CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-007
21-039

PHARMACOLOGY REVIEW(S)

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HFD-530

Sponsor: Glaxo Wellcome Co.
Five Moore Drive
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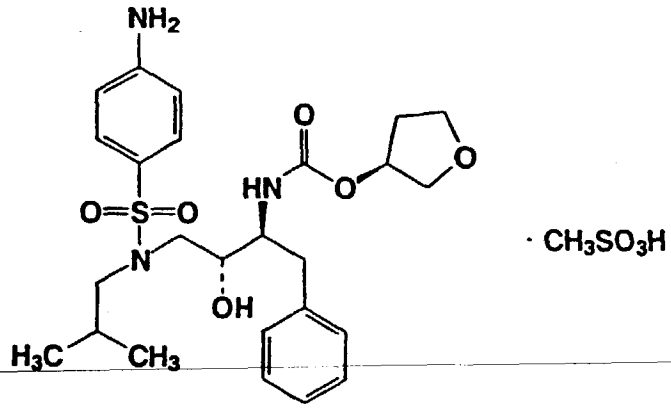

Drug: Agenerase (amprenavir)
Alternative names: 916.D, VX-478, 141W94
Chemical name: (3S)-tetrahydro-3-furyl N-[(1S,2R)-3-(4-amino-N-isobutylbenzenesulfamido)-1-benzyl-2-hydroxypropyl] carbamate.

Molecular formula: $C_{25}H_{35}N_3O_6S$
Molecular Weight: 505.64

Formulation:

Each capsule contains, amprenavir, (150 mg), and the inactive ingredients TPGS (tocopheryl polyethylene glycol 1000 succinate ———), polyethylene glycol 400, (PEG 400) propylene glycol. The capsule shell contains d-sorbitol and sorbitans solution, gelatin, glycerin and titanium dioxide. The soft gelatin capsules are printed with edible ink.

Related IND's: ———

Proposed Indication: Treatment of HIV infection.

Introduction:

141W94 inhibits the HIV-specific protease, an enzyme critical to the posttranslational processing of some viral proteins. The K_i values for the inhibition of HIV-1 and HIV-2 proteases were 0.6 and 0.9 nM respectively while the K_i values against host cellular aspartyl proteases were as follows: Pepsin, 3200 nM; cathepsin D, > 10,000 nM; Renin, 1750 nM.

In HIV-1 (strain IIIB) in MT4 cells (a human leukemia cell line transformed with HTLV I) and peripheral blood lymphocytes (PBL's), the mean IC_{50} 's are 0.084 and 0.08 μM respectively. In a chronically infected HIV-1- producing cell line (H9IIIB), the mean IC_{50} for 141W94 was 0.4 μM μM. In PBL's, the mean IC_{50} against six zidovudine-sensitive strains was 0.012 μM and the mean IC_{50} value against three zidovudine resistant isolates was 0.019 μM . Inhibition of the virally encoded protease is therefore assumed to be the mechanism of action of 141W94 as an anti-HIV agent.

Toxicology Studies Summary

1. Acute oral toxicity study in the mouse with 141W94 ——
2. Acute oral toxicity study in Sprague Dawley rat with 141W94 ——
3. Acute intravenous toxicity study in the mouse with 141W94 ——
4. Intravenous toxicity study in Sprague Dawley rat with 141W94 ——
5. Single dose toxicity study in monkey.
6. A two-phased oral dose rangefinding with 141W94 in Sprague Dawley rats
7. One-month oral toxicity study in rats given 141W94 ——
8. Twenty-eight day repeat dose oral gavage study in rats with 141W94.
9. Oral gavage three month study in rats
10. Oral gavage six month study in rats
11. Twenty-eight day oral gavage study in dogs
12. Oral gavage study in dogs for three months
13. Six month oral gavage study in dogs
14. Twelve month oral gavage study in dogs
15. Twenty-eight day oral gavage study in monkey
16. Insemination study of amprenavir in male rats.
17. Fertility study in male SD rats after administration of 141W94 ——
18. Oral female fertility and embryo fetal development study in the Han Wistar rat.
19. Dose rangefinding study in the nonpregnant female rabbit.
20. Oral embyro-fetal development study in New Zealand White rabbits after dosing with 141W94.
21. Pre and Post-natal development study in Han Wistar rats after oral dosing with 141W94.
22. *Salmonella*/Mammalian-microsome assays with 141W94 ——
23. 141W94: *Salmonella* and *E.coli*/microsome reverse mutation preincubation and standard.
24. 141W94: Microbial mutagenicity study: liquid preincubation and standard plate incorporation assay.
25. 141W94 (spiked): *Salmonella* and *E.coli*/microsome reverse mutation plate incorporation and preincubation assays.
26. L5178Y/tk$^{\pm}$ mouse lymphoma mutagenesis study with 141W94.
27. An in vitro cytogenetic study in cultured human lymphocytes with 141W94

Toxicology Studies Review

1. An Acute Oral Toxicity Study in the mouse with 141W94 —— GLP study. Report TTEP/94/0052. Study # ACU 605. Glaxo Wellcome Co. 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709. July 1994. Drug reference # 94/0239-105-A.

Groups of Crl:Cd-1(CR)BRVAF/Plus mice (5 male mice per group except for Group 1 which also had 5 female mice) were treated orally, by gavage, with a single dose of 141W94 —— as follows: Group 1, 3000 mg/kg; Group 2, 2000 mg/kg; Group 3, 1500 mg/kg; Group 4, 1000 mg/kg. Drug was dissolved in PEG 400/Na acetate/methanesulfonic acid. Animals were

observed for 14 days and records were kept of mortality, clinical signs and body weights. Animals were sacrificed on postdose day 14 and gross pathology findings recorded.

Only the 1000 mg/kg group was observed for clinical signs beyond the day of dosing. Signs observed included rough coat, prostration, shallow, labored breathing, dehydration, prolapsed penis, scabs on tail or hind-quarter and one animal was cool to the touch.

Table 1. Clinical signs seen on the day of dosing of mice with oral 141W94

Dose of 141W94 (mg/kg)	1000	1500	2000	3000	3000
Sex	male	male	male	male	female
Number of animals	5	5	5	5	5
Deaths	1	3	2	3	none
Clinical signs on day of dosing:					
Eyes partially closed	2	2	3	3	1
Salivation			5	1	1
Decreased activity	2	2	2	3	
Yellow anal-genital stains		3	3	1	
Cool to touch				2	
Labored or shallow breathing		2	2	1	
Bodyweight change	NR	NR	NR	NR	NR
Gross Pathology					
White granular material in stomach			2	2	
Enlarged spleen	1				

NR: not remarkable

Conclusion

The oral minimum lethal dose of 141W94 was 1000 mg/kg for male and >3000 mg/kg for female Crl:Cd-1(CR)BRVAF/Plus mice.

2. An Acute Oral Toxicity Study in the Sprague Dawley rat with 141W94 — GLP study. Report TTEP/94/0053. Study # ACU 606. Glaxo Wellcome Co. 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709. July, 1994. Drug reference number 94/0239-105-A.

Groups of Sprague Dawley rats, 5 per sex per group, were treated with a single dose of 141W94 — via oral gavage. Drug was dissolved in PEG 400/Na acetate/methanesulfonic

acid. Animals were observed on the day of dosing and daily thereafter for 14 days and were sacrificed on postdose day 14. Animals were necropsied and gross pathology findings recorded.

Apart from salivation, seen in all rats for 8 to 23 minutes following dosing, no clinical signs were observed. There were no deaths, changes in bodyweight or treatment-related gross findings. Median lethal oral dose > 3000 mg/kg in Sprague Dawley rats.

3. An Acute Intravenous Toxicity Study in the Mouse with 141W94 ——. GLP study. Report TTEP/94/0050. Study # ACU 603. Glaxo Wellcome Co. 3030 Cornwallis Road, Research Triangle Park, North Carolina 27709. August 1994. Drug reference number 94/0239-105-A.

Groups of Charles River CD-1 mice, 5 per sex per group, were treated with a single intravenous dose of 0, 50, 75 or 100 mg/kg 141W94 —— Drug was dissolved in a PEG 400/Na acetate/methanesulfonic acid vehicle. Control animals received vehicle only. Animals were observed on the day of dosing and daily thereafter for 14 days and were sacrificed on postdose day 14. Animals were necropsied and gross pathology findings recorded.

Mortality

There were five deaths during this study: one (male) in the 75 mg/kg group and four (one male and 3 females) in the 100 mg/kg group. All deaths occurred within 2 minutes of dosing and were preceded by clonic convulsions and ataxia. The median intravenous lethal dose of 141W94 was calculated to be 130 mg/kg for males and 99 mg/kg for females.

Toxicity

Onset of clinical signs was immediate or up to 43 minutes postdose. Clinical signs included clonic convulsions, labored breathing, ataxia, prostration and began immediately after dosing or up to 43 minutes thereafter. Since labored breathing, ataxia and prostration were also seen in the control group, these were not thought to be drug related. However, clonic convulsions seemed to be drug-related since they were restricted to the 75 mg/kg group (one male) and the 100 mg/kg group (2 males and 3 females). Animals returned to normal within 5 hours of dosing and, apart from scabs associated with the injection sites, did not show any drug-related symptoms for the duration of the 14-day observation period. There were no changes in bodyweight and no remarkable findings in gross pathology. The median intravenous lethal dose of 141W94 in mice was calculated to be 130 mg/kg for males and 99 mg/kg for females.

Table 2. Number of mice showing clinical signs after intravenous 141W94

Clinical Sign	Dose of 141W94 (mg/kg)			
	0	50	75	100
Clonic convulsion			1/10	5/10
Ataxia	10/10	9/10	9/10	10/10
Prostration	2/10			2/10
Labored breathing	3/10	3/10	3/10	3/10

4. An Intravenous Toxicity Study in the Hsd:Sprague Dawley SD rat with 141W94 ——— GLP study. Report TTEP/94/0051. Study ACU 604. Glaxo Wellcome Co. 3030 Cornwallis Road, Research Triangle Park, North Carolina, 27709. August, 1994. Drug reference # 94/0239-105-A..

Sprague Dawley rats (5/sex/group) were given single intravenous doses of 0, 0, 100 or 200 mg/kg 141W94. Drug was dissolved in a PEG 400/Na acetate/methanesulfonic acid vehicle. Control animals received vehicle only. There were two control groups (5 rats/sex/group) which were established to control for the differences in dose volume in the drug treated groups. Dose volume was 5 ml/kg for control group A and the 100 mg/kg group. Dose volume was 10 ml/kg for control group B and the 200 mg/kg group. Animals were observed for 13-14 days and records were kept of clinical signs, bodyweights, mortality and gross pathology.

Mortality

There were 9 deaths in this study, one control male from control group B and 8 (4 males and 4 females) from the 200 mg/kg group. All deaths occurred within 3 minutes of dosing and were associated with labored breathing, clonic convulsions and prostration.

Toxicity

Clinical signs began up to 3 minutes after dosing and included clonic convulsions, ataxia, prostration, gasping and labored breathing. Surviving animals appeared normal approximately 3 hours after dosing. There were no changes in body weight or gross pathology.

Table 3 . Incidence of clinical signs after intravenous 141W94 in Sprague Dawley rats.

Dose of 141W94	0 mg/kg		100 mg/kg		200 mg/kg	
Sex	M	F	M	F	M	F
Clonic convulsions	1/10				5/5	1/5
Ataxia	9/10	10/10	3/5	5/5	1/5	
Prostration	1/10		5/5	4/5	1/5	5/5
Gasping					1/5	
Labored breathing	4/10	5/10	5/5	5/5	5/5	5/5

The median lethal intravenous dose of 141W94 was 189.3 mg/kg for male and female Harlan SD rats.

5. Single dose toxicity study in cynomolgus monkeys. Study —53/934056. Study performed by [redacted]. GLP Study. August 1994. Drug batch number 916.D.94.501.

This study was designed to determine the toxic effects of a single oral gavage dose of KVX-478 (hereafter referred to as 141W94) in cynomolgus monkeys. Three groups of monkeys, one/sex/dose group, were dosed with either 500, 1000 or 2000 mg/kg 141W94. Drug was prepared as a 200 mg/ml solution in 20 % water/PEG 400 vehicle for oral gavage. For two weeks after dosing, records were kept of clinical signs (daily), bodyweight (once predose and twice weekly after dosing), food consumption (daily), urinalysis (predose, and on day 10/11) hematology and biochemistry (predose and day 14). Animals were sacrificed after the end of the study, but were not examined further.

Mortality: There were no deaths during the study.

Toxicity

Salivation and vomiting were the most common toxic effects. In the low dose group, the male vomited 40 minutes after dosing, while the mid- dose monkeys vomited after an hour (male) or overnight (female). High dose animals vomited within two minutes of dosing (male) or after half an hour (female). Salivation was noted in low and high dose animals. High dose animals were unusually quiet between one and four hours postdose. Pale and/or loose and/or liquid feces were noted in one low dose, two mid dose and one high dose animals.

Significant changes associated with 141W94 also included an increase in total protein (mean increase, 7 %) and increased urea (mean increase, 22 %).

Summary and Conclusion

Single dose administration of 141W94 to cynomolgus monkeys resulted in salivation, vomiting and loose feces. Slight changes in total protein (+7%) and urea (+22%) were also noted. It is unclear how much the vomiting reduced the effective dose administered to the animals as toxicokinetic evaluations were not performed. These results may therefore not reflect the full profile of toxicological effects of these doses in cynomolgus monkeys.

6. A Two-phased oral dose-range finding study with 141W94 in Hsd:Sprague Dawley SD rats. Non-GLP study. Report # TTDR/94/0025. Study DRF 680. Glaxo Wellcome Co. Five Moore Drive. P.O. Box 13398. Research Triangle Park, North Carolina, 27709. May, 1994. Drug batch # 94/0236-005-A. Vehicle: PEG 400.

This study was designed to characterize the toxic effects of amprenavir after a single dose and after 7 days of dosing. Groups of 2-3 animals were treated with single doses of 25, 50, 100, 250 or 500 mg/kg (groups 1-5) or 1500 (750x2), 3000 (1500x2), 3000 (3000x1). Dose volumes were 5 ml/kg/day for groups 1-5, and 10 ml/kg/dose initially until day 4 when dose volume was reduced to 5 ml/kg/dose.

Dosing at 1500 mg/kg/dose, twice daily, resulted in the deaths of one male and one female. All animals dosed at the highest dose volume had stomach filled with food and precipitated drug. Deaths were ascribed to regurgitation and aspiration of drug. Other sign included postdose salivation (all dose groups), coolness to the touch, and soft/watery feces(doses 500 mg/kg and above). Animals dosed above 1500 mg/kg showed labored breathing, decreased activity, brown stains in the facial/genital area and urine stains.

Table 4. Pharmacokinetics data

Dose (mg/kg/dose)	AUC (0-2h) (μM.hr)	Cmax (μM)	Tmax (hr)
Phase 1			
25	1.8	1.8	1.0
50	6.6	5.2	0.5
100	10	7.7	1.0
250	17	12	0.7
500	24	18	0.5
Phase 2			
750	15	16	0.7
1500	27	28	0.5
3000	79	48	0.5

There was a greater than dose proportional increase in AUC between 25 and 50 mg/kg/day. At the higher doses there was great variability in plasma levels, but there seemed to be saturation above 500mg/kg/dose.

The maximum dose to be used for further toxicology studies was determined to be 1500 mg/kg.

7. One month oral toxicity study in HSD:Sprague Dawley SD rats given 141W94 ——— GLP study. Report TTEP/94/0054/01. Study # TOX 686. Glaxo Wellcome Co. 3030 Cornwallis Road, Research Triangle Park, North Carolina, 27709. July, 1994. Drug reference # 94/0239-105A.

Five groups of Sprague Dawley rats were treated daily by oral gavage for 32-33 days according to the following protocol:

Table 5. Treatment protocol for one month oral toxicity study of 141W94 in rats

Group #	Treatment	Dose (mg/kg)	# of rats per sex per dose
1	vehicle[a]	5[b]	15
2	141W94	100	10
3	141W94	500	10
4	141W94	1000	15
5	0.5 % methylcellulose	5[b]	5

a: vehicle control consisted of (20 % (v/v) water in PEG 400, methanesulfonic acid and sodium acetate. b: vehicle and control solutions were given at 5 ml/kg.

Animals were dosed twice daily with 6 hours between doses. Food was removed approximately 2 hours after the first daily dose to increase bioavailability. Animals were generally sacrificed at the end of the study (day +1). However, five animals of each sex from groups 1 and 4, and 2 animals per sex from group 5 were randomly preselected to be subjected to a two weeks drug-free recovery period. These animals were sacrificed on study day +15 and these animals allowed the sponsor to determine the reversibility of toxicities. Records were kept of clinical signs, body weights, food consumption, ophthalmoscopic examinations, plasma drug levels, hematology, clinical chemistries, urinalyses, organ weights, gross pathology and histopathology. Tissues examined included adrenal glands, aorta, bone marrow, brain, cervix, epididymes, esophagus, eyes, femur, harderian gland, heart, kidneys, large intestines, larynx,

liver, lungs, lymph nodes, mammary glands, ovaries pancreas, parathyroid gland, prostate gland, salivary glands, sciatic nerves, skeletal muscle, skin, small intestine, spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus and vagina. Three separate groups of animals (12/sex/group) were treated with 100, 500 and 1000 mg/kg 141W94 for pharmacokinetic analyses only. Plasma was drawn on study day #2 (predose and 0.5, 1, 2, 4 and 6 hours after the first daily dose and prior to the second daily dose) and on study day # 29 (predose, 0.5, 1, 2, 4, 6, 8, and 10 hours after the first daily dose, prior to a second dose.

Mortality: There were no deaths in this study.

Toxicity

The administration of 141W94 was associated with a number of reversible changes including post-dose salivation, decreased triglycerides, decreased cholesterol (males), increased cholesterol (females), increased urine output, thyroid follicular hyperplasia and/or hypertrophy and increased ovary weights. Among the irreversible changes were increased adrenal weights (females only) and increased liver weights. All of the above changes were seen in rats given 141W94 at 500 and 1000 mg/kg/day. At the 100 mg/kg level, the reversible decrease in cholesterol (males) was not observed and neither were the thyroid changes. At the lowest dose level (100 mg/kg, equivalent to a human dose of 15.9 mg/kg based on body surface area) irreversible changes in the adrenals (females) and the liver were still being observed. Changes observed upon ophthalmoscopic examination included nuclear cataracts and/or anterior uveitis, which were detected in two group 4 male and two group 3 females.

Table 6 . Mean adrenal weights in female SD rats administered 141W94 for 30 days with 14 day recovery group

Group	Adrenal weight (mg)	
(mg/kg 141W94)	Study day +1	Study day +15
0	57.5	65.4
100	68	
500	73.8	

Table 7. Mean adrenal weights in female SD rats administered 141W94 for 30 days with 14 day recovery group

Group	Adrenal weight (mg)	
(mg/kg 141W94)	Study day +1	Study day +15
0	64.7	60
1000	76.0	78.0

Comment: Sponsor has been vigilant in screening for changes in ophthalmoscopic parameters in patients receiving this drug.

Male rats and female rats respond differently to this drug. Cholesterol levels increase or decrease depending on the sex of the rat and some toxicities such as increased adrenal weights, occur only in female rats.

8. Twenty-eight day repeat-dose oral gavage study in rats with VX-478. GLP study. Study # N001768B. October 1995. Drug lot # GP-336-34-1.

Four groups of Sprague Dawley rats (eighteen/sex/group) received either vehicle (Group 1) or formulated test article at doses of 250 (Group 2), 500 (Group 3) or 1000 mg/kg (Group 4), by oral gavage, twice per day, approximately twelve hours apart, for twenty eight days.

Twelve animals in each group were designated toxicokinetics animals. Blood for plasma level determinations was taken from a subgroup of 4 toxicokinetics rats/sex/dose group on day 1 (prior to dosing and at 0.5, 1, 2, 4, 6, 8, and 12 hours postdose), from a separate subgroup of 4 rats/sex at 2 and 8 hours postdose on days 7, 14 and 21, and from a third subgroup of 4 rats/sex/dose on day 28 (prior to dosing, and at 1, 2, 4, 8 and 12 hours postdose). Each subgroup of toxicokinetics animals were euthanized following the terminal bleed and discarded. Records were kept of clinical signs, mortality, moribundity, body weights, food consumptions, hematology, serum chemistry, gross examination, organ weight evaluations and histological examinations for core toxicology animals only.

Tissues examined included adrenal glands, aorta, bone marrow, brain, cervix, epididymes, esophagus, eyes, femur, harderian gland, heart, kidneys, large intestines, larynx, liver, lungs, lymph nodes, mammary glands, ovaries pancreas, parathyroid gland, prostate gland, salivary glands, sciatic nerves, skeletal muscle, skin, small intestine, spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus and vagina.

Mortality

Eight animals died during the course of this study. Deaths included one control, two low dose, one mid dose, and four high dose rats. Pathology reports were only filed for three of these animals which belonged to the core toxicology group.

The sponsor contends that 7 of the 8 deaths were related to accidental trauma. However, histopathology findings were only reported for three animals, which belonged to the core toxicology group (see Table 1.)

Table 8: Histology findings in animal deaths from 28-day rat study with 141W94

Dose group	Day	Findings
Control	2	Perforated esophagus
250 mg/kg	5	No lesions
1000 mg/kg	8	Inflamed, dilated, degenerated esophagus

In the absence of pathology reports for five of the eight deaths in this study, the sponsor's claim (that death in these instances were due to punctures of the esophagus) cannot be substantiated. Twice as many rats died in the high dose group, than in any other group, and, in the absence of evidence to the contrary, these deaths must be assumed to be drug related.

Toxicity

The sponsor reports problems with gavage of the drug and reports that animals were very resistant to the gavage process. This was ascribed to a potentially unpleasant taste or odor associated with the drug substance.

Clinical signs included red nasal discharge, salivation, urine stains and/or rough haircoat. Soft stool and or diarrhea were noted in animals from all groups and may be related to the stool softening properties of the vehicle. Statistical differences in clinical chemistry values included elevated total protein, albumin, globulin, phosphorus and calcium values and were ascribed to dehydration associated with the chronic soft stool/diarrhea. Depressions were also found in aspartate aminotransferase, creatinine phosphokinase, triglycerides, lactate dehydrogenase and alkaline phosphatase. Increased mean glucose values may be due to stress-related increased adrenal cortical activity. Gamma glutamyl transferase was slightly elevated, and mean cholesterol values were increased in mid and high dose females, suggesting that the liver was affected by drug therapy.

In addition to the esophageal trauma, reported above, gross changes were observed in one group 4 male, which had calculi in its urinary bladder, associated with severe urocystitis and

hydronephrosis. Mean absolute and relative liver and adrenal weights were also significantly elevated. Increased lung weight was slight and was not dose related and was probably associated with a pulmonary reaction to test substance aspirated during the gavage process. Liver enlargement was associated with centrilobular hepatocellular hypertrophy, with affected cells showing increased cytoplasm. Tubular degeneration in male but not female rats (1,4,4,5 of 6 male rats affected and 1, 1, 2, 1 female rats from 0, 250, 500, and 1000 mg/kg doses. There were no histopathological correlates with increased adrenal weights.

Ophthalmic Findings

In both sexes, there was an increase in the occurrence of mild corneal crystals seen in one or both eyes during the study. One male and one female showed a bubble-like cataract. Irregular translucent appearance of the cornea was noted in some male and female rats. Since these findings were also noted in the control animals, these findings may be associated with the vehicle.

Pharmacokinetics

141W94 was rapidly absorbed, with a Tmax between 1 and 4 hours. Plasma levels remained fairly constant up to 12 hours post dose. Cmax and AUC values increased with dose, but were not dose proportional. Plasma concentration (Cmax) decreased over time, and was lower on day 28 than on day 1 (see Table 2) in rats. This may indicate that the drug induces its own metabolism, and so is eliminated at a faster rate following repeated administration. The AUC's in the mid and high dose rats were higher than the exposures obtained in the high dose humans in the sponsor's phase one study (see Table 3). In fact, since the low dose AUC in the rat study was similar to the high dose AUC for the human study, and due to the autoinduction phenomenon, then 28 day administration of 141W94 to humans given 1200 mg (see Table 3) may be reasonably expected to produce a toxicity profile similar to that of the low dose rats in the 28-day study.

Table 9. Pharmacokinetic parameters in rats receiving 141W94

Dose (mg/kg)	Tmax (h)		Cmax (μg/ml)		AUC(hr*μg/ml)	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
250	3.2	2.5	5.0	4.1	41	26
500	1.4	3.6	7.4	4.1	56	34
1000	2.1	3.2	11	6.9	89	51

Table 10. Pharmacokinetics of 141W94 in Humans

Dose (mg)	Dose mg/kg	AUC (hr*μg/ml)
150	2.5	4.0
300	5.0	9.1
600	10	21
900	15	32
1200	20	47

Summary and Conclusions

This drug product seems to target the liver, adrenals, eyes and kidneys. Deaths occurred among the animals treated at 1000 mg/kg, a dose equivalent to a human dose of 159 mg/kg (or 9.5 grams/day) for 30 days. Based on pharmacokinetics data, humans receiving 1200 mg/day for 28 days may be expected to have exposures similar to rats receiving 250 mg/kg/day for 28 days. Adequate monitoring of these organs, incorporated into the clinical study protocol, should allow detection of any changes before serious harm is done to patients.

9. 141W94: Oral gavage 3-month toxicity study in rats. GLP study. Report TTEP/96/0017. Study TOX 771. Glaxo Wellcome Co. 3030 Cornwallis Road, Research Triangle Park, North Carolina, 27709. October 1995. Drug batch numbers 95/0351-110A, 95/5350-044 and 95/5350-045).

Groups of Han Wistar rats were treated with 141W94 as described in Table 11.

Group	Treatment	Dose (mg/kg/day)	Number of animals/sex
1*	vehicle	0	20
2	water	0	20
3	141W94	50	20
4	141W94	160	20
5*	141W94	500	20
6	141W94	50	12
7	141W94	160	12
8	141W94	500	12

*10 rats from 1 and 5 kept for 30 day recovery study.
Rats dosed twice daily 6 hours apart.

Animals were dosed twice per day, approximately 6 hours apart for 91 to 95 days. Animals were subjected to terminal necropsy one day after the end of the dosing period (main study group) or 32 days after the end of dosing (recovery group). Tissues examined included adrenal glands, aorta, bone marrow, brain, cervix, epididymes, esophagus, eyes, femur, harderian gland, heart, kidneys, large intestines, larynx, liver, lungs, lymph nodes, mammary glands, ovaries pancreas, parathyroid gland, prostate gland, salivary glands, sciatic nerves, skeletal muscle, skin, small intestine, spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus and vagina. Drug concentrations were measured in plasma on days 1, 28, 56 and 84. On those days blood was taken from the rats pre-dose, and approximately 1, 2, 6 (just prior to the second daily dose) and 7, 8, 10 and 24 hours following the first daily dose.

Mortality

There were no excess deaths among the treated animals. One group 1 male (vehicle control) was found dead during week 8 and one group 5 male (500 mg/kg) was found dead during week 13.

Comment: There is a reduction in the number of pharmacokinetics female animals from 12 to 11 between weeks 1 and 2. There is no explanation for the absence of this animal. The sponsor also summarized that there were no drug-related deaths.

Toxicity

Salivation was associated with drug administration and was seen in all animals postdose, beginning on study day 1 and persisting throughout the study.

Hyperproteinemia (dose related) was observed at doses greater than 160 mg/kg/day and increases ranged from 3 to 17%. Albumin was increased in a dose-related fashion by 3-15%. Globulin increased 5-25%. Triglycerides were decreased by 25-57% in males only. ALT was increased by 54 to 142 % but the increase was not dose related and was not seen in females. All clinical chemistry changes were reversible.

Mean values for urine quantities were increased in drug-treated animals and the increases were in the range of 50% for males and 190% for females on day 85. Increases were not dose related.

Drug administration was associated with dose related increases in absolute and relative liver adrenal, ovarian and thyroid weights. Absolute and relative liver weights were increased by approximately 20 to 70 % at doses of 160 mg/kg/day and above. Hypertrophy was generally

centrilobular but became panlobular in some rats at the highest dose. Hypertrophic hepatocytes had more cytoplasm and larger nuclei than normal and cytoplasm was more basophilic. Male rats only showed multinucleated hepatocytes (3 or more nuclei) at all doses, and the incidence and degree of vacuolation was dose related. There was also slight fat accumulation in periportal hepatocytes and an increased incidence of micro granulomas (reversible) and lipofuscin-containing macrophages (still present at the end of the recovery period). Absolute and relative adrenal weights were increased by approximately 10 to 30 % at doses of 160 mg/kg/day and above. There was a slight increase in the incidence and degree of vacuolation in the adrenal cortex at doses above 160 mg/kg/day in females and at 500 mg/kg in males. Absolute and relative thyroid weights were increased by approximately 20% at 500 mg/kg/day and thyroid findings were associated with thyroid follicular hypertrophy. Absolute and relative ovarian weights were increased by up to 20 % at doses of 160 mg/kg/day and above.

There was increased NADPH cytochrome P450 reductase activity in all doses in females and at 500 mg/kg/day in males. Other changes seen only in one sex included increased pentoxyresorufin O-dealkylation at all doses in females, increased testosterone 6B-hydroxylation in females, and slightly decreased total cytochrome P450 content at 160 mg/kg/day in females.

Pharmacokinetics

Cmax for the study was dose dependent, but less than dose-proportional. Cmax (which occurred approximately 1 hr after the second dose) was 3.0, 5.8 and 9.6 μg/ml in the low, mid and high-dose animals. While AUC's were stable throughout the dosing period at the low dose, AUC's decreased by as much as 50 % in the mid and high groups between days 1 and 28 then stabilized at the lower level. Plasma half-life was approximately 3 hours at all doses. Plasma clearance was increased (doubled) at the high dose although it remained stable at the two lower doses. 141W94 seems to induce its own metabolism

10. 141W94: Oral gavage six month toxicity study in rats. GLP study. Report RD1996/00584/00. Study TOX 773. Glaxo Wellcome Inc. 3030 Cornwallis Dr. Research Triangle Park, NC 27709. USA. December 1995. Drug reference numbers 95/5350-044 and 95/5350-045.

This study was designed to determine the effects of 141W94 when given twice daily, by oral gavage to rats for six months. 141W94 was formulated in a solution containing PEG 400, vitamin E-TPGS (D-alpha-tocopheryl polyethylene glycol 1000 succinate), propylene glycol and Han Wistar rats, from were divided into treatment groups as follows:

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Table 12. Treatment protocol

Group no.	Substance	141 Dose (mg/kg)	No. animals/sex
1	vehicle	0	30
2	water	0	20
3	141W94	50	20
4	141W94	190	20
5	141W94	750	30
6	141W94	50	12
7	141W94	190	12
8	141W94	750	12

Animals in groups 1-5 were assigned for toxicology and pathology evaluation. Animals in groups 6-8 were assigned for drug plasma determinations only (3 animals/sex/group/time point). Ten animals of each sex assigned to groups 1 and 5 were randomly selected before the start of dosing for a thirty-day post dose recovery period.

Records were kept of clinical signs (daily), food consumption (weekly), body weights (weekly), clinical pathology (study days 30 (males), 31 (females), 87 (males) 89 (females), 178 (males), 179 (females) and +27), urinalysis (study days 33, 93, 184 and + 29), ophthalmic examinations (study days -20, 17, 36, 59, 93, 122, 183 and +26). Plasma drug levels were measured predose and approximately 1, 2, 6, (just before the second dose), 7, 8, 10 and 24 hours following the first daily dose on study days 1, 31, 93 and 183). Rats were sacrificed on study days +1 and +33 and animals subjected to gross examination. Necropsy was performed and organ weights recorded. The following tissues were examined microscopically from all animals in the vehicle control and high dose groups. Target organs were examined at the low and intermediate doses as well as in all recovery animals.

Tissues examined histologically:

Adrenals, aorta, bone, bone marrow smear, brain, caecum, cervical lymph node, cervix, colon, duodenum, esophagus, eye, Harderian gland, femur, heart, ileum, jejunum, joint, kidney, liver, lung/bronchii, mammary glands, mesenteric lymph nodes, ovaries, oviduct, pancreas, parathyroids, pituitary, salivary glands, sciatic nerve, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, thymus, thyroid, tongue, trachea, urinary bladder, uterine body, uterine horn, vagina.

Mortality

Mortality was as follows (Groups 1-5 respectively): 2 (vehicle control group), 1 (water control group), 3, 3, 3 deaths. Most groups had early deaths and late deaths and numbers were similar between, males (7 deaths) and females (5 deaths). All 3 deaths in group 4 were ascribed to dosing accidents and one group 3 female, died after struggling during dosing. Cause of death was determined for all other animals.

Clinical signs

The most noticeable clinical sign was salivation, which was found in all drug-dosed groups. Salivation was noted postdose and was attributed to palatability of the dosing preparation containing 141W94. Yellow, brown or red stains in the anogenital areas as well as soft, watery or liquid feces were found intermittently.

There were no drug-related changes in body weights or food consumption, although all female groups including controls, showed higher food consumption (gram/kg/day) compared to males.

Percent and absolute eosinophils were decreased in high dose rats compared to control by as much as 30% during dosing but this change reversed by the end of the recovery period. Platelets were increased up to approximately 20% during dosing, but this trend was largely recovered during the reversibility period. There was a transient decrease in alkaline phosphatase in rats on the order of 20 % on day 30, but this decrease was less pronounced on the other study days. CPK was reduced at the two higher doses in the order of 30 %, but the values were approaching control levels by the end of dosing. While males returned to control levels by the end of the recovery period, values in the high dose females were about 60 % of control values. A slight increase in total protein, associated with a slight increase in globulin, was seen during dosing in females and returned to control levels by the end of the recovery period. Cholesterol levels were increased (by up to 92 %) in females only at the mid- and high doses. Levels remained 27 % higher than control levels at the end of the recovery period. LDH levels were about 50 % lower than controls on day 30 for males, but remained lower than controls throughout dosing in females. Reduction was typically seen in the high dose females only, but by day 178, reductions were seen in both mid dose animals (52 % of controls) and high dose animals (36 % of controls). High dose animals had LDH values 60 % of controls at the end of the recovery period. An increase in adrenal weight (27 %), detected in males at the high dose at the end of dosing, was not reproduced in the female rats. There was also a slight increase in relative adrenal weight. Mid and high dose animals showed an increase in absolute (a mean of about 25 and 60 % increase respectively) and relative liver weights (approximately 30 and 64 %, respectively) compared to controls. Weights remained increased at the end of the recovery period. Relative and absolute ovary weights were slightly increased (10 % range for all doses tested) and remained increased after recovery period.

AST and ALT activities were elevated at all doses by the end of the study. Changes were first seen at the 90 day time point in low dose males. In these animals, the magnitude of the increase was inversely proportional to dose and, by the end of the dosing period was equivalent to three times control levels for AST and four times control for ALT at the low dose. In females, AST and ALT activities were not increased during dosing, but these activities were increased at the end of the recovery period (AST and ALT were 1.3 and 2 times control respectively). A dose related inhibition of the age-related increase in triglycerides levels was observed in all drug treated animals, beginning at day 30. Levels were on the order of 50 % compared to controls at the high dose and remained 25 % lower than controls at the end of the recovery period. Vehicle associated changes (which were seen in all rats given vehicle with or without controls) included higher urine specific gravity, and higher electrolyte concentrations (increased sodium, potassium and chloride, 2 to 3 times control levels at all doses).

Absolute and relative liver weights were increased at all doses and the increases ranged from 9 % at the low dose, to about 80 % in high dose females. Adrenal weights were also elevated at all doses, ranging from 10 % to 28 %. Thyroid weights were elevated at the high dose up to 13 % above vehicle controls. Ovary weights were also about 10 % higher than control levels at all doses.

Histopathological changes were prominent in the liver and included reversible, dose-dependent hepatocellular hypertrophy at all doses in both sexes. Multinucleated hepatocytes were seen at all dose groups and (although only in females given 190 mg/kg and above). Granular brown pigment was seen in hepatocytes, at doses above 190 mg/kg (only at 750 mg/kg in females) and seemed to be partially reversible. Hepatocyte vacuolation was seen at doses above 190 mg/kg/day in both sexes but was absent in the reversibility group. Hepatocellular necrosis was seen at all doses and in both sexes, although necrosis did not become apparent until the end of the recovery period for high-dose animals. Granular, golden-brown pigment was detected in hepatocytes at the end of the recovery period. Irreversible thyroid follicular hypertrophy was present in most rats at the end of the recovery period. Fine brown pigment was also seen in tubular epithelial cells of the kidney at the end of the recovery period, and this was not reversible.

Pharmacokinetics

There were no clear differences in the pharmacokinetics of this drug between male and female rats. Cmax levels increased with dose but were less than dose proportional with mean Cmax values of 2.3, 5.1 and 15.9 μg/ml for 50, 190 and 750 mg/kg doses respectively. AUC values increased with dose but were less than dose-dependent. These $AUC_{0\text{-}24}$ values decreased by 40 to 80 % between study days 1 and 31 in all dosed groups except low dose males, in which they remained constant. After day 31, the $AUC_{0\text{-}24h}$ values remained constant throughout the remainder of the study. Mean AUC values, averaged over the 93 and 182 day time points were 15, 46 and 102 h*μg/ml for 50, 190 and 750 mg/kg doses. Estimated half life was between 3 and 3.5 hours for all doses.

There was no NOAEL level for this drug. A number of changes including liver necrosis

and increased liver enzymes were observed at all doses. The NOAEL level was therefore less than 50 mg/kg/day for rats which is equivalent to a human dose of 8 mg/kg or a dose of less than 400 mg/day.

11. Twenty-eight day repeat-dose oral gavage study in dogs with VX-478. GLP study. Study N001768C. October 1995. Drug batch number GP 336-34-1.

Four groups of beagle dogs, (4/sex/group) received either vehicle (Group 1) or formulated test article at doses of 100 (Group 2), 200 (Group 3) or 400 mg/kg (Group 4), by oral gavage, twice per day, approximately twelve hours apart, for twenty-eight days.

Records were kept of clinical signs (twice daily), body weights (weekly), food consumption (daily), ophthalmic examinations (weekly) and plasma drug levels. On day 1, blood samples were collected predose and approximately 0.5, 1, 2, 4, 6, 8 and 12 hours postdose. On study days 7, 14 and 21, blood was collected at 2 and 8 hours postdose. On study day 28, blood was collected predose and at 1, 2, 4, 8, and 12 hours postdose. After 28 days, animals were bled for hematology and serum chemistries and sacrificed. Records were kept of organ weights as well as gross and microscopic findings. Tissues examined histologically included adrenals, aorta, bone, bone marrow smear, brain, caecum, cervical lymph node, cervix, colon, duodenum, esophagus, eye, Harderian gland, femur, heart, ileum, jejunum, joint, kidney, liver, lung/bronchii, mammary glands, mesenteric lymph nodes, ovaries, oviduct, pancreas, parathyroids, pituitary, salivary glands, sciatic nerve, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, thymus, thyroid, tongue, trachea, urinary bladder, uterine body, uterine horn, vagina.

Effects of 141W94 included emesis, diarrhea and soft feces. An initial depression in body weight was detected in all groups, including controls, and resolved by the second week of dosing. This is probably related to the excipient and no significant differences were detected between the treated and control groups. Although there were significant changes in food consumption over the dose period, the relationship to drug dose was not straightforward. Group 4 males had food consumption which was consistently higher than all other male dose groups (Table 1), but food consumption in group 3 females was higher than in other female dose groups (Table 2).

Table 13. Mean daily food consumption (g) in dogs treated with 141W94: Males

	Week 1	Week 2	Week 3	Week 4
Group 1	176	242	268	284
Group 2	178	256	264	257
Group 3	194	235	262	269
Group 4	308	376	358	354

Table 14. Mean daily food consumption (g) in dogs treated with 141W94: females

	Week 1	Week 2	Week 3	Week 4
Group 1	140	157	149	164
Group 2	148	160	155	174
Group 3	242	194	200	197
Group 4	108	131	141	171

Cardiac Changes

Administration of 141W94 was also associated with increases incidence of flattening of the T-wave of the electrocardiogram. This increase was seen at all doses (see Table 3).

Table 15. Incidence of flattened T waves in treated dogs

Dose group (mg/kg)	Number of animals
Control	1
100	1
200	4
400	3

Histology was performed on hearts from control and 400 mg/kg group. Hearts from affected animals were normal.

Other Changes

Drug administration was also associated with increased absolute and relative liver weights (15 to 35 % increases) for both sexes, and at all doses. The enlarged livers were associated with hepatocellular hypertrophy, which was found in all rats treated with the drug. This change was characterized by slight enlargement of the hepatocytes adjacent to or near to the central veins of the hepatic lobules. Other changes associated with drug administration were generally mild, not clinically significant and not dose related.

Pharmacokinetics

In the dog, 141W94 was rapidly absorbed, with a Tmax between 1 and 2 hours post dose (see Table 4). Cmax levels increased with dose (but were not dose proportional) and were greater on day 28 than on day 1. Interpretation of the data is confounded by the fact that most of the animals vomited between 1 and 2 hours post dose, and this may have resulted in the incorrect estimation of a number of pharmacokinetic parameters.

Table 16. Pharmacokinetic parameters in Dogs receiving 141W94

Dose (mg/kg)	Tmax (h)		Cmax (μg/ml)		AUC(hr*μg/ml)	
	Day 1	Day 28	Day 1	Day 28	Day 1	Day 28
100	0.8	1.0	8	8	23	22
200	1.25	1.2	11	17	38	49
400	1.1	1.7	12	21	41	91

12. Oral Gavage Toxicity study in dogs dosed with 141W94. GLP study. Report # RD1996/00213/00. Study # TOX 772. Glaxo Wellcome Co. Five Moore Drive. P.O. Box 13398. Research Triangle Park, North Carolina, 27709. December, 1995. Drug batch # 95/0351-11A, 95/5350-044, 95/5350-045. Vehicle: PEG 400 (—— lot # J06617, ——— propylene glycol (—— lot # H42640, ——— , vitamin E TPGS (—— , lot # AA04-0295. ———

This study was designed to determine the toxic effects of 141W94 administered by oral gavage to dogs for three months.

Groups of beagle dogs, 4/sex/dose group were treated with amprenavir at 0 (water control), 0 (vehicle control), 50, 160 or 500 mg/kg/day for 94 to 97 days. Two additional dogs/sex/dose group were added to the vehicle control and high dose groups to determine the reversibility of any findings that were seen at the end of dosing. Dosing was discontinued in the high dose animals on day 11 because of overt clinical signs and dosing resumed on day 15 at 350 mg/kg for the rest of the study. Supplemental canned food was offered to enhance condition of these high dose dogs.

Emesis, fecal alterations (soft/liquid stools) and salivation were seen in all doses. In the high dose group given 500 mg/kg/day, signs included decreased activity, dehydration, body tremors, exaggerated heart beat. After the dose was reduced clinical signs included pale gingiva, coolness to the touch and body tremors. Red areas and scabs on the abdomen and ears were also seen in the high dose. Records were kept of clinical observations, bodyweights, food consumption, clinical pathology, urinalysis, toxicokinetics, electrocardiograms, ophthalmoscopy,

liver electron microscopy, liver microsome analysis, organ weights and histology. Tissues examined histologically included the adrenals, aorta, bone, bone marrow smear, brain, caecum, cervical lymph node, cervix, colon, duodenum, esophagus, eye, Harderian gland, femur, heart, ileum, jejunum, joint, kidney, liver, lung/bronchii, mammary glands, mesenteric lymph nodes, ovaries, oviduct, pancreas, parathyroids, pituitary, salivary glands, sciatic nerve, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, thymus, thyroid, tongue, trachea, urinary bladder, uterine body, uterine horn and vagina.

There were no significant increases in drug related deaths from amprenavir. Drug-related changes included reduced body weights, (bodyweight gains were -75 % at 160mg/kg and -157 % at 350 mg/kg, compared to controls), reduced hematocrit (-16 % at the high dose, compared to controls), reduced hemoglobin (-16 and 14 % at 160 and 350 mg/kg/day), reduced red blood cell count (-16 and -15 % reduced at mid and high doses), increased alkaline phosphatase (two to four times control levels at mid and high doses after 8 weeks and three months), reduced urine output (reduced by 16 % at the high dose at the end of dosing), increased relative liver weights (+43 % compared to controls at the high dose), decreased relative thymus weights (20 % reduced at the end of the study at the high dose), increased relative adrenal weights (+17-28 % at the mid and high dose) and increased relative thyroid weights (+20 to +40 % increased at mid and high doses). Histological changes seen at the end of dosing included mild hepatocellular hypertrophy (all high dose animals), mild to moderate fatty change in the medullary epithelium of the kidney (2 of 4 vehicle control animals, all mid and high dose animals) and minimal to mild atrophy of the thymus (1/3, 2/4 and 4/4 animals at the low, mid and high doses). At the end of a 33 day recovery period the following changes were still apparent at the high dose (the only dose examined): increased alkaline phosphatase (2 times control levels), urine output (now *increased* by 56 % compared to controls), relative liver weights (increased by 24 %) relative thymus weight (now *increased* by 68 %), relative thyroid weight (+ 43 %), fatty change in kidney (seen in 1 of 2 vehicle control and high dose animals) and thymus atrophy (seen in 1 of 2 vehicle control animals).

Table 17. Pharmacokinetics data

Amprenavir dose (mg/kg/day)	C_{max} (µg/ml)		AUC_{0-24} (µg.hr/ml)	
	Males	Females	Males	Females
50	7.0	3.4	61	24
160	23	35	170	311
350	45	47	380	452

Cmax and AUC values increased with increasing dose and increased over time between days 1 and 35 (except in the low dose). Plasma clearance in the mid- and high dose groups was decreased during the first month after which it remained consistent. AUC values increased 2- to 3 fold during the first month of dosing. Amprenavir did not produce any consistent changes in the levels of cytochrome P450 isozymes CYP1A, CYP2B and CYP3A. However, cytochrome P450-

dependent monooxygenase enzyme activity was increased in mid dose females and high dose animals.

Amprenavir produced minimal effects in dogs treated at 50 mg/kg/day for 3 months. At higher doses significant changes were noted in bodyweight (reduced), hematocrit, hemoglobin and rbc's (reduced), urine output (decreased), liver, adrenal and thyroid weights (increased) and thymus weight (decreased). Fatty change in the medullary epithelium of the kidney was also observed as well as thymic atrophy. Changes in the liver, thyroid, kidney and thymus were not reversed after a 33-day recovery period.

13. 141W94: Six month oral gavage toxicity study in dogs. Study # RD 1996/00752/00 Glaxo Wellcome Inc. Medicines Safety Evaluation, Research Triangle Park North Carolina.March 1996. Drug lot #95/5350-075 and R0428/22/1

This study was designed to determine the toxic effects of six months dosing with amprenavir at 50, 130 or 350 mg/kg/day. Drug was dissolved in PEG 400 (——— , vitamin E TPGS (——— and propylene glycol (——— . Five groups of dogs, 4/sex/dose, were used including one water control group and one vehicle control group. Two extra dogs were added to the vehicle control group and the high dose group to determine the reversibility of toxic effects observed at the end of the study. Record were kept of clinical observations, body weights food consumption, clinical pathology, urinalysis, electrocardiograms, toxicokinetics findings, ophthalmoscopy, organ weights, histology.

The following tissues were examined:Brain, spinal cord, nerve-peripheral, kidneys, liver, spleen, lungs, pituitary, mesenteric lymph nodes, thymus/thymic area, heart, aorta, tongue, trachea, oesophagus, thyroid, parathyroids, adrenals, testes, epididymes, ovaries, stomach, duodenum, pancreas, jejunum, colon, caecum, rib/bone marrow, ileum, rectum, skin, mammary, salivary, mediastinal lymph node, uterus, vagina, prostate, eyes, lacrimal glands, bone marrow, skeletal muscle, gall bladder, mandibular lymph node, femur/bone marrow, urinary bladder, cervix.

Animal deaths during this study consisted of one water control and one vehicle control animals and four high dose animals (three females and one male). The three high dose deaths appeared to be drug related. Animals died following tremors and ataxia, epileptiform convulsions, decreased activity, dehydration, bloody diarrhrea, prostration, pale gingiva and very low serum potassium levels. As a result of these deaths and poor condition, the high dose was stopped on day 36 and resumed at 225 mg/kg/day on day 42.

Toxic effects included emesis, soft/liquid stools, salivation but high dose animals also showed decreased activity, dehydration, body tremors, weakness, reduced movement, pale gingiva, labored breathing, pupils dilated and staggering. Epileptiform convulsions were noted in one high dose female before it died. High dose animals also showed a higher frequency of rough coat, scabs and dermatitis. Weight loss was significantly greater than for water controls in treated animals. Males lost 0.15, 0.78 and 1.5 kg during the first 36 days while females lost .17, 0.32 and 1.5 kg at the low mid and high doses. At the end of the study, body weight gain was 42 and 23 %

of that seen in control animals. Hematocrit (-13 %), hemoglobin (-13 %) and red blood cells were reduced in males only at the end of the study. Alkaline phosphatase was increased by up to 1.7 times control, 2.6 times control and 4.4 times control at low mid and high doses starting during week 4. It was as much as 2.4 times control levels at the end of the recovery period. Urine output was reduced by up to 23 and 42 % at mid and high doses starting at week 6. All dose groups treated with drug or vehicle showed exaggerated T waves. Mid and high dose aniimals showed stress type electrocardiograms, These changes wee absent in the recovery animals.

Liver weights were increased at all doses, increasing by 14, 42 and 36 % in low mid and high dose groups. At the end of the recovery period, no such increase was maintained. Relative thymus weight was reduced by 12 to 30 % at the mid- and high doses. Relative adrenal weight increased by 13 and 33 % at the mid- and high doses. In the liver, centrilobular hydropic degeneration (1/3 high dose males), centrilobular hypertrophy (most mid and high dose animals), cytoplasmic alterations (some mid and high dose females), single cell necrosis (one of two high dose females), hepatocellular brown pigment (low dose males and in mid and high-dose groups). Kidney changes included tubular dilatation (2/5 high dose animals), tubular lipid deposition (in vehicle control animals, females at all doses and in one high dose male). Thymic involution was seen at all dose groups and was more common in females.

Table 18. Pharmacokinetics data

Dose Mg/kg/day	Cmax (μg/ml) Males	Cmax (μg/ml) females	$AUC_{(0\text{-}24h)}$ μg*hr/ml (males) Day 176	$AUC_{(0\text{-}24h)}$ μg*hr/ml (females) Day 176
50	5	8	30	49
130	19	22	144	145
225	27	22	247	155

Brown pigment, thymus involution, adrenal weight, thymus weight, alkaline phosphatase did not revert to control levels at the end of the recovery period.

Dogs treated at 50 mg/kg/day had exposure to drug (AUC 30 or 49 μg*hr/ml, males or females respectively) similar to humans exposed to 1200 mg of amprenavir/day. This exposure is associated with increases in alkaline phosphatase, liver weight, hepatocellular brown pigment, kidney tubular lipid deposition, exaggerated T waves and thymus involution.

14 Twelve month (oral gavage) toxicity study in dogs with 141W94. Study # TOX 779. Report # RD1997/01249/00. Glaxo Wellcome Inc. Medicines Safety Evaluation, Research Triangle Park North Carolina. Drug batch #'s 95/5350-075and R/0428/22/1. March 1996. GLP study.

This study was designed to determine the toxic effects of twelve months of dosing of

amprenavir in dogs. Drug was dissolved in a vehicle containing — PEG 400, —— vitamin E- TPGS (D-alpha tocopheryl PEG 1000 succinate) and ~ propylene glycol. Dogs were treated with amprenavir at doses of 50, 130 or 350 mg/kg/day or water or vehicle. There were 4 dogs/sex/dose group except in the vehicle control and high dose groups where two extra dogs per sex were added to assess reversibility over a 60 day period. Record were kept of clinical observations, physical examinations, body weight, food consumption, hematology, clinical chemistry, urinalysis, ophthalmoscopy, organ weights, gross pathology and histopathology, electrocardiography and toxicokinetics.

The following tissues were examined histologically:

Brain, spinal cord, nerve-peripheral, kidneys, liver, spleen, lungs, pituitary, mesenteric lymph nodes, thymus/thymic area, heart, aorta, tongue, larynx, trachea, oesophagus, thyroid, parathyroids, adrenals, testes, epididymes, ovaries, stomach, duodenum, pancreas, jejunum, colon, caecum, femur/bone marrow, ileum, rectum, skin, mammary glands, salivary glands, mediastinal lymph node, uterus, vagina, prostate, eyes, lacrimal glands, sternum/bone marrow, skeletal muscle, gall bladder, mandibular lymph node, femur/bone marrow and urinary bladder.

One high dose male was euthanized after the 15th dose because of poor condition. This animal showed evidence of a dosing accident : red discharge from mouth and nose, severe, diffuse dark discoloration in lungs, with slight emphysema in the left anterior lobe.

Clinical signs included soft feces and emesis, which were seen in vehicle control and drug-treated animals and were ascribed to the vehicle. Drug related effects included salivation, dehydration, decreased activity and rough coat. Loss of appetite and dehydration, noted between days 9 and 28, in 2 males and 4 females at the high dose and one male at 130 mg/kg/day resulted in a decision to halt dosing while these animals were given fluid therapy (lactated Ringer's solution) and one male given feed supplements (——— canine diet). Supplemental food was offered to dogs for the remainder of the study.

Body weight gain was reversibly reduced by 38, 61 and up to 89 % at the low mid and high dose animals. Platelets were reduced by as much as 20 % at the low dose but increased at the high dose up to an increase of 30 %. Reticulocyte counts were decreased by up to 52 % at the high dose but high dose females retained this deficit even after the recovery period. Changes in hematocrit were seen at all doses (maximum 17 % decrease), partial thromboplastin time was decreased at the mid and high dose (up to 11% decrease) and alkaline phosphatase increased up to 2.6, 3.1 and 4.7 times control levels at the low mid and high doses at the end of dosing. Albumin levels decreased by up to 21 % in the high dose animals only while globulin levels increased by up to 16 % in the high dose. Potassium levels were decreased in the mid and high dose animals (up to 18 % below control levels), and urine output was affected by drug therapy, but increases at one time point were often as large as decreases at another time point. Relative liver weight increases were more prominent in males and were up to 29, 36 and 57 % higher than controls. Relative adrenal weights were up to 19 and 33 % higher at the mid and high doses.

Relative prostate weight was 15 and 29 % less than control values. Histopathological findings in the liver included hepatocellular hypertrophy and hepatocellular brown pigment in most animals at all doses. At the end of the 54 day recovery period, platelets (-18%), reticulocytes (females, -21 %) hematocrit (females, -10 %), alkaline phosphatase (males, 1.9 times control), globulin (-12 %), phosphorus (-28 %, males), urine output (6.4 times control levels in males), liver weights (+23 %) and prostate weight (+46 %) remained affected in the high dose animals.

Table 19. Pharmacokinetics data

Dose Mg/kg/day	Cmax µg/ml				AUC (0-24h) µg*h/ml			
	Males		Females		Males		Females	
Day	Day1	D364	Day 1	d364	Day 1	D364	Day1	D364
50	6	10	8	9	39	62	48	49
130	11	20	9	25	73	157	69	188
350/225	16	42	13	38	93	351	80	278

Peak plasma concentration and exposure (AUC) increased with dose and as the number of doses increased over time. Exposure remained relatively consistent after day 181 except at 50 mg/kg/day, where a doubling of exposure was seen after this time point. Elimination half life was approximately 2.5 hours.

Exposure at the lowest dose is similar to the exposure seen in the clinic. Based on the effects seen at the lowest dose, long term therapy with amprenavir is expected to affect body weight gain, alkaline phosphatase, urine output, liver weights and adrenal weights. This dose may also be associated with hepatocellular hypertrophy and brown pigment.

In all dose groups except the water control group, some dogs had exaggerated T and U waves on days 92, 177 and 363. This could be related to hypokalemia associated with emesis and diarrhea. Exaggerated T waves were also seen in the 6 month dog study and seem to be associated with the vehicle. The potential for cardiac effects in humans underscores the need for further study of this phenomenon.

15 Twenty eight-day repeat-dose oral gavage toxicity study in Cynomolgus monkeys with 141W94 . Study # BW TOX 685 (HWA 309-247) GLP study.). September 1994.

Groups of Cynomolgus monkeys (*Macaca fascicularis)* were treated with 141W94 twice daily by oral gavage for at least 28 days according to the following protocol.

Table 20. Treatment protocol for monkey toxicology study #BWTOX685

Group #	141W94 dose (mg/kg/day)	Number of animals (male/female)
1	0	5/5
2	50	3/3
3	200	3/3
4	400	5/5

Two males and two females in groups 1 and 4 were observed for an additional 14-day recovery period. Drug was dissolved in a solution of sodium acetate and a mixture of deionized water and PEG 400. The control vehicle contained methanesulfonic acid to mimic the concentration of the high-dose treatment. Drug was administered in two equal portions, approximately 6 hours apart. During the study, records were kept of clinical findings, body weights, ophthalmology, electrocardiography, hematology, clinical chemistry, urinalysis, and plasma concentration. At the end of dosing, 3 monkeys per group were sacrificed and subjected to gross examination, after which selected organs were removed, weighed and tissues removed for histopathological examination. Tissues examined included adrenal glands, aorta, bone marrow, brain, cervix, epididymes, esophagus, eyes, femur, Harderian gland, heart, kidneys, large intestines, larynx, liver, lungs, lymph nodes, mammary glands, ovaries pancreas, parathyroid gland, prostate gland, salivary glands, sciatic nerves, skeletal muscle, skin, small intestine, spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus and vagina. The two monkeys observed for an additional 14 days were sacrificed and examined at the end of this recovery period.

Mortality

One male monkey from group 4 (400 mg/kg/day) died on dose day 10. Autopsy findings are consistent with death from chronic aspiration pneumonia or gavage accident.

Toxicity

Soft stool and decreased appetite were noted frequently in animals from all groups throughout the study and were attributed to the vehicle since these conditions were not observed during the recovery period. There were also isolated instances of salivation following dosing (one high dose female and two high dose males). There were no drug-related changes in bodyweight, hematology, clinical chemistry, urinalysis, ophthalmoscopy, electrocardiography, organ weights, gross pathology or histopathology.

Reproductive Toxicology Studies

16. Insemination study in male rats with administration of amprenavir (KVX-478). Study # 2387 (005-009) [redacted] April 1995.

141W94 was administered orally to five groups of twenty two male rats at doses of 0 (vehicle control), 100, 500 and 1000 mg/kg/day. Male rats were treated for a twenty eight day pre mating period, after which they were placed in a cage with a female which was recorded to be in estrous during the previous 14 days. Vaginal smears were performed daily, and, the day on which sperm was observed was designated day 0 of gestation. On day 20 of gestation dams were anesthetized with ether and sacrificed by exsanguination. Records were kept of the number of corpora lutea, implantation rate, preimplantation loss rate, number of live and dead fetuses, resorption rate, sex ratio, fetal individual body weight, external abnormalities, and placental weights. Male rats were sacrificed between days 30 and 43 of administration and the effects of the drug on the reproductive performance of male rats were assessed. Records included the weight of the testes, left caudal epididymis, sperm motility, sperm viability and sperm numbers. Records were also kept of general condition, body weights, food consumption, mating and necropsy findings.

There were no negative effects on copulation and fertility that could be ascribed to this drug. Indices of copulation and fertility were consistently greater than 90%. Sperm count and motility score was comparable across all dose groups. Sperm viability increased by 20% at the 500 mg/kg dose, but this increase was not detected at the 1000 mg/kg dose. Drug treatment was not associated with any adverse reproductive findings in dams or their fetuses.

Treatment of males rats with drug at doses up to 1000 mg/kg/day for 28 days did not produce any negative effects on reproductive function in the treated males, the mated females or the resulting F1 generation of fetuses. This dose is equivalent to a human dose of 159 mg/kg for 28 days.

17. Fertility study in male SD rats after administration of 141W94 [redacted] Report 3028. Study number 2387. [redacted] Drug batch number 916.D.94.501. August 1994. GLP study.

This study was designed to determine the effects of amprenavir on the reproductive performance of male rats. Five groups of male rats, 22 male rats/dose group, were used. Three groups were treated with amprenavir (dissolved in [redacted] water/PEG400 with sodium acetate) at 100, 500 or 1000 mg/kg/day for 28 days before they were mated with female rats. One control group consisted of untreated rats, while the other was made up of vehicle treated rats. Male rats were sacrificed after successful copulation (between 30 and 43 days of dosing) for macroscopic examinations. To assess the effect of amprenavir on the offspring, dams were allowed to live

until day 20 of gestation when they underwent cesarean section to determine gestational parameters and fetal effects. Records were kept of general condition, body weight, food consumption, mating activity, necropsy findings, sperm evaluations, observations of Fo females and observations of F1 fetuses.

Amprenavir treatment was associated with salivation, enlarged liver and increased liver weights as seen in other toxicology studies. There were no effects of amprenavir on sperm motility, viability or numbers. The total number of females inseminated, pregnant females, fetuses evaluated, corpora lutea, implantations, live fetuses, resorptions, dead fetuses, placental weights, fetus weights and sex ratio were not significantly different when treated animals were compared to controls.

18. Oral female fertility and embryo fetal development study in the Han Wistar rat. Report WD1997/00284/00. Study number R21330. Glaxo Wellcome Research and Development, Ware Hertfordshire, UK. Drug batch number R0428/22/1. January 1997. GLP Study.

This study was designed to determine the effects of amprenavir in the pregnant rat. Groups of female rats, 24 rats per dose, were dosed for 15 days before pairing, during the mating period and up to day 17 of gestation. Females were killed on day 21 of gestation and after cesarean sections, fetuses were examined for external, visceral and skeletal effects. Dose groups received amprenavir at 50, 190 or 750 mg/kg/day or water or vehicle in two equally divided doses. The vehicle consisted of a solution of vitamin E TPGS, PEG400 and propylene glycol. Records were kept of body weights, food consumption, estrous cycles, mating rate, copulation index, fertility index, uterine parameters, fetal/placental weights, and fetal observations. Toxicokinetics evaluations were also recorded.

Mortality

Two high dose animals died. One death was attributed to a dosing accident (fluid in thoracic cavity) and the cause of death of the other was undetermined.

Toxicokinetics

Cmax levels increased with increased doses of amprenavir, but while the increases were dose proportional on day 1, they were less than dose proportional on day 17. AUC(0-24h) also increased with dose in a less than dose proportional fashion.

Table 21. Pharmacokinetics data

Cmax day 1 μg/ml	Cmax day 12 μg/ml	AUC day17 μg*hr/ml
1.6	2.2	12
5.7	4.6	52
19	6.3	87

Dams treated with amprenavir showed increased bodyweight gain (6, 15 and 36 % greater than controls in low mid and high dose groups) in the pre-pairing treatment period. Placental weight at necropsy was 20 % greater in the 750 mg/kg group compared to controls. Amprenavir treated rats were more likely to have fetuses with moderate thymic elongation than vehicle control animals (1.5, 1.5 and 3.8 % at low mid and high doses compared to 0.4 % in the controls). Incomplete ossification of the skull bones was also associated with amprenavir treatment. At 750 mg/kg/day, the affected bones included the parietal, interparietal, occipital, squamosal and zygoma.. See table below.

Table 22. Percentage of rats affected by incomplete ossification of skull bones due to amprenavir

	Vehicle	190 mg/kg amprenavir	750 mg/kg amprenavir
Parietal	19	24	32*
Interparietal	29	40*	50*
Occipital	14	25*	32*
Squamosal	2	5	11*
Zygoma	1	2	5

*statistically significant $p < 0.05$

The following changes were associated with amprenavir administration but because the increased incidences of the findings remained within the background incidence, they were not considered causally related to amprenavir dosing.

Amprenavir administration was also associated with the increased incidence of slight bilateral dilatation of the lateral brain ventricles (0.4, 2.9 and 1.7 % of fetuses at low mid and high groups compared to 0.4 and 0.8 in water and vehicle controls respectively). Amprenavir treatment was also associated with increased incidences of incomplete ossification of nasal and frontal bones.

Table 23. Incidences of incomplete ossification of nasal and frontal bones.

Parameter	Vehicle control	Water control	50	190	750
Frontal-unilateral % fetuses affected	1.5	3.3	6.5	4.9	5.6
Nasal-bilateral % fetuses affected	0.0	0.8	1.4	2.8	4.0
Bilateral cervical rib % fetuses affected	0.8	0.0	3.6	3.5	2.4
4th sacral vertebral arch incompletely ossified (% fetuses affected)	0.0	1.6	0.7	2.1	4.0

Conclusion

Since thymic elongation was observed in all rats, no NOAEL could be determined for amprenavir. It is estimated at less than 50 mg/kg. Amprenavir dosing is associated with incomplete ossification of a number of skull bones.

19. Dose rangefinding study in the nonpregnant female rabbit. Study # DRF 750. Report RD 1996/00371/00. Glaxo Wellcome Research Institute. Medicines Safety Evaluation Division, Glaxo Wellcome Inc. Five Moore Drive, Research Triangle Park, North Carolina. August 1996. Non-GLP study. Drug Batch number R0428/22/1.

Groups of nonpregnant female rabbits, 3/group were treated with 141W94 at 0, 100, 200, 400 and 1000 mg/kg/day in equally divided doses, six hours apart by oral gavage for 14 days.

Mortality

All three animals at the 1000 mg/kg dose died. Two control animals died as well as one of the 100 mg/kg and one of the 400 mg/kg animals. Slight to severe loose feces were observed in all animals.

Table 24: Toxicokinetics

Dose	Cmax µg/ml	AUC µg*h/ml
100	0.5	3.3
200	2.3	13
1000	12	81

Plasma Cmax and AUC values were as shown above. Cmax and AUC increased with dose but were not dose proportional. The high mortality seen at 1000 mg/kg suggests that the maximum recommended dose for repeated dosing in rabbits should be approximately 400 mg/kg/day.

20. Oral embryo-fetal development study in New Zealand White rabbits after dosing with 141W94. Report # RD1997/04032/00. Study # TOX 815. Medical Safety Evaluation Unit. Glaxo Wellcome ResearchMarch 1997.

Groups of pregnant New Zealand White rabbits (24/dose group) were treated with amprenavir at 25, 50 and 100 mg/kg in divided doses, six hours apart. Rabbits were treated from day 8 to 20 of pregnancy inclusive. Records were kept of clinical signs, mortality, abortions, number of live/dead fetuses, fetal abnormalities, corpora lutea, implantations, resorptions, fetus weights, placental weight and sex ratio.

Mortality

Two, three and four animals died from the 25, 50 and 100 mg/kg doses during the course of the study. Animals that died showed ataxia, decreased activity, labored breathing and gasping.

Toxicity

Dams treated with amprenavir or vehicle showed soft feces and feces stained anogenital areas. This effect was thought to be related to the vehicle. Only the 100 mg/kg/day group had drug levels above the limit of detection — mcg/ml). AUC was 1.2 µg*h/ml and Cmax was 0.3 µg/ml.

Mean placental weights were increased by 20 % in 100 mg/kg animals compared to controls. Abortions occurred in 2, 1 and 1 animals from the 25, 50 and 100 mg/kg/day groups. An increase in preimplantation loss was observed in treated animals which showed losses of 0.4, 0.4 and 0.5 % in low, mid and high dose groups compared to controls which showed loss of 0.3 %. An increase in fetal variations was also observed with amprenavir treatment. All amprenavir

treated animals showed increases in the number of fetuses with the head of the humerus not ossified. At the 50 mg/kg dose, there was an increase in the number of fetuses with no ossification at the distal end of the femur (26 % vs 17 % in controls). At the 100 mg/kg dose, there was an increase in the number of fetuses with the trochlea not ossified (12 % at 100 mg/kg compared with 0 % in controls).

Since abortions and increased numbers of fetuses with humerus not ossified were found at 25 mg/kg/day, no NOAEL could be determined. It is estimated to be less than 25 mg/kg/day. The rabbit seems to be particularly sensitive to this drug's adverse effects since toxic effects are seen at doses below the limit of quantification for this drug — µg/ml).

21. Pre and Post natal development study in Han Wistar rats after oral dosing with 141W94. Report # WD1997/00420/00. Study # R21629.

Groups of pregnant female rats, 24 rats per dose group, were treated between day 7 of pregnancy and day 22 of lactation with 141W94. Drug was administered by oral gavage at 50, 190 or 750 mg/kg/day in two equally divided doses approximately 6 hours apart. 141W94 was formulated in a mixture of vitamin E-TPGS, PEG 400 and propylene glycol and the two control groups consisted of untreated rats and vehicle treated rats. Plasma concentrations of drug were measured in the dams 7 hours after the first dose on day 12 of lactation and dams were sacrificed after day 22 of lactation. On day 23 of lactation, pups were separated into cages, by sex and litter. One female and one male were selected from each litter, (where possible) to form the F_1 group. Upon vaginal opening (females) or balano-preputial separation (males), the unselected animals were sacrificed. The selected animals were subjected to sensory examinations (auditory function, nocturnal activity, learning ability, neuromuscular function, ophthalmoscopy) and later paired and mated. The F1 females were allowed to litter and gestation and litter data collected. On day 4 of lactation, these dams, along with their offspring sacrificed. The F1 males were killed after the female autopsies and reproductive organs were examined.

Table 25: Mean peak plasma concentrations were as follows:

Dose (mg/kg)	Mean plasma concentration (mcg/ml)
0	-
50	2.3
190	5.5
750	10

Plasma level of amprenavir increased with dose but the increase was less than dose proportional.

A single dam died after receiving one day of dosing. The cause of death was not determined but autopsy results revealed congested lungs and slightly enlarged thymus with punctate large areas.

Dose-related increases in salivation were observed in drug treated animals. Changes in the F1 generation were restricted to reductions in bodyweight and bodyweight gain. The bodyweights of males were 7 to 14 % lower than control animals between days 8 and 28 with bodyweight gains being 14 to 19 % less than controls. The bodyweights of females were 10 to 14 % lower than controls between days 12 and 28 with the bodyweight gains being 11 to 19 % less than in controls. Subsequently bodyweights were slightly lower than controls, but bodyweight gains remained comparable to controls.

There were no drug-related effects on offspring survival, sensory development or reproductive performance.

Conclusion

Dams treated with 141W94 between day 7 of pregnancy and day 22 of lactation showed increased dose related salivation at all doses. F_1 pups from amprenavir-treated dams showed reduced bodyweights (10 to 19 %) compared to controls. Litter parameters were not significantly different between treated and untreated animals.

Genotoxicity Testing

22. *Salmonella*/Mammalian-microsome assays with 141W94 —— Report TTEP/94/0057. Study # MUT 230. GLP Study. Drug reference # 94/0239-105-A. —— September 1994.

141W94 was tested for its ability to induce gene mutations in the Ames *Salmonella*/mammalian mutagenicity assay at doses between 100 and 5,000 μg/plate. Tester strains were TA1535, TA1537, TA98 and TA100 and drug was tested in the presence and absence of Aroclor-induced rat liver S-9 metabolic activation

Vehicle controls were plated for all tester strains using a 50 μl aliquot of vehicle (equal to the maximum aliquot of test article dilution plated) along with a 100 μl aliquot of the appropriate tester strain and a 500 μl aliquot of S9 mix (when necessary) on agar.

Using both the *Salmonella* plate incorporation assay and the *Salmonella* preincubation modification assay (20 minute preincubation), 141W94, did not cause any positive increases in the number of histidine revertants per plate in either the presence or absence of microsomal enzymes derived from Aroclor-induced rat liver. Positive controls, shown below, produced the expected changes.

Table 26. Positive controls used in Ames *Salmonella*/mammalian mutagenicity assay.

Tester Strain	S9 mix	Positive control	Conc. per plate • g
TA98	+	2 aminoanthracene	2.5
TA98	-	2-nitrofluorene	1.0
TA100	+	2 aminoanthracene	2.5
TA100	-	sodium azide	2.0
TA1535	+	2 aminoanthracene	2.5
TA1535	-	sodium azide	2.0
TA1537	+	2 aminoanthracene	2.5
TA1537	-	ICR-191	2.0
TA1538	+	2 aminoanthracene	2.5
TA1538	-	2 nitrofluorene	1.0

Conclusion

141W94 does not produce gene mutations in the Ames Salmonella/mammalian-microsome mutagenicity assay either by the plate incorporation or preincubation method.

23. 141W94: *Salmonella* and *E.coli*/microsome reverse mutation preincubation and standard plate incorporation assay. Report TTEP/1996/00380/00. Study # V40197. GLP Study. Drug batch # 1008.D.96.2. Glaxo Wellcome Inc. Medicinals Safety Evaluation Division and Analytical Sciences Department. Five Moore Drive, Research Triangle Park, NC 27709. USA. June 1996.

This study was essentially a repeat of the above study MUT 230 performed at another facility and using drug batch of 141W94. (1008.D.96.2). 141W94 demonstrated no potential to cause gene mutations in the presence or absence of metabolic activation.

24. 141W94: Microbial mutagenicity study: liquid preincubation and standard plate incorporation assay. Report WPT/96/082. Study # V21300. GLP Study. Drug batch # 95/0351-110A . Glaxo Wellcome Research and Development, Park Road, Ware, Hertfordshire, SG120DP.

This study was designed to test the possible genetic hazards of amprenavir. *E. coli.* strains WP2(pKM101) and WP2*uvrA*(pKM101)were used to detect base change mutagens and were tested in the presence and absence of metabolic activation. Drug was tested using standard plate incorporation assay and liquid preincubation assay. Drug levels ranged between 100 and 5000 µg of 141W94 per plate.

Positive controls performed as expected and amprenavir demonstrated no consistent evidence of mutagenic activity at doses up to 5000 µg per plate.

25. 141W94 (spiked): *Salmonella* and *E.coli*/microsome reverse mutation plate incorporation and preincubation assays. Report TTEP/1996/00380/00. Study # V40197. GLP Study. Drug batch # 1008.D.96.2. Glaxo Wellcome Inc. Medicinals Safety Evaluation Division and Analytical Sciences Department. Five Moore Drive, Research Triangle Park, NC 27709. USA. June 1996.

Due to a change in the chemical synthesis of 141W94 and a more complete chemical analysis, it was found that 141W94 contained a number of —— This study was designed to test the mutagenicity of 141W94 spiked with the

[ ]

The standard or preincubation Ames assay was used in the presence or absence of metabolic activation. Salmonella trains tested were TA1535, TA1537, TA98 and TA100 and *E.coli* strain [WP2*uvrA*(pKM101). 141W94 levels ranged up to 5000 µg per plate. Spiked 141W94 demonstrated no potential to cause gene mutations in the presence or absence of metabolic activation.

26 L5178Y/tk$^{\pm}$ mouse lymphoma mutagenesis study with 141W94. Report # TTEP/95/0002. Study number MUT246. Division of Medicines Safety Evaluation, Research Triangle Park, NC, USA.. Drug lot number 94/0239-105-A. GLP study. Septrmber 1994.

This study was designed to determine the potential of amprenavir to induce mutations affecting the heterozygous thymidine kinase (tk$^{\pm}$) locus in the L5178Y/tk$^{\pm}$ mouse lymphoma assay in the presence and absence of metabolic activation.

L5178Y/tk$^{\pm}$ mouse lymphoma cells were exposed to amprenavir for 4 hours at 4 degrees centigrade in the presence or absence of S-9 mix followed by centrifugation and resuspension. Suspensions were diluted daily and relative suspension growths calculated at 23 to 24 and 47 to 48 hours after the start of treatment. The cell populations were then maintained in tubes for a 2 day expression. At the end of the expression period, the cell suspensions were plated to determine post expression time, viability and mutation frequency. Plates were incubated at 37 degrees centigrade for 9 to 13 days before scoring for surviving and mutant colonies.

Mutation frequencies in amprenavir treated cells were comparable to rates in DMSO treated cells (control). Amprenavir levels tested were up to 400 µg/ml in the presence of metabolic activation and 500 µg/ml in the absence of metabolic cativation. Positive controls produced the expected increases in mutation rates.

27. An in vitro cytogenetic study in cultured human lymphocytes with 141W94. Report TTEP/95/0050. Study # MUT 236. Glaxo Wellcome Inc. Division of Medicines Safety Evaluation, Research Triangle Park, NC, USA.. Drug lot number 94/0239-105-A. GLP study. September 1994.

This study was designed to determine the potential of amprenavir to produce chromosome aberrations in cultured human lymphocytes both in the presence and absence of metabolic activation. Cells were incubated with amprenavir at doses up to 1000 µg/ml. Incubations were 4 hours long in the presence of metabolic activation and 21 hours long in the absence of metabolic activation. Positive controls were provided by cells exposed to mitomycin C (for incubation without metabolic activation) and cyclophosphamide (for incubations with metabolic activation).

There were no increases in chromosomal damage in cells exposed to amprenavir when compared to cells exposed to DMSO in the presence or absence of metabolic activation. The validity of the assay was confirmed by the marked increases in chromosomal damage seen with positive controls.

Pharmacokinetic Studies Review

28. Pharmacokinetics of 141W94 —— in Hsd:Sprague Dawley SD rats.

Groups of four Hsd:Sprague Dawley rats were given 141W94 at 10 and 50 mg/kg by intravenous injection or by oral gavage. 141W94 was given as the ——— dissolved in PEG-400. Plasma drug levels were determined by HPLC from samples obtained predose and from 2 minutes to 7 hr postdose.

Table 27. Pharmacokinetics of 141W94 in Hsd:Sprague Dawley rats.

	Dose			
	10 mg/kg		50 mg/kg	
	oral	i.v.	oral	i.v.
Mean C_{max} (µg/ml)	1.3	21.5	4.0	54
T_{max}	0.4 h	2 min	0.9 h	2 min
AUC (h*µg/ml)	1.3	4.6	9.2	36
Clearance (L/h/kg)	2.2	2.2	1.4	1.4
T1/2 (h)		0.4		0.3

Bioavailability was estimated to be 28 and 26 % in the 10 and 50 mg/kg doses respectively.

29. Preliminary disposition studies of 141W94 in Hsd:Sprague Dawley SD rats.

The first study in this report was designed to determine the effect of three different excipients on the pharmacokinetics of 141W94. Four groups of three Hsd:Sprague Dawley rats were given four different formulations of 141W94 in hard gelatin microcapsules containing 21 to 24 mg/kg 141W94. Rats were cannulated and plasma samples obtained predose and from 0.5 to 6 hours postdose. Plasma concentrations were determined by ———

Table 9. Effect of formulation on 141W94 Pharmacokinetics

Formulation	Dose (mg/kg)	T_{max} (h)	C_{max} μg/ml	AUC h*μg/ml
PEG 400[1]	24.1	2.5	0.4	1.3
TPGS[2]	21.0	2.7	1.4	2.6
TPGS/ ———	21.5	1.2	1.0	2.4
TPGS/ ———	24.2	0.8	0.9	1.7

1. PEG 400 polyethylene glycol 400
2. Tocopheryl polyethylene glycol 1000 succinate
3. Tocopheryl polyethylene glycol 1000 succinate with ——— ratio)
4. Tocopheryl polyethylene glycol 1000 succinate with ——— ratio)

In the second study, groups of three male Hsd rats were dosed intravenously by gavage with 20 mg/kg ^{14}C-labelled 141W94 dissolved in ethanol and saline. Samples of urine and feces were collected up to 48 hours postdose.

Table 28. Recovery of ^{14}C-labelled 141W94 in urine (48 hour collection period) after a single dose of drug.

	0-24 hours (μCi)	25-48 hours (μCi)	Total[1] (%)
Intravenous	3.3	0.1	5.3
Oral gavage	2.7	0.4	4.9

Table 29. Recovery of ^{14}C-labelled 141W94 in feces (48 hour collection period) after a singles dose of drug.

	0-24 hours (μCi)	25-48 hours (μCi)	Total[1] (%)
Intravenous	33.8	1.1	54.7
Oral gavage	24.7	3.9	44.8

1. expressed as a percentage of total administered dose.

Comment

Only 60 % of the radiolabelled drug was recovered in urine and feces after a 48 hour collection period. Please send information on the fate of the other 40 %.

30. Toxicokinetics of 141W94 in Harlan rats in a one-month oral toxicity study.

Three groups of rats (12/sex/group) were treated with 100, 500 and 1000 mg/kg 141W94 for one month for pharmacokinetic analyses only. Animals were dosed twice daily with 6 hours between doses. Food was removed approximately 2 hours after the first daily dose to increase bioavailability. Plasma was drawn on study day # 2 (predose and 0.5, 1, 2, 4 and 6 hours after the first daily dose and prior to the second daily dose) and on study day # 29 (predose, 0.5, 1, 2, 4, 6, 8, and 10 hours after the first daily dose, prior to a second dose.)

Table 30. Pharmacokinetics of 141W94 in a one-month rat oral toxicity study

Dose	Sex	Day 2			Day 29		
		C_{max} (μg/ml)	T_{max} (hr)	AUC μg/ml*hr)	C_{max} (μg/ml)	T_{max} (hr)	AUC μg/ml*hr)
100	male	4.2	1	5.8	3.7	1	7.3
	female	4.2	1	13	4.0	0.5	10.6
500	male	6.0	2	29	7.3	0.5	35.8
	female	5.5	1	23.8	11.5	1	44.8
1000	male	6.6	1	27.9	8.4	1	38
	female	19.5	1	43.4	10	0.5	50

31. Toxicokinetics of 141W94 in Cynomolgus monkeys in a 28-day oral toxicity study.

Groups of Cynomolgus monkeys (*Macaca fascicularis*) were treated with 141W94 in two divided doses by oral gavage daily for at least 28 days. Total daily doses were 50, 200 and 400 mg/kg. Drug was dissolved in a solution of sodium acetate and a mixture of deionized water and PEG 400. Drug was administered in two equal portions, approximately 6 hours apart. Plasma levels of 141W94 were measured by -

Table 31. Pharmacokinetics of 141W94 in Cynomolgus monkeys in a 28-day toxicity study

Dose	Sex	Day 2			Day 26		
		C_{max} (μg/ml)	T_{max} (hr)	AUC μg/ml*hr)	C_{max} (μg/ml)	T_{max} (hr)	AUC μg/ml*hr)
100	male	id	id	id	id	id	id
	female	id	id	id	id	id	id
500	male	2.1	2.7	7.0	1.8	1.7	5.2
	female	2.8	2.3	10.6	2.1	1.7	6.1
1000	male	4.1	2.4	16	2.3	2.0	8.3
	female	5.3	2.6	22	2.6	1.5	8.5

id: insufficient data, due to drug levels below the sensitivity of the assay system.

At the lowest dose, drug levels were generally low or below the limit of detection • M) indicating little absorption at this dose level. Mean peak plasma concentrations were not dose proportional at the higher doses. Although there is no clear difference in plasma levels between males and females, the drug tends to be more bioavailable in females than in males (higher C_{max} 's and AUC's). Based on the AUC's there was a decline in drug exposure between days 2 and 26.

Comment

The decline in AUC's suggests that the drug is being eliminated at a faster rate on day 26. Although the normal rat shows very little metabolism of this drug, there is some metabolism in the monkey. The increased metabolism later in the study may reflect the drug's induction of its own metabolizing enzymes. However, since no elimination half-lives were provided, no further conclusions can be reached regarding the mechanism behind the lower AUC's.

32. Binding of 141W94 to plasma proteins by equilibrium dialysis.

Using an equilibrium dialysis apparatus, the binding of ^{14}C 141W94 to human serum

proteins was determined. At a concentration of 1 μM 141W94, plasma protein binding was 93 %. Percentage binding of other blood components are shown below.

Table 32 Binding of 141W94 to plasma proteins by equilibrium dialysis.

Protein	Concentration (μM)	% 141W94 Bound
Plasma		93
Serum		91
Fibrinogen	11.7	5
IgG	9.4	2
• $_2$-Macroglobulin	3.6	3
Serum Albumin	580	42
Glycoprotein • $_1$-acid	18.2	89
Apolipoprotein B	1.9	31
HDL	12.8	8
LDL	<0.4	13

33. Transport of 141W94 into CEM/CD4+ cells.

The transport of 141W94 was studied in an HIV infectable cell line, CEM/CD4+ cells to determine how it permeates cell membranes.

Cell association was rapid, with equilibrium being reached in 60 seconds in 34 • M 141W94. The rate of association was concentration-dependent (as determined between 4 and 99 μM) and not inhibited by 95 μM unlabelled 141W94. Drug associated with cells was concentrated 3.8 fold. 141W94 was partly membrane bound (40 %) and partly cytosolic protein-bound 20%).

Thus 141W94 rapidly associates with CEM/CD4+ cells in a concentrative, concentration-dependent manner, with significant membrane and cytosolic protein-binding.

34. Exploratory *in vitro* metabolism of 141W94 using rat, monkey and human liver S9:An interspecies comparison.

Metabolism of 141W94 was studied *in vitro* in the presence of S9 fractions from four different sources: Arochlor-induced rat, normal (untreated) rat, cynomolgus monkey, and human livers.

Results indicate that there was very little turnover of the substrate under *in vitro* incubation conditions. Comparing the three species, there was very little metabolism seen in the case of the noninduced rat. Incubation of 141W94 with the induced rat liver S9 fraction yielded nine metabolites.

Oxidative-reductive opening of the THF ring of 141W94 produced one diol and carboxylic acid derivative. Cyclic oxidation of the THF ring produced a mixture of three compounds, all positional isomers of the dihydrofuran. Two monohydroxylated products were obtained, one hydroxylation on the aniline ring (which is subsequently glucuronidated to an additional metabolite) and one hydroxylated at the benzylic position (which is further metabolized to a styrene). The parent compound was also metabolized into an additional glucuronide and a deamidification product. The profile of the induced rat, monkey and human were similar except that the monohydroxylated glucuronide and the deamidification product were unique to the induced rat.

Overall conclusions

There are no toxicology data submitted to this NDA that would preclude the approval of Agenerase to treat HIV infection. The toxicity of the drug has been characterized by the sponsor and consists of changes which can be monitored in patients. The sponsor has ongoing carcinogenicity studies in rats and mice and will submit such data to the agency as soon as they become available. This has been entered into the list of phase 4 agreements.

/S/

Owen G. McMaster, Ph.D.
Pharmacology Toxicology Reviewer, DAVDP

Concurrences:
HFD-530/JFarrelly 4/29/99
HFD-530 WDempsey 5/13/99

Disk:
HFD-530/JFarrelly

cc:
HFD-530 Original IND
HFD-530 Division File
HFD-340
HFD-530/Chem/GLunn
HFD-530/CSO/MTruffa
HFD-530/Micro/NBattula
HFD-530/MO/JMartin
HFD-530/Pharm/OMcMaster