		0.236 ± n.a.	
Placenta maternal	plm	16.6 ± 3.02		11.2 ± 0.546		0.468 ± 0.002	
LLOQ		0.493-0.300		0.348-0.225		0.283-0.177	

2.6.4.5 Metabolism

Study Report AL67: Metabolism and excretion of ^3H -2F-ara-A in male and female SD rats following a single intravenous and intragastric liquid dose of 20 mg/kg ^3H -fludarabine phosphate/kg Module 4.2.2.4.1

Following i.v. and i.g. administration, fludarabine phosphate (2F-ara-AMP) was rapidly hydrolyzed to 2F-ara-A, the principal metabolite in plasma, serum and tissues, which is actively taken up by cells and intracellularly phosphorylated to its cytotoxic triphosphate, 2F-ara-ATP. Metabolic pathways of fludarabine phosphate are similar in laboratory animals and man as in Figure below.

Figure 2.6.4: Metabolic Pathways of Fludarabine Phosphate in Animal Species

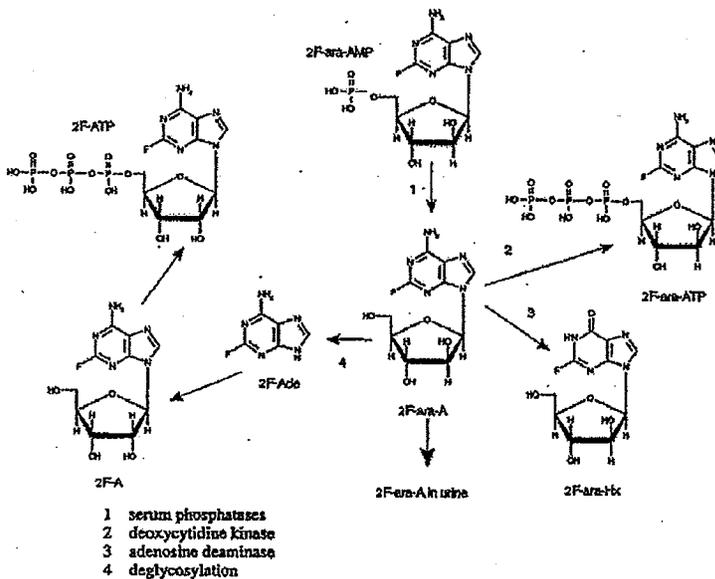


Figure excerpted from sponsor's submission

Wistar rats (5/sex/dose) received a single i.v. (18.75 mg/kg) or i.g. (20 mg/kg) bolus dose of ^3H -2F-ara-AMP (~5 MBq/kg) fludarabine phosphate. Urine and feces were collected in 24 hr intervals at the end of days 1, 2, 3, 4, 5, 6, 7, 8, and 9 post-dose. ^3H in feces, blood and intestines was determined by combustion and LSC after lyophilization and homogenization. ^3H in urine was determined by LSC. Metabolite patterns were recorded by radiochromatography. Several reference standards were used for metabolite identification.

Results:

The prodrug ^3H -2F-ara-AMP was rapidly converted to dephosphorylated ^3H -2F-ara-A. No parent drug was detected in urine or feces. Excretion of total ^3H and metabolites after i.v. and i.g. doses is summarized below.

Table 4: Mean recovery of ^3H -label in urine, feces, washing fluids, blood, GI-tract after single i.v. and i.g. ^3H -2F-ara-AMP/doses in rats

Route, Dose	i.v., 18.75 mg/kg		i.g., 20 mg/kg	
	Male	Female	Male	Female
	%			
Urine	79.8±5.18	76.8±9.28	69.5±10.8	68.0±8.74
Feces	8.82±4.22	10.4±4.87	12.1±2.29	12.4±4.71
Washing fluids	5.83±2.22	6.41±0.85	6.16±2.43	6.04±3.19
Blood	0.041±0.019	0.029±0.003	0.038±0.008	0.033±0.008
GI-tract	0.084±0.025	0.088±0.017	0.106±0.015	0.113±0.034
Not recovered	5.45	6.29	12.1	13.4

Table excerpted from the sponsor

Metabolic patterns were similar in both genders after i.v. and i.g. administration. ^3H -fludarabine was primarily renally excreted during the first 24 hr after a single dose. A minor portion was recovered in feces. Absorption from the GI tract was almost complete following i.g. (oral) administration. Only traces of radiolabel (<0.1%) were found in blood and GI tract on day 9.

The metabolic pattern of ^3H -fludarabine in urine did not show any presence of the prodrug ^3H -fludarabine. Approximately 50% of the recovered ^3H -activity was ^3H -2F-ara-A. The remaining excreted dose portion was ^3H -M1 (Text Table 3).

Text Table 3: Mean portions of fludarabine metabolites in urine cumulatively excreted over 24 h following single i.v. (18.75 mg/kg) and i.g. (20 mg/kg) ^3H -2F-ara-AMP/doses in male and female rats (n=5)

route	i.v.		i.g.	
	male	female	male	female
	[% of radioactivity of evaluable peaks]			
^3H -2F-ara-AMP	0	0	0	0
^3H -2F-ara-A	52.6 ± 9.2	49.9 ± 4.3	44.5 ± 2.4	49.3 ± 5.8
^3H -M1	47.4 ± 9.2	50.1 ± 4.3	55.5 ± 2.4	50.7 ± 5.8

Table excerpted from the sponsor

Study Report A21917: Metabolic patterns of ^{14}C -ZK 153851 (Fludarabine) in plasma and urine from female and male rats after intravenous and intragastric administration
Module 4.2.2.4.2

Metabolite patterns of ^{14}C -ZK 153851 (Fludarabine) in plasma, and urine were determined following intravenous injection and intragastric administration of 20 mg/kg ^{14}C -ZK 153851 to SD rats. Each individual sample was collected and combined into

pool samples at time points (for plasma: 0.5, 1.5 and 6 h) or time intervals (for urine samples: 0-8, 8-24 and 0-24 h). Plasma samples were analyzed by HPLC and radiodetection. The metabolic patterns of ZK 153851 (fludarabine phosphate) in different biological samples were generated after HPLC separation with subsequent radiometric online detection of ^{14}C -radioactivity. Fludarabine corresponds to Fludarabine phosphate (2F-ara-AMP, ZK 153851).

Results:

Table 2.6.24: Metabolite Profile (% of Dose) in Urine from Rats After Single IV or PO Doses of ^{14}C -fludarabine phosphate.

Metabolite	Relative HPLC Retention Time (min)	% of peak in radio-chromatogram after i.g. administration	% of dose in pool after i.g. administration	% of peak in radio-chromatogram after i.v. administration	% of dose in pool after i.v. administration
2F-ara-AMP	n.d.	n.d.	n.d.	n.d.	n.d.
2F-ara-A	1.0	43.85	20.66	45.86	38.54
2F-ara-Hx	0.24-0.25	48.68	22.94	40.43	33.97
RU2	0.71-0.72	2.98	1.40	3.43	2.88
RU3	0.83-0.84	2.48	1.17	2.91	2.45
RU5	1.23-1.28	2.02	0.95	7.38	6.20

Table 2.6.24: Distribution [% of dose] of major ^{14}C -labeled compounds in urine samples (0-1 d pool) after intragastric and intravenous administration of 20 mg/kg ^{14}C -ZK 153851 (2F-ara-AMP) to male rats. Abbreviations: n.d., not detected; RTT, Relative Retention Time (range) [min]; ZK 159155 was set as reference for the calculation of the relative retention times. Data from Study Report A21917.

Table excerpted from the sponsor

Plasma:

As in Table 2.6.24, after i.v. and i.g. administration, no intact 2F-ara-AMP (the parent drug) was detected in plasma. The parent compound (fludarabine phosphate, ZK 153851) was extensively dephosphorylated to major metabolite ZK 159155 (2F-ara-A) which could be detected at highest concentrations in all plasma samples. In addition to 2F-ara-A (major metabolite), 2F-ara-Hx (hypoxanthine-derivative; ZK 180059) was detected in plasma. No gender differences in metabolite profile were found.

Urine:

The majority of administered radioactivity was excreted in urine within 24 hrs. No intact 2F-ara-AMP was detected in urine. ZK 180059 (2F-ara-Hypoxanthine) and ZK 159155 (2-Fluoro-ara-adenine) were detected as major metabolites. In addition, three minor metabolites (RU2, RU3 and RU5) were detected in the urine.

2.6.4.6 Excretion

Study Report A21908: Formation of $^{14}\text{CO}_2$, excretion and mass balance of ^{14}C -ZK-153851 after single intravenous and intragastric administration to male and female rats
Module 4.2.2.5.1

The study was to determine the formation of $^{14}\text{CO}_2$, the excretion and mass balance of radioactivity derived from ^{14}C -ZK 153851 (fludarabine phosphate) in male and female rats after i.v. and i.g. dose of 20 mg/kg ^{14}C -ZK 153851.

Results:

As in Table TT7, total recovery of ^{14}C (% of dose) was high for both i.v. and i.g. doses, ranging from 84.8% in female rats after 20 mg/kg i.g. to 93.1% in male rats after the i.v. dose. Most of the recovered dose was excreted in urine, ranging from ~47 to 85%. Negligible amounts were excreted in expired air. Fecal excretion was minor after i.v. doses (4-13%), but was greater after i.g. dose (~25-41%). Complete excretion of ^{14}C -radiolabeled compounds was observed after i.v. and i.g. doses within 7 days although the majority was excreted within 24 hrs reflecting both unabsorbed drug and biliary excretion.

TT 7: Mean total recovery of ^{14}C -radiolabeled compounds (% of dose) after single intravenous and intragastric administration of 20 mg/kg ^{14}C -ZK 153851 to male and female rats over a sampling period of 7 days (168 h)

Matrix	i.v. M	i.v. F	i.g. M	i.g. F
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
Urine	85.4 \pm 7.66	69.4 \pm 5.59	48.2 \pm 6.79	46.6 \pm 1.22
Cage wash	2.31 \pm 0.890	8.10 \pm 2.65	1.36 \pm 0.38	8.03 \pm 5.31
Feces	4.33 \pm 0.771	12.7 \pm 5.68	41.5 \pm 6.06	25.5 \pm 10.3
Carcass	0.901 \pm 0.0621	1.17 \pm 0.164	0.987 \pm 0.297	1.55 \pm 0.0513
Exhaled air	0.197 \pm 0.0491	0.230 \pm 0.116	0.910 \pm 0.147	3.17 \pm 1.12
Total	93.1 \pm 7.36	91.6 \pm 1.01	93.0 \pm 2.73	84.8 \pm 5.44

i.v. M intravenous administration in male rats
 i.v. F intravenous administration in female rats
 i.g. M intragastric administration in male rats
 i.g. F intragastric administration in female rats
 SD standard deviation (n = 3 rats)

Table excerpted from the sponsor

Urinary excretion was the major pathway for the elimination of both i.v. and i.g. doses. Fecal excretion was greater after i.g. doses, reflecting both unabsorbed drug and biliary excretion.

2.6.4.6 Inhibition of CYP enzymes

The study was conducted to determine which CYP450 isoforms are responsible for metabolism of [^{14}C]-fludarabine phosphate and [^{14}C]-fludarabine (2F-ara-A). [^{14}C]-fludarabine phosphate was incubated with human liver microsomes in the presence or absence of selective inhibitors of CYP450 isoforms. The radioactive concentration profiles of the test article and its metabolites were measured using both HPLC-Radioactive Measurement (HPLC-RAM) and fractionation and LSC (Report A07088) Module 5.3.2.2.2.

Results:

Following the incubation of [¹⁴C]-2F-ara-A with or without human liver microsomes for 60 minutes, no metabolite peaks were observed. After incubation of [¹⁴C]-fludarabine phosphate with human liver microsomes in presence of the cofactor of CYP450, one metabolite was identified as 2F-ara-A by comparison with radioactivity profile of the referenced standard.

Results of incubations with selective CYP450 isoform inhibitors suggested that human liver microsomal CYP1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4 are not responsible for the formation of the metabolite.

2.6.4.7 Pharmacokinetic drug interactions

No studies were submitted.

2.6.4.8 Other Pharmacokinetic Studies

No studies were submitted.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

A previously approved intravenous fludarabine phosphate NDA (NDA 20-038) included a complete package of single and repeat dose, genotoxicity, and developmental toxicity studies. Studies to support oral fludarabine phosphate include those studies conducted by both intravenous and oral routes of administration. Six new oral studies include acute toxicity (rats) and repeat-dose toxicity (up to 4 weeks administration in rats and dogs).

Two single dose oral toxicity studies using intragastric (i.g.) administration were conducted in the rat. In the first study (Study report AB98), SD rats (n=3/sex/group) were given i.g. doses of 10, 50 and 100 mg/kg and were subsequently observed for one week. No mortality occurred in this study. With the exception of a transient hypoactivity in some males, no indications of compound-related toxic effects were reported at the tested dose levels. In the second study (Study Report A22092), SD rats received single doses of 1500 or 2000 mg/kg (9000 or 12000 mg/m²) fludarabine phosphate. Transient dose-related clinical signs of gastrointestinal and general toxicity were observed. These toxicities include diarrhea, discoloration of feces, emaciation, decreased body weight gain and retarded growth. Hematological, clinical pathology or histopathological evaluations were not performed.

Repeat-dose oral toxicity studies were conducted to detect possible influences of the route of administration on the toxicological profile of fludarabine phosphate in the comparative (i.v. versus i.g.) toxicity studies in rats over 14 days (Study report AA95) and dogs over 5 days (Study report AB94). Two additional 4-week oral toxicity studies were conducted in rats (Study report A22369) and dogs (Study report A22370).

In the 14-day rat study (Study report AA95), SD rats (n=10/sex/group) received either IV doses of 20, 75, and 200 mg/kg (120, 450, and 1200 mg/m²) or PO doses of 20, 75, 200, and 250 mg/kg (120, 450, 1200, and 1500 mg/m²) daily. Mortality was observed following 200 mg/kg IV (n=40/40) and following 200 mg/kg (n=15/40) and 250

mg/kg (n=11/20) PO doses during the 14-day treatment period. Mortality appeared higher after intravenous dosing. This difference could be associated with lower systemic exposure after intragastric administration (bioavailability ~67%). At a dose of ≥ 75 mg/kg/day (450 mg/mg/day) IV or PO, dose-related clinical signs included hunched posture, ruffled coat, diarrhea/soft feces, nasal discharge, decreased physical activity and reductions in body weight gain and food consumption. Hemoconcentration, lymphopenia, lymphoid depletion in thymus, spleen, and lymph nodes as well as leukocytosis and acute inflammatory changes in the GI tract were observed. Increased ALT, AST, and ALP levels in conjunction with increased liver weights suggested an effect on the liver, despite the lack of histological findings. No relevant effects were observed at the low dose of 20 mg/kg/day independent of route of administration.

In the 5-day dog study (Study report AB94), fludarabine phosphate was administered to beagle dogs (n=1/sex/dose) intravenously at a dose level of 150 mg/kg (3000 mg/m²), and orally at dose levels of 1.5 (30), 15 (300) and 150 mg/kg (3000 mg/m²) for 5 consecutive days. Toxicological effects were only observed at the HD (150 mg/kg/day) IV and PO and did not differ from the known toxicity pattern in the dog. All dogs survived to their scheduled sacrifice (day 6 and 20). Emesis was seen on day 5 in the male dog given i.v. dose of 3000 mg/m²/day fludarabine and general body trembling was observed in the female given oral doses of 3000 mg/m²/day on days 1 and 2. A mild to moderate lymphopenia was associated with lymphoid depletion in the thymus and mild lymphocytic necrosis in thymus and mesenteric lymph nodes, bone marrow hypocellularity and inflammatory changes of the intestinal mucosa. Increased ALP, AST, and ALT levels following both IV and PO doses 3000 mg/m² was observed whereas increased BUN and creatinine were limited to a male administered 3000 mg/m² intravenously. The same animals showed an increase in segmented neutrophil counts and decreased lymphocyte counts. In this study, the HNSTD was 30 mg/m²/day (1.5 mg/kg/day).

In the 4-week oral toxicity study (Study report A22369), SD rats (n=10/sex/dose) were given i.g. doses of 10, 25 and 75 mg/kg (60, 150 and 450 mg/m²) daily for 28 days. One HD male in the satellite group died on day 23. Clinical signs of piloerection were noted mostly in the HD animals from day 12 onward. Reductions of body weight gain with transient body weight loss (weeks 1-4) and increase of food consumption (weeks 2-3) were noted in the males and females of the HD group. Hematological findings in the HD group revealed increased neutrophil granulocytes and decreased lymphocyte counts with increased large unstained cells and fibrinogen values. Hepatic enzymes were elevated at both MD and HD groups, however no signs of histopathology were found in the liver of these animals. Drug-induced microscopic findings were mostly in the lymphoid system, gastrointestinal mucosa and reproductive organs. At the end of 4 week recovery period, most of the drug-induced changes were resolved, however, testicular changes (tubular atrophy, degeneration of spermatids, delayed spermatogenesis, oligospermia) were still present. The NOAEL dose was 10 mg/kg/day when administered PO daily for 4 weeks.

In the 4-week oral toxicity study (Study report A22370), beagle dogs were administered 4, 16 or 60 mg/kg (80, 320 or 1200 mg/m²) daily for 28 days. No mortality or clinical signs were observed. Body weight was not affected by oral doses of fludarabine phosphate, however, a statistically significant reduction of food consumption

Results:

- No mortality or other drug-related toxic effects were observed.
- Transient hypoactivity was noted ~1.5 hr post dose in one male at 10 mg and all males given with 50 mg fludarabine phosphate tablet suspension; however, they returned to normal within an hour.
- Body weight changes were not remarkable in the treated groups.
- No drug-related gross findings were observed at necropsy.

Study title: Acute toxicity of SHL 573A (ZK 153851, fludarabine phosphate) in male and female rats after single i.g. administration

Key study findings:

- Single oral doses up to 2000 mg/kg, the highest dose administered in this study, were well tolerated.
- Clinical symptoms of ruffled fur, emaciation, decreased body weight gain and GI toxicity was observed.
- No compound-related gross findings were observed.

Study no: Schering study report A22092
Volume # and Page #: Module 4.2.3.1.2
Conducting laboratory and location: Schering AG, Experimental Toxicology
 D-13342 Berlin, Germany
Date of study initiation: Feb 11, 2004
GLP compliance: Yes
QA report: Yes
Drug, lot #, radiolabel, and % purity: Fludara™, batch #34171
Formulation/vehicle: 50 mg Mannitol, 2N NaOH ad pH 7.7, ad 1 mL water per injection

Methods (unique aspects): Single intragastric administration of aqueous solution of ZK 153851 (fludarabine phosphate) at doses of 1500 mg/kg and 2000 mg/kg were given to two groups of rats. Control animals received the vehicle.

Dosing:
Species/strains: SD rats
#/sex/group: 3/sex/group
Age: ~5 weeks
Weight: Males 122-149 g; females 111-127 g
Route/volume/infusion rate: Single oral dose via gavage
Dose/Treatment: 1500 or 2000 mg/kg fludara aqueous solution
Assessments: Mortality, clinical sign, body weight and gross necropsy

Results:

- No mortality or other toxic effects were observed.

- Clinical signs at 2000 mg/kg in females included emaciation (d 5-6), ruffled fur (d1-9), hypoactivity (d3), decreased weight gain (d7, 14) and ruffled fur (d3-4).
- Gastrointestinal impairment (diarrhea, discolored feces) was observed in male animals on day 1. At 1500 mg/kg dose, GI impairment (increased defecation, discolored feces, diarrhea) and decreased weight gain were noted on days 5-7 in one female.
- Slight decreases in body weight gain (8-10%) were seen in males at the HD on days 8 and 15.
- No treatment-related gross findings were observed.

2.6.6.2 Repeat-dose toxicity

Study title: A 4-week systemic toxicity study of SHL 573A (fludarabine phosphate) by repeated oral administration to SD rats with a subsequent recovery period of 4-weeks (Module 4.2.3.2.2)

Key findings:

- One male, administered 75 mg/kg, died prematurely on test day 23.
- Piloerection was noted mostly in the HD group from test day 12 onwards.
- At the HD, a statistically significant decrease in body weight gain was noted in both male and female in weeks 1-4. The relative food consumption was increased from week 2 (males) and week 3 (females).
- Increased neutrophils and decreased lymphocyte counts and slightly decreased red cell parameters were observed mostly in the HD group. A statistically significant increase in fibrinogen (>50%) was noted in males and females (both $p \leq 0.01$) in the MD and HD groups.
- Histopathologic lesions included lymphoid depletion in various lymph nodes, spleen and thymus, inflammation in large intestine, testicular atrophy, degeneration of spermatids, Sertoli-cell vacuolation and delayed spermatogenesis in the testes.
- The NOAEL dose was 10 mg/kg fludarabine phosphate/day by oral administration.

Study no:	Shering report no. A22369
Volume/Pages:	Module 4.2.3.2.2
Conducting laboratory and location:	✓
Date of study initiation:	Dec 21, 2001
Date of final report:	Nov 10, 2004
GLP compliance:	Yes
QA report:	Yes
Drug, lot #, and % purity:	ZK 153851 (fludarabine phosphate) Batch# 131570, 97% purity
Formulation/vehicle:	50 mg mannitol; 1N NaOH pH 7.7 q.s water for injection

b(4)

Dosing: Once Daily for 4 weeks
 Species/strains: SD/—CD@BR rats
 #/group for main study: 10/sex/group (5/sex/group for non-treatment recovery period)
 #/group for satellite study: 6/sex/group for TK and for the 4 week recovery period
 Initial Age: ♂: 29 days, ♀: 31 days
 Weight: ♂: 117-144 g, ♀: 112-132 g
 Route: Oral
 Frequency: Once daily for 4-weeks

b(4)

TT 1: Treatment schedule

Group / Dose level	Number of animals/sex MS + SA	Compound	Dose per day [mg/kg]	Administration volume [mL/kg]	Animal number and sex	
					MS	SA
1 (control)	10 + 6 ♂	Vehicle	N/A	10	1 - 10 ♂	11 - 16 ♂
	10 + 6 ♀				17 - 26 ♀	27 - 32 ♀
2 10 mg/kg	10 + 6 ♂	Fludarabine phosphate	10	10	33 - 42 ♂	43 - 48 ♂
	10 + 6 ♀				49 - 58 ♀	59 - 64 ♀
3 25 mg/kg	10 + 6 ♂	Fludarabine phosphate	25	10	65 - 74 ♂	75 - 80 ♂
	10 + 6 ♀				81 - 90 ♀	91 - 96 ♀
4 75 mg/kg	10 + 6 ♂	Fludarabine phosphate	75	10	97 - 106 ♂	107 - 112 ♂
	10 + 6 ♀				113 - 122 ♀	123 - 128 ♀

MS: main study
 SA: satellite animals for toxicokinetics and the recovery period.
 N/A: not applicable

Table excerpted from the sponsor

Observations and times:

Clinical signs: Daily
 Body weights: Weekly
 Food consumption: Weekly
 Ophthalmic exam: Pre-dose, Days 29 and 54
 Hematology: Days 26 and 56
 Bone marrow exam: Days 29 and 57
 Clinical chemistry: Days 26 and 56
 Gross Pathology: Days 29 and 57
 Histopathology: Days 29 and 57
 Toxicokinetics: Blood samples were collected at 0.5, 1, 1.5, 3, 6, 12 and 24 on days 1 and 25. Samples were analyzed for fludarabine in rat plasma.

Results:

Mortality:

One male satellite animal died at 75 mg/kg/day on day 23. At necropsy, small prostate and seminal vesicles and lymphocytic depletion was noted in spleen, thymus and various lymph nodes as well as inflammation in the large intestine.

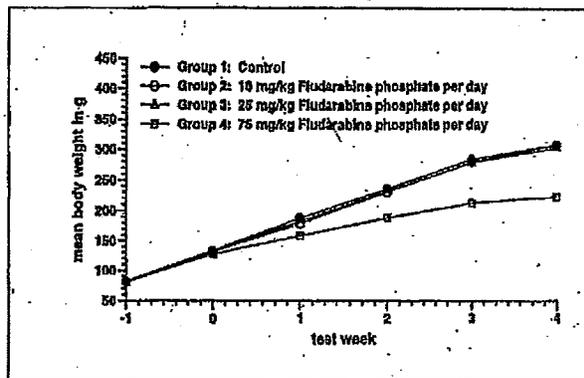
Clinical signs:

In main study animals, piloerection was noted in 3/10 HD♂ and 2/10 HD♀ from days 12 to 28, but piloerection was not noted in MD or LD groups.

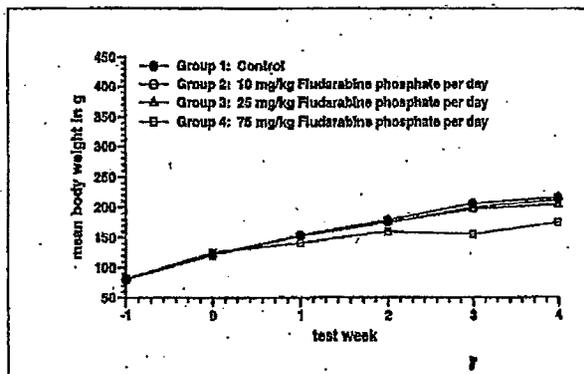
Body weight/food consumption:

A statistically significant reduction in body weight gain with a transient body weight loss was noted in all test weeks (tw 1-4) in the HD group. The maximum decrease was noted in week 4 in males (-28%) and week 3 in females (-24% each) compared to the control.

TF 5: Body weight of male animals (main study)
mean values per group (n=10)



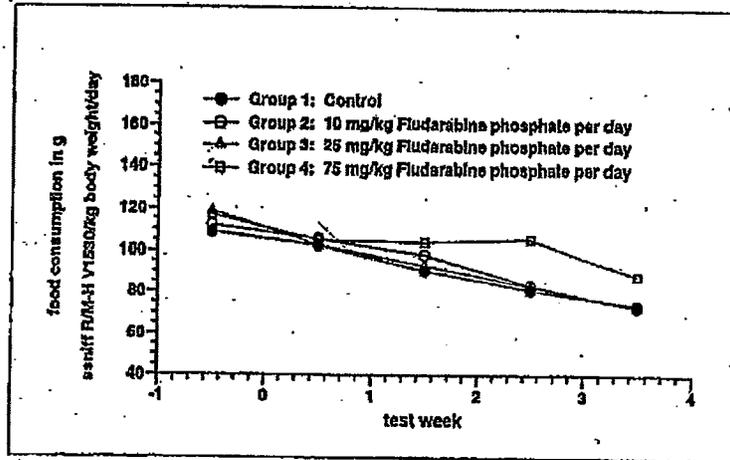
TF 6: Body weight of female animals (main study)
mean values per group (n=10)



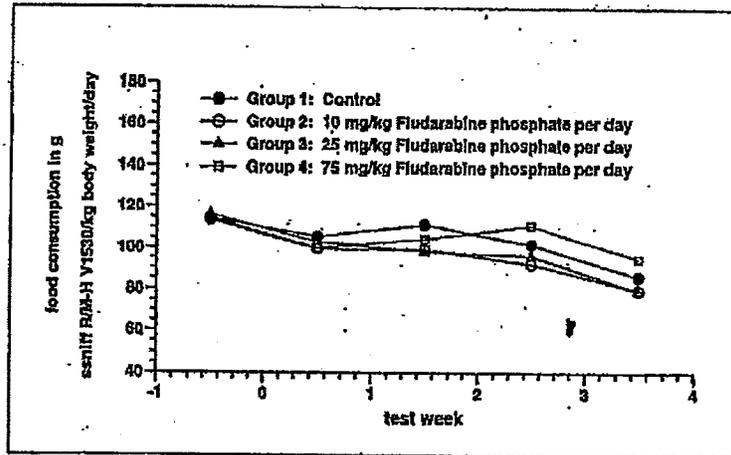
Figures excerpted from the sponsor

Fludarabine resulted in statistically significant increase in relative food consumption in HD males (28%) and in females (22%) at week 2-3 compared to the control values.

TF 1: Food consumption of male animals (main study)
mean values per group (n=10)



TF 2: Food consumption of female animals (main study)
mean values per group (n=10)



Figures excerpted from the sponsor

Ophthalmologic examination:

Not remarkable

Hematology:

Dose, mg/kg/day Sex of Animals	Hematology Findings 4-Week Oral Gavage Toxicity Study with ZK 15385 in Rats with 4-Week Recovery Period							
	0		10		25		75	
	M	F	M	F	M	F	M	F
Number of Animals	10	10	10	10	10	10	10	10
Day 26								
Hematocrit (%)	45.0	43.5	43.9	42.9	44.2	43.8	43.5	41.5
Neutrophils	0.76	1.03	1.15	1.10	1.27	1.26	2.13	1.77
Lymphocytes	8.93	8.45	9.39	7.45	8.96	6.55	6.88	5.13
LUC (cells x 10 ⁹ /L)	0.11	0.12	0.12	0.11	0.16	0.10	0.32	0.35
TPT (sec)	8.9	8.1	8.5	7.7	8.9	8.3	8.6	8.3
APTT (sec)	17.5	18.9	15.6	15.2	17.2	16.2	17.5	15.8
FIB (mg/dL)	145	133	143	145	163	156	217	251
Day 56								
Hematocrit	47.5	41.0	47.3	44.8	46.3	44.2	47.2	44.0
RBC (10 ¹² /L)	8.77	7.67	8.77	8.25	8.67	8.33	8.22	7.97
Reticulocytes (%)	23.5	24.8	24.2	20.3	25.5	20.8	32.0	24.3
Neutrophils	1.13	0.83	1.25	0.93	1.42	0.71	1.39	6.57
Lymphocytes	8.00	6.92	8.63	6.56	10.60	7.15	6.93	6.57
LUC	0.10	0.06	0.09	0.07	0.08	0.08	0.06	0.07
TPT (sec)	8.9	8.6	8.6	8.0	9.9	8.5	8.3	8.0

Note: LUC=Large unstained cells; TPT=Thromboplastin time; Bold indicates a significant difference from the control (p≤0.05).

Clinical chemistry:

Dose, mg/kg/day Sex of Animals	Serum Chemistry Findings Following 4-Week Oral Gavage Toxicity Study with ZK 15385 in Rats with 4-Week Recovery Period							
	0		10		25		75	
	M	F	M	F	M	F	M	F
Number of Animals	10	10	10	10	10	10	10	10
Serum Chemistry Day 26								
ALT (U/L)	29	24	32	31	47	35	78	71
AST (U/L)	73	73	92	100	124	115	202	249
AP (U/L)	404	240	438	263	478	273	528	384
Bilirubin (umol/L)	2.6	2.9	3.0	2.8	2.8	2.7	3.2	3.3
Cholesterol (mmol/L)	1.60	1.89	1.59	1.74	1.58	1.56	1.61	1.86
Total Protein (g/L)	60	65	63	66	63	66	64	67
Glucose (mmol/L)	7.3	7.5	7.1	7.6	6.7	6.5	6.6	6.9
Creatinine (umol/L)	34	36	35	38	35	39	35	38
Albumin (g/L)	29	31	29	32	30	32	29	29
Globulin (g/L)	32	33	33	34	33	34	35	38
A/G Ratio	0.91	0.95	0.89	0.97	0.91	0.94	0.85	0.77
Gamma-GT (U/L)	10.9	10.0	10.6	10.2	10.3	10.9	13.4	14.0
Inorganic phosphate	2.8	2.5	2.9	2.3	2.6	2.2	2.5	2.3
Sodium (mmol/L)	141	140	141	141	141	141	142	142
Chloride (mmol/L)	99.2	100.4	98.9	100.2	100.2	102.6	101.1	101.0
Recovery: Day 56								
Total protein	64	65	63	70	63	71	64	69
Cholesterol	1.54	1.41	1.54	1.77	1.58	1.93	1.71	1.80
Albumin	31	32	31	35	31	34	32	33
Globulin	33	33	34	36	33	38	34	36

Note: Bold indicates a significant difference from the control (p≤0.05).

Urinalysis: Not remarkable

Gross pathology:

Post mortem examination revealed enlarged black-brown discolored lymph nodes (popliteal, iliosacral) in one female treated with HD. The animal (#109) which died prematurely from the satellite group had a small prostate and seminal vesicles.

Dose (mg/kg/day)	0		10		25		75	
# of Animals Examined								
MS: main study	10M	10F	10M	10F	10M	10F	10M	10F
SA: satellite animals	6M	6F	6M	6F	6M	6F	5M	6F
Prostate:								
-reduced in size	-	NA	-	NA	-	NA	1M(SA)*	NA
Seminal vesicles:								
-reduced in size	-	NA	-	NA	-	NA	1M(SA)*	NA
Lymph node (iliosacral):								
-increased in size	-	-	-	-	-	-	-	1F(MS)
-black-brown discolored	-	-	-	-	-	-	-	1F(MS)
Lymph node (popliteal):								
-increased in size	-	-	-	-	-	-	-	1F(MS)
-black-brown discolored	-	-	-	-	-	-	-	1F(MS)

Note- *= Animal died prematurely; NA=Not available

Organ weight:

Fludarabine phosphate caused statistically significant organ weight changes. The HD dose increased relative weights of the brain, lungs, spleen, and thyroid and decreased the relative weights of the thymus in males and females at the terminal sacrifice. The MD dose increased relative weights of thyroid and decreased the relative thymus weight in female animals at terminal sacrifice.

At MD and HD dose, a statistically significant increase of relative weight of the thymus was noted in females at the recovery sacrifice (40 and 65%, respectively). Also, a statistically significant increase in relative brain weights (in females only, 22%) and lungs (both males, 20% and females, 17%) were observed in the HD group.

Dose, mg/kg/day	Percent Deviation from Control							
	Control		10		25		75	
# of Animals/Sex	10M	10F	10M	10F	10M	10F	10M	10F
Main Study								
Body weight/Autopsy								
-absolute (g)	296/22	207/15	-	-	-	-	-25%	-15%
Brain								
-absolute (g)	1.9/0.1	1.8/0.1	-	-	-	-		
-relative (g/kg b.w.)	6.5/0.5	8.9/0.7	-	-	-	-	+28%	+16%
Lungs								
-absolute	1.6/0.3	1.5/0.2	-	-	-	-	-10%	-
-relative	5.6/1.0	7.2/1.3	-	-	-	-	+22%	+14%
Spleen								
-absolute	0.7/0.1	0.6/0.1	-	-	-	-	+21%	+28%
-relative	2.4/0.3	3.0/0.4	-	-	-	-	+64%	+50%
Thymus								
-absolute	0.5/0.1	0.5/0.1	-	-	-	-17%	-39%	-38%
-relative	1.8/0.2	2.3/0.3	-	-	-	-12%	-19%	-27%

Thyroid								
-absolute	0.013	0.010	-	-	+23%	+50%	+23%	+70%
	/0.003	/0.004						
-relative	0.044	0.048	-	-	+25%	+54%	+71%	+94%
	/0.012	/0.018						
# of Animals/Sex	6M	6F	6M	6F	6M	6F	5M	6F
Satellite Study								
Body weight/Autopsy								
-absolute (g)	375/31	262/21	-	-	-	-	-	-20%
Brain								
-absolute	-	-	-	-	-	-	-	-
-relative	5.4/0.4	7.4/0.6	-	-	-	-	-	+22%
Lungs								
-absolute	-	-	-	-	-	-	+15%	-
-relative	4.9/0.1	5.8/0.7	-	-	-	-	+20%	+17%
Thymus								
-absolute	-	-	-	-	+12%	+32%	-	+32%
-relative	1.3/0.2	1.4/0.3	-	-	-	+40%	+9%	+65%

Note- Bold=Statistically significant changes; -=No change; b.w.=body weight.

Histopathology:

Dose, mg/kg/day	0		10		25		75	
No. Animals Examined	10	10	10	10	10	10	10	10
Sex of Animals	M	F	M	F	M	F	M	F
Terminal Sacrifice (Day 30)								
Cervical lymph node								
-lymphocytic depletion					1/10	2/10	10/10	9/10
-macrophage accumulation							2/10	1/10
-plasmacytosis	3/10	8/10	9/10	6/10	9/10	6/10	10/10	10/10
Mesenteric lymph node								
-lymphocytic depletion					3/10	2/10	10/10	7/10
-macrophage accumulation						1/10	5/10	7/10
-plasmacytosis			2/10		1/10	3/10	7/10	7/10
-multinucleated giant cells							1/10	2/10
Mandibular lymph node								
-lymphocytic depletion							8/10	6/10
-macrophage accumulation							3/10	
-plasmacytosis	1/10	1/10					9/10	8/10
Spleen								
-increased no. of macrophages					5/10	4/10	10/10	9/10
-lymphoid depletion						2/10	10/10	9/10
Thymus								
-lymphoid depletion							8/10	6/10
Intestine (large)								
-inflammation cell infiltration		1/10			3/10	6/10	10/10	8/10
-mucosal hyperplasia						2/10	9/10	9/10
Peyer's patches								
-lymphoid depletion							4/10	5/10
Epididymides								
-abnormal cells/cell debris							1/10	
-oligospermia							1/10	
Testes								
-tubular atrophy							2/5	
-multinucleated giant cells					1/10		1/10	

-Sertoli-cell vacuolation			7/10		10/10		9/10	
Recovery (Satellite) animals	6M	6F	6M	6F	6M	6F	5M	6F
Cervical lymph node								
-plasmacytosis	4/6	4/6	4/6	6/6	5/6	3/6	5/5	3/6
Mesenteric lymph node								
-plasmacytosis	2/6		3/6	3/6	1/6	2/6	2/5	1/6
Mandibular lymph node								
-plasmacytosis	4/6	5/6					5/5	3/6
Testes								
-tubular atrophy							1/5	
-degeneration of spermatids							5/5	
-delayed spermatogenesis							5/5	
-Sertoli-cell vacuolation	4/6		4/6		4/6		5/5	
Epididymides								
-abnormal cells/cell debris							5/5	
-oligospermia							5/5	

Toxicokinetics:

Administration of fludarabine phosphate at daily oral doses of 10, 25 and 75 mg/kg resulted in a dose related increase of systemic exposure for the metabolite ZK 159155 (2F-ara-A) in AUC_(0-6h) values in male and female rats. The dose-proportion factor revealed an increase of systemic exposure corresponding with increased dose on day 1, and on day 25 from medium to the HD after single and repeated administration (Table TT 13). No accumulation was observed after repeated administration at all dose levels.

TF 9: Dose-dependent systemic exposures (AUC_(0-6h)) of ZK 159155 (2F-ara-A) after oral administration of Fludarabine phosphate (2F-ara-AMP) to rats on day 1 and day 25

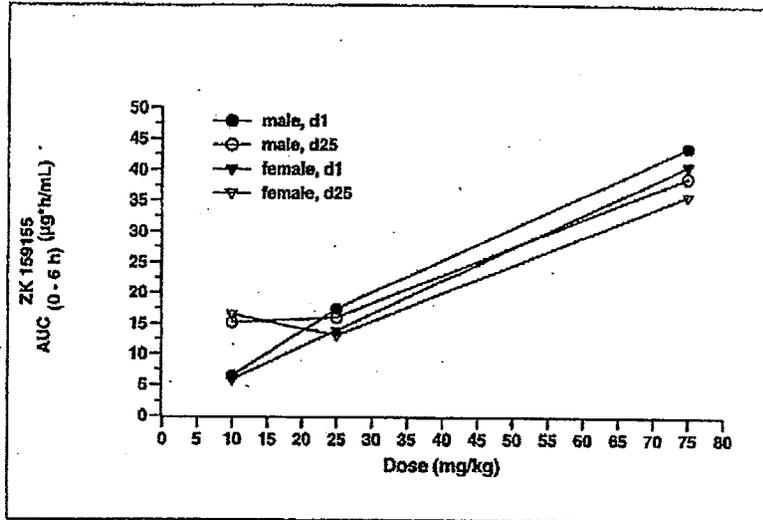


Figure excerpted from the sponsor

TT 13: Mean toxicokinetic parameters of ZK 159155 (2F-ara-A) in rats after oral treatment with Fludarabine phosphate (2F-ara-AMP) on test day 1 and day 25

Parameters	[unit]	Time [day]	Dose (mg Fludarabine phosphate/kg)					
			10		25		75	
			m	f	m	f	m	f
C _{max}	[µg/mL]	1	2.34±0.357	1.42±0.222	4.60±0.348	3.77±0.854	10.6±2.05	10.1±1.60
		25	3.74±0.852	4.00±0.748	4.48±1.15	4.70±0.543	10.9±2.32	8.35±3.71
t _{max}	[h]	1	0.5	1.5	1.5	0.5	1.5	1.5
		25	0.5	1.5	1.5	0.5	1.5	1.5
AUC _(0-6h)	[µg x h/mL]	1	6.40	5.84	17.4	13.9	43.7	40.8
		25	15.2	16.5	16.0	13.1	38.9	35.9
AUC _(0-24h)	[µg x h/mL]	1	n.e.	n.e.	n.e.	n.e.	80.8	71.9
		25	n.e.	n.e.	n.e.	n.e.	60.6	70.2
R			2.38	2.83	0.92	0.94	0.89	0.88
DPF		1	1	1	1.09	0.95	0.91	0.93
	25	1	1	0.42	0.32	0.34	0.29	

m male

f female

n.e. not evaluable

C_{max} maximum measured concentration of drug in plasma after drug administrationt_{max} time-point to reach C_{max}AUC_(0-nh) area under the concentration versus time curve from dosing time to 6 h or 24 h post administrationR accumulation factor (AUC_(0-6h) d25/AUC_(0-6h) d1)

DPF dose normalized dose proportionality factor:

[(AUC(x mg/kg)/AUC(10 mg/kg))/(x mg/kg/10 mg/kg)] for the same day

Excerpted from the sponsor

Study title: Multidose oral toxicity study (pilot) of fludarabine in dogs

Key study findings:

- Toxic effects without mortality were observed following HD (3000 mg/m²) independent of route of administration (IV vs PO).
- Clinical signs related to the test article were emesis (d5) in the male given the HD intravenously and general body trembling (d1-2) in female given HD orally.
- Increased segmented neutrophil counts and decreased lymphocytes were seen following the HD both routes of administration.
- No drug-related abnormalities were seen in these dogs by physical and neurological evaluations.
- Microscopic findings were similar in IV and PO HD group.

Study no:

Schering report no. AB94

Volume/Pages:

Module 4.2.3.2.3

Conducting laboratory and location:

[]

b(4)

Date of study initiation:

April 13, 1988

Date of final report: May 24, 1989
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: Fludarabine phosphate, #AP-02-287
Formulation/vehicle: Saline

Dosing: Daily for 5 days
Species/strains: Beagle dogs
#/group: 1/sex/group
Initial Age: ~7-8 months
Weight: M: 13.0-13.6 kg; F: 9.5-12.0 kg

Treatment Regimen:

Species	Number/Sex per group	Route	Dose Level mg/kg (mg/m ²)	Duration
Beagle dogs	1M/1F	IV	150 (3000)	5 days
	1M/1F	Intragastric (gelatin capsule)	1.5, 15, 150 (30, 300, 3000)	5 days

Observation: Animals were observed daily following drug administration (daily x 5) with 14 days recovery period. Mortality, clinical signs, body weight, hematology, clinical pathology, and gross/histopathology were assessed at termination.

Results:**Mortality:**

No mortality, all animal survived scheduled sacrifices on days 6 and 20.

Clinical signs:

Clinical signs included diarrhea (soft/mucoid), inappetence, salivation, and ocular discharge in dogs given both 150 mg/kg intravenously and orally. Only slight soft stool and lacrimation were noted in dogs given 15 and 1.5 mg/kg/day.

Body weight/food consumption:

Body weight loss (>10%) was seen in both dogs given i.v. and oral dose of 150 mg/kg/day fludarabine which was not remarkable. Food consumption was not remarkable in both groups of animals.

Neurological evaluations:

Physical and neurological evaluations revealed that no-drug-related abnormalities were observed in these dogs.

Hematology:

Increased segmented neutrophil counts (↑34% i.v., ↑51% i.g.) and decreased lymphocyte counts (↓85% i.v., ↓52% i.g.) were seen only in males given i.v. and oral dose

of 150 mg/kg/day on day 6. No drug-related changes in red cell parameters or in total leukocyte counts were observed. All hematological changes were reversible on day 20.

Clinical chemistry:

Increased ALP, AST, ALT, BUN and creatinine values were observed in the male given i.v. 150 mg/kg/day on day 6. Similarly increased ALT, AST and ALP values were observed in the females given i.g. 150 mg/kg/day on day 6. Also decreased K (10%) and increased phosphorus (62%) and total protein (30%) levels were noted independent of routes. These drug-related changes were reversed on day 20. Clinical chemistry values for the remaining animals were not remarkable.

Urinalysis: Not remarkable

Gross pathology:

Dose-dependent gross pathology findings were not observed. However, skin nodules were seen in dog given 150 mg/kg/day orally; an oral nodule was noted in a dog given 150 mg/kg/day intravenously; and corneal opacity was noted in dog given 15 mg/kg/day orally.

Histopathology:

Drug-induced lesions in both male and female dogs sacrificed on days 6 and 20 included mild to moderate inflammation of GI tracts, mild necrosis of mesenteric lymph nodes, necrosis or lymphoid depletion in thymic and mesenteric region and moderate bone marrow hypocellularity at HD. Similar lesions were seen in the male dog treated with the HD orally sacrificed on day 6. These changes were less severe after intragastric dosing. Only minimal inflammation of the GI tract and thymic change were seen in 15 and 1.5 mg/kg/day groups.

Incidence and Severity^a of Microscopic Findings in dogs following 5-Day IV or PO doses

Dose, mg/m2/day	3000/IV		3000/PO		300/PO		30/PO	
	1M	1F ^b	1M	1F ^b	1M ^b	1F	1M ^b	1F
Bone marrow								
-hypocellularity	3	0	1	0	0	0	0	0
Gastrointestinal tracts								
-inflammation	3, 2	1	2, 1	0	0	1	0	0
Mesenteric lymph node								
-necrosis	2	0	0	0	0	0	0	0
Thymic region								
-necrosis or lymphoid depletion	2	1	1	0	0	0	0	0

Note- ^a 0=normal, 1=trace, 2=mild, 3=moderate; ^b =Sacrificed on day 20, all others sacrificed on day 6.

Study title: A 4-week systemic toxicity study of SHL 573A (fludarabine phosphate) by repeated oral administration to Beagle dogs with a subsequent recovery period of 4 weeks (Module 4.2.3.2.4)

Key findings:

- No mortality or drug-related clinical signs were observed.
- At MD and HD, slight decreases in red cell parameters and leukocyte counts and increased fibrinogen values were observed.
- Macroscopically, increased adrenal and decreased thymus weight were noted.
- Microscopic findings from the MD and HD included lymphoid depletion in the thymus and atrophy of testicular seminiferous tubules, increased vacuolation of Sertoli cells, accumulation of degenerated spermatids, and cell debris and oligospermia in the epididymides.

Study no: Study report no. A22370
Volume/Pages: Module 4.2.3.2.4
Conducting laboratory and location: -7
Date of study initiation: Jan 14, 2002
Date of final report: Aug 12, 2004
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: ZK 153851(fludarabine phosphate) Batch # 131570
Formulation/vehicle: 50 mg mannitol; 1N NaOH (pH=7.6-7.8); water for injection
Dosing: Once Daily for 4 weeks
Species/strains: Beagle dogs
#/group for main study: 3/sex/group
#/group for satellite study: 2/sex/group
Initial Age: 7 months
Weight: ♂: 6.5-9.5 kg, ♀: 5.3-7.1 kg

b(4)

TT 1: Treatment schedule

Group/ Dose level	Number of animals/sex MS + SA	Compound	Dose per day [mg/kg]	Administration volume [mL/kg]	Animal number and sex	
					MS	SA
1 control	3 + 2 ♂	Vehicle	N/A	4	1-3 ♂	4-5 ♂
	3 + 2 ♀				6-8 ♀	9-10 ♀
2 4 mg/kg	3 + 2 ♂	Fludarabine phosphate	4	4	11-13 ♂	14-15 ♂
	3 + 2 ♀				16-18 ♀	19-20 ♀
3 16 mg/kg	3 + 2 ♂	Fludarabine phosphate	16	4	21-23 ♂	24-25 ♂
	3 + 2 ♀				26-28 ♀	29-30 ♀
4 60 mg/kg	3 + 2 ♂	Fludarabine phosphate	60	4	31-33 ♂	34-35 ♂
	3 + 2 ♀				36-38 ♀	39-40 ♀

MS: main study
 SA: satellite animals for the recovery period N/A: not applicable

Excerpted from the sponsor

Observations and times:

Clinical signs:	Daily
Body weight:	Weekly
Food consumption:	Weekly
Ophthalmic exam:	Days predose (test day 0) and 56
ECG:	Predose (before the 1 st dose) and 1 hr after the dose, and days 21, 28, and 56
Hematology:	Weekly
Bone marrow examination:	Days 29 and 57
Clinical chemistry:	Weekly
Urinalysis:	Days 29 and 56
Gross Pathology:	Day 29
Histopathology:	Day 29
Toxicokinetics:	Blood samples were collected on days 0 and 28 at 0.5, 1, 3, 6, 12, and 24 hrs post-dose.

Results:

Mortality: None died

Clinical signs: Not remarkable

Body weight/food consumption:

The body weight of male and female dogs were not influenced by oral treatment with either 4, 16 or 60 mg/kg fludarabine phosphate per day in the main study or in the satellite animals.

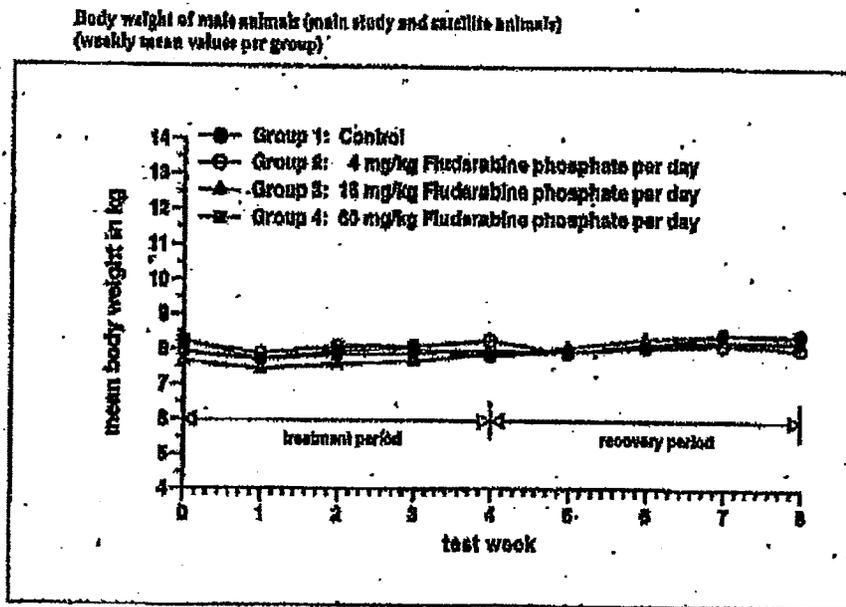


Figure excerpted from the sponsor

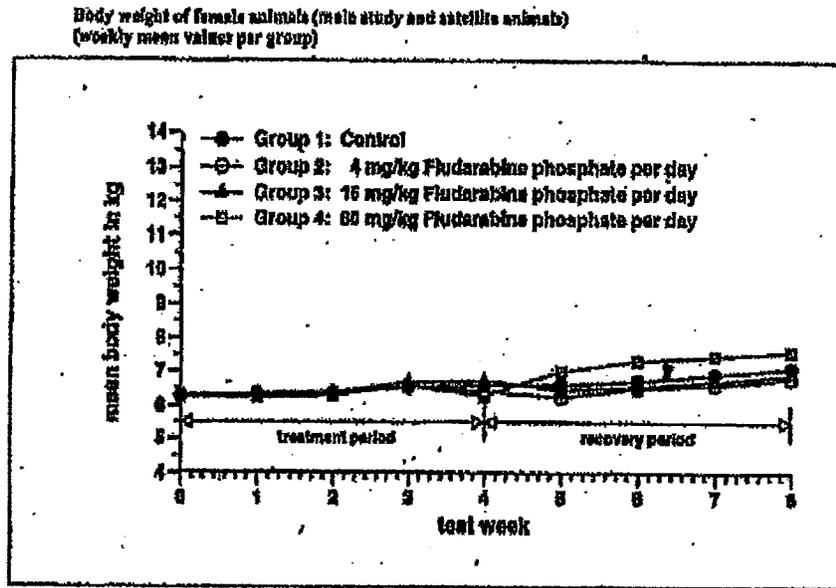


Figure excerpted from the sponsor

Food consumption:

The daily relative food consumption was not affected by oral treatment with either 4 or 16 mg/kg fludarabine phosphate per day. At a dose of 60 mg/kg, the food consumption of males was significantly ($p \leq 0.05$) reduced in test week 3. Relative food consumption was not affected in female animals.

TF 1: Daily relative food consumption of male animals (main study and satellite animals)
(weekly mean values per group)

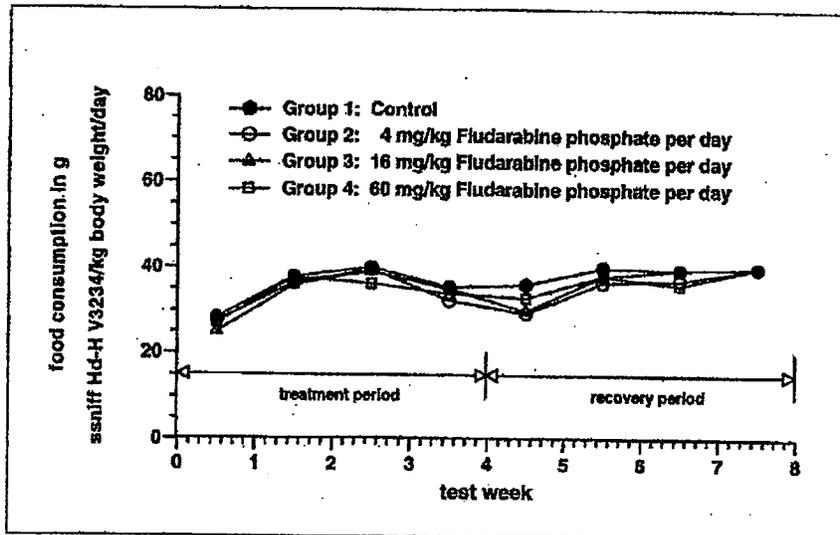


Figure excerpted from the sponsor

TF 2:

Daily relative food consumption of female animals (main study and satellite animals)
(weekly mean values per group)

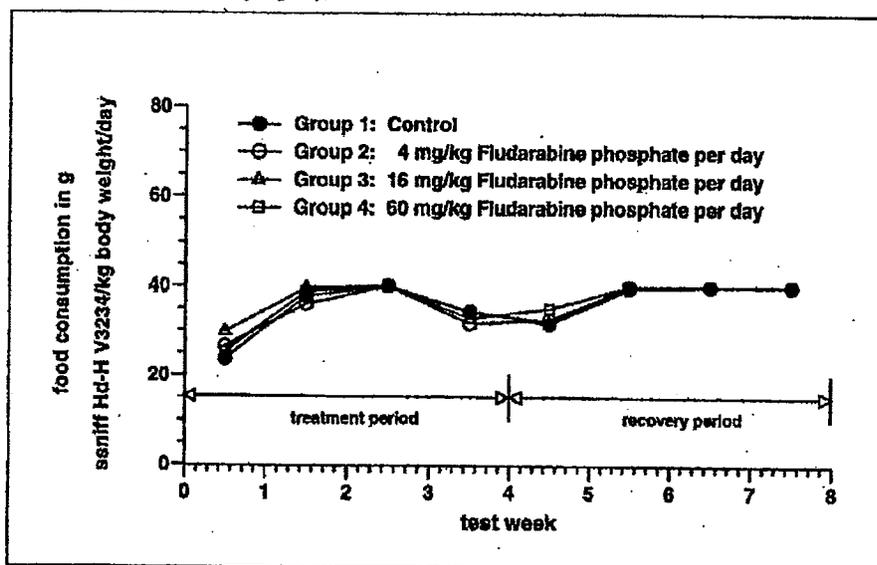


Figure excerpted from the sponsor

Ophthalmic examination: Not remarkable

Reflex testing/auditory examination:

Examination of the patella reflex revealed fludarabine phosphate had no effects on the reflexes after the 4-week treatment in the main study and satellite animals. There was no indication of any auditory effect after the 4-week treatment period (test day 28) in main and satellite animals.

ECG examination:

ECG examinations were conducted during the study weeks 1, 3, 4, and 8 (recovery period) pre-dose and 1 hr post-dose. Based on an ECG assessment, there were no fludarabine phosphate effects on heart rate or ECG parameters including QRS and QT- intervals at all dose levels.

Hematology:

No treatment-related hematological changes were noted in the LD or MD group. At HD of 60 mg/kg, decreased red cell parameters, in addition, an increase in fibrinogen was noted in the HD males and females.

		Hematology Findings 4-Week Oral Gavage Toxicity Study with ZK 15385 in Dogs with 4-Week Recovery Period							
		0		4		16		60	
Dose, mg/kg/day	Sex of Animals	M	F	M	F	M	F	M	F
Number of Animals		3	3	3	3	3	3	3	3
RBC (10 ¹² /L)	Tw 4	5.50	5.66	5.50	5.56	5.82	6.12	4.80	5.12
	Tw 8	5.30	5.45	5.10	5.05	5.75	5.50	4.95	5.30
Hematocrit (%)	Tw 4	38	37	39	37	42	40	38	33
	Tw 8	35	37	35	35	40	40	34	38
Hemoglobin (mmol/L)	Tw 4	7.84	8.22	7.96	7.94	8.56	8.08	7.74	8.22
	Tw 8	7.40	8.2	7.20	7.35	8.25	8.05	6.75	7.60
WBC (10 ⁹ /L)	Tw 4	8.66	8.82	8.38	8.38	7.62	9.60	5.92	5.22
	Tw 8	10.00	10.55	9.10	9.90	10.10	12.65	8.80	14.40
Fibrinogen (mg/dL)	Tw 4	123	104	133	93	137	134	140	159
	Tw 8	107	97	104	64	114	116	101	113

Note: Tw=Test week

Table excerpted from the sponsor

Clinical chemistry: Not remarkable**Urinalysis:** Not remarkable**Gross pathology:**

Treatment-related visible macroscopic changes in main study animals were limited to reduced thymus size in one HD female. Scarred retractions of the spleen were noted in one male each at MD and HD, and thickening, black-red discolored spleen was noted in 2 males at MD group in the satellite animals.

Organ weight:

At terminal sacrifice, dose-related decreases in thymus weights and increases in adrenal weights were noted in the main study.

Dose, mg/kg/day	Percent Deviation from Control							
	Control		4		16		60	
# of Animals/Sex	3M	3F	3M	3F	3M	3F	3M	3F
Main study								
Body weight								
-absolute (kg)	7.90	5.97	-	-	-	-	-	-
Thymus:								
-absolute (g)	7.5	5.0	-	-	-28%	-22%	-41%	-40%
-relative (g/kg bw)	1.0	0.8	-	-	-30%	-25%	-40%	-38%
Adrenals:								
-absolute (g)	1.73	1.56	-	-	-	+21%	+25%	+32%
-relative (g)	0.22	0.26	-	-	-	+15%	+23%	+12%

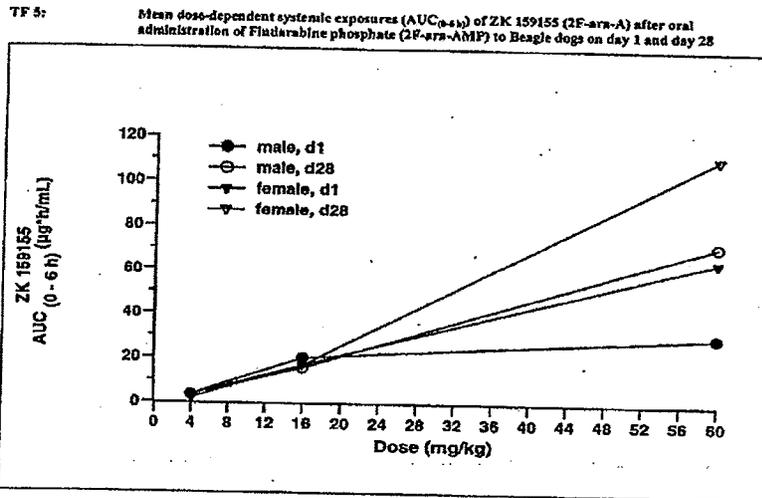
Note: bw=body weight

Histopathology:

Dose, mg/kg/day	0		4		16		60	
No. Animals Examined	3	3	3	3	3	3	3	3
Sex of Animals	M	F	M	F	M	F	M	F
Terminal Sacrifice (Day 30)								
Thymus								
-lymphoid depletion					3/3	3/3	3/3	3/3
Epididymides		NA		NA		NA		NA
-abnormal cells/cell debris					1/3			
-oligospermia					2/3			
Testes		NA		NA		NA		NA
-tubular atrophy					2/3		2/3	
-Sertoli-cell vacuolation					1/3		2/3	
Satellite animals (Day 56)	2M	2F	2M	2F	2M	2F	2M	2F
Epididymides		NA		NA		NA		NA
-abnormal cells/cell debris					1/2		2/2	
-oligospermia							2/2	
Testes		NA		NA		NA		NA
-tubular atrophy							2/2	
-Sertoli cell vacuolation							2/2	

Toxicokinetics:

Toxicokinetic evaluation revealed a dose-dependent increase for the metabolite 2F-ara-A in AUC_(0-6h) values after single and repeated administration of oral doses of Fludarabine phosphate (ZK 159851). Although the mean AUC values are different, the individual plasma concentrations are similar, therefore, no gender differences in systemic exposure of 2F-ara-A were observed.



Excerpted from the sponsor's submission

TT 15: Mean (\pm SD) toxicokinetic parameters of ZK 159155 (2F-ara-A) in Beagle dogs after oral treatment with Fludarabine phosphate (2F-ara-AMP) on test day 1 and day 28 (mean values of n=3 per gender)

Parameters	[unit]	Time [day]	Dose (mg/kg Fludarabine phosphate)					
			4		16		60	
			m	f	m	f	m	f
C_{max}	[μ g/mL]	1	0.670*	0.450 \pm 0.004	5.92 \pm 1.29	5.60 \pm 0.414	6.46**	15.8 \pm 7.37
		28	0.860 \pm 0.255	1.02 \pm 0.556	4.63 \pm 1.09	6.29 \pm 3.29	21.9 \pm 2.94	30.8**
t_{max}	[h]	1	0.5*	1.5 \pm 0	0.5 \pm 0	0.83 \pm 0.58	1.5**	1.5 \pm 0
		28	1.5 \pm 0	0.83 \pm 0.58	1.17 \pm 0.58	0.83 \pm 0.58	0.5 \pm 0	1.5**
$AUC_{(0-6h)}$	[mgxh/mL]	1	3.48*	1.87 \pm 0.082	19.9 \pm 2.28	16.7 \pm 2.44	29.9**	64.4 \pm 24.6
		28	3.38 \pm 0.822	3.41 \pm 1.15	15.4 \pm 1.43	16.6 \pm 5.59	71.7 \pm 8.80	111**
$AUC_{(0-24h)}$	[mgxh/mL]	1	n.e.	n.e.	n.e.	n.e.	78.8*	109 \pm 18.2
		28	n.e.	n.e.	n.e.	n.e.	99.7 \pm 11.5	178*
R			1.23	1.82 \pm 0.59	0.78 \pm 0.12	1.03 \pm 0.47	2.40	1.94
DPF		1	1	1	1.43	2.23	0.57	2.29
		28	1	1	1.14	1.22	1.41	2.17

* n = 1 animal
 ** n = 2 animals
 n.e. not evaluable
 m male
 f female
 C_{max} maximum measured concentration of drug in plasma after drug administration
 t_{max} time-point to reach C_{max}
 $AUC_{(0-6h)}$ area under the concentration versus time curve from dosing time to 6 h or 24 h post administration
 R accumulation factor ($AUC_{(0-6h)}$ d28 / $AUC_{(0-6h)}$ d1)
 DPF dose normalized dose proportionality factor:
 $[(AUC(x \text{ mg/kg})/AUC(4 \text{ mg/kg})) / (x \text{ mg/kg}/4 \text{ mg/kg})]$ for the same day
 SD standard deviation

Excerpted from the sponsor

2.6.6.4 Local tolerance

No studies submitted

2.6.6.5 Special toxicology studies

No studies submitted

2.6.6.6 Genetic toxicology

No studies submitted

2.6.6.7 Carcinogenicity studies

No studies submitted

2.6.6.8 Reproductive and developmental toxicology

No studies submitted

2.6.7 TOXICOLOGY TABULATED SUMMARY

Summary of Acute and Repeat dose toxicology studies

Acute Toxicity Studies				
Species/ Study No.	Route/Duration	N/sex/ dose	Doses, mg/kg (mg/m ²)	Significant findings
Rat AB98	i.g. (intragastric) single	3	Fludarabine phosphate: 250-1000 (1500-6000)	6000 mg/m ² : Hypoactivity and ↓ body weight gain
Rat A22092	i.g. single	3	Fludarabine phosphate: 1500-2000 (9000-12000)	12000 mg/m ² : Transient signs of GI toxicity noted and the LD50 was >12000 mg/m ² .
Repeat Dose Toxicity Studies				
Species/ Study No.	Route/Duration	N/sex/ dose	Doses, mg/kg/day (mg/m ² /day)	Significant findings
Rat AA95	i.g. and i.v. 14-days	10 or 20	Fludarabine phosphate: i.v.: 0*(0), 20 (120), 75 (450), 200*(1200) i.g.: 0*(0), 20 (120), 75 (450), 200*(1200), 250 (1500) * indicates 20/sex/dose	1500 mg/m ² : 11/20 i.g. rats died, ↑ ALT, AST, AP and BUN by both routes 1200 mg/m ² : 40/40 i.v., and 15/40 i.g. rats died, elevated liver enzymes by both routes. 450 mg/m ² : 1/20 i.g. rat died, ↑ red cell mass (♀ only by i.v.), ↓ leukocytes and lymphocytes by both routes, histopath. changes in thymus, spleen, lymph node, enteropathy, bone marrow and atrophy of salivary gland by both routes. Qualitatively similar toxic profiles were observed after i.v. or i.g. doses.
Dog AB94	i.g.** and i.v. 5 days	1	Fludarabine phosphate: i.v.: 150 (3000) i.g.: 1.5 (30), 15 (300), 150 (3000)	3000 mg/m ² : body trembling, emesis, and signs of GI toxicity by both routes, ↑ segmented neutrophils, ↓ lymphocytes, ↑ liver enzymes, histopath. changes in GI tracts, lymphoid tissues and bone marrow. Overall, similar drug-related changes were noted in both routes.
Rat A22369	i.g.	10	Fludarabine phosphate: 0 (0), 10 (60), 25 (150), 75 (450)	450 mg/m ² : 1/6 ♂ dead, ≥150 and 450 mg/m ² : piloerection, ↓ body weight gain and affected tissues included the peripheral blood, GI tract, lymphatic organs and genital tract. Most test-related changes were reversed by the end of recovery period, however, testicular changes were noted in the recovery males.
Dog A22370	i.g.		Fludarabine phosphate: 0 (0), 4 (80), 16 (320), 60 (1200)	1200 mg/m ² : ↓ RBC parameters, ↑ fibrinogen, and ↓ thymus size. ≥320 mg/m ² : ↑ adrenal weight, ↓ thymus weight, thymic lymphocytic depletion, and testicular atrophy.

Note: **=oral capsule

OVERALL CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: Oral fludarabine phosphate is approvable from pharmacology/toxicology perspective.

Unresolved toxicology issues (if any): None

Recommendations: This NDA is approvable from a pharmacology/toxicology perspective.

Suggested labeling: Separate review will be conducted.

Reviewer Signature _____

Supervisory Signature _____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS

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this page is the manifestation of the electronic signature.**

/s/

Doo Young Lee-Ham
8/25/2008 01:57:47 PM
PHARMACOLOGIST

Leigh Verbois
8/25/2008 02:09:43 PM
PHARMACOLOGIST

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