Table 25 Gestational parameters in rats treated with 1592U89

	Control	500	1000	1500
% pre-implantation losses	2.1	3.3	5.5	10
% resorptions per litter	1	0	5	80
% litters with resorptions	17	0	50	100
% non-live fetuses/litter	1	0	5	81
% litters with non-live fetuses	17	0	50	100
Number of live fetuses/litter	15	15	13	5
Fetal body weight	3.6	3.4	2.8	2.4

All litters treated at the high dose of 1592U89 showed fetal malformations.

Table 26 : Fetal malformations observed in rats treated with 1592U89 at 1500 mg/kg/day on days 6-14 of gestation

Finding	% Litters	% Fetuses
Any malformation	100	48
Anasarca	75	38
Cleft palate	75	29
Micrognatha	50	10
Short and curly tail	75	38
Kinked tail	25	5

Conclusion

1592U89 produces maternal toxicity when administered at 1000 and 1500 mg/kg including reductions in food consumption and body weight gain. At these doses, animals experienced increased fetal loss and reduced fetal body weight. Fetal abnormalities were observed only at the highest dose, but these were seen at all litters.

20.Developmental toxicity study in CD rats given 1592U89 succinate by gavage: Oral embryo-fetal development study. (Segment I). Glaxo Wellcome report # RD1997/01056/00. Glaxo study # TOX 775. Reproductive and Developmental Toxicology Laboratory.Research Triangle Institute. PO Box 12194, Research Triangle Park, NC 27709. Contract house study # 65C-6479-400. Drug reference # 95/5431-009-S Drug Batch # 5X3226. GLP study. January 1996.

Four groups of mated female rats, (30 rats/dose) were treated with 1592U89 succinate (dissolved on 0.5 % methyl cellulose) at 0, 100, 300 and 1000 mg/kg/day (in two divided doses of 0, 50, 150 and 500 mg/kg given 6 hours apart) from day 6 to 17 of pregnancy. Control animals received vehicle only. On day 20, all surviving dams were scarificed and necropsied and evaluated for body weight changes, liver weight and gravid uterine weight. Records were kept of clinical signs, bodyweights and food consumption as well as gestational parameters.

Administration of 1592U89 succinate was associated with rooting in the bedding after dosing. This was interpreted as taste aversion. There was also a 15 % decrease in food consumption during the first 3 days of treatment and this was acompanied by a 10 % decrease in body weight over the study period. As in other studies, there was a subsequent increase in food consumption compared to control, but the timing of this rebound effect was not consistent since it occurred late in dosing and/or after dosing in the different groups.

Relative maternal liver weights were increased by 5, 10 and 18 % at 100, 300 and 1000 mg/kg/day.

Dosing at 1000 mg/kg was associated with a 13 % decrease in fetus weight, a slight (7%) decrease in fetal length, increased malformations (3 times more litters were affected than in control animals), anasarca (whole body edema, 17 % of litters compared to 0 in control animals) and skeletal malformations (28 % of litters compared to 0 in control animals). Skeletal malformations included rib on lumbar 1 (93 % of litters affected at 1000 mg/kg versus 60 % in control animals) left full rib (34 % of litters at 1000 mg/kg/day versus 0 in controls), bilateral full rib (28 % of litters at 1000 mg/kg/day versus 0 % in control animals) bilateral rudimentary rib (62 % of litters at 1000 mg/kg/day versus 20 % in controls) and short rib XIII (21 % of litters at 1000 mg/kg versus 0% in controls). At 300 mg/kg/day there was an increase in the incidence left full rib on lumbar 1(15 % of litters compared to 0 % in controls) and anasarca was seen in 7 % of litters compared to control which had no cases. Although the anasarca was not seen at 300 mg/kg/day, it was seen in 7 % of the litters treated with 100 mg/kg/day (compared with 0% in controls).

Conclusions

1592U89 administration to rats at 1000 mg/kg during organogenesis was associated with increased liver weight as well as reduced food intake, body weight gain

and body weight. Increased skeletal malformations and anasarca were also seen. Extra rib (at 300 mg/kg) and anasarca (at 100 mg/kg) were also produced by the lowest doses of 1592U89.

21 Oral toxicokinetic study in pregnant CD rats given 1592U89. Glaxo Report # [redacted] Triangle Park, NC 27709. (Study # 65C-6479-500. February 1996. GLP study.

This study contains toxicokinetics data from animals treated with 1592U89 at doses identical to those in study RD1997/01056.

1592U89 succinate was dissolved in 0.5 % methylcellulose and administered by oral gavage to groups of 15 pregnant CD Sprague Dawley rats during organogenesis (gestation days 6 to 17). Doses were 100, 300 and 1000 mg/kg/day 1592U89 succinate (65, 194 and 648 mg/kg 1592U89 base) and were administered in two equally divided doses, six hours apart. Control animals received vehicle (0.5 % aqueous methyl cellulose). Records were kept of body weights, food consumption, clinical signs. On gestation day 17, blood was collected from 2 to 8 rats /group at time 0 (just before the last dose, 0.5, 1, 2, 4, and 8 hours post dosing. At necropsy, after the last dose on day 17, animals were again bled, and necropsied for macroscopic post mortem examination and the status of uterine implantation sites. Plasma concentrations of 1592U89 base were recorded.

Table 27. Mean toxicokinetics parameters recorded on gestational day 17 with 1592U89 succinate.

Dose (1592 succinate) Mg/kg/day	Dose (1592 base) Mg/kg/day	AUC (µg*h/ml)	Cmax (*µg/ml)
0	0	Not quantifiable	Not quantifiable
100	65	42	5.3
300	194	159	13
1000	648	427	23

Mortality

One dam treated at 1000 mg/kg/day was found dead on day 8 and 3 animals (one from the 1000 mg/kg dose group and two from the 300 mg/kg dose group) were removed from the study due to gavage errors.

Toxicity

Drug treated animals showed predose salivation and post dose rooting (interpreted as a taste aversion). At 300 and 1000 mg/kg/day, body weight gain decreased by 48 and 77 % respectively between gestational days 6 and 9. A rebound increase in body weight gain occurred at 300 and 1000 mg/kg/day (+ 19 and + 25 % increased compared to control, respectively) between days 12 and 15. Food consumption was down by 10 and 18 % at mid and high doses on days 6-9 and there was a slight rebound increase in food consumption on days 12-15. Liver weight (absolute and relative) increased by 18 % during dosing.

Pharmacokinetics

As seen in Table 27, Cmax values increased with dose, but were less than dose proportional. AUC values were approximately dose proportional.

Embryo toxicity

1592U89 succinate, given at 300 and 1000 mg/kg/day to pregnant female CD rats, resulted in an increase in the number of dead fetuses (1 and 5 % dead fetuses respectively, compared with 0 % in controls). There was also a corresponding increase in the number of litters with deaths (8 and 42 % respectively at the mid and high doses versus 0 % in controls).

Conclusions

Exposure to 1592U89 succinate doses producing 13 times the anticipated human exposure (AUC, 159 hr*μg/ml at 300 mg/kg/day) and above produced increased fetal deaths, pre-implantation losses, resorptions and reduced fetal body weights in rats.

22. Oral fertility and embryofetal Development Study. Glaxo Wellcome report #

Contract house study # 65C-6752-100. Drug lot # A96L700 and A96L701. Drug Batch # B5/R1/P3 and B6/R1/P3. GLP study. March 1997.

Four groups of rats, (28 rats/sex/group) were treated with 1592U89 hemisulfate, suspended in 0.5% methyl cellulose, at 0, 60, 160 and 500 mg/kg/day (in two divided doses of 0, 30, 80 and 250 mg/kg given 6 hours apart). Doses were equivalent to 51, 137, and 427 mg/kg/day of abacavir free base. Control animals received vehicle only. Four rats/sex/dose group were designated as satellite animals for toxicokinetics evaluation.

Males were dosed for 70 consecutive days prior to and during an initial mating with treated females , a second mating to untreated, naïve females and until termination after 14 weeks of treatment. After completion of a second mating to untreated females, 16 (of the 24 remaining) males per group were killed. Reproductive organs were weighed and histologic evaluation of reproductive organs and seminology (sperm number, motility and morphology) were done. Eight males per dose group were then held for a four week post dose recovery period during which no drug was administered. These animals were killed at the end of this recovery period and reproductive organs were weighed and retained.

Treated females were dosed for 14 consecutive days prior to pairing, throughout pairing and to day 16 of pregnancy. These were then killed on day 20, and necropsied. Untreated females mated with treated males were killed on day 15 for examination of uterine contents. Records were kept of clinical signs, bodyweights and food consumption as well as liver weight, gravid uterine weight and other gestational parameters

Females treated at 160 mg/kg showed an 11 % reduction in implantations and a 9 % reduction in the number of live fetuses. At 500 mg/kg/day there was no reduction in implantations but there was a slight (6 %) reduction in the number of live fetuses. At 500 mg/kg there was also a 13 % increase in resorptions and an 11 % reduction in fetus weights.

There were no effects in seminology parameters or the histology of the reproductive organs in treated males. No effects on the fertility of treated males or gestational parameters of the untreated females (with which these males were mated) were observed.

The 500 mg/kg dose of abacavir hemisulfate produced transient reductions in food intake and body weight gains. Liver weights were increased by about 10 % at this dose.

Table 28. Pharmacokinetics of abacavir hemisulfate in male and female rats.

Dose	Males $AUC_{(0\text{-}24h)}$ (µg*h/ml)	Females $AUC_{(0\text{-}24h)}$ (µg*h/ml)	Males C_{max} (*µg/ml)	Females C_{max} (*µg/ml)
0	-	-	-	-
60	39	49	7	5
160	123	118	13	12
500	435	393	25	26

AUC and Cmax values for abacavir hemisulfate increased with increasing dose and did not differ between male and female rats. The exposures produced by the 160 and

500 mg/kg dose are approximately 10 and 35 times the exposures seen in humans at the clinically recommended dose.

Conclusions

Abacavir hemisulfate produces reductions in the number of implantations and the number of live fetuses at doses 10 times the exposures seen with the clinically recommended dose. At 35 times the clinical exposures there are increases in resorptions and reductions in fetus weights.

23 Dose range finding study of 1592U89 succinate administered by gavage to nulliparous New Zealand White rabbits. Glaxo Report # RD1996/00156. Glaxo Study # DRF 744. Reproductive and Developmental Toxicology Laboratory, Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709. (Study # 65C-6479-100. November 1996. Non-GLP study.

This study was designed to determine the toxicity of 1592U89 succinate in nulliparous New Zealand White rabbits, in order to aid in dose selection for an embryo fetal toxicology study. 1592U89 succinate was dissolved in 0.5 % methylcellulose and administered by oral gavage to 5 groups of 2 female New Zealand White rabbits for ten days. Doses were 250, 500, 750, 1500 and 2000 mg/kg/day 1592U89 succinate, administered in two equally divided doses, six hours apart. Control animals received vehicle (0.5 % aqueous methyl cellulose). Records were kept of body weights, clinical signs, liver weights and gross necropsy findings.

Toxicity

Animals treated with 1592U89 succinate at 750 mg/kg and above showed red foci in lungs. Dosing with 1592U89 at 1500 and 2000 mg/kg/day resulted in flushed ears, while dosing at the higher dose resulted in the death of the two animals so treated. In the animals that died, lethargy, yellow eyes and ears, lacrimation, salivation, rapid respiration, soft stool, diarrhea, yellow/white streaks in papilla of kidneys, yellow, empty bladder and large intestines were noted. Relative liver weight was increased by 50 % in the 2000 mg/kg dose group.

Conclusion

New Zealand White rabbits, treated with 1592U89 succinate showed flushed ears and red foci in lungs at doses between 750 and 1500 mg/kg/day. 1592U89 succinate at oral doses of 2000 mg/kg/day produced moribundity and mortality in nulliparous New Zealand White rabbits. Doses of 750, 1000 and 1500 mg/kg were therefore chosen to use in the dose range finding study in pregnant rabbits.

24 Dose range finding study of 1592U89 succinate administered by gavage to timed mated New Zealand White rabbits. Glaxo Report # RD1996/00155. Glaxo Study # DRF 745. Reproductive and Developmental Toxicology Laboratory, Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709. (Study # 65C-6479-200. January 1996. Non-GLP study.

This study was designed as a pilot study to determine the embryo fetal toxicity of 1592U89 succinate in New Zealand White rabbits, and to aid in dose selection for the definitive embryo fetal toxicology study. 1592U89 succinate was dissolved in 0.5 % methylcellulose and administered by oral gavage to 3 groups of 7 female New Zealand White rabbits for 14 days (gestational days 6-20). Doses were 750, 1000 and 1500 mg/kg/day 1592U89 succinate, administered in two equally divided doses, six hours apart. Control animals received vehicle. Records were kept of body weights, clinical signs, liver weights gross necropsy findings and gestational parameters (including gravid uterus weight, ovarian corpora lutea, status of all ovarian implantation sites, total, nonlive and live fetuses). All live fetuses were euthanized, weighed, examined for external malformations and variations and discarded.

Mortality

Deaths/moribund sacrifices among treated animals included 1 of 7 animals at 750 mg/kg, 3 of 7 animals at 1000 mg/kg and 5 of 7 animals at 1500 mg/kg. Animals which died showed lethargy, prone positioning, stiffness of hind limb and failure to right. Necropsy findings included red, pale or mottled kidneys, liquid In the gastrointestinal tract, pale congested lungs with multiple red foci and blood around mouth, nose and/or perianal area.

Toxicity

Drug treatment was associated with a dose-related incidence of red ears post dose and rapid respiration. Shallow, audible/labored respiration was seen at 1500 mg/kg/day. Maternal body weight gain was decreased by 624 and 441 % (days 18 – 20) and food consumption was decreased by 72 and 53 % (days 20-21) respectively at 750 and 1000 mg/kg/day. At 1500 mg/kg/day, body weight gain (+47 %) and food consumption (+19 %) were increased compared to controls.

The number of fetal deaths was increased at 1500 mg/kg/day (9 % compared with 0 % in controls) and the number of resorptions was increased (mean of 1.33 and 0.33 resorptions/litter at 750 and 1000 mg/kg/day versus 0 at controls. At 1500 mg/kg/day there were no resorptions but fetal body weight was reduced by 18 %.

Two does from the 750 mg/kg dose group delivered early and one animal in the 1500 mg/kg dose aborted.

Conclusion

Mortality at 1000 mg/kg/day indicates that this dose is too high to be useful in a definitive embryo toxicity study. Doses to be used in the definitive study will be 125, 350 and 700 mg/kg/day of 1592 succinate, doses equivalent to 81, 227 and 453 mg/kg/day 1592U89 base.

25 Oral embryo fetal developmental study of 1592U89 succinate in pregnant New Zealand White rabbits. Glaxo Report # RD1997/01058/00. Glaxo Study # DRF 777. Reproductive and Developmental Toxicology Laboratory, Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709. (Study # 65C-6479-600). March 1996. GLP study. 1592U89 reference number 95/5431-009-S. Batch # 5X3226.

This study was designed to determine the embryo fetal toxicity of 1592U89 succinate in New Zealand White rabbits. 1592U89 succinate was dissolved in 0.5 % methylcellulose and administered by oral gavage to 3 groups of 27 pregnant female New Zealand White rabbits for 14 days (gestational days 6-20). Doses were 125, 350 and 700 mg/kg/day 1592U89 succinate, administered in two equally divided doses, six hours apart. Control animals received vehicle. Records were kept of clinical signs, body weights, food consumption, gross necropsy findings and gestational parameters (including gravid uterus weight, ovarian corpora lutea, status of all ovarian implantation sites, total, nonlive and live fetuses). All live fetuses were euthanized, weighed, examined for external malformations and variations and discarded.

Table 29. Mortality

Dose	Maternal deaths
0	4
125	1
350	7
700	7

The high mortality rate in this study (15 % in controls and 25 % at 350 and 700 mg/kg/day) is unexplained, but not assumed to be related to dosing accidents. Necropsy findings included dark red/brown congested lungs at all doses. At higher doses, trachea was red and/or had blood present and there was blood in the thoracic cavity. At 350 mg/kg, there were capillaries hemorraging on the pancreas and at 700 mg/kg/day ovarian cysts and irregular, pale heart surface.

Toxicity

1592U89 dosing was associated with a dose related increase in red ears and increased respiration.

At 700 mg/kg/day, there was a 22 to 32 % reduction in food consumption between days 12 and 21. Body weight gain was reduced by 68, 93 and 133 % at low, mid and high doses between days 15-18. There was a slight non-significant increase in the number of fetuses with skeletal variations (62 % at 700 mg/kg versus 42 % in controls) and litters with skeletal variations (94 % at 700 mg/kg versus 75 % in controls). There was also a slight non-significant increase in resorptions (mean 0.39 resorptions/litter at 700 mg/kg versus 0.25 resorptions/litter in controls. Resorption was observed in five of the seven dead does. There were no differences in corpora lutea, implanatations, live fetuses, dead fetuses.

Conclusion

1592U89 succinate produces some embryo-fetal toxicity in New Zealand White rabbits at 700 mg/kg/day, a dose which produces exposures 8 times the human exposure (see the following study report).

26 Oral toxicokinetics study of 1592U89 succinate in pregnant New Zealand White rabbits. Glaxo Report # RD1997/01059/00. Glaxo Study # DRF 778. Reproductive and Developmental Toxicology Laboratory, Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709. (Study # 65C-6479-700). March 1996. GLP study. 1592U89 reference number 95/5431-009-S. Batch # 5X3226.

This study was designed to determine the toxicokinetics of 1592U89 succinate in New Zealand White rabbits. 1592U89 succinate was dissolved in 0.5 % methylcellulose and administered by oral gavage to 3 groups of 5 pregnant female New Zealand White rabbits for 14 days (gestational days 6-20). Doses were 125, 350 and 700 mg/kg/day 1592U89 succinate, administered in two equally divided doses, six hours apart. Control animals received vehicle. Records were kept of drug levels, clinical signs, body weights, food consumption, gross necropsy findings and gestational parameters (including gravid uterus weight, ovarian corpora lutea, status of all ovarian implantation sites, total, non-live and live fetuses). All live fetuses were euthanized, weighed, examined for external malformations and variations and discarded.

Toxicokinetics

Table 30. Mean toxicokinetics parameters in New Zealand White rabbits on gestational day 20

Dose	$AUC_{0\text{-}24h}$ (µg*h/ml)	C_{max} (*µg/ml)
125	15	6.5
350	33	11
700	102	20

1592U89 produced two deaths at 125 mg/kg/day. One animal was found dead on day 16 after vaginal bleeding was observed the previous day while a second animal was sacrificed moribund on day 17 (blood was found in the animal's pan.)

Maternal toxicity was similar to those in the main study with red ears in all groups, rapid respiration and weight loss (up to 13 % loss in body weight on day 15 in low and high dose groups). Animals lost 7 % of body weight on day 9 in mid dose group. Relative liver weights were increased by 15 to 18%.

Embryo-fetal toxicity

High dose animals (700 mg/kg/day) produced more dead fetuses (0.4 dead fetuses/litter versus 0.2 dead fetuses/litter in controls) and more resorptions (0.4 resorptions/litter versus 0.2 resorptions/litter for controls.

Conclusion

Treatment of rabbits with 1592U89 succinate at doses which produce exposures (AUC's) eight times the expected clinical exposures produces maternal deaths, rapid respiration, reduction in body weight and body weight gain and red ears. Embryo fetal toxicity included slight increases in resorptions and skeletal variations. The most common skeletal variation was bilateral full extra rib on lumbar 1(48 at 700 mg/kg versus 40 at controls) and right rudimentary limb on lumbar 1 (11 and 13 fetuses affected at 350 and 700 mg/kg/day compared with 4 in control animals). Since reductions in body weight were observed at all doses, these findings may have been influenced by general maternal health.

27. Oral pre and post-natal developmental toxicology study of 1592U89 hemisulfate in CD-1 rats. Segment III. Glaxo Report # RD1997/040233. Glaxo Study # R40233. Reproductive and Developmental Toxicology Laboratory, Research Triangle Institute, PO Box 12194, Research Triangle Park, NC 27709. (Study # 65C-6752-200). May 1997. GLP study. 1592U89 lot number A96L630. Batch # B4/R1/P3.

This study was designed to determine the effects of 1592U89 hemisulfate in the rat when administered from the time of embryonic implantation in maternal females through weaning. 1592U89 hemisulfate was administered orally, by gavage to four groups of 24 inseminated female CD rats (F0) from gestational day 6 through postnatal day 20. Mating was designated gestational day 0 and birth was designated postnatal day 0. After weaning on postnatal day 21, a minimum of one male and one female (F1) was selected from each litter while all remaining pups and dams were killed for postmortem examinations. The selected offspring were allowed to reach sexual maturity and mated within treatment groups. The females were allowed to litter and their F2 offspring were observed for four days before dams and pups were sacrificed and examined. F1 males were also sacrificed at this point. Drug was given at doses of 60, 150 and 500 mg/kg/day

(doses equivalent to 51, 137 and 427 mg/kg/day of 1592U89 base) to the F0 generation females only. Plasma drug levels were measured in F0 females one hour after the second dose on postnatal day 12.

Results

Treatment with 1592U89 hemisulfate resulted in rooting post dose (interpreted as a taste aversion) in all treated groups. At 500 mg/kg/day F0 females showed a clear reduction in food consumption (-10 %) and bodyweight change (-47 %) between gestational days 6 and 9. The mean number of still births was 0.42 per litter at the high dose compared to 0.09 in controls. The still birth index (number of dead pups on postnatal day 0 divided by total number of pups on postnatal day 0) was significantly increased at 500 mg/kg, (2.63) compared to control (0.59). Pup weight on postnatal day 0 was decreased at the high dose (6.1 g versus 6.6 g in controls). Average pup weight was reduced by about 10 % at all time points in high dose dams. Body weights of F1 males and F1 females were decreased (on the order of 15 %) at all observation periods (pre breeding, mating, sacrifice) although this did seem to affect reproductive performance in these animals. F2 offspring were not affected by the treatment of the F0 generation with 1592U89.

Table 31. Plasma 1592U89 levels

Dose 1592U89 hemisulfate Mg/kg/day	Dose 1592U89 (base) Mg/kg/day	[1592U89] in plasma µg/mL
60	51	4.1
160	137	9.4
500	427	22

Conclusion

Rats treated with 1592U89 hemisulfate at 500 mg/kg/day (a dose which produces plasma levels seven times the expected clinical plasma level) from the time of embryonic implantation in maternal females through weaning showed reduced food consumption and body weight gain between gestational days 6 and 9. There was no significant difference in body weight in these F0 dams when compared to controls. There is also an increase in the number of still births at this dose and average pup weights were reduced. The weight of these F1 offspring remained reduced compared to control animals even though reproductive performance of this group was not affected. The F2 generation produced from these F1 animals was normal.

The sponsor claims that these effects were observed at a maternotoxic dose since body weight gains were reduced at the high dose. However, since the maternal body weight did not in fact fall, the reduction in body weight gain must be seen as a part of its fetotoxic profile.

28 Salmonella/Mammalian microsome assay with 1592U89. Report TTEP/93/0034.

[redacted]

1592U89UW 0.6 H_2O. GLP Study

This experiment was designed to examine if 1592U89 would induce gene mutations in the Ames Salmonella/mammalian microsome mutagenicity assay. The drug was tested at six concentrations between 100 and 5000 ug per plate in the presence or absence of metabolic activation (Aroclor-induced rat liver S9) using tester strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100. Three plates were used per dose level, along with appropriate positive and negative controls.

The drug 1592U89 did not induce reverse mutations at the histidine locus in any of the tester strains in the Salmonella plate incorporation assay or in the preincubation modification assay. Appropriate controls, both positive and negative responded as expected and met the criteria for a valid assay.

29. 1592U89 succinate (GI265235A): *In vitro* assay for chromosomal abberrations in cultured human whole blood lymphocytes. Glaxo Wellcome Study # V40177. Report

[redacted]

Batch number 5X3226. CAPPS lot # A95L300. GLP study.

Human venous blood was used in the chromosome aberration assays to determine whether 1592U89 or its metabolites can induce chromosome breaks. Replicate cultures were used at each dose level, as well as for the negative, solvent and positive control. The aberration assays were conducted at 30-hour harvest time in the initial trial (Trial 1) and with 30 (Trial 2) and 53 hour (Trial 2) harvest times in the confirmatory trial.

Table 32. Chromosome aberrations in human lymphocytes in 1592U89 without S9

Treatment	Trial 1 27exp/30fix*	Trial 2 27exp/30fix*	Trial 2 50exp/53fix**
Negative control	1.0	1.0	0.5
DMSO	0.5	1.5	0.0
mitomycin C	43.3	35.3	
25 (µg/ml)	1.0	1.0	1.5
50 (µg/ml)	4.0	1.0	1.0
100 (µg/ml)	2.5	1.0	7.5
125 (µg/ml)	1.0	3.0	11.5

*exposed for 27.1h, fixed after 30 hours. ** exposed for 50.3 hours and fixed after 53 hours.

Table 33.Chromosome aberrations in human lymphocytes exposed to 1592U89 for 3 hours

	Initial	Initial	Confirm	Confirm	Confirm
Treatment (µg/ml)	**Trial 1 3exp/30fix +S9**	**Trial 1 3exp/30fix -S9**	**Trial 2 3exp/30fix -S9**	**Trial 2 3exp/30fix +S9**	**Trial 2 3exp/53fix -S9**
negative control	0.5	0.0	0.0	0.0	1.0
DMSO	1.5	0.0	0.5	0.0	1.0
mitomycin C	30.5	59.0	26.0	42.0	
1200	2.5			0.5	
2000	2.5	1.5	1.0		1.0
2400	1.5	0.0	1.0	0.0	1.0
2800	5.5	3.3	1.3	1.0	1.0
3200	6.5	5.0	1.5	4.5	3.5

In the initial assays, a statistically significant increase in chromosome aberration occurred in the cells exposed to the test article in the absence of S9 metabolic activation at 2800 and 3200 µg/ml, (3 hour exposure and 30 hour harvest) and at 50 µg/ml (27 hour exposure and 30 hour harvest). In the presence of S9 activation, increases were recorded at 2800 and 3200 µg/ml, (3 hour exposure and 30 hour harvest).

In the confirmatory assays, in the absence of S9 metabolic activation, significant increases in chromosome aberrations were observed at 100 and 125 µg/ml (50.3 hour exposures and 53 hour harvest). In the presence of S9, chromosome aberrations were increased at 3200 µg/ml (3 hour exposure, 30 hour harvest).

Conclusion:

1592U89 was clastogenic in the human whole blood lymphocyte chromosomal aberration assay. Statistically significant increases in the frequency of cells with chromosomal aberrations were observed with this drug with and without metabolic activation.

30. Bone marrow micronucleus assay in male and female CD-1 mice dosed orally with 1592U89 succinate Drug lot number 94/5977-045. September 1995. (TTEP/95/0073)

The study is designed to determine the ability of 1592U89 succinate to cause chromosome breaks and/or spindle malformations as assessed by the incidence of micronucleated polychromatic erythrocytes in the bone marrow of mice dosed daily with 1592U89 for three days.

Groups of mice (5/sex/dose group) were dosed with three daily oral doses of 1592U89 at 0 (vehicle, 0.5 % methylcellulose), 250, 500, 750, 1000 mg/kg/day or a single dose of cyclophosphamide (positive control) and were sacrificed 24 hours after the final dose. A satellite group of animals (4/sex/dose group) were treated with 1592U89 at 0, 250, 500 or 1000 mg/kg but were used for pharmacokinetic analysis. Plasma samples were taken from these animals 30 minutes after the final dose to monitor 1592 levels.

Immediately following sacrifice, both femurs of each mouse were exposed, separated from the surrounding tissue and rinsed free of any blood with Dulbecco's phosphate buffered saline. A cell suspension was obtained from the marrow canal and slides were prepared. Two thousand to four thousand polychromatic erythrocytes (PCE's) per animal were scored for the incidence of micronuclei. The ratio of PCE's to normochromatic erythrocytes was determined by counting a total of 1000 erythrocytes. Slides were analyzed by a person blinded to the treatment regimen and results were not decoded until all slides had been analyzed.

Results

Table 34A. Mean plasma concentrations (µg/ml) of mice treated with 1592U89

Dose (mg/kg)	[1592U89] (µg/ml)	
	Male	Female
250	22	18
500	14	27
750	42	40
1000	42	72

Table 34B. Polychromatic erythrocytes (PCE) and micronucleated polychromatic erythrocytes (MPCEs) in male mice treated with 1592U89

Treatment	Dose	% PCE	MPCEs
0.5 % methylcellulose	10 mL/kg	42	1.7
1592U89 succinate	500 mg/kg	41	1.6
1592U89 succinate	750 mg/kg	39	2.4
1592U89 succinate	1000 mg/kg	32	3.9
Cyclophosphamide	75 mg/kg	39	36

% PCE = 100 x PCE / (PCE + NCE [normochromatic erythrocytes]) values are based on 1000 erythrocytes per animal.
MPCEs= micronucleated polychromatic erythrocytes per 1000 PCEs; values are based on the scoring of 2000 PCEs per animal

Table 35 Polychromatic erythrocytes (PCE) and micronucleated polychromatic erythrocytes (MPCEs) in female mice treated with 1592U89

Treatment	Dose	% PCE	MPCEs
0.5 % methylcellulose	10 mL/kg	40	1.5
1592U89 succinate	500 mg/kg	43	2.1
1592U89 succinate	750 mg/kg	41	2.1
1592U89 succinate	1000 mg/kg	34	1.6
Cyclophosphamide	75 mg/kg	36	55

% PCE = 100 x PCE / (PCE + NCE [normochromatic erythrocytes]) values are based on 1000 erythrocytes per animal.
MPCEs= micronucleated polychromatic erythrocytes per 1000 PCEs; values are based on the scoring of 2000 PCEs per animal

Conclusion

Oral treatment with 1000 mg/kg 1592U89 resulted in a significant increase in micronucleated polychromatic erythrocytes (MPCEs) in male mice. The mean plasma concentrations for animals exposed to 1000 mg/kg 1592U89 were 42 and 72 µg/ml for males and females respectively.

Pharmacokinetic Studies

Mouse Studies

The pharmacokinetics of 1592U89 was studied in male CD-1 mice which were treated by intragastric intubation and by intravenous injection. Five independent mice were used for each time point, route of administration and dose. In mice, between 14 and 77 mg/kg, the pharmacokinetics of 1592U89 appears to be dose dependent (see Tables 36 and 37). The higher intravenous dose is associated with a longer t1/2 and higher clearance, while the AUC disproportionately higher and the Cmax lower after the higher oral dose. The lower bioavailability at the 77 mg/kg dose probably reflects saturation of absorption, since the clearance of this drug is primarily metabolic. Glaxo Wellcome Reports TEIN/94/0004/00, TEIN/94/0005/01, TEIN/94/0015.

Monkey Studies

The pharmacokinetics of 1592U89 was studied in groups of four male Cynomolgus monkeys given a single intravenous and oral dose of 14 or 35 mg/kg of drug in a crossover design study with a two week washout period between doses. Select pharmacokinetic parameters are shown in Tables 9 and 10. Of note, the Vdss of 1. 1 L/kg suggests that the drug is distributed in total body water. The pharmacokinetics of 1592U89 seem to be dose independent since the increase in mean AUC seen at the higher dose (35 mg/kg) was approximately proportional to the dose escalation. After oral dosing, oral bioavailability was determined to be 76 % and the pharmacokinetics again appeared to be dose-independent within the range tested.

Table 36. Summary of intravenous pharmacokinetic data

Species	1592U89 dose (mg/kg)	$T_{1/2}$ (hr)	Clearance (L/hr/kg)	Vdss (L/kg)	AUC (μM*hr)
Mouse	14	0.27	2.65	0.91	13.2
	77	0.78	1.86	1.34	103
Monkey	14	1.06	0.87	1.12	41
	35	1.56	0.71	1.16	129

Table 37. Summary of Oral pharmacokinetic data

Species	1592U89 dose (mg/kg)	Cl/F (L/hr/kg)	AUC0- (μM.hr)	C_{max} (μM)	T (hr)	F (%)
Mouse	14	2.87	12.2	15	0.17	92
	77	2.45	78.5	52	0.17	76
Monkey	14	1.37	31.8	15	1.5	77
	35	1.02	101	29	2.0	76

Table 38. Protein binding of 1592U89 in human, monkey and mouse plasma

1592U89 concentration (μM)	Percent bound in plasma		
	Human	Monkey	Mouse
0.10948.8	36.9		20.1
0.284	50.6	39.0	17.3
0.85	50.5	39.3	17.8
2.34	49.2	38.8	18.5
5.43	49.3	38.9	18.2
13.4	49.3	39.2	19.4
36.0	48.1	38.5	18.5
Means	**49.4**	**38.7**	**18.5**

The pharmacokinetics of this drug seems to be species specific with dose dependent pharmacokinetics in mice and dose independent pharmacokinetics in monkey.

Table 38 shows that protein binding of this drug ranged from a low of 19 % in mouse to a moderate 49% in human plasma. Drug interactions involving protein binding displacement are therefore not anticipated.

Table 39. Urinary and fecal recovery of 14C-1592U89 from mice given a single oral dose of 54 mg/kg.

Collection Period	Average % of radioactive dose recovered		
	Urine	Feces	Urine & Feces
0-8 hr	83.	15.8	98.8
8-24 hr	5.6	3.1	8.7
4-48 hr	1.3	1.7	3.0
Total (0-48h)	89.8	20.6	110

Table 39 summarizes the urinary and fecal recovery of radioactivity from mice given a single oral dose of 1592U89 dispositional studies conducted with 1592U89. The table shows that a radioactive dose of 1592U89, administered orally to mice, was almost completely recovered in the urine and feces within 8 hours. Metabolism was the major route of elimination of the drug in both species with only 11-13 % of the drug being excreted unchanged in the urine.

Other Studies

Study report RD1997/01258/00 describes the bioequivalence of the succinate and hemisulfate salts when groups of 4 monkeys were treated with a single dose of 1592U89 succinate 30 mg (base)/kg or 1592U89 hemisulfate 22mg (base)/kg. There was no statistical difference between the succinate and hemisulfate salt forms in plasma exposure $AUC_{(0-\infty)}$, C_{max}, $T_{1/2}$, MRT or T_{max}.

Table 40. Mean Pharmacokinetics parameter estimates for male cynomolgus monkeys after a single oral dose of 1592U89 succinate or 1592U89 hemisulfate.

Salt Form	Dose (base) [mg/kg]	Cmax µg/ml	T max h	$AUC_{(0-\infty)}$ (µg*h/ml)	$T_{1/2}$ (h)	MRT
Succinate	30	11	1.8 (± 0.5)	39	1.2	3.0
Hemisulfate	22	14	1.0 (± 0.7)	42	1.2	2.4

Succinate and hemisufate forms of the drug produce similar pharmacokinetics and so studies of exposure to the abacavir succinate provides useful information about the toxicity of 1592U89 hemisulfate.

A number of other studies were performed during the investigation of the pharmacokinetics of this drug. If these studies were performed in conjunction with toxicity studies, the information is included in the toxicology report.

Overall conclusions

Abacavir toxicity has been characterized by Glaxo Wellcome and no findings herein preclude the approval of this drug product. Toxic effects included liver toxicity, mutagenicity and fetotoxicity and these have been adequately described in the product label.

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

Concurrences:

HFD-530/WDempsey 11/26/99
HFD-530/JFarrelly 11/21/99
HFD-590/OMcMaster
Disk
HFD-530/JFarrelly

cc:
Original NDA 20-977 and NDA 20-978
Division File
HFD-340
HFD-530/SMO/Kukich
HFD-530/MD/Cvetkovich
HFD-530/Micro/Mishra
HFD-530/Pharm TL/Farrelly
HFD-590/Pharm/McMaster
HFD-530/Chem/Kambhampati
HFD-830/CChen
HFD-530/Biopharm/Rajagopalan
HFD-530/CSO/Truffa

Appendix I. In the aforementioned studies, the following tissues were examined by light microscopy whenever a full panel of histology was performed.

Kidney	Urinary Bladder	Aorta
Heart	Trachea	Lung
Liver	Gall Bladder	Pancreas
Fat	Salivary Gland	Spleen
Cervical Lymph Nodes	Mesenteric Lymph Nodes	Thymus
Tongue	Esophagus	Stomach
Duodenum	Jejunum	Ileum
Cecum	Colon	Mammary Gland
Skin	Skeletal Muscle	Sciatic Nerve
Parathyroid	Thyroid	Adrenal
Pituitary	Prostate	Testis
Epididmyis	Ovaries	Oviducts
Uterine Horns	Uterine Body	Cervix
Vagina	Brain	Spinal Cord
Eye	Sternum	Bone
Harderian Gland	Bone Marrow Smear	All Gross Lesions
Seminal Vesciles		

Executive CAC
December 23, 1997

Committee: Joseph DeGeorge, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-900, Member
, Alternate Member
, Presenting Reviewer
, Division Team Leader

Owen McMaster, Pharmacology Reviewer, DSPIDP:
The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

IND/NDA #
Name of Drug: 1592U89

Sponsor: Glaxo Wellcome Inc.

The compound 1592U89 succinate is a carboxylic nucleoside analog which has potent *in vitro* activity against HIV. The sponsor plans to conduct carcinogenicity studies of this drug in rats and mice.

The mouse study duration is to be 84 weeks. Crl:CD®-1(ICR)BRVAP/Plus™ mice will be dosed orally in the diet at 0 (control 1), 0 (control 2), 55, 110, and 330 mg/kg of 1592U89 and there will be 60 mice/dose group. Toxicokinetics mice (60 mice/dose group) will be dosed along with these animals but will not be subject to post-dose evaluation.

In a six month toxicology study in mice, there were no deaths recorded at 330 mg/kg dose (the highest dose). In a three month study excess deaths were seen in the 1000 mg/kg/day group (mortality incidence was 1, 1, 2, and 8 [of thirty animals] from animals treated with 1592U89 at 0, 110, 330 and 1000 mg/kg respectively). 1592U89 was associated with increased cholesterol (21 to 30 % increase at 330 mg/kg and 61 to 129 % increase at 1000 mg/kg/day) , increased triglyceride (+ 59% at 1000 mg/kg) and increased ALT (+ 210 % at 1000 mg/kg). Drug related histopathological findings were reversible and included minimal to mild hepatocellular hypertrophy, single cell necrosis (males at 330 mg/kg and male and female mice at 1000 mg/kg), bile stasis (in one of 30 animals at 330 mg/kg/day and 11 of 30 animals at 1000 mg/kg/day).

The CAC requested further information about the liver changes reported in this study. Histopathology changes (in particular, single cell necrosis), liver enzyme changes, and biliary stasis are observed beginning at 330 mg/kg, but there is no decrease in body weight. At 1000 mg/kg, these effects increase in magnitude and 8 of 30 animals died. The sponsor is proposing a high dose of 330 mg/kg/day but a high dose of 660 mg/kg/day should be tolerable.

Rat Carcinogenicity Study

Han Wistar (Glx:Han:WlfBR) rats will be treated with drug at 0, 0, 30, 120 and 600 mg/kg/day for 104 weeks by gavage. There were no excess deaths at 530 mg/kg in the three month study. Toxic effects were slight and included increases in liver weights (~ 20 %) with

centrilobular hypertrophy in rats. In acute toxicity studies, only 1 of 10 animals treated orally with 2000 mg/kg died. The sponsor has no further studies in rats as most of the toxicology has been performed in mice and monkeys. The CAC did not have enough information to concur with the sponsors choice of doses since the doses tested did not produce sufficiently toxic effects in rats to allow the determination of a maximum tolerated dose.

Joseph DeGeorge, Ph.D.
Chair, Executive CAC

cc:\

/Division File, HFD ###
/Presenting Reviewer
/Team Leader
/Project Manager, HFD-024