

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

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**PHARMACOLOGY REVIEW(S)**

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR ONCOLOGY DRUG DEVELOPMENT

**PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION**

<b>NDA Number</b>	21-877
<b>Serial Number</b>	001
<b>Received by CDER</b>	December 17, 2004
<b>Product</b>	ARRANON® (Nelarabine)
<b>Clinical Indication</b>	The treatment of patients with T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma whose disease has not responded to or has relapsed following treatment with at least two chemotherapy regimens
<b>Sponsor</b>	GlaxoSmithKline King of Prussia, PA 19406
<b>Documents Reviewed</b>	EDR December 17, 2004 RRZ
<b>Review Division</b>	Division of Oncology Drug Products (HFD-150)
<b>Reviewer</b>	W. David McGuinn, Jr., Ph. D., D.A.B.T.
<b>Supervisor</b>	David Morse, Ph. D.
<b>Division Director</b>	Robert Justice, M.D.
<b>Project Manager</b>	Sheila Ryan, Pharm. D. Nicholette Hemingway, M.P.H.
<b>Medical Officer</b>	Martin Cohen, M.D.
<b>Clinical Pharmacologist</b>	
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- I. Pharmacodynamics:
  1. 506U87 Inhibited the growth of CEM cells in beige nude Xid mice.
  2. Clarification of data on the Xid nude mouse CEM tumor experiments
- II. Pharmacokinetics
  1. Pharmacokinetics of Nelarabine in the dog.
  2. Pharmacokinetics of araG and Nelarabine in the monkey.
  3. Pharmacokinetic parameters for Nelarabine and ara-G in monkeys after i.v. administration of 60 mg/kg Nelarabine.
- Toxicology
  1. Acute murine toxicity study.
  2. A report for AUC 512--an acute single dose i.v. study in beagle dogs with Nelarabine.
  3. Monkey toxicity study
  4. An acute single dose i.v. toxicity study in the mouse with Nelarabine.
  5. An acute 5 day i.v. toxicity study in the mouse with Nelarabine.
  6. An i.v. dose range finding study with Nelarabine in cynomolgus monkeys.

7. An acute 5 day i.v. toxicity study in cynomolgus monkeys with Nelarabine including unaudited draft copied of pathology.
8. A 30 day intravenous toxicity study in Cynomolgus monkeys with Nelarabine (with recovery period). Serial # 036.
9. Nelarabine: An acute single dose intravenous study in Beagle dogs with Nelarabine. Serial # 049.

These studies were contained in five reviews.

#1 Original review of IND 42,788 completed June 24, 1993

#2 Completed March 16, 1994

#3 Review of submission serial number 002, completed April 7, 1994

#4 Review of submission serial numbers 036, 045 and 049, completed April 28, 1998

#5 Review of submission serial numbers 110 and 113, completed March 7, 2001

#### ***Studies not reviewed***

- 1) METHOD VALIDATION: Analysis of Nelarabine, Fludarabine des-Phosphate, and Ara-G in Cynomolgus Monkey, New Zealand White Rabbit, and HanWistar Rat Plasma, using HPLC  
Study RD1997/02506/00
- 2) The Validation of a Method for the Determination of GI262250 (Nelarabine) and GI186S98 (ara-G) in Stabilized Rabbit Plasma (ranges — ng/mL and — µg/mL) using HPLC. — Study RD2004/00764/00
- 3) Comparison of ara- G and Nelarabine Plasma Levels in Rats Dosed Orally with ara- G, Nelarabine or 1493U89. Study TEZZ/95/0004/00
- 4) Nelarabine is a more potent anti-VZV agent than 96u68. Study TEZA/90/0010

## **Executive Summary**

### ***Recommendations***

See Labeling below

### ***Recommendation on approvability***

I find no pharmacological or toxicological issues that would prevent the approval of Nelarabine for the proposed medical indication.

### ***Recommendation for Non-clinical studies***

None for the current indication

### ***Recommendations on labeling***

1 Page(s) Withheld

       § 552(b)(4) Trade Secret / Confidential

       § 552(b)(5) Deliberative Process

✓ § 552(b)(5) Draft Labeling

## Summary of Non-clinical findings

### *Brief overview of Non-clinical findings*

Early research determined that rats, mice and dogs were not good models for Nelarabine toxicology studies because they eliminated ara-G, the clinically active metabolite, much more quickly than humans. Thus, the sponsor did most of the non-clinical development of Nelarabine in the monkey.

Nelarabine is a potent toxin. It is cytotoxic *in vitro* at micromolar concentrations in human bone marrow progenitor cell lines. Experiments *in vitro* suggest it is more toxic to human malignant T-cell lines than it is to malignant B-cells.

Solutions of Nelarabine were not hemolytic and did not cause protein flocculation at clinically relevant concentrations. Only 7 to 20% of a dose of Nelarabine is bound to human plasma and binding did not increase with concentration over a range of 6 to 600  $\mu\text{M}$ . Binding of ara-G increased from 7 to 24% over this same concentration range.

Nelarabine did not inhibit any major human cytochrome P450 isoenzyme. Nelarabine did not inhibit the P-glycoprotein transporter nor was it a substrate.

Based on limited information, single doses of 300 mg/kg or less in the monkey cause little toxicity but higher doses appear to cause some saturation of metabolism. The increase in AUC becomes greater than dose normal. Single doses of 400 or 500 mg/kg result in irreversible neurotoxicity, manifested as a deepening loss of consciousness within hours after dosing. This neurotoxicity is distinct from the Guillain-Barre like syndrome seen in patients receiving Nelarabine chronically.

Nelarabine distributes widely and rapidly to all major tissues. Concentrations in metabolic and excretory organs were usually higher than the concentrations in blood. Concentrations in the brain and CNS were lower than the concentrations in blood. The concentrations in other major organs were approximately equal to those in blood. Monkeys eliminate most of an IV dose of Nelarabine in the urine (>62%) within 24 hours. The monkeys excreted less than one percent of total radioactivity in the bile and less than 2% in the feces. Less than 80% of the radiolabel was recovered after 10 days from the intact animals suggesting biological incorporation.

In humans, the half-life of ara-G is between 2 and 4 hours,  $T_{\text{max}}$  occurred after about two hours,  $Cl/F$  was about 0.3 L/h/kg,  $V_{\text{ss}}/F$  was about 1. At the recommended clinical dose for adults, the AUC was 23  $\mu\text{M}\cdot\text{h}$ ,  $C_{\text{max}}$  was 14  $\mu\text{M}$ , clearance was about 8 L/h/kg, half-life was about 16 minutes and the volume at steady state was about 9 L/kg. AUC and clearance increased roughly linearly with dose but there was considerable variability.

With the exception of the neurotoxicity, Nelarabine causes a spectrum of toxicities that one would expect with a purine antimetabolite. Monkeys tolerated single IV doses of 3600 mg/m<sup>2</sup> with few signs of toxicity, but higher doses caused fatal neurotoxicity. Monkeys that died showed loss of palpebral reflex, shallow respiration, flaccid limbs, and minimal jaw tone resulting in death within hours. Monkeys tolerated five daily doses of 1800 mg/m<sup>2</sup> showing dose dependent decreases in WBC and platelets and a decrease in thymus weight. At this schedule, a dose of 3600 mg/m<sup>2</sup> killed 3 of 4 monkeys by day 13. All monkeys at this dose had tremors and convulsions, neurotoxicity distinct from that seen after a single dose. Body weight decreased as did WBC and platelets. AST and ALT increased 2 to 12 fold. BUN, triglycerides and glucose rose. Microscopic damage was found in proliferative tissues, GI, spleen, marrow, thymus. There was congestion in the lungs.

In an IV study with daily X30 dosing, monkeys tolerated doses of only 120 mg/m<sup>2</sup> with decreased red and white cell parameters. 240 mg/m<sup>2</sup> killed one of six monkeys on day 28. A dose of 480 mg/m<sup>2</sup> killed three of 10 monkeys despite the secession of dosing on day 23. Red and white cell parameters decreased significantly. Significant damage was seen in the brain (cerebellar degeneration, perivascular cuffing in the cerebrum and cerebellum) and the spine (vacuolization and myelopathy, not reversible in surviving monkeys). Changes in clinical chemistry parameters were suggestive of liver damage. There was lymphoid infiltration in the bladder, kidney, trachea, salivary gland, lacrimal gland and heart. In females, there was myocardial degeneration and liver damage (cholangitis, edema and vacuolization).

An IV dose of 3600 mg/m<sup>2</sup>/d or about twice the adult recommended dose given to pregnant rabbits during organogenesis was toxic to the does, causing mortality, abortion, decreased body weight gain, and labored breathing. The high dose was not embryolethal but it did cause toxicity, which manifest as low fetal weight. All dose levels including 1200 mg/m<sup>2</sup>/d or 360 mg/m<sup>2</sup>/d were associated with fetal abnormalities including cleft pallet, absent polices, absent gall bladders, accessory lung lobes and fused or extra sternbrae. Lower doses were not tested.

Nelarabine was mutagenic (>10X controls) in the mouse lymphoma TK test.

### ***Pharmacological activity***

Nelarabine is a pro-drug of deoxyguanosine analogue 9-β-D-arabinofuranosylguanine (ara-G). Adenosine deaminase demethylates Nelarabine to form the active compound. Intracellular deoxyguanosine kinase and deoxycytidine kinase phosphorylate ara-G sequentially for form ara-GTP. ara-GTP substitutes for GTP in numerous biological processes including the replication of DNA. This substitution leads to inhibition of DNA synthesis and cell death. Malignant T-cells are killed by this process because they are rapidly replicating. Nevertheless, ara-G has the potential to kill any replicating cell. This mechanism appears so well accepted that little research has been done to determine other mechanisms. The acute lethal neurotoxicity seen in monkeys is probably caused by a different mechanism.

### ***Non-clinical safety issues relevant to clinical use***

Nelarabine is a potent toxin. It is cytotoxic *in vitro* at micromolar concentrations. It causes dose dependent myelosuppression in humans and animals and toxicities that suggest acute liver damage. The unusual neurotoxicity seen clinically and non-clinically is possibly due to a secondary pharmacology and not the intracellular formation of ara-GTP. The toxic dose response curve for lethal neurotoxicity in the monkey is relatively steep, 300 mg/kg is non-lethal but 400 mg/kg (4800 mg/m<sup>2</sup>) killed one monkey within 4 hours. Nelarabine is a mutagen. It is also toxic to the developing fetus.

## **PHARMACOLOGY AND TOXICOLOGY REVIEW**

### **Introduction and Drug History**

Dr. Joanne Kurtzberg submitted IND 42,778 in June of 1993 specifying “Compound 506, ara-G pro-drug” for \_\_\_\_\_ Dr. Wendelyn Schmidt reviewed the initial submission. GlaxoWellcome acquired the drug some time after 1994 and continued development

During early clinical studies, nelarabine demonstrated some activity in patients with multiple types of hematological malignancies. Investigators observed the greatest activity in patients with T-cell disease. Results of the first Phase I trial, Study PGAA1001 [RM2002/00406/00], showed an overall response rate of 35% (33/93), including complete responses in four adults and six children with relapsed or refractory T-cell acute lymphoblastic leukemia or lymphoblastic lymphoma (sponsor’s estimates). Complete and partial responses were observed in patients with other T-cell malignancies, and PRs were observed in subjects with various B-cell malignancies.

Phase II studies included patients with a variety of hematological malignancies and included both children and adults. GlaxoSmithKline collaborated with the Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis at the National Cancer Institute during the nelarabine clinical trial program. Both GSK and NCI hold INDs for this compound. Between 1994 and February 2005, over 900 patients received nelarabine on clinical trials or special exception protocols under these two INDs. There are five phase I studies, nine phase II studies and a compassionate use program either completed or ongoing. GSK included full study reports on four Phase I and three Phase II studies with this submission. They have partial information on the other studies. The following tables (sponsor’s) summarize these clinical studies.

**Table 1 Listing of Clinical Studies**

Protocol No.	Type of Study	Study Objective(s)	Study Design	Key Inclusion Criteria of Subjects	No. of Subjects: Gender M/F: Mean Age (Range)	Treatment Details (Drug/Dose/Form/Route/Frequency/Duration)	Study Status: Type of Report	Location of Study Report
PGAA1001	Dose escalating, PK, safety	Determine MTD and toxicities, define PK, establish preliminary efficacy	Open-label, Dose-rising	Refractory hematologic malignancy in relapse in adult subjects ( $\geq 18$ y at time of enrollment)	39 leukemia patients 32 male/7 female 44.7 y (18-75 y)	5, 10, 20, 40, 60 mg/kg, and 1000, 1200 mg/m <sup>2</sup> nelarabine by intravenous infusion over one hour once daily for five consecutive days.	Completed; Final	m5.3.3.2.3
				26 lymphoma patients 16 male/10 female 46.3 y (19-74 y)				
				Refractory hematologic malignancy in relapse in pediatric subjects <18 at time of enrollment)	26 leukemia patients 18 male/8 female 9.3 y (3-17 y)	5, 10, 20, 40, 60, 75 mg/kg, and 1200 mg/m <sup>2</sup> nelarabine by intravenous infusion over one hour once daily for five consecutive days.		
				2 lymphoma patients 2 female 13.5 y (13-14 y)				
PGAA1002	Dose escalating, PK, safety	Determine MTD and toxicities, define PK	Open-label, Dose-rising	Refractory hematologic malignancies in adult subjects ( $\geq 18$ y at time of first dose)	17 patients 13 male/4 female 42.4 y (18-71 y)	900, 1200, or 1500 mg/m <sup>2</sup> nelarabine by intravenous infusion over a two-hour period once daily for three consecutive days	Completed; Final	m5.3.3.2.1
				Refractory hematologic malignancies in pediatric subjects (<18 y at time of first dose)	10 patients 7 male/3 female 12.7 y (6-16 y)			
						900, 1200, or 1500 mg/m <sup>2</sup> nelarabine by intravenous infusion over a 2-hour period once daily for either 3 or 5 consecutive days		

Protocol No.	Type of Study	Study Objective(s)	Study Design	Key Inclusion Criteria of Subjects	No. of Subjects: Gender M/F: Mean Age (Range)	Treatment Details (Drug/Dose/Form/Route/Frequency/Duration)	Study Status: Type of Report	Location of Study Report
PGAA1003	Dose escalating, PK, safety	Determine MTD, toxicities, PK profile and preliminary antitumor efficacy and intracellular concentrations of ara-GTP.	Open-label, Dose-rising	Refractory hematologic malignancies in adult patients ( $\geq 18$ y at time of enrollment)	46 patients 28 male/18 female 53.2 y (19-77 y)	1200, 1500, 1800, 2200, 2500, or 2900 mg/m <sup>2</sup> nelarabine by intravenous infusion over a 2-hour period on Day 1, 3, and 5	Completed; Final	m5.3.3.2.2
				Refractory hematologic malignancies in pediatric subjects (<18 y at time of enrollment)	2 patients 2 male 9.5 y (7-12 y)	1200 or 1500 mg/m <sup>2</sup> nelarabine by intravenous infusion over a 2-hour period on Day 1, 3, and 5		
PGAA1005	PK/PD, safety	Evaluate effect of fludarabine on intracellular concentrations of ara-GTP, cyto-reduction and toxicity after administration of Nelarabine in patients with refractory leukemia.	Open-label	Chronic lymphocytic leukemia refractory to prior regimens with purine analogs or alkylating agents or both (age $\geq 15$ y)	13 patients 9 male/4 female 59.7 y (27-83 y)	1200 mg/m <sup>2</sup> nelarabine by intravenous infusion over a 2 hour period on Days 1, 3, and 5 and 30 mg/m <sup>2</sup> fludarabine by intravenous infusion over a 30-minute period 4 hours before the nelarabine infusion on Days 3 and 5 in course 1 and Days 1, 3, and 5 on second and subsequent courses.	Completed; Final	m5.3.3.2.4

Protocol No.	Type of Study	Study Objective(s)	Study Design	Key Inclusion Criteria of Subjects	No. of Subjects: Gender M/F: Mean Age (Range)	Treatment Details (Drug/Dose/Form/Route/Frequency/Duration)	Study Status: Type of Report	Location of Study Report
COG P9673 PGAA2001	Efficacy, safety	Determine response rate, further define toxicities, determine impact of nelarabine on survival, duration of response, and time to response.	Open-label	Refractory or recurrent T-ALL or T-NHL, with adequate hepatic and renal function (age $\leq 21$ y at time of initial diagnosis).  Stratum 01: T-ALL or T-NHL in first relapse/refractory.  Stratum 02: T-ALL or T-NHL in second or later relapse/refractory.  Stratum 03: T-ALL or T-NHL with positive bone marrow and CNS involvement, first or greater relapse/refractory.  Stratum 04: Extramedullary relapse and <25% blasts in marrow, first or greater relapse/refractory.	151 patients 110 male/41 female 11.58 y (0.6 – 21.7 y).  Stratum 01: 37 patients. 31 male/6 female 11.39 y (0.6 – 21.7 y).  Stratum 02: 48 patients. 29 male/19 female 11.24 y (1.8 – 20.0).  Stratum 03: 32 patients. 24 male/8 female 10.48 y (1.2 – 19.2 y).  Stratum 04: 34 patients. 26 male/8 female 13.31 y (2.4 – 21.3 y).	400, 650, 900, or 1200 mg/m <sup>2</sup> as a 1 hour infusion daily for 5 days, to be repeated in 21-day cycles.	Completed; Final	m5.3.5.2.1

Protocol No.	Type of Study	Study Objective(s)	Study Design	Key Inclusion Criteria of Subjects	No. of Subjects: Gender M/F: Mean Age (Range)	Treatment Details (Drug/Dose/Form/Route/Frequency/Duration)	Study Status: Type of Report	Location of Study Report
CALGB 19801 PGAA2002	Efficacy, safety	Determine rates of complete and partial response, time to response, duration of response, overall survival and one-year survival, evaluate safety of treatment, determine impact of treatment on survival.	Open-label	Relapsed or refractory T-ALL or T-LBL, positive for 2 of 7 specified cell surface antigens, with CLcr $\geq 50$ ml/min and bilirubin $\leq 2 \times$ ULN, without CNS disease requiring intrathecal or craniospinal radiation therapy or history of seizure disorder or $\geq$ grade 3 neurologic toxicity during prior treatment of ALL/LBL, and no pre-existing neuropathy $\geq$ grade 2 at time of registration (age $\geq 16$ y).	39 patients 32 male/7 female 35.0 y (16 – 66 y).	1500 mg/m <sup>2</sup> as a two-hour infusion administered on days 1, 3, 5 of a 21-day cycle. Three subjects received a starting dose of 2200 mg/m <sup>2</sup> .	Completed; Final	m5.3.5.2.2
PGAA2003	Efficacy, safety, PK	Evaluate rates of complete and partial response, time to maximal response, duration-free response, progression-free survival and overall survival, define safety, and evaluate PK of nelarabine and metabolites in multiple treatment courses.	Open-label	Chronic lymphocytic leukemia refractory to fludarabine and at least one alkylator or alkylator-containing regimen (age $\geq 18$ y).	87 patients at 2 dosage levels (2200 mg/m <sup>2</sup> , 1500 mg/m <sup>2</sup> ).  2200 mg/m <sup>2</sup> : 20 patients 15 male/5 female 58.9 y (44 – 77 y).  1500 mg/m <sup>2</sup> : 67 patients 46 male/21 female 60.9 y (43, 80 y).	2200 mg/m <sup>2</sup> (subsequently modified by amendment to 1500 mg/m <sup>2</sup> ) administered as a two-hour infusion on Day 1, 3, and 5.	Completed; Final	m5.3.5.2.3

Protocol No.	Type of Study	Study Objective(s)	Study Design	Key Inclusion Criteria of Subjects	No. of Subjects: Gender M/F: Mean Age (Range)	Treatment Details (Drug/Dose/Form/Route/Frequency/Duration)	Study Status: Type of Report	Location of Study Report
CALGB 69803	Dose-escalating, safety, PK	Determine MTD, safety, pharmacokinetics of nelarabine in subjects with renal or hepatic impairment	Open label Dose-rising	Hematologic malignancies that have failed standard therapy or for which no standard therapy exists (age $\geq 18$ y).	10 patients (control arm with normal hepatic and renal function)	1 cohort at 1500 mg/m <sup>2</sup> /day IV infusion over 2 hours on days 1, 3, and 5, repeated every 21 days. 4 cohorts starting at 1000 mg/m <sup>2</sup> /day IV over 2 hours on days 1, 3, and 5, repeated every 21 days.	Closed; Study Summary	m5.3.5.4.1 Section 3.1
SWOG S0010	Safety, efficacy	Investigate safety and efficacy of nelarabine	Open label	Adult subjects with relapsed or refractory non T-cell ALL with morphological characteristics of ALL as determined by FAB class L1 – L2, having failed standard induction therapy or having relapsed following a successful induction therapy (age $\geq 16$ y).	20 patients	1500 mg/m <sup>2</sup> /day IV administered as a two-hour infusion on days 1, 3, and 5, repeated every 21 days.	Closed; Study Summary	m5.3.5.4.1 Section 3.2
CALGB 59501	Safety, efficacy	Investigate safety and efficacy of nelarabine	Open label	Previously systemically untreated cutaneous T-cell lymphoma (CTCL) or refractory or relapsed non-cutaneous peripheral T-cell lymphoma (PTCL). Age $< 70$ y.	19 patients	1500 mg/m <sup>2</sup> /day administered by 2-hour IV infusion on days 1, 3, and 5 repeated every 21 days.	Closed; Study Summary	m5.3.5.4.1 Section 3.3
MDACC 86	Safety, efficacy, PK	Investigate safety, efficacy and pharmacokinetics of nelarabine	Open label	Subjects with previously treated T-cell lymphoma. Age $\geq 16$ y.	2 patients	1500 mg/m <sup>2</sup> /day by 2-hour infusion on days 1, 3, 5 repeated every 28 days.	Closed; Study Summary	m5.3.5.4.1 Section 3.4



**Information to sponsor**  
**Sponsor**

No  
GlaxoSmithKline  
King of Prussia, PA

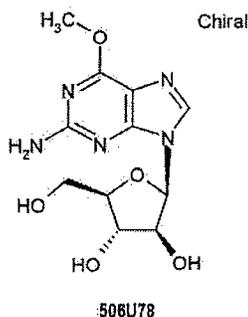
**Reviewer name**  
**Division name**  
**Review completion date**

W. David McGuinn, Jr., M.S., Ph. D., D.A.B.T.  
Division of Oncology Drug Products, HFD-150  
October 3, 2005

**Drug**

Trade name: ARRANON®  
Generic name: Nelarabine  
Code Name: 506U78  
Chemical Name: 2-amino-9-β-D-arabinofuranosyl-6-methoxy-9H-purine  
FW 297.27 g/mole CAS not given C<sub>11</sub>H<sub>15</sub>N<sub>5</sub>O<sub>5</sub>

**Structure**



Relevant INDs 42,778 (GSK) and 52611 (NCI)  
Drug class cytotoxin  
Intended clinical population patients with T-cell acute lymphoblastic leukemia and T-cell lymphoblastic lymphoma whose disease has not responded to or has relapsed following treatment with at least two chemotherapy regimens  
  
Clinical formulation sterile solution in glass vials  
Each vial contains 250 mg of Nelarabine (5 mg/mL)  
Sodium chloride 4.5 mg/mL  
50 mL Water for injection, USP  
Route of administration IV, 2 hour infusion  
Dose (adult) 1500 mg/m<sup>2</sup>  
Schedule days 1, 3, and 5 every 21 days  
Course 21 days  
  
Dose (pediatric) 650 mg/m<sup>2</sup> IV over 1 hour for 5 consecutive days repeated every 21 days  
  
Disclaimer: I have copied and inserted all tabular and graphical information directly from the sponsor's electronic submission unless otherwise specified. In most of the tables

and graphs I have constructed, I converted the sponsor's mass units from grams to moles. I calculated the percentage differences in tables as  $(\text{value in exposed animal} - \text{value in control}) \div (\text{value in control})$ . I have excerpted some of the information from Dr. Wendylen Schmidts reviews of IND 42,778.

## Pharmacology

### 1) Human *in vitro* marrow progenitor cell toxicity of 155U70UL (ara-G) and Nelarabine

In this study investigators determined the cytotoxicity of 155U70UL (ara-G), AZT and Nelarabine *in vitro* in two human bone marrow progenitor cell lines, CFU-GM and BFU-E. The cells were from three different donors. The following table (sponsor's) shows that Nelarabine, a prodrug of ara-G, was toxic at concentrations about twice those that were toxic with ara-G.

Table 1	150 $\mu\text{M} \pm \text{SE}$ (standard error)		
	CFU-GM	BFU-E	# expts
155U70UL (ara-G)	4 $\pm$ 1	0.7 $\pm$ 0.2	3
506U78UQ	8 $\pm$ 1	2 $\pm$ 0.3	3
AZT (509U81UBJ)	6 $\pm$ 5	0.4 $\pm$ 0.1	3

Study number	TEZZ/95/0017
Laboratory	Burroughs Wellcome, Co., RTP, NC
Date	February 1995
GLP	No
Audited	No
Drug	Nelarabine, purity and lot not specified

### 2) T-cell versus B-cell selectivity of Nelarabine

In this experiment, investigators tested the ability of Nelarabine to inhibit the growth of a human T-cell line (Molt 4) relative to its ability to inhibit a human B-cell line (IM9) *in vitro*. They also compared the toxicity of Nelarabine with that of other purine arabinosides. The following table (sponsor's) shows that Nelarabine was considerably more toxic to T-cells than to B-Cells, as were the other arabinosides. It was more toxic to T-cells than was ara-G.

Compound	150 $\mu$ M (% of Control growth)	
	Molt 4 cells	IM9 cells
506U78	0.8 $\pm$ 0.1	>10 (85%)
araG	1.4 $\pm$ 0.08	>10 (84%)
araH	>10 (72%)	>10 (90%)
araA	20 $\pm$ 6	>10 (76%)
araA+ EHNA	0.8 $\pm$ .06	11 $\pm$ 1.5
506U78 + EHNA	>10 (72%)	>10 (87%)

Study number           TEXR/ 90/ 0006  
Laboratory            Burroughs Wellcome, Co., RTP, NC  
Date                    February 1990  
GLP                    No  
Audited                No  
Drug                    Nelarabine, purity and lot not specified

### 3) Comparison of ara-G and 506U in T-cell and B-cell lines

Investigators again tested the relative toxicity of ara-G and Nelarabine against various human T-Cell and B-Cell lines *in vitro*. They also tested the ability of two inhibitors of adenosine deaminase, cofomycin and EHNA (erythro-9-(2-hydroxy-3-nonyl) adenine) to diminish Nelarabine toxicity by blocking its conversion to ara-G. The following table (sponsor's) shows that in most T-cell lines the two compounds are approximately equipotent and in the  $\mu$ M range. Neither compound was toxic to B-Cells (IM-9). The presence of either inhibitor of adenosine deaminase diminished the toxicity of Nelarabine as a function of concentration as one would expect.

Table 1. *In vitro* toxicity (IC<sub>50</sub>) of ara-G and 506U with several cell lines  
The IC<sub>50</sub> for ara-G and 506U in human T-cell (CEM, CEM CD4+, MOLT4), B-cell (IM-9) and macrophage (U937) cell lines

Compound	Cell Line	COFORMYCIN ( $\mu$ M)			
		0	0.1	1	10
506U	CEM	1.9 $\pm$ 0.2	1.8 $\pm$ 0.3	2.4 $\pm$ 0.2	15 $\pm$ 3
ara-G	CEM	0.70 $\pm$ 0.08	0.60 $\pm$ 0.05	0.43 $\pm$ 0.06	0.60 $\pm$ 0.05
506U	CEM CD4+	3.44 $\pm$ 0.9	13.7 $\pm$ 0.9	75 $\pm$ 7	54 $\pm$ 13
ara-G	CEM CD4+	5.0 $\pm$ 0.2	3.1 $\pm$ 0.2	3.5 $\pm$ 0.2	2.6 $\pm$ 0.2
506U	IM-9	> 200 $\mu$ M	> 200 $\mu$ M	> 200 $\mu$ M	> 200 $\mu$ M
ara-G	IM-9	> 200 $\mu$ M	> 200 $\mu$ M	> 200 $\mu$ M	> 200 $\mu$ M
506U	MOLT4	1.6 $\pm$ 0.2	1.60 $\pm$ 0.02	1.7 $\pm$ 0.2	13 $\pm$ 3
ara-G	MOLT4	1.8 $\pm$ 0.2	1.7 $\pm$ 0.1	1.6 $\pm$ 0.3	1.7 $\pm$ 0.1
506U	U 937	1.3 $\pm$ 0.1	3.9 $\pm$ 0.5	11 $\pm$ 1	57 $\pm$ 5
ara-G	U 937	0.46 $\pm$ 0.43	0.62 $\pm$ 0.11	0.61 $\pm$ 0.16	0.60 $\pm$ 0.03

Compound	Cell Line	EHNA ( $\mu$ M)			
		0	0.1	1	10
506U	CEM	1.6 $\pm$ 0.1	2.4 $\pm$ 0.4	8 $\pm$ 1	27 $\pm$ 8
ara-G	CEM	0.60 $\pm$ 0.01	0.50 $\pm$ 0.06	0.50 $\pm$ 0.02	0.60 $\pm$ 0.02
506U	CEM CD4+	4.4 $\pm$ 0.3	12 $\pm$ 10	44 $\pm$ 4	51 $\pm$ 5
ara-G	CEM CD4+	3.2 $\pm$ 0.2	2.0 $\pm$ 0.2	2.1 $\pm$ 0.1	1.1 $\pm$ 0.1
506U	IM-9	> 200 $\mu$ M			
ara-G	IM-9	> 100 $\mu$ M			
506U	MOLT4	1.40 $\pm$ 0.02	1.9 $\pm$ 0.4	7.8 $\pm$ 1.2	14.3 $\pm$ 1.1
ara-G	MOLT4	2.3 $\pm$ 0.2	2.30 $\pm$ 0.03	1.9 $\pm$ 0.1	1.7 $\pm$ 0.1
506U	U 937	1.0 $\pm$ 0.1	9.2 $\pm$ 0.2	25 $\pm$ 4	-
ara-G	U 937	0.44 $\pm$ 0.04	0.54 $\pm$ 0.10	0.48 $\pm$ 0.07	-

\*IC<sub>50</sub>, Concentration that inhibited growth 50% compared to untreated control cells.

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Study number TEZA/94/0071/00  
 Laboratory GlaxoSmithKline., RTP, NC  
 Date August 1994  
 GLP No  
 Audited No  
 Drug Nelarabine, purity and lot not specified

#### 4) Anabolism of Guanosine Arabinoside [155U70, ara-G] and 2-Amino-6-Methoxypurine Arabinoside [Nelarabine] in CEM Cells

In this experiment, the investigators incubated CEM cells with either radiolabeled Nelarabine or radiolabeled ara-G in the presence or absence of deoxycoformycin, an inhibitor of adenosine deaminase. They then measured the intracellular concentration of radiolabeled ara-GTP by HPLC. The table below (sponsor's) shows that the cells took up both compounds and phosphorylated them to about the same concentrations in the absence of deoxycoformycin. Deoxycoformycin blocked the formation of ara-GTP by blocking the conversion of Nelarabine to ara-G.

Table 1. Intracellular Concentration of [<sup>3</sup>H]ara-G 5'-Triphosphate in CEM Cells Incubated for 4 hr with 100 μM [<sup>3</sup>H]ara-G or [<sup>3</sup>H]506U78 with and without 1 μM Deoxycoformycin

Compound	Deoxycoformycin	ara-GTP (pmol/10 <sup>6</sup> cells) <sup>a</sup>
100 μM ara-G	---	55.9 ± 10.4
100 μM ara-G	1 μM	65.1 ± 22.1
100 μM 506U78	---	54.7 ± 11.3
100 μM 506U78	1 μM	6.9 ± 0.7 <sup>b</sup>

<sup>a</sup> Mean ± standard deviation, N=3.

<sup>b</sup> Statistically significant difference, students T-test, p<0.05.

Study number TEZZ/94/0037/00  
 Laboratory Burroughs Wellcome Co, RTP, NC  
 Date May 1994  
 GLP No  
 Audited No  
 Drug [<sup>3</sup>H] Nelarabine (Lot #100-287-022, radiochemical purity > —  
 [<sup>3</sup>H] ara-G (Lot #67035, radiochemical purity > —

#### 5) Growth inhibition of human leukemic cell lines by Nelarabine and 155U70

This study is similar to those above. The investigators treated human T-cell leukemia cell lines (Molt4, CEM and CEM-CD4), three cell lines expressing monocyte markers (U937, — and THP-1) and a B-cell line (IM9) to determine the relative toxicity of Nelarabine and ara-G. The following table (sponsor's) shows that the drugs were more toxic to the T-cells than the B-cells.

**Table 1. Inhibition of Five Human Leukemic Cell Lines by 506U78 or 155U70 in the Presence and Absence of Coformycin or EHNA**

Data are presented as the mean 50% inhibitory concentration ( $\mu\text{M}$ )  $\pm$  standard deviation (n=4).

		[COFORMYCIN], $\mu\text{M}$			
Compound	Cell Line	No Drug	0.1	1	10
506U78UD	CEM	1.9 $\pm$ 0.2	1.8 $\pm$ 0.3	2.4 $\pm$ 0.2	14.5 $\pm$ 2.7
155U70UH	CEM	0.7 $\pm$ 0.08	0.6 $\pm$ 0.05	0.43 $\pm$ 0.06	0.6 $\pm$ 0.05
506U78UD	Molt4	1.6 $\pm$ 0.2	1.6 $\pm$ 0.02	1.7 $\pm$ 0.2	13 $\pm$ 3.4
155U70UH	Molt4	1.8 $\pm$ 0.2	1.7 $\pm$ 0.1	1.6 $\pm$ 0.3	1.7 $\pm$ 0.05
506U78UD	U 937	1.27 $\pm$ 0.05	3.9 $\pm$ 0.5	11 $\pm$ 1	57 $\pm$ 5
155U70UH	U 937	0.5 $\pm$ 0.4	0.6 $\pm$ 0.1	0.6 $\pm$ 0.2	0.6 $\pm$ 0.03
506U78UD	CEM CD4+	3.4 $\pm$ 0.9	13.7 $\pm$ 0.9	75 $\pm$ 7	50 $\pm$ 10
155U70UH	CEM CD4+	5.0 $\pm$ 0.2	3.1 $\pm$ 0.2	3.5 $\pm$ 0.2	2.6 $\pm$ 0.2
506U78UD	IM-9	> 200 $\mu\text{M}$	> 200 $\mu\text{M}$	> 200 $\mu\text{M}$	> 200 $\mu\text{M}$
155U70UH	IM-9	> 200 $\mu\text{M}$	> 200 $\mu\text{M}$	> 200 $\mu\text{M}$	> 200 $\mu\text{M}$

		[EHNA], $\mu\text{M}$			
Compound	Cell Line	No Drug	0.1	1	10
506U78UD	CEM	1.6 $\pm$ 0.14	2.4 $\pm$ 0.4	8 $\pm$ 1.2	27 $\pm$ 7.6
155U70UH	CEM	0.6 $\pm$ 0.01	0.5 $\pm$ 0.06	0.5 $\pm$ 0.02	0.6 $\pm$ 0.02
506U78UD	Molt4	1.4 $\pm$ 0.02	1.9 $\pm$ 0.4	8 $\pm$ 1	14 $\pm$ 1
155U70UH	Molt4	2.3 $\pm$ 0.2	2.3 $\pm$ 0.03	1.9 $\pm$ 0.1	1.7 $\pm$ 0.05
506U78UD	U 937	1 $\pm$ 0.1	9.2 $\pm$ 0.2	25 $\pm$ 4	N.D.*
155U70UH	U 937	0.44 $\pm$ 0.04	0.5 $\pm$ 0.1	0.48 $\pm$ 0.07	N.D.
506U78UD	CEM CD4+	4.4 $\pm$ 0.3	12 $\pm$ 1.4	44 $\pm$ 4	51 $\pm$ 5
155U70UH	CEM CD4+	3.2 $\pm$ 0.2	2.0 $\pm$ 0.2	2.1 $\pm$ 0.1	1.07 $\pm$ 0.09
506U78UD	IM-9	> 200 $\mu\text{M}$	> 200 $\mu\text{M}$	> 200 $\mu\text{M}$	> 200 $\mu\text{M}$
155U70UH	IM-9	> 100 $\mu\text{M}$	> 100 $\mu\text{M}$	> 100 $\mu\text{M}$	> 100 $\mu\text{M}$

\* not determined because of inhibition of U937 cell growth by 10  $\mu\text{M}$  EHNA

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**Table 2. Inhibition of Three Human Leukemic Cell Lines by 506U78 or 155U70 in the Presence and Absence of Deoxycytosine or EHNA**

Data are presented as the mean 50% inhibitory concentration ( $\mu\text{M}$ )  $\pm$  standard deviation (n=4).

		[DEOXYCYTOSINE], $\mu\text{M}$			
Compound	Cell Line	No Drug	0.1	1	10
506U78UD	CEM	0.36 $\pm$ 0.05	0.48 $\pm$ 0.06	1.42 $\pm$ 0.06	8.4 $\pm$ 0.3
155U70UH	CEM	0.39 $\pm$ 0.07	0.37 $\pm$ 0.03	0.40 $\pm$ 0.02	0.56 $\pm$ 0.05
506U78UD	Molt4	0.71 $\pm$ 0.06	0.71 $\pm$ 0.06	2.1 $\pm$ 0.2	19.7 $\pm$ 0.5
155U70UH	Molt4	0.65 $\pm$ 0.07	0.74 $\pm$ 0.09	0.71 $\pm$ 0.08	0.9 $\pm$ 0.1
506U78UD	U937	1.3 $\pm$ 0.2	12 $\pm$ 2	>50	>50
155U70UH	U937	0.67 $\pm$ 0.04	0.60 $\pm$ 0.02	0.49 $\pm$ 0.05	0.53 $\pm$ 0.01

		[EHNA], $\mu\text{M}$			
Compound	Cell Line	No Drug	0.1	1	10
506U78UD	CEM	0.307 $\pm$ 0.006	0.62 $\pm$ 0.05	1.2 $\pm$ 1	3.0 $\pm$ 0.4
155U70UH	CEM	0.31 $\pm$ 0.06	0.32 $\pm$ 0.05	0.32 $\pm$ 0.02	0.11 $\pm$ 0.001
506U78UD	Molt4	0.70 $\pm$ 0.09	1.2 $\pm$ 0.1	2.8 $\pm$ 0.3	7.8 $\pm$ 0.9
155U70UH	Molt4	0.63 $\pm$ 0.05	0.55 $\pm$ 0.02	0.48 $\pm$ 0.04	0.34 $\pm$ 0.02
506U78UD	U937	3.9 $\pm$ 0.3	13 $\pm$ 1	25 $\pm$ 7	N.D.*
155U70UH	U937	0.64 $\pm$ 0.06	0.75 $\pm$ 0.01	0.64 $\pm$ 0.04	N.D.

\* not determined because of inhibition of U937 cell growth by 10 $\mu\text{M}$  EHNA

**Table 3. Susceptibility of Three Human Monocytic Leukemic Cell Lines to 506U78 and 155U70**

Data are presented as the mean 50% inhibitory concentration ( $\mu\text{M}$ )  $\pm$  standard deviation (n = 6 for U937 and n = 4 for THP-1)

Compound	Cell Line	IS <sub>50</sub> ( $\mu\text{M}$ )
506U78UD	U937	1.5 $\pm$ 0.4
155U70UH	U937	1.0 $\pm$ 0.4
506U78UD	/	0.8 $\pm$ 0.5
155U70UH	/	0.8 $\pm$ 0.4
506U78UD	THP-1	>50
155U70UH	THP-1	>50

Again, this experiment demonstrates that inhibition of adenosine deaminase decreases the toxicity of Nelarabine.

Study number           TEZZ/95/0001  
 Laboratory            Burroughs Wellcome Co, RTP, NC  
 Date                    January 1995  
 GLP                    No  
 Audited                No  
 Drug                    Nelarabine and ara-G, lot and purity not specified

## 6) Efficacy of 506V78 and Ara-G Purging on Malignant T-, R-, or Stem Cells

Investigators in Dr. Kutzberg's laboratory evaluated the ability of Ara-G and Nelarabine to purge malignant T-lymphoid cells in culture. Both compounds killed comparable numbers of cells at concentrations between 100 and 500  $\mu\text{M}$  against wild-type T-lymphoidblastoid cells. Nelarabine was less toxic to malignant B- and stem cells consistent with other results above.

Study number	RR1998/00119/00
Laboratory	—
Date	August 1998
GLP	No
Audited	No
Drug	Nelarabine and ara-G, lot and purity not specified

### ***Pharmacology summary***

Nelarabine is cytotoxic *in vitro* at micromolar concentrations in human bone marrow progenitor cell lines. Experiments *in vitro* suggest it is more toxic to human malignant T-cell lines than it is to malignant B-cells, in some cases by at least a factor of 10. In some cell lines it is more toxic than ara-G. This is probably due to greater uptake of the pro-drug and not a separate toxicity. Nevertheless, a separate mechanism of toxicity is certainly a possibility and one that has not been investigated.

### **Safety Pharmacology**

#### **1) Nelarabine: Skin sensitization (Magnusson and Kligman Maximization) study in the albino Dunkin-Hartley guinea pig.**

##### Major findings

Nelarabine caused some irritation during the induction phase of this experiment but it did not cause skin sensitization upon re-challenge in the guinea pig.

Study number	G22214
Laboratory	Glaxo Wellcome Research and Development, Derby, UK
Study date	April 1998
GLP	Yes
Audited Report	Yes
Drug	Nelarabine, Batch MBR 027/02/01A
Method	
Animal	albino Dunkin-Hartley guinea pigs
N	10 control, 20 treated
Formulations	
Intradermal Induction	
a - 1: 1 Freund ' s Complete Adjuvant (FCA) and distilled water	
b - 5% w/ v Nelarabine (higher concentrations caused local necrosis – sighting tests)	
c - 5% w/ v Nelarabine in 1: 1 FCA and distilled water	
d - 50% w/ v Arachis oil BP in 1: 1 FCA and distilled water	
Topical Induction	
e - 50% w/ w Nelarabine	
Topical Challenge	
f - 25% w/ w Nelarabine	

g - 50% w/ w Nelarabine  
Vehicle - Arachis oil BP

#### Study Design

##### Intradermal Induction: Day 0

Test Animals - 3 intradermal injections (0.1 mL each of formulation a, b or c on each side of mid line shoulder region.

Controls – treated as for test animal except injections were a, vehicle, or d.

##### Topical Induction: Day 7

Application on same site as intradermal injections.

Test Animals - formulation e applied under occlusive dressing for 48 hours.

Controls – treated as for test animals except used vehicle alone.

##### Topical Challenge: Day 21.

Test and control animals – application on flanks. Formulation f and g applied to left and right flanks respectively under an occlusive dressing for 24 hours.

#### Observations

Body weights:	Day 0 and 24
Skin reactions after injection or patch removal ( hours):	
Intradermal Induction:	24 and 48
Topical Induction:	1 and 24
Topical Challenge:	24 and 48

#### Results

Intradermal Induction: Well-defined or moderate to severe erythema was noted at the induction sites of all test group animals at the 24 and 48-hour observations. Very slight erythema was noted at the induction sites of all control group animals at the 24-hour observation and in 6 control group animals at the 48 hour observation.

Topical Induction: Very slight or well-defined erythema was noted at the induction sites of all test group animals at the 1 hour observation. Very slight edema was also noted at the induction sites of five test group animals. Very slight erythema was noted at the induction sites of 9 test group animals with very slight edema at the induction sites of 6 test group animals at the 24 hour observation.

Topical Challenge: No skin reactions were noted at the challenge sites of any test and control group animals at the 24 and 48-hour observations.

## **2) Nelarabine: acute eye irritation study in the New Zealand White Rabbit**

#### Major findings

A single application of 61 mg of Nelarabine in 0.1 mL of solution (about 0.67 M) resulted in a maximum overall mean score of 6.7 on the Draize scale. Thus, Nelarabine caused negligible eye damage and is classified as a Grade 1 ocular toxin.

Study number	L22213
Laboratory	Glaxo Wellcome Research and Development, Derby, UK
Study date	March 1998
GLP	Yes
Audited	Yes
Drug	Nelarabine, Batch MBR 027/02/01A
Methods	
Animal	Male New Zealand White rabbits
Doses	10 mg powder – 1 rabbit
Formulation	0.1 ml containing 61 mg – 3 rabbits
Measurement	Draize scale

## Results

### 10 mg application

The investigators noted a slight initial local pain reaction along with a very slight conjunctival reaction 1 and 3 hour after dosing. The treated eye appeared normal at the 6-hour observation.

### 0.1 ml (61 mg, or about 0.67 M) application

This high concentration caused a slight initial local pain reaction immediately after application. It caused a slight conjunctival reaction up to the 3-hour observations, with a very slight conjunctival reaction up to 6 hours. The treated eyes appeared normal at the 24-hour observation.

## 3) An *in vitro* hemolysis and protein flocculation study with Nelarabine

### Major findings

Solutions of Nelarabine were not hemolytic and did not cause protein flocculation or increased turbidity at ratios that simulated total doses ranging from 35 to 140 mg/kg.

Study Number	Tox 509
Laboratory	Burroughs Wellcome Co., RTP, NC
Study date	June 1992
GLP	Yes
Audited	Yes
Drug	Nelarabine, reference number 91/0927-124-W
Methods	

The investigators diluted Nelarabine with either 5% dextrose in water or in normal saline to a final concentration of 10 mg/ml. They then added various amounts of this to type O positive human blood mixtures (plasma and 50% suspension of washed human erythrocytes in normal

saline). The following table (sponsor's) shows that Nelarabine did not cause protein flocculation or hemolysis at any of the concentrations.

Table 2  
In Vitro Hemolysis and Protein Flocculation Study with 506U78  
In Vitro Protein Flocculation Results

Ratio Solution:	Immediate	Turbidity
<u>Plasma</u>	<u>Visual Flocculation</u>	<u>NTU* at 37 C</u>
	<u>506U78 (10 mg/ml) in 5% Dextrose in Water</u>	
1:4	none	74
1:4	none	74
1:10	none	74
1:10	none	76
	<u>506U78 (10 mg/ml) in 0.9% saline</u>	
1:4	none	68
1:4	none	69
1:10	none	77
1:10	none	75
Plasma Blank/Plasma Blank		80/80

\* NTU = Nephelometric Turbidity Units

### *Safety Pharmacology Summary*

As one would expect Nelarabine is a mild topical irritant but it did not cause skin sensitization on re-challenge. It caused only negligible irritation in the Draize test. Solutions of Nelarabine were not hemolytic and did not cause protein flocculation or increased turbidity at ratios that simulated total doses ranging from 35 to 140 mg/kg.

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## Pharmacokinetics and Toxicokinetics

### 1) An *In Vitro* Investigation into the Inhibition of Human Cytochrome P450 Enzymes by Nelarabine (GI262250X) and GI186898X

#### Major finding

Nelarabine did not inhibit any of the major human cytochrome P450 isoenzymes tested. Cytochromes P450 are probably not involved in the metabolism of Nelarabine.

Study number	CD2004/ 01142/ 00, 04DMM014
Laboratory	GlaxoSmithKline, King of Prussia, PA
Date	March 31, 2004
GLP	No
Audited	No
Drug	Nelarabine, batch #2, chemical purity — GI186898X, batch 1,
Test tissue	pooled human liver microsomes
Concentration range	0, 0.1, 0.33, 1, 3.3, 10, 33, 100 $\mu$ M
Incubation	NADPH regenerating system, 37 C
Probe substrates	appropriate to the cytochrome P450
Positive control inhibitors	appropriate to the cytochrome P450
Detection	LC/MS/MS

The following tables (sponsor's) summarize the results of this experiment:

P450	Inhibition of cytochrome P450 enzymes by 506U78		
	Direct Inhibition IC <sub>50</sub> ( $\mu$ M)	Metabolism-dependent inhibition: IC <sub>50</sub> ( $\mu$ M)	
		Control pre-incubation <sup>1</sup>	NADPH pre-incubation <sup>2</sup>
1A2	> 100	> 100	> 100
2A6	> 100	> 100	> 100
2B6	> 100	> 100	> 100
2C8	> 100	> 100	> 100
2C9	> 100	> 100	> 100
2C19	> 100	> 100	> 100
2D6	> 100	> 100	> 100
3A4 (atorvastatin)	> 100	> 100	> 100
3A4 (midazolam)	> 100	> 100	> 100
3A4 (nifedipine)	> 100	> 100	> 100

P450	Inhibition of cytochrome P450 enzymes by GI186898X		
	Direct Inhibition IC <sub>50</sub> ( $\mu$ M)	Metabolism-dependent inhibition: IC <sub>50</sub> ( $\mu$ M)	
		Control pre-incubation <sup>1</sup>	NADPH pre-incubation <sup>2</sup>
1A2	> 100	> 100	> 100
2A6	> 100	> 100	> 100
2B6	> 100	> 100	> 100
2C8	> 100	> 100	> 100
2C9	> 100	> 100	> 100
2C19	> 100	> 100	> 100
2D6	> 100	> 100	> 100
3A4 (atorvastatin)	> 100	> 100	> 100
3A4 (midazolam)	> 100	> 100	> 100
3A4 (nifedipine)	> 100	> 100	> 100

1. Microsomes, buffer and 506U78 or GI186898X pre-incubated for 20 minutes with probe substrate prior to initiation of reaction with NADPH.
2. Microsomes, buffer and 506U78 or GI186898X pre-incubated for 20 minutes with NADPH prior to initiation of reaction with probe substrate.

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**2) Nelarabine: Pilot developmental toxicity study in non-pregnant rabbits**

Summary

Grp	Plasma AUC ( $\mu\text{M}\cdot\text{h}$ )						Plasma $C_{\text{max}}$ ( $\mu\text{M}$ )					
	506U78			ara-G			506U78			ara-G		
	Day1	Day12	Day16	Day1	Day12	Day16	Day1	Day12	Day16	Day1	Day12	Day16
2	1.74	1.84		15.7	14.0		10.9	10.4		15.2	14.0	
3	4.15	4.33		25.3	25.3		19.8	19.9		26.8	26.0	
4	7.96	7.81	31.7	63.4	63.3	278	54.3	44.6	138	57.3	52.9	202

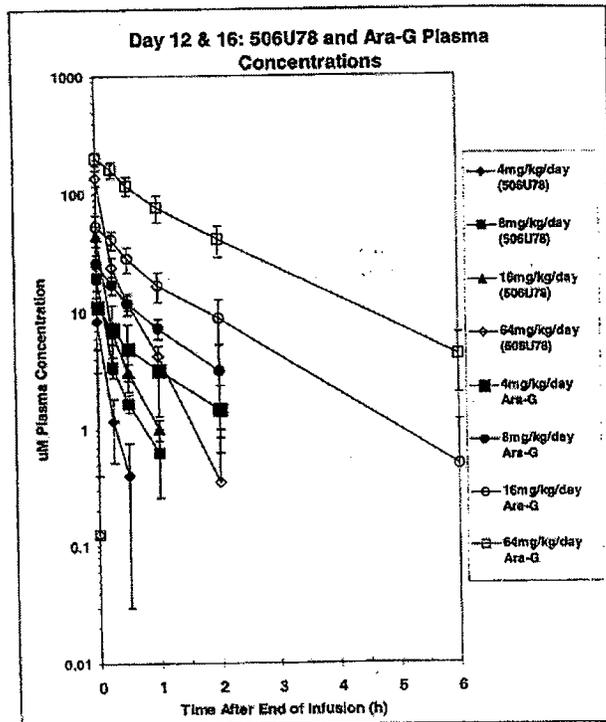
The values above show that most of the drug was rapidly converted to ara-G. On day 1,  $C_{\text{max}}$  and AUC increased linearly with dose. On day 12,  $C_{\text{max}}$  and AUC also increased proportional to the increase in dose. The decrease in both Nelarabine and ara-G concentrations was biphasic. None of these doses caused observable toxicity.

Study Number	L40321, RD1998/00903/00
Laboratory	
Date	December 1998
GLP	Yes
Audited	Yes
Drug	Nelarabine, Batch A97L317
Methods	
Animal	female New Zealand White rabbits
Doses	0, 4, 8, or 16 mg/kg (0, 48, 96, or 192 mg/m <sup>2</sup> ) The doses were too low so the dose in the high dose group was increased to 64 mg/kg (768 mg/m <sup>2</sup> ) days 13–16
N	5
Schedule	Daily days for 16 days
IV	10 or 20 minute infusion
Formulation	0.45% saline
Pharmacokinetics	days 1 and 12 (and 16 in high dose group) Sample times 0, 5, 15, 30 min, 1, 2, 6 hours

See toxicology study of same study number below.

The following graph (sponsor's) shows that the concentration of ara-G was considerably higher than that of the parent drug Nelarabine. The decrease in plasma concentration was biphasic for both compounds.

Figure 2 Day 12 & 16: Average 506U78 and Ara-G Plasma Concentrations\*



\* - only 64mg/kg/day samples were collected on day 16, only 4,8 and 16mg/kg/day samples were collected on Day 12

### 3) Pharmacokinetics of Nelarabine and Ara-G in New Zealand White Rabbits Following a 1 Hour Intravenous Infusion of Nelarabine at 20mg/kg.

Major findings

	AUC <sub>0-∞</sub> μM*hr	C <sub>max</sub> μM	CL L/hr	t <sub>1/2</sub> hr
Nelarabine	6.3	7.7	10.8	0.29
sd	1	1.5	1.9	0.03
ara-G	77	46	0.88	0.87
sd	3.6	2.6	0.4	0.16

Study Number	RD1997 03410/00; 97/APK/0009
Laboratory	GlaxoWellcome, RTP, NC
Date	November 1997
GLP	No
Audited	No
Drug	Nelarabine, Batch not stated
Methods	
Animal	male New Zealand white rabbits
Doses	20 mg/kg (240 mg/m <sup>2</sup> )
N	3
Schedule	single dose

Route IV, one hour, ear vein  
 Formulation 0.45% saline  
 Pharmacokinetics Sample times pre-infusion, 0, 15, 30, 45 min, 1, 2, 4, 6, 8, 12 hours, after the end of the infusion  
 Analysis HPLC, —

I found a problem with this study. In the listings for the individual animal data, the first value, that one labeled 0 time-point and supposedly the first sample after the end of the infusion, is in all cases listed as below the limit of quantitation (bql). The next value at the 15-minute time-point is in each case a reasonable value and a maximum in the curve. I do not understand why the value taken at the end of a one-hour infusion would be bql and that 15 minutes later would be a maximum. The investigators do not address this problem. Including this point would make it very difficult for WinNonLin to regress to a solution.

**4) Pharmacokinetics of Nelarabine and Ara-G in Female Rat Following Intravenous Administration of Nelarabine at 62.5 mg/kg**

Major findings

	AUC <sub>0-∞</sub> μM*hr	C <sub>max</sub> μM	CL L/hr	t <sub>1/2</sub> hr
506U78	97.9	605	2.2	0.18
sd	12.3	78.7	0.3	0.2
ara-G	24	39	9	0.25
sd	3	4	1.3	0.02

Study Number RD 1997/03414/00, 97/APK/0144  
 Laboratory GlaxoWellcome, RTP, NC  
 Date November 1997  
 GLP No  
 Audited No  
 Drug Nelarabine  
 Method  
 Animal Hann Wistar female rats  
 N 5  
 Dose 62.5 mg/kg  
 Route IV cannula, femoral vein  
 Collection Jugular vein  
 0, 0.25, .5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24 hours  
 Analysis HPLC — analysis

**5) Pharmacokinetics of 2-amino-6-methoxypurine arabinoside (Nelarabine) given intravenously to Cynomolgus monkeys**

Major findings

The half-life of Nelarabine is somewhat greater on day 3 than on day 1 but the difference is insignificant, 12.5 and 10.5 minutes, respectively. The plasma half-life for ara-G is short but considerably longer than that of Nelarabine, 1.8 hours and 1.6 hours on days 3 and 1 respectively. Despite the fact that the predose measurement of [ara-G] is more than nine half-lives after the previous dose, the concentration remains more than 1% of  $C_{max}$ . This suggests a longer-term elimination rate constant not detectable by the non-compartmental analysis.  $T_{max}$  for ara-G is rapid, 30 to 45 minutes, and the  $C_{max}$  values for ara-G and Nelarabine are comparable. But, the AUC values for Nelarabine are 7 to 10 times less than those of ara-G, again reflecting the much slower elimination of the latter compound. Nelarabine is a true pro-drug. The average  $C_{max}$  for ara-G was higher on day 3 than on day 1, 68 vs 57  $\mu\text{M}$ , but the difference did not reach significance. Nevertheless, this schedule was short; concentrations did not have time to reach steady-state. I think the study provides a suggestion for some accumulation of ara-G.

The investigators say that "(t)here was no toxicity seen in this study."

Study Number	TEZA/93/0096-1; P221 92/0192-175
Laboratory	Burroughs Wellcome Co, RTP, NC
Date	May 1992
GLP	Yes
Audited	Yes
Drug	Nelarabine, Batch A97L317
Methods	
Animal	male Cynomolgus monkeys
Doses	25 mg/kg BID (300 mg/m <sup>2</sup> /dose, 600 mg/m <sup>2</sup> total)
N	4
Schedule	BID for three days, 6 hours between doses
Route	IV bolus saphenous vein
Formulation	0.45% saline
Pharmacokinetics	Sample times 0, 5, 15, 30, 45 min, 1, 2, 4 hours, immediately before the second dose (6 hr), 5 and 15 minutes after the second dose. This schedule was repeated on day three
Analysis	HPLC, —

The following table (sponsor's) shows the results of this experiment.

Table 2. Plasma pharmacokinetics of 506U78 given i.v. to cynomolgus monkeys. Day 1.

Monkey	C <sub>max</sub> ara-G (µM)	T <sub>max</sub> ara-G (h)	C <sub>max</sub> 506U78 (µM)	ara-G AUC (µM·h)	506U78 AUC (µM·h)	ara-G T <sub>1/2</sub> (h)	506U78 T <sub>1/2</sub> (min)
IM01							
IM02							
IM03							
IM04							
Avg. ±S.D.	57.0±7.9	0.5±0.2	67.9±8.5	155.5±22.4	24.3±7.2	1.8±0.2	10.5±0.6

Table 3. Plasma pharmacokinetics of 506U78 given i.v. to cynomolgus monkeys. Day 3

Monkey	µM ara-G *at predose	C <sub>max</sub> ara-G (µM)	T <sub>max</sub> ara-G (h)	C <sub>max</sub> 506U78 (µM)	ara-G AUC (µM·h)	506U78 AUC (µM·h)	ara-G T <sub>1/2</sub> (h)	506U78 T <sub>1/2</sub> (min)
IM01								
IM02								
IM03								
IM04								
Avg. ±S.D.	0.8±0.2	67.9±8.5	0.6±0.1	82.5±8.5	199.1±21.9	29.4±3.8	1.6±0.4	12.5±2.1

Samples taken predose, at 5, 15, 30, 45 min and 1, 2, 4 h after the first dose. Samples also taken immediately prior to the second dose (approximately 6 h after first dose).

\*18 h after last dose on day 2

The following figure (sponsor's) demonstrates the elimination profiles for Nelarabine and ara-G in two of the monkeys on day 3. Elimination was biphasic for both compounds.

Figure 2 shows the plasma pharmacokinetics of 506U78 and ara-G in monkeys on day 3 after i.v. doses of 25 mg/kg 506U78.



6) ***In Vitro* Investigation of the Potential for Nelarabine (GI262250) and ara-G (GI186898) to Inhibit Human P-glycoprotein Heterologously Expressed in MDCKII Cells**

Major findings

At all tested concentrations, the investigators measured no inhibition of <sup>3</sup>H-digoxin transport by P-glycoprotein by either Nelarabine or ara-G. The data demonstrate that Nelarabine and ara-G, at concentrations up to 100 µM, are not inhibitors of digoxin transport via P-glycoprotein under these assay conditions.

Study number 04DMR020  
 Laboratory GlaxoSmithKline, RTP, NC  
 Date April 2004  
 GLP No  
 Drug Nelarabine, Batch R11341/14/1, purity > —

**7) *In Vitro* Investigation of Both the Transport via Heterologously Expressed Human P-glycoprotein and the Passive Membrane Permeability of Nelarabine (GI262250) and ara-G (GI156898) in MDCKII-MDR1 Cells**

Under the conditions of this experiment, neither <sup>14</sup>C-Nelarabine nor <sup>14</sup>C-ara-G were substrates for human p-glycoprotein. Both compounds have a low passive permeability.

Study number R2004/00140/00, 04DMR013  
 Laboratory GlaxoSmithKline, RTP, NC  
 Date April 2004  
 GLP Not required  
 Drug Nelarabine, Batch R10940/178/8, purity —

**8) *In Vitro* Protein Binding of Nelarabine and GI186898 (ara-G) to Human Plasma Proteins**

Major findings

The following table shows that neither Nelarabine nor ara-G bound appreciably to human plasma. I reconstructed this table from the sponsor's data.

Conc. µM	% bound in pooled human plasma (mean ± SD)		
	506U78	506U78 + dcF	ara-G
6	10 ± 5	14 ± 5	7 ± 7
60	20 ± 15	10 ± 9	12 ± 8
600	7 ± 4	6 ± 5	24 ± 17

The investigators also measured the extent of protein binding of Nelarabine in the presence of deoxycoformycin (dcF, 2.5 µM). dcF is an adenosine deaminase inhibitor used to prevent Nelarabine from converting to ara-G in plasma. dcF did not have an effect on the binding of Nelarabine. The variability in this experiment is unusually large due to small sample size and possibly technique. Nevertheless, the experiment does establish that Nelarabine is not significantly bound to human plasma and that there is no dose relationship associated with its binding.

Study number	RD1998/02142/01, 98/AVT/0013
Laboratory	GlaxoSmithKline, RTP, NC
Date	April 1999
GLP	No
Audited	No
Drug	Nelarabine, lot 92/0950-063-A, —, pure
Methods	
Tissue	Pooled human blood plasma (two men and two women)
Concentrations	6, 60 and 600 $\mu$ M
Time	30 minutes incubation
Separation	ultrafiltration
Detection	HPLC/ —

**8) Studies of the guanine arabinoside (ara-G) prodrug, 2-amino- 6-methoxypurhae arabinoside (Nelarabine), in monkey; pharmacokinetics following 300 mg/kg i.v. dose, and effect of Ketamine anesthetic**

Major finding

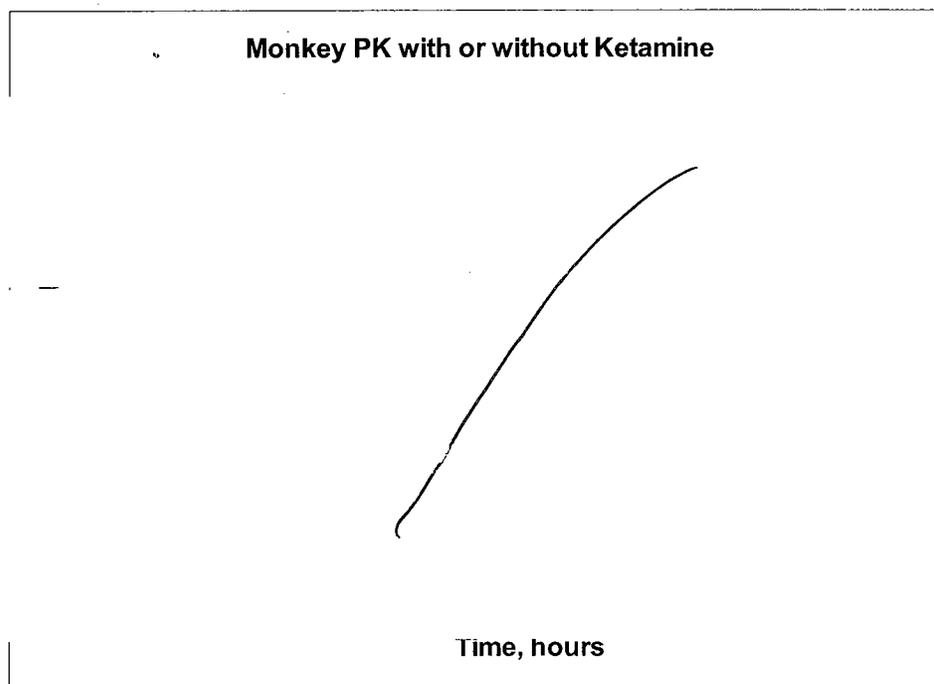
Ketamine anesthesia may slow the conversion of Nelarabine to ara-G in monkeys.

Study	TEZA/93/0115, DRF600, SER579
Laboratory	Burroughs Wellcome Co., RTP, NC
Date	November 1993
GLP	No
Audited	No
Drug	Nelarabine, lot and purity not specified
Animal	Cynomolgus monkey
N	1 with ketamine (10 mg/kg IM prior to dosing), 2 without
Dose	500 mg/kg/d for two days
Route	IV, bolus
Analysis	HPLC, —

In previous studies, monkeys given IV doses of Nelarabine appeared to descend into a deeper state of unconsciousness when they received Nelarabine after ketamine anesthesia. In this study, the investigators attempted to establish whether ketamine anesthesia exacerbated the toxicity of Nelarabine. The following table (mine) shows the major results of this study. The monkey that was given ketamine died after the second of five planned daily doses of Nelarabine. One of the two monkeys not given ketamine anesthesia also died after the second dose but the other survived.

Route		Dose		Nelarabine		ara-G	
		mg/kg	mg/m <sup>2</sup>	AUC <sub>0-∞</sub> μM*hr	t <sub>1/2</sub> hr	AUC <sub>0-∞</sub> μM*hr	t <sub>1/2</sub> hr
IV Bolus no Ketamine	Lethal	500	6000	2030	0.5	7490	3.1
IV Bolus no Ketamine		500	6000	1570	0.3	10000	4.4
IV Bolus with Ketamine	Lethal	500	6000	6290	0.5	5220	2.3

The study failed to determine whether Ketamine affected Nelarabine toxicity because of the small number of monkeys used. Nevertheless, the investigators say “[k]etamine anesthesia did not appear to affect the metabolism of Nelarabine.” I cannot agree with this conclusion. The numbers above and the following graph of [Nelarabine] with time suggest that the Nelarabine may be converted to ara-G more slowly in the presence of ketamine. The concentration of ara-G is lower in the presence of ketamine.



Humans and other species metabolize ketamine HCl by N-dealkylation (metabolite I). The enzymes that accomplish this metabolism are cytochromes P450 3A4 and 2B6 and to a lesser extent 2C9 (Y Hijazy and R Boulieu, *Drug Metab Dispos.* 2002 Jul;**30**(7):853-8.). Other metabolic modifications include hydroxylation of the cyclohexone ring (metabolites III and IV), conjugation with glucuronic acid and dehydration of the hydroxylated metabolites to form the cyclohexene derivative (metabolite II) (Mosby's Drug Consult™ - 15th Ed. (2005). None of these mechanisms would appear to influence with Nelarabine metabolism.

If one considers ara-G the ultimate toxin, one might expect Nelarabine to be less toxic in the presence of ketamine, but monkeys died with or without ketamine anesthesia after the second dose. But, this assumes that the mechanism of toxicity is that postulated for the pharmacological application of ara-G, the substitution into DNA. This toxicity would be slow and would affect

multiple organs causing death several days after the toxic insult. Here the monkeys died hours after the second dose. They descended into a deeper level of unconsciousness, had no palpebral reflex, respiration grew shallow, limbs were flaccid, and jaw tone was minimal (below). The description suggests they died due to CNS depression and respiratory arrest. This is evidently an uncharacterized neurotoxicity distinct from the dose limiting neurotoxicity seen clinically. The rapid progression to death after the second dose is curious. The Nelarabine from the first dose should have been eliminated from the plasma, thus some residual effect must remain at some site high up in the CNS. Some of the symptoms are similar to those seen in Guillain-Barre syndrome, but recovery from Guillain-Barre syndrome usually takes weeks or months. Monkeys that survive the second dose fully recover within a day.

The parent compound, Nelarabine, or ara-G may be phosphorylated and then interfere with some neurotransmitter site that involves ketamine, GTP or both, but I found no direct evidence for this in the literature.

### 9) Pharmacokinetics of 2-amino-6-methoxypurine arabinoside (Nelarabine) given intravenously to dog

#### Major finding

The average half-life of ara-G was  $13.6 \pm 0.4$  minutes after an IV dose of 25 mg/kg of Nelarabine to beagle dogs. The average half-life of Nelarabine was  $2.8 \pm 1$  minutes. Urinary recovery of ara-G plus Nelarabine was 14% of the original dose of Nelarabine. Dogs eliminate both drugs more quickly than rats, monkeys or humans.

Study	TEZA/92/0073/00
Laboratory	Burroughs Wellcome Co., RTP, NC
Date	June 1992
GLP	No
Audited	No
Drug	Nelarabine, lot and purity not specified
Method	
Animal	female beagle dogs
N	4
Dose	25 mg/kg, 10 mg/ml
Route	IV, cannula in the saphenous vein, bolus
Analysis	HPLC. —

#### Results

The following table (sponsor's) shows the results of this experiment.

Table 1. Plasma pharmacokinetics of 506U78 given i.v. to dogs.

Dog	C <sub>max</sub> ara-G (µM)†	C <sub>max</sub> 506U78 (µM)†	ara-G AUC (µM·h)	506U78 AUC (µM·h)	ara-G T <sub>1/2</sub> (min)	% dose in Urine
1206028 (#1)						
1205277 (#2)						
1174479 (#3)						
*1335472 (#4)†						
Average±S.D.	93.4±5.5	8.8±4.2	31.3±4.1	1.5±0.8	13.6±0.4	13.7±1.4

\*Plasma data from dog #4 has been omitted from the averages.

†Maximum plasma concentrations of both ara-G and 506U78 were obtained at the first time point post-dose (5 min) except in dog #4. Dog # 4 was anomalous in having an initial low

This study demonstrated that dogs eliminate both Nelarabine and ara-G very quickly. With these results and others, the sponsor decided that dogs were not an appropriate species for the development of Nelarabine.

## 10) Pharmacokinetic of guanine arabinoside (ara-G) and 1493U89 (5'-O-acetyl-2-amino-6-methoxypurine arabinoside) given orally to B<sub>6</sub>D<sub>2</sub> Mice.

### Major findings

B<sub>6</sub>D<sub>2</sub> mice dosed orally with 100 mg/kg/day of ara-G had peak plasma concentrations of ara-G of about 36-µM and a t<sub>1/2</sub> of 26 minutes. An equivalent dose of Nelarabine gave a peak ara-G plasma concentration about 19 µM with a half-life of about 50 minutes. The peak plasma concentration of Nelarabine was about 16 µM.

Study	TEZA/91/0092
Laboratory	Burroughs Wellcome Co., RTP, NC
Date	July 1991
GLP	No
Audited	No
Drug	Nelarabine, lot and purity not specified
Method	
Animal	B <sub>6</sub> D <sub>2</sub> Mice
N	3 per time point
Dose	100 mg/kg of ara-G PO or 123 mg/kg Nelarabine (molar equivalent of 100 mg/kg ara-G) PO
Route	IP, PO or SC
Analysis	HPLC, —

### Results

Table 1. Comparison of pharmacokinetic parameters in mice dosed with 100 mg/kg/day of ara-G or a molar equivalent dose of 1493U89.

Mice	Compound	*AUC µM·h	T <sub>1/2B</sub> (h)	C <sub>max</sub> (µM)	T <sub>max</sub> (h)
<b>Ara-G</b>					
Average	ara-G	47.1	0.441	36.3	0.5
<b>1493U89</b>					
average	ara-G	38.2	0.84	19.0	0.5
average	506U78	16.4	0.66	14.8	0.25

\* AUC's calculated over 4 hours.

## 11) Pharmacokinetics of Pro-drugs of Guanine Arabinoside (AraG) in Cynomolgus Monkeys

### Major findings

In this exploratory study, monkeys given a low oral dose of Nelarabine of about 28 mg/kg had peak ara-G concentrations of about 3  $\mu$ M,  $T_{max}$  of about two hours, and an elimination half-life of about 2.7 hours. The investigators did not quantify the parent compound.

Study	TEZA/89/0213/00
Laboratory	Burroughs Wellcome Co., RTP, NC
Date	January 1990
GLP	No
Audited	No
Drug	Nelarabine, lot and purity not specified
Method	
Animal	Cynomolgus monkeys
N	2
Dose	94 $\mu$ mol/kg (about 28 mg/kg or 336 mg/m <sup>2</sup> )
Route	Single dose PO
Analysis	HPLC, -

### Results

The following table (sponsor's) shows the results of this study.

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ON ORIGINAL**

PHARMACOKINETICS OF GUANINE ARABINOSIDE IN CYNOMOLGUS MONKEYS AFTER ORAL ADMINISTRATION OF ARA-G PRODRUGS

Compound	Monkey#	Solubility (mM) in PBS at 25°C	AraG Peak Level(µM)	Plasma		AUC 0 to 7 h	Urine	
				Peak Time	T <sub>1/2</sub> (h)		% recovered as AraG	Avg.% araG
AraG	169 170							4.1
AraG Intravenous	169 170							29.8
506U78 2-amino-9-methoxy purine arabinoside	161 165							8.6
1493U89 5'-acetyl-506U78	245 246							20.9
1M88U89 5'-propionyl-506U78	169 170							7
2049U88 5'-butyryl-506U78	165 172							17.4
1492U89 5'-valeryl-506U78	310 311							13.3
1719U88 5'-butyryl-2,6-diamino- purine arabinoside	302 303							17.5

Two cynomolgus monkeys were used for each drug. They were fasted for 8 hours and then given a suspension of drug by oral gavage at a dose of 94 µmol/kg. Blood samples were taken over the following 24 hours and urine was collected over the same 24 hours. Concentrations of araG in plasma extracts or urine were determined by HPLC.

Tox study # SER 391 and 424  
Macfie, Cyno. araG AUC table

12) Plasma Levels of Nelarabine and ara-G Cynomolgus monkeys on Days 3 and 28 of a 30-day Toxicity Study

Major findings

The following table (mine) shows that there is little change in the pharmacokinetics of Nelarabine and ara-C change little with repeat dosing in the monkey. AUC increases linearly with dose.

Day	Dose mg/kg	mg/m <sup>2</sup>	AUC <sub>0-∞</sub> µM*hr	C <sub>max</sub> µM	CL L/kg/hr	t <sub>1/2</sub> hr	T <sub>max</sub>
506U78							
3	10	120	22				
3	20	240	57				
3	40	480	130				
28	10	120	22				
28	20	240	52				
28	40	480	dosing stopped on day 23				
ara-G							
3	10	120	85	25	0.4		0.5
3	20	240	177	51	0.4		0.5
3	40	480	307	93	0.4		0.5
28	10	120	69	24	0.47		0.5
28	20	240	161	51	0.41		0.5
28	40	480	dosing stopped on day 23				

**Study** TEIN/95/0010/01, TOX740  
**Laboratory** GlaxoWellcome Co., RTP, NC  
**Date** September 1996  
**GLP** No  
**Audited** No  
**Drug** Nelarabine, lot and purity not specified  
**Method**  
**Animal** Cynomolgus monkeys  
**N** Control and high-dose groups – 5 male and 5 female  
 Low and mid dose groups – 3 male and 3 female  
**Dose** 0, 10, 20, or 40 mg/kg/day (0, 120, 240, 480 mg/m<sup>2</sup>; 2.5, 5, or 10 mg/ml)  
**Formulation** normal saline  
**Dose volume** 4 ml/kg  
**Route** IV bolus  
**Schedule** daily for 30 days  
**Samples** days 3 and 28, toxicology study reviewed by Dr. Schmidt (see below, study number RD1996/00319/00)  
 Immediately after infusion, 0.5, 1, 3, 8 and 24 hours  
 Dosing was stopped on day 23 on day in the high dose monkeys due to toxicity so there are no day 28 PK results  
**Analysis** HPLC. —

**13) Plasma levels of Nelarabine and ara-G in Cynomolgus Monkeys on days 1 and 5 of a five day toxicity study (ACU 515)**

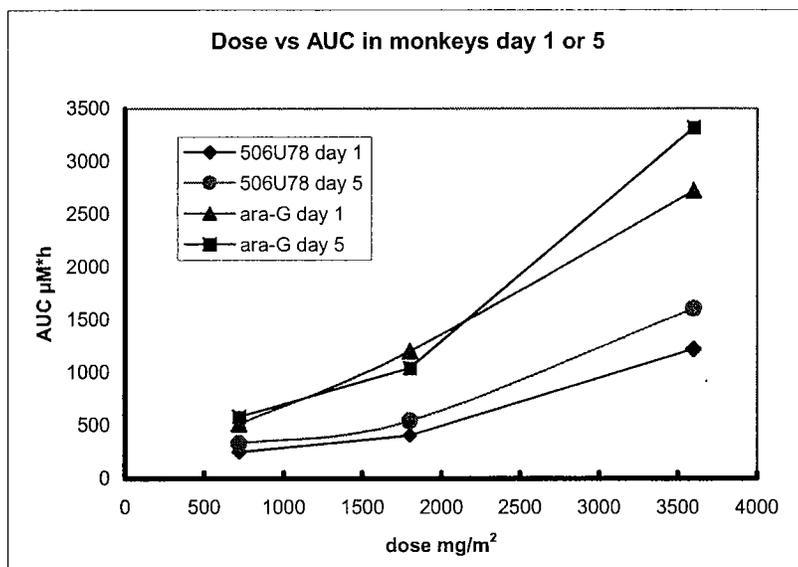
Major findings

The following table (mine) shows that the AUC of neither Nelarabine nor ara-G changed appreciably between day one and five after five days of IV dosing in the monkey. The AUC at the high dose was somewhat higher than a linear model would predict suggesting some saturation of metabolism (see my graph below). The AUC of ara-G was about twice that of Nelarabine.

Day	Dose mg/kg	mg/m <sup>2</sup>	AUC <sub>0-∞</sub> μM*hr	C <sub>max</sub> μM	T <sub>max</sub>
506U78					
1	60	720	248		
1	150	1800	407		
1	300	3600	1223		
5	60	720	330		
5	150	1800	545		
5	300	3600	1608		
ara-G					
1	60	720	513	130	0.5
1	150	1800	1203	288	0.8
1	300	3600	2721	534	1
5	60	720	582	124	0.5
5	150	1800	1042	262	0.9
5	300	3600	3320	495	1.5

Study TEIN/94/0043/01, ACU 515

Laboratory	GlaxoWellcome Co., RTP, NC
Date	November, 1994
GLP	No
Audited	No
Drug	Nelarabine, Lot and purity not specified
Method	
Animal	Cynomolgus monkeys
N	2/sex/dose group
Dose	0, 60, 150, or 300 mg/kg/day (0, 720, 1800, 3600 mg/m <sup>2</sup> )
Formulation	Normal saline, 10 mg/mL
Route	IV bolus
Schedule	daily for five days
Samples	days 1 and 5, toxicology study reviewed by Dr. Schmidt (see below, study number TTEP/94/0087)
	Immediately after infusion, 0.5, 1, 3, 8 and 24 hours
Analysis	HPLC —



**14) An *in vitro* Study to Investigate the Metabolism of [<sup>14</sup>C]-Nelarabine (Nelarabine/ GI262250) and [<sup>14</sup>C]-ara-G (GI186898) in Mouse, Rabbit, Monkey and Human Hepatocytes**

**Major findings**

Human hepatocytes demethylate Nelarabine to ara-G more rapidly than monkey hepatocytes. The conversion to ara-G is only slightly slower in rabbit and mouse hepatocytes than with human, but total conversion is comparable if one includes the formation of allantoin by

these species. Humans do not form appreciable allantoin, but convert ara-G to uric acid more rapidly than any of the species tested.

Study	RD2004/00449/00, 04DMR037
Laboratory	GlaxoSmithKline, RTP, NC
Date	April, 2004
GLP	No
Audited	No
Drug	Nelarabine, lot and purity not stated
Method	
Tissue	human, monkey, mouse, and rabbit hepatocytes
N	duplicate
Formulation	0.1 N HCl
Incubation conc.	150 $\mu$ M
Incubation time	4 and 24 hours
Analysis	HPLC.

The following table (sponsor's) shows the percentages of the various metabolites formed by the different hepatocytes *in vitro*.

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ON ORIGINAL**

**Table 2** Percentage of each Metabolite of [<sup>14</sup>C]-506U78 in Radiochromatograms of Mouse, Rabbit, Monkey and Human Hepatocyte Samples

Peak ID	RT (min) <sup>1</sup>	Percent of Total <sup>14</sup> C (%) in Chromatogram				
		Fresh Human <sup>2</sup>	Cryo Human <sup>3</sup>	Cryo Monkey <sup>3</sup>	Cryo Rabbit <sup>2</sup>	Cryo Mouse <sup>2</sup>
Allantoin	3.4	<0.5	ND <sup>4</sup>	<0.5	1.22	7.78
Uric acid	5.8	12.1	2.20	ND	ND	ND
Xanthine	8.6	<0.5	0.77	ND	0.98	ND
ara-G	10.7	44.4	43.7	11.8	37.2	26.1
506U78	19.3	41.5	52.3	87.2	58.5	64.7
Total		98.0	99.0	99.0	97.9	98.6

1. Retention time is  $\pm$  1 minute.
2. Mouse and rabbit metabolites assignments were inferred by comparison to human and monkey metabolite retention times.
3. Sample fractionated and submitted for structural identification.
4. Not detected.

The following table (sponsor's) shows that human fresh hepatocytes oxidize ara-G to uric acid. Monkeys metabolize ara-G only slowly.

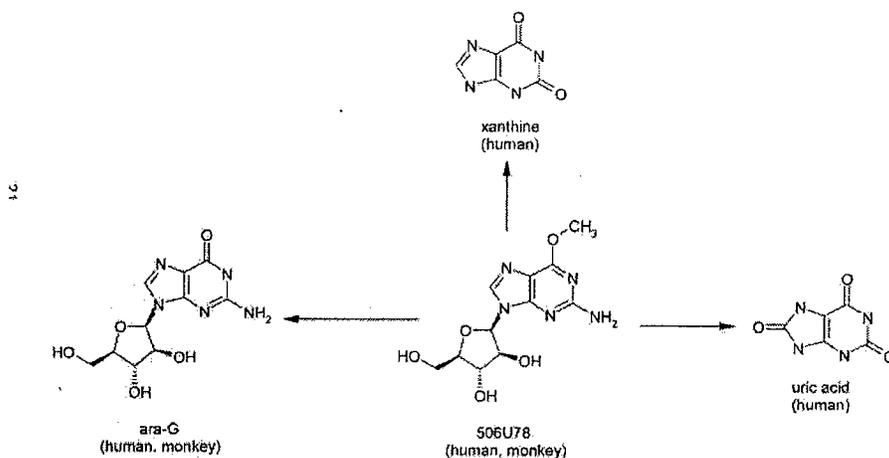
**Table 3** Percentage of each metabolite of [<sup>14</sup>C]-ara-G in Radiochromatograms of Mouse, Rabbit, Monkey and Human Hepatocyte Samples

Peak ID	RT (min) <sup>1</sup>	Percent of Total <sup>14</sup> C (%) in Chromatogram				
		Fresh Human <sup>2</sup>	Cryo Human <sup>2</sup>	Cryo Monkey <sup>2</sup>	Cryo Rabbit <sup>2</sup>	Cryo Mouse <sup>3</sup>
Allantoin	3.3	<0.5	ND <sup>4</sup>	1.43	9.37	87.5
Uric acid	5.8	27.4	5.13	0.57	<0.5	ND
Xanthine	8.6	<0.5	0.61	ND	1.80	ND
ara-G	10.7	70.4	93.5	96.1	85.2	10.8
Total		97.8	99.2	98.1	96.4	98.3

1. Retention time is ± 1 minute.
2. Human, monkey, and rabbit metabolite assignments were inferred by comparison to mouse metabolite retention times for ara-G (where applicable), and to human and monkey metabolite retention times for 506U78 (where applicable).
3. Sample fractionated and submitted for structural identification.
4. Not detected.

The sponsor proposes the following scheme for the metabolism of ara-G metabolism by humans and monkeys. The scheme does not show pathways but only products. One must suppose the conversion of Nelarabine to uric acid involves three steps, O-demethylation, N-oxidation and hydrolysis of the arabanose.

**Figure 1** Proposed Metabolic Scheme for the Identification of Metabolites in Human and Monkey Hepatocytes Administered [<sup>14</sup>C]-506U78



**15) (<sup>14</sup>C)-Nelarabine: Quantitative Tissue Distribution of Test Substance-Related Material Using Whole-Body Autoradiography Following Single intravenous Administration of (<sup>14</sup>C)-Nelarabine (100 mg/kg) to Male and Female B6C3F1 (Pigmented) Mice**

Major findings

After a single dose of radiolabeled Nelarabine (100 mg/kg) pigmented mice showed no clinical signs of toxicity. The drug distributed rapidly to all major tissues. Immediately after the injection and for the first six hours the highest concentrations occurred in the urinary bladder. Concentrations in metabolic and excretory organs were higher than the concentrations in blood at most time points throughout the experiment (35 days). Significant detectable concentrations of radioactivity remained in these organs throughout the experiment suggesting that some of the radioactivity was incorporated into structural biomolecules. Concentrations in the CNS were lower than the concentrations in blood throughout the experiment. The concentration in brain was less than three percent that of blood at the first time point (immediately after the end of the infusion). The concentration in brown fat, gonads, secretory organs, endocrine organs, muscle, gastric mucosa marrow, pancreas, spleen and the contents of the GI tract were approximately equal to those in blood throughout the experiment. The concentrations in white fat were somewhat higher than those found in blood at time points past 1 hour. There were no differences between the sexes.

Study	RD2004/00135/00, 1990/436
Laboratory	
Date	February, 2004
GLP	Yes
Audited	Yes
Drug	<sup>14</sup> C-Nelarabine, Batch number R10940/178/8 6.96 MBq/mg (188.1 µCi/mg), 2082 MBq/mmol (56.28 mCi/ mmol) radiochemical purity —, Nelarabine, Batch BU44733 purity not stated
Method	
Animal	male and female G6C3F1/ — pigmented mice
N	10 per sex, one per sex per time point
Dose	100 mg/kg (300 mg/m <sup>2</sup> )
Formulation	0.45% w/v saline, 10 mg/mL
Route	IV 10 minutes
Schedule	daily for five days
Samples	0, 1, 3, 6 and 12 hours and 1, 7, 14, 28 and 35 days
Analysis	whole body autoradiography

The following tables (sponsor's) show the results of this experiment.

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ON ORIGINAL**



**Table 1, continued. Concentrations of Test Substance-Related Radioactivity in Tissues of Male Pigmented Mice at Various Times After a Single Intravenous Administration of (<sup>14</sup>C)-506U78 at 100 mg/kg (group A)**

Tissue type	Tissue	Animal Number and Sex	µg equivalents of 506U78/g of tissue																			
			Sampling Time	0 h	1 h	3 h	6 h	12 h	1 d	7 d	14 d	28 d	35 d									
Muscular	Muscle																					
	Myocardium																					
	Tongue																					
Ocular	Lens																					
	Uveal tract																					
Unclassified	Bone marrow																					
	Lung																					
	Pancreas																					
	Pigmented skin																					
	Spleen																					
	Tooth pulp																					
	Nasal mucosa																					
	Gastrointestinal	Oesophagus																				
Stomach mucosa																						
Small intestine mucosa																						
Caecum mucosa																						
Large intestine mucosa																						
Rectum mucosa																						
Stomach contents																						
Small intestine contents																						
Caecum contents																						
Large intestine contents																						
Rectum contents																						

Upper Limit of Quantification = — µg equiv/g  
 Lower Limit of Quantification = / µg equiv/g

BLQ - Tissue radioactivity concentration below the lower limit of quantification  
 NS - Tissue not sectioned

**APPEARS THIS WAY  
ON ORIGINAL**

**Table 2. Concentrations of Test Substance-Related Radioactivity in Tissues of Female Pigmented Mice at Various Times After a Single Intravenous Administration of (<sup>14</sup>C)-506U78 at 100 mg/kg (group B)**

Tissue type	Tissue	Animal Number and Sex	µg equivalents of 506U78/g of tissue														
			Sampling Time	0 h	1 h	3 h	6 h	12 h	1 d	7 d	14 d	28 d	35 d				
Vascular/ lymphatic	Blood																
	Aorta																
	Mandibular lymph nodes																
Metabolic/ excretory	Gall bladder																
	Kidney cortex																
	Kidney medulla																
	Kidney pyramid																
	Liver																
	Urinary bladder (contents)																
	Urinary bladder (wall)																
CNS	Brain																
	Choroid plexus																
	Meninges																
	Pineal body																
	Spinal cord																
Endocrine	Adrenal cortex																
	Adrenal medulla																
	Pituitary																
	Thymus																
	Thyroid																
Secretory	Harderian gland																
	Intra-orbital lachrymal gland																
	Salivary gland																
Fatty	Brown fat																
	White fat																
Gonads	Ovary																
	Uterus																
	Clitoris																

Upper Limit of Quantification = — µg equiv/g  
 Lower Limit of Quantification = — µg equiv/g

BLQ - Tissue concentration below lower limit of quantification  
 NS - Tissue not sectioned

**APPEARS THIS WAY  
ON ORIGINAL**

**Table 2, continued. Concentrations of Test Substance-Related Radioactivity in Tissues of Female Pigmented Mice at Various Times After a Single Intravenous Administration of (<sup>14</sup>C)-506U78 at 100 mg/kg (group B)**

Tissue type	Tissue	Animal Number and Sex	µg equivalents of 506U78/g of tissue														
			Sampling Time	0 h	1 h	3 h	6 h	12 h	1 d	7 d	14 d	28 d	35 d				
Muscular	Muscle																
	Myocardium																
	Tongue																
Ocular	Lens																
	Uveal tract																
Unclassified	Bone marrow																
	Lung																
	Pancreas																
	Pigmented skin																
	Spleen																
	Tooth pulp																
	Nasal mucosa																
	Gastrointestinal	Oesophagus															
Stomach mucosa																	
Small intestine mucosa																	
Caecum mucosa																	
Large intestine mucosa																	
Rectum mucosa																	
Stomach contents																	
Small intestine contents																	
Caecum contents																	
Large intestine contents																	
Rectum contents																	

Upper Limit of Quantification = — µg equiv/g  
 Lower Limit of Quantification = / µg equiv/g

BLQ - Tissue radioactivity concentration below the lower limit of quantification  
 NS - Tissue not sectioned

**16) Elimination of Radioactivity Following a Single Intravenous (100 mg/kg) Administration of [<sup>14</sup>C] Nelarabine (GI262250) to Male and Female Intact and Male Bile Duct-Cannulated Cynomolgus Monkeys**

**Major findings**

Cynomolgus monkeys eliminate most of an IV dose of Nelarabine in the urine (>62%). Most of the dose was eliminated within 24 hours. The monkeys excreted less than one percent of total radioactivity in the bile (96 hours) and less than 2% in the feces (240 hours). Less than 80 % of the radiolabel was recovered after 10 days from the intact animals suggesting biological incorporation. The investigators did not collect samples of expired air.

Study RD2004/00267/00, 7274- 512  
 Laboratory  
 Date April 2004  
 GLP Yes

Audited	Yes
Drug	<sup>14</sup> C-Nelarabine, Batch No. R10940/178/8, 188 µCi/mg, — radiochemical purity Nelarabine, Batch No. BU4473RB, purity —
Method	
Animal	Cynomolgus monkeys
N	Group 1 = 3 intact females and three intact males, Group 2 = 3 bile duct cannulated (BDC) males
Dose	100 mg/kg (1200 mg/m <sup>2</sup> ), 50 µCi/kg
Formulation	0.9% w/v saline, 10 mg/mL
Route	IV 2 minutes
Schedule	single dose
Samples	From Group 1, urine was collected pre-dose, 0-6 h, 6-12 h, 12-24 h and at 24 h intervals up to 240 h post-dose. Feces and cage rinse was collected pre-dose at 24 hours intervals. Blood was collected pre- dose and at 0.5 h, 2 h, 6 h, 24 h, 168 h and 240 h post-dose From Group 2, bile and urine were collected pre-dose and at 0-6 h, 6- 12 h, 12- 24 h and at 24 h intervals up to 96 h post-dose. Feces and cage wash was collected daily. Blood was collected at 96 h.
Analysis	Liquid scintillation counting

Additional samples of blood, plasma, urine, feces, and bile were frozen and stored for further analysis (metabolic profiling). See the following study, [RD2004/00784/00](#).

#### Results

Dosing caused no mortality. Two group one females had low food consumption at 48 hours. One of these animals suffered emesis 24 hours post dosing and abnormal feces at 72 and 168 hours. All other animals appeared normal and healthy throughout the experiment.

The following table (sponsor's) shows that monkeys excrete most of a radioactive dose in the urine (>62%) over the course of this experiment. They excreted less than two percent in the feces or feces and bile combined. The small amount of compound in the feces of cannulated animals is probably due to sloughing in the GI tract. The intact animals eliminated only about 80% of the compound 10 days after dosing, suggesting biological incorporation of the drug or its metabolites. Nevertheless, the experiment did not test for respiratory elimination.

**APPEARS THIS WAY  
ON ORIGINAL**

**Table 2 Total Mean Recovery of Radioactivity Following an Intravenous Administration of [<sup>14</sup>C]506U78 (100 mg/kg) to Male and Female Intact and Male Bile Duct-Cannulated Monkeys**

Matrix	Percent of Administered Dose		
	Group		
	Intact Males	Intact Females	BDC Males
Urine	66.9 ± 11.6	62.5 ± 13.6	71.4 ± 12.4
Feces	1.01 ± 0.73	1.54 ± 0.87	0.77 ± 0.20
Bile	-	-	0.84 ± 0.04
Cage Pan/Screen Rinse	8.51 ± 1.41	13.3 ± 6.17	5.55 ± 3.04
Cage Debris	1.19 ± 0.44	0.54 ± 0.29	1.05 ± 0.99
Cage Wash	0.31 ± 0.16	0.52 ± 0.27	1.19 ± 0.68
Cage Wipe	1.66 ± 1.92	0.98 ± 0.94	2.01 ± 1.11
Bile Cannula	-	-	0.02 <sup>a</sup>
Jacket Rinse	-	-	0.20 ± 0.12
<b>Total</b>	<b>79.5 ± 8.15</b>	<b>79.4 ± 6.62</b>	<b>83.0 ± 6.66</b>

Notes: Values are the mean ± standard deviation (n=3).

- Not determined

BDC Bile duct-cannulated

a: At least one value was below the limit of quantitation, therefore, the standard deviation was not calculated.

The following table (sponsor's) shows the time course of elimination from urine and feces by the intact animals. Most of the compound was eliminated within 24 hours.

**Table 3 Mean Recovery of Radioactivity in Urine and Feces During Each Collection Interval Following an Intravenous Administration of [<sup>14</sup>C]506U78 (100 mg/kg) to Male and Female Intact Monkeys**

Collection Interval (h)	Percent of Administered Dose			
	Male		Female	
	Urine	Feces	Urine	Feces
0-6	43.5 ± 14.0	-	34.4 ± 20.5	-
6-12	8.10 ± 3.46	-	12.1 ± 3.28	-
12-24	8.65 ± 2.10	-	5.15 ± 2.65	-
0-24	-	0.39 ± 0.42	-	0.74 <sup>b</sup>
24-48	3.97 ± 1.81	0.29 ± 0.12	3.61 ± 1.30	0.30 ± 0.09
48-72	1.14 ± 0.45	0.14 ± 0.06	3.13 ± 2.61	0.16 ± 0.08
72-96	0.51 ± 0.18	0.06 ± 0.06	2.07 ± 1.60	0.07 ± 0.02
96-120	0.23 ± 0.05	0.05 ± 0.04	0.35 ± 0.14	0.07 ± 0.04
120-144	0.22 ± 0.09	0.02 ± 0.01	0.45 ± 0.37	0.05 ± 0.04
144-168	0.18 ± 0.08	0.02 ± 0.02	0.38 ± 0.20	0.04 ± 0.02
168-192	0.15 ± 0.09	0.01 ± 0.02	0.18 ± 0.02	0.04 ± 0.02
192-216	0.10 ± 0.03	0.01 ± 0.01	0.31 ± 0.31	0.04 ± 0.04
216-240	0.12 ± 0.08	0.01 <sup>a</sup>	0.16 ± 0.07	0.02 ± 0.02
<b>Total</b>	<b>66.9 ± 11.6</b>	<b>1.01 ± 0.73</b>	<b>62.5 ± 13.6</b>	<b>1.54 ± 0.87</b>

Note: Values are the mean ± standard deviation (n=3).

- Not determined

a: At least one value was below the limit of quantitation, therefore, the standard deviation was not calculated.

b: N=2; one animal did not have a sample at this timepoint.

The following table (sponsor's) shows that bile cannulated animals also eliminated most of the radioactivity within 24 hours.

**Table 4 Mean Recovery of Radioactivity in Urine, Feces and Bile During Each Collection Interval Following an Intravenous Administration of [<sup>14</sup>C]506U78 (100 mg/kg) to Male Bile Duct-Cannulated Monkeys**

Collection Interval (h)	Percent of Administered Dose		
	Urine	Feces	Bile
0-6	44.7 ± 17.8	-	0.81 ± 0.04
6-12	11.8 ± 2.57	-	0.02 ± 0.01
12-24	6.06 ± 3.59	-	0.01 <sup>a</sup>
0-24	-	0.45 ± 0.20	-
24-48	5.16 ± 2.58	0.19 ± 0.06	0.00 <sup>a</sup>
48-72	2.56 ± 2.61	0.08 ± 0.02	0.00 <sup>a</sup>
72-96	1.13 ± 0.66	0.04 ± 0.02	0.00 <sup>a</sup>
Total	71.4 ± 12.4	0.77 ± 0.20	0.84 ± 0.04

Notes: Values are the mean ± standard deviation (n=3).  
 Values of zero represent <0.005% of the administered dose.  
 - Not determined  
 a: At least one value was below the limit of quantitation, therefore, the standard deviation was not calculated.

**17) Quantitative Metabolic Profiling and Metabolite Identification of 506U7S (GI262250/Nelarabine) in Male and Female Intact and Male Bile Duct-Cannulated Cynomolgus Monkeys after a Single Intravenous Administration of [<sup>14</sup>C]-Nelarabine at 100 mg/kg**

Major findings

Monkeys rapidly hydrolyze Nelarabine to ara-G; the parent compound was undetectable in plasma within two hours. They further metabolize ara-G to xanthine and then allantoin, and much of this metabolism is probably extrahepatic. Significant amounts of the radiolabel are incorporated into biological molecules within 24 hours. Respiration is probably not a major route of elimination.

Study RD2004/00784/00, 04DMR052  
 Laboratory GlaxoSmithKline, RTP, NC  
 Date July 2004  
 GLP No  
 Audited No

All the samples in this experiment came from the previous experiment, RD2004/00267/00, reviewed above. — sent the samples to the sponsor for further analysis after the end of the experiment. See above for the experimental parameters.

Methods Liquid scintillation counting and HPLC

Results

The following tables (sponsor's) show that monkeys rapidly convert most of the given dose to ara-G, the parent compound is not detectable after 2 hours. The monkeys further convert some of the dose to allantoin. Xanthine is a minor metabolite in the urine (<2%) and feces (<0.07%).

**Table 1** Concentration ( $\mu\text{g eq/g}$ ) of 506U78 and Metabolites in Plasma Collected from Male and Female Cynomolgus Monkeys after Intravenous Administration of [ $^{14}\text{C}$ ]506U78 at 100 mg/kg

Peak ID	RT (min)	% Radioactivity <sup>1</sup>					
		$(\mu\text{g eq/g})$					
		Male			Female		
		0.5 h	2 h	6 h	0.5 h	2 h	6 h
Allantoin	3.3	1.56 (1.19)	8.24 (2.92)	25.8 (1.54)	2.17 (1.89)	9.09 (3.78)	25.5 (2.06)
Ara-G	10.6	60.9 (46.5)	76.7 (27.2)	68.3 (4.07)	47.3 (41.3)	79.1 (32.9)	70.6 (5.70)
506U78	19.3	32.8 (25.1)	ND	ND	43.6 (38.0)	2.01 (0.84)	ND
Total		95.3 (72.8)	84.9 (30.1)	94.1 (5.61)	93.1 (81.2)	90.2 (37.5)	96.1 (7.76)

1. Plasma was pooled from 3 animals per time point.  
ND = Not detected

**Table 2** Mean Percent of Dose Excreted as 506U78 and Metabolites in Urine, Feces and Bile from Intact Male and Female, and BDC Male Cynomolgus Monkeys after Intravenous Administration of [ $^{14}\text{C}$ ]506U78 at 100 mg/kg

Peak ID	RT (min)	Mean % of Dose <sup>1</sup>									
		Male			Female			BDC Male			
		Urine <sup>2</sup>	Feces <sup>3</sup>	Total	Urine <sup>2</sup>	Feces <sup>3</sup>	Total	Urine <sup>2</sup>	Feces <sup>3</sup>	Bile <sup>4</sup>	Total
Allantoin	3.4	2.57	0.01	2.68	3.70	ND	3.70	3.31	ND	0.02	3.33
Xanthine	8.6	1.35	0.07	1.42	1.99	0.05	2.04	2.26	0.04	ND	2.30
Ara-G	10.7	49.4	0.20	49.6	44.9	0.64	45.5	59.9	0.13	0.19	60.2
506U78	19.3	11.5	0.02	11.5	7.67	0.09	7.76	3.74	ND	0.40	4.14
Total		64.9	0.30	65.2	58.3	0.78	59.0	69.2	0.17	0.61	70.9

1. Mean of 3 cynomolgus monkeys each for intact males and females, and BDC males.  
2. Urine samples for each cynomolgus monkey were pooled from urine collected from 0 to 96 hours post-dose.  
3. Fecal samples were from 0 to 24 hours, or 24 to 48 hours  
4. Bile samples were from 0 to 6 hours  
ND = Peak not detected

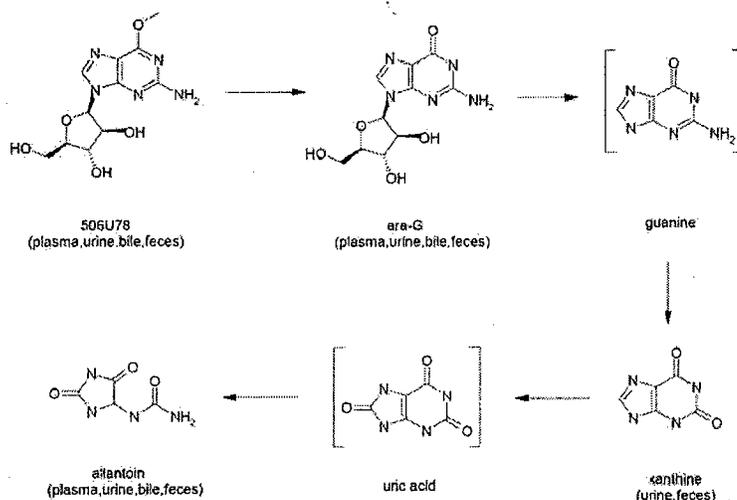
The following table (sponsor's) shows that through the first six hours of the experiment, most of the radiolabel found in the plasma remained extractable. At 24 hours and later, much of the dose was not extractable, strongly suggesting that some of the radiolabel is incorporated into biological molecules.

**Table 3 Nonextractability of Radioactivity from Pooled Male and Female Plasma from Cynomolgus Monkeys Following Intravenous Administration of [<sup>14</sup>C]-506U78 at 100 mg/kg**

	nmole equivalents/g (%)									
	Male					Female				
	0.5 h	2 h	6 h	24 h	168 h	0.5 h	2 h	6 h	24 h	168 h
Extractable	257 (96.1%)	119 (93.6%)	21.2 (95.6%)	1.10 (52.9%)	BQL	294 (92.9%)	140 (91.2%)	29.2 (92.2%)	1.23 (29.7%)	BQL
Non-Extractable	ND	ND	0.42 (1.90%)	0.60 (28.7%)	0.40 (70.2%)	ND	ND	1.87 (5.90%)	2.15 (51.9%)	1.39 (67.5%)
Total	257 (96.1%)	119 (93.6%)	21.6 (97.7%)	1.70 (81.6%)	0.40 (70.2%)	294 (92.9%)	140 (91.2%)	31.1 (98.1%)	3.38 (81.6%)	1.39 (67.5%)

1. Plasma is pooled from three cynomolgus monkeys per sex per time point.  
 ND = Not determined. Greater than 90% extractability was obtained with initial extraction using 0.1% formic acid in methanol.  
 BQL = Below quantitation limit.

The sponsor suggested the following metabolic pathway for Nelarabine in the monkey. The radiolabeled carbon is not removed in this sequence suggesting that respiratory elimination is probably minimal.



**18) Collection of Plasma and Excreta Samples for the Determination of the Metabolite Profiles of [<sup>14</sup>C] Nelarabine ( GI262250) Following a Single Intravenous Administration to a Male Cynomolgus Monkey**

**Major findings**

This dose was not toxic. — did the dosing and sampling in this study then sent the samples to GlaxoSmithKline for analysis. These are only the experimental parameters of the study. GlaxoSmithKline reported the results in study RD2004/00370/00, the review of which follows immediately.

Study RD2004/00244/00, 7274- 540  
 Laboratory

Date	March 2004
GLP	No
Audited	No
Drug	<sup>14</sup> C-Nelarabine, Batch No. R10940/178/8, 188 µCi/mg, > — radiochemical purity Nelarabine, Batch No. BU4473RB, purity —
Method	
Animal	Cynomolgus monkey
N	1 male
Dose	2 mg/kg (24 mg/m <sup>2</sup> ), 86 µCi/kg
Formulation	0.9% w/v saline
Route	IV 1.5 minutes
Schedule	single dose
Samples	Blood, predose, 1, 30 minutes, 2, 24, and 48 hours Urine, 0-6, 6-12, 12-24, and 24-48 hours Feces, 0-24, 24-48
Analysis	HPLC. —

**19) Quantitative Metabolic Profiling and Metabolite Identification of 506U7S (Nelarabine GI262250) in a Male Cynomolgus Monkey after a Single Intravenous Administration of [<sup>14</sup>C]-Nelarabine at 2 mg/kg**

Major findings

Here again the monkey rapidly metabolizes Nelarabine to ara-G and thence to guanine, xanthine, and allantoin. These latter three compounds are rapidly excreted and are found in appreciable concentrations only in the urine.

Study	RD2004/00370/00, 04DMR030
Laboratory	GlaxoSmithKline, RTP, NC
Date	April 2004
GLP	No
Audited	No

— dosed one male monkey and took urine, feces, and blood samples. They sent these samples for analysis to GlaxoSmithKline. For the parameters of this experiment see previous study report, RD2004-00244-00, reviewed above.

Results

The monkey suffered no ill effects from this small dose. The following table (sponsor's) shows that this monkey had excreted 42.7% of the radiolabel in the urine after 48 hours.

**Table 1** Percent of Dose Recovered in Urine of a Male Cynomolgus Monkey after Intravenous Administration of [<sup>14</sup>C]-506U78 at 2 mg/kg

Collection Period (h)	% of Dose Recovered
0-6	
6-12	
12-24	
24-48	
Total	

The following table (sponsor's) shows that the radiolabel was eliminated rapidly from the plasma.

**Table 2** Total Concentration (µg eq/L) of 506U78 and Metabolites in Plasma of a Male Cynomolgus Monkey after Intravenous Administration of [<sup>14</sup>C]-506U78 at 2 mg/kg

Time	Plasma Conc (µg eq/mL)
1 min	
0.5 h	
2 h	
24 h	
48 h	

The following table (sponsor's) shows that the monkey rapidly converted Nelarabine to ara-G in the plasma.

**Table 3** Summary of the HPLC Analysis of 506U78 and Its Metabolites in Plasma Collected from a Male Cynomolgus Monkey after Intravenous Administration of [<sup>14</sup>C]-506U78 at 2 mg/kg

Peak ID	RT (min)	% Radioactivity (µg eq/mL)		
		1 min	0.5 h	2 h
Ara-G	8.2			
506U78	16.4			
Total				

ND = Not Detected

The following table (sponsor's) shows the metabolic profile in the urine. As in the previous experiment ara-G is the major metabolite and it is further converted to xanthine, guanine and allantoin.

**Table 4** Percentage of Dose Excreted as 506U78 and Metabolites in Urine from a Male Cynomolgus Monkey after Intravenous Administration of [<sup>14</sup>C]-506U78 at 2 mg/kg

Peak ID	RT (min)	% of Dose
allantoin		
guanine		
xanthine		
ara-G		
506U78		
Total		

**20) Elimination of Radioactivity Following a Single Intravenous ( 100 mg/ kg) Administration of [ 14C] Nelarabine ( GI262250) to Male and Female Intact and Male Bile Duct- Cannulated Mice**

Major findings

Like monkeys, mice eliminate most of a dose of Nelarabine in the urine (>60%). Less than 6% of a radiolabeled dose is found in the feces and less than 2% is recovered in the bile. Most of the radiolabel is cleared from the plasma within 24 hours.

Study	RD2004/00241/00, 7274-478
Laboratory	
Date	March 2004
GLP	No
Audited	No
Drug	[ <sup>14</sup> C]Nelarabine (GI262250A; Batch No. R10940/178/8), radiochemical purity — , 188 µCi/mg
Method	
Animal	CD-1 intact females and intact males (Groups 1 and 3), 5 bile duct cannulated (BDC) males (Group 2)
Dose	100 mg/kg (300 mg/m <sup>2</sup> )
Formulation	0.9% w/v saline, 10 mg/mL
Route	IV
Schedule	single dose
Samples	Urine and feces – 24 hour intervals, four per sex (Group 1) Urine, feces and blood – see tables below (Group 2 BCD mice) Blood – 0.5, 3 and 24 hours post dosing, 3 per sex per time point Liver and blood all groups at termination
Analysis	Liquid scintillation counting

Results

The following table (sponsor's) shows that mice eliminate over 60% of the radiolabel in the urine. They eliminate 3 to 6% in the feces and less than 2% in the bile. Unlike the monkey

most of the radiolabel was eventually recovered. Tables 3 and 4 (sponsor's, below) show that most of the dose was eliminated within 24 hours in both intact and bile duct cannulated mice.

**Table 2 Total Mean Recovery of Radioactivity Following an Intravenous Administration of [<sup>14</sup>C]506U78 (100 mg/kg) to Male and Female Intact and Male Bile Duct-Cannulated Mice**

Matrix	Percent of Administered Dose		
	Group		
	Intact Males	Intact Females	BDC Males
Urine	77.8 ± 10.2	62.3 ± 22.9	72.0 ± 19.4
Feces	3.51 ± 0.87	4.15 ± 1.94	5.47 ± 7.29
Bile	-	-	1.72 ± 1.27
Cage Rinse	4.25 ± 2.17	17.1 ± 19.3	2.08 ± 1.78
Cage Wash	0.83 ± 0.47	1.60 ± 1.17	4.11 ± 6.44
Cage Wipe	3.15 ± 2.59	7.18 ± 3.33	3.24 ± 2.17
Carcass	4.40 ± 0.96	4.08 ± 0.27	6.75 ± 0.35
Bile Cannula	-	-	0.00 <sup>a</sup>
Jacket Rinse	-	-	0.00 <sup>a</sup>
Total	93.9 ± 9.19	96.4 ± 5.98	95.4 ± 8.69

Notes: Values are the mean ± standard deviation (n=4 for intact and n=5 for BDC).

Values of zero represent <0.005% of the dose.

- Not determined

BDC: Bile duct-cannulated

a: All values were below the limit of quantitation; therefore, the standard deviation was not reported.

**Table 3 Mean Recovery of Radioactivity in Urine and Feces During Each Collection Interval Following an Intravenous Administration of [<sup>14</sup>C]506U78 (100 mg/kg) to Male and Female Intact Mice**

Collection Interval (h)	Percent of Administered Dose			
	Male		Female	
	Urine	Feces	Urine	Feces
0-24	73.0 ± 11.5	2.61 ± 0.76	56.5 ± 25.2	3.11 ± 1.81
24-48	2.95 ± 1.01	0.49 ± 0.13	3.85 ± 2.17	0.50 ± 0.09
48-72	1.07 ± 0.24	0.26 ± 0.04	1.21 ± 0.48	0.38 ± 0.10
72-96	0.79 ± 0.39	0.15 ± 0.05	0.72 ± 0.17	0.16 ± 0.02
Total	77.8 ± 10.2	3.51 ± 0.87	62.3 ± 22.9	4.15 ± 1.94

Notes: Values are the mean ± standard deviation (n=4).

**Table 4 Mean Recovery of Radioactivity in Urine, Feces and Bile During Each Collection Interval Following an Intravenous Administration of [<sup>14</sup>C]506U78 (100 mg/kg) to Male Bile Duct-Cannulated Mice**

Collection Interval (h)	Percent of Administered Dose		
	Male		
	Urine	Feces	Bile
0-12	-	-	1.70 ± 1.27
0-24	70.4 ± 19.3	4.96 ± 6.91	-
12-24	-	-	0.01 ± 0.00
24-48	1.56 ± 0.30	0.51 ± 0.41	0.01 ± 0.00
Total	72.0 ± 19.4	5.47 ± 7.29	1.72 ± 1.27

Notes: Values are the mean ± standard deviation (n=5).

- Not determined

The following table (sponsor's) shows that concentrations decline rapidly in blood and plasma but significantly more slowly in the liver.

**Table 5 Pooled Blood, Plasma and Liver Concentrations of Radioactivity and Blood to Plasma and Liver to Blood Concentration Ratios at Selected Times Following an Intravenous Administration of [<sup>14</sup>C]506U78 (100 mg/kg) to Male and Female Intact and Male Bile Duct-Cannulated Mice**

Matrix	Sampling Time (h)	Group		
		Intact Males	Intact Females	BDC Males
		Concentration of Radioactivity (µg equiv 506U78/g)		
Blood	0.5	47.1	55.4	-
	3	7.53	17.9	-
	24	1.19	1.03	-
	48	-	-	0.714
	96	0.612	0.478	-
Plasma	0.5	55.9	64.1	-
	3	8.12	20.1	-
	24	0.987	0.937	-
	48	-	-	0.189
	96	0.103	0.097	-
Liver	0.5	83.0	92.1	-
	3	27.3	40.6	-
	24	30.7	42.7	-
Ratio				
Blood:Plasma	0.5	0.844	0.863	-
	3	0.928	0.890	-
	24	1.20	1.10	-
	48	-	-	3.78
	96	5.95	4.91	-
Liver:Blood	0.5	1.76	1.66	-
	3	3.62	2.27	-
	24	25.9	41.4	-

Note: Values are for pooled samples for each sex at the given time point (n=10 for intact animals at 0.5 h, 3 h and 24 h post-dose; n=4 for intact animals at 96 h post-dose; and n=5 for BDC animals).

- Not determined

BDC Bile duct-cannulated

**21) Quantitative Metabolic Profiling and Metabolite Identification of Nelarabine (GI262250/Nelarabine) in Male and Female Intact and Male Bile Duct-Cannulated Mice after a Single Intravenous Administration of [<sup>14</sup>C]-Nelarabine at 100 mg/kg**

Major finding

As with the monkey, — did the in-life portion of this study and sent the samples to GlaxoSmithKline for analysis. See the previous study for the experimental parameters of this study.

As with the monkey, mice rapidly convert the parent compound to ara-G. Unlike monkeys they convert most of the dose to uric acid and allantoin within 3 hours (>88%). This is probably why mice are not a good experimental model for this drug.

Study RD2004/00450/01, 04DMR038  
 Laboratory GlaxoSmithKline  
 Date April  
 GLP No  
 Audited No

The following tables (sponsor's) show the results of this experiment.

**Table 1 Summary of the HPLC Analysis of 506U78 and Its Metabolites in Plasma Collected from Male and Female Mice after Intravenous Administration of [<sup>14</sup>C]-506U78 at 100 mg/kg**

Peak ID	RT (min)	% Radioactivity † (µg eq/g)					
		Male			Female		
		0.5 h	3 h	24 h	0.5 h	3 h	24 h
Allantoin	3.3	43.5 (23.6)	88.5 (6.63)	76.3 (0.54)	44.9 (27.6)	91.9 (17.3)	83.0 (0.63)
Uric acid	6.0	9.49 (5.14)	3.85 (0.29)	6.10 (<0.1)	9.92 (6.10)	2.47 (0.46)	10.4 (<0.1)
Ara-G	10.6	31.4 (17.0)	6.44 (0.48)	9.54 (<0.1)	31.1 (19.1)	5.31 (1.00)	4.12 (<0.1)
506U78	19.3	15.3 (8.28)	ND	ND	13.9 (8.53)	ND	ND
Total		99.7 (54.0)	98.8 (7.40)	91.9 (0.62)	99.8 (61.4)	99.7 (18.8)	97.5 (0.74)

† 10 mice per sex per time point.  
 ND = Not detected.

**Table 2 Mean Percent of Dose Excreted as 506U78 and Metabolites in Urine, Feces and Bile from Intact Male and Female, and BDC Male Mice after Intravenous Administration of [<sup>14</sup>C]-506U78 at 100 mg/kg**

Peak ID	RT (min)	Mean % of Dose <sup>1</sup>									
		Male <sup>2</sup>			Female <sup>2</sup>			BDC Male <sup>3</sup>			
		Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Bile	Total
Allantoin	3.4	24.1	0.08	24.2	19.6	0.46	20.1	19.8	1.00	0.06	20.9
Uric acid	5.2	1.43	0.05	1.48	1.21	0.09	1.30	2.15	ND <sup>4</sup>	ND <sup>4</sup>	2.15
Guanine	8.1	0.20	0.18	0.38	0.03	0.10	0.13	0.29	0.13	ND <sup>4</sup>	0.42
Xanthine	8.5	0.96	0.63	1.59	0.28	0.32	0.60	0.59	0.56	ND <sup>4</sup>	1.15
Ara-G	10.6	21.7	0.30	22.0	19.2	0.53	19.7	22.5	1.42	0.06	24.0
Methyl-guanine	14.2	ND <sup>4</sup>	0.28	0.28	ND <sup>4</sup>	0.24	0.24	ND <sup>4</sup>	ND <sup>4</sup>	0.16	0.16
506U78	19.3	26.7	0.06	26.8	21.5	0.35	21.9	27.9	1.81	1.32	31.0
Total		75.1	1.58	76.7	61.8	2.09	63.9	73.2	4.92	1.60	79.7

1. Mean of 4 mice each for intact male and females, and 5 mice for BDC males.
2. Urine samples for each mouse were pooled from urine collected from 0 to 72 hour post-dose. Fecal samples were from 0 to 24 hour.
3. Urine samples for each mouse were pooled from urine collected from 0 to 48 hour post-dose. Fecal samples were from 0 to 24 hour. Bile was from 0 to 12 hour.
4. Peak not detected.

**Table 3 Nonextractability of Radioactivity from Pooled Male and Female Mouse Plasma Following Intravenous Administration of [<sup>14</sup>C]-506U78 at 100 mg/kg**

	nmole equivalents [ <sup>14</sup> C]-506U78/g (%)					
	Male			Female		
	0.5 h	3 h	24 h	0.5 h	3 h	24 h
Extractable	182 (96.9%)	25.2 (92.3%)	2.80 (83.3%)	207 (95.9%)	63.4 (93.6%)	2.68 (88.3%)
Non-Extractable	ND	ND	0.42 (12.6%)	ND	ND	0.21 (7.0%)
Total	182 (96.9%)	25.2 (92.3%)	3.23 (95.9%)	207 (95.9%)	63.4 (93.6%)	2.89 (95.3%)

n = 10 mice per sex per time point.  
 ND = Not determined where >90% extractability was obtained.

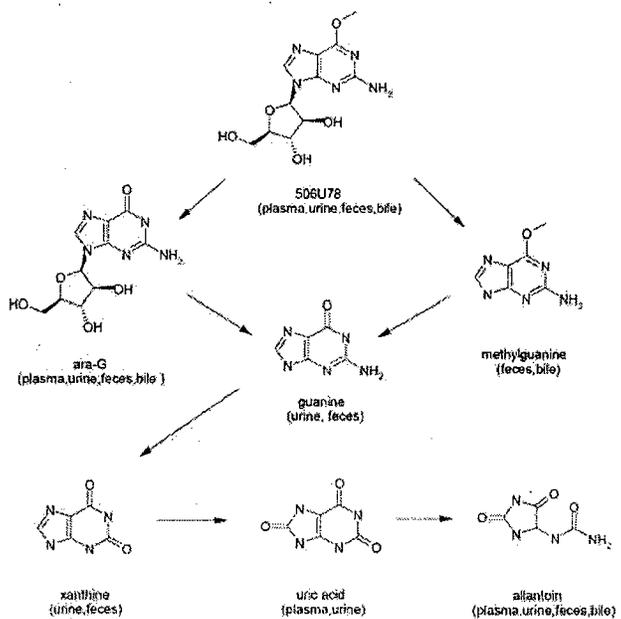
**Table 4 Nonextractability of Radioactivity from Pooled Male and Female Mouse Liver Following Intravenous Administration of [<sup>14</sup>C]-506U78 at 100 mg/kg**

	nmole equivalents [ <sup>14</sup> C]-506U78/g (%)					
	Male			Female		
	0.5 h	3 h	24 h	0.5 h	3 h	24 h
Extractable	917 (99.1%)	148 (73.3%)	58.9 (19.4%)	889 (102%)	207 (78.2%)	87.9 (20.5%)
Non-Extractable	42.6 (4.60%)	53.6 (26.0%)	226 (74.5%)	38.5 (4.40%)	58.0 (21.9%)	291 (67.9%)
Total	959 (104%)	201 (99.9%)	285 (93.9%)	927 (106%)	265 (100%)	379 (88.4%)

n = 10 mice per sex per time point.

The sponsor proposed the following metabolic pathway for the elimination of Nelarabine in the mouse.

Figure 6. Proposed Metabolic Pathways for [<sup>14</sup>C]-506U78 in Mice



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**Pharmacokinetic and Toxicokinetics Summary**

**Nelarabine**

Study	Species	Route	Toxicity	Dose mg/kg	mg/m <sup>2</sup>	AUC <sub>0-∞</sub> μM*hr	C <sub>max</sub> μM	CL L/hr	t <sub>1/2</sub> hr
RD1997/03410/00 97/APK/0009	Rabbit	1 hour infusion		20	240	6.3	7.7	10.8	0.29
L40321, RD1998/00903/00	Rabbit	10-20 min infusion day 1	none	4	48	1.74	10.9		
		day 1	none	8	96	4.15	19.8		
		day 1	none	16	192	7.96	54.3		
		day 12	none	4	48	1.84	10.4		
		day 12	none	8	96	4.33	19.9		
		day 12	none	16	192	7.81	44.6		
RD 1997/03414/00 97/APK/0144	Rat	IV bolus		62.5	375	97.9	605	2.2	0.18
TEZA/91/0092	mouse	PO		123	369	16.4	14.8		0.66
TEZA/92/0073/00	Dog	IV bolus		25	500	1.5	8.8		0.05
DRF600, SER579	Monkey	IV Bolus no Ketamine	Lethal	500	6000	2030			0.5
		IV Bolus no Ketamine		500	6000	1570			0.3
		IV Bolus with Ketamine	Lethal	500	6000	6290			0.5
TEZA/93/0096-1; P221 92/0192-175	Monkey	IV BID for 3 days - day 1	none	25	300	24	67		0.18
		IV BID for 3 days - day 3	none	25	300	29	82		0.21
TEZA/89/0213/00	Monkey	single dose PO	none	28	336	ND	ND		ND
TEIN/94/0043/01, ACU 515	Monkey	IV daily for five days day 1		60	720	248			
		day 1		150	1800	407			
		day 1		300	3600	1223			
		day 5		60	720	330			
		day 5		150	1800	545			
		day 5		300	3600	1608			
TEIN/95/0010/01 Day 3 parameters	Monkey	IV daily for 30 days		10	120	22			
				20	240	57			
				40	480	130			
TEIN/95/0010/01 Day 28 parameters	Monkey	IV daily for 30 days		10	120	22			
				20	240	52			
		dosing stopped day 23	Toxic, dosing stopped d23	40	480				

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ara-C

Study	Species	Route	Toxicity	Dose		AUC <sub>0-∞</sub> μM*hr	C <sub>max</sub> μM	CL L/hr	t <sub>1/2</sub> hr	T <sub>max</sub> hr
				mg/kg	mg/m <sup>2</sup>					
RD1997/03410/00 97/APK/0009	Rabbit	1 hour infusion		20	240	77	46	0.88	0.87	
L40321, RD1998/00903/00	Rabbit	10-20 min infusion day 1	none	4	48	15.7	15.2			
		day 1	none	8	96	25.3	26.8			
		day 1	none	16	192	63.4	57.3			
		day 12	none	4	48	14	14			
		day 12	none	8	96	25.3	26			
		day 12	none	16	192	63.3	53			
RD 1997/03414/00 97/APK/0144	Rat	IV bolus		62.5	375	24	39	9	0.25	
TEZA/91/0092	mouse	PO		123	369	38	19		0.84	0.5
TEZA/92/0073/00	Dog	IV bolus		25	500	31.3	93.4		0.23	
TEZA/89/0213/00	Monkey	single dose PO	none	28	336	12	3		2.7	2
DRF600, SER579	Monkey	IV Bolus no Ketamine	Lethal	500	6000	7490			3.1	
		IV Bolus no Ketamine		500	6000	10000			4.4	
		IV Bolus with Ketamine	Lethal	500	6000	5220			2.3	
TEZA/93/0096-1; P221 92/0192-175	Monkey	IV BID for 3 days - day 1	none	25	300	155	57		1.8	0.5
		IV BID for 3 days - day 3	none	25	300	199	67.9		1.6	0.6
TEIN/94/0043/01, ACU 515	Monkey	IV daily for five days day 1		60	720	513	130			0.5
		day 1		150	1800	1203	288			0.8
		day 1		300	3600	2721	534			1
		day 5		60	720	582	124			0.5
		day 5		150	1800	1042	262			0.9
		day 5		300	3600	3320	495			1.5
TEIN/95/0010/01 Day 3 parameters	Monkey	IV daily for 30 days		10	120	85	25	0.4 *		0.5
				20	240	177	51	0.4 *		0.5
				40	480	307	93	0.4 *		0.5
TEIN/95/0010/01 Day 28 parameters	Monkey	IV daily for 30 days		10	120	69	24	0.47 *		0.5
				20	240	161	51	0.41 *		0.5
			Toxic, dosing stopped d23	40	480					

\* Units in L/kg/h

Only 7 to 20% of a dose of Nelarabine is bound to human plasma and binding did not increase with concentration over a range of 6 to 600 μM. Binding of ara-G increased from 7 to 24% over this same concentration range.

Nelarabine did not inhibit any major human cytochrome P450 isoenzyme. Cytochromes P450 are probably not involved in the metabolism of Nelarabine. Human hepatocytes demethylate Nelarabine to ara-G more rapidly than monkey hepatocytes. The conversion to ara-G is only slightly slower in rabbit and mouse hepatocytes than with human, but total conversion is comparable if one includes the formation of allantoin by these species. Humans do not form appreciable allantoin, but convert ara-G to uric acid more rapidly than any of the other species tested. Nelarabine did not inhibit the P-glycoprotein transporter nor was it a substrate.

All species rapidly convert Nelarabine to ara-G after IV dosing. Plasma ara-G concentrations were consistently greater than Nelarabine concentrations. In the rabbit, the ara-G AUC was 7 to 10 fold higher than that of Nelarabine; AUC and C<sub>max</sub> for both compounds increased linearly with dose and did not change significantly with repeated dosing. The decrease in plasma concentration of both compounds was biphasic. In the rat, the AUC of ara-G was about 6 times greater than that of Nelarabine. The half-life of Nelarabine was only about 12 minutes while that of ara-G was only about 15 minutes, so the rat is not a good species for predicting human toxicity. Likewise, the average half-life of ara-G was 14 minutes after an IV dose of 25 mg/kg of Nelarabine to beagle dogs. The average half-life of Nelarabine was 3

minutes. Dogs eliminate both drugs more quickly than rats, monkeys or humans. Mice dosed orally with 100 mg/kg/day of ara-G had peak plasma concentrations of ara-G of about 36- $\mu$ M and a  $t_{1/2}$  of 26 minutes. An equivalent dose of Nelarabine gave a peak ara-G plasma concentration about 19  $\mu$ M with a half-life of about 50 minutes. Since all these species eliminate ara-G much more quickly than humans do, the sponsor chose to do the major toxicology studies for the development of Nelarabine in the monkey. Unfortunately, they did not do a comprehensive study of pharmacokinetics in the monkey. Based on limited information, single doses of 300 mg/kg or less in the monkey cause little toxicity but higher doses appear to cause some saturation of metabolism. The increase in AUC becomes greater than dose normal.

After a single dose of radiolabeled Nelarabine (100 mg/kg) the drug distributed to all major tissues in pigmented mice. Immediately after the injection and for the first six hours the highest concentrations occurred in the urinary bladder. Concentrations in metabolic and excretory organs were usually higher than the concentrations in blood. Significant detectable concentrations of radioactivity remained in these organs throughout the experiment (240 hrs) suggesting that some of the radioactivity was incorporated into structural biomolecules. Concentrations in the brain and CNS were lower than the concentrations in blood. The concentrations in brown fat, gonads, secretory organs, endocrine organs, muscle, gastric mucosa, marrow, pancreas, spleen and the contents of the GI tract were approximately equal to those in blood. There were no differences between the sexes.

Cynomolgus monkeys, the most tested species, eliminate most of an IV dose of Nelarabine in the urine (>62%). The urine contained little radioactivity after 24 hours. The monkeys excreted less than one percent of total radioactivity in the bile and less than 2% in the feces. Less than 80% of the radiolabel was recovered after 10 days from the intact animals suggesting biological incorporation. Monkeys rapidly hydrolyze Nelarabine to ara-G; the parent compound was undetectable in plasma within two hours. They further metabolize ara-G to xanthine and then allantoin, and much of this metabolism is probably extrahepatic. Significant amounts of the radiolabel are incorporated into biological molecules within 24 hours. Respiration is probably not a major route of elimination.

As with the monkey, mice rapidly convert the parent compound to ara-G. Unlike monkeys they convert most of the dose to uric acid and allantoin within 3 hours (>88%). This is probably why mice are not a good experimental model for this drug.

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## Toxicology

### *Single dose*

#### **1) An Intravenous Dose Range Finding Study with Nelarabine in Cynomolgus monkeys**

##### Major finding

Investigators at GlaxoWellcome gave a single dose of 100, 200, 300, 400 or 500 mg/kg (1200, 2400, 3600, 4800 or 6000 mg/m<sup>2</sup>) of nelarabine to one female monkey at each dose level. They anesthetized the monkeys with ketamine before dosing. Doses of 300 mg/kg or less caused no observable effects. The two higher doses caused significantly deeper anesthesia but the monkeys recovered and were clinically normal within 24 hours.

Study number	RD1997-1903-00, 474A-402-020-92
Research facility	—
Study Date	October 26, 1992
GLP	No
Audited	No
Drug	Nelarabine, lot and purity not specified

### *Multiple dose*

#### **1) An Acute Five Day Intravenous Toxicity and Pharmacokinetic Study in a Cynomolgus monkey with Nelarabine**

##### Major finding

Investigators gave one female Cynomolgus monkey an IV (15 min) dose of 500 mg/kg (6000 mg/m<sup>2</sup>) of Nelarabine for two consecutive days. They anesthetized the monkey before dosing with ketamine. The first dose caused no treatment related clinically detectable symptoms, but after the second dose the monkey's state of anesthesia deepened. The monkey died within four hours after the second dose.

Study number	RD1997/02378/00, DRF 600
Research facility	—
Study Date	November 2, 1992
GLP	No
Audited	No
Drug	Nelarabine, lot and purity not specified

## 2) An intravenous toxicity study in a Cynomolgus monkey with Nelarabine

### Major finding

Investigators gave one female Cynomolgus monkey a single IV (11 min) dose of 400 mg/kg (4800 mg/m<sup>2</sup>) of Nelarabine. They anesthetized the monkey before dosing with ketamine. Very near the end of dosing, the monkey had no palpebral reflex, respiration grew shallow, limbs were flaccid, and jaw tone was minimal. The monkey died within five hours after this single dose. The investigators had intended this to be a daily for five days dosing study.

The investigators modified the protocol to dose another single monkey daily for four days. This monkey received 300 mg/kg IV (10 min). This monkey suffered some emesis and deepening of anesthesia but recovered within four to five hours after each dose.

Study number	TTDR-92-0042, 474E-405-501-92
Research facility	_____
Study Date	November 13, 1992
GLP	No
Audited	No
Drug	Nelarabine, lot and purity not specified

## 3) Nelarabine: Pilot developmental toxicity study in non-pregnant rabbits

### Summary

The doses (48, 96, or 192 mg/m<sup>2</sup>) in this ranging experiment were too low to cause toxicity in the rabbit. There were no signs of weight loss, hematological changes, clinical observations or gross pathology.

Study Number	L40321, RD1998/00903/00
Laboratory	
Date	December 1998
GLP	Yes
Audited	Yes
Drug	Nelarabine, Batch A97L317
Methods	
Animal	female New Zealand White rabbits
Doses	0, 4, 8, or 16 mg/kg (0, 48, 96, or 192 mg/m <sup>2</sup> ) The doses were too low so the dose in the high dose group was increased to 64 mg/kg (768 mg/m <sup>2</sup> ) days 13-16
Schedule	Daily days for 16 days
Route	IV, marginal ear vein, 10 or 20 minute infusion
Formulation	0.45% saline
Pharmacokinetics	days 1 and 12 (and 16 in high dose group) see above
Parameters	clinical signs, body weight, food consumption, hematology, gross pathology

**4) Nelarabine: An intravenous dose toxicity study in non-pregnant New Zealand white rabbits.**

Major findings

Doses of 64 or 128 mg/kg/d for 13 days caused no significant toxicity in female rabbits.

Study Number CD2004/00040/00, D03323, Project 900252  
 Laboratory GlaxoSmithKline, Ontario, Canada  
 Date November, 2003  
 GLP No  
 Audited No  
 Drug Nelarabine, lot C049439, purity not specified  
 Methods  
     Animal female New Zealand White rabbits  
     Doses 64 and 128 mg/kg (768 and 1536 mg/m<sub>2</sub>/d)  
     N 4  
     Schedule Daily days for 13 days  
     Route IV marginal ear vein, 20 to 40 minute infusion, 38.4 mL/kg/hr  
     Formulation 0.45% saline  
     Pharmacokinetics not done  
     Observations clinical signs, body weight, food consumption, gross pathology (d14)

The following table (sponsor's) shows the results of this experiment.

Daily Dose (mg/kg)	64 (Group 1)	128 (Group 2)
Noteworthy findings	Slight tremors were seen during dosing in % Group 1 animals.  There were no effects on body weights or food consumption.	There were no treatment-related clinical signs and no effects on body weights or food consumption.

**5) Nelarabine: Intravenous dose toxicity and toxicokinetic study in non-pregnant New Zealand white rabbits.**

Major findings

Doses of 300 mg/kg/d (8-hour infusion) or 360 mg/kg/d (24 hours infusion) given to rabbits daily for 14 days caused no mortality, but the rabbits lost a significant amount of body weight (6% and 11% respectively). There were dose dependent decreases in WBC, lymphocytes, neutrophils, RBCs, Hbg, Hct and reticulocytes.

Study Number CD2004/00497/00, D04089  
 Laboratory  
 Date April 7 2004  
 GLP No  
 Audited No

Drug	Nelarabine, lot C049441, purity not specified
Methods	
Animal	female New Zealand White rabbits, 27 wks old, 3.3 to 3.9 kg
Doses	300 mg/kg/d (3600 mg/m <sup>2</sup> /d) 8 hour infusion = group 1 360 mg/kg/d (4320 mg/m <sup>2</sup> /d) 24 hour infusion = group 2
N	4
Schedule	Daily days for 13 days
Route	IV marginal ear vein, 7.5 mL/kg/h group 1, 3 mL/kg/h group 2
Formulation	0.45% saline, 5 mg/mL
Pharmacokinetics	not done
Observations	clinical signs, body weight, food consumption, gross pathology (d14)

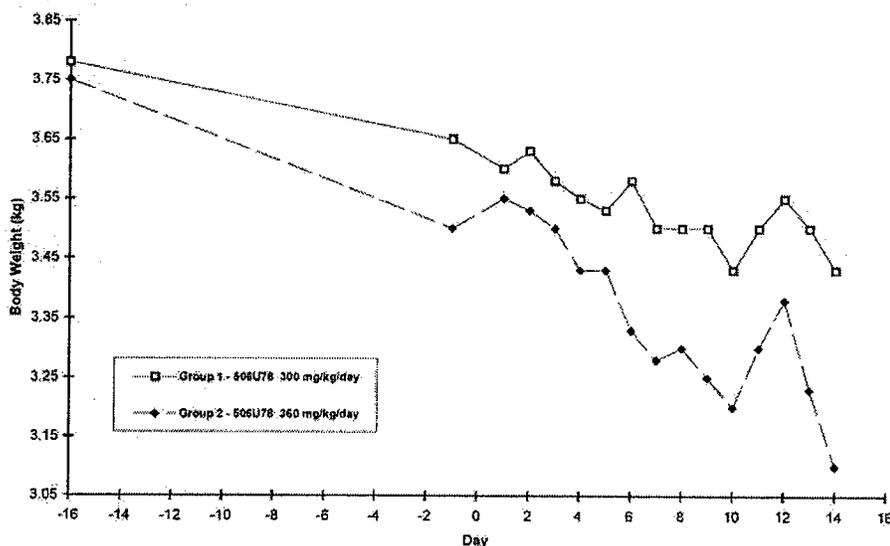
The previous study showed no toxicity at any of the tested doses so this study is really a continuation of that study at higher doses. Both studies were designed to determine appropriate doses for the reproductive toxicology study below.

The following table (sponsor's) shows that at the C<sub>max</sub> in the rabbits of group 1 was 2.3 times higher than that seen in humans at the recommended dose (14 µM) and that the AUC was 7.5 times higher than that seen in humans (23 µM\*h). But, the C<sub>max</sub> and AUC for ara-G were only 1.1 and 1.5 times higher than those seen in humans at the recommended dose (111 µM and 579 µM\*h).

	506U78		ara-G	
	AUC <sub>(0-24)</sub> (h.µM)	C <sub>max</sub> (µM)	AUC <sub>(0-24)</sub> (h.µM)	C <sub>max</sub> (µM)
300 mg/kg/8 hr/day	174	31.9	859	126
360 mg/kg/24 hr. /day	369	16.3	1608	72.7

Observation	Time	Group 1	Group 2
Mortality		None	None
Clinical Signs	twice daily	Decreased appetite, decreased feces	Decreased appetite, decreased feces
Body weight	daily	-6.3%	-11.4%
Food consumption	daily	significantly decreased	severely decreased particularly toward the end of the study
Hematology	Pre-dose and end of study		
WBC		-29%	-57%
Lymphocyte		-42%	-53%
Neutrophil			-68%
RBC		-21%	-17%
Hbg		-19%	-20%
Hct		-19%	-20%
Reticulocyte		-42%	-99%
Gross Pathology		None	None

The following graph (sponsor's) demonstrates the weight loss in these animals. Note that there was significant weight loss between randomization and the start of dosing. In the table above I have compared the weight loss to day -1.



### Toxicology Summary

With the exception of the neurotoxicity, Nelarabine causes a spectrum of toxicities that one would expect with a purine antimetabolite. In mice the LD<sub>10</sub> for dailyX5 dosing is about 1500 mg/m<sup>2</sup>; this dose is associated with shallow breathing, decreased activity and tremor. Consistent with their rapid elimination of the drug dogs tolerated single doses of 7200 mg/m<sup>2</sup> with no toxicity. In rabbits given daily doses for 13 days (CIV) the MTD was 4320 mg/m<sup>2</sup>. This dose was associated with reduced appetite and decreased fecal output, decreased total body weight (~11%), decreased white and red cell parameters.

Monkeys tolerated single IV doses of 3600 mg/m<sup>2</sup> with few signs of toxicity. As mentioned above higher doses caused fatal neurotoxicity in some individuals. Monkeys that died from this toxicity showed loss of palpebral reflex, shallow respiration, flaccid limbs, and minimal jaw tone. The monkeys that did not recover died hours after the first or second dose. Monkeys tolerated five daily doses of 1800 mg/m<sup>2</sup> showing dose dependent decreases in WBC and platelets and a decrease in thymus weight. At this schedule, a dose of 3600 mg/m<sup>2</sup> killed 3 of 4 monkeys by day 13. Moribund monkeys had tremors and convulsions. Ketamine anesthesia time increased on day 5. All monkeys at this dose had tremors and convulsions. Body weight decreased as did WBC and platelets decreased. AST and ALT increased 2 to 12 fold. BUN, triglycerides and glucose increased. Microscopic damage was found in proliferative tissues, GI, spleen, marrow, thymus. There was congestion in the lungs.

In an IV study with dailyX30 dosing, monkeys tolerated doses of only 120 mg/m<sup>2</sup> with decreased red and white cell parameters. 240 mg/m<sup>2</sup> killed one of six monkeys on day 28. A dose of 480 mg/m<sup>2</sup> killed 3 of 10 monkeys despite the cessation of dosing on day 23. Red and white cell parameters decrease significantly. Significant damage was seen in the brain (cerebellar degeneration, perivascular cuffing in the cerebrum and cerebellum) and the spine (vacuolization and myelopathy, not reversible in surviving monkeys). Changes in clinical chemistry parameters were suggestive of liver damage. There was lymphoid infiltration in the bladder, kidney, trachea, salivary gland, lacrimal gland and heart. In females, there was myocardial degeneration and liver damage (cholangitis, edema and vacuolization).

TTEP/83/0033/00 ACU 507	mouse	single dose IV	6/sex	0	0	none	Decreased activity for the first hour following dosing. Ptosis observed in males	
				300	900	none		
				400	1200	none		
				500	1500	none		
				600	1800	none		
TTEP/84/0015/00 ACU 509	mouse	IV dailyX5	6/sex	0	0		shallow breathing, decreased activity, coolness to touch, body tremors and ptosis and persisted following dosing for up to 5 hours. Decreased body weight gain.	
				200	600			
				300	900			
				400	1200 NOEL	1 F d13		
				500	1500 -LD10	2 F, d9 d12		
RD1997/01966/02 ACU 512	Dog	Single dose IV	1	80	1800	none	non-toxic	
				180	3600	none	non-toxic	
				360	7200	none	non-toxic	
RD1998/00903/00	Rabbit	IV, daily for 16 days	4	0	0		None	
				4	4	48	None	
				4	8	96	None	
				4	16	182 NOEL	None	
CD2004/00040/00	Rabbit	IV dailyX13 days	4	64	768		Slight tremor during dosing in 3/4 animals	
				4	128	1536	None	
CD2004/00497/00	Rabbit	dailyX13 days, 8 hour IV	300	3600	none	none	Reduced appetite in 3/4 animals, decreased fecal output in 2/4 females. Decreased food consumption (< 70 g/d) starting Day 12. Females lost about 6% body weight. Decreased WBC, Lymphocytes, Reticulocytes, RBC, Hbg, and Hct.	
		dailyX13 days, CIV					360	4320 MTD
None	Monkey	IV dailyX5	2/sex	0	0	none	none	
Under Ketamine anesthesia				60	720	none	Muscle twitching, ataxia in females, decreased body weight, visual disturbance, decreased red cell parameters, decreased WBC and platelets, increased AST, ALT, BUN, Bil	
RD1997-1903-00 Under Ketamine anesthesia	Monkey	Single IV dose	1	100	1200	None	No effect	
				1	200	2400	None	No effect
				1	300	3600	None	No effect
				1	400	4800	None	deepening anesthesia, recovered
				1	500	6000 MTD	None	deepening anesthesia, recovered
RD1997/02378/00 Under Ketamine anesthesia	Monkey	IV 15 minute, dailyX5	1	500	6000 Lethal	Lethal after first dose	The state of ketamine anesthesia deepened after the first dose but the monkey recovered. The monkey died within four hours after the second dose.	
TTDR-92-0042 Under Ketamine anesthesia	Monkey	IV 15 minute, dailyX5	1	300	3600	Lethal after first dose	This monkey suffered some emesis and deepening of anesthesia but recovered within four to five hours after each dose. Very near the end of dosing, the monkey had no palpebral reflex, respiration grew shallow, limbs were flaccid, and jaw tone was minimal. The monkey died within five hours after this single dose.	
TTEP/84/0087 ACU 515	Monkey	IV 15 minute, dailyX5	2/sex 2/sex	0 150	0 1800		Dose dependent decrease in WBC and Pli. Decreased thymus wt.	
Under Ketamine anesthesia				300	3600 Lethal	3/4 days 6 to 13	Moribund monkeys had tremors and convulsions. Ketamine anesthesia time increased on day 5. Surviving monkey also had tremors and convulsions. Decreased body weight. Dose dependent decrease in WBC and Pli. Increased AST and ALT (2 to 12 fold). Increases	
RD1996/00319/00	Monkey	IV dailyX30	5/sex	0	0		Decreased red cell parameters, WBC, increased Pli. One female had an axillary mass (lymphoid hyperplasia and inflammation), deteriorating condition, tremor, seizure. Decreased body wt gain. Decreased red cell parameters, WBC, increased Pli.	
Under Ketamine anesthesia		dosing stopped day 23	5/sex	40	480 Lethal	2 M, 1 F d23 to 28	Deteriorating condition, tremor, seizure. Decreased food consumption. Weight loss. Decreased red cell parameters, WBC, increased Pli. Cerebellar degeneration. Perivascular cuffing in the cerebrum and cerebellum. In the spine vacuolization and myelomat	
				RD1997/0403/00	monkey	IV dailyX30	2/sex	0
		dose fludara mg/m2 Fludara given days 1, 3, 5, 7, 9, 13		0	200	2400		severe convulsions, hunched posture, tremors, hypoactivity, emesis after dosing, and ataxia. Increased AST and ALT.
				60	0	0		
				60	50	600		
				60	100	1200	1M 1F d15-d19	severe convulsions, hunched posture, tremors, hypoactivity, emesis after dosing, and ataxia. Increased AST and ALT.
				60	200	2400	1M 1F d15-d19	severe convulsions, hunched posture, tremors, hypoactivity, emesis after dosing, and ataxia. Increased AST and ALT.

## Genotoxicity

### 1) Mouse Lymphoma mutagenicity report (non-GLP)

#### Major finding

In this preliminary study, Nelarabine was mutagenic in the mouse lymphoma assay in the absence of metabolic activation. The investigators did not do the test with metabolic activation. The effect was dose dependent with a maximum increase in mutations of 10 fold at a

concentration of 5000 µg/ml. The highest concentration tested caused a decrease in relative total growth of between 80 and 90%. The highest concentration tested did not precipitate.

Study number	V40346, report RD1998/01791/00
Laboratory	Glaxo Wellcome Inc.
Study date	February 1998
GLP	No
Audited	No
Drug	Nelarabine, MBR027/01/0ZA, Purity unknown
Methods	
Test system	L5178Ytk+/- mouse lymphoma cells
Concentrations	0, 25, 50, 100, 250, 500, 1000, 2500, 5000 µg/mL
Schedule	3-hour exposure
Formulation	DMSO
Positive controls	None

## 2) Nelarabine: L5178Ytk+/- mouse lymphoma mutagenesis study

### Major finding

In the absence of metabolic activation, Nelarabine caused a clear dose dependent increase in mutation frequency in mouse lymphoma cells *in vitro*. The highest concentration tested, 5000 mg/mL, caused a 10-fold increase in mutations while causing an 86% decrease in relative growth. In the presence of metabolic activation, Nelarabine caused a four-fold increase in mutation frequency with an 89% decrease relative growth. This suggests that the S9 preparation does not activate the compound but instead metabolizes it to less mutagenic compounds. The highest concentration tested did not precipitate.

Nelarabine is highly mutagenic but this effect is measurable in this assay only at non-physiological concentrations.

Study number	RD1998/02484/00, V40408
Research facility	GlaxoWellcome Inc. RTP, NC
Study Date	September 28, 1998
GLP	Yes
Audited	Yes
Drug	Nelarabine, lot MBR027/02/01A, purity not specified
Methods	
Test system	L5178Ytk+/- mouse lymphoma cells
Concentrations	0, 50, 100, 250, 500, 1000, 2500, 5000 µg/mL
Schedule	3-hour exposure
Basis of dose selection	results of study RD1988/01791/00
Formulation	DMSO
Positive controls	Methyl methanesulfonate (MMS) without activation 3-methylcholanthrene (3MCA) with activation
Metabolic activation	Aroclor 1254 induced male rat liver

### Results

The following table shows that in the absence of metabolic activation, Nelarabine caused a clear dose dependent increase in mutation frequency in mouse lymphoma cells *in vitro*. The highest concentration tested, 5000 mg/mL, caused a 10-fold increase in mutations while causing an 86% decrease in relative growth. The effect was greater than the positive control. In the presence of metabolic activation, Nelarabine caused a four-fold increase in mutation frequency with an 89% decrease relative growth. This suggests that the S9 preparation does not activate the compound but instead metabolizes it to less mutagenic compounds.

**Table 1: Summary Data of Mouse Lymphoma Mutagenicity Assays for 506U78**

506U78 Concentration (µg/mL)	Without S9		With S9	
	Mutant Frequency x 10 <sup>-8</sup>	Relative Total Growth (%)	Mutant Frequency x 10 <sup>-8</sup>	Relative Total Growth (%)
<b>3 hour exposure</b>				
Solvent Control <sup>a, b</sup>	125	100	95	100
100	134	88	76	68
250	77	116	83	81
500	314	62	205	45
1000	541	39	204	18
2500	1072	12	242	19
5000	1241	14	477	11
MMS <sup>c</sup>	882	33	ND	ND
3-MCA <sup>c</sup>	ND	ND	1866	11
Study in compliance with GLP: Yes    Experimental work dates: 29 September 1998 - 13 October 1998				
<b>Assay Results</b>				
506U78, MBR027/02/01A, was mutagenic in the absence and presence of S9 metabolic activation. 506U78 did not form a visible precipitate at any dose level. The highest dose level examined for mutagenicity was based on relative total growth (cytotoxicity). The 50mg/mL and 500mg/mL dosing solutions used in the mutagenesis assays were 79 and 85% of the nominal concentration. Dose levels are expressed in terms of 506U78. Positive controls gave the appropriate response, in terms of the increase in mutant frequency and generation of small colonies.				

**Key for Table**

<sup>a</sup> The values reported are the mean of two replicate cultures.

<sup>b</sup> The solvent was dimethyl sulfoxide (DMSO) which was at a final concentration in tissue culture media of 1% (vol/vol).

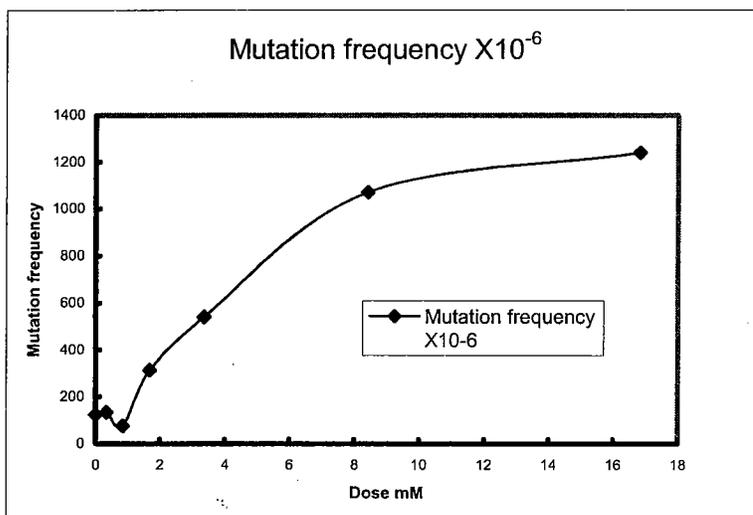
<sup>c</sup> The positive controls were methyl methanesulfonate (MMS) at 20.0µg/mL in the absence of S9 metabolic activation and 3-methylcholanthrene (3-MCA) at 5.0µg/mL in the presence of S9.

ND indicates not done.

Values showing a positive response are boxed in heavy lines. One of the criteria for a positive response is a dose level which shows an increase in mutant frequency of  $\geq 100 \times 10^{-8}$  over that of the solvent control.

The following graph (mine) demonstrates that this increase in mutation frequency in the absence of S9 is hyperbolic, approaching an asymptote at doses that are decidedly toxic.

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The sponsor did not report the full ICH battery of genotoxicity tests. There are no studies of mutagenicity with the Ames test or the rodent micronucleus test. I gather the sponsor assumed that this single resoundingly positive GLP test was sufficient to establish the genotoxicity of Nelarabine. I concur with this judgment.

## Reproductive toxicology

### 1) Nelarabine: Intravenous Infusion Embryo-Fetal Development Dose- Range Study in Rabbits

#### Major findings

Nelarabine was given to mated female New Zealand White rabbits by intravenous infusion from Days 7 to 19 of gestation, at dose levels of 1, 5, 25 and 125 mg/kg/day. There was no evidence of maternal toxicity, fetotoxicity, embryo-lethality or teratogenicity at any dose level.

Study number	CD2004/ 00514/ 00 D03408
Research facility	GlaxoSmithKline, Ontario, Canada
Study Date	February 7, 2004
GLP	No
Audited	No
Drug	Nelarabine, Batch number C049440, purity "on file"
Methods	
Animal	New Zealand white rabbits
Age and Wt	24 weeks, 2.8 to 3.3 kg
Doses	0, 1, 5, 25, 125 mg/kg (0, 12, 60, 300 or 1500 mg/m <sup>2</sup> )
N	5/dose group (one vehicle control)

Route	IV marginal ear vein, 20 or 40 minutes
Schedule	daily from day 7-19 post artificial insemination
Formulation	0.45% sodium chloride, USP
Day of C-section	29

## 2) Nelarabine: Intravenous Infusion Embryo- Fetal Development Study in Rabbits

### Major findings

An IV dose of 300 mg/kg/d (3600 mg/m<sup>2</sup>/d or about twice the adult recommended dose on a mg/m<sup>2</sup> basis) given to pregnant rabbits during organogenesis was toxic to the does, causing mortality, abortion, decreased body weight gain, decreased food consumption and labored breathing in various animals. The high dose was not embryo-lethal but it did cause toxicity, which manifested as low fetal weight. All dose levels including 100 mg/kg/d and 30 mg/kg/d were associated with delayed ossification. Increased incidences of fetal abnormalities were seen in all treated groups, these abnormalities included cleft palate at the high dose, absent palates at the mid and high dose and absent gall bladders, accessory lung lobes and fused or extra sternbrae at all doses. The low dose was not a NOAEL. Thus, Nelarabine is fetotoxic at clinically relevant doses.

Study number	CD2004/01225/00, G03324
Research facility	GlaxoSmithKline, Ontario, Canada
Study Date	July 1, 2004
GLP	Yes
Audited	Yes
Drug	Nelarabine, Batch number C049440, purity "on file"
Methods	
Animal	New Zealand white rabbits
Age and Wt	20-21 weeks, 2.3 to 3.5 kg
Doses	0, 30, 100, 300 mg/kg (0, 360, 1200, 3600 mg/m <sup>2</sup> )
N	22/dose group
Route	IV via indwelling catheter, 8 hour
Infusion	7.5, 0.75, 2.5 or 7.5 ml/kg/hr, groups 1, 2, 3, and 4 respectively
Schedule	daily from day 7-19 post artificial insemination
Formulation	0.45% sodium chloride, USP
Day of C-section	29

The following table (sponsor's) shows the pharmacokinetics of Nelarabine and ara-G on day 11 post insemination. The increase in concentration with dose was first order. The exposure to ara-G was about four-fold greater than the exposure to Nelarabine. The experiment establishes that the exposures in these rabbits were significantly higher than those achieved clinically.

Analyte	Dose-level <sup>1</sup> (mg/kg/day)	AUC <sub>(0-1)</sub> <sup>2</sup> (ng.h/mL)	AUC <sub>(0-1)</sub> <sup>3</sup> (μM.h)	C <sub>max</sub> (ng/mL)	C <sub>max</sub> <sup>3</sup> (μM)	t <sub>max</sub> (h)	AUC Ratio <sup>4</sup>
506U78	30	6950	23.4	1090	3.67	1.0	NA
	100	23620	79.5	3235	10.9	4.0	NA
	300	66236	223	9662	32.5	1.0	NA
ara-G	30	25687	90.8	3683	13.0	4.0	3.88
	100	91628	324	12771	45.1	4.0	4.07
	300	274411	970	32755	116	4.0	4.35

- Doses of 30, 100, and 300 mg 506U78/kg were given once-daily via intravenous infusion over 8 hours
  - AUC from the start of dosing to the last quantifiable time-point
  - Molecular weight of 506U78 and ara-G are 297 and 283, respectively
  - Ratio calculated by dividing AUC value of ara-G by AUC value of 506U78
- N = 2 or 3 rabbits per time point  
NA = not applicable

The following tables (sponsor's) present the results of this study.

Daily Dose (mg/kg)	0 (Control)	30	100	300
<b>Dams:</b>				
Toxicokinetics: 506U78 AUC (ng.h/mL)		6950	23457	64424
No. Animals/Group	22	22	22	22
No. Pregnant	22	21	20	17
No. Pregnant Evaluated	22	21	20	17
No. Died or Euthanized	1	0	2	3 <sup>2</sup>
No. with Total Resorption of Litter	0	0	0	0
<b>Clinical Observations</b>				labored breathing (No. 4514), reduced appetite (Nos. 4513, 4517 and 4518), thinness (Nos. 4513 and 4514) and decreased fecal output (No. 4514)

Daily Dose (mg/kg)	0 (Control)	30	100	300
<b>Necropsy Observations</b>	No treatment-related changes were seen during the gross examination of rabbits following the administration of 506U78 by intravenous infusion.			
Body Weight Gain (Days 7 to 29 pc) <sup>1</sup>	40g	0%	2.5%	-47.5%*
Corrected Body Weight Gain (Days 7 to 29 pc), kg	0.22	0.006	0.035	-0.110*
Food Consumption (Days 0 to 28 pc) <sup>1</sup>	125 g/day	0%	0%	-3.2%
Mean No. Corpora Lutea	9.0	9.8	9.1	9.6
Mean No. Implantations	6.9	7.5	7.2	8.1
Mean % Preimplantation Loss	23.7%	23.2%	18.8%	14.4%

(-) No noteworthy findings. + Mild \*\* Moderate \*\*\* Marked pc - postcoitum \*p<0.05 [Statistical significance (p<0.05) is based on raw data (not on the percent differences)]

- For controls, group means are shown. For treated groups, percent differences from controls are shown.
- Included one animal (4519) that was not pregnant, and also euthanized on Day 11 pc in poor condition.

Daily Dose (mg/kg)	0 (Control)	30	100	300
<b>Litters: No. Litters Evaluated<sup>1</sup></b>	21	21	18	15
Mean No. Live Fetuses	6.7	6.7	6.4	6.9
Mean No. Resorptions (early, middle and late)	0.2	0.8*	0.8	1.1*
Mean No. Dead Fetuses	0	0	0	0
Mean Total Postimplantation Loss - No.	4.3	12.1	11.4	14.0
Mean Fetal Body Weight (g) - Males	40.2g	39.3g	40.1g	30.4g*
Mean Fetal Body Weight (g) - Females	40.3g	38.8g	37.9g	30.2g*
% Male Fetuses	47.5%	53.8%	48.8%	47.4%

(-) No noteworthy findings  
\*p<0.05

- Method of fetal evaluation external, visceral, skeletal

Daily Dose (mg/kg)	0 (Control)	30	100	300
<b>Cleft Palate</b>				
No. Fetuses (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	58 (60.3%)*
No. Litters (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	14 (93.3%)*
<b>Gallbladder: Absent</b>				
No. Fetuses (ratio, %)	1 (0.7%)	2 (1.9%)	6 (5.3%)	10 (9.4%)*
No. Litters (ratio)	1	2	4	8*
<b>Accessory lung lobe: Absent</b>				
No. Fetuses (ratio, %)	9 (6.7%)	26 (20.4%)	37 (33.3%)*	65 (66.1%)*
No. Litters (ratio)	4	10	12*	15*
<b>Spleen: Reduced</b>				
No. Fetuses (ratio, %)	0 (0%)	1 (1%)	0 (0%)	23 (25.2%)*
No. Litters (ratio)	0	1	0	11*
<b>Pollex: Absent</b>				
No. Fetuses (ratio, %)	0 (0%)	0 (0%)	1 (0.9%)	35 (40.2%)*
No. Litters (ratio)	0	0	1	12*

Daily Dose (mg/kg)	0 (Control)	30	100	300
<b>Cleft Palate</b>				
No. Fetuses (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	58 (60.3%)*
No. Litters (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	14 (93.3%)*
<b>Gallbladder: Absent</b>				
No. Fetuses (ratio, %)	1 (0.7%)	2 (1.9%)	6 (5.3%)	10 (9.4%)*
No. Litters (ratio)	1	2	4	8*
<b>Accessory lung lobe: Absent</b>				
No. Fetuses (ratio, %)	9 (6.7%)	26 (20.4%)	37 (33.3%)*	65 (66.1%)*
No. Litters (ratio)	4	10	12*	15*
<b>Spleen: Reduced</b>				
No. Fetuses (ratio, %)	0 (0%)	1 (1%)	0 (0%)	23 (25.2%)*
No. Litters (ratio)	0	1	0	11*
<b>Pollex: Absent</b>				
No. Fetuses (ratio, %)	0 (0%)	0 (0%)	1 (0.9%)	35 (40.2%)*
No. Litters (ratio)	0	0	1	12*

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Daily Dose (mg/kg)	0 (Control)	30	100	300
<b>14<sup>th</sup> Rib(s): Extra</b>				
No. Fetuses (ratio, %)	0 (0%)	1 (0.8%)	7 (4.9%)*	53 (53.8%)*
No. Litters (ratio)	0	1	4*	14*
<b>Pubic Bones: Incomplete Ossification</b>				
No. Fetuses (ratio, %)	1 (0.6%)	0 (0%)	6 (4.7%)	55 (54.8%)*
No. Litters (ratio)	1	0	4	14*
<b>Pubic Bones: Unossified</b>				
No. Fetuses (ratio, %)	0 (0%)	0 (0%)	0 (0%)	7 (6.1%)*
No. Litters (ratio)	0	0	0	6*
<b>Forepaws: Reduced Number of Phalanges</b>				
No. Fetuses (ratio, %)	0 (0%)	0 (0%)	3 (2.5%)	56 (57.6%)*
No. Litters (ratio)	0	0	2	14*
<b>Pollex: Reduced Number of Phalanges</b>				
No. Fetuses (ratio, %)	0 (0%)	0 (0%)	1 (0.9%)	46 (50.3%)*
No. Litters (ratio)	0	0	1	13*

Daily Dose (mg/kg)	0 (Control)	30	100	300
<b>Hindpaws: Reduced Number of Phalanges</b>				
No. Fetuses (ratio, %)	0 (0%)	0 (0%)	0 (0%)	12 (13.9%)*
No. Litters (ratio)	0	0	0	7*
<b>Bilateral 13<sup>th</sup> rib</b>				
No. Affected Fetuses/Litter (Mean %)	27.3	96.1*	97.8*	98.1*
<b>Ribs - total 13<sup>th</sup></b>				
No. Affected Fetuses/Litter (Mean %)	41.8	96.7*	96.5*	99.4*

\* p<0.05

## Overall Summary

Nelarabine is a pro-drug of the deoxyguanosine analogue 9- $\beta$ -D-arabinofuranosylguanine (ara-G). Adenosine deaminase rapidly demethylates Nelarabine to form the active compound. Intracellular deoxyguanosine kinase and deoxycytidine kinase phosphorylate ara-G sequentially to form ara-GTP. ara-GTP then substitutes for GTP in numerous biological processes including the replication of DNA. This substitution leads to inhibition of DNA synthesis resulting in cell death. Malignant T-cells are killed by this process because they are rapidly replicating. Nevertheless, ara-G has the potential to kill any replicating cell. This mechanism appears so well accepted that little research has been done to determine other potential mechanisms. The acute lethal neurotoxicity seen in monkeys is probably caused by a very different mechanism.

Nelarabine is a potent toxin. It is cytotoxic *in vitro* at micromolar concentrations in human bone marrow progenitor cell lines. Experiments *in vitro* suggest it is more toxic to human malignant T-cell lines than it is to malignant B-cells, in some cases by at least a factor of 10.

Nelarabine is a topical irritant but it did not cause skin sensitization on re-challenge. It caused only negligible irritation in the Draize test. Solutions of Nelarabine were not hemolytic and did not cause protein flocculation at clinically relevant concentrations.

Only 7 to 20% of a dose of Nelarabine bound to human plasma and binding did not increase with concentration over a range of 6 to 600  $\mu\text{M}$ . Binding of ara-G increased from 7 to 24% over this same concentration range and binding increased with dose.

Nelarabine did not inhibit any major human cytochrome P450 isoenzyme. Cytochromes P450 are probably not involved in the metabolism of Nelarabine. Human hepatocytes demethylate Nelarabine to ara-G more rapidly than monkey hepatocytes. The conversion to ara-G is only slightly slower in rabbit and mouse hepatocytes than with human but total conversion is comparable if one includes the formation of allantoin by these species. Humans do not form appreciable allantoin, but convert ara-G to uric acid more rapidly than any of the species tested. Nelarabine did not inhibit the P-glycoprotein transporter nor was it a substrate.

All species rapidly convert Nelarabine to ara-G after IV dosing. Plasma ara-G concentrations were consistently greater than Nelarabine concentrations across species. In the rabbit, the ara-G AUC was 7 to 10 fold higher than that of Nelarabine; AUC and  $C_{\text{max}}$  for both compounds increased linearly with dose and did not change with repeated dosing. The decrease in concentration of both compounds was biphasic. In the rat, the AUC of ara-G was about 6 times greater than that of Nelarabine. The half-life of Nelarabine was only about 12 minutes while that of ara-G was only about 15 minutes, so the sponsor did not consider the rat a good species for predicting human toxicity. Likewise, the average half-life of ara-G was 14 minutes after an IV dose of 25 mg/kg of Nelarabine to beagle dogs. The average half-life of Nelarabine was 3 minutes. Dogs eliminate both drugs more quickly than rats, monkeys or humans. Mice dosed orally with 100 mg/kg/day of ara-G had peak plasma concentrations of ara-G of about 36- $\mu\text{M}$  and a  $t_{1/2}$  of 26 minutes. An equivalent dose of Nelarabine gave a peak ara-G plasma concentration about 19  $\mu\text{M}$  with a half-life of about 50 minutes. Since all these species eliminate ara-G much more quickly than humans do, the sponsor chose to do the major toxicology studies for the development of Nelarabine in the monkey; but, they did not do a comprehensive study of pharmacokinetics in the monkey. Based on limited information, single doses of 300 mg/kg or less in the monkey cause little toxicity but higher doses appear to cause some saturation of metabolism. The increase in AUC becomes greater than dose normal. Single doses of 400 or 500 mg/kg result in irreversible neurotoxicity, manifested as a deepening loss of consciousness. I believe that an overdose of Nelarabine would probably result in a similar saturation of metabolism and similar neurotoxicity in humans. This neurotoxicity is distinct from the Guillain-Barre like syndrome seen in patients receiving Nelarabine clinically. The ketamine anesthesia used in most of the monkey studies may slow the conversion of Nelarabine to ara-G in monkeys and exacerbate the neurotoxicity.

In humans the, half-life of ara-G is between 2 and 4 hours,  $T_{\text{max}}$  occurred after about two hours,  $Cl/F$  was about 0.3 L/h/kg,  $V_{\text{ss}}/F$  was about 1. At the recommended clinical dose for humans the AUC was 23  $\mu\text{M}\cdot\text{h}$ ,  $C_{\text{max}}$  was 14  $\mu\text{M}$ , clearance was about 8 L/h/kg, half-life was about 16 minutes and the volume at steady state was about 9 L/kg. AUC and clearance increased roughly linearly with dose but there was considerable variability.

After a single dose of radiolabeled Nelarabine (100 mg/kg) the drug distributed to all major tissues in pigmented mice. Immediately after the injection and for the first six hours the highest concentrations occurred in the urinary bladder. Concentrations in metabolic and excretory organs were usually higher than the concentrations in blood. Significant detectable concentrations of radioactivity remained in these organs throughout the experiment suggesting that some of the radioactivity was incorporated into structural biomolecules. Concentrations in the brain and CNS were lower than the concentrations in blood. The concentrations in brown fat, gonads, secretory organs, endocrine organs, muscle, gastric mucosa marrow, pancreas, spleen and the contents of the GI tract were approximately equal to those in blood. There were no differences between the sexes.

Cynomolgus monkeys, the most tested species, eliminate most of an IV dose of Nelarabine in the urine (>62%). The urine contained little radioactivity after 24 hours. The monkeys excreted less than one percent of total radioactivity in the bile and less than 2% in the feces. Less than 80% of the radiolabel was recovered after 10 days from the intact animals suggesting biological incorporation. Monkeys rapidly hydrolyze Nelarabine to ara-G; the parent compound was undetectable in plasma within two hours. They further metabolize ara-G to xanthine and then allantoin, and much of this metabolism is probably extrahepatic. Significant amounts of the radiolabel are incorporated into biological molecules within 24 hours. Respiration is probably not a major route of elimination.

As with the monkey, mice rapidly convert the parent compound to ara-G. Unlike monkeys they convert most of the dose to uric acid and allantoin within 3 hours (>88%). This is probably why the sponsor did not consider mice a good experimental model for this drug.

With the exception of the neurotoxicity, Nelarabine causes a spectrum of toxicities that one would expect with a purine antimetabolite. In mice the LD<sub>10</sub> for dailyX5 dosing is about 1500 mg/m<sup>2</sup>; this dose is associated with shallow breathing, decreased activity and tremor. Consistent with their rapid elimination of the drug dogs tolerated single doses of 7200 mg/m<sup>2</sup> with no toxicity. In rabbits given daily doses for 13 days (CIV) the MTD was 4320 mg/m<sup>2</sup>. This dose was associated with reduced appetite and decreased fecal output, decreased total body weight (~11%), decreased white and red cell parameters.

Monkeys tolerated single IV doses of 3600 mg/m<sup>2</sup> with few signs of toxicity. As mentioned above higher doses caused fatal neurotoxicity in some individuals. Monkeys that died from this toxicity showed loss of palpebral reflex, shallow respiration, flaccid limbs, and minimal jaw tone. The monkeys that did not recover died hours after the first or second dose. Monkeys tolerated five daily doses of 1800 mg/m<sup>2</sup> showing dose dependent decreases in WBC and platelets and a decrease in thymus weight. At this schedule, a dose of 3600 mg/m<sup>2</sup> killed 3 of 4 monkeys by day 13. Moribund monkeys had tremors and convulsions. Ketamine anesthesia time increased on day 5. All monkeys at this dose had tremors and convulsions. Body weight decreased as did WBC and platelets decreased. AST and ALT increased 2 to 12 fold. BUN, triglycerides and glucose increased. Microscopic damage was found in proliferative tissues, GI, spleen, marrow, thymus. There was congestion in the lungs.

In an IV study with dailyX30 dosing, monkeys tolerated doses of only 120 mg/m<sup>2</sup> with decreased red and white cell parameters. 240 mg/m<sup>2</sup> killed one of six monkeys on day 28. A dose of 480 mg/m<sup>2</sup> killed 3 of 10 monkeys despite the cessation of dosing on day 23. Red and white cell parameters decrease significantly. Significant damage was seen in the brain (cerebellar degeneration, perivascular cuffing in the cerebrum and cerebellum) and the spine (vacuolization and myelopathy, not reversible in surviving monkeys). Changes in clinical chemistry parameters were suggestive of liver damage. There was lymphoid infiltration in the bladder, kidney, trachea, salivary gland, lacrimal gland and heart. In females, there was myocardial degeneration and liver damage (cholangitis, edema and vacuolization).

An IV dose of 300 mg/kg/d (3600 mg/m<sup>2</sup>/d or about twice the adult recommended dose on a mg/m<sup>2</sup> basis) given to pregnant rabbits during organogenesis was toxic to the does, causing mortality, abortion, decreased body weight gain, decreased food consumption and labored breathing in various animals. The high dose was not embryo-lethal but it did cause toxicity, which manifest as low fetal weight. All dose levels including 1200 mg/m<sup>2</sup>/d or 360 mg/m<sup>2</sup>/d were associated with delayed ossification. Increased incidences of fetal abnormalities were seen in all treated groups, these abnormalities included cleft palate at the high dose, absent palates at the mid and high dose and absent gall bladders, accessory lung lobes and fused or extra sternbrae at all doses. Thus, Nelarabine is fetotoxic at clinically relevant doses.

Thus, Nelarabine causes dose dependent myelosuppression in humans and animals and toxicities that suggest acute liver damage, neurotoxicity that is possibly irreversible in some cases

and possibly cardiac damage. The unusual neurotoxicity seen clinically and non-clinically is possibly due to a secondary pharmacology and not the intracellular formation of ara-GTP. The toxic dose response curve for lethal neurotoxicity in the monkey is relatively steep, 300 mg/kg is non-lethal but 400 mg/kg (4800 mg/m<sup>2</sup>) killed one monkey within 4 hours. Nelarabine is profoundly genotoxic. It is also toxic to the developing fetus.

### **Recommendation**

I find no pharmacological or toxicological problems that would prevent the approval of ARRANON for the proposed indication.

### **Information requests to the sponsor**

None

### **Labeling Issues**

See Page 5

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

**APPEARS THIS WAY  
ON ORIGINAL**

## ***Appendix 1: Review 1***

### REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA ORIGINAL

IND 42,778

Reviewer: Wendelyn J. Schmidt, Ph.D.

Received by reviewer: 6/17/93

Completed: 6/24/93

Sponsor: Joanne Kurtzberg, M.D.

Drug Name: Compound 506, ara-G pro-drug

Chemical Name: 2-amino-9-B-D-arabinofuranosyl-6-methoxy-9H-purine

Indication: \_\_\_\_\_

Related Drugs and IND/NDA's: none

Information to be relayed to Sponsor: Y/N

Proposed Clinical Study: Objectives include determination of MTD as i.v. bolus infusion 1-2X daily for 5 days in children and adults, definition of toxicity profile of the drug, preliminary efficacy assessment, determination of deoxycytidine kinase immunoreactive protein in sensitive and resistant blasts cells and correlate with clinical response. Patients will be administered an i.v. infusion of Crmpd 506 over 1 hour daily for 5 days starting at 5 mg/kg (185 mg/m<sup>2</sup>) every 4 weeks. PK determinations will also be performed.

Dosage Forms and Route of Administration: i.v.; sterile lyophilized powder

Studies Received:

None; summaries of toxicologic and pharmacologic studies were submitted.

Note - Portions of this review were excerpted directly from the sponsor's submission.

#### Overall Summary and Evaluation

The toxicologic information submitted was inadequate. From the summaries provided, it was impossible to determine the drug vehicle, drug lot, volume injected, length of observation, peak and total exposure to drug (dog), parameters examined and timing of examination (body weight, hematology, serum chemistry, organs examined grossly and histopathologically), PK values measured and method of measurement, as well as whether the studies were conducted according to GLP. The pharmacodynamic studies did not contribute to the toxicologic profile as none were performed in an in vivo model with Compound 506.

With a series of assumptions, it is possible to draw several conclusions. Compound 506 does have activity, mostly in the 1-10 uM range, in a series of T-cell cultured cell lines. Compound 506 and Ara-G are detectable in dog, monkey and human following i.v. administration in dog and monkey, oral administration in human. The half-life was shortest in the dog (2.8 minutes), longer in monkey (10 minutes with 25 mg/kg, 30 minutes with 500 mg/kg), and still longer in human (2.8 hours by oral route). Ara-G half lives in dog and monkey were 13.5 and 2½-3 hours respectively. Toxicity was minimal in the mouse and dog with lethality seen only in 3 female mice (1 at 1500 mg/m<sup>2</sup>, 2 at 1800 mg/m<sup>2</sup>) which the sponsor attributed to volume effects. Monkeys given a single dose of 400 mg/kg or greater showed deep anesthesia in combination with ketamine; when administered multiple doses of 400 or 500 mg/kg, monkeys never regained consciousness. Three hundred mg/kg for 4 consecutive days in the monkey resulted in anorexia (degree unknown from data provided) and convulsions/ataxias, incoordination etc.; deaths also occurred at this dose at days 5-7. CNS effects were also

noted at doses as low as 150 mg/kg in the monkey, though recovery did occur by day 17. WBC counts in these groups were reduced as low as 1400 (no units given) but rebounded by day 14-21; thrombocyte number was also decreased. No serum chemistry parameters were discussed, nor were necropsy/histopathology results. Several papers discussing the effects of Ara-G in marrow purging were also included, but did not offer additional data on the safety of Compound 506 in the human, other than Ara-G did purge leukemic marrow, allowed marrow engraftment, and increased survival of the engrafted mice.

#### Recommendations

1. As the toxicity data presented did not allow for a complete assessment of the types and extent of toxicity of Compound 506, the study may not proceed.
2. The sponsor should provide complete data for each toxicity study performed including 1) the batch of drug used, 2) the vehicle and concentration of drug used, 3) the volume of drug injected, 4) the observations and measurements performed and the timing of these measurement, 5) the duration of the observation/measurement period, and 6) the measurement/data obtained from the animals. The type of measurements made should include body weight, clinical signs and gross necropsy for the acute lethality study in mice; body weight, clinical signs, and plasma levels of drug (including C<sub>max</sub> and AUC levels of Compound 506 and Ara-g) in the dog, and if available, hematology and serum chemistry values; and body weight, clinical signs, ECG, hematology, serum chemistry, necropsy reports, histopathology reports, and plasma drug and Ara-G levels in the monkey. Complete tables of the data, both mean values and individual animal values should be included, as well as a description of the technique used to measure plasma levels of the compound and Ara-G. For example, because no serum ALT/AST levels or histopathologic reports were submitted, it cannot be determined if there were toxicities to the liver or if the sponsor even examined the possibility. Since none of the pharmacology data was gathered in the whole animal, complete pharmacokinetic data would allow extrapolation of effective levels of drug in culture to actual plasma levels achieved. From the summaries, it seems that most of the data exists, but was not submitted.
3. From the data submitted, it is not clear that 150 mg/kg (1800 mg/m<sup>2</sup>) in the monkey is a non-lethal dose (1/4 150 mg/kg monkeys showed convulsions on days 5 and 6 and was sacrificed according to schedule on day 7; thus, the lethality of that dose is in doubt). A lower dose in the monkey should be tested to establish a minimally toxic dose. A dose (daily X 5) of 60-75 mg/kg in the monkey (720-900 mg/m<sup>2</sup>) with a recovery period of several weeks should give an adequate profile of non-lethal toxicity, and allow an acceptable safety margin in humans given the longer half-life of compound 506 in the human.

Wendelyn J. Schmidt, Ph.D.

cc:

IND ORIG.

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/WSchmidt

/CSO

/MO

/JDeGeorge

**Appendix 2: Review 2**

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA  
Review #2

IND 42,778

Reviewer: Wendelyn J. Schmidt, Ph.D.  
CDR Stamp Date: 2/17/94  
Received by reviewer: 2/22/94  
Completed: 3/16/94

Sponsor: Joanne Kurtzberg, MD

Drug Name: Compound 506, ara-G pro-drug

Chemical Name: 2-amino-9-B-D-arabinofuranosyl-6-methoxy-9H-purine

Indication:

Related IND/NDA's or DMF's: none

Proposed Clinical Study: Objectives include determination of MTD as i.v. bolus infusion 1-2X daily for 5 days in children and adults, definition of toxicity profile of the drug, preliminary efficacy assessment, determination of deoxycytidine kinase immunoreactive protein in sensitive and resistant blasts cells and correlate with clinical response. Patients will be administered an i.v. infusion of Cmpd 506 over 1 hour daily for 5 days starting at 5 mg/kg (185 mg/m<sup>2</sup>) every 4 weeks. Escalation to 75 mg/kg will be performed. PK determinations will also be performed. Allopurinol at 300 mg/m<sup>2</sup>/day will be administered q 8 hours starting 24 hours prior to Compound 506.

Information to be relayed to Sponsor: Y/N

Dosage Forms and Route of Administration: i.v.

Studies Received:

I. Pharmacodynamics:

1. 506U87 Inhibited the growth of CEM cells in beige nude Xid mice.

II. Pharmacokinetics

1. Pharmacokinetics of Nelarabine in the dog.

2. Pharmacokinetics of araG and Nelarabine in the monkey.

Toxicology

1. Acute murine toxicity study.

2. A report for AUC 512--an acute single dose i.v. study in beagle dogs with Nelarabine.

3. Monkey toxicity study

Note - Portions of this review were excerpted directly from the sponsor's submission.

## **I. Pharmacodynamics:**

### **1. 506U87 Inhibited the growth of CEM cells in beige nude Xid mice.**

CEM human t-cell (solid tumor) were transplanted into Xid mice, and the mice treated for 25 consecutive days beginning the day after transplanted either by i.p. or p.o. routes with 50 or 100 mg/kg 506U87. There was a dose dependent decrease in tumor weight with no significant difference in efficacy between p.o. and i.p. dosing. As data on lethality, body weight, or necropsy results was not included, no information on the toxicity of multiple doses in the mouse may be inferred.

Figure not available

## **II. Pharmacokinetics**

### **1. Pharmacokinetics of Nelarabine in the dog.**

The pharmacokinetics of a single i.v. dose of 25 mg/kg were investigated in 4 female dogs. Samples were collected at predose, 5, 15, 30, 45 minutes and 1, 2, 4, 6, 8, and 12 hours post-dose. Samples were analyzed by HPLC for both Nelarabine and araG content.

The half-life of araG in the dog was 13.6 minutes with a terminal half-life in the range of 1 hour. The half-life of Nelarabine was less than 5 minutes, with a terminal elimination on the order of 10 minutes. No other metabolites were detected with this HPLC method. Almost 14% of the dose was recovered in urine as parent compound or araG.

### **2. Pharmacokinetics of araG and Nelarabine in the monkey.**

Plasma samples from monkeys treated with either 150 or 300 mg/kg/day for 5 days (both doses resulting in lethality) were analyzed for Nelarabine and araG. Both Cmax and AUC were linear with dose. The approximate half-life for Nelarabine was under 30 minutes, 6-7 hours for araG (reviewer's eyeball method of calculation). No raw data or information on analysis method was provided.

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ON ORIGINAL**

### **III. Toxicology**

#### **1. Acute murine toxicity study.**

The study was performed at Burroughs Wellcome Co., Research Triangle Park, NC in 1992. Six mice/sex/dose were administered 0, 300, 400, 500, or 600 mg/kg NelarabineUD in 0.9% saline (10 mg/ml). Body weight and food consumption were monitored weekly, clinical signs were monitored daily. Gross necropsies were performed at sacrifice on day 14.

All mice survived to scheduled sacrifice. In the 600 mg/kg males and females, decreased activity for the first hour following dosing. Ptosis was also noted in the HD males. No significant changes in body weight or food consumption were noted with treatment. No significant changes were noted at necropsy.

#### **2. A report for AUC 512--an acute single dose i.v. study in beagle dogs with Nelarabine.**

The study was not performed according to GLP. A single dog (1 male and 2 females) were administered either 90, 180 or 360 mg/kg Nelarabine in saline and observed for 3-11 days. No clinical signs were observed and no other data was collected.

#### **3. Monkey toxicity study**

The study was performed according to GLP at Burroughs Wellcome, Research Triangle Park, NC, in 1993. Two Cynomolgous (*Macaca fascicularis*) monkeys/ sex/dose were administered either saline or 60 mg/kg/day NelarabineUH under ketamine anesthesia daily for 5 consecutive days. Monkeys were then observed for 59 days.

##### **Measurements and Observations:**

Twice daily: clinical observations

Weekly (including 2 weeks prior to dosing): body weight

Pretest, day 6 and 30: ophthalmoscopy

Days 1, 2, 5, 6: PK samples

Pretest, post-dose days 1, 9, 15, 22, 28, 36, 43, 50, 57: hematology, serum chemistry, urinalysis (last day for urinalysis, day 28).

Day 5, 11, and 35: ECG

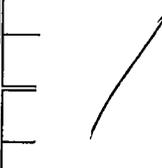
##### **Clinical Observations:**

All monkeys survived until scheduled sacrifice. Muscle twitching was noted in 1 treated male from postdose day 9 through 57 (noted particularly when monkey was stimulated or handled). Two treated females were ataxic from postdose day 10 through day 21 or 36. The sponsor noted that ataxia was "attributable to depth perception deficits, and neither animal was blind". Muscle twitching was also observed in one treated female during ketamine anesthesia for obtaining ECG on post-dose day 30. There were no significant differences between treated and control animals in time to recovery from ketamine anesthesia.

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ON ORIGINAL**

**Body weight:**

Body weight gain was decreased in the treated monkeys as compared to the saline controls (see following table).

Weight change (g) from pretest week 1 to week 9				
	Males		Females	
	Individual	Mean	Individual	Mean
Control		-112		+79
Treated		-364		-81

ECG: There were no significant changes in ECG parameters with treatment.

Ophthalmoscopy: There were no changes in ophthalmic observations with drug.

**Urinalysis:**

Ketones were present in the urine of both male and female treated monkeys from post-dose day 9 through day 22. Urine phosphorus was also elevated by >100 fold from postdose day 1-28 in at least 2 treated monkeys at all timepoints.

**Hematology:**

The RBC # (hematocrit and hemoglobin levels) in the treated males were decreased between 15 and 24% as compared to controls by postdose day 28, recovery was complete by postdose day 36. No effects on RBC's were noted in females. The WBC # was decreased in both males and females: males had a nadir at postdose day 9 of 52% as compared to controls, females had a nadir between postdose days 1 and 15 of 61% as compared to controls. WBC decreases were primarily in the lymphocytes. Platelets showed a decrease in males at day 1 of 55% of controls. Both males and females had platelet number above those in controls by 157% by day 22 with complete recovery by day 36.

**Serum Chemistry:**

Due to the small number of monkeys involved, interpretation of data, particularly enzyme levels, was difficult to interpret. However, AST and/or ALT were increased in the males 2-3 fold on postdose days 1, 9, 22. Lipase levels were decreased up to 50% as compared to controls through day 22 in both sexes. BUN was increased by 25% on post-dose day 22 in males, post-dose days 9-28 in females. Bilirubin was increased in males by 2 fold on day 1-9. Both males and females had a borderline (approximately 10%) decrease in albumin and increase in globulin after post-dose day 15. Finally, glucose was decreased by 25% on post-dose day 1 with recovery by day 28 in both sexes; although males showed an increase of 55% over controls on postdose day 15.

**Summary and Evaluation of toxicology:**

The sponsor has provided minimal data on the preclinical toxicology of Nelarabine, a prodrug for araG. The current submission included more-or-less complete data on a single dose study in the mouse and a daily X 5 administration of 60 mg/kg/day drug in the monkey, as well as minimal data on a single dose study in the dog. From the previous submission, where only

Summaries were presented, data on a daily X 5 administration of drug in the mouse, as well as single dose, and daily X 5 at 150 and 300 mg/kg/day Nelarabine in the monkey was included. However, no histopathology data in any species has been submitted to date.

There were essentially no effects that could not be attributed to large dosage volume in the single dose mouse. From the previous submission, daily X 5 dosing resulted in the deaths of 3 females: 1/6 @ 500 mg/kg, 2/6 @ 600 mg/kg, suggesting a starting dose on the daily X 5 schedule of 150 mg/m<sup>2</sup>.

In the monkey, lethality was noted at 150 mg/kg/day for 5 days. Neurotoxicity was present in all monkeys; somnolence, lack of recovery from anesthesia (i.e. died sleeping following the ketamine), and muscle tremor/convulsions were observed. But, recovery was noted within 2 weeks. At 60 mg/kg/day for 5 days, muscle twitching or ataxia was noted in 3/4 monkeys. WBC decreases and slight alterations in liver enzymes were also noted. Questionable effects on the kidney were observed as well (BUN, increased urine phosphorus). Alterations in glucose metabolism were also noted (altered glucose levels and ketones in the urine). The monkey was the most sensitive species to the effects of Nelarabine, which may be associated with the significantly longer residence time of the drug as compared to that in the dog.

#### Overall Summary and Evaluation

The most sensitive species to the effects of the ara-G prodrug, Nelarabine, was the monkey. In comparison with the dog, the half-life of distribution and elimination were an order of magnitude larger for both Nelarabine and ara-G. Murine efficacy studies demonstrated that oral and ip doses were similar, suggesting that bioavailability approaches 100%. A linear dose dependent decrease in tumor volume was noted in these mouse studies with a 94% decrease in tumor at 100 mg/kg (300 mg/m<sup>2</sup>). In the dog, approximately 14% of the dose was detected in the urine.

Toxicities in the monkey were primarily CNS (ataxia and muscle tremors noted through day 57 with daily X 5 administration of 60 mg/kg/day (720 mg/m<sup>2</sup>). The sponsor noted that the ataxia present in the 2 treated females was associated with alterations in depth perception and resolved by post-dose day 36 (first noted post-dose day 10). Higher doses (150 and 300 mg/m<sup>2</sup>) produced convulsions and death without awakening from anesthesia. It is not possible from the data provided to rule out that CNS effects are a product of the combination of ketamine and Nelarabine; however, high doses of ara-C have been demonstrated to produce coma, personality changes, and paralysis in children (PDR labeling). The other primary target of toxicity was the marrow (WBC depression in both males and females, RBC depression in males only). There were some alterations in glucose levels, liver enzymes and BUN, but in the absence of larger numbers of monkeys and histopathology, the interpretation of these results remains fuzzy.

The requesting starting dose (\_\_\_\_\_ in adults, approximately \_\_\_\_\_ in children) leaves at least a 3 fold margin in adults, 7 fold in children when compared with the monkey dose at which mild ataxia was observed. Based on the murine data, this is approximately 1/10 the LD<sub>10</sub>, however, the lethality in that study was probably due to the large volume injected. In a telephone conversation, the sponsor stated that she believed histopathology had been performed in the monkey and there were no drug related findings.

#### Recommendations

Information requested:

- 1) complete data on the daily X 5 mouse experiment.
- 2) complete data on the previous monkey experiments (single dose and daily X 5 @ 150 and 300 mg/kg.
- 3) Any histopathologic data on the monkeys (or any other species).

4) More complete text on the efficacy study in CEM.

The study remains on hold until the sponsor submits histopathologic data on the drug, which must then be reviewed for untoward findings.

Wendelyn J. Schmidt, Ph.D.

cc:

IND ORIG.

HFD-150

/WSchmidt

/CSO

/MO

/JDeGeorge

**Appendix 3: Review 3**

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Review #3

IND 42,778, serial #002

Reviewer: Wendelyn J. Schmidt, Ph.D.

Letter Date: 3/18/94

Received by reviewer: 3/22/94

Completed: 4/7/94

Sponsor: Joanne Kurtzberg, M.D.

Drug Name: Compound 506, ara-G pro-drug

Chemical Name: 2-amino-9-B-D-arabinofuranosyl-6-methoxy-9H-purine

Indication: —

Related IND/NDA's or DMF's: none

Proposed Clinical Study: Objectives include determination of MTD as i.v. bolus infusion 1-2X daily for 5 days in children and adults, definition of toxicity profile of the drug, preliminary efficacy assessment, determination of deoxycytidine kinase immunoreactive protein in sensitive and resistant blasts cells and correlate with clinical response. Patients will be administered an i.v. infusion of Cmpd 506 over 1 hour daily for 5 days starting at 5 mg/kg (185 mg/m<sup>2</sup>) every 4 weeks. Escalation to 75 mg/kg will be performed. PK determinations will also be performed. Allopurinol at 300 mg/m<sup>2</sup>/day will be administered q 8 hours starting 24 hours prior to Compound 506.

Information to be relayed to Sponsor: Y/N

Dosage Forms and Route of Administration: i.v.

Studies Received:

Pharmacodynamics:

1. Clarification of data on the Xid nude mouse CEM tumor experiments.

Pharmacokinetics:

1. Pharmacokinetic parameters for Nelarabine and ara-G in monkeys after i.v. administration of 60 mg/kg Nelarabine.

Toxicology

1. An acute single dose i.v. toxicity study in the mouse with Nelarabine.
2. An acute 5 day i.v. toxicity study in the mouse with Nelarabine.
3. An i.v. dose range finding study with 506 U78 in cynomolgus monkeys.
4. An acute 5 day i.v. toxicity study in cynomolgus monkeys with Nelarabine including unaudited draft copied of pathology.

Previously Reviewed:

1. An acute single dose i.v. toxicity study in the mouse with Nelarabine. (The study was performed according to GLP.)
2. An i.v. dose range finding study with 506 U78 in cynomolgus monkeys.

Note - Portions of this review were excerpted directly from the sponsor's submission.

### I. Pharmacodynamics:

#### **1. Clarification of data on the Xid nude mouse CEM tumor experiments.**

The sponsor clarified that the number of mice/group implanted with tumor was 6/group in the males, 8/group in the females. In the experiment with CEM in females, 2 mice at 50 mg/kg were euthanized/found dead on days 21 and 23 (one with paralyzed hindquarters) while a total of 4 mice treated at 100 mg/kg were euthanized/found dead (deaths on days 4 and 19, moribund sacrifice with paralyzed hindquarters on days 18 and 24. None of the 100 mg/kg mice had evidence of tumor. At least 1 control mouse had hindquarter paralysis which the sponsor suggested was due to either invasion of tumor or implantation technique.

### II. Pharmacokinetics:

#### **1. Pharmacokinetic parameters for Nelarabine and ara-G in monkeys after i.v. administration of 60 mg/kg Nelarabine.**

The pharmacokinetic parameters in the monkeys are summarized in the following tables.

Pharmacokinetic Parameters For 604U78 and ara-g in monkeys After IV Administration of 60 mg/kg 604U78

Day 1

Sex	Monkey ID	604U78 AUC	ara-g Cmax (µM)	ara-g Tmax (h)	ara-g AUC 0 to 24h
M	2M01	171.91	132.41	0.50	539.73
M	2M02	280.29	155.00	0.50	857.10
	Average	218.10	143.71	0.50	698.42
	SD	82.48	15.97	0.00	82.89
F	2F01	328.38	119.05	0.50	391.48
F	2F02	329.74	118.47	0.50	403.85
	Average	278.05	117.24	0.50	427.72
	SD	59.73	2.53	0.00	51.24
	Total ave.	247.56	130.48	0.50	513.07
	SD	85.14	17.90	0.00	173.51

Day 5

Sex	Monkey ID	604U78 AUC	ara-g Cmax (µM)	ara-g Tmax (h)	ara-g AUC 0 to 24h
M	2M01	288.17	126.88	0.50	512.48
M	2M02	355.51	147.95	0.50	842.27
	Average	311.84	137.41	0.50	737.38
	SD	81.76	14.91	0.00	162.48
F	2F01	421.00	107.48	0.50	418.27
F	2F02	278.91	115.84	0.50	463.87
	Average	348.98	111.88	0.50	428.07
	SD	101.89	5.77	0.00	25.17
	Total ave.	332.42	124.44	0.50	581.72
	SD	72.05	17.55	0.00	182.13

### III. Toxicology

#### **1. An acute 5 day i.v. toxicity study in the mouse with Nelarabine.**

The study was performed according to GLP at Burroughs Wellcome, RTP, NC in 1992. Six CD-1 mice/sex/dose were administered either 0, 200, 300, 400, 500 or 600 mg/kg/day (0, 600, 900, 1200, 1500 or 1800 mg/m2/day) of Nelarabine i.v. at 10 mg/ml in sterile saline daily for 5 days. Animals were observed for 14 days after dosing (total 19 days).

Measurements and Observations:

Daily: clinical signs

Weekly: body weight and food consumption

#### Termination (day 19): gross necropsy

##### Mortality and clinical signs:

A total of 3 female mice died during the experiment: 1 500 mg/kg/day female on day 13, and 2 600 mg/kg/day females on days 9 and 12. No clinical signs were noted in mice treated at less than 600 mg/kg/day. In the HD males and females, signs included shallow breathing, decreased activity, coolness to touch, body tremors and ptosis and persisted following dosing for up to 5 hours. Closed eyes, dehydration and alopecia were present in 1-3 mice/sex at HD between days 8 and day 19. Two HD females also showed "high carriage" from day 8 through day 17. The LD10's for males and females were >600 and 493 mg/kg respectively.

##### Body weight and food consumption:

There was a dose dependent decrease in body weight gain to a maximum of 90% in males, 60% in females at the highest dose as compared to controls. The food consumption was decreased in the HD groups during the dosing period and the first week of observation.

##### Gross necropsy:

No differences were found between treated and control mice with the exception of 1 HD female which died on day 9 which had red lungs and tarry material in the g.i. tract.

#### **2. An acute 5 day i.v. toxicity study in cynomolgus monkeys with Nelarabine.**

The study was performed according to GLP at \_\_\_\_\_ but was not signed (study # 474C-403-520-92, ACU 515). Two cynomolgus monkeys/sex/dose were administered Nelarabine in saline at 0, 150 or 300 mg/kg/day (0, 1800, or 3600 mg/m<sup>2</sup>/day) i.v. for 5 consecutive days while under ketamine anesthesia. Animals were observed for either 7 or 62 days post-dose.

##### Measurements and Observations:

Daily: clinical signs

Days -15, -7, 1, 5, weekly: body weight

Pretest, day 6, 58 post-dosing: ophthalmoscopy

Days 1, 2, 5 and 6: Samples for PK (multiple time points)

Day -15, post-dose days 1, 6; weekly: hematology, serum chemistry, urinalysis

Day -17, day 5, post-dose day 6 and 58: ECG

Termination: gross necropsy, organ weight, histopathology

##### Clinical observations:

Three of the 4 monkeys in the 300 mg/kg group died or were sacrificed moribund during the study: 1 male was found dead on post-dose day 8; 1 male and female were sacrificed moribund on post-dose days 1 and 3. The 2 monkeys sacrificed moribund showed tremors and convulsions. During dosing, the time to recovery from ketamine anesthesia was increased at day 5 in the 300 mg/kg monkeys. Other clinical signs in the surviving monkeys included emesis during dosing, periodic tremors/convulsions--particularly after ketamine anesthesia, and red crusty material around the nose/ respiratory difficulty. All signs had resolved (with the exception of ketamine response) by study day 22.

##### Body weight and food consumption:

The 300 mg/kg/day monkeys lost approximately 10% of their body weight during the dosing period. All monkeys lost weight by the end of 14 days post-dose, with no statistically significant difference between treated and controls; the body weight remained depressed in the 300 mg/kg female at post-dose day 56. Food consumption did not differ dose dependently.

ECG: There were no significant changes with treatment.

Ophthalmoscopy: There were no changes with treatment.

Urinalysis:

During the dosing period, the treated monkeys showed increased WBC and RBC's in the blood; ketones were also present in the urine. Through week 7, amorphous urates and phosphates were increased in the 150 and 300 mg/kg monkeys.

Hematology:

The RBC# and associated parameters were decreased by 12-35% in both the controls and treated monkeys (both sexes) with no apparent dose-dependence; recovery was complete in all surviving animals by day 33. WBC # was decreased dose-dependently to a nadir at days 8-11 of 88% of control; WBC's recovered within 4 weeks of treatment. Platelets were decreased by 37-50% as compared to controls at 300 mg/kg.

Serum Chemistry:

The following serum chemistry parameters were altered in the dogs that died at 300 mg/kg during the week following dosing: increased AST/ALT (2-12 fold in 1 male and female), increased glucose (61% in 1 male, 100% in 1 female), increased bile acids (10 fold in 1 male) and increased BUN (115% in 1 male, 380% in 1 female). In the surviving 300 mg/kg female, triglycerides were increased to a maximum of 10 fold above pretest at post-dose day 14, then returned to pretest levels by postdose day 21. An increase of >50% was noted in serum potassium levels at postdose day 6 in 1 150 mg/kg female and 1 300 mg/kg male.

Gross pathology and organ weights:

The only notable findings at necropsy were enlarged and reddened adrenals and gi reddening in 1 male at 300 mg/kg, and mottled lungs in the 2 males at 300 mg/kg.

The absolute and relative weight of the thymus was decreased by approximately 60% in both the male and female at 150 mg/kg at interim sacrifice. At terminal sacrifice, the absolute weigh of the thymus had recovered in the 150 mg/kg male, decreased by 26% and 65% as compared to control in the 150 and 300 mg/kg females. While there were apparent decreases in reproductive organ weight at interim sacrifice (increases at terminal), the control weights at both intervals were significantly different preventing interpretation. The absolute and relative weight of the lungs in the females was decreased at terminal sacrifice by approximately 50% at 300 mg/kg.

Histopathology:

There were no significant histopathologic findings in any monkeys except the 3 @ 300 mg/kg which died on study (the remaining 300 mg/kg female recovered completely). Findings in these three monkeys included lung congestion/edema/hemorrhage, lymphoid depletion, thymic atrophy (mild to severe), marrow hypocellularity, and injection site edema/hemorrhage. One male also had splenic congestion/involution, minimal gi hemorrhage, and adrenal congestion.

#### Overall Summary and Evaluation

The sponsor has finally provided sufficient preclinical data to evaluate the safety of the proposed starting dose. Using the proposed daily X 5 schedule, the LD10 in the mouse was >600 mg/kg (1800 mg/m<sup>2</sup> in the male and 493 mg/kg (1479 mg/m<sup>2</sup>) in the female. Deaths occurred during the first week following dosing; and could conceivably be due to marrow depletion

(although there is no evidence for this assumption). In the monkey, daily X 5 administration to monkeys in combination with ketamine anesthesia resulted in the deaths of 3/4 monkeys at 300 mg/kg (3600 mg/m<sup>2</sup>); minimal toxicity was noted at 150 mg/kg (1800 mg/m<sup>2</sup>), suggesting that mice were more sensitive than monkeys. Hematologic, serum chemistry and histopathologic observations suggest that the major toxicity in the monkey is to hematopoietic cells, particularly WBC's. Although liver and kidney parameters were altered, no histopathologic damage was noted. Neurologic changes (slow recovery from ketamine anesthesia, convulsions, tremors) were noted at 150 and 300 mg/kg as well as at 60 mg/kg in a separate set of experiments. This could correlate with the hindquarter paralysis noted in the CEM nude mouse efficacy study. Finally, glucose levels were increased in two moribund monkeys as well. There was no significant damage at the end of the recovery period in the remaining 300 mg/kg female, suggesting reversibility of drug damage.

The pharmacokinetics in the 60 mg/kg monkeys suggest no accumulation of either the Nelarabine or ara-g with multiple administration. Males may have a slightly higher exposure to drug which correlated with the greater number of deaths in males at 300 mg/kg in the monkey.

#### Recommendations

1. The study may proceed.
2. The sponsor should be aware that in the future, evaluation of an IND submission will proceed more rapidly if the following data is submitted in the original package: a study justifying the drug's use in an oncology setting, one toxicology study in rodents (usually establishing the mouse LD10), and a non-rodent toxicity study. Both rodent and non-rodent studies should be performed using the route, schedule and duration of administration proposed for human use; an observation period following dosing is helpful to track the recovery from drug toxicity. At least one study should include information on the hematologic, serum chemistry and histopathologic effects of the drug (usually the non-rodent study). Other parameters to monitor should include body weight, organ weight, clinical signs; urinalysis, ophthalmoscopy, and ECG may be useful. Pharmacokinetic determinations are not required, but are helpful in determining dose escalations and dosing intervals.

Wendelyn J. Schmidt, Ph.D.

cc:

IND ORIG.

HFD-150

/WSchmidt

/CSO

/MO

/JDeGeorge

**Appendix 4: Review 4**

IND 42778 review 4

1

**Division of Oncology Drug Products, HFD-150**  
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA  
Review No. 4

IND No. 42778

Serial No(s):	036	Type:	IT	Date(s) of Submission:	4/21/97
	045		IT		6/9/97
	049		IT		8/7/97

Information to be Conveyed to Sponsor: Yes (X), No ( )

Reviewer: Wendelyn J. Schmidt, Ph.D.

Date Review Completed: 4/28/98

Sponsor: Glaxo Wellcome      Manufacturer (if different):

Drug Name: Primary: 506U78      Other Names: pro-ara-G

Chemical Name: 9-β-arabinofuranosyl-6-methoxy-9H-purine

CAS Number: 121032-29-9

Structure:

Molecular Weight (and Formula optional): C<sub>11</sub>H<sub>15</sub>N<sub>5</sub>O<sub>5</sub>, mw= 297.27

Related INDs/NDAs/DMFs: none

Class: antineoplastic (nucleotide analog)

Indication: \_\_\_\_\_

Clinical Formulation: 250 mg 506U78 in 50 mL of 0.45% NaCl (5 mg/mL).

Route of Administration: intravenous

Proposed Clinical Protocol:

Objectives: Phase II studies to determine efficacy (toxicity) in CLL patients

Starting dose: 1.2 g/m<sup>2</sup> on days 1, 3, 5, daily X5 as 1- 2 hour infusion

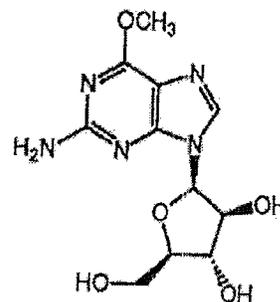
Frequency: every 28 days

Total duration of drug exposure or cycles: max 8 cycles

Escalation doses: no

Age of patient population: >18 years in most studies, at least 1 pediatric study

Previous Review(s), Date(s) and Reviewer(s): W. Schmidt, #1: 6/24/93, #2: 3/16/94, #3: 4/7/94



**Studies Reviewed in this submission:**

1. A 30 day intravenous toxicity study in Cynomolgus monkeys with 506U78 (with recovery period). Serial # 036.
2. 506U78: An acute single dose intravenous study in Beagle dogs with 506U78. Serial # 049.

**Studies Not Reviewed in these submissions:** none

**Studies Previously Reviewed from these submissions:**

1. An intravenous dose range finding study with 506U78 in Cynomolgus monkeys. Serial # 044. (Previously included in initial submission).
2. An acute five day intravenous toxicity and pharmacokinetic study in a cynomolgus monkey with 506U78. Serial # 049 (conducted in 1992, single ♀ monkey @ 500 mg/kg/day, died on D2, included in initial submission).

**Other Studies Previously Reviewed:**

## I. Pharmacodynamics:

1. 506U87 Inhibited the growth of CEM cells in beige nude Xid mice.
2. Clarification of data on the Xid nude mouse CEM tumor experiments

## II. Pharmacokinetics

1. Pharmacokinetics of 506U78 in the dog.
2. Pharmacokinetics of araG and 506U78 in the monkey.
3. Pharmacokinetic parameters for 506U78 and ara-G in monkeys after i.v. administration of 60 mg/kg 506U78.

## Toxicology

1. Acute murine toxicity study.
2. A report for AUC 512--an acute single dose i.v. study in beagle dogs with 506U78.
3. Monkey toxicity study
4. An acute single dose i.v. toxicity study in the mouse with 506U78.
5. An acute 5 day i.v. toxicity study in the mouse with 506U78.
6. An i.v. dose range finding study with 506U78 in cynomolgus monkeys.
7. An acute 5 day i.v. toxicity study in cynomolgus monkeys with 506U78 including unaudited draft copied of pathology.

*Note: Portions of this review were excerpted directly from the sponsor's submission.*

**INTRODUCTION/ DRUG HISTORY and PREVIOUS CLINICAL EXPERIENCE**

The IND for pro-ara-G (506U78) has been at the FDA since 1994. The phase II dose is not yet determined. CNS toxicities are most prevalent and include changes in mental status, somnolence, photophobia, seizures, neuropathy, paralysis, myoclonic jerks, and a Guillian Barre-like syndrome. Other reported toxicities include myelosuppressive effects (fungal infection, fever), and increased heart rate. The purpose of this review is to examine animal toxicities at longer durations than previous.

**IV. TOXICOLOGY****1. A 30 day intravenous toxicity study in Cynomolgus monkeys with 506U78 (with recovery period). Serial # 036.**

The study was conducted according to GLP at \_\_\_\_\_ in 1995. Cynomolgus monkeys (*Macaca fascicularis*) were dosed intravenously with either 0 (5/sex), 10 (3/sex), 20 (3/sex) or 40 (5/sex) mg/kg 506U78 (lot # 506U78UH in 0.9% saline) (0, 120, 240 or 480 mg/m<sup>2</sup>) once daily for 30 consecutive days. The 40 mg/kg dose was discontinued on day 23 due to excess toxicity. Three monkeys/sex/dose monkeys were killed on day 31, the remaining 2/sex control and HD monkeys were observed through the day 63-70 recovery period.

**Measurements and Observations:**

Twice daily: clinical signs

Weekly: body weight

Pretest, "near the end of the dosing phase": ophthalmoscopy

Day 3, 28: blood for PK @ 0.5, 1, 3, 8 and 24 hrs. post-infusion (reported as summary only)

Pretest, day 30-31: urinalysis of fasted monkeys

Pretest, day 15/16 and 30/31; post dose days 7/8, 13, 22, 37 and 64: hematology, serum chemistry

Pretest, day 7/8 and 28/29: ECG

Termination: gross pathology, organ weights, histopathology

**Mortality and clinical signs:**

One HD male was sacrificed moribund on day 23, another on day 25 and 28. One HD female was sacrifice moribund on day 28. Beginning on day 19, monkeys treated at 20 and 40 mg/kg showed coarse muscle tremors and convulsive activity. By day 44, muscle tremors were less severe and classed as fine muscle tremors. Additionally, one 10 mg/kg female had an axillary mass 1-3 cm on days 29-30. Ketamine administration triggered seizure-like activity.

**Ophthalmoscopy and ECG:**

No significant changes were noted with treatment.

**PK:**

Only a summary table was submitted and is reproduced below. No significant differences in AUC or C<sub>max</sub> were noted with time. AUC and C<sub>max</sub> were also linear with dose.

<i>Dose (mg/kg day):</i>	<i>ara-G</i>			<i>506U78</i>		
	<i>10</i>	<i>20</i>	<i>40</i>	<i>10</i>	<i>20</i>	<i>40</i>
<i>(mg/m<sup>2</sup>/day):</i>	<i>125</i>	<i>250</i>	<i>500</i>	<i>125</i>	<i>250</i>	<i>500</i>
<b>C<sub>max</sub> (µM)</b>						
Day 3	25	51	93	n.c.	n.c.	n.c.
Day 28	24	51	—	n.c.	n.c.	—
<b>AUC (µM·hr)</b>						
Day 3	85	177	307	22	57	130
Day 28	69	161	—	22	52	—

n.c. = not calculated

**Body weight and food consumption:**

Although there were no statistically significant differences in mean body weight during the course of the experiment, changes in body weight gain were evident and highly variable. (See following table). Statistically significant decreases in body weight gain were noted at day 28 in the

HD females. Food consumption was decreased inconsistently on a day-to-day basis with dose.

Absolute change (g) in body weight (versus day -1)				
	Males		Females	
	D28	D91	D28	D91
Control	106 ± 124	366 ± 163	70 ± 87	274 ± 131
10 mg/kg	47 ± 124	---	98 ± 128	---
20 mg/kg	-181 ± 336	---	30 ± 115	---
40 mg/kg	-148 ± 18	187 ± 416	-248 ± 235	79 ± 243

#### Urinalysis:

Urinary values were widely variable, but the sponsor claimed they were within expected values.

#### Hematology:

Changes in hematologic values are shown in the following table. Hematocrit and hemoglobin values paralleled RBC #; reticulocyte # increased at day 30 to reflect marrow repopulation. Most parameters resolved by either day 45 or 60. WBC subsets which were decreased included eosinophils, neutrophils, monophils and lymphocytes (less severely than other types). Platelet numbers increased at day 30.

% change as compared to control							
Parameter	Day #	Males			Females		
		LD	MD	HD	LD	MD	HD
RBC #	15	---	113%	113%	121%	122%	122%
	30	120%	124%	127%	128%	131%	125%
Platelet #	15	---	---	133%	---	---	125%
	30	134%	162%	1119%	123%	124%	152%
reticulocyte #	30	---	---	13X	---	---	1>5X
WBC #	15	---	155%	129%	143%	145%	149%
	30	132%	145%	179%	135%	145%	119%

#### Serum Chemistry:

Significant changes were noted only at day 30 (with the exception of K<sup>+</sup> levels at day 15 in females, which were similar to those seen on day 30). Changes are summarized in the following table. Most of the damage, with the exception of glucose and cholesterol in the males, resolved by day 45.

% change as compared to controls at day 30					
Parameter	Males			Females	
	LD	MD	HD	MD	HD
glucose	↑10%	↑18%	↑26%	—	—
cholesterol	↑15%	↑15%	↑28%	—	↑26%
K <sup>+</sup>	↑16%	↑20%	↑10%	↑10%	↑14%

## Organ weights:

Organ weights are summarized in the following table. A single HD male monkey was sacrificed at week 4. The most significant changes were noted in the thymic weights in both sexes. Although only the control and HD animals were carried through the recovery period, most changes resolved by the end of the recovery period.

% change as compared to controls									
Organ	Dose	Males				Females			
		Treatment		Recovery		Treatment		Recovery	
		Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Adrenal	LD	↑15%	—	—	—	↑23%	—	—	—
	MD	↑9%	—	—	—	—	—	—	—
	HD	↑12%	—	↑14%	—	↑29%	↑35%	—	—
Liver	HD	↑39%	↑19%	↑16%	—	↑24%	—	—	—
Kidney	HD	↑65%	↑36%	—	—	↑12%	↑18%	—	↑16%
Heart	HD	↑36%	↑19%	—	—	↑18%	↑14%	—	↑19%
Spleen	LD	↑37%	↑22%	—	—	—	—	—	—
	MD	—	—	—	—	↑25%	↑19%	—	—
	HD	↑56%	↑29%	—	↑23%	↑48%	↑54%	—	—
Testes	LD	↑53%	↑48%	—	—	—	—	—	—
	MD	↑57%	↑51%	—	—	—	—	—	—
	HD	↑25%	↑29%	↑5.4 X <sup>⊗</sup>	↑4.2 X <sup>⊗</sup>	—	—	—	—
Thymus	LD	—	↑23%	—	—	↑74%	↑77%	—	—
	MD	↑54%	↑60%	—	—	↑75%	↑76%	—	—
	HD	↑18%	↑38%	↑40%	↑41%	↑56%	↑53%	↑11%	↑20%
Thyroid	HD	↑68%	—	—	—	↑83%	↑92%	—	—

⊗ one of two dogs testes weight did not differ from controls, while the other had these reported values.

**Gross Pathology:**

In the monkeys sacrificed moribund, the MD male had no appreciable alterations. In the HD moribund animals, enlarged spleen and enlarged thyroid were found in 1/3 monkeys. In the scheduled sacrifice group there were ovarian cysts at the end of treatment in 1 LD and MD females and at the end of recovery in 2 HD females.

**Histopathology:**

The most worrisome toxicities were to the CNS (cerebellar degeneration, perivascular cuffing in the cerebrum and cerebellum, and vacuolation/myelopathy in the spinal cord) were seen in the HD group of both sexes (1-2/2 monkeys/sex), and persisted through the recovery period. No pathology was noted in the lymph nodes or marrow, with the exception of a minimal degree of lymphoid infiltration. One female at HD had thymic lymphoid depletion. Other organs at HD with lymphoid infiltration included urinary bladder, kidney, trachea, salivary gland, lacrimal gland and heart. Two toxicities were peculiar to the HD females: myocardial degeneration in 1/2 treatment and recovery females, and liver/gall bladder damage (gall bladder edema, liver cholangitis, and hepatocyte vacuolation) at the end of the recovery period. One LD female had an axillary mass at the end of the dosing period which was lymphoid hyperplasia and inflammation.

**2. 506U78: An acute single dose intravenous study in Beagle dogs with 506U78. Serial # 049.**

The non-GLP study was conducted at Glaxo Wellcome, Research Triangle Park, NC in 1997. One beagle dog/dose was administered either 90, 180 or 360 mg/kg intravenous 506U78. Clinical signs were observed daily. After initiation of the study, the beagle was determined to be an inappropriate model. No signs were observed on day 1; no mortality was noted. It was not clear how long the dogs were actually observed.

**Histopathology Inventory for IND #42778**

Study	DX30						
Species	monkey						
Adrenals	X						
Aorta	X						
Bone Marrow smear	X						
Bone (femur)	X						
Brain	X						
Cecum	X						
Cervix	X						
Colon	X						
Duodenum	X						
Epididymis	X						
Esophagus	X						
Eye	X						
Fallopian tube							
Fat	X						
Gall bladder	X						
Gross lesions							
Harderian gland							
Heart	X						
Hypophysis							
Ileum	X						
Injection site	X						
Jejunum	X						
Kidneys	X						
Lachrymal gland	X						

Larynx									
Liver	X								
Lungs	X								
Lymph nodes, cervical	X								
Lymph nodes mandibular									
Lymph nodes, mesenteric	X								
Mammary Gland	X								
Nasal cavity									
Optic nerves									
Ovaries	X								
Pancreas	X								
Parathyroid	X								
Peripheral nerve									
Pharynx									
Pituitary	X								
Prostate	X								
Rectum									
Salivary gland	X								
Sciatic nerve	X								
Seminal vesicles									
Skeletal muscle	X								
Skin	X								
Spinal cord	X								
Spleen	X								
Sternum	X								
Stomach	X								
Testes	X								
Thymus	X								
Thyroid	X								
Tongue	X								
Trachea	X								
Urinary bladder	X								
Uterus	X								
Vagina	X								
Zymbal gland									

Data on tissues observed was not collected at the time of the first 3 reviews.

#### OVERALL SUMMARY AND EVALUATION

As with both previous non-clinical studies and human trials, CNS toxicity was the major finding in the 1 month monkey studies. The most noteworthy observation in the one month study is the microscopic evidence of damage in the spinal cord and brain in the HD group which persisted throughout the recovery period which correlated with observations of seizure, neuropathy, etc. in both the clinic and laboratory. An apparent threshold of toxicity exists for the permanent microscopic damage, as although tremors/convulsions were observed in the LD and MD groups, no microscopic damage was observed in these monkeys. Minimal changes in other organs were noted, but did include relatively mild reversible myelotoxicity, slight increases in potassium levels, possible testicular damage (no histopathologic evidence), and possible changes in thyroid in females only (based on organ weight).

In the daily X 5 mouse studies, minimal CNS toxicities (shallow breathing, "high carriage", tremors, decreased activity) were seen for up to 5 hours after dosing. No hematology or clinical chemistry values were collected in this species. In the daily X 5 monkey, 3/4 monkeys at the HD (300 mg/kg) died during the study. CNS toxicity (excessive response to ketamine anesthesia, tremors, convulsions) were noted. Myelosuppression was also noted for both RBC's and WBC's. Higher doses also resulted in some derangements of liver parameters which were not apparent with

lower doses over longer time periods. No new toxicities emerged with longer exposure to drug. From a 1996 abstract, AUC and half-life levels in humans and monkeys appear similar. A similar toxicity profile is also seen between humans and monkeys, suggesting that monkeys are an appropriate species to study for this drug.

The sponsor has requested an End-of-Phase-1 meeting with the FDA and would like to know if their proposal for preclinical data is sufficient for approval. In addition to the mouse, dog and monkey toxicity (reviewed in prior submissions or here), the sponsor has proposed 1) a hemolysis study, 2) a Segment II reprotox study in rabbit, and 3) a genotoxicity mouse lymphoma assay. The acute/subchronic toxicity program is sufficient for the sponsor's purposes. A second segment II toxicity study may be necessary if the rabbit proves negative. Finally, although not required, a more extensive genotoxicity assessment (including Ames test, chromosomal aberrations and mouse micronucleus) would be useful in delineating the genotoxic potential of 506U78.

**RECOMMENDATION** (will be communicated at sponsor's EOP1 meeting)

The acute/subchronic toxicity program is sufficient for the sponsor's purposes. A second segment II toxicity study may be necessary if the rabbit proves negative. Although not required, a more extensive genotoxicity assessment (including Ames test, chromosomal aberrations and mouse micronucleus) would be useful in delineating the genotoxic potential of 506U78.

Wendelyn J. Schmidt  
Wendelyn J. Schmidt, Ph.D.  
Pharmacologist/Toxicologist

5/19/98  
Date

Paul A. Andrews  
Paul A. Andrews, Ph.D.  
Pharm/Tox Team Leader

5/19/98  
Date

Original IND/NDA/DMF

c.c. /Division File  
/WSchmidt  
/PAndrews  
/GSchechter  
/MPelosi



I. Pharmacodynamics:

1. 506U87 Inhibited the growth of — cells in beige nude Xid mice.
2. Clarification of data on the Xid nude mouse — tumor experiments

II. Pharmacokinetics

1. Pharmacokinetics of Nelarabine in the dog.
2. Pharmacokinetics of araG and Nelarabine in the monkey.
3. Pharmacokinetic parameters for Nelarabine and ara-G in monkeys after i.v. administration of 60 mg/kg Nelarabine.

Toxicology

1. Acute murine toxicity study.
2. A report for AUC 512—an acute single dose i.v. study in beagle dogs with Nelarabine.
3. Monkey toxicity study
4. An acute single dose i.v. toxicity study in the mouse with Nelarabine.
5. An acute 5 day i.v. toxicity study in the mouse with Nelarabine.
6. An i.v. dose range finding study with Nelarabine in cynomolgus monkeys.
7. An acute 5 day i.v. toxicity study in cynomolgus monkeys with Nelarabine including unaudited draft copied of pathology.
8. A 30 day intravenous toxicity study in Cynomolgus monkeys with Nelarabine (with recovery period). Serial # 036.
9. 2. Nelarabine: An acute single dose intravenous study in Beagle dogs with Nelarabine. Serial # 049.

*Note: Portions of this review were excerpted directly from the sponsor's submission.*

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**INTRODUCTION and DRUG HISTORY:**

The IND for pro-ara-G (Nelarabine) has been at the FDA since 1994.

Nelarabine is a pro-drug for araG, an antimetabolite primarily for the treatment of leukemias and lymphomas. Toxicity has been investigated in mice, monkeys, and dogs, although dogs are not considered to be good models due to short half-life compared to humans. The major toxicities are hematologic and neurologic.

The sponsor would like to increase the impurities levels of — from —. The percentages of the impurity in drug substance batches ranged from —, while the impurity in the drug product ranged from — in stability testing.

**PREVIOUS CLINICAL EXPERIENCE:**

Nelarabine has been studied clinically in both adults and children for leukemias and lymphomas. Doses of greater than 1.2 g/m<sup>2</sup>/day on a daily X 5 schedule or 1.5 g/m<sup>2</sup> on day 1, 3, 5 schedule every 21 or 28 days have proven too toxic. The pediatric dose in at least 1 trial was set at 0.9 g/m<sup>2</sup> DX5. CNS toxicities are the most prevalent and include changes in mental status, somnolence, photophobia, seizures, neuropathy, paralysis, myoclonic jerks, and a Guillian Barre-like syndrome. These toxicities were observed as early as after a single course. Other reported toxicities include myelosuppressive effects (fungal infection, fever), and increased heart rate.

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**TOXICOLOGY:**

1. RD1997/0403/00. Nelarabine: an intravenous toxicity study in cynomolgus monkeys given 506 U78 and fludarabine. Serial # 113

Conducting laboratory and location: —

Date of study initiation: 3/14/97

GLP compliance: YES QA status: YES

Methods (if unusual): Fludara was administered iv 4 hours prior to Nelarabine on days 1, 3, 5, 7, 9, 11, and 13 (7 doses on alternate days). Monkeys were from a pool of previously treated animals that had not received another drug within the past 3 weeks.

Species and strain: Cynomolgus monkeys

#/sex/group: 2/sex/dose

age: approximately 4-9 years

weight: M: 3.1-5.1 kg; F: 2.0-3.7 kg  
 drug, lot#, radiolabel, % purity: Nelarabine: batch # 7A2793/PD13297; Fludara: lots W60153, W60178, W60201, W60155  
 formulation/vehicle: 0.9% NaCl for Nelarabine, sterile water for fludara, control = saline  
 dosage groups in actually administered units: fludara administered 4 hours prior to Nelarabine on days 1, 3, 5, 7, 9, 11, 13.

Group #	Fludara doses		Nelarabine doses	
	Mg/kg	Mg/m2	Mg/kg	Mg/m2
1	0	0	0	0
2	0	0	200	2400
3	5	60	0	0
4	5	60	50	600
5	5	60	100	1200
6	5	60	200	2400

route, form, volume, infusion rate: intravenous; 0.2 mL/kg bolus for fludara, 40 mL/kg as a slow injection (10 mL/min) for Nelarabine

**Observations:**

Clinical signs (twice daily): One male and one female monkey in G5 and G6 died between days 15 and 19. Clinical signs at doses of 100 to 200 mg/kg 506 U78 (with or without fludara) included severe convulsions, hunched posture, tremors, hypoactivity, emesis after dosing, and ataxia. These signs were seen primarily at/after day 13. Ataxia persisted through the end of the observation period.

Body weights (on dosing days and weekly thereafter): There were no noteworthy changes in body weight in the females. One G6 male lost 0.4 kg during the course of the recovery period (all other monkeys gained some weight).

Food consumption (daily): No significant differences were noted.

Ophthalmoscopy (prior to treatment, days 14, 27): There were no remarkable changes.

Hematology (pretest, day 13, 24, 36, 58): With only 1 or 2 animals/sex/dose, changes were difficult to interpret (and are represented by approximations). Changes in group 2 (Nelarabine only) and groups 5 and 6 (506 U78 + fludara) were usually similar. The findings are summarized in the following table. Values resolved by day 58.

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Parameter	Males		Females	
	Day of nadir	% change	Day of nadir	% decrease
RBC #	24	↓40%	24	↓25% (G6 63%)
Platelet #	24	↑2-3X	---	---
WBC #	13	↓75%	13	↓60%

Serum chemistry (pretest, day 13, 24, 36, 58): AST and ALT were increased by 6-8 fold at day 13 in G2, G5 and G6 monkeys of both sexes.

Gross pathology (only on animals found dead or sac'd moribund): There were no observations that were common to all 4 early death monkeys. In 3/4 (exception G5 female), dark areas of the colon and cecum were observed. The liver had pale areas or prominent reticular patterns in the G5 male and G6 female. The G6 male had a soft, dark grey area in the brain. The adrenals were enlarged in the G6 male and the G5 female.

Histopathology (only on animals found dead or sac'd moribund, saved but not analyzed).

Toxicokinetics (days 1, 13, prior to fludara, 1, 2, 4, hours post fludara, immediately following Nelarabine, and 0.5, 1, 3, 5 hours post-dose): The report was not included in this submission.

#### OVERALL SUMMARY AND EVALUATION

Nelarabine was tested in monkeys on an alternate day schedule for 7 doses (over 14 days) alone and in combination with fludara. Myelosuppression and neurotoxicity was observed both with Nelarabine by itself and with fludara; however, the myelosuppressive effects were more severe in the drug combination, resulting in some deaths. Neurotoxicity has also been observed in the DX5 and DX30 monkey studies. In the alternate day study in monkeys, CNS effects were seen at 100 mg/kg/day (1200 mg/m<sup>2</sup>) at the end of the cycle. Human neurotoxicity has been observed after a single cycle at 1500 mg/m<sup>2</sup>/day.

The sponsor would like to increase the impurities levels of \_\_\_\_\_ from \_\_\_\_\_ to \_\_\_\_\_. The percentages of the impurity in drug substance batches ranged from \_\_\_\_\_ while the impurity in the drug product ranged from \_\_\_\_\_ in stability testing. \_\_\_\_\_ according to the RTECS database, is a mutagen.

The concentrations (actual and proposed) of \_\_\_\_\_ are shown in the following table. The recommended human dose for the studies used was \_\_\_\_\_ on alternate days for 3 doses/cycle in adults and \_\_\_\_\_ daily for 5 consecutive days/cycle for children. The differences between the sponsor's calculation of cumulative dose of the impurity and the reviewer's dose are due to use of different conversion factors between body surface area and body weight. Note that the amount/dose is based on the assumption that \_\_\_\_\_ of the dose is \_\_\_\_\_. No data was provided on actual levels in the study.

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Actual and proposed levels of _____								
Species	Schedule	Dose Nelarabine		Amount/dose		Cumulative dose		Sponsor's cumulative dose
		Mg/kg	Mg/m2	Ma/ka	Ma/m2	Ma/ka	Mg/m2	Mg/kg
Monkey	Alt day X 7	200	2400					
Human child	DX5							
Human adult	Alt day X 3							/

Number in ( ) represents sponsor's calculation of daily dose of Nelarabine.

From the table above \_\_\_\_\_ has been tested in animals at levels approximately equivalent to those used in the clinical trials at the proposed impurity limits. Judging from the structure, the compound is \_\_\_\_\_

Given that impurities of up to \_\_\_\_\_ have already been found in the stability batches, one might assume that the clinical batches would have similar levels, and thus the purity might be qualified under ICH Q3A. There are no pharm/tox objections to increasing the current specifications.

**RECOMMENDATION** : There are no pharm/tox objections to increasing the current specifications.

a) **Comments for further studies:** none

**Draft Letter to the Sponsor:** none

\_\_\_\_\_  
Wendelyn J. Schmidt, Ph.D.  
Pharmacologist/Toxicologist

\_\_\_\_\_  
Date

Concurrence:

\_\_\_\_\_  
David Morse, Ph.D.  
Pharmacology Supervisor

\_\_\_\_\_  
Date

Original IND/NDA/DMF

c.c. /Division File  
/WSchmidt  
/DMorse  
/XHChen, chemist  
/Mpelsoi, project manager

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/s/

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William McGuinn  
10/27/2005 05:45:04 PM  
PHARMACOLOGIST

David Morse  
10/28/2005 09:05:57 AM  
PHARMACOLOGIST