CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022561Orig1s000

NON-CLINICAL REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	22561
Supporting document/s:	61
Applicant's letter date:	5/31/18
CDER stamp date:	5/31/18
Product:	Mavenclad (cladribine)
Indication:	Multiple sclerosis
Applicant:	Merck Serono, Inc.
Review Division:	DNP
Reviewer:	Melissa Banks-Muckenfuss, PhD
Supervisor:	Lois Freed, PhD
Division Director:	Billy Dunn, MD
Project Manager:	Sandy Folkendt, RPM

Disclaimer

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1 Executive Summary

1.1 Introduction

Cladribine is a purine antimetabolite, initially approved as Leustatin® as an IV treatment for hairy cell leukemia, that has been developed for the oral treatment of relapsing forms of multiple sclerosis (RMS).

1.2 Brief Discussion of Nonclinical Findings

The majority of the nonclinical information, and all studies required, to support NDA 22561 were previously submitted and reviewed (see nonclinical review dated February 18, 2011, as well as the secondary and tertiary reviews). This review acts as a supplemental review to the existing nonclinical review.

In the resubmission, ADME information and two non-pivotal combination toxicity studies (testing PO administration, up to 5 weeks of dosing cycles of cladribine in combination with SC Copaxone®) were submitted. The ADME information and relevant information from the two combination toxicity studies in mice (which tested a higher maximum dose of cladribine than in the chronic oral cyclical-administration toxicity study of cladribine) are summarized herein. It is noted that the toxicities observed with the higher dose of cladribine (i.e., 30 mg/kg) in the combination studies (e.g.., renal, testicular, and lymphoid tissue toxicity) were generally similar to those observed in previous studies by various routes.

1.3 Recommendations

1.3.1 Approvability

The nonclinical recommendation remains approvable. Based on the first cycle nonclinical reviews, additional nonclinical information was not required for approval.

1.3.3 Labeling

The following contains suggested text for labeling. Labeling was not addressed in the previous review cycle.

Under INDICATIONS AND USAGE
MAVENCLAD is a purine antimetabolite indicated for the treatment of patients with relapsing forms of multiple sclerosis (MS).

8.1 Pregnancy
Risk Summary

(b) (4)

(b) (4)

Data

Animal Data

(b) (4)

12.1 Mechanism of Action

(b) (4) The mechanism by which cladribine exerts its therapeutic effects in MS patient has not fully elucidated. (b) (4)

(b) (4)

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

(b) (4)

Mutagenicity

(b) (4)

Fertility

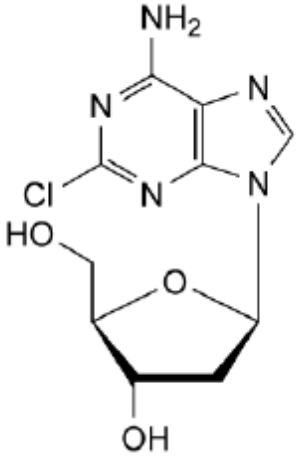
(b) (4)

(b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number	4291-63-8
Generic Name	cladribine
Chemical Name	2-Chloro-2'-deoxyadenosine
Molecular Formula/Molecular Weight	$C_{10}H_{12}ClN_5O_3$ / 285.69
Structure or Biochemical Description	(below from the sponsor)



Pharmacologic Class	purine antimetabolite

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 74634

2.3 Drug Formulation

The sponsor provided Table 1 below describing the composition of cladribine 10 mg tablets.

Table 1 Qualitative and Quantitative Composition of Cladribine Drug Product

Component	Quality Standard (and Grade, if applicable)	Function	Amount per Unit	
			[mg/Tablet]	[%/Tablet]
Cladribine	Ph. Eur.* / USP*	Active ingredient	10.00	(b) (4)
(b) (4)				
Hydroxypropyl betadex (2-Hydroxypropyl-β-cyclodextrin)	Ph. Eur.* / USP-NF*	(b) (4)		
Sorbitol	Ph. Eur.* / USP-NF*			
Magnesium stearate	Ph. Eur.* / USP-NF* (b) (4)			
Total Tablet Weight				

n/a = not applicable; NF = United States National Formulary; Ph. Eur. = European Pharmacopeia; USP = United States Pharmacopeia

* Current version

(b) (4)

2.4 Comments on Novel Excipients

Hydroxypropyl-beta-cyclodextrin (HPβCD) is used as an excipient in this oral formulation. The potential toxicity of this excipient was addressed in the previous nonclinical reviews for NDA 22561 (see review dated February 18, 2011).

2.5 Comments on Impurities/Degradants of Concern

Twelve potentially genotoxic impurities were identified as structurally-related compounds (confirmed by CMC) that shared the same structural alert with parent, which was not mutagenic. No other impurities were identified as requiring further assessment.

2.6 Proposed Clinical Population and Dosing Regimen

The recommended cumulative dose of cladribine is 3.5 mg/kg body weight over 2 years, administered as 2 yearly treatment courses of 1.75 mg/kg/year. Each yearly treatment course consists of two (b) (4) treatments, consisting 4 or 5 daily oral administrations of 10 mg or 20 mg of cladribine (1 or 2 tablets) depending on body weight, (b) (4) (b) (4). After the two yearly treatment courses, no further cladribine treatment is indicated in Years 3 and 4.

2.7 Regulatory Background

The sponsor stated that EMD Serono originally submitted NDA 22561 for cladribine oral tablets for the treatment of relapsing multiple sclerosis on May 27, 2010 (Seq. 0013); the Agency issued a Complete Response Letter, which the sponsor received on February 28, 2011. The sponsor subsequently requested that NDA 22561 be withdrawn on August 19, 2011 (Seq. 0056). NDA 22,561 was resubmitted on May 30, 2018.

3 Studies Submitted

3.1 Studies Reviewed

Studies 29061 and 29345
Various ADME studies

3.3 Previous Reviews Referenced

N22561 Nonclinical Review, dated 2/18/11

4 Pharmacology

Cladribine is a purine antimetabolite. The mechanism by which it exerts its effect in the treatment of multiple sclerosis is not fully elucidated.

Cladribine (also called 2-chloro-2′-deoxyadenosine, 2-CdA) is a synthetic chlorinated analogue of the naturally occurring nucleoside deoxyadenosine. Cladribine is phosphorylated to the active triphosphate form (2-CdATP), which causes impairment of DNA synthesis and cellular metabolism; this effect is presumed to generate its immunosuppressant effect via the destruction of immune cells.

5 Pharmacokinetics/ADME/Toxicokinetics

See the nonclinical review dated February 18, 2011 for most of the ADME information. The sponsor submitted several additional studies, summarized below.

An in vitro study investigating the distribution kinetics of radiolabeled cladribine into human lymphocytes and the intracellular accumulation of cladribine and/or its metabolites was conducted. Distribution of cladribine (0.1 mcM; "expected clinical Cmax") into human lymphocytes was time-dependent. The rate of accumulation was rapid (particularly over the first hr), with the maximum concentration of radioactivity in lymphocytes occurring at approximately 3 hr, and reached approximately 30-fold that in the whole lymphocyte suspension; radioactivity concentration remained stable or was slightly reduced over a 24 hr incubation period. The accumulation was reportedly concentration-dependent. While the absolute concentration in lymphocytes increased with increasing cladribine concentration, the relative concentration of total radioactivity (i.e., lymphocyte radioactivity to whole cell suspension radioactivity) was greater at the lowest concentration tested (38 to 47-fold at 0.03 mcM cladribine) than at the highest concentration tested (i.e., 19 to 20-fold 0.3 mcM).

Several non-GLP assays investigated the effects of cladribine on hepatocytes and CYP P450 enzymes. Cladribine was not actively taken up by human hepatocytes. Uptake was neither concentration-dependent nor saturable (approximately 1 to 251 mcM cladribine). Uptake was also not time-dependent and did not appear to involve the transporters tested (1 mcM cladribine; based on use of a "pan-mix inhibitor cocktail" containing rifampicin, cyclosporine A, and quinidine). Cladribine showed concentration-dependent toxicity in hepatocytes, particularly from 11.1 mcM (80% viability) to 251.1 mcM (43% viability). In an assay of cladribine's effects on CYP enzymes, concentration-dependent hepatocyte toxicity was observed (beginning as low as 10 mcM), as were effects on CYP1A2 (increase in mRNA; EC_{50}= 4.8 mcM, but "less than 8% as effective… as omeprazole"), CYP2B6 (reduction in mRNA), and CYP3A4 (reduction in mRNA). Inhibition of CYP3A4/5 activity (~20%) was observed as measured by testosterone 6beta-hydroxylation (estimated IC_{50} >390 mcM), but not midazolam 1'-hydroxylation or nifedipine oxidation.

It was determined during the first cycle that there were no circulating major human metabolites after oral administration of cladribine. The sponsor provided additional non-

GLP in vitro assays investigating the metabolism of cladribine. In plasma and blood cells from human, monkey, and mouse, several potential metabolites were observed after incubation with radiolabeled cladribine, including 2-chlorohypoxanthine (monkey and human; likely catalyzed by adenosine deaminase), 2-chlorodeoxyinosine, and 2-chloroadenine (2-CA; main metabolite identified in mouse). Formation of 2-chlorohypoxanthine suggested that cladribine might not be as resistant to adenosine deaminase, an enzyme involved in cladribine's mode of action in addition to deoxycytidine kinase, as previously believed. In hepatocytes from CD-1 mouse, SD rat, NZ rabbit, beagle dog, Cynomolgus monkey, and humans, little metabolism was observed for human, dog, and mouse (see the sponsor's table, below).

Metabolite	Male subject 1		Male subject 2		Male subject 3		Male monkey		Female dog		Female rabbit		Male rat		Male mouse	
	6h	24h	6h	24h	6h	24h	6h	24h	6h	24h	6h	24h	6h	24h	6h	24h
2-CAD[a]	-	-	-	-	-	-	-	-	-	-	3.8	-	1.4	-	-	-
M185	1.9	8.9	2.3	9.2	2.0	9.9	2.3	8.6	2.7	7.1	13.4	46.0	4.0	18.6	2.2	10.2
M461-1	-	1.1	-	1.6	-	1.3	6.3	-	-	-	-	-	1.4	3.2	-	-
M461-2	-	-	-	-	-	-	-	20.4	-	-	-	-	1.1	3.9	-	-
M461-3	1.4	5.2	-	3.6	-	3.8	6.1	23.1	-	4.7	-	-	-	-	-	-
M461-4	-	1.1	-	-	-	-	-	-	-	2.1	-	-	-	-	-	-
Cladribine	96.7	83.7	97.7	85.6	98.0	85.1	82.3	40.3	97.3	86.0	82.8	51.6	92.1	71.5	97.8	89.8
M301	-	-	-	-	-	-	3.0	7.6	-	-	-	2.5	-	-	-	-
Unknowns	-	-	-	-	-	-	-		-	-	-	-	-	2.8	-	-

Values are expressed as % of sample radioactivity (%ROI)

a: 2-Chloroadenine

-: not detected

Several non-GLP in vitro assays assessing interactions between cladribine and a number of transporters were submitted. In one assay, cladribine (0.05 to 1 mcM) was shown not to be a substrate for MRP2, MRP5, or MRP4 human proteins in transfected dog MDCKII or HEK293 cells. Cladribine was a weak inhibitor of the human BCRP protein in transfected MDCKII cells (IC_{50} >150 mcM). No clear inhibitory potential of cladribine (12 and 60 mcM) on human transporters hOATP1B1, hOATP1B3, and hOCT1 was observed in transfected HEK293 cells. A series of assays demonstrated that cladribine (ranging from 0.05 to 2.8 mcmol/L among experiments) did not act as a substrate for transporters OAT1, OAT3, OAT4, and OCT2.

10 Special Toxicology Studies

The sponsor submitted two combination studies of oral cladribine and SC Copaxone® in mice. These studies were reviewed for effects related to cladribine exposure (the maximum dose, 30 mg/kg, was greater than that used in the chronic cyclic oral dosing study reviewed previously), and not effects attributed to Copaxone® or to the combination; the sponsor indicated that they do not intend to pursue an indication for combination treatment.

IMP29061 – RE7970: Multi Site 4-week toxicity study of oral Cladribine administration in combination with Copaxone subcutaneous injection in mice followed by 4 weeks of recovery
GLP (OECD); Initiated 6/11/08
Conducted by (b) (4)

Sponsor's Summary Design Table (below)

Group (No. of animals)	Compounds	Doses (mg/kg/day)	Admin. route	Dosing regimen
1 (15M + 15F)	Control item 1+ Control item 2	0 0	os s.c.	5 days on + 23 days off for 4 cycles+ 5 days on after the last cycle Daily
2 (15M + 15F)	cladribine	30	os	5 days on + 23 days off for 4 cycles + 5 days on after the last cycle
3 (15M + 15F)	copaxone	20	s.c.	Daily
4 (15M + 15F)	cladribine + copaxone	10 20	os s.c.	5 days on + 23 days off for 4 cycles + 5 days on after the last cycle Daily
5 (15M + 15F)	cladribine + copaxone	30 20	os s.c.	5 days on + 23 days off for 4 cycles + 5 days on after the last cycle Daily

Control item 1: vehicle for cladribine tablet with concentration of HPβCD present in the cladribine high dose (30 mg/kg).
Control item 2: 40 mg/mL Mannitol in WFI.

Animals: Crl:CD-1(ICR) mice, 5 weeks old at arrival
Main: 10/sex/gp, Recovery 5/sex/gp
(TK was conducted in a satellite group.)
Drug: Cladribine tablets (IVAX), batch N0219A, 106.4%
Tablets were dissolved in deionized water
(Control item 1 was HPβCD in deionized water)

Mortality occurred in one cladribine-alone male animal (1/30; week 5), four Copaxone®-alone, and two animals treated with the combinations (one in each group); no cause of death was determined.

The sponsor reported cladribine-related slight reductions in alpha 1 globulin (sponsor's summary table, below).

Dose-level (mg/kg /day)		0a		30b		20c		10b+20c		30b+20c	
	Week	M	F	M	F	M	F	M	F	M	F
Alpha 1 globulin (%)	5	13.73	11.72	12.10*	10.98*	12.12*	10.52**	12.24*	9.93***	11.81**	9.92*
Alpha 2 globulin (%)	5	15.62	12.97	16.77	13.74	14.43	11.76	13.77**	11.97	13.95**	11.86
	10	15.41	12.95	15.86	13.30	16.32	10.78**	15.02	11.30	16.94	10.27 **
Beta globulin.(%)	5	18.01	15.62	17.57	17.29	20.12	19.94**	19.66	21.04**	21.92**	18.71**
	10	18.53	16.07	16.23	16.73	18.19	18.73	19.35	20.23*	23.34	18.82*

a: Vehicle; b: cladribine; c: copaxone

Statistical significance: * P<0.05; ** P<0.01; *** P< 0.001

No effects on lymphocyte subpopulations (by flow cytometry) were reported.

Reduced testes weight (~18% [ss]) was observed with cladribine alone, as well as in combination with Copaxone®; at recovery, mean testis weight (cladribine alone) was reduced approximately 10%. At the end of recovery, males previously treated with cladribine alone showed reduced relative kidney weights (~16% [ss], but was largely a result of reduced left kidney size in M#2310, which correlated with moderate cortical fibrosis histologically).

Histopathology was conducted by (b) (4). Cladribine 30 mg/kg (alone or in combination) increased the incidence of basophilic (regenerating) tubules and cortical fibrosis in the kidneys (see the summary data, below). Partial reversibility was observed after a 4-week recovery period. No clear histological correlate was observed for the reduced testicular weight.

TERMINAL

Group	1	2	3	4	5		1	2	3	4	5
Doses: Clad (PO) Copax (SC)	0 0	30 0	0 20	10 20	30 20		0 0	30 0	0 20	10 20	30 20
N=	10	10	10	10	10		10	10	10	10	10
Kidney											
Focus(i) of basophilic (regenerating) tubules	**4**	**6**	**1**	**2**	**6**		**2**	**7**	**2**	**1**	**2**
Minimal	*4*	*2*	*1*	*2*	*3*		*2*	*4*	*1*	*1*	*1*
Mild	*0*	*4*	*0*	*0*	*3*		*0*	*1*	*1*	*0*	*0*
Moderate	*0*	*0*	*0*	*0*	*0*		*0*	*2*	*0*	*0*	*1*
Cortical fibrosis	**1**	**3**	**0**	**1**	**4**		**0**	**3**	**1**	**0**	**1**
Minimal	*1*	*0*	*0*	*1*	*3*		*0*	*1*	*0*	*0*	*0*
Mild	*0*	*1*	*0*	*0*	*1*		*0*	*1*	*1*	*0*	*0*
Moderate	*0*	*2*	*0*	*0*	*0*		*0*	*1*	*0*	*0*	*1*
Tubular dilatation	**2**	**3**	**2**	**0**	**0**		**0**	**3**	**2**	**0**	**0**
Minimal	*2*	*2*	*1*	*0*	*0*		*0*	*2*	*1*	*0*	*0*
Mild	*0*	*1*	*1*	*0*	*0*		*0*	*1*	*0*	*0*	*0*
Moderate	*0*	*0*	*0*	*0*	*0*		*0*	*0*	*1*	*0*	*0*
Cortical Tubule Vacuolation, mild	**0**	**1**	**0**	**0**	**0**		**0**	**0**	**0**	**0**	**0**
Testes											
Focal germinal epithelial degeneration, bilateral, minimal	**0**	**1**	**0**	**0**	**0**		**-**	**-**	**-**	**-**	**-**

RECOVERY

Group	1	2	3	4	5		1	2	3	4	5
Doses: Clad (PO) Copax (SC)	0 0	30 0	0 20	10 20	30 20		0 0	30 0	0 20	10 20	30 20
N=	5	5	5	5	5		5	5	5	5	5
Kidney											
Focus(i) of basophilic (regenerating) tubules	**1**	**4**	**0**	**3**	**1**		**0**	**1**	**1**	**0**	**2**
Minimal	*1*	*2*	*0*	*3*	*1*		*0*	*1*	*1*	*0*	*2*
Mild	*0*	*1*	*0*	*0*	*0*		*0*	*0*	*0*	*0*	*0*
Moderate	*0*	*1*	*0*	*0*	*0*		*0*	*0*	*0*	*0*	*0*

Cortical fibrosis	**0**	**3**	**0**	**0**	**0**		**0**	**2**	**0**	**0**	**2**
Minimal	*0*	*0*	*0*	*0*	*0*		*0*	*1*	*0*	*0*	*1*
Mild	*0*	*2*	*0*	*0*	*0*		*0*	*1*	*0*	*0*	*1*
Moderate	*0*	*1*	*0*	*0*	*0*		*0*	*0*	*0*	*0*	*0*
Tubular dilatation	**0**	**1**	**0**	**0**	**0**		**0**	**1**	**1**	**0**	**1**
Minimal	*0*	*0*	*0*	*0*	*0*		*0*	*0*	*1*	*0*	*1*
Mild	*0*	*1*	*0*	*0*	*0*		*0*	*1*	*0*	*0*	*0*
Moderate	*0*	*0*	*0*	*0*	*0*		*0*	*0*	*0*	*0*	*0*
Focus(i) or hypertrophic tubular epithelium	**0**	**1**	**1**	**0**	**2**		**0**	**2**	**1**	**1**	**3**
Minimal	*0*	*0*	*1*	*0*	*1*		*0*	*1*	*0*	*1*	*2*
Mild	*0*	*1*	*0*	*0*	*1*		*0*	*1*	*1*	*0*	*1*

The sponsor provided the following TK summary table, below. The TK parameters for cladribine were calculated based on n=2/sex/time/group (at 0.5, 2, 5, and 24 hr postdose).

	Group 7		*Group 9*		*Group 10*	
Cladribine	**30 mg/kg/day**		**10 mg/kg/day**		**30 mg/kg/day**	
Copaxone	**-**		**20 mg/kg/day**		**20 mg/kg/day**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**
	Day 1					
C_{max} (ng/mL)	4580	3390	1440	1310	5160	4930
t_{max} (h)	1	1	1	1	1	1
C_z (ng/mL)	52.7	40.8	10.0	5.94	81.3	37.7
t_z (h)	5	5	5	5	5	5
AUC_{24} (h×ng/mL)	4108	3065	1236	994.7	4648	4310
AUC_{24} ratio	-	-	1	1	3.8	4.3
AUC_{24} ratio (comb. vs. alone)	1	1	-	-	1.1	1.4
GF AUC_{24} ratio	1.3	-	1.2	-	1.1	-
	Day 5					
C_{max} (ng/mL)	4880	3015	1880	4830	6725	7430
t_{max} (h)	1	1	1	1	1	1
C_z (ng/mL)	2.63	2.80	5.14	1.56	3.22	2.23
t_z (h)	24	24	5	24	24	24
AUC_{24} (h×ng/mL)	3498	4522	1351	3334	5022	5917
AUC_{24} ratio	-	-	1	1	3.7	1.3
AUC_{24} ratio (comb. vs. alone)	1	1	-	-	1.4	1.3
Rac AUC_{24}	0.9	1.5	1.1	3.4	1.1	1.4
GF AUC_{24} ratio	0.8	-	0.4	-	0.8	-
	Day 33					
C_{max} (ng/mL)	4750	3075	1300	696	8325	4225
t_{max} (h)	1	1	1	1	1	1
C_z (ng/mL)	1.13	73.2	2.96	4.70	1.67	2.55
t_z (h)	24	5	24	5	24	24
AUC_{24} (h×ng/mL)	4313	3190	1327	588.0	8362	4008
AUC_{24} ratio	-	-	1	1	6.3	6.8
AUC_{24} ratio (comb. vs. alone)	1	1	-	-	1.9	1.3
Rac AUC_{24}	1.0	1.0	1.1	0.6	1.8	0.9
GF AUC_{24} ratio	1.4	-	2.3	-	2.1	-

IMP29345 - RE7980: 4-month toxicity study of oral Cladribine administration in combination with Copaxone subcutaneous injection in mice followed by 8 weeks of recovery

GLP (OECD); Initiated 11/18/08

Conducted by (b) (4)

Sponsor's Summary Design Table (below)

Group/No. of animals		Compounds	Doses (mg/kg/day)	Admin. route	Dosing Regimen
1	18M+18F	control item 1*+	0	os	*b*
		control item 2**	0	s.c.	*c*
2	18M+18F	Cladribine Tablet	30[a]	os	*b*
3	18M+18F	Copaxone	20	s.c.	*c*
4	18M+18F	Cladribine Tablet +	10[a]	os	*b*
		Copaxone	20	s.c.	*c*
5	18M+18F	Cladribine Tablet +	30[a]	os	*b*
		Copaxone	20	s.c.	*c*
6	18M+18F	Cladribine drug substance +	30	os	*b*
		Copaxone	20	s.c.	*c*

**:Control item 1: 43.1 mg/mL of 2- hydroxypropil - β - cyclodestrin (HPβCD) in deionized water, vehicle used for Cladribine tablet with concentration of HPβCD present in the high dose (30 mg/kg) Cladribine.*

***: Control item 2: 40 mg/mL Mannitol in WFI, vehicle used for Copaxone.*

a: Doses are expressed as Cladribine drug substance equivalent present in tablets.

b: Cladribine was given to animals 5 days on + 23 days off for 4 cycles plus an additional 5-day dosing period at the end of 4 cycles just before sacrifice.

c: Copaxone was given to animals daily, until the day before sacrifice.

Animals: Crl:CD-1(ICR) mice, 5-6 weeks old at arrival (b) (4)
Main: 12/sex/gp, Recovery 6/sex/gp
(TK was conducted in a satellite group.)

Drug: Cladribine tablets (IVAX), batch N0219A, 106.4%
Cladribine drug substance (b) (4) batch 06P0557, 99.7-100.2%
Cladribine drug substance batch 07P0694, 100.3%
Cladribine was dissolved in ized water.

Mortalities occurred in all drug-treated groups during the study (see the summary table from the sponsor, below). Most of the deaths occurred in animals receiving 30 mg/kg cladribine alone or in combination.

No. of mice	Compounds administered	Week of death (range)
6M+4F	Cladribine 30 mg/kg/day	1 - 13
2F	Copaxone 20 mg/kg/day	2 - 3
2M	Cladribine 10 mg/kg/day + Copaxone 20 mg/kg/day	3
4M+1F	Cladribine 30 mg/kg/day + Copaxone 20 mg/kg/day	1-17 and 1st recovery
2M+1F	Cladribine 30 mg/kg/day (drug substance) + Copaxone 20 mg/kg/day	3 – 8th recovery

Clinical signs in these animals included piloerection, hypomotility, hypothermia, urine-stained fur, skin and mucosae pallor, skin sores, dyspnea, and/or decreased weight. Pale kidneys were observed in 2 of 6 male and 2 of 4 females from Gr#2 (i.e., 30 mg/kg cladribine) early decedents. Histologically, kidney alterations including cortical sclerosis (mild to moderate, 2 of 6 Gr#2 M) were observed in early decedent males receiving 30 mg/kg cladribine alone. The pathologist attributed the reduced survival in animals receiving 30 mg/kg cladribine alone in part to "the presence of cortical sclerosis in the kidneys in males but not females," and that in most instances "the cause of death was not identified."

Occasionally, similar clinical signs (e.g., hypomotility, hypothermia, dehydration and/or piloerection) were observed in surviving animals receiving cladribine 30 mg/kg, alone or in combination.

At the end of the dosing period, reduced body weights were observed in animals receiving 30 mg/kg cladribine alone or in combination (see the sponsor's table, below).

Mean body weight as % of the control value

Dose-level (mg/kg/day)	30a		20b		10a + 20b		30a + 20b		30c + 20b	
Sex	M	F	M	F	M	F	M	F	M	F
Week 1	0	-4.2	0.4	-2.0	-1.4	-1.4	-2.9	-1.5	-2.7	-4.3
Week 5	-0.4	1	-1.7	-3.3	-2.7	-2.4	-5.8	-5.4	-5.4	-4.3
Week 17	-7.4	1	-1.5	-5.3	-6.5	-2.6	-9.4	-7.7	-11.3	-7.1
Rec. Week 8	-7.1	10.6	-1.3	-4.9	-1.7	-4.9	-2.5	-2.5	-7.3	-1.7

a: Cladribine Tablet; b: Copaxone; c: Cladribine Drug Substance

In males, treatment with cladribine 30 mg/kg alone (or alone and in combination) resulted in reductions in RBC parameters (~20%, without clear recovery), increases in platelets (~1.5x, showed recovery), and increases urea (~3x, without clear recovery) and creatinine (~2x, partial recovery). See the sponsor's summary tables, below. These effects were not clearly observed in females. Clear reductions in lymphocytes were not observed. Urine pH was also reduced (pH of 6 versus 8 in controls).

Sex		Males					
Groups		1	2	3	4	5	6
Dose-level (mg/kg/day)		0	30 Clad.[a]	20 Copax.	10 Clad.[a]	30 Clad.[a]	30 Clad.[b]
Erythrocytes ($x10^6$/µL)	Week 17	8.66	* 7.25	8.54	9.02	* 7.74	8.21
	Week 25	8.68	* 7.15	8.14	8.44	7.81	* 7.14
Hemoglobin (g/dL)	Week 17	13.04	* 10.76	12.88	13.39	11.69	* 12.35
	Week 25	13.05	** 10.16	12.44	12.81	11.67	* 10.63

Sex		Males					
Groups		1	2	3	4	5	6
Dose-level (mg/kg/day)		0	30 Clad.[a]	20 Copax.	10 Clad.[a]	30 Clad.[a]	30 Clad.[b]
Hematocrit (%)	Week 17	44.30	* 37.11	43.92	45.68	* 39.52	42.44
	Week 25	44.59	** 34.66	42.53	43.91	39.84	* 36.25
Platelet ($x10^3$/µL)	Week 17	684.08	** 1019.00	752.58	728.30	** 863.50	** 859.45
	Week 25	725.17	867.67	781.83	772.83	834.17	974.20

ANOVA: Significance Level: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
(a): Cladribine Tablet; (b): Cladribine Drug substance

Sex		Males					
Groups		1	2	3	4	5	6
Dose-level (mg/kg/day)		0	30 Clad.[a]	20 Copax.	10 Clad.[a] 20 Copax.	30 Clad.[a] 20 Copax.	30 Clad.[a] 20 Copax.
Urea (mg/dL)	Week 17	52.20	** 160.20	49.84	57.25	** 102.03	* 87.27
	Week 25	53.38	** 131.42	54.25	59.58	111.75	124.42
Creatinine (mg/dL)	Week 17	0.35	** 0.74	0.34	0.35	* 0.45	* 0.44
	Week 25	0.34	0.50	* 0.30	** 0.30	0.42	0.46

ANOVA: Significance Level: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
(a): Cladribine Tablet

Cladribine 30 mg/kg alone or alone and in combination resulted in reduced testes weights (21% [ss]). At recovery, reduced testes weights were partially reversible.

At macroscopic examination, changes in kidney were observed (see tables below).

TERMINAL

	MALE							FEMALE					
Group	1	2	3	4	5	6		1	2	3	4	5	6
Doses: Clad (PO) Copax (SC)	0 0	30 0	0 20	10 20	30 20	30ds 20		0 0	30 0	0 20	10 20	30 20	30ds 20
N=	12	6	12	10	8	11		12	8	10	12	11	11
Kidney													
Pale	0	5	0	0	6	4		0	2	0	0	0	0
Decreased size	0	0	0	0	0	0		0	1	0	0	0	0

RECOVERY

	MALE							FEMALE					
Group	1	2	3	4	5	6		1	2	3	4	5	6
Doses: Clad (PO) Copax (SC)	0 0	30 0	0 20	10 20	30 20	30ds 20		0 0	30 0	0 20	10 20	30 20	30ds 20
N=	6	6	6	6	6	6		6	6	6	6	6	6
Kidney													
Cyst	0	2	0	0	0	1		0	0	0	0	0	0
Pale	0	4	0	0	2	1		0	0	0	0	0	0

Histopathological examinations were conducted by (b) (4) (b) (4); evaluation of specially-stained (i.e., PAS stained, counterstained with Meyer's hemalaun) slides of testes and epididymides was conducted by (b) (4) (b) (4). Treatment with 30 mg/kg cladribine alone or in combination resulted in drug-related adverse histological alterations in kidney and testis. Recovery histopathological assessment was limited to tissues showing drug-related alterations at the end of the dosing period. Alterations were also observed in salivary gland and lymphoid tissues, and heart in males.

In kidney, minimal to marked basophilic (regenerating) tubules and cortical sclerosis were observed (see selected in the tables, below). The pathologist provided a description of the extent of the lesions (below). After an 8-week recovery period, the incidence and severity of the renal lesions remained increased compared to controls.

> "The sclerotic lesions consisted of radial orientated, wedge-shaped lesions areas of fibrous tissue mixed with variable numbers of atrophic tubules, glomeruli and mononuclear cell infiltrates. They often caused depression of the capsule and in more severe cases distortion of the kidneys. Associated findings included diffuse dilatation of cortical tubules and intratubular inflammatory infiltration. The incidence and severity of sclerosis was marginally greater in animals given Cladribine in the tablet form than in those given drug substance."

TERMINAL

	MALE							FEMALE					
Group	1	2	3	4	5	6		1	2	3	4	5	6
Doses: Clad (PO) Copax (SC)	0 0	30 0	0 20	10 20	30 20	30ds 20		0 0	30 0	0 20	10 20	30 20	30ds 20
N=	12	12	12	12	12	12		12	12	12	12	12	12
Kidney													
Focus(i) of basophilic (regenerating) tubules	**3**	**11**	**2**	**2**	**9**	**10**		**0**	**5**	**1**	**0**	**3**	**3**
Minimal	*3*	*1*	*2*	*2*	*0*	*3*		*0*	*2*	*1*	*0*	*3*	*1*
Mild	*0*	*5*	*0*	*0*	*4*	*1*		*0*	*1*	*0*	*0*	*0*	*2*
Moderate	*0*	*4*	*0*	*0*	*5*	*5*		*0*	*2*	*0*	*0*	*0*	*0*
Marked	*0*	*1*	*0*	*0*	*0*	*1*		*0*	*0*	*0*	*0*	*0*	*0*
Cortical sclerosis	**2**	**10**	**0**	**0**	**10**	**8**		**0**	**6**	**0**	**1**	**2**	**7**
Minimal	*2*	*0*	*0*	*0*	*1*	*2*		*0*	*1*	*0*	*1*	*2*	*6*
Mild	*0*	*4*	*0*	*0*	*1*	*2*		*0*	*4*	*0*	*0*	*0*	*1*
Moderate	*0*	*6*	*0*	*0*	*7*	*3*		*0*	*1*	*0*	*0*	*0*	*0*
Marked	*0*	*0*	*0*	*0*	*1*	*1*		*0*	*0*	*0*	*0*	*0*	*0*
Diffuse tubular dilatation	**0**	**8**	**0**	**0**	**3**	**6**		**0**	**2**	**0**	**0**	**0**	**0**
Minimal	*0*	*1*	*0*	*0*	*0*	*1*		*0*	*1*	*0*	*0*	*0*	*0*
Mild	*0*	*4*	*0*	*0*	*2*	*4*		*0*	*1*	*0*	*0*	*0*	*0*
Moderate	*0*	*3*	*0*	*0*	*1*	*1*		*0*	*0*	*0*	*0*	*0*	*0*
Intratubular inflammatory infiltrates	**0**	**3**	**0**	**0**	**1**	**1**		**0**	**1**	**0**	**0**	**0**	**0**
Minimal	*0*	*2*	*0*	*0*	*1*	*1*		*0*	*1*	*0*	*0*	*0*	*0*
Mild	*0*	*1*	*0*	*0*	*0*	*0*		*0*	*0*	*0*	*0*	*0*	*0*

RECOVERY

	MALE							FEMALE					
Group	1	2	3	4	5	6		1	2	3	4	5	6
Doses: Clad (PO) Copax (SC)	0 0	30 0	0 20	10 20	30 20	30ds 20		0 0	30 0	0 20	10 20	30 20	30ds 20
N=	6	6	6	6	6	6		6	6	6	6	6	6
Kidney													
Focus(i) of basophilic (regenerating) tubules	**3**	**5**	**-**	**1**	**5**	**2**		**1**	**3**	**-**	**1**	**2**	**2**
Minimal	*2*	*1*	*-*	*1*	*1*	*0*		*1*	*0*	*-*	*1*	*2*	*2*
Mild	*1*	*0*	*-*	*0*	*1*	*1*		*0*	*3*	*-*	*0*	*0*	*0*
Moderate	*0*	*4*	*-*	*0*	*3*	*1*		*0*	*0*	*-*	*0*	*0*	*0*
Cortical sclerosis	**1**	**5**	**-**	**0**	**4**	**2**		**0**	**3**	**-**	**0**	**2**	**0**
Minimal	*1*	*1*	*-*	*0*	*0*	*0*		*0*	*0*	*-*	*0*	*2*	*0*
Mild	*0*	*0*	*-*	*-*	*1*	*1*		*0*	*2*	*-*	*0*	*0*	*0*
Moderate	*0*	*4*	*-*	*0*	*3*	*0*		*0*	*1*	*-*	*0*	*0*	*0*
Marked	*0*	*0*	*-*	*-*	*-*	*1*		*0*	*0*	*-*	*0*	*0*	*0*
Focal cortical inflamm. Cell infiltration	**0**	**0**	**-**	**0**	**0**	**0**		**1**	**5**	**-**	**3**	**1**	**2**
Minimal	*0*	*0*	*-*	*0*	*0*	*0*		*0*	*3*	*-*	*2*	*1*	*1*
Mild	*0*	*0*	*-*	*0*	*0*	*0*		*1*	*2*	*-*	*1*	*0*	*1*
Cortical cysts	**0**	**0**	**-**	**0**	**0**	**0**		**0**	**3**	**-**	**0**	**0**	**0**

In the testes, minimal to mild bilateral germinal epithelial atrophy was observed (see selected changes in the tables below). Partial reversibility was observed. There was little evidence of changes in the epididymides.

	TERMINAL							RECOVERY					
Group	1	2	3	4	5	6		1	2	3	4	5	6
Doses: Clad (PO) Copax (SC)	0 0	30 0	0 20	10 20	30 20	30ds 20		0 0	30 0	0 20	10 20	30 20	30ds 20
N=	12	12	12	12	12	12		6	6	6	6	6	6
Testes													
Bilateral germinal epithelial atrophy	**1**	**7**	**0**	**0**	**4**	**2**		**0**	**2**	**-**	**0**	**0**	**0**
Minimal	*1*	*4*	*0*	*0*	*1*	*1*		*0*	*2*	*-*	*0*	*0*	*0*
Mild	*0*	*3*	*0*	*0*	*3*	*1*		*0*	*0*	*-*	*0*	*0*	*0*
Epididymides													
Intratubular degenerate spermatozoa/spermatids	**1**	**1**	**0**	**0**	**1**	**1**							
Minimal	*1*	*0*	*0*	*0*	*1*	*1*							
Mild	*0*	*1*	*0*	*0*	*0*	*0*							
Bilateral hypospermia	**0**	**1**	**0**	**0**	**1**	**0**							
Minimal	*0*	*0*	*0*	*0*	*1*	*0*							
Mild	*0*	*1*	*0*	*0*	*0*	*0*							
Focal inflammatory cell infiltration	**0**	**1**	**0**	**0**	**0**	**1**							
Minimal	*0*	*0*	*0*	*0*	*0*	*1*							
Mild	*0*	*1*	*0*	*0*	*0*	*0*							

Changes were also observed in salivary gland and lymphoid tissues, and were suggested in the heart in males (see selected in the table below). These changes were not reported as drug-related by the sponsor; therefore, histopathology of these tissues was not conducted in Gp#2 recovery animals (only kidney and testes were examined).

TERMINAL

	MALE							FEMALE					
Group	1	2	3	4	5	6		1	2	3	4	5	6
Doses: Clad (PO) Copax (SC)	0 0	30 0	0 20	10 20	30 20	30ds 20		0 0	30 0	0 20	10 20	30 20	30ds 20
N=	12	12	12	2	12	13		12	12	12	-	12	12
Salivary gland, sublingual													
Diffuse acinar hypertrophy	**0**	**4**	**0**	**0**	**0**	**0**		**0**	**0**	**0**	**0**	**0**	**0**
Minimal	*0*	*3*	*0*	*0*	*0*	*0*		*0*	*0*	*0*	*0*	*0*	*0*
Mild	*0*	*1*	*0*	*0*	*0*	*0*		*0*	*0*	*0*	*0*	*0*	*0*
Salivary gland, submandibular													
Diffuse hypertrophy of mucus acini	**0**	**6**	**0**	**0**	**1**	**0**		**0**	**6**	**0**	**-**	**3**	**1**
Minimal	*0*	*3*	*0*	*0*	*1*	*0*		*0*	*1*	*0*	*-*	*1*	*1*
Mild	*0*	*3*	*0*	*0*	*0*	*0*		*0*	*5*	*0*	*-*	*2*	*0*
Spleen													
Lymphoid follicular atrophy	**0**	**6**	**0**	**0**	**4**	**1**		**0**	**0**	**0**	**0**	**0**	**0**

Mild	*0*	*4*	*0*	*0*	*0*	*1*		*0*	*0*	*0*	*0*	*0*	*0*
Moderate	*0*	*2*	*0*	*0*	*4*	*0*		*0*	*0*	*0*	*0*	*0*	*0*
Increased hemosiderosis, Mild	**0**	**1**	**0**	**0**	**0**	**0**		**0**	**0**	**0**	**0**	**0**	**0**
Thymus													
Decreased cortico-medullary ratio	**1**	**6**	**0**	**0**	**3**	**3**		**0**	**4**	**0**	**-**	**0**	**0**
Mild	*1*	*1*	*0*	*0*	*1*	*1*		*0*	*1*	*0*	*-*	*0*	*0*
Moderate	*0*	*1*	*0*	*0*	*1*	*2*		*0*	*3*	*0*	*-*	*0*	*0*
Marked	*0*	*4*	*0*	*0*	*1*	*0*		*0*	*0*	*0*	*0*	*0*	*0*
Heart													
Focal pericarditis	**0**	**3**	**1**	**0**	**0**	**2**		**0**	**0**	**0**	**0**	**0**	**0**
Minimal	*0*	*2*	*0*	*0*	*0*	*0*		*0*	*0*	*0*	*0*	*0*	*0*
Mild	*0*	*1*	*1*	*0*	*0*	*1*		*0*	*0*	*0*	*0*	*0*	*0*
Moderate	*0*	*0*	*0*	*0*	*0*	*1*		*0*	*0*	*0*	*0*	*0*	*0*

The sponsor provided the following summary table of TK data.

Table A. Cladribine main PK parameters (calculated from the mean profiles, n=2)
(p. 1)

CLADRIBINE								
Group	**8**		**9**		**10**		**11**	
Cladribine dose (mg/kg/day)	**30**		**10**		**30**		**30 (*)**	
Copaxone dose (mg/kg/day)	**0**		**20**		**20**		**20**	
Cladribine dose ratio	-		**1**		**3**		**3**	
	Day 1-Week 1-Cycle 1							
	Males	***Females***	***Males***	***Females***	***Males***	***Females***	***Males***	***Females***
t_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
C_{max} (ng/mL)	4937.1	6870.2	1172.8	1925.0	4285.3	4313.2	6294.2	5250.7
t_z (hr)	24	5	5	5	24	24	5	24
C_z (ng/mL)	2.5	32.2	6.7	9.0	1.6	1.1	29.9	2.3
AUC_z (hr*ng/mL)	5849.4	7351.9	1355.0	2075.5	5564.7	5223.3	6849.8	5613.0
AUC_{24} (hr*ng/mL)	5849.4	7657.8	1418.6	2161.0	5564.7	5223.3	7133.9	5613.0
AUC_{24} norm	195	255	142	216	185	174	238	187
M/F AUC_{24} ratio	0.8	-	0.7	-	1.1	-	1.3	-
AUC_{24} vs. Gr. 8	1	1	-	-	0.9	1.1	1.2	0.7
AUC_{24} vs. Gr. 11	-	-	-	-	0.8	0.9	1	1
AUC_{24} ratio	-	-	1	1	3.9	2.4	5.0	2.6
	Day 5-Week 1-Cycle 1							
t_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
C_{max} (ng/mL)	4862.2	4296.1	1623.5	934.4	9199.4	3091.8	5013.8	3250.2
t_z (hr)	24	24	24	24	24	24	24	24
C_z (ng/mL)	4.1	4.0	2.4	3.6	8.2	3.5	7.3	4.3
AUC_z (hr*ng/mL)	5619.1	5529.3	2058.5	1188.2	11220.1	3668.8	6780.9	3834.2
AUC_{24} (hr*ng/mL)	5619.1	5529.3	2058.5	1188.2	11220.1	3668.8	6780.9	3834.2
AUC_{24} norm	187	184	206	119	374	122	226	128
Rac AUC_{24}	1.0	0.7	1.5	0.5	2.0	0.7	1.0	0.7
M/F AUC_{24} ratio	1.0	-	1.7	-	3.1	-	1.8	-
AUC_{24} vs. Gr. 8	1	1	-	-	2.0	0.7	1.2	0.7
AUC_{24} vs. Gr. 11	-	-	-	-	1.7	1.0	1	1
AUC_{24} ratio	-	-	1	1	5.5	3.1	3.3	3.2

(*) Administration of Cladribine drug substance
Rac AUC_{24} = accumulation ratio
M/F AUC_{24} ratio = male/female exposure ratio (male/female AUC_{24} ratio)

CLADRIBINE								
Group	**8**		**9**		**10**		**11**	
Cladribine dose (mg/kg/day)	**30**		**10**		**30**		**30 (*)**	
Copaxone dose (mg/kg/day)	**0**		**20**		**20**		**20**	
Cladribine dose ratio	**-**		**1**		**3**		**3**	
	Day 89-Week 13-Cycle 4							
	Males	***Females***	***Males***	***Females***	***Males***	***Females***	***Males***	***Females***
t_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
C_{max} (ng/mL)	4313.9	4587.8	786.9	1257.7	4243.0	3865.5	3528.8	4754.2
t_z (hr)	24	24	24	5	24	24	24	24
C_z (ng/mL)	2.3	3.0	1.5	5.8	4.9	1.9	2.9	2.4
AUC_z (hr*ng/mL)	5427.0	5043.3	909.8	1362.3	5094.9	6352.0	5363.0	5521.5
AUC_{24} (hr*ng/mL)	5427.0	5043.3	909.8	1417.4	5094.9	6352.0	5363.0	5521.5
AUC_{24} norm	181	168	91	142	170	212	179	184
Rac AUC_{24}	0.9	0.7	0.6	0.7	0.9	1.2	0.8	1.0
M/F AUC_{24} ratio	1.1	-	0.6	-	0.8	-	1.0	-
AUC_{24} vs. Gr. 8	1	1	-	-	0.9	1.3	1.0	1.1
AUC_{24} vs. Gr. 11	-	-	-	-	1.0	1.2	1	1
AUC_{24} ratio	-	-	1	1	5.6	4.5	5.9	3.9
	Day 117-Week 17-Cycle 5							
t_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
C_{max} (ng/mL)	5008.0	3476.5	992.8	1974.3	5234.1	3451.6	5132.2	5402.1
t_z (hr)	24	24	5	24	24	24	24	24
C_z (ng/mL)	3.9	3.9	7.7	3.8	4.7	1.9	3.5	5.9
AUC_z (hr*ng/mL)	6127.7	4548.4	1118.7	2166.7	6449.3	4712.6	6146.6	6257.7
AUC_{24} (hr*ng/mL)	6127.7	4548.4	1191.8	2166.7	6449.3	4712.6	6146.6	6257.7
AUC_{24} norm	204	152	119	217	215	157	205	209
Rac AUC_{24}	1.0	0.6	0.8	1.0	1.2	0.9	0.9	1.1
M/F AUC_{24} ratio	1.3	-	0.6	-	1.4	-	1.0	-
AUC_{24} vs. Gr. 8	1	1	-	-	1.1	1.0	1.0	1.4
AUC_{24} vs. Gr. 11	-	-	-	-	1.0	0.8	1	1
AUC_{24} ratio	-	-	1	1	5.4	2.2	5.2	2.9

(*) Administration of Cladribine drug substance
Rac AUC24 = accumulation ratio
M/F AUC24 ratio = male/female exposure ratio (male/female AUC24 ratio)

11 Integrated Summary and Safety Evaluation

The nonclinical data required to support NDA 22561 were previously submitted and reviewed (see Nonclinical Review dated 2/18/11). In the resubmission, the sponsor included additional ADME studies and two combination toxicity studies.

The ADME studies submitted provide additional information about the disposition of cladribine; however, the information pivotal for nonclinical assessment remains unchanged. It was determined during the first review cycle that no major human metabolites were identified in vivo.

In the submitted combination toxicity studies, the dose of cyclic oral cladribrine administered alone (i.e., 30 mg/kg) resulted in increased mortality and adverse effects on kidney (i.e., cortical sclerosis, degenerating/regenerating tubules), testes (e.g., germinal epithelial degeneration and/or atrophy), and lymphoid tissues, as well as some evidence of alterations (pericarditis) in heart in males. In this 5-cycle study, 30 mg/kg of cladribine exceeded the MTD.

In previous toxicity studies reviewed in the original Nonclinical Review (dated 2/18/11), mortality, reduced body weights, clinical pathology, and histopathological alterations (i.e., kidney, liver, and lymphoid system) occurred at ≥ 30 mg/kg/day cyclical dosing in acute/subchronic studies in mice; in those studies deaths occurred in one male (of 6) at 30 mg/kg, and several animals at ≥ 50 mg/kg/day. Subacute inflammation of the epicardium was observed in 1 of 3 males evaluated at 30 mg/kg in the single cycle study (5 days of dosing followed by 22 days without dosing). The chronic (i.e., 4 to 7 monthly cycles of 7 days of dosing followed by 3 weeks without dosing) oral toxicity study tested a maximum dose of 20 mg/kg/day, which was identified as the NOAEL. In the chronic study, a single HDF mortality occurred, as well as increased cholesterol and low incidence alterations in kidney. In the 2-cycle study in non-transgenic mice, the observed MTD was 30 mg/kg; at this dose (and following a cycle at 5 mg/kg then 60 mg/kg), drug-related renal alterations were observed (i.e., minimal to marked tubular degeneration/ regeneration with luminal debris, showing increased creatinine and BUN after 60 mg/kg). Moreover, in the cyclical administration transgenic mouse carcinogenicity assay, kidney (e.g., tubular degeneration/regeneration; M and F), thymus (decreased cellularity; M), and testes (degeneration of the seminiferous tubules) were identified as target organs at the maximum dose of 30 mg/kg. Effects on male reproductive organs were also noted in the reproductive toxicology studies in mice and in toxicity studies in monkey.

Overall, the nonclinical information provided in the resubmission adds minor information to inform the overall safety profile for cladribine (supporting kidney, testes, and lymphoid tissues as target organs). The overall nonclinical recommendation is unchanged from the nonclinical review dated February 18, 2011.

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/s/

MELISSA K BANKS-MUCKENFUSS
03/06/2019 05:50:35 PM

LOIS M FREED
03/07/2019 05:35:24 PM
I concur that the new nonclinical data do not alter the original recommendation that the NDA is approvable from a nonclinical standpoint (see memo, dated February 23, 2011).

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: February 23, 2011

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 22-561, September 29, 2009; resubmission dated May 27, 2010
((b) (4) cladribine)

NDA 22-561 was originally submitted by EMD Serono, Inc. on September 29, 2009 to support approval of cladribine (b) (4) for treatment of relapsing forms of multiple sclerosis. That application was not filed due to clinical deficiencies. The NDA was resubmitted on May 27, 2010, and filed.

Cladribine for MS was developed under (b) (4) (b) (4) (b) (4) IND 74,634 (EMD Serono, Inc.), and submitted for marketing approval (b) (4) (b) (4) Cladribine is approved for intravenous administration in the treatment of hairy cell leukemia (Leustatin Injection; NDA 20-229; Ortho Biotech Products/Johnson and Johnson Pharmaceutical Research Development).

The sponsor provided in the NDA all the nonclinical studies conducted to support approval (b) (4) Some of these same studies were previously submitted under NDAs 20-229 and/or (b) (4) and were reviewed under those NDAs (b) (4) (b) (4) (b) (4). The additional nonclinical studies conducted to support oral administration were reviewed by Melissa Banks (*Pharmacology/Toxicology NDA Review and Evaluation Melissa K. Banks, Ph.D. 2/18/11*).

Based on her review, Dr. Banks has concluded that "…this application is approvable, with conditions/reservations." Specifically,

- The carcinogenic potential of the excipient, HPβCD, may not have been adequately evaluated if clinical use will allow unlimited 48-week courses of cladribine.
- Only limited *in vivo* metabolism data are available for humans; therefore, there may not be sufficient information to rule out the presence of major circulating metabolites. However, the OCBP team has determined that "major metabolites are unlikely…and, conduct of a traditional mass balance study is not possible…"
- The potential for "several relatively severe toxicities…" associated with cladribine administration. "…however, other approved treatments for multiple sclerosis have similar liabilities."
- Reports of "increased tumor/cancer incidence in patients previously treated with cladribine…"

Of these, Dr. Banks only recommends additional studies related to the carcinogenic potential of cladribine and HPβCD, as follows

- The sponsor should conduct a histopathology examination of "…Harderian gland tissue from the 7-month cyclic PO…toxicity study in mice…", if available.
- To support administration of (b) (4) in humans for longer than 96 weeks:
    - "…an adequate study of the cyclic administration of HPβCD for a period approximating lifetime exposure in rats…"
    - "Alternatively, if a more limited number of courses (but greater than 4) is to be permitted…, the sponsor could make efforts to confirm that humans are not systemically exposed to HPβCD….It is noted that these data would not evaluate any potential toxicity related to lifetime cyclic local GI exposure to HPβCD; the need for further evaluation of such effects should be discussed."

This memo briefly summarizes the nonclinical studies, and discusses a few issues in greater detail, including those raised by Dr. Banks.

Pharmacology

Cladribine (2-chloro-2'-deoxyadenosine) is a synthetic purine nucleoside analog, which is itself inactive. Following passive (and possibly active) transport into cells, it is phosphorylated by deoxycytidine kinase (to 2-chlorodeoxyadenosine 5'-monophosphate [2-CdAMP]) and other kinases to produce the active form, 2-chlorodeoxyadenosine 5'-triphosphate (2-CdATP). Once formed, 2-CdATP is trapped within the cell. Alternatively, 2-CdAMP can be metabolized by 5'-nucleotidase, which prevents accumulation of 2-CdATP. Intracellular accumulation of 2-CdATP induces apoptosis, resulting in impairment of DNA synthesis and repair. Cells with a high intracellular ratio of deoxycytidine kinase to 5'-nucleotidase (e.g., lymphocytes) are particularly sensitive to cladribine-induced cytotoxicity. (Cladribine is reported to be unique, in that it is cytotoxic to both proliferating and non-dividing cells.) In MS patients, cladribine has been demonstrated to produce sustained decreases in circulating CD4+ and CD8+ T cells,

with lesser effects on B cells and NK cells. This activity is the proposed mechanism underlying the therapeutic effects of cladribine in MS.

Cladribine demonstrated no evidence of efficacy in two separate studies in the experimental autoimmune encephalomyelitis (EAE) animal (SJL mouse) model of MS. According to the sponsor, there was also no evidence of cladribine-induced effects on lymphocyte subsets in either study, which may have explained the lack of efficacy.

PK/ADME

Two primary issues related to PK/ADME are (1) the extent of metabolism following parenteral (IV, SC) and oral administration and (2) interspecies differences in levels of endogenous deoxycytidine kinase substrate (i.e., deoxycytidine). Both have important implications for evaluating the adequacy of the nonclinical studies.

1. As previously noted, the majority of the pivotal nonclinical studies were conducted using parenteral (IV, SC) administration; only two pivotal bridging studies were conducted using the clinical (oral) route. According to published literature, 2-chloroadenine (2-CAde) is a "major" circulating metabolite in humans (cf. Lindemalm S *et al*., *Cancer Letts* 210:171-177, 2004). According to Lindermalm *et al*. (2004), plasma levels of 2-CAde were ≈4-5 times higher in humans following oral administration as compared with IV dosing. However, according to the OCBP review team (cf. *Clinical Pharmacology/Biopharmaceutics Review NDA 2256, Hristina Dimova Ph.D., Xinning Yang, Ph.D., Joo-Yeon Lee, Ph.D., Li Zhang, Ph.D., 11/23/2010*), the sponsor's data demonstrate that 2-CAde is not a major circulating metabolite in humans. This is important since minimal toxicity was evident in the nonclinical oral bridging studies. However, the absence of a major metabolite in humans increases the relevance of the nonclinical studies conducted using parenteral administration.

2. Mouse and monkey were the animals species selected for the pivotal nonclinical studies, since these species were determined to be the most similar to humans in terms of circulating levels of the endogenous ligand, deoxycytidine. It was presumed that species with higher endogenous levels of deoxycytidine (that would compete with cladribine for phosphorylation) would be more resistant to the cytotoxic effects of cladribine and, therefore, poor animal models for assessing human safety. In the study conducted by the sponsor, deoxycytidine was not detected in human or monkey plasma. Mean plasma levels of deoxycytidine ranged from 0.31 to 0.42 µg/mL in mouse, guinea pig, dog, and rabbit, but from 5.13 to 8.50 µg/mL in five strains of rat. These data were accepted as the basis for species selection. In Dr. Huff's review of [redacted] (b) (4) it is noted that the mouse may also underestimate the toxicity of cladribine since, for example, human deoxycytidine kinase has greater affinity for cladribine than does mouse deoxycytidine kinase and, although the difference is small, the circulating levels of deoxycytidine are higher in mouse than human. As reported by Reichelova *et al*. (Reichelova V *et al*. *Cancer Chemother Pharmacol* 36:524-529, 1995), the plasma cladribine exposures at "equitoxic" doses (defined as

mouse LD_{10}/human MTD) were ≈100 times higher in mouse, suggesting that "…human cells are much more sensitive than murine cells to the toxic effects of CdA". There may also be differences between animals and human in rate of tissue uptake of cladribine. These interspecies differences make selecting the best method for expressing interspecies comparisons somewhat challenging.

Toxicology

Toxicology studies were conducted in mouse, rat, and monkey; however, as noted previously, rat was considered an inappropriate (insensitive) animal model for humans. According to Dr. Huff's review (b) (4) findings in rat confirmed this conclusion; the primary drug-related toxicities attributable to cladribine's mechanism of action (e.g., leucopenia, thrombocytopenia, microscopic changes in bone marrow, spleen, testes) observed in mouse and monkey were not detected in rat. "Unique" toxicities observed in rat included cardiac (myocardial degeneration) and liver (biliary hyperplasia, centrilobular necrosis) changes. The pivotal toxicity studies were conducted in mouse and cynomolgus monkey. The parenteral studies were reviewed by Dr. Huff; the oral studies were reviewed by Dr. Banks.

[Although rat may be an insensitive species, related to cladribine's mechanism of action, it is not the case that the rat was insensitive to the toxicity of cladribine; in acute dose studies, cladribine was lethal in both mouse and rat at doses >100 mg/kg SC. And, as noted in Dr. Huff's review, cladribine induced clear toxicity in the rat with repeated dosing. For example, in a 13-week SC dose-ranging study in rat, deaths occurred at 10 and 30 mg/kg/day (but not 1 mg/kg). No definite cause of death was established; however, the sponsor concluded that deaths were probably due to heart failure resulting from intracardiac thrombi and associated myocardial degeneration. In comparison, in a 13-week SC dose-ranging study in mouse, deaths occurred at 30 mg/kg, but not at 1 or 10 mg/kg; no cause of death was determined. It is possible that rat may be relatively insensitive to pharmacologically-mediated toxicity, but not to off-target toxicity, of cladribine. However, only limited dose-ranging studies were conducted in rat.]

Mouse: the "pivotal" studies consisted of a 3-8 month intermittent toxicity study (0, 10, 30, 60, and 80 mg/kg SC; interim sacrifices after the 3rd and 6th dosing cycle) and a 4-7 month intermittent study (0, 0.2, 2, 20 mg/kg PO; interim sacrifice after the 4th dosing cycle). In both studies, cladribine was administered in monthly cycles; each cycle consisted of 7 consecutive daily doses followed by a 21-day withdrawal period.

The subcutaneous study was not a pivotal study; it was not conducted under GLP and only a very limited number of tissues were examined microscopically (and the tissue(s) examined varied among sacrifice times). Drug-related deaths were observed at 60 (1 M) and 80 mg/kg; in premature decedents, there was noted to be histopathological evidence of "infectious or septicemic processes". Male reproductive toxicity (testicular atrophy, degenerating cells and reduced sperm in the epididymal lumen) was also noted at 60 and 80 mg/kg. Lymphocyte depletion of thymus and spleen were observed at doses ≥30 mg/kg, although not consistently. Testicular (atrophy) and spleen (extramedullary

hematopoiesis, chronic interstitial inflammation) findings were reported at the lower doses. The oral study was a pivotal study; however, the doses tested produced minimal, if any, drug-related findings (i.e., slight increase in total cholesterol).

Toxicokinetic data were collected in the oral study, but not the subcutaneous study. TK data were, however, available from a 3-month daily subcutaneous study in mouse (0, 1, 10, 30 mg/kg/day). Based on these data, plasma cladribine exposures at the high dose in the oral study (C_{max} = 2640-2790 ng/mL; AUC = 2470-1620 ng·hr/mL) were lower than those at the mid dose in the subcutaneous study (C_{max} = 3425-3190 ng/mL; AUC = 3067-3049 ng·hr/mL). However, in the daily subcutaneous study, findings (e.g., lymphocyte depletion in spleen, fatty deposition in bone marrow) were observed at all doses. The LD was associated with plasma C_{max} and AUC of 273-257 ng/mL and 277-270 ng·hr/mL, respectively; therefore, it is somewhat surprising that some drug-related effects were not observed in the oral study.

The lack of toxicity in the oral study precludes a definitive comparison of toxicity between the two routes of administration, which was the purpose of the oral study. However, the doses tested were reasonable; in a 5-day pilot oral toxicity study in mouse, doses of ≥50 mg/kg/day were lethal in both males and females, and one male died following a 5th dose at 30 mg/kg/day (females were not tested at this dose).

Monkey: the pivotal studies consisted of a 14-month intermittent toxicity study (0, 0.15, 0.3, 1.0 mg/kg SC) and a 3-month (+ 3-month recovery) intermittent toxicity study (0, 0.2, 2, 20 mg/kg PO, 0.3 mg/kg SC) in cynomolgus monkey. In both studies, cladribine was administered in monthly cycles; each cycle consisted of 7 consecutive daily doses followed by a 3-week washout period.

In the 14-month subcutaneous study, animals were sacrificed moribund at the MD (1 F; Day 227) and HD (2 M, 1 F; Day 227 on); clear signs of toxicity (clinical signs, decreased body weight gain, reduced rbc and wbc parameters) were observed at the HD. Histopathology findings consisted of cellular depletion of the bone marrow and lymphoid depletion of the spleen at the MD and HD and testicular degeneration at the HD.

In the 3-month oral study (with an additional SC group), the only drug-related finding was a moderate reduction in motile sperm at 6 mg/kg PO and 0.3 mg/kg SC. The sponsor states that the HD of 6 mg/kg PO was the NOEL. (However, considering the sperm effects at that dose, the HD should be considered an NOAEL.) Unlike for the mouse, there are no data at higher oral doses in the monkey.

Only minimal TK data were obtained in the 14-month subcutaneous study; $C_{10\ min}$ on Day 343 was ≈22-28, 56-64, and 138-235 ng/mL, respectively. In an acute-dose PK study in monkey, the C_{max} and AUC associated with a 1-mg/kg subcutaneous dose were 352 ng/mL and 535 ng·hr/mL, respectively.

In the 3-month oral study, plasma cladribine exposures were as follows (M-F; Day 61/62, 3rd cycle):

PARAMETER	DOSE (mg/kg)			
	PO			SC
	1.5	3	6	0.3
Cmax	19.8-16.7	58.5-48.4	94.7-88.3	98.7-152.0
$AUC_{(0\text{-}24\ hr)}$	91.0-78.5	221.0-192.7	347.6-427.0	196.8-298.2

Based on the relative lack of drug-related toxicity in the 3-month oral study, the only pivotal chronic study in monkey is the 14-month subcutaneous study.

Conclusions: minimal or no drug-related toxicity was observed in the oral bridging studies conducted in mouse and monkey. The mouse study is acceptable since dose-ranging data suggest that substantially higher doses would not have been tolerated. However, the monkey study is inadequate since no data are available at higher oral doses to justify the doses used in the bridging study. The chronic subcutaneous study in mouse is inadequate by design (non GLP, histopathological evaluation of notably less than a standard battery of tissues). However, the chronic subcutaneous toxicity study in monkey and the subcutaneous carcinogenicity study in mouse (discussed in greater detail under **Carcinogenicity**) provide sufficient data on the general toxicity of cladribine since (1) no major circulating metabolites have been identified in humans following oral administration and (2) substantially higher plasma exposures to cladribine were achieved (or estimated to be achieved) in the subcutaneous studies in mouse and monkey.

The mouse may be a relatively insensitive animal model for assessing human safety (related to cladribine's presumed therapeutic PD effects), due to differences in intracellular PK/PD. The monkey may be the more relevant animal model, e.g., this species is reported to have greater similarity to humans in terms of the substrate specificity of deoxycytidine kinase (Arner ESJ, Eriksson S. *Pharmaco Ther* 67(2):155-186, 1995). However, similar target organs were identified in mouse and monkey (i.e., lymphoid tissue and testes). In contrast, unique toxicities (e.g., intracardiac thrombi, myocardial degeneration) were identified in subchronic studies in rat, a species considered less sensitive to cladribine (at least to PD-associated toxicities).

The complexity of cladribine's PK/PD related to its mechanism of action makes evaluation of the sensitivity of various animal models difficult; however, the available data suggest that the animal models may be relatively insensitive, rather than overly sensitive.

Reproductive and Developmental Toxicology

The following studies were conducted: mating and fertility in male (0, 1, 5, 10, 30 mg/kg SC) and female (0, 1, 2, 4, 8 mg/kg SC) mouse, embryofetal development in mouse (0, 0.5, 1.5, 3.0 mg/kg IV bolus) and rabbit (0, 0.3, 1.0, 3.0 mg/kg IV bolus), peri/postnatal development in mouse (0, 0.5, 1.5, 3 mg/kg IV bolus). The embryofetal development studies were reviewed under NDA 20-229, whereas Dr. Huff reviewed the remaining studies (b) (4). No oral studies were conducted.

According to Dr. Huff's review, the primary findings were as follows:

- No overall effect on fertility was observed in either male or female mouse. However, in males, testis and epididymis weights were reduced, as were sperm count and motility. In females, post-implantation loss was increased, resulting in a decrease in the number of live fetuses. The no-effect doses were 5 and 4 mg/kg in males and females, respectively.
- Cladribine was teratogenic in both mouse and rabbit at 3 mg/kg. Malformations included "…exencephaly, open eye lid, separated snout, cleft palate, micrognathia, thoracogastroschisis and skeletal malformations in mice, and dome head, gastroschiasis, cleft palate, malrotated limb, forelimb flexure, ectromelia, adactyly, oligodactyly and syndactyly in rabbits" (*Dr. Huff's NDA review*). Additional findings included decreases in fetal weight (mouse, rabbit) and increases in skeletal variations (mouse). No-effect doses were 0.5 and 1 mg/kg in mouse and rabbit, respectively.
- An increase in malformations and embryofetal lethality was also observed in the peri/postnatal study in mouse (3 mg/kg), as was an increase in skeletal variations (>0.5 mg/kg). No effects on physical development or reproductive performance were observed; however, performance on learning tasks was adversely affected at 3 mg/kg. The no-effect dose for developmental effects was 0.5 mg/kg.

Conclusions: these studies demonstrate that cladribine is teratogenic in both mouse and rabbit; they are adequate to support approval of (b) (4), with appropriate labeling.

The data from the sponsor's studies are consistent with published literature reports of malformations in mice (Charlap JH *et al*. *Birth Defect Res (Pt A)* 67:108-115, 2003; Lau C *et al*. *Teratology* 66:6-18, 2002; Mitala JJ *et al*. *Teratology* 53:116, 1996; Wubah JA *et al*. *Teratology* 64:154-169, 2001) and rabbit (Mitala JJ *et al*. *Teratology* 53:116, 1996). Adverse effects on the developing organism have also been reported in rat (Lau C *et al*. *Teratology* 66:6-18, 2002). Lau *et al*. (2002) demonstrated that the rat (dosed on GD 9.5) was less sensitive to the adverse developmental effects of cladribine (e.g., ocular malformations) compared to findings from previous studies in mouse (dosed on GD 8). Body trunk malformations (e.g., lumbar hernia, in some cases, accompanied by spina bifida) were observed only in rat, but at estimated fetal exposures 20-35 fold higher than those in the mouse, based on data previous reported in the mouse (Wubah *et al*., 2001). While these data suggest that rat may be less sensitive to the adverse developmental effects of cladribine, they do indicate that cladribine is teratogenic in a third animal species.

Genetic Toxicology

A standard battery of genetic toxicology studies was conducted (*in vitro* Ames, *in vitro* chromosomal aberration assay in CHO cells, *in vivo* micronucleus assay in mouse); these studies were reviewed under NDA 20-229. Cladribine was negative in the *in vitro* Ames assay (and an *in vitro* HPRT mutagenicity assay in CHO cells), but positive in the *in vitro* and *in vivo* (IV) clastogenicity assays. (In the *in vivo* clastogenicity study, cladribine was

positive at all doses tested.) Dr. Huff notes that "…literature references indicated that cladribine induced DNA strand breaks and inhibited DNA synthesis and repair", consistent with its known mechanism of action.

Conclusions: an adequate battery of genetic toxicology studies was conducted. The results of this battery, and numerous published studies, demonstrate the genotoxicity of cladribine. Genotoxicity is proposed to be a basis for cladribine's therapeutic effects in treatment of hairy cell leukemia and multiple sclerosis.

Carcinogenicity

The carcinogenic potential of cladribine was assessed in a 22-month SC bioassay in mouse and a 26-week Tg.rasH2 mouse assay. The 22-month study was submitted to (b) (4) and reviewed by Dr. Huff. The 26-week Tg.rasH2 mouse study was submitted to NDA 22-561, and was reviewed by Dr. Banks. Statistical evaluation of the 22-month study was conducted by Dr. Hung (b) (4). (b) (4) and Dr. Thomson (*Statistical Review and Evaluation. Carcinogenicity Study NDA 22,561, Steve Thomson, 1/18/2011*). Statistical evaluation of the 26-week study was conducted by Dr. Thomson. Both studies were evaluated by the ExeCAC (b) (4) *Executive CAC Minutes, NDA 22-561, 11/4/2010*).

In the 22-month study, cladribine (0, 0, 0.1, 1, and 10 mg/kg SC) was administered in cycles, each cycle consisting of 7 consecutive days of dosing followed by 21 days off drug. The primary tumor finding was an increase in Harderian gland tumors at 10 mg/kg, summarized below (VC = vehicle control, C = untreated control).

HARDERIAN GLAND	MALES					FEMALES				
	VC	UC	LD	MD	HD	VC	UC	LD	MD	HD
adenoma	6/65	11/65	3/65	8/65	28/65	1/65	4/65	2/65	2/65	11/65
adenocarcinoma	0/65	0/65	1/65	0/65	1/65	0/65	0/65	0/65	0/65	2/65
combined	6/65	11/65	4/65	8/65	29/65	1/65	4/65	2/65	2/65	13/65

Although humans do not have Harderian glands, the increase in Harderian gland tumors is a relevant finding (as concluded by Dr. Huff and the ExeCAC) since cladribine is genotoxic and there are documented cases of lack of target organ concordance for trans-species carcinogens.

In the 26-week Tg.rasH2 assay, cladribine (0, 5, 15, 30 mg/kg dissolved tablets, 15 mg/kg drug substance) or excipient (431 mg/kg 2-hydroxypropyl-β-cyclodextran [HPβCD]) was administered for 7 monthly cycles; each cycle consisted of 5 consecutive daily doses followed by a 23-day washout period, ending with a 7th final dosing cycle. The primary drug-related findings were microscopic changes in kidney (tubular degeneration), thymus (decreased cellularity), and testes (degeneration), observed at 30 mg/kg. There were no adverse effects on mortality, clinical signs, or body weight. No drug- or excipient-related tumors were observed.

Conclusions: The Tg.rasH2 study is inadequate by design since it has not been validated for this type of cyclic dosing regimen. (Agency concurrence on study design or dose selection was not requested.) Therefore, only the 22-month subcutaneous study in mouse provides an adequate assessment of carcinogenicity for cladribine. A lifetime bioassay in rat was not required at the time of review of [redacted] (b) (4) due to the presumed insensitivity of this species. This is an arguable position since cladribine could have toxicity, including carcinogenic potential, unrelated to its therapeutic mechanism of action, and toxicity was clearly demonstrated in the rat in repeat-dose toxicity studies. However, the positive finding in the 22-month mouse study and the positive genotoxicity data indicate that cladribine is a genotoxic carcinogen.

Dr. Banks has recommended that the sponsor examine Harderian gland tissue from the 7-month cyclic PO study in mouse, since Harderian gland tumors were identified in the 22-month SC carcinogenicity study. However, because of the 22-month SC study findings, I do not believe an evaluation of Harderian gland tissue from the 7-month PO study is necessary.

In my opinion, no additional studies are needed to address the carcinogenic potential of cladribine. It is possible that additional investigative studies might aid the evaluation of the apparent signal for carcinogenicity in humans; however, at this time what the nature of those studies would be is unclear.

Excipient

In the to-be-marketed product, cladribine is formulated in 2-hydroxypropyl-β-cyclodextran [HPβCD], an excipient demonstrated to cause pancreatic acinar cell tumors in rats at all doses tested (500, 2000, 5000 mg/kg PO for 2 years) (Gould S, Scott RC *Food Chem Toxicol* 43:1451-1459, 2005). As a result, there is currently no drug product approved for chronic oral administration that contains this excipient. The to-be-marketed drug product contains 10 mg cladribine and 143.76 mg HPβCD per tablet; the maximum daily dose is 20 mg (cumulative dose over 96 weeks is ≈3.5 mg/kg). This would result in a maximum daily dose of HPβCD of 287.52 mg and a cumulative dose over 96 weeks of ≤6 gms or ≈50-60 mg/kg).

Early in development, the sponsor was advised to re-formulate the drug product to remove HPβCD. However, the sponsor argued that the intermittent nature of dosing for [redacted] (b) (4) would avoid the concern. To demonstrate a lack of an effect on the pancreas with intermittent dosing, the sponsor conducted a chronic study of HPβCD in rat. (Although other oral studies in mouse and monkey used cladribine formulated in HPβCD, these are not of sufficient duration to provide relevant information and HPβCD-induced pancreatic tumors have not been detected in the mouse.)

In the chronic rat study, HPβCD was administered orally at doses of 0, 100, 500, and 5000 mg/kg according to a cyclic regimen (each cycle consisted of 5 consecutive days of dosing followed by a 23-day washout period) for four 28-day cycles (5 daily doses followed the 4th cycle) or at doses of 0 and 5000 mg/kg daily for 4 or 12 consecutive

months. For the cyclic regimen, main study animals were sacrificed 24-48 hrs after the last dose; recovery animals were maintained for an 8-month recovery period. For the daily dosing regimen, animals were sacrificed at the end of either 4 or 12 consecutive months of dosing. BrdU (200 mg/kg IP) was administered for 3 consecutive days prior to sacrifice of animals treated according to the cyclic regimen (end of dosing and 8-month recovery) and by daily dosing (end of 4- or 12-month dosing period). Since the primary concern is effects on the pancreas, discussion will be limited to that organ.

No effects on pancreas (histopathology, BrdU staining) were observed in animals receiving HPβCD by cyclical or daily administration for 4 months; however, in animals dosed daily for 1 year with this excipient, there were increases in both pancreatic acinar cell hyperplasia (acknowledged to be "an early sign of proliferation leading to tumors") and pancreatic BrdU staining (in randomly selected areas and hyperplastic foci). The data from animals treated daily are consistent with previous findings of HPβCD-induced pancreatic acinar cell hyperplasia at 12 months, leading to tumors at 24 months, with daily administration (Gould & Scott, 2005).

The sponsor also argues that the pancreatic acinar cell tumors induced by HPβCD in the rat is species-specific, resulting from increases in CCK (due to local GI effects) and subsequent mitogenic effects on the rat pancreas acinar cell; no investigative studies were conducted in support of this hypothesis. Two publications are cited in support of a CCK-mediated phenomenon for HPβCD-induced tumors (Gould & Scott, 2005; Stella VJ, Quanren H *Toxicol Pathol* 36:30-42, 2008). However, Stella and Quanren (2008) cite Gould and Scott (2005); Gould and Scott (2005) reference personal communication ("Van Cauteren, personal presentation, 1997"). To date, the Van Cauteren data do not appear to have been published. The sponsor also states that, at the proposed clinical doses, there is no systemic exposure to HPβCD in humans. No data were provided to support this statement, and there was clear (although limited) evidence of systemic exposure in the 1-year study of HPβCD in rat (at HD [M-F], C_{max} and AUC at the end of the last cycle were 17394-7845 ng/mL and 78069-99029 ng∗hr/mL, respectively), although at the low dose tested, plasma AUC could not be reliably estimated due to the low level of systemic exposure.

Conclusion: There are insufficient data to conclude that HPβCD-induced pancreatic acinar cell tumors are not relevant to humans; however, the data provided by the sponsor suggest that the clinical intermittent dosing regimen (limited to 4 cycles over 2 years) is unlikely to cause concern regarding HPβCD-induced pancreatic acinar cell tumors. According to the sponsor's draft labeling, (b) (4) is to be administered to humans in two (b) (4) cycles, each cycle involving daily doses of 10 or 20 mg cladribine for the first 4-5 days of each of two (b) (4) cycle during the course of a (b) (4) period, with the 2-cycle regimen being repeated at the beginning of a subsequent (b) (4) period. Whether or not there will be additional (b) (4) courses of treatment is unclear. Dr. Banks's concern is that the sponsor's data do not address whether or not proliferative changes to the pancreas would be observed with additional cycles of dosing.

Certainly, the lack of pancreatic findings in a 2-year study in rat, with monthly dosing cycles, would have been a more definitive evaluation. However, if positive, it would have been difficult to judge the relevance of the findings since the frequency of dosing would be greater than that proposed for humans (it is unclear how monthly dosing in rat would relate to yearly dosing in humans), and the risk of pancreatic acinar cell tumors may increase with greater frequency (or the resulting increase in total dose for a chronic indication).

In my opinion, the data from the 1-year rat study of HPβCD provide some reassurance regarding the carcinogenic potential of unlimited yearly administration in humans. With daily dosing for one year, HPβCD clearly resulted in a proliferative response in pancreatic acinar cells. In contrast, with either cyclic or daily dosing of rats with HPβCD for 4 months, no microscopic changes were detected in the pancreas. In addition, no pancreatic changes were evident at the end of an 8-month recovery period in animals dosed cyclically, indicating a lack of delayed effects on the pancreas. The maximum daily dose, given by either regimen, was 5000 mg/kg, which is ≈170-280 times the maximum recommended daily dose of HPβCD in humans (287.52 mg; 60-100 kg individual), on a mg/m^2 basis. The total dose of HPβCD over the four months of cyclical or daily dosing was ≈7-350 and ≈34-1700 times, respectively, the total dose in humans over the 96-week period, on a mg/m^2 basis. While it is true that there are no data on pancreatic effects at 1 year following 4 months of daily dosing, the lack of delayed pancreatic findings in the cyclically dosed animals and the lack of positive genotoxicity findings with HPβCD (Gould & Scott, 2005) suggest a reduced concern.

No PK/ADME data for HPβCD were provided by the sponsor. However, Zhou *et al*. (Zhou H *et al*. *J Clin Pharmacol* 38:593-602, 1998) reported that plasma levels of HPβCD were undetectable by 12 hrs following IV administration of itraconazole (200 mg BID for 2 days; 200 mg QD for 5 days) formulated in 40% HPβCD, with 93-110% being excreted in urine by that time and no evidence of accumulation. Although minimal, these data (as do the data in rat) suggest that, with the cyclic dosing regimen proposed, accumulation of HPβCD in tissues would not be expected with yearly courses.

Itraconazole (Sporanox; an antifungal product; NDA 20-657) is approved as an oral solution containing HPβCD. The marketed oral solution contains 10 mg/mL of Sporanox and 400 mg/mL of HPβCD. The maximum recommended daily dose (MRDD) of Sporanox is 100-400 mg/day, depending on the indication. The recommended duration of treatment with the oral solution also varies (≈1-5 weeks) according to the indication; however, for treatment-resistant oropharyngeal candidiasis, labeling states "Limited data on the safety of long-term use (>6 months) of SPORANOX® Oral Solution are available at this time." At a dose of 200 mg/day, the daily dose of HPβCD would be 8000 mg or 8.0 gm. In comparison, the maximum daily dose of HPβCD at the MRDD of cladribine is 287.52 mg. Given according to the proposed dosing regimen, the total amount of HPβCD administered over 96 weeks would be 2.3-6 gm, i.e., less than the daily amount administered in the Sporanox oral solution. At this level, it would take decades to achieve

the same cumulative dose of HPβCD as obtained with a 5-week course of Sporanox (at 200 mg/day).

Taking into consideration the available information, including comparisons of the cumulative dose of HPβCD resulting from (b) (4) and Sporanox administration, I do not believe that additional studies of the excipient in animals (or studies to assess systemic exposure to HPβCD in humans, as Dr. Banks proposes) are necessary.

Recommendation

The nonclinical studies submitted by the sponsor are sufficient to support approval, with appropriate labeling (to include tumor findings reported for the excipient, HPβCD).

It is my understanding that, due to concerns regarding the high incidence of cancer reported in the clinical database, (b) (4) is currently not being approved. Therefore, there are no labeling recommendations at this time.

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/s/

LOIS M FREED
02/23/2011

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	N 22-561
Supporting document/s:	SDN14, SDN13, SDN1, SDN35
Applicant's letter date:	05/27/10 (SDN14), 04/27/10 (SDN13), 09/29/09 (SDN1), 9/22/10 (SDN35)
CDER stamp date:	05/28/10 (SDN 14), 04/28/10 (SDN13), 09/30/09 (SDN1), 09/22/10 (SDN 35)
Product:	(b) (4) cladribine oral formulation
Indication:	Treatment of Multiple Sclerosis
Applicant:	EMD Serono
Review Division:	Division of Neurology Products
Reviewer:	Melissa K. Banks, Ph.D.
Supervisor/Team Leader:	Lois M. Freed, Ph.D.
Division Director:	Russell G. Katz, M.D.
Project Manager:	Hamet Toure, LT, PharmD, MPH

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1 Executive Summary

1.1 Introduction

This application for an oral formulation of cladribine received fast-track and priority review status on the basis of its oral administration for the treatment of multiple sclerosis; no oral treatments were available at the time of NDA submission. The drug has been developed as an IV formulation for the treatment of hairy cell leukemia (i.e., Leustatin®, approved at a dose of 0.09 mg/kg/day for 7 days), (b) (4) (b) (4) and now as an oral formulation for the treatment of multiple sclerosis. The drug's primary mechanism of action is as an anti-metabolite, which functionally leads to relatively selective cell destruction (i.e., notably lymphocytes, as well as other cells with higher rates of cell turnover); through its action on lymphocytes, cladribine acts as an immunosuppressant/myelosuppressive. By mechanism, the drug causes DNA damage and inhibits DNA repair; it is known to be a genotoxic agent. Cladribine's mechanism of action is consistent with carcinogenic potential, and it was shown to cause Harderian gland tumors in mice following 22 monthly 1-week cycles of treatment.

1.2 Brief Discussion of Nonclinical Findings

Cladribine is an anti-metabolite purine nucleoside analog that acts as an immunosuppressant via the destruction of immune cells; however, it is notable that the drug did not demonstrate efficacy in nonclinical models of multiple sclerosis. With regard to toxicity, cladribine is known to show several toxicities (i.e., hematological, neurological, renal, reproductive [including teratogenicity], mutagenesis and a potential for carcinogenesis). A number of these toxicities have been demonstrated clinically (cf. the label for Leustatin®, an IV cladribine formulation by a different manufacturer, and the clinical safety review by Dr. Mentari dated 1/4/11, as well as the recent EMA "Refusal of Marketing Authorisation" Question and Answer sheet dated 9/23/10). The oral bridging toxicity studies conducted with cladribine did not identify new toxicities; however, the maximum doses tested were NOAELs and safety margins (based on AUC or estimated AUC exposures) are negligible. It is not known whether studies using higher doses might have identified new toxicities. Notably, a small AUC safety margin was observed following the 4-7 cycle oral administration in the mouse (at the maximum tested dose, an NOAEL) compared to the maximum daily AUC exposure in humans; this margin was approximately one-half of the margin demonstrated at the NOAEL in the 3-8 cycle SC toxicity study (~15x). The 3-cycle oral toxicity study in monkey provided a slightly higher safety margin than the NOAEL in the 14-cycle SC toxicity study, but the exposures were similar (0.8-1.5x) to those achieved after the maximum daily dose in humans. The SC toxicity studies in mice and monkeys demonstrated: bone marrow depletion (with anemia), lymphocytic depletion of the spleen and/or thymus, extramedullary hematopoiesis in the spleen, testicular atrophy (with degenerated cells and/or hypospermia in the epididymis), single cell necrosis in the duodenum, renal toxicity, adrenal toxicity and/or CNS toxicity; mortalities were noted to result from anemia, leukopenia and/or infection. Although the recently conducted cyclic oral transgenic mouse carcinogenicity assay did not demonstrate cladribine-induced tumors, cladribine

was previously shown to cause Harderian gland tumors in a 22-month cyclic SC dosing mouse carcinogenicity study and is considered carcinogenic. The safety margin for tumors of the Harderian gland in mice (estimated AUC at the highest dose that did not produce tumors, compared to the extrapolated AUC at a human dose of 20 mg) was approximately 1.3-fold; that is, cladribine has been shown to produce tumors at near clinically-relevant exposures. While a clear potential for relatively severe toxicities (including teratogenicity and carcinogenesis) has been demonstrated for cladribine, the immunosuppressants/immunomodulators approved and/or commonly-used clinically to treat multiple sclerosis are also known to cause relatively severe toxicities, including reproductive toxicity (ranging from abortifacient effects and/or embryolethality to teratogenicity; cf. the Gilenya® package insert) and carcinogenic potential (ranging from a general theoretical concern for immunosuppressants as a mechanistic class to an increased incidence of hematologic cancer demonstrated in humans; cf. secondary acute myelogenous leukemia with Novantrone® treatment, as stated in the package insert).

Notably, the clinical formulation of cladribine uses an excipient (hydroxypropyl-beta-cyclodextrin; HPβCD) known to cause tumors of the exocrine pancreas in rats. Following numerous discussions with the Division, the sponsor made an effort to assess the potential for toxicity of the HPβCD excipient with additional nonclinical studies; however, the ability of these limited studies to detect the toxicity (given the cyclic dosing regimen and the limited duration of treatment compared to the possible unlimited clinical regimen) remains questionable. Although an overall NOAEL (100 mg/kg, cyclic) was demonstrated in the sponsor's 4 to 12 month study of cyclic- and/or daily orally administered HPβCD in rats, the adequacy of study cannot be fully verified since the 10 mg/ml dose formulation was not subjected to concentration analysis.

1.3 Recommendations

1.3.1 Approvability

From a Pharmacology/ Toxicology perspective, this application is approvable, with conditions/reservations. The conditions/reservations involve persisting questions about the total duration of the recommended clinical dosing regimen, and also about the adequacy of the assessment of cladribine's metabolism.

With regard to HPβCD, the exposures achieved in the sponsor's rat toxicity study are expected to exceed those that would be experienced clinically in the 4 courses/2-year regimen, but do not provide an adequate assessment of additional courses. It is not clear from the sponsor's submission/proposed labeling whether additional cycles (after the four courses over two years) are recommended or permissible. The sponsor also asserts that HPβCD does not pose a safety concern in humans (i.e., because humans are not systemically exposed and the mechanism for exocrine tumor formation is rat-specific; cf. Gould & Scott, 2005, Stella & He, 2008 and Irie et al., 1997); however, systemic exposure is not required to produce toxicity. Toxicities of the cladribine drug product may be related to local effects generated by the presence of HPβCD in the GI

tract. For this reason, the study conducted can be used to support up to 5 cycles (i.e., the four courses over 2 years).

Although the Clinical Pharmacology review states that the application is approvable, a note to the Pharmacology / Toxicology review staff states that the *in vivo* metabolism of cladribine "has not been reliably assessed." An adequate assessment of the *in vivo* metabolism of cladribine in humans (and, if there are major human metabolites, in the animal species) is necessary to make a recommendation regarding the adequacy of the nonclinical studies. It should be noted that the oral toxicity studies did not achieve MTDs; however, the toxicity of cladribine has been explored via SC and/or IV administration. Only limited data are available in animals for metabolites (i.e., the sponsor provided only limited data because they assert that there are no major human metabolites). Although the Clinical Pharmacology review makes this statement, discussion with the Clinical Pharmacology team leader indicated that the sponsor's assessment is, in fact, adequate since: major metabolites are unlikely, the IV and SC PK is similar, and conduct of a traditional mass balance study is not possible (i.e., the active drug is formed post-cell entry).

The potential for several relatively severe toxicities (i.e., hematologic, renal, CNS, genotoxic, reproductive and carcinogenic) has been demonstrated. Observed safety margins are small (i.e., carcinogenicity) or nonexistent (i.e., teratogenicity); however, other approved treatments for multiple sclerosis have similar liabilities. Strictly taking the nonclinical data into consideration, the teratogenic and carcinogenic effects demonstrated would not preclude an approvable recommendation, due to the similar toxicities demonstrated with other approved drugs for the indication. Labeling would be used to clearly address the toxicities, especially teratogenicity and carcinogenicity. Although not within the purview of this nonclinical review, one should note that there have been reports of increased tumor/cancer incidence in patients previously treated with cladribine (see Dr. E. Mentari's review, dated 1/4/11). These tumors do not appear to be localized to a type, organ, or tissue (unlike the carcinogenic effect noted in the package insert of previously approved treatment Novantrone®; i.e., secondary acute myelogenous leukemia). These findings in the presence of a clear mechanistic basis for carcinogenic activity and a positive signal for carcinogenesis in the nonclinical studies are disconcerting.

1.3.2 Additional Non Clinical Recommendations

It does not appear that this application will receive an approval decision this cycle; the sponsor should address the following issues:

If available, Harderian gland tissues from the 7-month cyclic PO administration toxicity study in mice (study 25329) should be histologically evaluated. This tissue showed neoplastic effects in a 22-month cyclic SC administration carcinogenicity study in mice.

Although the sponsor states that HPβCD does not pose a safety concern in humans (i.e., because humans are not systemically exposed and the mechanism for exocrine

pancreas tumor formation is rat-specific; cf. Gould & Scott, 2005, Stella & He, 2008 and Irie et al., 1997), these assertions have not been adequately documented. No data were provided to demonstrate the lack of systemic exposure in humans using this dosing regimen, and the mechanism of the exocrine tumor formation is not generally accepted as species-specific. The cyclic administration toxicity study of HPβCD in rat performed by the sponsor provides support for 5 (monthly) cycles of treatment (and chronic daily dosing) with HPβCD.

It is not clear from the sponsor's application that the marketed treatment regimen is limited to 4 courses (a total of 3.5 mg/kg) (b) (4), and that further treatment courses are not permitted. To support additional courses up to intermittent lifetime exposures, a full evaluation of the potential toxicity of HPβCD would need to investigate the potential toxicity related to both systemic exposure and local effects in the GI system (i.e., not requiring systemic exposures). It is recommended that, since lifetime exposure was not evaluated in the sponsor's study, Section 13.1 of the label should discuss the carcinogenic potential of the HPβCD excipient, in addition to the carcinogenic activity of cladribine. To address this concern and to support a significant increase in the number of permitted courses (as much as lifetime cyclic exposure), an adequate study of the cyclic administration of HPβCD for a period approximating lifetime exposure in rats should be conducted. Alternatively, if a more limited number of courses (but greater than 4) is to be permitted (e.g., according to the dosage and administration section of labeling), the sponsor could make efforts to confirm that humans are not systemically exposed to HPβCD. If systemic exposure is observed, that exposure level should be compared to that at the NOAEL for exocrine pancreas hyperplasia in rats (it is noted that only limited exposure data at the NOEL are available from the rat HPβCD toxicity study). It is noted that these data would not evaluate potential toxicity related to lifetime cyclic local GI exposure to HPβCD; the need for further evaluation of such effects would require further discussion.

1.3.3 Labeling
[Labeling will not be addressed at this time.]

2 Drug Information

2.1 Drug

CAS Registry Number	4291-63-8
Generic Name	cladribine
Code Name	2CdA, RWJ-26251-000 (J&J) EMD 280922 (Merck)
Chemical Name	2-chloro-2'-deoxy-β-D-adenosine 2-chloro-6-amino-9-(2-deoxy-β-D-erythropento-furanosyl) purine

Molecular Formula/Molecular Weight $C_{10}H_{12}ClN_5O_3$ / 285.69

Structure or Biochemical Description (from the sponsor's submission)

The structure of cladribine is presented below. It is a molecule with three stereogenic centers (indicated by the asterisks).

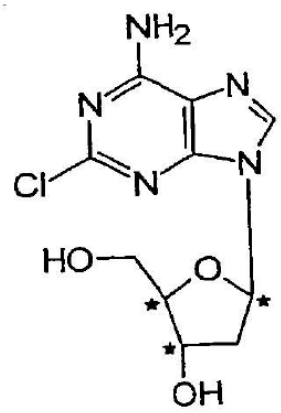


Figure 1: Representation of the structure of cladribine ($C_{10}H_{12}ClN_5O_3$)

Pharmacologic Class: immunosuppressant, chlorinated purine analogue, cytotoxic nucleoside anti-metabolite

2.2 Relevant INDs, NDAs, BLAs and DMFs

DMFs

(b) (4) (type II)

INDs (commercial)

74,634, cladribine PO for the treatment of MS, EMD Serono Inc.

(b) (4)

NDAs

(b) (4)

20-229 (Approved), Leustatin®, IV injection, Hairy Cell Leukemia, Ortho Biotech

2.3 Drug Formulation

Table 1: Composition of the drug product

Name of Ingredients	Unit Formula (mg/tablet)	Function	Reference to Standards
Drug substance			
Cladribine	10.00	Active Ingredient	Ph.Eur / USP
Other ingredients			
2-hydroxypropyl-β-cyclodextrin	(b) (4)		Ph.Eur / USP-NF
Sorbitol (b) (4)			Ph.Eur / USP-NF
Magnesium Stearate			Ph.Eur / USP-NF
Total			----

2.4 Comments on Novel Excipients

The Division informed the sponsor during a 10/26/07 teleconference that the inclusion of hydroxypropyl-β-cyclodextrin (HPβCD) as an excipient in the oral drug product could be problematic. Orally administered HPβCD has been shown to cause tumors of the exocrine pancreas in rats at all doses tested. The sponsor opted to continue development of the HPβCD oral formulation. To support the use of HPβCD in the formulation at the proposed clinical dose and cyclic regimen, the sponsor performed additional studies of the formulation and a study of HPβCD alone in rats using a cyclic dosing regimen.

2.5 Comments on Impurities/Degradants of Concern

None.

2.6 Proposed Clinical Population and Dosing Regimen

(b) (4) is indicated for the treatment of relapsing forms of multiple sclerosis (MS) to decrease the frequency of clinical exacerbations. (b) (4) is administered in treatment courses, each course consisting of daily oral administration of 1 or 2 of the 10 mg tablets given for the first 4 or 5 days of a 28-day period. Treatment is initiated with 2 consecutive courses at the beginning of the first 48-week period. Two additional consecutive courses are started at the beginning of a subsequent 48-week period. This corresponds to a cumulative dose of ≈3.5 mg/kg over 96 weeks. Hematological criteria for starting and continuing therapy have to be met before initiating (b) (4) or administration of any subsequent treatment course.

2.7 Regulatory Background

(b) (4) has had several sponsors, with EMD Serono eventually purchasing rights and reformulating to achieve a PO formulation. A significant portion of the nonclinical data submitted to (b) (4) has been previously reviewed Dr. R. Huff (Pharmacology/Toxicology review for (b) (4)). EMD Serono requested both fast-track designation and priority review of this NDA.

3 Studies Submitted

3.1 Studies Reviewed

Study 8920203: *In Vitro* Pharmacology- Study of MSC1326724A [Large Receptor Profile (Cerep)].

Study 8920216: *In Vitro* Pharmacology- Study of MSC1326724A and MSC2312582A [Adenosine Assays (Cerep); cladribine and metabolite 2CdA].

Study 25785, RC9840: Evaluation of effects on HERG current in stably transfected HEK-293 cells.

Study 26515, RD5310: Evaluation of effect on cardiac action potential in isolated canine Purkinje fibers.

Study No.: GSP0009ECG: Measurement of heart rate, arterial blood pressure, ECG, platelet aggregation and clotting time in awake dogs after oral administration of MSC1326724A.

Study No. 9MERCDP8R2, DMPK 114-09: Permeability Assessment of Cladribine in two Different Formulations Using Caco-2 Cell Monolayers.

ASLP Study No.: 8MERCDP7: In vitro Permeability Assessment of Cladribine using Caco-2 Monolayers.

Study DM91026: Report DM91026 - Determination of the in-vitro plasma protein binding of 2-chlorodeoxyadenosine.

Study No. DMPK 137-08: In Vitro Distribution of [14C]-EMD 280922 in Whole Blood of CD-1 and C57BL6J Mice, Monkey and Human.

Study No.: 9MERCDP6R1, DMPK 58-09: BCRP Substrate Assessment of the Customer Test Compound, Cladribine, Using Caco-2 and CellPort CPTB1 (BCRP-knock-down) Cell Monolayers.

ASLP Study No. 8MERCDP2R1, DMPK 109-08: P-Glycoprotein Substrate and Inhibitor Assessment of Cladribine in MDR1-MDCK, MDCK, and Caco-2 Cell Lines.

Study DMPK 131-09: *In vitro* Interaction Studies of Cladribine with human MRP2 (ABCC2), MRP4 (ABCC4), and MRP5 (ABCC5) ABC (efflux) Transporters, and with human OCT2, OAT1 and OAT3 (SLC22A8) Uptake Transporters.

Study Number of Sponsor: DMPK 133-09: Transport assay using OAT4 expressing cells. *(incomplete study report.*

Study 25329, RC4660 and RC4661(TK): Chronic oral toxicity study in mice.

Study 28732, RE7750 and RE7751: Five-day oral toxicity study in mice followed by a 22-day off-dosing period.

Study 29006, RE7460: Toxicity study in Wistar rats treated by oral route with 2- hydroxypropyl-β-cyclodextrin (HPβCD) for 4 cycles, followed by 8-month recovery.

Study Number 28853, (b) (4).281.01: A Five-Week Oral Repeat Dose Range Finding Study of Dissolved Cladribine tablets in CByB6F1-Tg(HRAS)2Jic (+/- hemizygous c- Ha-Ras) Mice and a Toxicokinetic Study in Non-Transgenic CByB6F1 Hybrid Mice.

Study (b) (4).281.02, RE8680 (IMP29174): A 26-Week Oral Dose Carcinogenicity Study of Cladribine in CByB6F1-Tg(HRAS)2Jic Hemizygous Mice and Toxicokinetic Study in CByB6F1-Tg(HRAS)2Jic Wild Mice.

3.2 Studies Not Reviewed

Studies in (b) (4) previously reviewed (see below) were not reviewed in full.

3.3 Previous Reviews Referenced

(b) (4) P/T Review #1, Dr. R. Huff, dated 12/15/1998
P/T Review #2, Dr. R. Huff, dated 1/26/1999
Statistical Consultation, Dr. H.M.J. Hung, dated 4/27/1999

4 Pharmacology

4.1 Primary Pharmacology

Cladribine is a chlorine-substituted purine nucleoside analog of naturally-occurring deoxyadenosine that functions as an anti-metabolite. Cladribine enters cells (passively and/or possibly involving nucleoside transporters; e.g., CNT, ENT), is phosphorylated by deoxycytidine kinase (DCK) to 2-chloro-2'-deoxy-ß-D-adenosine monophosphate (2-CdAMP), and is subsequently phosphorylated to form the triphosphate (2-CdATP). The cytotoxic nucleotide accumulates selectively in cells, such as monocytes and lymphocytes, which have a high ratio of deoxycytidine kinase to deoxynucleotidase activity; accumulation is also enhanced because cladribine, due to the chlorine substitution, is resistant to deamination by adenosine deaminase. Accumulation of the triphosphate form results in an imbalance of intracellular deoxynucleotide triphosphates; this leads to disruption of cellular metabolism, impairment of DNA synthesis and repair, and ultimately cell death. Both dividing and quiescent cells are damaged by accumulation of 2-CdATP. As detailed in Dr. Huff's review, 2-CdATP is believed to impair DNA synthesis in dividing cells by being incorporated directly into the DNA; in quiescent cells, repair of single strand DNA breaks is believed to be hampered by deoxynucleotide accumulation because cellular stores of NAD and ATP are depleted when the DNA breaks initiate poly (ADP-ribose) polymerase activity (e.g., see Seto et al., 1985; Griffig et al., 1989). Cladribine's main metabolite, 2-chloroadenine (2-CA), is reported in the literature to also be cytotoxic, albeit 8x less so than cladribine (cf. Lindemalm et al., 2004). In addition, cladribine and the main metabolite, 2-chloroadenine, were found to weakly bind adenosine receptors in standard receptor binding assays.

Studies to demonstrate efficacy in nonclinical models of multiple sclerosis failed. In 2 mouse (SJL/J mice) EAE models, the effects of cladribine on disease amelioration were evaluated using an injection of whole myelin and complete Freund's adjuvant and pertussis toxin. No positive effect on the clinical outcomes of EAE was seen in either study. The sponsor attributed this lack of an efficacious response to "pharmacogenetic differences affecting PK and PD between rodents and humans… it is very likely that the level of cladribine necessary to suppress the immune response was either not achieved or not sustained for sufficient time to have an effect."

4.2 Secondary Pharmacology

In response to the Divisions' request (cf. correspondence dated 07/14/09), the sponsor provided additional information about the actions of cladribine at other than the desired pharmacological target.

Cladribine showed binding at human adenosine (A) receptors and phosphodiesterases (PDE). At the A_1 receptor, the IC_{50}s for the inhibition of antagonist and agonist binding ranged from $1.7x\ 10^{-5}$ to 3.6×10^{-6} M, respectively; the K_is for the A_1 receptor were 1.1×10^{-5} to 1.5×10^{-6} M. In the initial assay, cladribine binding did not meet significance for inhibition of agonist binding at A_{2A} or A_3 receptors. Cladribine and its metabolite 2-chloroadenine (2-CA) were later tested at human A_1, A_{2A}, A_{2B} and A_3 receptors. Cladribine was shown to activate the *h*A_1 receptor and compete for the antagonist binding site of the *h*A_{2A} receptor. Cladribine demonstrated EC_{50}s (agonist effects) of 0.59 μM at *h*A_1 and 59 μM at *h*A_3, and an IC_{50} of 10 μM at *h*A_{2A}. For 2-CA, the IC_{50}s ranged from 13 to 88 μM. Additionally, inhibition of binding at PDE_{2A} and PDE_{4D} showed IC_{50}s of 5.5×10^{-6} and 3.8×10^{-6}, respectively.

4.3 Safety Pharmacology

The majority of the safety pharmacology studies were reviewed previously. The potential for respiratory and cardiovascular effects was observed; see the summary below from Dr. Huff's review for withdrawn (b) (4). Additionally, the sponsor conducted a canine Purkinje fiber assay, a hERG assay and a cardiovascular study in awake dogs (briefly reviewed below).

> When cladribine was evaluated in safety pharmacology studies, most endpoints were unaffected, with the exception of mild to moderate effects on respiration and cardiovascular parameters in anesthetized dogs. Neurological parameters (Irwin test), thiopental-induced sleep time, nociception and gastrointestinal motility were unpertubed by a 10 mg/kg i.v. dose in mice. Likewise, this dose did not produce analgesia, or alter diuresis or saluresis in rats. *In vitro*, cladribine concentrations up to 100 μg/ml did not hemolize rabbit blood or affect coagulation or platelet aggregation. When the affinity for adenosine receptors was assessed *in vitro*, cladribine was shown to have low affinity for endogenous rat A1 and A2 receptors, and no affinity for recombinant A3 receptors (species origin unspecified); the concentrations of cladribine required to inhibit A1 and A2 ligand binding were ≥ 3.5 μM. Similarly high concentrations were required to inhibit adenosine uptake in guinea pig tissue.

Although the affinity of cladribine for adenosine binding sites is low, the cardiovascular effects observed in anesthetized dogs during the 30 min after cladribine injection were consistent with A2 receptor-mediated vasodilatation. Intravenous doses of ≥ 5 mg/kg slightly decreased arterial pressure (~10 and 20% at 5 and 10 mg/kg, respectively) and increased heart rate (~30% at both 5 and 10 mg/kg) and cardiac output (~50 and 40% at 5 and 10 mg/kg, respectively). Stroke volume was unaffected. Doses of ≥ 0.5 mg/kg increased respiration rate (~100, 40, 160 and 100% at 0.5, 1, 5 and 10 mg/kg, respectively) and volume (~40, 20, 70, and 70% at 0.5, 1, 5 and 10 mg/kg, respectively). A 0.1 mg/kg dose did not affect cardiac or respiration parameters, but it is noted that ECG's were not recorded in this study. Although no pharmacokinetic data were collected in the dog study, it is likely that the Cmax attained after bolus administration of cardioactive doses far exceeds the plasma levels achieved in humans after subcutaneous administration of the significantly lower 0.07 mg/kg clinical dose.

Study 26515, RD5310: Evaluation of effect on cardiac action potential in isolated canine Purkinje fibers

Cladribine (batch 01P0358) was tested for effects at concentrations of 10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M. Formulation analysis indicated that concentrations were within ± 10% of nominal. There were no statistically significant effects on action potential parameters under either normal (1 Hz) or low (0.33 Hz) stimulation rates. Neither early nor delayed after-depolarization was observed. Cladribine, up to a concentration of 10^{-4} M, had no effect on action potential duration.

Study 25785, RC9840: Evaluation of effects on HERG current in stably transfected HEK-293 cells

Cladribine (batch 01P0358) was tested at 10^{-4} M for effects on the hERG tail current. Cladribine was dissolved in DMSO (stock solution of 10^{-1}M) and subsequently diluted in Tyrode's solution. Cladribine solution concentrations were within ±10% of nominal. Cladribine produced a very slight inhibition on hERG tail current (13 ± 2% inhibition, n=6, p≤0.05). Since cladribine failed to produce more than 70% inhibition of the hERG tail current, no IC_{50} value was determined.

Study No.: GSP0009ECG: Measurement of heart rate, arterial blood pressure, ECG, platelet aggregation and clotting time in awake dogs after oral administration of MSC1326724A

(b) (4) dated 12/1/09

GLP, except: 1) measurement of the total plasma concentrations of cladribine (MSC1326724A) and its metabolite, 2-chloroadenine, 2) measurement of the *ex vivo* collagen-induced platelet aggregation and clotting time and 3) two-way statistical analysis of variance for repeated measurements

The effects of cladribine (80 mg, as clinical tablets, batch N0800818) on heart rate, arterial blood pressure, ECG parameters, *ex vivo* collagen-induced platelet aggregation, and clotting time parameters were investigated in 10 awake male and female mongrel dogs (5 placebo animals, 5 cladribine animals; 2-9 years, 21-27 kg). Measurements were taken for ~30 min prior to treatment and for ~240 min after treatment. There were few significant effects on general condition, behavioral, cardiovascular or other

parameters. Slightly decreased heart rate was observed between 150-180 min, with a very slight decrease in systolic blood pressure. Rectal temperature was very slightly decreased at 120-240 min, and venous pH, partial O_2 pressure and O_2 saturation were very slightly increased at 240 min. (Notably, respiration rate appeared slightly reduced at around the same time, 105-240 min, in both control and drug-treated animals.) At 120 min, prothrombin time was very slightly increased (8.5 vs. 8.3 at -20 min), the number of platelets was very slightly decreased (17.5 vs. 19.0) and maximal collagen-induced platelet aggregation was decreased (48.2 vs. 59.6). Following administration of 80 mg cladribine, C_{max} was 1775 ± 1128 ng/ml and AUC_{0-24hr} was 4530 ± 5514 ng.hr/ml for the parent drug. A C_{max} of 3.2 ± 1.6 ng/ml and an AUC_{0-24hr} of 3.2 ± 3.1 ng.hr/ml were observed for the metabolite, 2-chloroadenine. The sponsor indicated that the exposure was ~46-fold above (parent drug) or approximately in the range (metabolite) of the exposures in patients with multiple sclerosis following a 10 mg dose (i.e., C_{max} of 29 ± 10 ng/ml and $AUC_{0-\infty}$ of 99 ± 29 ng.hr/ml).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Summary

The TK of cladribine has been investigated in mice and monkeys (as well as on a very limited scale in dogs and rats) after PO administration, which is the intended route for human use, and in mice, rats, rabbits, dogs and monkeys after parenteral administration (SC and/or IV). Limited ADME characteristics of cladribine were evaluated in mice and monkeys, the species selected for evaluation in toxicity studies. Species selection was based on plasma concentrations of deoxycytidine. According to the sponsor, the highest deoxycytidine levels were observed in rats (between 5.13 to 8.5 μg/ml); this is in contrast to the concentrations found in mice, guinea pigs, dogs and rabbits (ranged 0.31 to 0.42 μg/ml) and in monkeys and humans (could not be measured). Deoxycytidine and cladribine compete for phosphorylation by deoxycytidine kinase, which was believed would result in an altered toxicity profile in rats (i.e., decreased formation of cytotoxic cladribine nucleotides and, therefore, lessened effects on blood cell lines and rapidly dividing tissues). As noted in Dr. Huff's review, rats showed fewer hematologic effects and some unique target organ toxicities (e.g., intracardiac thrombi and myocardial degeneration of the heart, biliary hyperplasia and single cell/multifocal hepatic necrosis of the liver). Therefore, rat was not considered an appropriate animal model. Oral bioavailability was also determined in dogs, since they were used for the cardiac safety pharmacology evaluation. For repeat-dose oral toxicology studies in mice and monkeys, cladribine was formulated in 5% HPβCD (2-hydroxypropyl beta-cyclodextrin) in water solution to mimic the intended formulation for humans (HPβCD-tablets). Oral bioavailability in rat and monkey was determined using cladribine in HPβCD water solution, while oral bioavailability in dog was determined using an HPβCD-tablet prototype. See the reviewer-modified sponsor's PK summary Table 2.6.4-2, detailing exposures in the toxicology species.

Table 2.6.4- 2 Comparative Summary of Main Pharmacokinetic Parameters of Cladribine Administered i.v. (or s.c.) and p.o. (Mean (Sd))

Species	Study report (summary table no.)	Route of administration (Dose)	Cmax (ng/mL)	Tmax (h)	AUC0-t (ng·h/mL)	t1/2 (h)	CL (mL/min/kg)	Vss (L/kg)	F (%)
Mouse	DM94033 (2.6.5.16C)	s.c. (1 mg/kg)	316 [a]	0.5 [a]	334 [a]	-	-	-	-
	25329-RC4660 (2.6.5.16D)	**p.o. (2 mg/kg) cladribine-HPβCD water solution**	**118 [a]**	**0.5 [a]**	**148 [a]**	-	-	-	-
	28853-RE7990 (2.6.5.16K)	**p.o. (5mg/kg) cladribine HPβCD water solution**	**717 [a]**	**0.5 [a]**	**757 [a]**	-	-	-	-
	29174-RE8680 (2.6.5.16L)	**p.o. (5mg/kg) cladribine HPβCD water solution**	**505 [a]**	**0.5 [a]**	**457 [a]**	-	-	-	-
Rat	DM94032 (2.6.5.16E)	s.c. (1 mg/kg)	225 [a]	-	270 [a]	-	-	-	-
	28215-RE3000 (2.6.5.3A)	i.v. (2 mg/kg)	3035 [b]	-	686 [c]	0.92	48.6	1.54	NA
		p.o. (5 mg/kg) cladribine-HPβCD water solution	**382**	**0.5**	**439 [c]**	**1.06**	-	-	**26**
Rabbit	DM95067 (2.6.5.3B)	i.v. infusion (1 mg/kg)	-	-	1099 (54)[c]	0.64 (0.20)	15.2 (0.7)	0.465 (0.025)	-
		s.c. (1 mg/kg)	616 (93)	0.5 (0.0)	933 (191)[c]	0.58 (0.11)	-	-	85 (17)
Dog	DM95312 (2.6.5.3B)	i.v. infusion (1 mg/kg)	992 (248)	0.25 (0.0)	689 (74)[c]	0.8 (0.1)	24.4 (2.7)	1.16 (0.16)	-
		s.c. (1 mg/kg; 1 mg/mL)	428 (75)	0.6 (0.2)	723 (124)[c]	0.8 (0.0)	-	-	105 (18)
	0315 (2.6.5.3C)	i.v. 5mg (0.40 mg/kg)	-	-	382 (42)[c]	10.3 (1.0)	17.3 (0.9)	-	-
		p.o. (0.40 mg/kg) 5 mg HPβCD tablet	**132 (58)**	**0.9 (0.5)**	**174 (26) [c]**	**13.7 (2.2)**	-	-	**44.8 (5.4)**
Monkey	DM96006 (2.6.5.3B)	i.v. (1 mg/kg)	760 (103)	0.2 (0.1)	666 (100)[c]	1.2 (0.1)	25.4 (3.7)	1.62 (0.18)	-
		s.c. (1 mg/kg)	352 (44)	0.4 (0.2)	535 (90)[c]	1.4 (0.2)	-	-	81 (8)
	26112-RD2770 (2.6.5.3D)	i.v. (1 mg/kg)	1345 (523)	-	794 (130)[c]	5.7 (0.9)	21.5 (3.8)	2.20 (0.66)	-
		s.c. (1 mg/kg)	628 (150)	0.5	1031 (235)[c]	5.1 (0.4)	-	-	130 (19)
		p.o. (2 mg/kg) cladribine-HPβCD water solution	**44.7 (19.3)**	**1.75**	**170 (66.7)[c]**	**3.6 (1.5)**	-	-	**11 (3)**
Monkey	26210 (2.6.5.16F)	**p.o. (2 mg/kg cladribine-HPβCD water solution)**	**22.6 (3.18) [a]**	**1 [a]**	**103 (12.6) [a]**	**-**	-	-	**-**
	26992 (2.6.5.16H)	s.c (0.3 mg/kg)	99.3 [a]	0.5 [a]	159 [a]	-	-	-	-
		p.o. (1.5 mg/kg cladribine-HPβCD water solution))	**9.3 (M); 27 (F) [d]**	**3 (M): 1 (F) [a]**	**41.2 (M); 93.7 (F) [d]**	**-**	**-**	**-**	**-**

a: Mean from Males and Females on first day of dosing (Day 1)

b: C0 (First sampling time)

c: AUC0-∞

d: Mean from first day of dosing (Day 1)

The sponsor stated that bioanalytical methods were developed and validated to support the nonclinical program for cladribine. Liquid chromatography-mass spectrometry (LC-MS) with atmospheric pressure chemical ionization (APCI) was used. The method was validated for the quantification of cladribine in mouse, rat, rabbit, dog and monkey plasma, as well as for the quantification of cladribine in dog and monkey cerebrospinal fluid. Quantification of cladribine and its metabolite, 2-chloroadenine (2-CA), was validated in rat plasma and in monkey plasma and urine. However, most studies were not conducted as GLP studies (with the exception of mouse and rat plasma).

Oral absorption of cladribine in mice, rats, dogs and monkeys was fairly rapid, generally reaching C_{max} within 1 hr after administration. Absolute oral bioavailability was ~45% in dogs (following administration of a cladribine-HPβCD tablet), but was lower in rats (~26%, after administration of cladribine-HPβCD dissolved in deionized water) and monkeys (11%, following administration of cladribine-HPβCD dissolved in deionized water). The lower oral bioavailability in monkey was believed to result from high first pass metabolism. In comparison, oral bioavailability of the cladribine tablet in humans was ~40%. (For reference, bioavailability following SC administration was nearly complete in rabbits, dogs, monkeys and humans [80-100%]). According to the sponsor, no major human metabolites were observed after oral administration to humans. Following PO administration of cladribine, 2-chloroadenine (2-CA) was found to be a minor metabolite in rat and human plasma with exposures <5% of the parent compound; however, 2-CA exposure was around 60% that of cladribine following PO administration of cladribine in the monkey, consistent with a much higher first pass metabolism. After oral administration of tablets to dogs, the mean elimination half-life ranged between 12 and 15 hr. After oral administration in monkeys, cladribine was eliminated, with a terminal half-life of 3.6 hr. In monkeys, urinary excretion was negligible and variable for the parent compound and for 2-CA. In comparison, cladribine showed moderate oral bioavailability (~43%), moderate IV clearance (~52% of liver blood flow) and rapid excretion (58%-66% within the 24-hr urine fraction) after administration in humans. Excretion in mice was investigated following SC administration; cladribine elimination was rapid (~74% within 24 hr) and predominately renal (64%, vs. 3% fecal; recovery was incomplete). The sponsor provided the following summary Table 2.6.4-8 to detail exposures at the NOAELs for repeated dosing toxicity studies. (Sponsor's Table 2.6.4-9 detailing human exposures is also provided for comparison.)

Table 2.6.4- 8 **Summary of Cmax and AUC0-24h Values for Cladribine at the NOAEL in Toxicity Studies with Repeated Dosing.**

Study number (report number)	Species	Route of admin.	Duration of dosing / Day of sampling	NOAEL (mg/kg)	Mean Cmax (ng/mL) at NOAEL	Mean AUC_{0-24h} (ng.h/mL) at NOAEL
25329 (RC4660)	Mouse	p.o.	Four to seven 28-days dosing courses [a] / Course 7 Day 7 (Day 169)	20	**2715**	**2045**
28853 (RE7990)	Mouse	p.o.	Two 28-days dosing courses [b] / Course 1 Day 1	30 [c]	**4345** [c]	**3992** [c]
DS94005 (DM94031)	Mouse	i.v.	4 weeks - Daily dosing	ND [d]	**ND**	**ND**
DS94125 (DM94033)	Mouse	s.c.	13 weeks- Daily dosing	ND [d]	**ND**	**ND**
DS94126 (DM94032)	Rat	s.c.	13 weeks- Daily dosing / Day 1	1	**225**	**270** [e]
26210 (RD3030)	Monkey	p.o.	One 28-days dosing courses [a] / Day 7	5	**73.2**	**405**
26669 (TK report 26992)	Monkey	p.o.	Three 28-days dosing courses [a] / Day 61-62	6	**91.5**	**387**
		s.c.		0.3	**125**	**248**
DS95311 (DM95358)	Monkey	s.c.	Fourteen 28-day dosing courses [a] / Day 343	0.15 [f]	**25.1** [g]	**ND**

ND: Not determined

a: One "28-days dosing course" corresponds to 7 days of daily dosing followed by 21-days without dosing.

b: One "28-days dosing course" corresponds to 5 days of daily dosing followed by 23-days without dosing.

c: MTD for cladribine drug substance (Maximal Tolerated Dose)

d: NOAEL not established in this study

e: Mean AUC(0-7h)

f: NOAEL with the exception of dermal scaling

g: Mean plasma concentration 10 min post dose

Table 2.6.4- 9 **Metabolite (2-CA) to Parent Compound (Cladribine) Ratios After Oral and i.v. Administration of Cladribine to 16 Patients with MS (refer to clinical study 25803)**

Oral Dose / Formulation	Matrix (No. for Mean PK Parameter)	Mean PK Parameter	Cladribine	2-CA	2-CA / Cladribine Ratio
10 mg / Tablet	Plasma (N=16)	C_{max} (ng/mL)	29 (101.5 nM)	0.67 (3.95 nM)	**0.04**
		AUC_t (ng.h/mL)	94.9 (332 nM)	2.62 (15.4 nM)	**0.05**
	Urine (N=16)	% of dose	28.5	1.37	**0.05**
3 mg / 1 h infusion	Plasma (N=16)	C_{max} (ng/mL)	20.6 (72.1 nM)	0.38 (2.24 nM)	**0.03**
		AUC_t (ng.h/mL)	65.2 (228 nM)	1.03 (6.07 nM)	**0.03**
	Urine (N=16)	% of dose	57.5	3.91	**0.07**

The sponsor submitted a number of ADME studies (mostly non-GLP) with the NDA. Cladribine showed low plasma protein binding in all species tested (see table from the sponsor, below). The in vitro distribution of cladribine in whole blood was investigated in mouse (2 strains), cynomolgus monkey and human. Cladribine was found in greater concentration in plasma in CD-1 mouse and Cynomolgus monkey, but in blood cells in C57BL6J mouse and human. Taking into account the species differences in the hematocrit, these results translate into similar average distribution coefficients $K_{(BC/plasma)}$ of 1.1 (C57BL6J mouse), 1.2 (CD-1 mouse), 1.2 (monkey) and 1.3 (human). The distribution was found to be concentration-independent. See sponsor's Table 1 for results. After SC administration, cladribine was distributed throughout the body of mice (see sponsor's table, below, from Study DM97002). Bladder, kidney, spleen, adrenal, liver and/or heart showed high concentrations early; prolonged concentrations were demonstrated in liver, GI tract, bladder, spleen and/or thymus (although interpretation of the data is complicated by the usage of "ND" for both tissue levels <LLOQ and the lack of data).

	Concentration of 2-CdA*		
Matrix	6.1 ng/mL	61.1 ng/mL	6.1 ug/mL
Rat Plasma	14.4 ± 1.10	13.8 ± 1.21	12.6 ± 1.64
Dog Plasma	9.2 ± 1.60	10.3 ± 2.21	9.2 ± 1.01
Monkey Plasma	15.9 ± 0.75	15.8 ± 1.70	17.1 ± 0.40
Human Plasma	19.4 ± 1.33	19.9 ± 2.88	20.8 ± 0.38
Human Serum	13.1 ± 2.41	19.0 ± 3.25	20.3 ± 1.74

Values = %bound ± S.D.

* Theoretical spiked concentration; at equilibrium and assuming that 2-CdA is 20 percent protein bound; the 6.1 ng/mL, 61.1 ng/mL and 6.1 µg/mL concentrations would equate to 3.4 ng/mL, 33.9 ng/mL and 3.4 µg/mL in the matrix, respectively.

Table 1 Summary of Results

Species	mouse CD-1		mouse C57BL6J		monkey		human	
EMD 280922	0.1 µM	10 µM	0.1 µM	10 µM	0.1 µM	10 µM	0.1 µM	10 µM
fraction in plasma (%)	54	54	48	49	52	52	47	46
fraction in BC (%)	46	46	52	51	48	48	53	54
K(BC/plasma)	1.2	1.2	1.1	1.1	1.2	1.2	1.3	1.3
max. uncertainty of K	0.08	0.04	0.06	0.08	0.23	0.18	0.20	0.17

Table SD2: Tissue Concentrations (µg·equivalents/g) in Male and Female Mice Following Subcutaneous Injection of ^{3}H-Cladribine (DM97002)

Tissue	Females								
	10 min	30 min	1 h	2 h	3 h	4 h	8 h	24 h	48 h
Adrenal glands	7.13	3.07	ND	NM	NM	ND	ND	NM	ND
Heart Blood	2.70	2.20	0.87	ND	ND	ND	ND	ND	NM
Salivary Gland	3.22	1.51	2.72	NM	NM	ND	ND	NM	NM
Brain	0.91	0.65	0.63	ND	ND	ND	ND	NM	NM
GIT	3.59	2.39	2.91	1.97	2.35	3.88	3.32	1.10	ND
Heart Muscle	4.53	2.22	1.59	ND	ND	ND	NM	NM	NM
Kidneys	8.32	6.72	2.90	0.67	ND	ND	ND	ND	NM
Liver	5.26	4.01	3.15	1.00	0.62	ND	ND	ND	ND
Lungs	3.72	1.78	1.23	ND	ND	ND	ND	ND	NM
Skeletal Muscle	2.00	1.63	1.04	ND	ND	NM	ND	ND	NM
Skin	2.30	1.86	1.45	ND	ND	ND	ND	ND	ND
Spleen	8.04	4.44	3.04	0.84	0.71	0.77	0.71	ND	NM
Thymus	3.15	2.40	1.36	ND	NM	ND	ND	NM	ND
Bladder	12.83	37.85	31.82	5.63	3.10	4.85	NM	2.62	NM

Tissue	Males								
	10 min	30 min	1 h	2 h	3 h	4 h	8 h	24 h	48 h
Adrenal glands	3.05	5.55	1.58	ND	ND	ND	ND	ND	ND
Heart Blood	3.29	4.65	0.63	ND	ND	ND	ND	ND	ND
Salivary Gland	4.84	14.54	3.05	ND	ND	ND	ND	ND	ND
Brain	0.85	1.06	ND	ND	ND	ND	ND	ND	ND
GIT	3.92	6.27	3.29	3.14	2.31	3.76	1.46	ND	ND
Heart Muscle	4.64	6.40	1.34	0.60	ND	ND	ND	ND	ND
Kidneys	6.86	16.74	5.65	0.72	0.73	ND	ND	ND	ND
Liver	5.39	8.75	2.67	1.02	0.64	ND	ND	ND	ND
Lungs	3.64	5.51	0.91	ND	ND	ND	ND	ND	ND
Skeletal Muscle	2.38	3.90	0.80	ND	ND	ND	ND	ND	ND
Skin	1.94	4.59	0.87	ND	ND	ND	ND	ND	ND
Spleen	6.93	15.02	2.26	0.70	1.09	1.00	0.61	NM	ND
Testes	1.50	2.86	ND	ND	NM	ND	ND	NM	ND
Thymus	3.51	5.97	1.45	ND	ND	0.65	ND	ND	ND
Bladder	12.53	45.68	132.62	4.86	5.31	6.81	NM	2.04	ND

LOQ = 0.6 µg·equivalents/g
ND = Tissue not present in one or more sections or below LOQ.
NM = Tissue present in one or more sections, but not visible/below LOQ.
Data rounded to 2 significant figures.

Cladribine was shown to be a moderately permeable drug (as measured by comparative unidirectional (A-to-B) P_{app} value). The efflux ratio of cladribine suggested that at least one transporter is involved in the cladribine permeation through Caco-2 monolayers. Cladribine formulations did not affect the permeability of the low permeability marker, atenolol, but reduced the P_{app} values of the high permeability marker, minoxidil, across Caco-2 cell monolayers. Cladribine was also shown to be a substrate of human BCRP, MRP5, OCT2 and OAT3, but not of P-gp (tested at 10-50 µM). See Table 8, below, from the sponsor. Cladribine did not appear to be a substrate for OAT4.

Table 8. Calculated inhibition parameters from vesicular transport and uptake assays.

TA	TA concentration range [µM]	Assay	Maximal Inhibition [%]	IC_{50} [µM]
Cladribine	0.14 - 100	MRP2 VT	22	-
	0.14 – 100	MRP4 VT	-	-
	0.14 – 100	MRP5 VT	58	64
	0.14 – 100	OAT1 Uptake	14	-
	0.14 – 100	OAT3 Uptake	39	-
	0.14 – 100	OCT2 Uptake	-*	-

* Additional stimulation was observed up to 35% comparing to the control at 33 µM.

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: RE7750 (and RE7751): Five-day oral toxicity study in mice followed by a 22-day off-dosing period.

Study no.: 28732
Study report location: EDR, eCTD
Conducting laboratory and location: (b) (4)
Date of study initiation: January 23, 2008
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: Cladribine tablets (composition as below); batch N0219A

Composition of cladribine tablet 10.0 mg

Cladribine Active ingredient	10.00 mg/tablet
Hydroxypropylbetadex(2-hydroxypropyl-ˇ-cyclodextrin)	(b) (4)
Sorbitol (b) (4)	
Magnesium stearate	

Key Study Findings

- MTD= 20 mg/kg
- Deaths occurred at ≥ 30 mg/kg, in a dose-related fashion
- Causes of death involved kidney and/or lymphoid toxicities

Methods (in addition to the excerpted sponsor's summary table, below)

Route of administration: PO, gavage
Dose volume: 15 ml/kg
Formulation/Vehicle: In deionized water, as a suspension
Species/Strain: Crl:CD-1(ICR) mice (M and/or F, as below)
Age: 6 weeks old, at receipt
Weight: 26-29g for males and females, at receipt

	Sex	Group (No. of animals)	Doses (mg/kg/day)	effective dosing period days	planned dosing period days
Phase 1	Male	1 (6M)	0 (vehicle)	3*	5
		2 (6M)	50		
		3 (6M)	100		
		4 (6M)	200		
		5 (6M)	320	2*	
	Female	1 (6F)	0 (vehicle)	5	5
		2 (6F)	50		
		3 (6F)	100	3*	
		4 (6F)	200		
		5 (6F)	320	2*	
Phase 2**	Male	6 (5M)	0 (vehicle)	7	7
		7 (5M)	10		
		8 (5M)	20		
		9 (6M)	30	5	5
		10 (6M)	50	3*	

*: surviving animals of treatment group were sacrificed for humane reason or for comparative reasons.

**: raw data generated from this phase were collected under RBM internal n° RE7751.

At the end of the 5-day or 7-day dosing periods, 3 animals from groups 1, 2, and 9 or 2 animals/groups 6, 7 and 8 were kept off treatment for 22 days and then sacrificed for recovery evaluation. Pathology investigations were performed only in animals of phase 2.

Observations and Results

Mortality (2x/day) and Clinical Signs (Daily)

Most Phase 1 animals died or were sacrificed early (see sponsor's summary table below). All animals given 320 mg/kg were found dead or were sacrificed for humane reasons after the second dose; these animals showed sedation and dyspnea. At 200 mg/kg, death occurred in all males and 4/6 females after the second dose; these animals also showed piloerection and a single case of hypothermia. At 100 mg/kg, 5 males and 2 females died after the first or the second dose; clinical signs also included skin/mucous membrane pallor and hypothermia in 1 male. At 50 mg/kg, all males died and 2 females died after 4 or 5 doses, showing hypomobility and sedation (1F) or hunched posture, hypothermia, skin/mucous membrane pallor and piloerection starting at the early stage of the recovery period and continuing to death (day 12); 3 females died during the 2nd week (during recovery). The remaining animals dosed at 50, 100 and 200 mg/kg (with the exception of the 50 mg/kg females) were sacrificed early on day 3 (the animals received a total of 2 administrations) after showing

hypomobility/sedation and piloerection; the control males were also sacrificed early to provide a similarly-timed comparator group.

Phase 1								
	Group	dose	Number of Death					Number of Death
	(n. of animals)	mg/kg/day	Day 1	Day 2	Day 3	Day 4	Day 5	Recovery
Males	1 (6)	0	/	/	/*			
	2 (6)	50	/	2	1*			
	3 (6)	100	/	4	/*			
	4 (6)	200	/	3	3*			
	5 (6)	320	/	*				
Females	1 (6)	0	/	/	/	/	/	/
	2 (6)	50	/	/	/	/	2	1
	3 (6)	100	/	/	2*			
	4 (6)	200	/	/	4*			
	5 (6)	320	/	2 *				

*: sacrifice for humane reasons.

In Phase 2 animals, mortality occurred at ≥30 mg/kg. See the sponsor's summary table (below). Histological examinations were performed only in Phase 2 animals. Given the findings observed, alterations in kidney (tubular dilation and presence of amorphous proteinaceous material in the tubular lumen and tubular basophilia) and in lymphoid system (depletion of lymphoid elements) were believed to be the main causes of the death. Dose-related clinical signs were similar to those observed at higher doses: sedation (1/6 at 30 mg/kg, 3/6 at 50 mg/kg), hypomobility (1/6 at 30 mg/kg, 3/6 at 50 mg/kg) and piloerection (1/6 at 30 mg/kg, 3/6 at 50 mg/kg). One male at 30 mg/kg also showed thinness.

Phase 2										
	Group	dose	Number of Death							Number of Death
	(n. of animals)	mg/kg/day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Recovery
Males	6 (5)	0	/	/	/	/	/	/	/	/
	7 (5)	10	/	/	/	/	/	/	/	/
	8 (5)	20	/	/	/	/	/	/	/	/
	9 (6)	30	/	/	/	/	1			
	10 (6)	50	/	/	3*					

*: sacrifice for humane reasons.

Body Weights (prior to start, days 3 and 5/7, as well as weekly for Phase 2)

Due to the early mortality observed in Phase 1, body weight was measured in limited groups, i.e., primarily in females dosed at 50 mg/kg (Phase 1) and in males dosed up to 30 mg/kg (Phase 2).

In Phase 1 animals, average body weights showed dose-related losses at all doses (for which data were available) over day 0-3 (M) and 0-7 (F). On day 5, average body weight loss (~8%) was observed for the 5 surviving females at 50 mg/kg; this loss recovered during the 2nd week. In Phase 2 males, dose-related slight weight losses occurred in animals given 50 and 30 mg/kg; average body weights recovered at 30 mg/kg. Normal body weight and food intake were recorded in the Phase 1 surviving females during the recovery period and in Phase 2 males treated with 10 and 20 mg/kg throughout the study period.

Feed Consumption (weekly)

Markedly decreased food consumption was observed in animals (with severe clinical signs) that were dosed at 50, 100, 200 and 320 mg/kg. The values were approximately 50% lower than that of controls.

Hematology (Routine: Phase 2, day 5 in 3/group and at the end of recovery)

Unscheduled blood sampling was performed on many animals because they were sacrificed *in extremis*. The sponsor noted the following hematology changes (below, from the sponsor) among groups:

- **At 200 and 320 mg/kg/day:** marked increase in neutrophils, lymphocytes (both doses) and reticulocytes (higher dose only)
- **At 50 and 100 mg/kg/day:** moderate increase in neutrophils (females dosed at 100 mg/kg/day, males dosed at 50 mg/kg/day)
- **At 20 and 30 mg/kg/day:** marked decrease in lymphocytes at the end of the recovery period
- **At 10 mg/kg/day:** no relevant findings were noted.

Sex	Males					Females				
Dose-level (mg/kg /day) (n. of animals)	0 (6)	50 (3)	100 (1)	200 (0)	320 (4)	0a (3)	50a (1)	100 (4)	200 (2)	320 (6)
Early sacrifice (Day 2/3)										
Neutrophils (%)	19.92	24.23	26.60	-	25.00	17.70	27.20	39.10	37.90	49.92
Neutrophils (x 10E3 /mcL)	0.36	0.23	0.39	-	0.67	0.12	0.15	0.65	0.99	1.21
Lymphocytes (x 10E3 /mcL)	1.32	0.80	0.99	-	2.02	0.53	0.38	0.92	1.46	1.37
Reticulocytes (%)	4.44	5.69	1.78	-	7.87	4.64	0.89	2.35	2.01	7.15

[a]: In the females, the results in the controls and in the 50 mg/kg/day group were obtained at the scheduled sacrifice on Day 6.

Sex		Males				
Dose-level (mg/kg /day) (n. of animals)		**0** (3)	**10** (3)	**20** (3)	**30** (3)	**50b** (3)
*End of treatment period (Day 6 or 8)**						
Neutrophils	(%)	12.70	20.03	12.70	28.90	32.53
Neutrophils	(x 10E3 /mcL)	0.14	0.22	0.08	0.20	0.35
End of recovery period (Day 28 or 30)						
Neutrophils	(%)	16.15	24.35	22.95	37.80	-
Neutrophils	(x 10E3 /mcL)	0.23	0.42	0.25	0.37	-
Lymphocytes	(%)	83.45	74.85	76.30	60.55	-
Lymphocytes	(x 10E3 /mcL)	1.17	1.23	0.82	0.61	-

[b] : In the second part of the study, the results in the dose 50 mg/kg/day group were obtained from animals that were sacrificed early on Day 3.

Although not noted by the sponsor, rbc parameters (i.e., erythrocyte count, hemoglobin and hematocrit) were reduced 10-20% on day 3 in the 50 mg/kg males; rbc parameters were very slightly increased in treated females (5-10%). The sponsor's stated increase in lymphocyte counts at 200 could not be verified (data for 200 mg/kg were not presented in the summary table). Neutrophils were increased ~80% at 320 m/kg, but were decreased ~40% at 50 mg/kg. Monocytes were increased (>2-8x) at 320 and 100 mg/kg, on day 2 and 3 (respectively). Eosinophils and basophils were decreased. Reticulocytes appeared decreased in males at 100 mg/kg on day 3 (but only 1 animal was affected).

In Phase 2 males, leukocyte counts were reduced up to 30% at 20 and 30 mg/kg. Neutrophil counts were increased at 30 and 50 mg/kg (~60%). Lymphocyte counts were reduced 30-50%at 20-50 mg/kg; this effect did not show clear recovery. Monocyte, eosinophil and basophil counts appeared to be increased slightly at 30 and 50 mg/kg.

Clinical Chemistry

The sponsor noted the following clinical chemistry changes (text and tables from the sponsor):

- **At 100, 200 and 320 mg/kg/day:** slight to moderate increase in creatinine in most of the animals, moderate to marked elevation in AST activity, slight increase in total protein and globulins (α2-globulin) with a corresponding slight decrease in albumin; moderate increment in potassium and marked elevation in inorganic phosphorus in females given 200 mg/kg/day. Slight (females) to marked (males) reduction in calcium also occurred

- **At 50 mg/kg/day:** changes in total protein, albumin and globulins (α2-globulin) occurred as in the higher dose groups often without dose-relation-ship. Moreover, moderate or marked increase in triglycerides, urea, sodium and chloride also occurred

- **At 30 mg/kg/day:** changes in urea, creatinine, α2-globulin, sodium and chloride were found as at the higher doses, often less severe. The changes in urea, sodium and chloride did not recover after the withdrawal period

- **At 10 and 20 mg/kg/day:** changes in sodium and chloride were seen at the end of the treatment period.

Sex		Males					Females				
Dose-level (mg/kg /day) (n. of animals)		**0** (6)	**50** (2)	**100** (1)	**200** (0)	**320** (1)	**0a** (3)	**50a** (2)	**100** (4)	**200** (2)	**320** (1)
Early sacrifice (Day 2/3)											
Creatinine	(mg/dL)	0.36	0.38	1.73	-	1.63	0.37	-	0.79	1.36	0.38
SGOT/AST	(U/L)	50.67	53.50	272.00**	-	331.00	78.00	-	63.50	123.50	759.00
Total Protein	(g/dL)	4.88	5.34*	6.55**	-	-	5.19	-	5.46	6.17	-
Albumin	(%)	51.95	49.73	-	-	50.01	60.08	-	55.15	-	51.02
Globulins	(%)	48.05	50.27	-	-	49.99	39.92	-	44.85	-	48.98
Alpha 2 Globulin	(%)	13.95	17.77*	-	-	15.10	12.21	-	14.68	-	16.26
Potassium	(mEq/L)	4.46	4.56	-	-	8.02	4.08	-	5.71	7.65	8.21
Calcium	(mg/dL)	9.24	9.81*	0.24**	-	0.02	9.67	-	7.26	5.81	-
Inorg.Phosph.	(mg/dL)	7.90	8.00	-	-	-	7.99	-	8.70	13.99	-

[a]: In the females, the results in the controls and in the 50 mg/kg/day group were obtained at the scheduled sacrifice on Day 6.

Statistical significance: * P<0.05; ** P<0.01

Sex		Males				
Dose-level (mg/kg /day) (n. of animals)		**0** (3)	**10** (3)	**20** (3)	**30** (3)	**50b** (3)
*End of treatment period (Day 6 or 8)**						
Triglycerides	(mg/dL)	78.00	70.33	57.67	51.67	111.33
Urea	(mg/dL)	39.37	40.03	35.10	80.43	105.60
Creatinine	(mg/dL)	0.41	0.40	0.43	0.52	0.57
Alpha 2 Globulin	(%)	16.18	17.09	16.10	22.36	18.66
Sodium	(mEq/L)	138.90	144.17**	143.67**	151.23	144.67
Chloride	(mEq/L)	101.67	108.67*	106.33	107.67	106.33
End of recovery period (Day 28 or 30)						
Urea	(mg/dL)	49.45	46.20	45.55	85.25	-
Sodium	(mEq/L)	144.60	147.25	145.05	154.05	-
Chloride	(mEq/L)	106.00	109.00	109.50	117.00	-

[b]: the survival animal treated at the dose 50 mg/kg/day were sacrificed in the day 3.

Statistical significance: * P<0.05; ** P<0.01

In males and/or females given 100, 200 and/or 320 mg/kg, increased creatinine (3-5x) and AST (up to 10x), but not ALT, were observed; in males, blood calcium was reduced nearly 100%. Although not noted by the sponsor, blood glucose was mildly-markedly reduced (15-80%) in individual males and females at 100-320 mg/kg (this may be related to reduced food consumption). Males given 100 and 320 mg/kg also tended to show slight reductions in sodium, chloride, and/or ALP (~40%, only 1 animal at 100 mg/kg). Urea was increased (~3x) in a single surviving female at 200 mg/kg on day 3.

In Phase 2 males, urea was increased (up to ~2x) at 30 and 50 mg/kg; this did not recover at 28 days. Creatinine was increased (~40%) at these doses through day 6, but showed recovery at 30 mg/kg. Total protein was slightly increased at 30 and 50 mg/kg, but appeared to recover at 30 mg/kg. Observed increases in sodium and chloride at the end of the dosing period were generally slight (5-10%). Although not noted by the

sponsor, AST appeared slightly increased (~40-50%) at 20 and 30 mg/kg, through day 8 or day 28, respectively.

Organ Weights

Not performed.

Gross Pathology

At necropsy, drug-related changes were observed in the spleen (moderate-severe decreased size of the organ) of early mortalities and in the kidneys and/or thymus of some animals. In addition to the findings below, one of two recovery females showed decreased kidney size.

Dead or Moribund Sacrifice

Male

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 50	Gr# 3 100	Gr# 4 200	Gr# 5 320
no. of animals	0	3	5	6	6
no. of animals without appreciable lesions	0	0	1	1	1
Kidneys					
pale	-	0	0	2(1.0) 33.33%	2(1.0) 33.33%
Spleen					
decreased size	-	3(1.7) 100.00%	4(1.8) 80.00%	5(1.6) 83.33%	5(2.4) 83.33%
pale	-	3(1.3) 100.00%	3(1.7) 60.00%	3(1.3) 50.00%	2(1.0) 33.33%
Thymus					
decreased size	-	0	0	0	2(1.0) 33.33%

Female

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 50	Gr# 3 100	Gr# 4 200	Gr# 5 320
no. of animals	0	3	2	4	6
no. of animals without appreciable lesions	0	0	2	4	0
Kidneys					
pale	-	1(1.0) 33.33%	0	0	2(1.0) 33.33%
Spleen					
decreased size	-	3(1.7) 100.00%	0	0	6(1.8) 100.00%

Early Sacrifice

Male

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 50	Gr# 3 100	Gr# 4 200	Gr# 5 320
no. of animals	6	3	1	0	0
no. of animals without appreciable lesions	6	1	0	0	0
Kidneys					
pale	0	0	1(1.0) 100.00%	-	-
Spleen					
decreased size	0	1(3.0) 33.33%	1(3.0) 100.00%	-	-
pale	0	0	1(1.0) 100.00%	-	-
Thymus					
decreased size	0	2(2.0) 66.67%	1(2.0) 100.00%	-	-

Female

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 50	Gr# 3 100	Gr# 4 200	Gr# 5 320
no. of animals	0	0	4	2	0
no. of animals without appreciable lesions	0	0	3	1	0
Spleen					
decreased size	-	-	0	1(1.0) 50.00%	-
Thymus					
decreased size	-	-	1(1.0) 25.00%	1(2.0) 50.00%	-

In the Phase 2 animals, findings were observed in spleen and kidney. No gross abnormalities were observed in animals surviving to final sacrifice. Multifocal whitish areas were observed in the kidneys of the early mortality (M) at 30 mg/kg.

Dead or Moribund Sacrifice

Dose (mg/kg/day)	Gr# 6 0	Gr# 7 10	Gr# 8 20	Gr# 9 30	Gr# 10 50
no. of animals	0	0	0	1	6
no. of animals without appreciable lesions	0	0	0	0	2
Spleen					
decreased size	-	-	-	1(2.0) 100.00%	3(3.0) 50.00%
pale	-	-	-	0	1(1.0) 16.67%

Recovery sacrifice

Dose (mg/kg/day)	Gr# 6 0	Gr# 7 10	Gr# 8 20	Gr# 9 30	Gr# 10 50
no. of animals	2	2	2	2	0
no. of animals without appreciable lesions	2	2	2	1	0
Kidneys					
whitish area	0	0	0	1(1.0) 50.00%	-

Histopathology

Adequate Battery- Only Phase 2; only a partial battery (see list below)

(adrenal, bone with knee joint and marrow, heart, large intestine [cecum, colon, rectum], small intestine [duodenum, jejunum, ileum], kidney, liver, lung, lymph nodes [mandibular, mesenteric], Peyer's Patches, reproductive organs [ovary, oviduct, uterus, vagina, testis, epididymis, prostate, seminal vesicle], spleen, stomach, thymus, and all gross lesions)

Peer Review- No.

Histological Findings

In the Phase 2 males, clearly drug-related changes were observed in the kidneys, spleen, thymus, mesenteric and submandibular lymph nodes, liver and stomach. In the kidneys, several alterations were observed; partial reversibility was observed after 22 days of recovery; however, changes in the kidneys were seen in 1/2 animals given 30 mg/kg. Lymphoid depletion was observed in several organs. Changes were also observed in liver and stomach (inflammation and/or damage), and were suggested in heart, testes and possibly lung.

Dead or Agonal Sacrifice

Dose (mg/kg/day)	Gr# 6 0	Gr# 7 10	Gr# 8 20	Gr# 9 30	Gr# 10 50
no. of animals	0	0	0	1	6
Kidneys	-	-	-	-1-	-6-
intratubular, inflammation acute	-	-	-	1(1.0) 100.00%	0
tubule(s), altered tinctorial properties-increased basophilia	-	-	-	1(2.0) 100.00%	0
tubule(s), cast(s)	-	-	-	0	1(1.0) 16.67%
tubule(s), dilatation	-	-	-	1(1.0) 100.00%	3(1.3) 50.00%
tubule(s), hyperplasia	-	-	-	1(1.0) 100.00%	0
tubule(s), necrosis	-	-	-	1(1.0) 100.00%	0
tubule(s), vacuolization	-	-	-	0	3(2.0) 50.00%
tubule(s), crystals	-	-	-	1(2.0) 100.00%	1(0.0) 16.67%
Kidneys	-	-	-	-1-	-6-
tubule(s), amorphous material	-	-	-	1(2.0) 100.00%	4(1.8) 66.67%
Liver	-	-	-	-1-	-6-
inflammation acute	-	-	-	1(2.0) 100.00%	0
glycogen content increased	-	-	-	0	3(1.0) 50.00%
hepatocellular, necrosis	-	-	-	1(2.0) 100.00%	0
Lungs	-	-	-	-1-	-6-
mineralization	-	-	-	1(1.0) 100.00%	0
alveoli, edema	-	-	-	0	1(2.0) 16.67%
alveoli, red blood cells	-	-	-	1(1.0) 100.00%	0

Mandibular lymph nodes	-	-	-	-1-	-4-
depletion of lymphoid elements	-	-	-	1(3.0) 100.00%	2(2.0) 50.00%
Mesenteric lymph nodes	-	-	-	-1-	-6-
depletion of lymphoid elements	-	-	-	1(3.0) 100.00%	5(1.2) 83.33%
Spleen	-	-	-	-1-	-6-
depletion of lymphoid elements	-	-	-	1(3.0) 100.00%	4(2.0) 66.67%
extramedullary hematopoiesis	-	-	-	0	3(1.7) 50.00%
Stomach	-	-	-	-1-	-6-
inflammation acute	-	-	-	1(1.0) 100.00%	0
glandular, mucosa, inflammation acute	-	-	-	0	1(0.0) 16.67%
glandular, mucosa, mineralization	-	-	-	1(2.0) 100.00%	0
mucosa, necrosis	-	-	-	1(1.0) 100.00%	0
Thymus	-	-	-	-1-	-6-
depletion of lymphoid elements	-	-	-	1(3.0) 100.00%	5(1.8) 83.33%
Testes	-	-	-	-1-	-6-
multinucleated spermatids	-	-	-	0	1(1.0) 16.67%

Final Sacrifice

Dose (mg/kg/day)	**Gr# 6 0**	**Gr# 7 10**	**Gr# 8 20**	**Gr# 9 30**	**Gr# 10 50**
no. of animals	3	3	3	3	0
Heart	-3-	-3-	-3-	-3-	-
epicardium, inflammation-subacute	0	0	0	1(1.0) 33.33%	-
Kidneys	-3-	-3-	-3-	-3-	-
interstitium, aggregate(s) of mononuclear (predominantly lymphoid) cells	2(0.5) 66.67%	1(0.0) 33.33%	0	0	-
tubule(s), altered tinctorial properties-increased basophilia	0	0	0	2(2.0) 66.67%	-
tubule(s), cast(s)	0	0	0	2(1.0) 66.67%	-
tubule(s), dilatation	0	0	0	2(2.0) 66.67%	-

Liver	-3-	-3-	-3-	-3-	-
inflammation acute	0	0	0	1(0.0) 33.33%	-
glycogen content increased	3(1.3) 100.00%	3(0.7) 100.00%	3(1.3) 100.00%	3(1.3) 100.00%	-
centrilobular, vacuolization consistent with fatty change	0	0	1(1.0) 33.33%	0	-
Spleen	-3-	-3-	-3-	-3-	-
extramedullary hematopoiesis	3(1.7) 100.00%	3(2.0) 100.00%	3(1.7) 100.00%	3(2.0) 100.00%	-
Thymus	-3-	-2-	-3-	-3-	-
depletion of lymphoid elements	0	0	0	3(1.3) 100.00%	-

Recovery

Dose (mg/kg/day)	Gr# 6 0	Gr# 7 10	Gr# 8 20	Gr# 9 30	Gr# 10 50
no. of animals	2	2	2	2	0
Kidneys	-2-	-2-	-2-	-2-	-
inflammation-chronic	0	0	0	1(2.0) 50.00%	-
tubule(s), altered tinctorial properties-increased basophilia	0	0	0	1(2.0) 50.00%	-
tubule(s), cast(s)	0	0	0	1(2.0) 50.00%	-
Liver	-2-	-2-	-2-	-2-	-
glycogen content increased	2(1.0) 100.00%	2(1.0) 100.00%	2(1.5) 100.00%	2(2.0) 100.00%	-
interstitium, aggreg. of mononuclear cells adjacent to deg. or necrotic cells	0	0	1(0.0) 50.00%	1(0.0) 50.00%	-
Spleen	-2-	-2-	-2-	-2-	-
extramedullary hematopoiesis	2(1.5) 100.00%	2(1.5) 100.00%	2(2.0) 100.00%	2(2.0) 100.00%	-

Dose Analysis
Not performed.

Study title: Chronic oral toxicity study in mice

Study no.:	IMP 25329, RC4660
Study report location:	SDN, SN18, 08/24/06
Conducting laboratory and location:	(b) (4)
Date of study initiation:	5/4/04
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	cladribine, lot 01P0358, 99.8%

Key Study Findings

- According to the sponsor, NOAEL= 20 mg/kg, the maximum dose tested in this study
- While there were no clear histological effects, the spontaneous death of a single HDF without apparent cause suggests that the NOEL= 2 mg/kg in females.
- Notably, Harderian gland was not histologically assessed; the 22-month SC administration carcinogenicity study in mice (conducted 1995-1998) identified an increased incidence of tumors in this organ.

Note: The sponsor indicated that the doses were selected on the basis of previous toxicity studies carried out by subcutaneous route; however, information from a 5-day oral dosing study in mice suggested that 60 mg/kg may exceed an MTD. In that study, 30 mg/kg was considered an MTD, though there was one death at 30 mg/kg for 5 days.

Methods

Doses:	0.2, 2 and 20 mg/kg/day
Frequency of dosing:	Each monthly course consisted of 7 consecutive days of treatment followed by a 3-week withdrawal period.
Route of administration:	PO, gavage
Dose volume:	10 ml/kg body weight
Formulation/Vehicle:	5% HPβCD water solution Solutions were administered within 4 hours of preparation.
Species/Strain:	Crl:CD-I (ICR) BR mice
	(b) (4)
Number/Sex/Group:	30/sex/gp
Age:	4 weeks old, at arrival
Weight:	16-21 g, at arrival
Satellite groups:	TK, 60/sex/gp; gps 5, 6 & 7
Study design details:	Four to seven monthly courses followed by 6 weeks of recovery Pathology investigations (organ weights, gross pathology and histology) were performed on 10/sex/group sacrificed after 4 cycles, on 15/sex/group sacrificed after 7 cycles and on 5/sex/group sacrificed at the end of the recovery period. *Ad libitum* diet and water Individually caged
Deviation from study protocol:	TK on day 1 for males was performed on serum instead of plasma

Observations and Results

Mortality [twice a day (early in the morning and late in the afternoon)]

One HDF died during treatment (#7835; week 16, during withdrawal from the 4th course); markedly decreased body weight was recorded before death. No drug-related lesions were reported; autolysis precluded histologic assessment, and the cause of death was unknown.

Clinical Signs [daily]

No drug-related signs were reported. Skin sores or crusts were observed in a few animals across groups.

Body Weights [day 0, then weekly]

No clear drug-related effects on body weight were reported.

Feed Consumption [weekly, calculated g/animal/day]

No clear drug-related effects on food consumption were reported.

Ophthalmoscopy [visual inspection and indirect ophthalmoscope; before treatment, wk 12, wk 24 and end of recovery]

No drug-related eye abnormalities were reported.

Clinical Pathology [before interim, terminal & recovery sacrifices; fasted 2 hours prior to the scheduled bleeding from the abdominal aorta]

Hematology

No drug-related changes were reported. Leukocyte counts were dose-dependently reduced in males in week 26 (up to ~40%); consistent changes were not observed in week 32. Neutrophil counts were reduced in MD and HD males through week 26 (up to ~30%), and lymphocyte counts were reduced in MD and HD males in week 26 (up to 40%; only ~10% in MD and HD F). Platelet counts tended to be reduced in treated males.

Clinical Chemistry

An increase in total cholesterol (up to 50%) was noted in both males and females treated at 2 and 20 mg/kg/day; this effect showed some recovery (~24% in M in week 32). Triglycerides were reduced (~20%) in MD and HD males at weeks 14 and 26. Glucose was slightly increased in treated males (up to ~50%) in weeks 26 and 32. Beta-globulin percentage was increased up to 20% in treated females. The sponsor provided a summary table of the statistically significant changes in blood chemistry parameters (see below). Changes in AST and ALT were not consistently dose-related, but were seen in males and females.

Parameters	Males									Females								
	Gr. 2			Gr. 3			Gr. 4			Gr. 2			Gr. 3			Gr. 4		
	14	26	32	14	26	32	14	26	32	14	26	32	14	26	32	14	26	32
Glucose	\	\	\	\	\	\	\	+	\	\	-	\	\	\	\	\	\	\
Total Cholesterol	\	\	\	\	+	\	\	+	\	\	\	\	\	+	\	+	\	\
Creatinine	\	\	\	\	\	\	\	\	\	\	\	\	\	\	-	\	\	\
SGOP/AST	\	+	\	\	+	\	\	\	\	\	+	\	\	+	\	\	\	\
SGPT/ALT	\	+	\	\	+	\	\	\	\	\	+	\	\	+	\	\	\	\
Total Bilirubin	\	\	\	\	\	\	\	+	\	-	\	\	\	\	\	\	\	\
Albumin	\	\	\	\	\	\	-	\	\	\	\	-	\	+	\	\	-	\
Globulin	\	\	\	\	\	\	+	\	\	\	\	+	\	-	\	\	+	\
A/G ratio	\	\	\	\	\	\	-	\	\	\	\	-	\	\	\	\	\	\
Alpha 1Globulins	\	\	\	\	\	\	\	\	\	\	-	\	\	-	\	\	\	\
Alpha 2Globulins	\	\	\	\	\	\	\	\	\	\	\	+	\	\	\	\	\	\
Beta Globulins	\	\	\	-	-	\	+	-	\	\	+	+	\	\	\	\	+	\
Gamma Globulins	\	\	\	\	\	\	\	\	\	\	+	\	\	\	\	\	+	\
Sodium	+	+	\	+	+	\	\	\	\	\	\	\	\	\	\	\	\	\
Potassium	\	+	\	\	+	\	\	\	\	\	\	\	\	\	+	\	\	\
Chloride	-	+	+	-	+	+	\	\	\	\	\	\	\	\	\	\	\	\

Legend:

Gr. 2 = 0.2 mg/kg/day
Gr. 3 = 2 mg/kg/day
Gr. 4 = 20 mg/kg/day
14 = week 14 (after four monthly course)
26 = week 26 (after seven monthly course)
32 = week 32 (after 6 weeks of recovery)

\+ = statistically significant increase, when compared to control group
\- = statistically significant decrease, when compared to the control group
\ = no statistically significant changes, when compared to the control group

Urinalysis

No clear drug-related urinalysis changes were reported. Urine protein was reduced up to ~90% in HDM, but was increased (up to 2x) in treated females in week 25. Ketones were slightly increased in HDF in week 25; treated females showed average increases during week 3, but these were not dose-related.

Gross Pathology

No drug-related findings were reported. There were few potentially drug-related gross changes. Changes in single HD animals at terminal sacrifice were seen in kidney (HDM, roughened surface and/or cortex whitish area), lymph nodes (HDF, increased size), clitoral gland (HDF, increased size) and uterus (HDF, elevated firm area of the horn).

Organ Weights (absolute and body-weight corrected)

Relative thymus weights were slightly increased (~12%) in HDM in the intermediate sacrifice, and relative thymus weights were dose-dependently increased up to 55% in treated females at the intermediate sacrifice. Relative spleen weights were dose-dependently reduced up to 45% in males at terminal sacrifice; reduced spleen weight was also observed at recovery (but was not clearly dose-related). Relative liver weights tended to be very slightly increased (8%) in HDF at terminal sacrifice. Relative ovary weights were reduced (20% to 40%) in HDF at intermediate and recovery sacrifices. Relative kidney weights were slightly increased (9%) in HDM at recovery.

Histopathology

Adequate Battery Yes, except that Harder's lacrimal (Harderian) gland was not assessed
(See list taken from the sponsor, below)

Peer Review: **Histology by** (b) (4)
Internal peer review by (b) (4)

11.3.2 Post-mortem: Organ/Tissue Examinations and Processing

ORGAN/TISSUE COLLECTED	ORGAN WEIGHT	FIXATIVE	Histological examination
skin and mammary gland		F	+
urinary bladder		F	+
prostate		F	+
testes	*	B	+
epididymides		B	+
seminal vesicles		F	+
vagina		F	+
uterus with uterine cervix		F	+
ovaries	*	F	+
oviduct		F	+
spleen	*	F	+
stomach		F	+
duodenum		F	+
jejunum		F	+
Ileum with Peyers Patches		F	+
cecum		F	+
colon		F	+
rectum		F	+
mesenteric lymph nodes	*	F	+

pancreas		F	+
liver (two samples for histology)	*	F	+
gall bladder		F	+
kidneys	*	F	+
ureters		F	+
adrenals		F	+
mandibular salivary glands		F	+
parotid salivary glands		F	+
sublingual salivary glands		F	+
mandibular lymph nodes	*	F	+
sternum with bone marrow		F	+
heart	*	F	+
thymus	*	F	+
lungs with bronchi and bronchioles		F	+
aorta		F	+
larynx		F	+
trachea		F	+
esophagus		F	+
thyroid with parathyroid, if present in the thyroid section		F	+
eyes and optic nerves		D	+
Harder's lacrimal glands		F	
tongue		F	
brain (coronal section at three levels, to include cerebrum, cerebellum and brain stem)	*	F	+
pituitary		F	+
skeletal muscle biceps femoris		F	+
peripheral nerve: sciatic nerve		F	+
spinal cord: thoracic		F	+
spinal cord: cervical		F	
spinal cord: lumbar		F	

Histological Findings

The sponsor reported no drug-related changes; however, at the intermediate and final sacrifices, changes in incidence and/or severity of alterations in the adrenals and liver (M) were observed. Changes in lymph nodes (M), kidney, lung (F), stomach and

thymus (F) were also suggested. Generally, recovery was demonstrated. See excerpts from the sponsor's summary tables, below.

Table legend (excerpted from the sponsor)

(no. of cases, mean severity, %)

- (not examined)
Severity: 0(very slight) 1(slight) 2(moderate) 3(severe)

Intermediate killing — Males

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 0.2	Gr# 3 2	Gr# 4 20
no. of animals	10	10	10	10
Adrenals	-10-	-	-	-10-
cortex, hyperplasia-spindle cell	1(1.0) 10.00%	-	-	2(1.0) 20.00%
cortex, accessory nodule	1(0.0) 10.00%	-	-	4(0.0) 40.00%
Liver	-10-	-	-	-10-
aggreg. of mononuclear cells adjacent to deg. or necrotic cells	3(0.0) 30.00%	-	-	6(0.2) 60.00%
Mandibular lymph nodes	-9-	-2-	-	-9-
plasmocytosis	1(2.0) 11.11%	2(2.0) 100.00%	-	2(2.0) 22.22%
follicle(s), hyperplasia-lymphoid	1(2.0) 11.11%	2(2.0) 100.00%	-	2(2.0) 22.22%

Intermediate killing — Females

Dose (mg/kg/day)	Gr# 1 0	Gr# 2 0.2	Gr# 3 2	Gr# 4 20
no. of animals	10	10	10	10
Adrenals	-10-	-	-	-10-
cortex, hyperplasia-spindle cell	2(1.0) 20.00%	-	-	3(1.0) 30.00%
Kidneys	-10-	-	-1-	-10-
tubule(s), cast(s)	1(0.0) 10.00%	-	0	3(0.0) 30.00%
Lungs	-10-	-	-	-10-
alveoli, histiocytosis	0	-	-	1(1.0) 10.00%
Uterus	-10-	-	-	-10-
endometrial glands, cystic dilatation	0	-	-	1(1.0) 10.00%

Final killing	Males			
Dose (mg/kg/day)	Gr# 1 0	Gr# 2 0.2	Gr# 3 2	Gr# 4 20
no. of animals	15	15	15	15
Adrenals	-15-	-	-	-15-
hyperplasia-spindle cell	1(1.0) 6.67%	-	-	3(1.0) 20.00%
cortex, accessory nodule	0	-	-	1(1.0) 6.67%
zona fasciculata, hypertrophy	0	-	-	1(1.0) 6.67%
Kidneys	-15-	-1-	-	-15-
glomerulosclerosis	0	0	-	1(1.0) 6.67%
cortex, dilatation	0	0	-	1(1.0) 6.67%
tubule(s), altered tinctorial properties-increased basophilia	6(0.2) 40.00%	0	-	6(0.8) 40.00%
tubule(s), cast(s)	1(1.0) 6.67%	0	-	2(1.0) 13.33%
tubule(s), fibrosis	0	0	-	1(1.0) 6.67%
Liver	-15-	-	-	-15-
aggreg. of mononuclear cells adjacent to deg. or necrotic cells	6(0.3) 40.00%	-	-	10(0.2) 66.67%
centrilobular, vacuolization consistent with fatty change	5(0.4) 33.33%	-	-	6(0.8) 40.00%
Stomach	-15-	-	-	-15-
nonglandular, mucosa, hyperplasia-epithelial	0	-	-	1(2.0) 6.67%
nonglandular, mucosa, inflammation acute	0	-	-	1(1.0) 6.67%
Thymus	-12-	-	-	-15-
cortex, atrophy	0	-	-	1(1.0) 6.67%

Final killing	Females			
Dose (mg/kg/day)	Gr# 1 0	Gr# 2 0.2	Gr# 3 2	Gr# 4 20
no. of animals	15	15	15	15
Adrenals	-15-	-	-	-15-
hyperplasia-spindle cell	8(1.0) 53.33%	-	-	8(1.0) 53.33%
cortex, accessory nodule	1(1.0) 6.67%	-	-	3(1.0) 20.00%
Kidneys	-15-	-	-	-15-
glomerulosclerosis	1(0.0) 6.67%	-	-	1(1.0) 6.67%
Kidneys	-15-	-	-	-15-
interstitium, aggregate(s) of mononuclear (predominantly lymphoid) cells	5(0.4) 33.33%	-	-	4(1.0) 26.67%
tubule(s), altered tinctorial properties-increased basophilia	4(0.0) 26.67%	-	-	5(0.2) 33.33%
tubule(s), cast(s)	5(0.2) 33.33%	-	-	5(0.4) 33.33%
Lungs	-15-	-	-	-15-
alveolar histiocytosis	0	-	-	2(1.0) 13.33%
Stomach	-15-	-	-	-15-
nonglandular, mucosa, hyperplasia	0	-	-	2(2.0) 13.33%
nonglandular, mucosa, ulcer(s)	0	-	-	1(1.0) 6.67%
Thymus	-13-	-	-	-15-
cortex, atrophy	2(1.0) 15.38%	-	-	3(1.7) 20.00%

Toxicokinetics [Days 1 and 7 of the 1st, 4th and 7th courses, at 0.5, 1.5, 3, 8 and 24 hours, in two animals/sex/group/time point]

Plasma exposures throughout the study were generally roughly proportional to the dose administered (some tendency towards a greater than dose-proportional increase in AUC was observed at HD), and some sex differences were observed (males tended to show slightly higher exposures, but this was not entirely consistent). T_{max} occurred at 0.5 hr. Some LD and MD animals showed exposures below the LLOQ at 8 and/or 24 hr after dosing. See the sponsor's summary table of C_{max} and AUC for cladribine exposures in males and females, below.

		Cmax (ng/mL)					
Dose		Course 1		Course 4		Course 7	
(mg/kg/day)	Sex	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0.2	M	19.4	6.21	5.80	4.91	6.25	14.3
0.2	F	5.27	4.48	6.98	5.42	3.32	6.69
2	M	107	162	71.7	86.2	80.8	73.2
2	F	128	77.2	90.0	69.0	79.1	137
20	M	2600	3240	1660	2550	1380	2640
20	F	2140	1090	1700	1720	1510	2790

		AUC_{0-24} (h·ng/mL)					
Dose		Course 1		Course 4		Course 7	
(mg/kg/day)	Sex	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
0.2	M	17.8	7.05	16.5	9.68	9.84	13.1
0.2	F	28.9	5.71	10.3	8.18	4.49	10.9
2	M	113	128	74.8	97.1	117	79.7
2	F	182	86.0	80.0	86.7	71.1	107
20	M	1780	2070	1180	1800	1840	2470
20	F	1740	914	1290	1290	1480	1620

Dosing Solution Analysis

Precision was demonstrated for the analytical method (± 15%). Stability for 7 days was also shown (range 0.02 - 2 mg/ml). Concentration analyses showed some deviations from the expected range, but these generally resulted in slightly increased concentrations.

	Concentration (µg/mL)		
Formulate preparation day	**Expected**	**Observed**	**Δ%**
May 06, 2004	20	20.2	1.0
	200	197.8	-1.1
	2000	2022.8	1.1
July 01, 2004	20	23.7	18.3
	200	230.3	15.1
	2000	2120.1	6.0
October 21, 2004	20	23.8	18.8
	200	231.4	15.7
	2000	2113.2	5.7

Study title: Cladribine. Subchronic toxicity study in Cynomolgus monkey treated by oral or subcutaneous routes for 3 one per month weekly cycles, followed by 3 months of recovery

Study no.:	RD5880, also IMP 26669.1 Also, Study 26662: TK report And Study 26866: Bioanalytical report
Study report location:	SN26, 11/27/06; NDA N000, 9/29/09
Conducting laboratory and location:	(b) (4) Study number 0388-2005
Date of study initiation:	12/20/05
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Cladribine, lot 04P0146 (b) (4) [lot 1012315 given for raw material], 100.1%

Key Study Findings

- According to the sponsor, NOEL= 6 mg/kg PO, the maximum dose tested, or 0.3 mg/kg SC
- Oral doses were selected based on information from a previous oral dose-range-finding study in the same animal species (Study RD3030, IMP26210).
- Although not clearly indicated in data here, 0.3 mg/kg SC has been shown to approximate an MTD with 14 treatment cycles (1 mg/kg SC for 14 monthly cycles caused drug-related mortalities [2/3HDM and 1/3 HDF] in Study 95311; cf. Dr. Huff's (b) (4) review)

Methods

Doses:	1.5,3.0 and 6.0 mg/kg/day, Plus 0.3 mg/kg/day (SC)
Frequency of dosing:	Three 7-8 day, monthly cycles
Route of administration:	PO, gavage SC, flank region
Dose volume:	PO: 3 ml/kg, SC: 0.5 ml/kg
Formulation/Vehicle:	PO: (b) (4) HPβCD (2-Hydroxypropyl)-β-cyclodextrin, water solution) SC: (b) (4) Sodium Chloride solution
Species/Strain:	Monkey, Cynomolgus (b) (4)
Number/Sex/Group:	Main: 3/sex/gp Recovery: 2/sex/gp
Age:	"young adult"
Weight:	Female: 2.9 - 5.4 kg / Male: 2.7 - 6.8 kg
Unique study design:	The first two cycles consisted of 7 consecutive days of dosing followed by 3 consecutive weeks of withdrawal. The third/last cycle consisted of 7 or 8 days of dosing for main study animals (scheduled sacrifice on Day 62, 63 or 64, at ~24-30 hr after the last administration). Recovery monkeys were kept off treatment for 3 months, then sacrificed.

Observations and Results

Mortality [daily]

No mortality occurred in any group.

Clinical Signs [daily]

No clinical signs were reported.

Body Weights [twice pretest, then weekly]

No changes in body weight were reported. By D61, the SCM group showed a 2% weight loss, as compared to pretest; other groups showed little difference. PO- and SC-treated females tended to show slightly reduced weight gain by D61. Recovery treated (PO and SC) males tended to gain slightly more weight then controls from day 66-154 (~3-5%); recovery females did not show consistent changes.

Feed Consumption [daily]

Slightly to moderately reduced food intake was observed in 1 ConF (4 days), 1 LDF (2 days), 3 MDF (6, 2 & 2 days), 2 HDF (1 & 34 days), 1 HDM (3 days), 3 SCF (2, 23 & 12 days) and 2 SCM (1 & 6 days). The incidence and severity was dose-related (although only occasional, moderate reduction occurred at MD, HD and/or SC).

Ophthalmoscopy

[pretest, end of the third cycle (end of .dosing period) & end of recovery period]
No drug-related abnormalities were observed.

ECG

[pretest, 2 and 24 hours after the 1st & 7th treatment of the first and third treatment cycle, and end of recovery]
The sponsor reported no drug-related changes. QTc was calculated according to Bazett's formula. The SCF appeared to show increased heart rates starting day 57. P wave amplitude was reduced [ss] on D1, but it was similar at pre-test. The sponsor provided the following summary table.

Main ECG Parameters – Differencies in Comparison with Pretest – Mean Values

		Males					Females				
			mg/kg/day					mg/kg/day			
	Days	Control	1.5 os	3 os	6 os	0.3 sc	Control	1.5 os	3 os	6 os	0.3 sc
Heart rate	**Pretest**	**227**	**248**	**254**	**267**	**261**	**247**	**262**	**247**	**244**	**241**
bpm	1	14	9	3	0	10	-7	-12	-5	-10	-9
	2	21	21	17	0	-1	-6	-11	-8	0	-4
	7	11	9	7	-15	-5	-2	-6	3	15	10
	8	17	13	9	-3	3	-5	-3	-2	-4	-2
	55	15	16	11	1	-3	-	-	-	-	-
	56	24	21	6	4	14	-9	-7	-1	4	2
	57	-	-	-	-	-	3	5	7	7	12
	61	15	19	12	5	-6	-	-	-	-	-
	62	12	10	4	-7	-6	-8	-6	-3	-6	1
	63	-	-	-	-	-	-3	-10	-3	-4	7
	153	5	0	-5	-7	-6	-40	-25	-18	-2	13
P-R interval	**Pretest**	**72**	**63**	**59**	**61**	**60**	**65**	**63**	**63**	**63**	**61**
ms	1	-7	-2	3	0	0	-1	2	0	-2	3
	2	-8	-4	0	-1	-3	0	1	-4	0	-1
	7	-7	-2	2	2	-1	-1	4	0	1	1
	8	-7	-1	-2	0	2	2	0	0	-1	0
	55	-6	-1	1	0	2	-	-	-	-	-
	56	-6	-5	-1	1	-5	-1	2	-1	1	-2
	57	-	-	-	-	-	0	-1	-2	2	-2
	61	-6	-3	0	0	1	-	-	-	-	-
	62	-8	-2	2	1	-1	3	-1	0	3	0
	63	-	-	-	-	-	-2	0	1	0	-4
	153	-2	2	-3	1	0	2	6	8	1	-4
QRS complex duration	**Pretest**	**47**	**46**	**47**	**46**	**44**	**44**	**46**	**46**	**46**	**43**
ms	1	0	1	-4	0	1	3	1	-1	1	3
	2	-1	2	-6	-1	1	2	-3	1	-2	3
	7	1	3	-4	-3	1	0	0	-1	-1	1
	8	0	0	-5	-3	1	0	1	-1	0	2
	55	-1	0	-4	1	0	-	-	-	-	-
	56	0	3	-1	-1	1	0	1	-1	1	6
	57	-	-	-	-	-	0	-1	0	-1	5
	61	-1	-2	-4	0	1	-	-	-	-	-
	62	-1	2	-3	1	1	1	3	2	2	3
	63	-	-	-	-	-	2	0	0	3	0
	153	-4	2	-2	2	4	5	1	0	1	1

Main ECG Parameters – Differencies in Comparison with Pretest – Mean Values

		Males					Females				
			mg/kg/day					mg/kg/day			
	Days	Control	1.5 os	3 os	6 os	0.3 sc	Control	1.5 os	3 os	6 os	0.3 sc
Q-T interval	**Pretest**	**168**	**160**	**163**	**155**	**154**	**158**	**154**	**152**	**163**	**161**
ms	1	-1	-2	-7	-5	-1	6	5	5	2	6
	2	-9	-8	-11	-7	-3	4	-1	4	-1	3
	7	-4	-2	-9	3	0	4	3	1	-9	-1
	8	-10	-9	-9	-5	-3	2	2	2	-1	-2
	55	-7	-6	-9	-5	-4	-	-	-	-	-
	56	-8	-7	-9	-6	-7	3	1	2	-2	2
	57	-	-	-	-	-	-5	0	-1	-5	-2
	61	-6	-9	-7	-5	3	-	-	-	-	-
	62	-3	-5	-5	0	3	3	6	8	-1	-3
	63	-	-	-	-	-	1	5	2	4	-4
	153	-10	-5	-14	3	1	10	12	17	-5	-2
Q-T interval (Bazett)	**Pretest**	**327**	**325**	**335**	**326**	**321**	**320**	**321**	**308**	**329**	**322**
ms	1	8	1	-12	-9	4	7	3	6	-3	6
	2	-3	-4	-13	-13	-7	5	-9	2	-3	4
	7	0	1	-13	-4	-2	8	3	5	-9	5
	8	-9	-10	-13	-11	-3	1	4	3	-5	-4
	55	-4	-2	-12	-8	-10	-	-	-	-	-
	56	0	-1	-15	-9	-7	1	-2	4	-2	6
	57	-	-	-	-	-	-8	3	2	-6	4
	61	-2	-6	-8	-6	2	-	-	-	-	-
	62	2	-3	-8	-2	3	1	7	14	-8	-4
	63	-	-	-	-	-	1	5	4	5	-3
	153	-17	-11	-31	3	-1	-8	9	23	-12	4

Pretest data (Day –7) is reported as absolute value.

M = males; F = females; Rec. = recovery phase; os = oral; sc = subcutaneous

Hematology [pretest, end of each cycle of dosing, end of each washout period, and end of recovery; except coagulation: pretest and at the end of the third cycle and at the end of recovery]

No clear drug-related changes were reported. Platelets counts tended to be increased in treated males (up to ~30%) throughout dosing. White blood cell counts were variable. One LDM reportedly showed a frank neutrophilia on D8. SCF showed a 30% reduction in % LYM on D27. On D27, HDF and SCF showed increased neutrophils (up to ~2x), but neutrophils were reduced (~50%) in HDF on D35. On D54, HDM showed increased neutrophils and HDF showed increased lymphocytes. On D153, a moderate decrease in neutrophils was reported in MDM, HDM and SC animals, without a clear dose relationship. A few cases of neutrophilia were also noted in control animals.

Clinical Chemistry [pretest, at the end of each washout period, at the end of the third cycle, and at the end of recovery]

The sponsor reported that sporadic slight increases in AST, ALT, urea and creatinine were observed in individual animals. Alkaline phosphatase was increased in treated males on D62 (2-3x). On D153, AST was increased in treated males (up to 2x), and ALT was increased in MDM, HDM and SCM (1.5-2x). Total bilirubin was slightly increased in MD, HD and SC male and females (25-70%). Total cholesterol was increased 30-40% in SC animals on D153. Phosphorus was increased in treated males and MD, HD and SC females.

Urinalysis [pretest and at the end of the third cycle and at the end of recovery]

Urine ketone bodies were slightly increased on D62 in MDF, HD males and females, and SC males and females.

Gross Pathology

At terminal sacrifice, 1 HDM, 1 HDF and 1 SCM showed abnormal colored areas on the liver; a SCF was noted to show yellowish discoloration of the liver. One HDM showed reddening of the mucosa of the colon. Single black-colored areas were observed on the ileum of 1 HDM and on the rectum of 1 MDM. The testes were small (bilaterally) in 1 HDM. At recovery sacrifice, abnormal colored areas and/or prominent lobular pattern in the liver was observed in 1 SCF.

Organ Weights [see histopathology list, from the sponsor]

Few drug-related changes were reported. Relative adrenal weight was reduced 15% in treated males on D62. Relative (to body) thymus and spleen weights were increased in HDF and reduced in SCF (20-30%) on D154. Relative adrenal weight was decreased in HDF and SCF (~25%) on D154. SCF showed decreased relative pituitary weight (~30%) on D154. Decreased relative (to body) ovary weight (~20%) was seen in HDF and SCF.

Histopathology

Adequate Battery Yes, list below from the sponsor
Peer Review Yes, (b) (4)

Organ/Tissue	Main study and recovery animals		Examined (mg/kg/dose) All animals				
	Collected	Weighed	0	1.5	3	6	0.3
Aorta	X		E	E	E	E	E
Application site	X		E				E
Adrenal glands (both)	X	X	E	E	E	E	E
Bone, sternum (with marrow)	X		E	E	E	E	E
Bone marrow smear	X		E	E	E	E	E
Brain	X	X	E	E	E	E	E
Diaphragm	X		E	E	E	E	E
Epididymides (both)	X		E	E	E	E	E
Esophagus	X		E	E	E	E	E
Eyes, optic nerve (both)	X		E	E	E	E	E
Heart	X	X	E	E	E	E	E
Duodenum	X		E	E	E	E	E
Jejunum	X		E	E	E	E	E
Ileum with Peyer Patches	X		E	E	E	E	E
Cecum	X		E	E	E	E	E
Colon	X		E	E	E	E	E
Gall bladder	X		E	E	E	E	E
Femur Head	X		E	E	E	E	E
Kidneys (both)	X	X	E	E	E	E	E
Lymph node, mandibular	X		E	E	E	E	E
Lymph node, mesenteric	X		E	E	E	E	E
Lymph node, mediastinal	X						
Lymph node, inguinal	X		E				E
Mammary gland	X		E	E	E	E	E
Ovaries	X	X	E	E	E	E	E
Pancreas	X		E	E	E	E	E
Pituitary	X	X	E	E	E	E	E
Prostate	X	X	E	E	E	E	E
Salivary glands (mandibular, sublingual, parotids)	X		E	E	E	E	E
Sciatic nerve	X		E	E	E	E	E
Seminal vesicles	X		E	E	E	E	E
Skeletal muscle	X		E	E	E	E	E
Skin	X		E	E	E	E	E
Spinal cord (thoracic)	X		E	E	E	E	E
Spinal cord (cervical, lumbar)	X						
Spleen	X	X	E	E	E	E	E
Stomach	X		E	E	E	E	E
Rectum	X		E	E	E	E	E
Testes (both)	X	X	E	E	E	E	E
Thymus	X	X	E	E	E	E	E
Thyroid glands (with parathyroid glands)[a]	X		E	E	E	E	E
Tongue	X		E	E	E	E	E
Trachea	X		E	E	E	E	E
Urinary bladder	X		E	E	E	E	E
Uterus	X		E	E	E	E	E
Vagina	X		E	E	E	E	E
Lesions	X		E	E	E	E	E

E: Examined by microscopy
a Parathyroid glands examined microscopically if included in the section of thyroid glands

Fixatives:
Eyes and Testes: Davidson's solution
Bone Marrow Smears: Methanol-ether
All Other Tissues: 10% neutral buffered formalin

Histological Findings

Overall, the sponsor reported few histologic changes; liver was identified as a potential target organ. Additionally, the sponsor reported no significant changes at the injection site from animals of either sex receiving 0.3 mg/kg/day subcutaneously. Hepatocellular vacuolation, indicative of fatty change, and/or hepatocellular clear cell change, reflecting the glycogen content, correlated with the single or multiple yellow areas and/or yellowish discoloration or prominent lobular pattern noted on gross examination (except one HDF that showed a granuloma). The moderate focal hemorrhages, correlating with the single black areas noted on gross examination, in the rectum from the MDM and in the ileum from the HDM were considered due to trauma during semen collection and, in the HDM, probably a consequence of the spontaneous arteriopathy observed in that animal. The sponsor attributed the histological changes noted in the biceps femoris muscle from treated animals at both scheduled sacrifices to the injections of ketamine before ophthalmoscopic examination and sperm collection. A few thyroid findings were noted in treated males. Inflammation of the heart and/or surrounding tissues was noted in a few animals at end of dosing and/or recovery. Salivary glands and/or lymph nodes sometimes showed infiltrates. Low incidence findings at HD included papillary mineralization and/or lung changes in HDF. Also, an unusual finding of intradermal nevocellular nevus was noted in the nipple region of the skin from a recovery MDM; the sponsor provided literature indicating that similar findings have been previously documented in a young rhesus monkey (Frazier et al., 1993).

CONTROLS FROM GROUP(S): 1	ANIMAL SEX:	Males					Females				
TISSUES WITH DIAGNOSES	DOSAGE GROUP:	1	2	3	4	5	1	2	3	4	5
	NO. IN GROUP:	3	3	3	3	3	3	3	3	3	3
AORTA	NUMBER EXAMINED:	3	3	3	3	3	3	3	3	3	3
INFLAMMATION OF ADJACENT TISSUES											
	Nad>	3	3	3	3	3	3	3	3	2	3
	Minimal>	0	0	0	0	0	0	0	0	1	0
COLON	NUMBER EXAMINED:	3	3	3	3	3	3	3	3	3	3
MICROHEMORRHAGES OF MUCOSA											
	Nad>	3	3	3	2	3	3	3	3	3	3
	Slight>	0	0	0	1	0	0	0	0	0	0
DIAPHRAGM	NUMBER EXAMINED:	3	3	3	3	3	3	3	3	3	3
CHRONIC INFLAMMATION											
	Nad>	3	3	3	3	3	3	3	3	2	3
	Minimal>	0	0	0	0	0	0	0	0	1	0
HEART	NUMBER EXAMINED:	3	3	3	3	3	3	3	3	3	3
MYOCARDITIS											
	Nad>	1	3	3	2	1	2	3	3	3	2
	Minimal>	2	0	0	0	2	1	0	0	0	1
	Slight>	0	0	0	1	0	0	0	0	0	0
ILEUM	NUMBER EXAMINED:	3	3	3	3	3	3	3	3	3	3
HEMORRHAGE											
	Nad>	3	3	3	2	3	3	3	3	3	3
	Moderate>	0	0	0	1	0	0	0	0	0	0
KIDNEYS	NUMBER EXAMINED:	3	3	3	3	3	3	3	3	3	3
ARTERITIS/PERIARTERITIS											
	Nad>	2	3	3	1	3	3	3	3	3	3
	Minimal>	1	0	0	2	0	0	0	0	0	0
LIVER	NUMBER EXAMINED:	3	3	3	3	3	3	3	3	3	3
GRANULOMA/S											
	Nad>	3	3	3	3	3	3	3	3	2	3
	Moderate>	0	0	0	0	0	0	0	0	1	0
CLEAR CELL CHANGE,HEPATOCELLULAR											
	Nad>	0	0	0	0	0	0	0	0	1	1
	Slight>	2	2	2	1	1	1	2	2	1	0
	Moderate>	1	1	1	2	2	2	1	1	1	2
FOCAL HEPATOCELLULAR VACUOLATION											
	Nad>	3	3	3	2	2	3	3	2	2	3
	Minimal>	0	0	0	0	0	0	0	1	1	0
	Moderate>	0	0	0	1	0	0	0	0	0	0
	Marked>	0	0	0	0	1	0	0	0	0	0
DIFFUSE HEPATOCELLULAR VACUOLATION											
	Nad>	3	3	3	3	3	3	3	2	2	1
	Slight>	0	0	0	0	0	0	0	0	1	1
	Moderate>	0	0	0	0	0	0	0	1	0	1

SUBLINGUAL S.G. . . . . .NUMBER EXAMINED:	3	3	3	3	3		3	3	3	3	3
LYMPHOCYTIC INFILTRATION											
Nad>	2	1	0	2	1		1	2	1	0	0
Minimal>	0	1	0	0	1		1	0	2	0	3
Slight>	1	1	3	1	1		1	1	0	2	0
Moderate>	0	0	0	0	0		0	0	0	1	0
MANDIBULAR S.G. . . . . .NUMBER EXAMINED:	3	3	3	3	3		3	3	3	3	3
LYMPHOCYTIC INFILTRATION											
Nad>	3	1	3	1	2		2	2	2	2	0
Minimal>	0	2	0	2	1		1	1	1	1	3
THYROIDS . . . . . .NUMBER EXAMINED:	3	3	3	3	3		3	3	3	3	3
CYSTIC FOLLICLES											
Nad>	2	2	2	1	2		3	3	3	3	3
Slight>	1	1	1	2	0		0	0	0	0	0
Moderate>	0	0	0	0	1		0	0	0	0	0
ULTIMOBRANCHIAL CYST/CYSTS											
Nad>	3	3	3	0	2		3	3	3	3	3
Slight>	0	0	0	2	0		0	0	0	0	0
Moderate>	0	0	0	1	1		0	0	0	0	0

Recovery

AORTA . . . . . .NUMBER EXAMINED:	2	2	2	2	2		2	2	2	2	2
INFLAMMATION OF ADJACENT TISSUES											
Nad>	2	2	2	2	2		2	2	2	2	1
Slight>	0	0	0	0	0		0	0	0	0	1
HEART . . . . . .NUMBER EXAMINED:	2	2	2	2	2		2	2	2	2	2
MYOCARDITIS											
Nad>	1	2	1	2	0		2	2	0	1	2
Minimal>	1	0	1	0	2		0	0	2	1	0
PERICARDITIS											
Nad>	2	2	2	2	1		2	2	2	2	2
Minimal>	0	0	0	0	1		0	0	0	0	0
KIDNEYS . . . . . .NUMBER EXAMINED:	2	2	2	2	2		2	2	2	2	2
PAPILLARY MINERALIZATION											
Nad>	2	2	2	2	2		2	2	2	1	2
Minimal>	0	0	0	0	0		0	0	0	1	0
MESENTERIC L.N. . . . . .NUMBER EXAMINED:	2	2	2	2	2		2	2	2	2	2
EOSINOPHILIC INFILTRATION OF ADJACENT TISSUES											
Nad>	2	2	2	2	2		2	2	2	1	1
Minimal>	0	0	0	0	0		0	0	0	0	1
Slight>	0	0	0	0	0		0	0	0	1	0
LUNG . . . . . .NUMBER EXAMINED:	2	2	2	2	2		2	2	2	2	2
GRANULOMAS WITH CHOLESTEROL CLEFTS											
Nad>	2	2	2	2	2		2	2	2	1	2
Minimal>	0	0	0	0	0		0	0	0	1	0
PLEURAL FIBROSIS											
Nad>	2	2	2	2	1		2	2	2	1	2
Minimal>	0	0	0	0	1		0	0	0	0	0
Slight>	0	0	0	0	0		0	0	0	1	0
SKELETAL MUSCLE . . . . . .NUMBER EXAMINED:	2	2	2	2	2		2	2	2	2	2
MYOFIBER REGENERATION											
Nad>	2	2	2	2	1		2	2	2	2	2
Slight>	0	0	0	0	1		0	0	0	0	0
SUBLINGUAL S.G. . . . . .NUMBER EXAMINED:	2	2	2	2	2		2	2	2	2	2
LYMPHOCYTIC INFILTRATION											
Nad>	0	0	0	1	0		0	0	0	1	0
Minimal>	2	1	1	1	0		1	1	1	1	1
Slight>	0	1	1	0	2		1	1	1	0	1
SKIN . . . . . .NUMBER EXAMINED:	2	2	2	2	2		2	2	2	2	2
GRANULOMA/S,DERMAL											
Nad>	2	2	2	2	2		2	2	2	2	2
INTRADERMAL NEVOCELLULAR NEVUS (BENIGN MELANOMA)											
Nad>	2	2	1	2	2		2	2	2	2	2
Moderate>	0	0	1	0	0		0	0	0	0	0

Special Evaluation

Sperm analysis was performed pretest, at the end of the third cycle and at the end of recovery. At the end of treatment, HDM and SCM showed a moderate decrease in spermatozoa with rapid progressive motility (56.2 and 60.8 %, respectively), compared to controls and to pretest measures of the same animals. This decrease was characterized by an increase in the percentage of spermatozoa with slow and/or non-progressive motility (percentages of spermatozoa with slow progressive motility plus those with non-progressive motility were 22.1 and 15.8% for groups HDM and SCM, respectively). The percentage of immotile spermatozoa was similar to controls. Alterations in sperm motility were not observed at the end of recovery.

Toxicokinetics

Blood samples for TK were drawn from the same animals, after the first and the last administration of the first and third cycles. The average T_{max} was 1-3 hrs. Following the SC dose, cladribine exposure peaked at ~0.5 hr. Metabolite 2-Chloroadenine showed T_{max} within 3-6 hr after PO dosing and at ~0.5 hr after SC dosing. Comparing cladribine AUC_{24} values following oral and subcutaneous dosing, an oral dose of 1.5- 3 mg/kg/day in male animals provided approximately the same exposure levels as those achieved at the 0.3 mg/kg/day SC dose. In contrast, while exposures after ~3 mg/kg PO were similar to those after 0.3 mg/kg SC on day 1 in females, repeated doses of 0.3 mg/kg SC provided exposures similar to those after 3-6 mg/kg PO repeated doses. Plasma levels of metabolite 2-chloroadenine after oral dosing were higher than after SC dosing, suggesting a first pass-effect. The sponsor provided the following TK summary tables.

Cladribine

	Dose as Cladribine (mg/kg/day)							
	1.5		3		6		0.3	
Route	ORAL						SUBCUTANEOUS	
Dose ratio	1		2		4		0.2	
	Day 1							
	Males	Females	Males	Females	Males	Females	Males	Females
C_{max} (ng/mL)	9.3	27.0	23.0	54.8	86.2	123.4	87.9	110.7
t_{max} (h)	3	1	1	1	3	1	0.5	0.5
AUC_{24} (h*ng/mL)	41.2	93.7	92.4	181.7	265.2	350.6	140.2	177.1
AUC_{24} ratio	–	–	2.2	1.9	6.4	3.7	–	–
GF ratio	0.4	–	0.5	–	0.8	–	0.8	–
	Day 7							
C_{max} (ng/mL)	25.6	22.9	96.4	46.5	65.5	128.1	65.9	156.4
t_{max} (h)	1	1	3	1	1	1	0.5	0.5
AUC_{24} (h*ng/mL)	106.5	87.0	368.5	134.8	219.5	402.2	126.7	240.8
AUC_{24} ratio	–	–	3.5	1.6	2.1	4.6	–	–
GF ratio	1.2	–	2.7	–	0.5	–	0.5	–
Rac	5.5	1.0	5.3	0.8	1.4	1.2	0.9	1.4
	Day 55/56							
C_{max} (ng/mL)	20.7	26.0	73.5	76.3	119.8	108.4	109.1	152.5
t_{max} (h)	1	1	1	1	1	1	0.5	0.5
AUC_{24} (h*ng/mL)	78.9	74.6	226.8	220.9	424.9	374.9	171.0	246.2
AUC_{24} ratio	–	–	2.9	3.0	5.4	5.0	–	–
GF ratio	1.1	–	1.0	–	1.1	–	0.7	–
Rac	3.8	0.9	3.1	1.3	2.3	1.1	1.3	1.4
	Day 61/62							
C_{max} (ng/mL)	19.8	16.7	58.5	48.4	94.7	88.3	98.7	152.0
t_{max} (h)	1	1	1	1	1	1	0.5	0.5
AUC_{24} (h*ng/mL)	91.0	78.5	221.0	192.7	347.6	427.0	196.8	298.2
AUC_{24} ratio	–	–	2.4	2.5	3.8	5.4	–	–
GF ratio	1.2	–	1.1	–	0.8	–	0.7	–
Rac	4.1	0.9	2.9	1.1	1.7	1.1	1.4	1.7
Rac D61/D55	1.2	1.2	1.0	1.0	0.8	1.1	1.1	1.2

2-Chloroadenine

	Dose as 2-Chloroadenine equivalent (mg/kg/day)							
	0.9		1.8		3.6		0.2	
Route	ORAL						SUBCUTANEOUS	
Dose ratio	1		2		4		0.2	
	Day 1							
	Males	Females	Males	Females	Males	Females	Males	Females
C_{max} (ng/mL)	2.9	3.4	3.2	10.3	22.8	24.6	3.5	4.0
t_{max} (h)	6	3	3	6	3	3	0.5	0.5
AUC_{24} (h*ng/mL)	32.9	38.2	41.2	113.4	193.4	254.2	5.8	6.8
AUC_{24} ratio	–	–	1.3	3.0	5.9	6.7	–	–
GF ratio	0.9	–	0.4	–	0.8	–	0.9	–
	Day 7							
C_{max} (ng/mL)	7.5	9.2	14.4	8.6	20.0	21.9	1.6	6.6
t_{max} (h)	6	3	3	3	6	3	0.5	0.5
AUC_{24} (h*ng/mL)	81.4	40.7	133.1	66.1	208.3	175.2	2.3	10.8
AUC_{24} ratio	–	–	1.6	1.6	2.6	4.3	–	–
GF ratio	2.0	–	2.0	–	1.2	–	0.2	–
Rac	3.3	1.1	3.3	0.6	1.2	0.8	0.5	1.6
	Day 55/56							
C_{max} (ng/mL)	5.4	3.6	8.9	11.9	46.2	18.3	2.3	2.5
t_{max} (h)	6	6	6	6	6	6	0.5	0.5
AUC_{24} (h*ng/mL)	63.8	40.5	107.1	128.7	396.8	168.4	3.9	3.5
AUC_{24} ratio	–	–	1.7	3.2	6.2	4.2	–	–
GF ratio	1.6	–	0.8	–	2.4	–	1.1	–
Rac	2.2	1.1	2.8	1.1	2.1	0.8	0.8	0.5
	Day 61/62							
C_{max} (ng/mL)	4.2	3.0	6.4	6.5	18.6	14.3	3.2	4.2
t_{max} (h)	3	6	3	6	6	6	0.5	1
AUC_{24} (h*ng/mL)	40.1	40.1	71.9	82.1	171.9	161.4	6.9	11.9
AUC_{24} ratio	–	–	1.8	2.0	4.3	4.0	–	–
GF ratio	1.0	–	0.9	–	1.1	–	0.6	–
Rac	1.5	1.1	1.8	0.8	0.9	0.8	1.3	1.8
Rac D61/D55	0.7	1.1	0.7	0.7	0.4	1.0	1.8	3.4

Rac = Accumulation Ratio (vs. Day 1)
Rac D61/D55 = Accumulation Ratio on Day 61/62 vs Day 55/56
GF ratio= Gender Factor (AUC_{24} male/female ratio)

Dosing Solution Analysis

Formulations were prepared at 0.5, 1 and 2 mg/ml (PO) and 0.6 mg/ml (SC); generally, concentrations were within ±10% of nominal.

Study title: A Five-Week Oral Repeat Dose Range Finding Study of Dissolved Cladribine tablets in CByB6F1-Tg(HRAS)2Jic (+/- hemizygous c- Ha-Ras) Mice and a Toxicokinetic Study in Non-Transgenic CByB6F1 Hybrid Mice

Study no.:	28853; also (b) (4) 281.01
Study report location:	EDR, SDN1, 9/28/09
Conducting laboratory and location:	(b) (4)
Date of study initiation:	3/10/08
GLP compliance:	Yes, except: - Test article characterization - Dose concentration and TK - Various pre-study activities
QA statement:	Yes
Drug, lot #, and % purity:	**Test Article 1 (TA1):** 10 mg cladribine tablets, lot N0219A, concentration 106.4% and total impurities <1% **Test Article 2 (TA2):** cladribine drug substance (b) (4) lot 06P0557; 99.7% **Test Article 3 (TA3):** hydroxypropyl-beta-cyclodextrin (HPβCD; (b) (4) (b) (4)), lot E0070, total impurities <1%

Key Study Findings

- According to the sponsor, the MTD was 30 mg/kg, the maximum dose tested for two cycles.
- At 30 mg/kg, renal effects were demonstrated that might have been dose-limiting for a longer duration study; these effects were more severe in males.
- Following single 5-day cycles at 5 mg/kg then 60 mg/kg, the severity of the histopathological findings in the kidney were more severe than at 30 mg/kg. Creatinine (1.5x in females and 2x in males) and BUN (2.5x in females and 4x in males) were increased following the 60 mg/kg cycle.

Methods (details not included in sponsor's summary table, below)

Frequency of dosing: administered 1x/day on days 1-5 & 29-33
Route of administration: PO, gavage, 10 ml/kg
Species/Strain: transgenic CByB6F1-Tg(HRAS)2Jic (±hemizygous c-Ha-Ras) mice (b) (4)
Age: 6 weeks of age, at randomization
Weight: 13.3 – 39.8 g, at randomization
Satellite groups naïve male and female non-transgenic (wild type) CByB6F1 hybrid mice
Special details *ad lib.* food and water

Study design

Group	Treatment	Dose Level (mg/kg/day)	Number of Animals (Male/Female) Toxicology	Toxicokinetic	Necropsy Toxicology (Male/Female)
1	Vehicle	0	10/10	6/6	10/10
2	Cladribine Tablet (TA1)	5/60a	10/10	26/24	10/10
3	Cladribine Tablet (TA1)	20	10/10	26/24	10/10
4	Cladribine Tablet (TA1)	30	10/10	24/24	10/10
5	Cladribine drug substance (TA2)	30	10/10	24/24	10/10
6a	HPβCD (TA3)	431[b]	5/5	20/21[d]	5/5
6b	HPβCD (TA3)	431/862[c]	5/5	4/4[d]	5/5

[a] All toxicology (tox) and all toxicokinetic (TK) animals were dosed with test article 1 at 5 mg/kg/day on days 1 – 5 and with test article 1 at 60 mg/kg/day on days 29 – 33.
[b] Tox animals (5 per sex) were dosed with 431 mg/kg/day of test article 3 on days 1 – 5 and 29 – 33.
[c] Tox animals (5 per sex) were dosed with test article 3 at 431 mg/kg/day on days 1 – 5 and with test article 3 at 862 mg/kg/day on days 29 – 33.

[d] TK animals (24 per sex) were dosed with test article 3 at 431 mg/kg/day on days 1 – 5 and TK blood samples were collected by terminal bleeds. Of the remaining 8 animals (per sex), four (per sex) were dosed at 431 mg/kg/day of test article 3 on days 29 – 33 (Group 6a) and four were dosed with 862 mg/kg/day of test article 3 on days 29 – 33 (Group 6b). Note: Total dose volumes (mL) were calculated based on the most recent body weight.

Three test articles (TAs) were used in this study. Test Article 1 (TA1, lot N0219A) consisted of 10 mg cladribine tablets complexed with 2-hydroxypropyl-β-cyclodextrin, TA2 (lot 06P0557) consisted of cladribine (drug substance), and TA3 (lot E0070) consisted of 2-hydroxypropyl-β-cyclodextrin (HPβCD), the main excipient in cladribine tablets. All three test articles were dissolved in deionized water.

Observations and Results

Mortality [twice daily]

There were no test article-related deaths.

Clinical Signs [twice daily, recorded for all main study and TK animals]

The sponsor indicated there were no drug-related clinical signs; however, moderate lack of skin turgor was noted in 3 male animals after 60 mg/kg (day 32 or 35). This clinical sign was also noted sporadically, generally of slight severity, in other animals across groups. Green/yellow feces were observed in 2 females at 20 mg/kg. One 30 mg/kg female had a wound on the lower right forelimb and swollen forefoot from days 13 - 28; this may reflect altered immune function and infection.

Body Weights [all main study and TK animals on the day before the first dosing, once weekly throughout the study and main study animals only at necropsy]

Body weights generally remained within 10% of controls; males and females given 60 mg/kg showed slightly reduced mean body weights (15 and 10%, respectively). See the sponsor's table below for details.

Mean body weights

Group	Treatment	Dose Level[1] (mg/kg/day)	Mean Body Weight (g) day 28 Females	day 28 Males	day 34/35[2] Females	day 34/35[2] Males
1	Vehicle	0/0	18.5	25.3	19.2	25.4
2	Cladribine Tablet	5/60	18.2	25.4	17.3	21.6
3	Cladribine Tablet	20/20	19.0	24.3	19.9	24.6
4	Cladribine Tablet	30/30	18.5	24.5	19.9	24.3
5	Cladribine Drug Substance	30/30	18.4	23.7	19.4	23.9
6a	HPβCD	431/431	18.3	24.1	19.2	24.4
6b	HPβCD	431/862	19.4	23.8	19.5	24.2

[1] Numbers represent the doses administered during the first treatment cycle/ and during the second treatment cycle.

[2] Odd numbered male and female animals in each group were selected for necropsy on day 34. All even numbered male and female animals in each group were selected for necropsy on day 35.

Feed Consumption [once weekly, main study animals only]

Food consumption was within approximately 10% of control, except on day 34 in females given 30 mg/kg TA2 (22% lower), females given 60 mg/kg (34% lower) and

males given 60 mg/kg (45% lower than controls). Food consumption was slightly increased in the HPβCD group females on day 15 (+18%) and day 22 (+36%).

Hematology [day 35 for even numbered animals in Groups 1 – 5, main study only]

The sponsor noted changes primarily in males given 60 mg/kg; these changes consisted of increased platelet (62%, [ss]) and neutrophil (~2.5x control, [nss]) counts, and decreased reticulocyte (62%, [nss]), eosinophil (almost 100%, [ss]) and lymphocyte (56%, [nss]) counts. In cladribine-treated females, a decrease in neutrophil count (~40-60%, [nss]) and a decrease in eosinophil count (75%, [nss]) were also noted. White blood cell counts appeared slightly reduced in animals given ≥ 30 mg/kg cladribine (~20-40%); however, the finding was not strictly dose-related in females. Lymphocyte count was reduced in males at ≥ 30 mg/kg (26-56%, [nss]) and in females treated with ≥ 30 mg/kg tablets (24-39%, [nss]). Monocyte counts were highly variable, but were increased 2.7-fold in males given 60 mg/kg (compared to control). Red cell distribution width was slightly increased in females given 60 mg/kg (9%, [ss]). Reticulocyte counts were slightly increased (10-20%) at 20-30 mg/kg cladribine in males and in cladribine-treated females (25-26%). Platelet count was also slightly increased in a dose-related manner in treated females (25-50%, [nss]).

Clinical Chemistry [day 34 for odd-numbered animals in Groups 1 – 5 and all Group 6 animals, main study animals only]

Due to insufficient blood volumes, electrolyte analysis (sodium, chloride, and potassium) could not be performed for 32 study animals. Also, samples from a number of animals showed slight-moderate hemolysis. The sponsor noted changes in BUN, creatinine, total cholesterol, calcium, globulin, triglyceride and glucose. Alkaline phosphatase was reduced 40% [ss] in animals given 60 mg/kg, and 10-20% in males given 30 mg/kg.

The following increases were observed in animals given 60 mg/kg, compared to controls: BUN was ~4.1 fold in males and ~2.5 fold in females, creatinine was ~2.0 fold in males and ~1.7 fold in females, globulin was ~1.6 fold in males and females, total protein was ~1.3 fold in males and females, total cholesterol was ~1.4 fold in males and ~1.7 fold in females, calcium was ~1.1 fold in males and females, sodium was +5% in males and potassium was +30% in males and females. At 30 mg/kg, total cholesterol was increased ~20-25% and globulin was increased ~20% in males. The following decreases were observed in animals given 60 mg/kg, compared to controls: albumin/globulin ratio was -31% in males and females, triglycerides were -55% in males and -37% in females, and glucose was -34% in males and -29% in females. Increased serum ALT (3.3x) and AST (2.3x) were observed in male mice treated with 862 mg/kg HPβCD; 20-60 mg/kg cladribine resulted in increases of 40% to 2.8-fold in female mice. Creatine kinase was increased ~80% in male mice given 862 mg/kg HPβCD.

Gross Pathology [days 34 and 35, main study animals only]

Changes in the kidneys of both sexes given 60 mg/kg were observed. Nine of 10 males and 6/10 females had mild to moderate dark red discoloration of the kidneys (a similar

finding was observed in 1 control female); the finding was generally bilateral (except for 2 animals). One additional female had an observation of "discoloration" (not otherwise specified); the discoloration appeared as large dark patches visible on the kidney surface. One male given 60 mg/kg also showed a mild, small, pale discoloration near the periphery on the right cranial lobe of the lung; microscopically, this was identified as a bronchioloalveolar adenoma. One 30 mg/kg (tablet) male showed mild, granular irregular surface of the kidneys.

Organ Weights (see table from the sponsor, below)

(Paired organs were examined grossly and weighed together.)

Brain	Spleen
Heart	Thyroids* (including parathyroids)
Kidneys*	Testes*
Liver	Thymus
Ovaries*	Uterus (horns and cervix)

*Paired organs

Kidney weights were significantly increased (relative to body weight) in males (53%, [ss]) and females (26%, [ss]) given 60 mg/kg. Although liver weights in animals given 60 mg/kg appeared decreased in terms of absolute weight and as a percent of brain weight, liver weight as a percent body weight was not affected. Testes weights (absolute) were decreased 5-20% at ≥ 20 mg/kg cladribine (although this was not strictly dose-related); relative to brain weight, testes weights were significantly decreased at 30 mg/kg cladribine (~15%, [ss]). Spleen weight (relative to body weight) was increased in males receiving ≥ 30 mg/kg cladribine (~20-25%, [ss]). Heart weight was slightly increased in males given 60 mg/kg cladribine (11%, [nss]) and HPβCD (8%, [nss]). Thymus weights (absolute, as a percent of body weight and as a percent of brain weight) were decreased in animals given 60 mg/kg (60%, [ss]). Ovary weights (relative to body weight) appeared decreased in all groups compared to controls (40-70%); uterus weights (relative to body weight) were variable.

The changes in kidney weights correlated to the gross and microscopic observations. Testes and thymus were not examined microscopically.

Histopathology

Adequate Battery: Incomplete list (see list from the sponsor, below)
All main study animals

Tissues collected for histopathology

Bone, femur (with marrow)	Ileum
Brain	Jejunum
Cecum	Kidneys*
Colon	Liver
Duodenum	Lymph node (mesenteric)
Esophagus (thoracic)	Lymph nodes (mandibular)*
Gross lesions	Pancreas
Heart	Spleen
Harderian glands*	Stomach

* Paired organs

Peer Review No

Histological Findings

See "Special Evaluation" for bone marrow smear examination results.

The primary dose-dependent, drug-related finding was in the kidney, and consisted of minimal to marked tubular degeneration/regeneration with luminal debris. The sponsor noted that the majority of the tubular epithelial cells had enlarged basophilic nuclei indicative of regeneration, and the luminal debris appeared to be remnants of epithelial cells. There was little cellular infiltrate associated with the tubular change. The distribution of the affected tubules was focal, and in the more severe cases involved large areas of the kidney. The renal findings were generally more severe in males, and with higher cladribine doses. Alterations were also observed sporadically in the spleen (marginal zone depletion), bone marrow (myeloid hyperplasia) and pancreas (infiltrates and/or hemorrhage). A few changes were observed in pancreas, heart and/or liver in animals treated with HPβCD.

Three neoplasms were observed in the study. Two neoplasms were present in the spleen: a hemangioma in a 30 mg/kg male and a hemangiosarcoma in a 30 mg/kg female. Additionally, a malignant bronchioloalveolar adenoma was present in the lung of a male given 60 mg/kg.

Controls from group(s): 1	MALES						FEMALES					
Group:	1	2	3	4	5	6	1	2	3	4	5	6
Dosage mg/kg:	0	60	20	30	30	431	0	60	20	30	30	431
No. in group:	10	10	10	10	10	10	10	10	10	10	10	10
Kidneys												
Tubular degeneration/regeneration with luminal debris												
Not noted>	10	1	10	2	3	10	10	1	10	10	5	10
Minimal>	0	0	0	2	5	0	0	0	0	0	3	0
Mild>	0	0	0	4	2	0	0	3	0	0	2	0
Moderate>	0	2	0	2	0	0	0	3	0	0	0	0
Marked>	0	7	0	0	0	0	0	3	0	0	0	0
Hyperplasia, tubular												
Not noted>	10	10	10	10	10	10	10	10	10	9	10	10
Mild>	0	0	0	0	0	0	0	0	0	1	0	0
Inflammation, chronic, peritubular												
Not noted>	10	10	10	10	10	10	10	10	10	9	10	10
Minimal>	0	0	0	0	0	0	0	0	0	1	0	0
Spleen												
Depletion, marginal zone												
Not noted>	10	10	10	10	10	10	10	9	10	10	10	10
Minimal>	0	0	0	0	0	0	0	1	0	0	0	0
B.marrow,fem.,L												
Myeloid Hyperplasia												
Not noted>	10	7	10	10	10	10	10	9	10	10	10	10
Minimal>	0	3	0	0	0	0	0	0	0	0	0	0
Mild>	0	0	0	0	0	0	0	1	0	0	0	0
Pancreas												
Mononuclear cell infiltration												
Not noted>	10	10	10	10	10	10	10	10	10	10	10	8
Minimal>	0	0	0	0	0	0	0	0	0	0	0	2
Mixed cell infiltration												
Not noted>	10	9	10	10	10	10	10	10	10	10	10	10
Minimal>	0	1	0	0	0	0	0	0	0	0	0	0
Hemorrhage												
Not noted>	10	9	10	10	10	10	10	10	10	10	10	10
Minimal>	0	1	0	0	0	0	0	0	0	0	0	0
Heart												
Infiltration, mixed cell, epicardium												
Not noted>	10	10	10	10	10	10	10	10	10	10	10	9
Minimal>	0	0	0	0	0	0	0	0	0	0	0	1

Liver												
Necrosis, single cell												
Not noted>	10	10	9	10	10	10	9	10	10	9	10	9
Minimal>	0	0	1	0	0	0	1	0	0	1	0	1
Focus, basophilic												
Not noted>	10	10	10	10	10	9	10	10	10	10	10	10
Minimal>	0	0	0	0	0	1	0	0	0	0	0	0

Special Evaluation

Bone marrow smears were examined the end of the dosing period (day 34 or 35, from 10/sex/group; see below). The parameters assessed were the myeloid: erythroid (M:E) ratio, megakaryocytes, bone marrow cellularity, storage pool, maturation pool, erythroid precursors, maturation arrest (erythroid, myeloid or megakaryocytic), plasma cells, mast cells, lymphoid cells, atypical cells, and iron stores. Notably, there were problems with the quality of the slides from the female groups (up to 3/10 unreadable). Increased myeloid:erythroid ratios (~50%) were observed for males that received 60 mg/kg (see sponsor's summary table, below). Mild decreases in bone marrow cellularity were suggested at ≥ 30 mg/kg cladribine. In animals receiving 60 mg/kg cladribine, proliferating myeloid cells (storage pool) were mildly or moderately increased in 6 males and 1 female, and non-proliferating myeloid cells (maturation pool) were mildly decreased in 2 males and 2 females. Also in this group, a mild increase in atypical cells (identified as proliferating myeloid precursors) were seen in 2 male animals, and erythroid precursors were mildly increased in 1 male animal.

Summary M:E ratio results

Group	Treatment	Dose Level (mg/kg)	M:E ratios (Mean ± SD, N)	
			Male	Female
1	Vehicle Control	0	1.53 ± 0.50, 10	1.38 ± 0.34, 7
2	Cladribine Tablet	5/60	2.32 ± 1.22, 10	1.01 ± 0.46, 7
3	Cladribine Tablet	20	1.18 ± 0.46, 10	1.10 ± 0.28, 8
4	Cladribine Tablet	30	1.16 ± 0.45, 10	0.93 ± 0.61, 8
5	Cladribine (Drug Substance)	30	1.16 ± 0.43, 10	0.92 ± 0.24, 9
6a	HPβCD	431	1.70 ± 0.24, 5	1.22 ± 0.39, 5
6b	HPβCD	431/862	1.38 ± 0.14, 5	1.62 ± 0.15, 5

Toxicokinetics

Blood sampling occurred at four time points (0.5, 2, 5 and 24 hours postdose) on days 1, 5, and 33 in Groups 2 – 6 and once (0.5 hour postdose) in Group 1.

TK parameters were assessed for the 1st and 5th day of the first cycle, and the 5th day of the second cycle. Plasma exposures were generally approximately dose-proportional. Exposure of animals to cladribine, following administration of solubilized tablets or cladribine drug substance, both at 30 mg/kg/day, was comparable. Following repeated administration of cladribine tablets (5th vs. 1st dose of first cycle); no accumulation was observed; however, a slight accumulation was found in males following cladribine drug substance treatment. Clear sex differences in exposure were not observed in the first cycle after cladribine tablets or drug substance treatment; however, males showed much higher exposures than females in the second cycle after cladribine tablets (but not cladribine drug substance). See sponsor's summary table, below, for details.

Cladribine		Dose (mg/kg/day)							
		Tablets						*Drug substance*	
		5		20		30		*30*	
		Dose ratio							
		1		4		6		-	
		Day 1 (Cycle 1)							
		Male	Female	Male	Female	Male	Female	*Male*	*Female*
C_{max}	(ng/mL)	748	686	3350	2710	4900	4190	*3905*	*4785*
t_{max}	(h)	0.5	0.5	0.5	0.5	0.5	0.5	*0.5*	*0.5*
AUC_{24}	(h.ng/mL)	882	632	2702	2861	4941	3843	*3617*	*4367*
AUC_{24} ratio		1	1	3.1	4.5	5.6	6.1	-	-
GF AUC_{24} ratio		1.4	-	0.9	-	1.3	-	*0.8*	-
Frel		-	-	-	-	1.4	0.9	*1.0*	*1.0*
		Day 5 (Cycle 1)							
C_{max}	(ng/mL)	529	387	1724	2085	5605	4415	*3600*	*3955*
t_{max}	(h)	0.5	0.5	0.5	0.5	0.5	0.5	*0.5*	*0.5*
AUC_{24}	(h.ng/mL)	640	425	1927	2001	5199	4211	*5892*	*3894*
AUC_{24} ratio		1	1	3.0	4.7	8.1	9.9	-	-
Rac AUC_{24} ratio		0.7	0.7	0.7	0.7	1.1	1.1	*1.6*	*0.9*
GF AUC_{24} ratio		1.5	-	1.0	-	1.2	-	*1.5*	-
Frel		-	-	-	-	0.9	1.1	*1.0*	*1.0*
		Day 33 (Cycle 2)							
		Dose (mg/kg/day)							
		60		20		30		*30*	
		Dose ratio							
		12		-		-		-	
C_{max}	(ng/mL)	16050	8110	3145	1590	4150	2375	*3215*	*2830*
t_{max}	(h)	0.5	0.5	0.5	0.5	0.5	0.5	*0.5*	*0.5*
AUC_{24}	(h.ng/mL)	34760	7333	3297	1778	5069	1855	*3787*	*3258*
AUC_{24} ratio		54.3	17.3	-	-	-	-	-	-
AUC_{24} cycle ratio		-	-	1.7	0.9	1.0	0.4	*0.6*	*0.9*
GF AUC_{24} ratio		4.7	-	1.9	-	2.7	-	*1.1*	-
Frel		-		-		1.3	0.5	*1.0*	*1.0*

Dosing Solution Analysis

With a few exceptions in single samples (17-25% lower at 4-6 hr stability samples), samples were within ±15% the nominal cladribine concentration. The sponsor reported no trends at the end of 4 – 6 hours stability testing. HPβCD formulations were slightly higher than the nominal value (up to 36% higher than the nominal concentration at the end of stability testing).

7 Genetic Toxicology

The genetic toxicology studies of cladribine were previously reviewed (see Dr. Huff's review for (b) (4) dated 12/15/98). Briefly, as described in that review, the sponsor evaluated the genotoxicity of cladribine in the standard test battery (an Ames test, a chromosomal aberration test in CHO cells, and an in vivo micronucleus test in mice), as well as in an HPRT mutagenicity test in CHO cells and an unscheduled DNA synthesis test in cultured rat hepatocytes. Cladribine was clastogenic *in vitro* and *in vivo*. The sponsor also provided a number of literature references that indicated that cladribine induced DNA strand breaks and inhibited DNA synthesis and repair (e.g., Seto et al, 1985; Seto et al., 1986). Cladribine did not induce mutations in either bacterial or mammalian cells.

8 Carcinogenicity

Study title: A 26-Week Oral Dose Carcinogenicity Study of Cladribine in CByB6F1-Tg(HRAS)2Jic Hemizygous Mice and Toxicokinetic Study in CByB6F1-Tg(HRAS)2Jic Wild Mice

Study no.:	Report #29174
Study report location:	EDR, SDN
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/17/08
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	*Test article 1:* cladribine tablets (IVAX Pharmaceuticals, IRL), lot N0219A, dissolved in deionized water. (Cladribine drug product is formulated as 10 mg white, round tablets; cladribine tablets consist of cladribine combined with the following excipients: 2-hydroxypropyl-β-cyclodextrin, sorbitol powder and magnesium stearate.) *Test Article 2:* cladribine drug substance (b) (4), lot 06P0557, expiration date 12/09 *Test Article 3:* 2-hydroxypropyl-β-cyclodextrin (b) (4), lot E0070, purity= >98% *Vehicle/control article:* deionized water (b) (4), multiple lots *Positive control article:* MNU (b) (4) (b) (4) lots 037K0690 & 048K1230,

	purity= 55%, water and acetic acid noted as the significant impurities, prepared in 0.9% (w/v) saline for injection, USP (b) (4), lot C711051
CAC concurrence:	No; sufficient information was not submitted for protocol review/concurrence

Key Study Findings

- No cladribine-related increase in tumor incidence.
- Target organs included: kidney, thymus and testes (possibly Harderian gland and bone marrow).

Adequacy of Carcinogenicity Study

The maximum dose of cladribine used for this study (30 mg/kg) appears to have been adequate, based on the renal histopathological alterations (i.e., tubular degeneration/regeneration with luminal debris, graded as moderate to marked) and concomitant clinical pathology (i.e., increased BUN and creatinine) observed at 60 mg/kg in the dose-ranging study. There is some question as to the adequacy of the cyclic dosing regimen used in this transgenic model. While the sponsor states that the 4-5 day monthly dosing pattern is adequate because it exceeds the experience in the clinic, the treatment period is already shortened in this alternative assay. Furthermore, it is unclear whether the cyclic dosing regimen is adequate to support multiple 2-year cycles of dosing in humans.

The adequacy of a cyclic dosing the regimen was also identified as a potential issue for the previous 22-month SC administration carcinogenicity assay in mouse; for that study, the Exec-CAC minutes (dated 1/28/99) indicated that the assay was considered adequate in spite of the intermittent dosing regimen used. The Exec-CAC indicated at that time that, on the basis of the 22-month study, "… cladribine should be considered carcinogenic" and "… labeling should reflect that the intermittent dosing schedule used in the study may not have been adequate to completely assess the carcinogenic potential of cladribine. Labeling should also state that the demonstrated genotoxicity of cladribine supports the likelihood that cladribine is a carcinogen."

Appropriateness of Test Models

The CByB6F1-Tg(HRAS)2Jic Hemizygous mouse is an acceptable alternative model for assessing the carcinogenic potential of nongenotoxic and genotoxic compounds. Although this is the second carcinogenicity assay conducted in mice (rat was not considered an appropriate species, based on endogenous levels of deoxycytidine, which competes with cladribine for phosphorylation by deoxycytidine kinase), previous Exec-CAC minutes (dated 1/28/99) indicated that a transgenic model would be appropriate as a second study.

Evaluation of Tumor Findings

There were no cladribine-related tumors.

Methods

Frequency of dosing:	Groups 1-6 (Drug & Vehicle Control): 7 dosing cycles (dosed 5 consecutive days, with a 23-day interval between cycles) [Dosing on D 1 – 5, 29 – 33, 57 – 61, 85 – 89, 113 – 117 and 141 – 145. Following the final non-dosing period, animals were again dosed from D169 – 173.] Group 7 (Positive Control- MNU): Animals received a single IP dose on D1.
Basis of dose selection:	MTD Doses were based on results from previous dose 5-week dose-ranging study (#28853)
Species/Strain:	Transgenic mice (*mus musculus*) Main: CByB6F1-Tg(HRAS)2Jic Hemizygous TK: CByB6F1–Tg(HRAS)2Jic Wild Type (b) (4)
Age, Weight:	6 – 7 weeks old at randomization Main: 18.8- 29.7 g M & 15.5-23.8 g F TK: 21.5- 32.0 g M & 16.9- 25.3 g F
Animal housing:	individually housed
Paradigm for dietary restriction:	n/a
Satellite groups:	TK
Deviation from study protocol:	Although several deviations were noted, none was noted to have an impact on the outcome or integrity of the study.

Groups/Route/Dose/Number of Animals (from the sponsor's submission):

Group assignments

Group	Test Article	Dose Route	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Animals (Main Study/TK) Females	Males
1	Control Article/ Vehicle	Oral Gavage	0	0	10	25/9	25/9
2	Test Article 1	Oral Gavage	5	0.5	10	25/24	25/24
3	Test Article 1	Oral Gavage	15	1.5	10	25/24	25/24
4	Test Article 1	Oral Gavage	30	3.0	10	25/24	25/24

5	Test Article 2	Oral Gavage	15	1.5	10	25/24	25/24
6	Test Article 3	Oral Gavage	431	43.1	10	25/9	25/9
7	MNU	IP Injection	75	7.5	10	25/0	25/0

Note: Total dose volumes (mL) were calculated based on the most recent body weight.

* NOTE: MNU dose for females was ~54 mg/kg, due to formulation issues.

Observations and Results

Mortality [once daily, performed no sooner than 2 hours postdose]

A number of animals in this study were either found dead or were sacrificed moribund. Only MNU-treated animals showed an increase in mortality; the increase was greater in males (who received the correct dose of MNU) than in females (who received a lower than nominal dose of MNU). Two TK animals were reported as unscheduled sacrifices (Group 1: 1M [no 33] on D62, Group 2: 1F [no 412] on D76).

Unscheduled deaths in main study animals

Group	SSAN (day of death)	Total Number of Early Deaths
1	6(167), 15(169)	2
2	47(172)	1
3	428(167)	1
4	479(32)	1
5	531(167), 537(139)	2
6	N/A	0
7	2(93), 17(107), 265(145), 267(162), 268(153), 269(143), 270(115), 271(165), 272(154), 273(122), 275(67), 276(106), 277(120), 278(123), 279(153), 281(126), 283(146), 284(89), 286(115), 287(128), 289(141), 596(93), 600(120), 602(119), 603(115), 605(156), 606(112), 607(93), 613(119), 614(112), 615(126), 618(82), 619(86)	33

Clinical Signs [once daily, performed no sooner than 2 hours postdose, with "mass tracking" once weekly for the presence of palpable masses in main study animals]

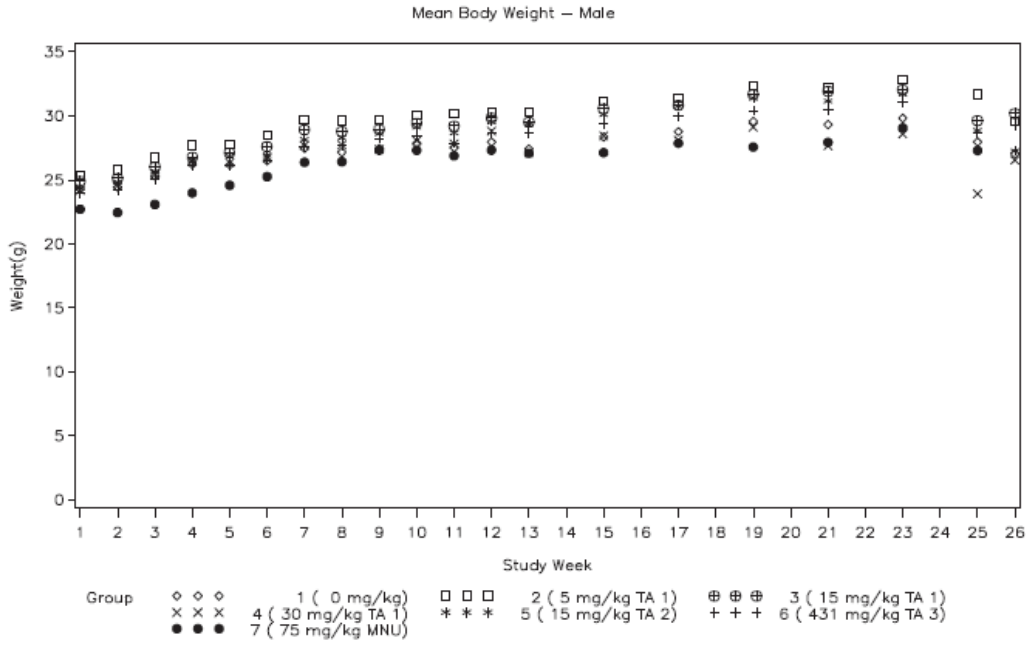
Treatment-related changes were observed in both males and females, but most frequently in HDM. These consisted of an increased incidence of hypoactivity/lethargy and hunched posture (males & females), as well as piloerection and lack of skin turgor (males only). Two MDF also showed hypoactivity/lethargy; in one case, the observation was secondary to a spontaneous tumor, and in the other the effect was transient. See table, next page. Similar clinical signs were observed in MNU-treated animals, with higher incidence and frequency. No test article-related changes in clinical observations were observed in other groups.

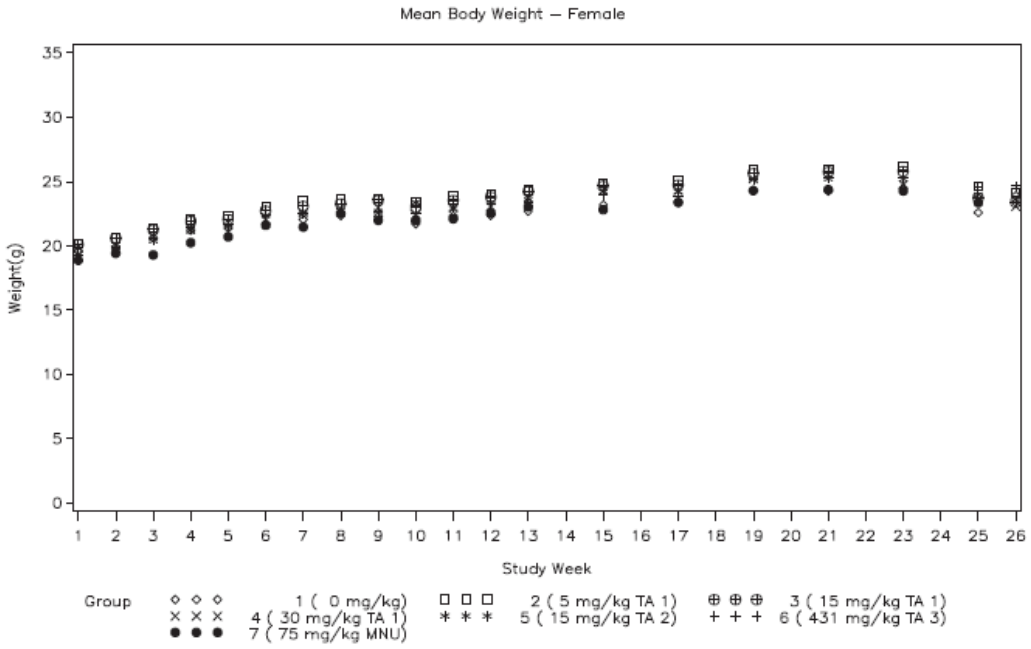
Sign	1- veh	2- 5 tab	3-15 tab	4- 30 tab	5- 15 DS	6- HPβCD	7- MNU
N= (M, F as incidence/ # of animals)	**25 + 9 (tox + TK)**	**25 + 24**	**25 + 24**	**25 + 24**	**25 + 24**	**25 + 9**	**25**
Hyperactive	- , -	- , -	- , -	2/1, -	- , -	- , -	- , -
Hunched	4/2, 9/2	- , -	2/1, 8/2	116/9, 16/4	- , 10/3	19/1, 10/2	101/15, 64/7
Lethargic	- , -	, -	- , 7/2	13/2, 3/2	- , -	- , -	74/8 , 35/3
Skin- Lack of Turgor	75/6, 64/8	115/10, -	171/13, 111/11	837/26, 49/6	118/11, 177/9	226/11, 92/9	521/24, 168/13
Skin- Loss of Fur	- , -	- , 101/1	- , 102/3	10/1, 135/1	34/1, 96/1	120/2, -	- , 52/3
Skin- Lump	73/1, 116/1	18/1, 136/2	- , 103/2	54/1, 82/1	128/2, 144/2	93/2, 95/1	462/8, 1466/23
Skin- Piloerection	- , -	- , -	- , -	9/4 , -	- , -	- , -	16/2, -
Skin- Swollen	8/3, 17/1	95/4, 117/2	135/4, 109/3	60/2, -	82/8, 207/4	52/7, -	- , -
Feces- abnormal color	- , -	- , -	- , -	- , -	- , 20/3	- , 4/1	- , 15/1

An increased incidence of palpable masses was detected only in MNU-treated male and females.

Body Weights [D1, once weekly for 13 wks and every other week thereafter]

No treatment-related changes in body weight were observed during the study. MNU-treated animals tended to have lower body weights. See the sponsor's Figures below.





Feed Consumption [estimated once weekly (average gram/animal/day calculated)]

Treatment-related changes in food consumption were not observed. Sporadic, statistically significant, differences from control were observed in all groups.

Necropsy:

Main study animals that were found dead were weighed and necropsied within 24 hours. Organs from animals found dead were not weighed. Main study animals euthanized early were necropsied and organ weights recorded. (Organ weights from unscheduled necropsy were reported, but the weights were not included in any statistical analyses.)

Necropsy was performed on all surviving main study animals on D174 – 177. TK animals were discarded without tissue collection. At necropsy, the external surfaces of the body, all orifices, and the cranial, thoracic, and abdominal cavities and their contents were examined. At the time of necropsy, bone marrow smears from the femur were prepared, fixed, and stained.

Hematology [at necropsy] See table from sponsor, below.

Hematology parameters*

Red Blood Cells	Mean Corpuscular Hemoglobin	Differential Leukocyte Absolute Count: Basophils Eosinophils Lymphocytes Monocytes Neutrophils
Hematocrit	Mean Corpuscular Hemoglobin Concentration	
Hemoglobin		
White Blood Cells	Platelets	
Mean Platelet Volume	Red Cell Distribution Width	
Mean Corpuscular Volume	Reticulocyte Count	

* Additional parameters beyond those specified above may have been collected due to hardware requirements. Those results are maintained in study and/or facility records and will not be reported herein.

No clear treatment-related changes were observed in hematology parameters, relative to controls, for Groups 2 – 5. In Group 4 males and females, increases in monocyte and neutrophil counts (and eosinophils, in females) were seen, usually due to individual animals. Group 4 females showed slightly decreased hematocrit and slightly increased mean platelet volume. Platelets were slightly increased in Group 4 males. Increases in reticulocyte counts, red blood cell distribution width, and neutrophils, and decreased red blood cells, hematocrit, and hemoglobin were seen in MNU-treated animals. Individual, sometimes substantial, increases in white blood cell, basophil, lymphocyte, and monocyte counts were observed in Group 6 and/or 7 males and females.

Organ Weights

Organs weighed

Brain	Ovaries*
Heart	Spleen
Kidneys*	Testes*
Liver	

(* Paired organs were examined grossly and weighed together.)

Treatment-related, statistically significant, decrease in testes weight (29%, relative to brain weight) was observed in HDM, compared to control males. The sponsor reported no other treatment-related changes in organ weights, except in MNU-treated animals. However, a few changes were apparent. Relative (to body weight) kidney (14%) and spleen (22%, with outlier data omitted) weights were increased in HDM. Relative liver weights were slightly decreased (~7%) in DS- and HPβCD-treated males. Relative ovary weight was decreased 21% in DS-treated females.

Statistically significant changes in organ weight were seen in the spleen (increased ~3-8x), liver (males only, increased 2x) and testes of MNU-treated animals.

Gross Pathology

Decreased size and discoloration of the kidneys were observed in HDM. The sponsor reported no other drug-related findings, except in MNU-treated animals. Liver, spleen and/or thymus changes were observed in a few HD animals. Numerous gross observations were seen in the MNU-treated animals. Skin masses, as expected, were reported with greater incidence in MNU-treated animals, especially females.

	Males							Females						
Group:	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Number of Animals in group:	23	24	25	25	25	25	4	25	25	24	24	23	25	13
Observed/No remarkable finding	20	19	24	8	21	23	1	22	23	23	22	21	22	-
Total.....:	20	19	24	8	21	23	1	22	23	23	22	21	22	-
Kidneys														
Decreased size	-	-	-	5	-	-	-	-	-	-	-	-	-	-
Discoloration	-	1	-	11	-	-	-	-	-	-	-	-	-	-
Enlargement	-	-	-	1	-	-	1	-	-	-	-	-	1	-
Irregular surface	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Total.....:	-	1	-	18	-	-	1	-	-	-	-	-	1	-
Liver														
Enlargement	-	-	-	-	-	-	1	-	-	-	-	-	1	-
Irregular Surface	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Total.....:	-	-	-	1	-	-	1	-	-	-	-	-	1	-
Skin mass(es)														
Mass	-	-	-	-	-	-	2	1	-	-	-	1	-	15
Nodule	-	-	1	-	-	-	1	-	1	-	-	-	-	5
Total.....:	-	-	1	-	-	-	3	1	1	-	-	1	-	20
Spleen														
Enlargement	-	-	-	-	-	-	2	-	-	-	-	-	1	3
Mass	1	1	-	2	-	2	-	-	-	-	1	-	1	-
Nodule	-	-	-	1	-	-	-	-	1	1	-	-	-	-
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Total.....:	1	1	-	3	-	2	2	-	1	1	1	-	2	4
Thymus														
Enlargement	-	-	-	1	-	-	1	-	-	-	-	-	-	1

Histopathology

Collected organs and tissues were stained with hematoxylin and eosin (H&E) and examined microscopically. For paired organs, both were examined (except for the bones and sciatic nerves, for which only the left was examined unless gross lesions were observed). See the sponsor's table below for details.

Tissues collected for histopathology

Adrenals*	Optic nerves*
Aorta (thoracic)	Ovaries*
Bone (sternum)	Oviduct*
Brain (cerebrum, cerebellum, brain stem)	Pancreas
Cecum	Pituitary
Clitoral gland	Preputial Gland
Colon	Prostate
Duodenum	Rectum
Epididymides*	Sciatic nerves*
Esophagus (thoracic)	Seminal vesicles*
Eyes*	Skeletal muscle
Gall Bladder	Skin (mammary)
Gross lesions	Spinal cord (cervical, thoracic, lumbar)
Harderian Gland	Spleen
Heart	Stomach (cardiac, fundic, pyloric)
Ileum	Salivary glands* (submandibular, parotid, sublingual)
Jejunum	Testes*
Joints (knee)*[1]	Thymus
Kidneys*	Thyroids (with parathyroids if possible)*
Liver	Trachea
Lung (with bronchi)	Urinary bladder
Lymph node (mesenteric)	Uterus (horns, corpus and cervix)
Lymph nodes (mandibular)*	Vagina
Mammary gland (females only)	

* Paired organs
[1] includes proximal tibia, distal femur (with marrow), articular surface, and growth plate (epiphysis)

Peer Review- Yes (b) (4).
The following tissues (see below from the sponsor) were examined. Necropsy and histopathology findings, with the interpretation of the primary pathologist, were available during the review. All differences in the diagnosis of tumors and focal hyperplastic lesions were discussed with the primary pathologist, and resolved.

- All sections from three animals per sex from Groups 1, 4, 5 and 6.
- All the sections from which the primary pathologist had made a diagnosis of a neoplasm in all groups
- All sections from which the primary pathologist made a diagnosis of hyperplasia in Groups 1-6
- All sections of kidneys, testes and thymus from Groups 1-6.

Neoplastic

There were no cladribine-related neoplastic findings. None of the neoplastic findings in cladribine- (or HPβCD-) treated animals occurred at an incidence that was dose-dependent and/or statistically different from control. A few sporadic tumors were observed in brain, pituitary, lung, Harderian gland, thymus and/or stomach (see excerpts from the sponsor's tables, below).

Controls from group(s): 1	MALES						FEMALES					
Group:	1	2	3	4	5	6	1	2	3	4	5	6
Dosage mg/kg:	0	5	15	30	15	431	0	5	15	30	15	431
No. in group:	23	24	25	25	25	25	25	25	24	24	23	25
Stomach												
B-Forestomach papilloma												
Not noted>	23	24	25	25	24	24	25	25	24	23	23	25
Incidental>	0	0	0	0	1	1	0	0	0	1	0	0
M-Hemangiosarcoma												
Not noted>	23	24	25	25	25	25	25	25	24	24	23	24
Incidental>	0	0	0	0	0	0	0	0	0	0	0	1
Thymus												
B-Thymoma												
Not noted>	22	23	24	22	24	23	23	21	21	21	20	23
Incidental>	0	0	0	0	0	1	0	0	0	1	0	1
Harderian gls.												
B-Adenoma												
Not noted>	23	24	25	25	24	24	24	25	24	22	23	25
Incidental>	0	0	0	0	1	0	0	0	0	0	0	0
Brain												
M-Lymphosarcoma												
Not noted>	23	24	25	24	25	25	25	25	24	24	23	25
Incidental>	0	0	0	1	0	0	0	0	0	0	0	0
Pituitary												
M-Lymphosarcoma												
Not noted>	22	24	23	22	23	25	25	24	24	23	23	25
Incidental>	0	0	0	1	0	0	0	0	0	0	0	0
Lungs/Bronchi												
M-Bronchiolar/alveolar carcinoma												
Not noted>	23	24	25	24	24	25	25	25	24	24	23	25
Incidental>	0	0	0	0	1	0	0	0	0	0	0	0

The male MNU-treated (Group 7, positive control) animals had a 52% incidence of lymphosarcoma, which the sponsor reported as in agreement with published values (Takaoka M, et al, 2003). The incidence of lymphosarcoma in female animals was only

32%, but these animals received a lower dose of MNU as compared to the males. The incidence of squamous cell papilloma/carcinoma of the forestomach was 68% in males and 64% in females; this is somewhat lower in both genders than previously published data reported by the sponsor (Takaoka M, et al, 2003). Several other tumors with sporadic incidence were also observed in the positive control group. See excerpts from the sponsor's tables, below.

Terminal sacrifices

Controls from group(s): 1	MALES		FEMALES	
Group:	1	7	1	7
Dosage mg/kg:	0	75	0	75
No. in group:	23	4	25	13
Reticuloendothel				
C-Lymphosarcoma				
Not noted>	23	3	25	11
Incidental>	0	0	0	2
Fatal>	0	1	0	0
Stomach				
B-Forestomach papilloma				
Not noted>	23	2	25	7
Incidental>	0	0	0	6
Probably incidental>	0	2	0	0
M-Squamous cell carcinoma				
Not noted>	23	4	25	12
Incidental>	0	0	0	1
Esophagus,thor.				
B-Papilloma				
Not noted>	23	4	25	12
Incidental>	0	0	0	1
Trachea				
B-Papilloma				
Not noted>	23	4	25	12
Incidental>	0	0	0	1
Spleen				
M-Hemangiosarcoma				
Not noted>	21	4	25	11
Incidental>	2	0	0	2
Lungs/Bronchi				
B-Alveolar bronchiolar adenoma				
Not noted>	23	4	24	12
Incidental>	0	0	1	1
M-Bronchiolar/alveolar carcinoma				
Not noted>	23	4	25	11
Incidental>	0	0	0	2

Unscheduled sacrifices

Controls from group(s): 1	MALES						FEMALES					
Group:	1	2	3	4	5	7	1	2	3	4	5	7
Dosage mg/kg:	0	5	15	30	15	75	0	5	15	30	15	75
No. in group:	3	1	0	0	0	21	0	1	1	1	2	12
Reticuloendothel												
C-Lymphosarcoma												
Not noted>	2	1	0	0	0	9	0	0	0	1	2	6
Probably fatal>	0	0	0	0	0	0	0	0	0	0	0	1
Fatal>	0	0	0	0	0	12	0	0	1	0	0	5
Number examined:	2	1	0	0	0	21	0	0	1	1	2	12
Stomach												
B-Forestomach papilloma												
Not noted>	2	1	0	0	0	10	0	0	1	1	2	2
Incidental>	0	0	0	0	0	6	0	0	0	0	0	6
Probably incidental>	0	0	0	0	0	1	0	0	0	0	0	4
Probably fatal>	0	0	0	0	0	4	0	0	0	0	0	0
M-Forestomach squamous cell carcinoma												
Not noted>	2	1	0	0	0	18	0	0	1	1	2	12
Probably fatal>	0	0	0	0	0	1	0	0	0	0	0	0
Fatal>	0	0	0	0	0	2	0	0	0	0	0	0
M-Squamous cell carcinoma												
Not noted>	2	1	0	0	0	20	0	0	1	1	2	12
Probably fatal>	0	0	0	0	0	1	0	0	0	0	0	0
Spleen												
M-Hemangiosarcoma												
Not noted>	2	0	0	0	0	20	0	0	1	1	2	10
Probably incidental>	0	0	0	0	0	1	0	0	0	0	0	0
Fatal>	0	1	0	0	0	0	0	0	0	0	0	2

Non-Neoplastic

Kidney, thymus and testes were clear target organs. Harderian gland and bone marrow showed some alterations that suggested they may be targets. See excerpts from the sponsor's tables, below. Tubular degeneration/regeneration of the kidneys (minimal to marked) was observed in HD males and females. This was characterized by shrunken and basophilic tubular epithelium in all animals affected, and by dilated tubules and proteinaceous casts in more severely affected animals. Mineralization and cellular infiltration of the kidneys were also observed in HDM. Decreased cellularity of the thymus and degeneration of seminiferous tubules of the testes were seen in HDM. In HDM, decreased cellularity of the thymus and degeneration of seminiferous tubules of the testes were considered to be test article-related changes. Harderian gland hyperplasia, though the severity was not dose-related, was observed in females given the MD and HD HPβCD-cladribine complex. Myeloid hyperplasia was observed in the bone marrow (sternum and knee joint) with increased incidence in HDM.

Controls from group(s): 1	MALES						FEMALES					
Group:	1	2	3	4	5	6	1	2	3	4	5	6
Dosage mg/kg:	0	5	15	30	15	431	0	5	15	30	15	431
No. in group:	23	24	25	25	25	25	25	25	24	24	23	25
Kidneys												
Mineralization												
Not noted>	23	24	25	12	25	25	25	25	24	23	23	25
Minimal>	0	0	0	12	0	0	0	0	0	1	0	0
Mild>	0	0	0	1	0	0	0	0	0	0	0	0
Degeneration/regeneration, tubular												
Not noted>	20	19	17	3	19	22	23	24	23	12	20	23
Minimal>	3	5	8	4	6	3	2	1	1	4	1	2
Mild>	0	0	0	2	0	0	0	0	0	8	2	0
Moderate>	0	0	0	5	0	0	0	0	0	0	0	0
Marked>	0	0	0	11	0	0	0	0	0	0	0	0
Mononuclear cell infiltration, interstitium												
Not noted>	23	24	22	23	22	23	25	25	24	22	22	25
Minimal>	0	0	3	1	3	2	0	0	0	2	1	0
Mild>	0	0	0	1	0	0	0	0	0	0	0	0
Hydronephrosis												
Not noted>	23	23	25	25	25	25	25	25	24	24	23	25
Moderate>	0	1	0	0	0	0	0	0	0	0	0	0
Cellular infiltration												
Not noted>	23	24	25	8	25	25	25	25	24	24	23	25
Minimal>	0	0	0	11	0	0	0	0	0	0	0	0
Mild>	0	0	0	6	0	0	0	0	0	0	0	0
Pelvic dilatation												
Not noted>	23	24	25	24	25	25	25	25	24	24	23	25
Minimal>	0	0	0	1	0	0	0	0	0	0	0	0
Thymus												
Decreased cellularity												
Not noted>	21	22	19	9	19	20	12	5	4	10	11	7
Minimal>	0	0	3	1	2	0	3	3	1	3	1	2
Mild>	1	1	1	6	3	2	4	8	10	7	6	14
Moderate>	0	0	1	6	0	2	4	3	4	2	2	0
Marked>	0	0	0	0	0	0	0	2	2	0	0	1
Hyperplasia, lymphocytes												
Not noted>	22	23	24	22	24	24	23	20	21	21	19	21
Marked>	0	0	0	0	0	0	0	1	0	1	1	3
Testes												
Degeneration, seminiferous tubules												
Not noted>	19	20	22	8	23	23						
Minimal>	4	4	3	10	2	2						
Mild>	0	0	0	5	0	0						
Moderate>	0	0	0	2	0	0						
Epididymides												
Increase, Degenerative Sperm Forms In Lumen												
Not noted>	23	24	25	23	25	25						
Mild>	0	0	0	1	0	0						
Harderian gls.												

Hyperplasia												
Not noted>	23	24	23	25	25	24	24	25	22	20	23	25
Minimal>	0	0	1	0	0	0	0	0	0	2	0	0
Mild>	0	0	1	0	0	0	0	0	2	0	0	0
Mononuclear cell infiltration												
Not noted>	23	23	25	24	25	24	24	25	24	22	23	25
Minimal>	0	1	0	1	0	0	0	0	0	0	0	0
Heart												
Cellular infiltration												
Not noted>	23	24	25	24	25	25	25	25	24	24	22	25
Minimal>	0	0	0	1	0	0	0	0	0	0	1	0
Degeneration												
Not noted>	23	24	25	23	25	25	25	25	24	24	23	25
Minimal>	0	0	0	2	0	0	0	0	0	0	0	0
Sk.ms.,quadr.												
Degeneration/regeneration												
Not noted>	3	0	0	0	3	3	1	0	1	0	1	0
Minimal>	18	17	22	19	18	20	16	17	16	15	17	22
Mild>	2	7	3	6	4	2	8	8	7	9	5	3
Polymorphonuclear cell infiltration												
Not noted>	14	9	9	13	10	16	11	14	13	13	14	14
Minimal>	7	13	16	11	14	8	13	9	10	11	9	10
Mild>	2	2	0	1	1	1	1	2	1	0	0	1
Fibroplasia												
Not noted>	23	24	24	25	25	25	25	25	24	24	23	25
Minimal>	0	0	1	0	0	0	0	0	0	0	0	0
Adrenals												
Subcapsular hyperplasia												
Not noted>	15	17	12	14	13	16	1	0	0	0	0	2
Minimal>	8	7	12	9	11	8	3	10	8	9	7	6
Mild>	0	0	1	2	1	1	20	14	16	14	15	17
Moderate>	0	0	0	0	0	0	1	1	0	1	1	0
Liver												
Cellular infiltration												
Not noted>	18	17	20	23	22	15	16	14	12	14	11	10
Minimal>	5	7	5	2	3	10	9	11	12	10	12	15
Necrosis												
Not noted>	21	24	25	24	25	24	25	21	21	24	23	24
Minimal>	2	0	0	1	0	1	0	4	3	0	0	1
Bone,sternum												
Myeloid hyperplasia, marrow												
Not noted>	21	23	25	19	25	22	25	25	23	24	20	25
Minimal>	0	0	0	1	0	1	0	0	0	0	0	0
Mild>	1	0	0	4	0	1	0	0	0	0	2	0
Joints,knee												
Myeloid hyperplasia, marrow												
Not noted>	22	24	25	21	25	23	25	25	24	24	21	25
Minimal>	0	0	0	0	0	1	0	0	0	0	0	0
Mild>	1	0	0	4	0	1	0	0	0	0	2	0
Lymph ns.,mand.												

Thrombus, mesenteric vessel												
Not noted>	22	23	23	24	21	23	24	25	24	24	23	25
Present>	0	0	0	1	0	0	0	0	0	0	0	0
Spleen												
Increase, brown pigment												
Not noted>	22	24	24	24	24	25	24	23	21	22	20	23
Minimal>	0	0	0	0	0	0	0	0	1	0	0	0
Mild>	1	0	0	1	1	0	1	2	2	2	3	2
Moderate>	0	0	1	0	0	0	0	0	0	0	0	0
Stomach												
Hyperplasia, glandular mucosa												
Not noted>	23	24	25	25	25	24	25	25	23	22	22	24
Minimal>	0	0	0	0	0	0	0	0	1	0	0	0
Mild>	0	0	0	0	0	1	0	0	0	2	1	1
Cellular infiltration												
Not noted>	23	24	25	25	25	24	25	25	23	22	22	24
Mild>	0	0	0	0	0	1	0	0	1	2	1	1
Hyperplasia, non-glandular mucosa												
Not noted>	23	24	25	25	25	25	25	25	24	23	23	25
Mild>	0	0	0	0	0	0	0	0	0	1	0	0
Uterus												
Cystic hyperplasia												
Not noted>							15	9	13	12	14	10
Minimal>							3	8	6	5	5	6
Mild>							6	7	5	7	4	9
Moderate>							1	0	0	0	0	0
Thyrs./paraths.												
Cellular infiltration												
Not noted>	22	24	25	22	24	25	24	25	24	22	23	25
Mild>	0	0	0	0	0	0	0	0	0	2	0	0

Toxicokinetics

Blood samples from Groups 1 and 6 (vehicle and HPβCD groups) were collected from 3 animals on D1, 169, and 173 at 30 minutes post dose. Blood samples from Groups 2 – 5 (cladribine) were collected from 2 animals/time point on D1, 169, and 173 at 0.5, 2, 5, and 24 hr post dose. Group 7 was not sampled.

Cladribine was rapidly absorbed, and exposure was demonstrated for all animals in Groups 2 – 5; no test article was detected in any sample from animals in Groups 1 (control) or 6 (HPβCD). The increase in exposure with increasing dose was roughly dose-proportional in females and slightly greater than dose-proportional in males. Only slight accumulation was observed in males at the end of the last cycle, with cladribine tablets or drug substance. Exposure of animals which received cladribine tablets or drug substance at a dose of 15 mg/kg cladribine (Groups 2 and 5) was comparable; few differences in exposure due to formulation were observed (slight difference in C_{max}). The summary table below was taken from the sponsor's submission.

Test article	TA1 (Cladribine tablets 10 mg, complexed with HP-β-CD)						TA2 (Cladribine drug substance)	
Dose	5		15		30		15	
Dose ratio	1		3		6		-	
	CYCLE 1 – DAY 1							
	Males	Females	Males	Females	Males	Females	Males	Females
C_{max} (ng/mL)	392	618	1640	1930	4630	4020	1230	2000
t_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
AUC_{24} (hr*ng/mL)	380	533	1480	1490	4120	3090	1180	1600
AUC_{24} ratio	1	1	3.9	2.8	10.8	5.8	-	-
GF AUC_{24}	0.7	-	1.0	-	1.3	-	0.7	-
Frel	-	-	1.3	0.9	-	-	-	-
	After 1st dosing of the last 5-day dosing period – DAY 169							
C_{max} (ng/mL)	397	707	2040	2750	3370	4860	1900	2090
t_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
AUC_{24} (hr*ng/mL)	431	598	1930	2030	3540	3560	2030	1750
AUC_{24} ratio	1	1	4.5	3.4	8.2	6.0	-	-
AUC_{24} Cycle ratio	1.1	1.1	1.3	1.4	0.9	1.2	1.7	1.1
GF AUC_{24}	0.7	-	0.9	-	1.0	-	1.2	-
Frel	-	-	0.9	1.2	-	-	-	-
	After 5th dosing of the last 5-day dosing period – DAY 173							
C_{max} (ng/mL)	785	577	2360	2030	4600	4340	2400	1800
t_{max} (hr)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
AUC_{24} (hr*ng/mL)	712	505	2320	1620	6340	3480	2320	1670
AUC_{24} ratio	1	1	3.3	3.2	8.9	6.9	-	-
Rac AUC_{24}	1.7	0.8	1.2	0.8	1.8	1.0	1.1	1.0
GF AUC_{24}	1.4	-	1.4	-	1.8	-	1.4	-
Frel	-	-	1.0	1.0	-	-	-	-

Stability and Homogeneity

Duplicate samples were collected (from the top, middle, and bottom of the container for each formulation containing test article) for homogeneity and dose concentration analysis on days 1, 57, 113, and 169.

Analysis of the concentrations of cladribine or HPβCD indicated that all preparations were within acceptance criteria from nominal concentrations at all sampling times. Analyses of the concentration of the MNU formulations were within acceptance criteria for males, but did not meet acceptance criteria ((b) (4)% of nominal concentration; (b) (4) (b) (4)) for females; however, both MNU formulations were uniform and stable under the conditions of use of the study.

9 Reproductive and Developmental Toxicology

Reproductive toxicity studies were conducted by parenteral (IV and/or SC) administration in mice and rabbits under (b) (4); no new studies (i.e., using oral administration) were conducted. Embryofetal development studies were conducted in mice and rabbits using IV administration; the results of these studies were excerpted in Dr. Huff's review for (b) (4), Appendix A (dated 12/15/98). Cladribine was teratogenic in both mice and rabbits, and safety margins were negligible if present at all. Fertility studies in male and female mice were conducted using SC administration, and a pre-/ post- natal development study in mice was conducted using IV administration

(although identified by "peri-/ post- natal" study #97279 in Dr. Huff's review, the report is also identified as pre-/ post- natal study #300315); these studies were reviewed by Dr. Huff (see review for (b) (4) dated 12/15/98). Generally, fertility parameters were not affected, but embryolethality was observed. Although fertility parameters in mice were not clearly affected, Dr. Huff noted damage to male reproductive organs and adverse effects on sperm (e.g., reduced testes and epididymides weight, reduced sperm count and motility). As noted by Dr. Huff, human fertility may be more sensitive than that of mice to such adverse effects on sperm count and/or motility (e.g., see Nallella et al., 2006). The pre-/post-natal study showed embryolethality, teratogenicity and effects on learning and memory. See Dr. Huff's review for details.

10 Special Toxicology Studies

The sponsor conducted a cyclic and/or daily oral administration toxicity study of HPβCD to justify the use of HPβCD as an excipient in the PO formulation. HPβCD has been shown to produce acinar exocrine pancreas hyperplasia and tumors in rats receiving ≥500 mg/kg daily for 12 and 24 months, respectively (in the diet; see Gould & Scott, 2005); a NOEL was not observed in this study. The sponsor hypothesized that cyclic PO administration would not demonstrate the pancreatic effects observed following daily dosing.

Study title: IMP29006 – RE7460: Toxicity study in Wistar rats treated by oral route with 2- hydroxypropyl-β-cyclodextrin (HPβCD) for 4 cycles, followed by 8-month recovery

Study no.:	29006
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 5, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	2-Hydroxypropyl- β-Cyclodextrin (HPβCD), batch E0070

Key Study Findings

- Overall NOEL/NOAEL= 100 mg/kg (Note: Plasma exposures were negligible at this dose, and dose analysis was not conducted on the 10 mg/ml formulation.)
- According to the sponsor, NOAEL= 500 mg/kg for the cyclic regimen.
- Focal exocrine pancreas hyperplasia was observed, generally at ≥500 mg/kg (daily-HD, plus cyclic-recovery animals); in addition to histopathology, BrdU staining was observed.
- After 5 cycles, histopathological findings were observed in large intestine (cecum, colon and rectum; diffuse mucosal hyperplasia; 500 and/or 5000 mg/kg) and

urinary bladder (transitional epithelium vacuolation; 5000 mg/kg); urinary bladder findings did not fully reverse after 8 months recovery.

- After 4 and/or 12 months of daily dosing at 5000 mg/kg, histopathological findings were observed in the large intestine (minimal-moderate diffuse mucosal hyperplasia), the kidneys (minimal-marked diffuse vacuolation and vacuolar degeneration) and the urinary bladder (minimal-marked diffuse vacuolation and vacuolar degeneration).

Methods (also see sponsor' s diagrams, below)
Doses and Frequency of Administration (from the sponsor's submission):

Groups 1, 2, 3 and 4 were dosed at 0, 100, 500 and 5000 mg/kg/day respectively for 4 consecutive cycles, each consisting of 5-day treatment followed by 23-day withdrawal (one complete cycle consisted of 28 days). At the end of the 4th cycle the rats were treated for 5 additional days, before interim sacrifice or start of the off-treatment period. Groups 5 and 6 were dosed daily at 0 and 5000 mg/kg/day, respectively for 4 (the first 10 animals/sex/group, subjected to the interim sacrifice) or 12 consecutive months (the remaining 20 animals/sex/group). Control animals, i.e. groups 1 (cyclic) and 5 (daily), received the vehicle alone.

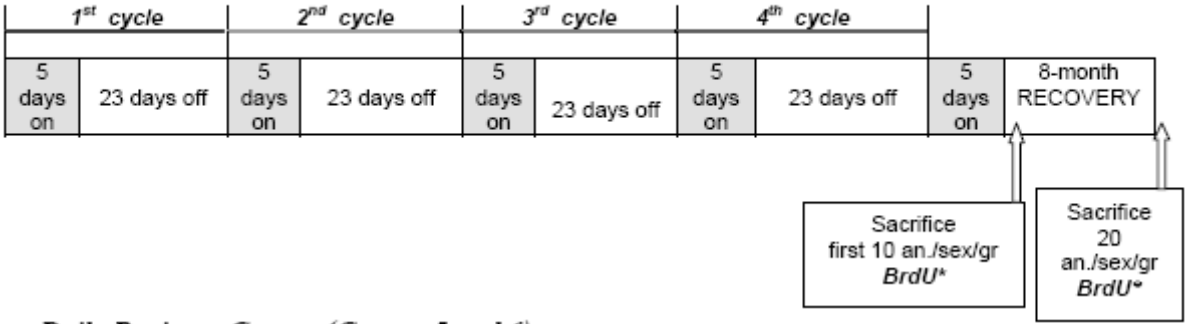
Clinical observations, body weight and food consumption recordings, ophthalmological and hematological examinations, blood chemistry tests and urinalyses were carried out.

At the end of the 4-month dosing period, 10 animals/sex from each group were interim sacrificed for pathology investigations. The remaining animals, 20 animals/sex for groups 1, 2, 3 and 4, were kept off treatment for an 8-month period, while the remaining 20 animals/sex in groups 5 and 6 were daily treated up to 12 months, and then sacrificed.

Route of administration:	PO, gavage
Dose volume:	10 ml/kg body weight
Formulation/Vehicle:	Deionized water
Species/Strain:	Crl: Wistar rat: (b) (4) (b) (4)
Number/Sex/Group:	30/sex/gp
Age:	4 weeks old at arrival
Weight:	90-100 g males and 71-90 g females at arrival
Satellite groups:	TK: 46 animals/sex

Groups 1-4

The dosing regimen is summarized in the scheme below:



Observations and Results

Mortality

There were a few deaths at all doses (see list taken from the sponsor's submission, below); a few deaths at doses ≥ 500 mg/kg were possibly HPβCD-related. The cause of death identified for two cyclic-HD animals involved urinary bladder findings (i.e., cystitis, peritonitis and focal ulceration). The cyclic-HDM (#4870) was euthanized moribund in the third cycle of treatment, showing sedation and hypothermia. The cyclic-HDF that died during week 2 was not reported to show clinical signs. Severe clinical signs were observed in one cyclic-MDM and one daily high-dosed male (the cause of death was listed as possible gavage accident for both animals). The cyclic-MDM (#4856) showed inguinal skin swelling, piloerection, dyspnea, incisors cut, hypomobility, hypothermia, thinness, pallor of skin/mucosa and perineum/abdomen stained with urine, together with a decrease in body weight gain during the last week before death (during the recovery period); the daily-HDM (#4944) showed piloerection starting from week 27 and progressively worsening, with salivation, dyspnea, abdomen dilatation and emaciation before death in week 38. No clinical signs were noted in the other animals found dead. Possible gavage accident was identified as the cause of death, in addition to #4944 above, for animals #4820, 4856, 4873, 4876, 4931 and 177. No cause of death could be found for the control males, for one of the females given cyclic regimen at 5000 mg/kg (#97), or for a female given daily dose at 5000 mg/kg (#158).

Animal No.	Week of death
Cyclic dose 0 mg/kg	
4794 M	Week 11 (3rd cycle)
4796 M	Week 50 (Week 33 of recovery period)
Cyclic dose 100 mg/kg	
4820 M	Week 53 (Week 36 of recovery period)
Cyclic dose 500 mg/kg	
4856 M	Week 49 (Week 32 of recovery period)
Cyclic dose 5000 mg/kg	
4870 M *	Week 9 (3rd cycle)
4873 M	Week 5 (2nd cycle)
4876 M	Week 9 (3rd cycle)
97 F	Week 5 (2nd cycle)
115 F	Week 2 (1st cycle)
Reference group: daily dose 5000 mg/kg	
4931 M	Week 11
4944 M	Week 38
158 F	Week 5
177 F	Week 22

(*) = sacrificed due to moribund conditions

Clinical Signs

Soft stools were observed in all cyclic and daily HD animals, starting after the first administration. Recovery was found within 24 hours of treatment withdrawal. Other signs observed in 1-5 animals/group did not appear to be drug- and/or dose-related. There were no treatment-related changes in the incidence of palpable masses.

Body Weights

Mean body weights were slightly reduced (up to ~8% vs. controls by the end of the dosing period) in the second half of the treatment period in males treated daily. No effects on body weight gain were seen in females treated daily or in animals treated with cyclic regimen.

Feed Consumption

Adverse effects on food consumption were not observed. Treated animals tended to eat slightly more than controls.

Ophthalmoscopy

There were no treatment-related ocular findings.

Hematology

Leukocytes were increased (~10-40%; generally greater in the daily-dosed animals) in the cyclic- and daily-dosed HD animals; this resulted primarily from increased neutrophils (up to ~2.5x respective controls). Lymphocyte counts tended to be slightly reduced in the cyclic-treated animals (10-30%). In the cyclic-dosed animals, the increase in neutrophils was observed through week 17, and resolved upon cessation of dosing. The daily-dosed animals did not show recovery. See the sponsor's summary tables, below.

Males Dose (mg/kg)		Gr# 1 0	Gr# 2 100	Gr# 3 500	Gr# 4 5000	Gr# 5 0	Gr# 6 5000
	Week						
Neutrophils (%)	17	24.06	19.57	21.88	41.26	16.93	31.39 ***
	35	25.52	23.44	24.32	21.49	25.71	25.96
	53	24.64	29.61	32.23	31.09	25.87	44.13 ***
Neutrophils (x10E3/mcL)	17	2.28	1.72	1.96	4.49 *	1.41	3.57 ***
	35	2.10	1.90	1.94	1.93	2.06	2.50
	53	1.81	1.99	2.57	2.40	1.70	3.30 **

Females Dose (mg/kg)		Gr# 1 0	Gr# 2 100	Gr# 3 500	Gr# 4 5000	Gr# 5 0	Gr# 6 5000
	Week						
Neutrophils (%)	17	18.18	18.59	19.99	32.24 *	20.37	21.29
	35	22.63	29.66	22.95	24.51	26.17	29.44
	53	23.69	18.47	25.02	27.02	21.38	42.04 ***
Neutrophils (x10E3/mcL)	17	0.88	1.03	0.95	2.01	0.90	1.14
	35	1.09	1.52	1.08	1.05	1.01	1.42 *
	53	0.91	0.76	0.97	0.97	0.88	1.77 ***

Statistical analyses of the data were performed for Gr. 2-3-4 vs Gr. 1 and for Gr. 6 vs Gr. 5
Significance Level: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Prothrombin time was increased 15% in cyclic-HDM at week 17, but appeared decreased (14%) at week 35. Platelets were increased 12% in daily-HDM.

Clinical Chemistry

GGT was not measured. Several alterations were suggested. The sponsor highlighted increases in AST, ALT and/or amylase in cyclic-treated animals. Total bilirubin was increased ~20% in cyclic- MDM and HDM [ss] on week 17 only. No clear effect on alpha-2-globulin was demonstrated. Blood sodium and chloride were slightly increased (3%, [ss]) in cyclic-HDF, in week 17. In week 17, AST levels appeared reduced ~26% [nss] in cyclic-HDM, but were highly variable. ALT was increased [nss] 23% in cyclic-MDM and HDM. In cyclic-HDF, AST and ALT were increased ~3.2x compared to controls in week 17 (slightly increased 20-30% at week 53, with high variability). Alkaline phosphatase was slightly decreased (19%, [ss]) in cyclic-HDM. Total cholesterol and triglycerides were variable, but appeared increased by week 53 in cyclic-HDF; amylase was increased 30-40% in weeks 35 and 53 in cyclic-HDF. Albumin was very slightly increased and globulin was very slightly decreased in cyclic-treated males. Gamma globulin appeared slightly decreased (~20%) in cyclic-HDM. The cyclic-HDM that died during week 9 showed very high blood urea (344 mg/dl, compared to ~50 mg/dl in controls during week 17), creatinine (1.36 vs. 0.64), alpha-2-globulin, and alkaline phosphatase (202 vs. 60 mg/dl) levels, and reduced AST and ALT.

In the daily-treated animals, glucose (~10%), total cholesterol (~40%) and triglycerides (~40%) were decreased in HDM; recovery was not clearly observed. A very slight decrease in potassium levels (~5%) was observed in HDM. Inorganic phosphorus was increased 23% in HDF at week 17. Slightly increased creatinine was observed in HDF (18%) at week 17 only. Increased AST (15-60%) and ALT (30-90%) were seen in HDM,

increasing with increasing weeks of dosing; increases in AST and ALT were also observed in HDF (20-90%). Increased amylase (~70%) was observed in HDF in weeks 35 and 53, and in HDM in week 53. Apparent, slight increases in alpha-2-globulin (10-20%) observed in weeks 17 and 35 in HDM were not seen at week 53.

Urinalysis

During week 17, urine volume was decreased 28% in cyclic-HDM. Protein was increased ~4x and amylase was increased ~5x in cyclic-HDM during week 17. Protein was increased 2x in cyclic-HDF during week 17 (and was increased ~30% at week 53). Bilirubin was >3x control, and amylase was increased 77%, in cyclic-HDF during week 17. Urobilinogen and blood appeared increased in cyclic-HD animals.

Urine volume was increased 23-50% in daily-HDM, but decreased ~50% in daily HDF. pH was increased (~30%, [ss]) and protein was increased up to 2-3x in daily-HD animals throughout the study. Urobilinogen and amylase (2-6x) were increased throughout the study in daily HD animals. Blood was increased in daily-HDF in week 17 and 35. Increased turbidity and darker color of the urine persisted in daily dosed animals.

Gross Pathology

The sponsor reported no HPβCD-related macroscopic findings.

Organ Weights

In cyclic-HDM, relative (to BW) adrenal and thyroid weights were increased ~20%; relative thyroid weight was still increased ~20% after recovery. Relative spleen weights were slightly increased (~20%) in cyclic-MDF and HDF at 4 months; relative liver weight was slightly increased (~8%) in cyclic MDF and HDF. At recovery, relative heart weight was slightly increased (18%, [nss]).

Daily-HDM dosed for 4 months showed increased relative kidney weight (~16%, [ss]) and adrenal weight (12%, [nss]). Daily-HDF (4 months) showed slightly increased relative liver weight (7%, [nss]). Daily-HDM (12 months) showed increased kidney (18%, [ss]) and adrenal (25%, [ss]) weights; slight increases were also observed for liver (8%, [ss]) and spleen (10%, [ss]) weights. Daily-HDF (12 months) showed increased relative liver (10%, [ss]), kidney (11%, [ss]), and spleen (10%, [nss]) weights.

Histopathology

Adequate Battery- Yes, see sponsor's table below for tissues examined.

Organ	Examination Group
Adrenal (2)	1 + 4 + 5 +6
Aorta	1 + 4 + 5 +6
Bone with knee joint (os femoris)	1 + 4 + 5 +6
Bone with bone marrow (sternum, femur)	1 + 4 + 5 +6
Brain (cerebrum, cerebellum, brain stem)	1 + 4 + 5 +6
Esophagus	1 + 4 + 5 +6
Eye (2)	1 + 4 + 5 +6
Heart	1 + 4 + 5 +6
Intestine, large Cecum Colon Rectum	 1 + 2 + 3 + 4 + 5 + 6 1 + 2 + 3 + 4 + 5 + 6 1 + 2 + 3 + 4 + 5 + 6
Intestine, small Duodenum Jejunum Ileum	 1 + 2 + 3 + 4 + 5 + 6 1 + 2 + 3 + 4 + 5 + 6 1 + 2 + 3 + 4 + 5 + 6
Kidney (2)	1 + 2 + 3 + 4 + 5 + 6
Larynx	
Liver (left lateral and right medial lobe)	1 + 2 + 3 + 4 + 5 + 6
Lung (with mainstem bronchi)	1 + 4 + 5 +6
Lymph nodes mandibular (2) mesenteric	 1 + 2 + 3 + 4 + 5 + 6 1 + 2 + 3 + 4 + 5 + 6
Mammary gland (inguinal)	1 + 4 + 5 +6
Muscle, skeletal (thigh)	1 + 4 + 5 +6
Nasal turbinates	
Nerve, optic (2)	1 + 4 + 5 +6
Nerve, sciatic	1 + 4 + 5 +6
Pancreas	1 + 2 + 3 + 4 + 5 + 6
Parathyroid (2)	1 + 4 + 5 +6
Peyer's Patches	1 + 2 + 3 + 4 + 5 + 6
Pituitary	1 + 4 + 5 +6
Reproductive organs, female Ovary (2) Oviduct (2) Uterus (cornu/corpus/cervix) Vagina	 1 + 4 + 5 +6 1 + 4 + 5 +6 1 + 4 + 5 +6 1 + 4 + 5 +6
Reproductive organs, male Testis (2) Epididymis (2) Prostate Seminal vesicle	 1 + 4 + 5 +6 1 + 4 + 5 +6 1 + 4 + 5 +6 1 + 4 + 5 +6
Salivary glands (2) (submandibular, parotid, sublingual)	 1 + 4 + 5 +6
Skin	1 + 4 + 5 +6
Spinal cord (cervical, thoracal, lumbal)	1 + 4 + 5 +6
Spleen	1 + 2 + 3 + 4 + 5 + 6
Stomach (proventricular, fundic, pyloric)	1 + 2 + 3 + 4 + 5 + 6
Thymus	1 + 4 + 5 +6
Thyroid (2)	1 + 4 + 5 +6
Tongue	
Trachea	1 + 4 + 5 +6
Ureter (2)	1 + 4 + 5 +6
Urinary bladder	1 + 2 + 3 + 4 + 5 +6
Zymbal's gland	
All tissues showing abnormality	All

Peer Review- The protocol indicates yes, but this could not be verified.

Histological Findings
Seven of the early mortalities (13 total; 2, 1, 1 and 5 cyclic control, LD, MD and HD animals, as well as 4 daily HD animals) were identified as possible gavage accidents. Although damage was not noted, gross changes in cyclic LDM (#4820), and cyclic HDMs (#4873 and #4876) were consistent with gavage accident. Other animals showed changes in addition to those considered gavage error-related. A cyclic-MDM (#4856) showed minimal diffusely increased intra-alveolar macrophages and a mild focus of intra-alveolar "pink material" in the lung, but was also reported to show mild centrilobular degeneration and necrosis in the liver, moderate extramedullary hematopoiesis and lymphocyte depletion in the thymus. Two cyclic-HDM (#4873 and #4876) showed hemorrhagic fluid in the thoracic and/or abdominal cavity; animal #4873 also showed moderate pericardial hemorrhage and acute inflammation, and animal #4876 also showed severe congestion in lung. A mild focus of intra-alveolar "pink material" in the lung, and minimal colon and urinary bladder changes, were reported for daily-HDM #4931. The daily-HDM (#4944) that died in week 38 showed a benign adrenal pheochromocytoma, mild inhalation pneumonia and congestion, and minimal-mild large intestine findings. Daily-HDF #177 demonstrated lung congestion and moderate inhalation pneumonia, as well as minimal chronic nephropathy. It is not clear whether all deaths attributed to gavage error were accurate/sole cause.

4-month assessment
Cyclic administration of HPβCD for 4 months (5 dosing periods) produced treatment-related changes in the large intestine (cecum, colon and rectum) of animals given MD and HD, and in the urinary bladder of animals given HD. No treatment-related findings were observed in animals given cyclic-LD. For animals treated with daily-HD, changes in the kidneys were observed in addition to changes in the target organs above. See descriptions below and the sponsor's summary table (following). There were a few findings of possible significance that occurred in only a few animals; these findings occurred in kidney, liver, lung, heart, pancreas, pituitary and/or female reproductive organs (see excerpts from the sponsor's submission, following the summary table).

Kidneys: Daily-HD animals had minimal to moderate vacuolation of the cortical tubular epithelium. In males, this finding was associated with foci of vacuolar degeneration of the cortical epithelium.
Large Intestine: Cyclic-MD, cyclic-HD and daily-HD animals showed minimal to moderate diffuse mucosal hyperplasia, present throughout the large intestine.
Urinary Bladder: In comparison to controls, minimal or mild epithelial vacuolation was present in about 1/2 of the cyclic-HD and nearly all of the daily-HD animals. Additionally, it is noted that urinary cystitis with ulceration of the bladder and peritonitis was identified as the cause of death in a cyclic-HDM euthanized moribund in the third cycle of treatment (#4870) and cyclic-HDF that died during week 2 (#115).

Administration regimen	Cyclic								Daily			
Daily dose (mg/kg)	0		100		500		5000		0		5000	
Histopathology												
Interim Sacrifice (at the end of 4th month)												
Number of animals examined	M:9	F:10	M:10	F:10	M:10	F:10	M:7	F:8	M:10	F:10	M:9	F:9
Caecum												
Diffuse mucosal hyperplasia												
Minimal	0	0	0	0	8	6	4	3	0	0	2	2
Mild	0	0	0	0	0	0	2	1	0	0	7	4
Moderate	0	0	0	0	0	0	1	2	0	0	0	3
Colon												
Diffuse mucosal hyperplasia												
Minimal	0	0	0	0	1	3	2	2	0	0	0	1
Mild	0	0	0	0	2	1	0	2	0	0	5	5
Moderate	0	0	0	0	0	0	4	3	0	0	2	3
Marked	0	0	0	0	0	0	1	1	0	0	2	0
Kidneys												
Diffuse cortical tubular vacuolation												
Minimal	0	0	0	0	1	0	0	0	0	0	1	1
Mild	0	0	0	0	0	0	0	0	0	0	2	2
Moderate	0	0	0	0	0	0	0	0	0	0	6	6
Focal vacuolar degeneration												
Minimal	0	-	0	-	0	-	0	-	0	0	2	0
Mild	0	-	0	-	0	-	0	-	0	0	3	0
Rectum												
Diffuse mucosal hyperplasia												
Minimal	0	0	0	0	1	1	2	3	0	0	3	5
Mild	0	0	0	0	1	0	3	1	0	0	5	4
Urinary bladder												
Epithelial vacuolation												
Minimal	0	0	0	0	0	0	2	2	0	0	6	3
Mild	0	0	0	0	0	0	1	1	0	0	2	4

- No noteworthy findings.

Statistical significance: * - $p < 0.05$ ** - $p < 0.01$ *** - $p < 0.001$

a: cyclic regimen: each cycle consisting of 5-day dosing+ 23-day withdrawal for 4 cyclies + 5 additional daily dosing + 8 months withdrawal; daily regimen: daily for 12 months

b: At end of dosing period. For controls, group means are shown. For treated groups, percent differences from controls are shown.

c: Moreover presence of reythrocytes and increased turbidity and darkness of urine were observed in males treated with either cyclic or daily regimen and in females (but less evident in degree).

d: Terminal sacrifice at the end of treatment (daily regimen groups); Recovery sacrifice (cyclic regimen groups).

na: Not applicable

Male

Dosage	0	100	500	5000	0	5000
Dosing Regime	5 days on + 23 days off, for 4 cycles + 5 days off				Daily	
number of animals	9	10	10	7	10	9
HEART						
number examined	9	-	-	7	10	9
Focus(i) of progressive cardiopathy						
(minimal)	1	-	-	3	2	3
(mild)	1	-	-	0	0	1
Total	2	-	-	3	2	4
KIDNEYS						
Cortical fibrosis						
(minimal)	0	0	0	1	0	1
Focus(i) of basophilic (regenerating) tubules						
(minimal)	2	3	5	1	3	4
(mild)	0	1	0	2	1	0
Total	2	4	5	3	4	4

LUNGS						
number examined	9	-	-	7	10	9
Focus(i) of alveolar macrophages						
(minimal)	1	-	-	2	2	4
(mild)	1	-	-	0	0	2
Total	2	-	-	2	2	6
PANCREAS						
number examined	9	10	10	7	10	9
Peri-islet pigment						
(minimal)	0	0	0	1	1	1
PITUITARY GLAND						
number examined	9	-	-	7	10	9
Cortical cyst(s)						
present	0	-	-	1	1	2
Vacuolated pituicytes						
(mild)	0	-	-	0	0	1

Female						
Dosage	0	100	500	5000	0	5000
Dosing Regime	5 days on + 23 days off, for 4 cycles + 5 days off				Daily	
number of animals	10	10	10	8	10	9
LIVER						
number examined	10	10	10	8	10	9
Focal capsular fibrosis						
(minimal)	0	1	0	0	0	0
(mild)	0	0	0	0	0	1
Total	0	1	0	0	0	1
LUNGS						
number examined	10	-	-	8	10	9
Inflammatory cell focus(i)						
(minimal)	0	-	-	0	0	1
(mild)	0	-	-	1	0	0
Total	0	-	-	1	0	1

12-month assessment

Cyclic Recovery Animals

There were no clearly drug-related findings for animals in recovery from cyclic-LD HPβCD administration, and very few following recovery from cyclic-MD administration. In the cyclic-HD animals, following a period of eight months without treatment, minimal vacuolation of the transitional epithelium of the urinary bladder and minimal vacuolation of the epithelium lining the ureters continued to be observed. The sponsor did not list the increase in uterine and ovarian findings in cyclic-HDF recovery animals. Additionally, there were some low incidence findings in a few organs (e.g., brain, GI tract, kidney, pancreas, liver, lung, mammary, pituitary, and reproductive organs). Mild hydronephrosis of the cerebrum was reported in 1 cyclic-HDM recovery animal; one cyclic-HDF recovery animal showed a meningeal sarcoma. Although not clearly dose-related, submucosal changes were observed in the glandular stomach of a few recovery cyclic males. In recovery cyclic-treated animals, minimal-mild focal exocrine hyperplasia in the pancreas was observed across groups; however, this finding was observed to be marked in 1 cyclic-MDF (#76, died week 53). Pituitary hyperplasia and lung alveolar macrophages were increased in incidence and/or severity in cyclic-HD recovery animals. There were increased incidences of squamous metaplasia of the

uterine endometrial glands and focal hyperplasia of the prostate. For details, see the excerpts from the sponsor's submission following the sponsor's summary table.

Daily-Dosed Animals

The daily-HD HPβCD administration for 12 consecutive months produced treatment-related changes in the kidneys, large intestine (cecum, colon and rectum), pancreas, and transitional epithelium of the ureters and urinary bladder of both sexes. Generally lower incidence findings were observed in a number of organs, including: liver, lung, seminal vesicles, ovaries, vagina, bone marrow, thymus, spleen, pituitary and lymph nodes. Liver vacuolation and/or fat and lung findings were increased in incidence in daily-HD animals. Focal inflammation (1) and/or reduced secretion (3) were observed in the seminal vesicles of daily-HDM. In female reproductive organs, increased incidences of "no corpora lutea present" and "squamous epithelium indicating persistent estrus" were observed. Mildly decreased cellularity, chondromucinous degeneration and/or degenerative joint were observed in the bone marrow of 1-3 of 19-20/sex daily-HD animals (and only 1 daily-ConF). Increased depletion of lymphocytes in the thymus was suggested in daily-HDF. Increased hemosiderin was observed in 2 daily-HDM. Although of low incidence, adenomas of the pars distalis of the pituitary (1 daily-ConM and 2 daily-HDF) and of the pars intermedia (1 daily-HDM) were observed. Some congestion and/or distension of lymph nodes were observed.

Kidneys: Minimal to marked diffuse vacuolation of the cortical tubular epithelium, vacuolated, swollen and vacuolated urothelium and foci of vacuolar degeneration were observed. In addition, minimal or mild vacuolation and swelling of the ureter urothelium was seen in both sexes. Focal urothelial hyperplasia showed increased incidence in daily-HDM.

Large Intestine: Minimal to moderate diffuse mucosal hyperplasia was present throughout the large intestine. This finding was often accompanied by a minimal or mild inflammatory cell infiltration of the lamina propria.

Pancreas: There was an increased incidence of focal exocrine hyperplasia in both sexes at daily-HD. (In contrast to the table below, the histopathology report indicates 1 minimal, 3 mild, 6 moderate and 1 severe in daily-HDM.) These lesions were described as "well-circumscribed, non-encapsulated spherical or oval foci containing acini with a prominent tubular glandular pattern" measuring <5mm in diameter. A pancreatic exocrine adenoma (identical morphology to the hyperplastic foci, but >5mm in diameter) was observed in a daily-ConM.

Urinary Bladder & Ureters: Minimal to moderate epithelial vacuolation was present.

Administration regimen	Cyclic								Daily			
Daily dose (mg/kg)	0		100		500		5000		0		5000	
Terminal / Recovery sacrifice[d] (at the end of 12th month)												
Number of animals examined	M:19	F:20	M:19	F:20	M:19	F:20	M:20	F:20	M:20	F:20	M:19	F:20
Caecum												
Diffuse mucosal hyperplasia												
Minimal	0	0	0	0	1	0	0	1	0	0	2	4
Mild	-	-	-	-	-	-	-	-	0	0	9	9
Moderate	-	-	-	-	-	-	-	-	0	0	8	6
Inflammatory cell infiltration of lamina propria												
Minimal	-	-	-	-	-	-	-	-	0	0	5	12
Mild	-	-	-	-	-	-	-	-	0	0	6	4
Colon												
Diffuse mucosal hyperplasia												
Minimal	0	-	0	-	1	-	0	-	0	0	3	6
Mild	-	-	-	-	-	-	-	-	0	0	9	5
Moderate	-	-	-	-	-	-	-	-	0	0	7	8
Inflammatory cell infiltration of lamina propria												
Minimal	-	-	-	-	-	-	-	-	0	0	9	9
Mild	-	-	-	-	-	-	-	-	0	0	7	1
Kidneys												
Swollen and vacuolated urothelium												
Minimal	-	-	-	-	-	-	-	-	0	0	8	12
Mild	-	-	-	-	-	-	-	-	0	-	1	-
Diffuse cortical tubular vacuolation												
Mild	-	-	-	-	-	-	-	-	0	0	11	7
Moderate	-	-	-	-	-	-	-	-	0	0	7	9
Focal vacuolar degeneration												
Minimal	-	-	-	-	-	-	-	-	0	0	3	4
Mild	-	-	-	-	-	-	-	-	0	0	4	7
Moderate	-	-	-	-	-	-	-	-	0	-	9	-
Marked	-	-	-	-	-	-	-	-	0	-	2	-
Papillary/medullary calculi												
Minimal	-	-	-	-	-	-	-	-	-	7	-	3
Mild	-	-	-	-	-	-	-	-	-	3	-	0
Focal urothelial hyperplasia												
Minimal	-	-	-	-	-	-	-	-	-	15	-	4
Mild	-	-	-	-	-	-	-	-	-	2	-	1
Pancreas												
Focal exocrine hyperplasia												
Minimal	-	-	-	-	-	-	-	-	-	0	-	3
Mild	-	-	-	-	-	-	-	-	0	0	2	3
Moderate	-	-	-	-	-	-	-	-	0	-	5	-
Severe	-	-	-	-	-	-	-	-	0	-	1	-
Rectum												
Diffuse mucosal hyperplasia												
Minimal	-	-	-	-	-	-	-	-	0	0	9	7
Mild	-	-	-	-	-	-	-	-	0	0	8	5
Moderate	-	-	-	-	-	-	-	-	0	0	2	5
Inflammatory cell infiltration of lamina propria												
Minimal	-	-	-	-	-	-	-	-	0	0	7	13
Mild	-	-	-	-	-	-	-	-	0	0	1	1
Ureters												
Vacuolated urothelium												
Minimal	-	0	-	-	-	-	-	4	0	0	7	12
Mild	-	-	-	-	-	-	-	-	0	0	8	2

Urinary bladder												
Epithelial vacuolation												
Minimal	0	0	0	0	0	0	12	17	0	0	7	6
Mild	-	-	-	-	-	-	-	-	0	0	12	9
Moderate	-	-	-	-	-	-	-	-	-	0	-	1
BrdU staining in exocrine pancreas	-	-	-	-	-	-	-	-	-	0	-	1
Number of animals examined	M:10	F:10	M:9	F:10	M:10	F:10	M:10	F:10	M:10	F:10	M:10	F:10
Number of animals showing positive to the BrdU staining	1	0	1	1	2	1	2	0	2	0	4	3

- No noteworthy findings.

Statistical significance: * - $p < 0.05$ ** - $p < 0.01$ *** - $p < 0.001$

a: cyclic regimen: each cycle consisting of 5-day dosing+ 23-day withdrawal for 4 cyclies + 5 additional daily dosing + 8 months withdrawal; daily regimen: daily for 12 months

b: At end of dosing period. For controls, group means are shown. For treated groups, percent differences from controls are shown.

c: Moreover presence of reythrocytes and increased turbidity and darkness of urine were observed in males treated with either cyclic or daily regimen and in females (but less evident in degree).

d: Terminal sacrifice at the end of treatment (daily regimen groups); Recovery sacrifice (cyclic regimen groups).

na: Not applicable

Recovery cyclic-treated animals

Male

Dosage	0	100	500	5000
Dosing Regime	5 days on + 23 days off, for 4 cycles + 5 days off			
number of animals	19	19	19	20
KIDNEYS				
Focal urothelial hyperplasia				
(minimal)	8	6	7	12
(mild)	2	0	0	1
Total	10	6	7	13
Diffuse urothelial hyperplasia				
(mild)	0	1	0	1
LUNGS				
Focus(i) of alveolar macrophages				
(minimal)	4	-	-	6
(mild)	0	-	-	2
Total	4	-	-	8
PANCREAS				
number examined	19	19	19	20
Focal islet cell hyperplasia				
(moderate)	0	0	0	1
PITUITARY GLAND				
Focal hyperplasia of the pars distalis				
(minimal)	2	-	-	2
(mild)	0	-	-	2
(moderate)	0	-	-	2
Total	2	-	-	6
PROSTATE GLAND				
number examined	19	-	-	20
Focal hyperplasia				
(minimal)	0	-	-	1
(mild)	0	-	-	1
Total	0	-	-	2

STOMACH, GLANDULAR				
number examined	19	19	19	20
Submucosal inflammatory cell infiltration				
(minimal)	0	3	2	1
(mild)	0	0	0	1
Total	0	3	2	2
Submucosal oedema present	0	0	1	2
Female				
Dosage	0	100	500	5000
Dosing Regime	5 days on + 23 days off, for 4 cycles + 5 days off			
number of animals	20	20	20	20
KIDNEYS				
number examined	20	20	20	20
Chronic nephropathy				
(minimal)	12	7	6	16
(mild)	2	6	5	2
(moderate)	0	0	0	1
Total	14	13	11	19
LIVER				
number examined	20	20	20	20
Bile duct hyperplasia				
(minimal)	3	8	9	8
(mild)	3	1	0	1
Total	6	9	9	9
Periportal fat vacuolation				
(minimal)	7	10	8	12
(mild)	1	0	0	1
Total	8	10	8	13
LUNGS				
number examined	20	-	-	20
Focus(i) of alveolar macrophages				
(minimal)	3	-	-	7
MAMMARY GLAND (INGUINAL)				
number examined	20	-	1	20
Diffuse lobular hyperplasia				
(minimal)	8	-	0	10
(mild)	4	-	0	6
(moderate)	1	-	0	0
Total	13	-	0	16
Dilated mammary ducts				
(minimal)	1	-	0	5
OVARIES				
number examined	20	-	-	20
Sex cord stromal hyperplasia				
(minimal)	0	-	-	1
(mild)	0	-	-	1
Total	0	-	-	2

PANCREAS				
number examined	20	20	20	20
Peri-islet pigment (minimal)	0	0	0	1
Focal exocrine hyperplasia				
(minimal)	0	1	0	0
(marked)	0	0	1	0
Total	0	1	1	0
Focal inflammatory cell infiltration (minimal)	0	0	0	1
UTERUS				
number examined	20	-	-	20
Squamous metaplasia - endometrial glands (minimal)	1	-	-	1
(mild)	0	-	-	1
Total	1	-	-	2
Metritis (minimal)	0	-	-	1
ENDOMETRIAL STROMAL TUMOURS one ENDOMETRIAL STROMAL POLYP	0	-	-	4
VAGINA				
number examined	20	-	-	20
Squamous epithelium - persistent oestrous present	2	-	-	4

Daily-treated animals

Male		
Dosage	0	5000
Dosing Regime	Daily	
number of animals	20	19
KIDNEYS		
Focal urothelial hyperplasia (minimal)	6	10
(mild)	1	1
Total	7	11
LIVER		
number examined	20	19
Periportal fat vacuolation (minimal)	2	6
Fatty focus(i) (minimal)	0	3
LUNGS		
number examined	20	19
Focus(i) of alveolar epithelialisation (minimal)	0	3

PANCREAS		
number examined	20	19
Focal exocrine hyperplasia		
(minimal)	0	1
(mild)	0	3
(moderate)	1	6
(severe)	0	1
Total	1	11
PITUITARY GLAND		
number examined	20	19
Vacuolated pituicytes		
(minimal)	0	1
Focus(i) of hypertrophic cells		
(minimal)	0	1
STOMACH, GLANDULAR		
number examined	20	19
Focal erosion		
(minimal)	0	1
Female		
Dosage	0	5000
Dosing Regime	Daily	
number of animals	20	19
LIVER		
Periportal fat vacuolation		
(minimal)	9	13
(mild)	1	0
Total	10	13
LUNGS		
number examined	20	19
Focus(i) of alveolar macrophages		
(minimal)	2	2
(mild)	0	5
(moderate)	0	1
Total	2	8
Inflammatory cell focus(i)		
(mild)	0	1
Focus(i) of alveolar epithelialisation		
(minimal)	0	1
(mild)	0	1
Total	0	2

OVARIES		
number examined	20	19
No corpora lutea present	2	6
Sex cord stromal hyperplasia (minimal)	0	1
Focal accumulation of pigmented macrophages (moderate)	0	1
PANCREAS		
number examined	20	19
Focal exocrine hyperplasia		
(minimal)	0	3
(mild)	0	3
Total	0	6
THYMUS		
number examined	20	19
Lymphocyte depletion		
(minimal)	9	12
(mild)	2	4
Total	11	16
VAGINA		
number examined	20	19
Squamous epithelium - persistent oestrous present	4	8

Toxicokinetics

HPβCD or water was administered for 4 consecutive cycles, each consisting of 5-day treatment followed by 23-day withdrawal to animals of Groups 7, 8, 9 and 10. At the end of the 4th cycle the rats were treated for 5 additional days. HPβCD or water was administered daily for 12 consecutive months to animals of Groups 11 and 12. Satellite treated groups consisted of 9 rats/sex (Groups 8, 9 and 10) and 12 rats/sex (Group 12). Toxicokinetic parameters (3 sampling times, 1 rat/sex/sampling time) were assessed on Day 1, 5, 117 and 364 (Group 12 only). Satellite vehicle control groups (Groups 7 and 11) consisted of 3 rats/sex and 4 rats/sex respectively.

HPβCD was not detected in plasma of 13/14 animals of the control groups. The main TK parameters are reported in the sponsor's summary table, below.

Group	8		9		10		12	
Dose (mg/kg)	100		500		5000		5000	
Regimen	Cycle		Cycle		Cycle		Daily	
	Day 1							
	Males	Females	Males	Females	Males	Females	Males	Females
t_{max} (h)	1	1	1	1	1	1	1	1
C_{max} (ng/mL)	236	304	890	1011	6460	8718	6272	9346
t_z (h)	1	1	5	1	24	5	5	5
C_z (ng/mL)	236	304	355	1011	233	5673	3530	3583
AUC_{24} (h*ng/mL)	591	760	2774	2528	44031	86599	55755	62760
M/F AUC_{24} ratio	0.8	-	1.1	-	0.5	-	0.9	-
	Day 5							
	Males	Females	Males	Females	Males	Females	Males	Females
t_{max} (h)	-	-	1	5	5	1	1	1
C_{max} (ng/mL)	-	-	583	664	3273	4642	3693	3079
t_z (h)	-	-	5	5	24	5	5	5
C_z (ng/mL)	-	-	219	664	225	2045	1552	1990
AUC_{24} (h*ng/mL)	-	-	3865	8760	35246	34425	26469	30425
M/F AUC_{24} ratio	-	-	0.4	-	1.0	-	0.9	-
Rac AUC_{24}	-	-	1.4	3.5	0.8	0.4	0.5	0.5
	Day 117							
	Males	Females	Males	Females	Males	Females	Males	Females
t_{max} (h)	1	-	5	1	1	5	1	5
C_{max} (ng/mL)	262	-	535	461	17394	7845	12668	26415
t_z (h)	1	-	5	5	24	5	24	24
C_z (ng/mL)	262	-	535	271	275	7845	360	232
AUC_{24} (h*ng/mL)	655	-	6424	4231	78069	99029	60608	181494
M/F AUC_{24} ratio	-	-	1.5	-	0.8	-	0.3	-
Rac AUC_{24}	1.1	-	2.3	1.7	1.8	1.1	1.1	2.9
	Day 364							
	Males	Females	Males	Females	Males	Females	Males	Females
t_{max} (h)	ns	ns	ns	ns	ns	ns	1	1
C_{max} (ng/mL)	ns	ns	ns	ns	ns	ns	11031	11851
t_z (h)	ns	ns	ns	ns	ns	ns	5	24
C_z (ng/mL)	ns	ns	ns	ns	ns	ns	5437	408
AUC_{24} (h*ng/mL)	ns	ns	ns	ns	ns	ns	88797	95645
M/F AUC_{24} ratio	ns	ns	ns	ns	ns	ns	0.9	-
Rac AUC_{24}	ns	ns	ns	ns	ns	ns	1.6	1.5

ns = Not Scheduled

Dosing Solution Analysis

For homogeneity and stability analyses, formulations at 25 and 500 mg/ml (in deionized water) were prepared in duplicate, and stored for 24 hours or one week at room temperature. The 10 mg/ml concentration was not analyzed. Analyses were performed in triplicate. All results were within ±15% of nominal.

11 Integrated Summary and Safety Evaluation

Cladribine was originally developed, and approved, for the treatment of hairy cell leukemia. The primary mechanism of the drug's action is as an anti-metabolite, which functionally leads to relatively selective cell destruction (e.g., lymphocytes, as well as other cells with higher rates of cell turnover). More specifically, cladribine is a synthetic chlorinated analogue of the naturally-occurring purine nucleoside deoxyadenosine. Cladribine enters cells (passively and/or possibly involving nucleoside transporters; e.g., CNT, ENT), is phosphorylated via deoxycytidine kinase (DCK) to 2-chloro-2'-deoxy-ß-D-adenosine monophosphate (2-CdAMP), and is subsequently phosphorylated to form the triphosphate (2-CdA TP). The cytotoxic nucleotide accumulates selectively in cells, such as monocytes and lymphocytes, which have a high ratio of deoxycytidine kinase to deoxynucleotidase activity. Accumulation is also enhanced due the chlorine substitution

in cladribine; the chlorine moiety yields resistance to deamination by adenosine deaminase. Accumulation of the triphosphate form results in an imbalance of intracellular deoxynucleotide triphosphates, which leads to disruption of cellular metabolism, impairment of DNA synthesis and repair, and ultimately cell death. Both dividing and quiescent cells are damaged by accumulation of 2-CdA TP. As detailed in Dr. Huff's review, 2-CdA TP is believed to impair DNA synthesis in dividing cells by being incorporated directly into the DNA; in quiescent cells, repair of single strand DNA breaks is believed to be inhibited by deoxynucleotide accumulation because when DNA breaks initiate poly (ADP-ribose) polymerase activity, cellular stores of NAD and ATP are depleted. The impairment and ultimate destruction of lymphocytes and monocytes is believed to underlie the therapeutic effect of cladribine for both hairy cell leukemia (approved as an IV formulation in 1993) and multiple sclerosis, an autoimmune demyelinating disorder and the indication for the current application. In addition to the described pharmacological mechanism, cladribine and the main metabolite 2-chlororadenine were found to weakly bind adenosine receptors in standard receptor binding assays.

As detailed above, cladribine is phosphorylated via DCK; deoxycytidine is an endogenous molecule that competes with cladribine for phosphorylation by DCK. The sponsor demonstrated that plasma concentrations of deoxycytidine were high in rats (~5-8 μg/ml), and low in mice, guinea pigs, dogs and rabbits (< 1 μg/ml). Deoxycytidine was not detected in monkey and human plasma. Although rats were used in early toxicology investigations of cladribine, the plasma deoxycytidine results indicated that rat may not be an appropriate species in which to investigate cladribine's effects; therefore, mice were used as the rodent species. However, as noted in Dr. Huff's review, the safety ratio estimates based on the mouse also may be overestimates because the mouse is less sensitive than either monkey or human because: 1) deoxycytidine kinase enzyme kinetics differ between human and mouse, such that the K_m for cladribine in mouse is 10-fold higher than that in human [Reichelova et al., 1995]; 2) cladribine is less efficiently converted to cytotoxic nucleotides in the mouse; 3) cladribine is more rapidly eliminated from mouse than human, which may decrease the duration of exposure to cytotoxic concentrations; and 4) although endogenous plasma deoxycytidine is fairly low in mouse [i.e., 0.31 μg/ml], plasma deoxycytidine is undetectable in monkey and human. Monkeys are believed to likely be more similar to humans, with respect to any differences in PK or PD, due to the lack of deoxycytidine in plasma (although, notably, the formation of metabolite 2-CA seems to be greater in monkeys than in humans). It is not only the differences in deoxycytidine plasma levels that limits the ability of the animal models to predict responses in humans; the active form of cladribine is formed intracellularly (relying on uptake processes), and the intracellular levels are noted to show variability even among humans.

Although the pharmacological mechanism demonstrated for cladribine should yield immunosuppressant effects, cladribine did not show efficacy in nonclinical experimental models of multiple sclerosis. The effects of cladribine on disease amelioration were evaluated in two EAE model studies using SJL/J mice (with an injection of whole myelin and complete Freund`s adjuvant and pertussis toxin). No positive effect on the clinical

outcomes of EAE was seen in either study. The sponsor indicated that this is likely due to pharmacogenetic differences affecting PK and PD between rodents and humans. The sponsor also suggested that the level of cladribine necessary to suppress the immune response was either not achieved and/or not sustained for a sufficient time in mice to have a beneficial effect. However, according to the clinical reviewer, the clinical data demonstrate efficacy in humans with multiple sclerosis (see review by Dr. J. Green).

The toxicology of cladribine has primarily been tested as IV (approved) and SC (b) (4) (b) (4) formulations; the AUC ratios between human exposures and nonclinical NOAELs have generally been low. The toxicities demonstrated for cladribine include hematological, neurological, renal, reproductive (including teratogenicity) and carcinogenic effects. In addition to the toxicities of cladribine itself, the sponsor's clinical/commercial formulation uses an excipient that has been allowed only limited use for oral administration due to demonstrated carcinogenic effects in rats.

The general toxicology of cladribine has been investigated in a number of studies in rats, mice, dogs (cardiovascular effects), monkeys and rabbits (reproductive effects). The majority of the studies have been conducted by IV or SC administration, using a cyclic dosing regimen. To support (b) (4) (b) (4) the 3 - 8 month intermittent study in mice and the 14 month intermittent study in monkeys were identified as the most relevant for human risk assessment (cf. Dr. Huff's review dated 12/15/98). Demonstrated toxicities for cladribine include hematologic changes (myelosuppression and/or anemia), suggestions of clinical chemistry alterations (e.g., increased glucose, decreased T4, or decreased phosphorus), and histologic effects in a number of organs and/or systems (spleen, thymus, bone marrow, male reproductive organs, GI tract, kidney, adrenal and CNS).

Although the 3-8 cycle mouse study was not conducted under GLP, it was used as the chronic rodent toxicity study (and a 22-mo. cyclic SC administration mouse carcinogenicity study was also conducted); the study tested 0, 10, 30, 60 and 80 mg/kg. Mortality occurred at doses ≥ 60 mg/kg. Myelosuppression and leukopenia were described as underlying the early deaths. Several histologic changes were observed, as early as 3 months: testicular atrophy (all doses; also decreased sperm), bone marrow depletion (all doses), lymphocytic depletion of the thymus (≥ 30 mg/kg), single cell necrosis in the duodenum (≥ 30 mg/kg) and lymphocytic depletion of the spleen (≥ 60 mg/kg); changes in the testes and spleen were noted to persist. At 225 days (i.e., after 21-77 days of recovery), testes and epididymides changes persisted at all doses, as did changes in spleen (i.e., chronic interstitial inflammation [10 and 30 mg/kg] and extramedullary hematopoiesis [30 mg/kg]; note that organs other than testes and epididymides were examined only at 10 and 30 mg/kg). No toxicokinetic data were collected in the intermittent SC dosing mouse study; however, data collected in another study (3 months of daily SC dosing) indicated that the AUC achieved at the maximum nonlethal dose of 30 mg/kg ($AUC_{0\text{-}7hr}$= 10050 ng.hr/ml) was approximately 50x the estimated AUC at the maximum 20 mg PO dose; no clear NOEL was demonstrated, but the lowest dose tested (10 mg/kg) yielded an AUC margin of approximately 15.

The sponsor conducted a GLP 14-month cyclic SC administration study in monkeys, testing doses of 0.15, 0.3, and 1 mg/kg. Mortalities occurred at 1 mg/kg; decreased red blood cell parameters, platelets and white blood cells were observed in animals that became moribund. At the end of 14 cycles, remaining animals at 1 mg/kg showed decreases of up to 20% in rbc parameters. Body weight gain was reduced in males at 1 mg/kg. Histologic changes were observed in a number of organs and/or systems: bone marrow cellular depletion (≥0.3 mg/kg), mild-marked lymphoid depletion of the spleen and/or lymph nodes (≥ 0.3 mg/kg), degeneration of the testes and hypospermia (≥ 0.3 mg/kg). Changes were also noted in the kidney (karyomegaly, tubular degeneration, and interstitial fibrosis; 1 mg/kg), CNS (multifocal necrosis and gliosis; 3/6 early mortalities; occlusion of small blood vessels suggested), and adrenal cortex (atrophy; ≥ 0.3 mg/kg). Dr. Huff also noted minimal hemorrhage in the heart of 2 early male mortalities at 1 mg/kg, one of which also showed multifocal degeneration. Toxicokinetic parameters were not determined. The NOAEL for death and hematological effects was 0.3 mg/kg; the overall NOAEL (including histopathology) was 0.15 mg/kg. In a more recent study in monkey, SC cyclic doses of 0.3 mg/kg for 3 months achieved an AUC of 159 ng.hr/ml, which provides no safety margin compared to the estimated AUC following the maximum 20 mg PO dose.

To support the PO formulation, the sponsor conducted a 4-7 cycle PO administration study in mice and a 3-month bridging toxicity study in monkeys. These studies demonstrated little toxicity (e.g., increased cholesterol and decreased WBC in mice, hepatocellular vacuolation and decreased sperm in monkeys), and the maximum doses tested were identified as the NOEL/NOAELs (20 mg/kg and 6 mg/kg, respectively). The calculated AUC margins (compared to the AUC following the maximum 20 mg/day dose in humans) were approximately 7.5 and 1.5, respectively, in mice and monkeys. Although the PO administration studies did not demonstrate toxicity defining MTDs, the doses tested were relatively close to doses (or achieved exposures) previously shown to be at or exceeding an MTD. Notably, although the 6 mg/kg PO dose exceeded plasma exposures achieved with the 0.3 mg/kg SC dose tested concomitantly in the 3-month bridging monkey toxicity study, the 0.3 mg/kg SC dose did not show the toxicities previously demonstrated in the 14-month SC administration toxicity study. It appears that 0.3 mg/kg SC demonstrated increased toxicity with longer duration dosing.

Reproductive toxicity

No new reproductive toxicity studies were submitted. Reproductive toxicology studies were conducted in mice and rabbits, by SC and/or IV administration. Cladribine demonstrated adverse effects on testes and epididymides and on sperm, as well as on embryo survival, and was teratogenic in two species; for details, please see Dr. Huff's NDA review dated 12/15/98 for withdrawn (b) (4). SC administration studies were conducted in male and female mice. Although fertility was not adversely affected in male mice, the findings in those studies (e.g., reductions in sperm count and motility) implied that human fertility, which is more sensitive to reductions in sperm count and motility, may be adversely affected by cladribine. The embryofetal development studies were conducted in mice (0.5, 1.5, 3.0 mg/kg IV) and rabbits (0.3, 1.0, 3.0 mg/kg IV; see

review excerpts in review by Dr. Huff). At 3 mg/kg, cladribine was teratogenic in both mice and rabbits, and skeletal variations were increased at 1.5 and 3.0 mg/kg in mice (also increased postimplantation loss). The peri- and postnatal development study was conducted via the intravenous route in mice (0.5, 1.5, 3.0 mg/kg); the study showed malformations, skeletal variations, embryolethality and neurobehavioral effects. The NOAELs for developmental effects were 0.5 mg/kg in mice and 1 mg/kg in rabbits; the safety margin for rabbits is small (approximately 5.5-fold, based on the AUC following a single IV dose in rabbit versus the AUC achieved following the MRHD of 20 mg PO in human) and the AUC exposure in mice exceeds that of humans following a dose of MRHD of 20 mg/day.

Carcinogenicity

By mechanism, cladribine causes DNA damage and inhibits DNA repair; it is known to be a genotoxic agent (clastogenic, not mutagenic, effects were demonstrated in the standard *in vitro* and *in vivo* assays), and can be expected to be carcinogenic. Cladribine was previously shown to cause Harderian gland tumors in a 22-month cyclic (SC) dosing mouse carcinogenicity study. Although the original review of the study (cf. Dr. Huff's review dated 1/26/99 of withdrawn (b) (4), summary table provided below) indicated Harderian gland tumors only were of toxicologic significance (uterine adenocarcinomas were also statistically significant, but the HD incidence was reportedly equivalent to the historical control range in 24 month studies), a comprehensive statistical analysis was requested for the study since only the review of uterine tumors could be obtained (cf. Dr. Hung's review dated 4/27/99; this review noted a statistically significant increase in adenocarcinomas of the uterus [trend and HD]).. This FDA statistical analysis found (in addition to statistically significant Harderian gland tumors in females and males) statistically significant or "close to" statistically significant increases in tumors of lung (i.e., carcinomas and/or adenoma/carcinoma combination; trend and/or HD) in females, compared to vehicle controls; see excerpts from Dr. Thomson's statistical review (dated 1/18/11) below for details. These findings were discussed with the ExeCAC, which found no other relevant drug-related tumors (other than Harderian gland). Although the increased incidences of lung carcinoma and adenoma/carcinoma in HDF were prominent, similar dose-related increases were not observed in HDM. It was notable that the female untreated controls also showed increased incidences compared to vehicle controls (e.g., in lung); these results complicated interpretation.

Tumor Type	Incidence									
	Vehicle		Untreated		LD		MD		HD	
	M	F	M	F	M	F	M	F	M	F
Harderian Gland										
-adenoma	6/65	1/65	11/65	4/65	3/65	2/65	8/65	2/65	**28**/65	**11**/65
-adenocarcinoma	0/65	0/65	0/65	0/65	1/65	0/65	0/65	0/65	**1**/65	**2**/65
Ovaries										
-luteoma		0/65		nd		**2**/65		**2**/65		**2**/64
Uterus										
-adenocarcinoma (endometrial)		0/65		nd		2/65		0/65		**4**/65

Organ/Tumor	Incidence					Significance Levels				
	Veh	No	Low	Med	Hi	Trend	High	Med	Low	
LUNG										
ADENOMA, BRONCHIOLO-ALVEOLAR	2	9	9	15	11	0.1847	0.0100	0.0008	0.0300	Veh
							0.4403	0.1580	0.6202	No
Adenoma/Carcinoma Bronch.-Alv.	5	14	10	17	20	0.0071	0.0013	0.0057	0.1501	Veh
							0.2197	0.3821	0.8889	No
CARCINOMA, BRONCHIOLO-ALVEOLAR	3	5	1	4	9	0.0058	0.0858	0.5226	0.9460	Veh
							0.2534	0.7762	0.9883	No

The sponsor also conducted a transgenic mouse carcinogenicity assay with cyclic dosing using the oral cladribine formulation. No drug-related increases in tumor incidence were observed in this study. Overall, cladribine demonstrated carcinogenic potential.

HPβCD

Hydroxypropyl-β-cyclodextrin is an excipient that has been allowed for only limited treatment durations (i.e., approximately 1 month) when given by oral administration due to the observed tumorigenic effect in exocrine pancreas; no approved drugs for chronic oral administration contain HPβCD. It has a few well-known toxicities, such as membrane alterations (especially on erythrocytes) and renal toxicity (see Irie et al., 1997). The sponsor provided justification for the use of HPβCD in IND 74,634 Serial No. 32, dated 12/18/06. Although published literature has indicated that oral exposure to HPβCD for 2 years caused pancreatic exocrine acinar tumors in rats (cf. Gould & Scott, 2005), the sponsor stated that the daily dose of HPβCD that produced these findings was 500 mg/kg (the low dose used in the carcinogenicity study), which would be a cumulative exposure of 356 g HPβCD/kg over the 24-month study. Notably, this study (as indicated in the sponsor's response) did not demonstrate a NOAEL for this tumorigenic effect (and therefore safety margins cannot be determined).

The dosing regimen for PO cladribine involves daily treatment of up to ~288 mg HPβCD for 4-5 days per month for 2 consecutive months per year. The yearly cumulative dose of HPβCD in oral cladribine over 2 years (i.e., a maximum of ~288 mg for 20 days over 2 years) totals almost 6 g. Literature was provided that indicated following oral administration of 1 or 3 g of HPβCD to healthy human volunteers, HPβCD could neither be detected in plasma nor in the urine, indicating that the compound is not bioavailable in humans following oral administration at these doses (e.g., Gould & Scott, 2005 and Stella & He, 2008). The sponsor argued that since HPβCD is not absorbed in human, the cumulative amount administered within a cycle is ~12.6 mg/kg (an average of 2.516 mg/kg x 5 days), and there is a washout period of 25 days between cycles, the sponsor does not expect any accumulation from one cycle to another. The sponsor expects the human systemic exposure to HPβCD within a cycle to be negligible and to not accumulate with multiple cycles; however, data to demonstrate this were not provided in the submission.

The sponsor made an effort to assess the potential for toxicity of the HPβCD excipient with an additional nonclinical study (i.e., treatment of up to 4 cycles or daily for up to 12 months); however, the ability of this limited study to detect the toxicity (given the cyclic

dosing regimen and the limited duration of treatment compared to the apparent unlimited clinical regimen) remains questionable. Although a NOAEL (100 mg/kg) was demonstrated in the sponsor's 4 to 12 month study of cyclic- and/or daily orally administered HPβCD in rats, the adequacy of study is questionable. The 10 mg/ml dose formulation was not subjected to concentration analysis. Also, it is not entirely clear from the study that pancreatic exocrine hyperplasia did not occur following cyclic dosing (see the recovery cyclic-treated animals). With regard to HPβCD, the plasma exposures achieved are expected to exceed those that would be experienced clinically in the 4 courses/2-year regimen (although exposures were not measured in humans), but may not provide adequate assessment of further cycles; it is not clear from the sponsor's application that cladribine treatment is limited to the 4 courses (for a total of 3.5 mg/kg) over 96 weeks, with no further courses. Additionally, the ability of the study to assess the local toxicity of cladribine (an effect generated by the presence of HPβCD in the GI tract) is limited; the study conducted can support up to five cycles (i.e., the four courses over 2 years).

Safety Margins

Safety margins to the NOAELs in the PO toxicity studies conducted are not large; notably, the maximum doses tested were the NOAELs. The sponsor calculated safety margins to the 7-cycle mouse and 3-cycle bridging monkey toxicity studies based on both intermittent-daily and projected yearly cumulative exposure in humans (see sponsor's Table 2.6.4-4).

In humans, the proposed oral clinical dose for MS is approximately 0.175 mg/kg/day, corresponding to 10 mg/day for a patient of body weight range of 50 ≤ 60 kg. Note that since one or two 10 mg tablets can be administered per day depending on human body weight (i.e., for body weights ≥60 kg, the dose on one or more days is doubled- 2 tablets of 10 mg), the C_{max} and $AUC_{0\text{-}24hr}$ ratios calculated by the sponsor on the 20 mg days would be reduced by one-half (i.e., only 56 and 1.9 and 56-fold and 2.3 and 12-fold, for C_{max} and AUC, respectively, in monkey and mouse).

Table 2.6.4- 4 Comparative Exposure to Cladribine After Oral Chronic Intermittent Administration in Mice and Monkeys vs. that After Oral Administration of 10 mg Tablet in Human Subjects

Study	Species	Dose (mg/kg/day)	Duration of dosing / day of sampling	Mean Cmax (ng/mL)	Cmax ratio (A/H)	Mean AUC_{0-24} (h·ng/mL)	AUC_{0-24h} ratio (A/H)	Cumulative AUC (based on AUC_{0-24h} x dosing days)	Cumulative AUC ratio (A/H)
25803, 26127, 26486, 27967, IXR-102-09-186	Human	10 mg HPβCD-tablet	1 day / day 1	24.2 [b]	-	86.4 [b]	-	864 [d]	-
25329	Mouse	20 (NOAEL)	Seven 28-day dosing courses [a] / day 169	2715	**112 (56 [1])**	2045 [c]	**24 (12 [1])**	100205 [e]	**116 (97 [1])**
26669	Monkey	6 (NOAEL)	Three 28-day dosing courses [a] / day 61-62	91.5	**3.8 (1.9 [1])**	387.3 [c]	**4.5 (2.3 [1])**	8133.3 [f]	**9.4 (7.8 [1])**

A/H: mean ratio of Animals to Human

a: One "28-days dosing course" corresponds to 7 days of daily dosing followed by 21-days without dosing

b Mean PK parameters (Cmax and AUC0-…) calculated from individual subject PK parameters across the following 5 clinical studies: 25803: 10 mg tablet (n=16); 26127: 10 mg tablet without food (n=16); 26486: 10 mg tablet monotherapy cladribine (n=15); 27967: 10 mg tablet monotherapy (n=17); IXR-102-09-186: 10 mg tablet (n=26)

c: Mean exposures of both gender

d: Calculated cumulative exposure in patients per year considering 5 days of dosing per course and 2 courses per year ($AUC_{0-\infty}$ times 5 days times 2 courses)

e: Calculated as: AUC0-24 times 7 dosing days times 7 courses

f: Calculated as: AUC0-24 times 7 dosing days times 3 courses

(1) Note: As one or two 10 mg tablets can be administered per day depending on the patient body weight (see *Table 2.6.4- 12*), for patients of body weight ≥60 kg the dose on one or more days is doubled (2 tablets of 10 mg). Therefore, based on cladribine dose-exposure linearity (refer to clinical section 2.7.2.3.6.1), the Cmax and AUC0-24 ratios on the 20 mg days need to be divided by 2 (corresponding values are presented in parenthesis). However, the cumulative AUC ratios decreases by less than 20% (calculated for patient of 60-<70 kg with 6 tablets per course times 2 courses per year; corresponding values are presented in parenthesis).

The NOAELs for developmental effects were 0.5 mg/kg in mice and 1 mg/kg in rabbits; the safety margin for rabbits is small (approximately 5.5-fold, based on the AUC following a single IV dose in rabbit versus the AUC achieved following the MRHD of 20 mg PO in human, 202 ng.hr/ml) and the AUC exposure in mice exceeds that of humans following a dose of MRHD of 20 mg/day.

The AUC safety margin for tumor production in mice (SC administration; mouse AUC derived from the lowest average exposure at the same dose in a 3-month daily SC administration toxicity study) is approximately 1.3-fold the AUC at the maximum recommended daily dose (20 mg) in humans.

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MELISSA K BANKS
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