N20-564 EA

20564

EA

ENVIRONMENTAL ASSESSMENT

REDACTIONS MADE BY APPLICANT

AND

FINDING OF NO SIGNIFICANT IMPACT

3TC™

(lamivudine)

Tablets

150 mg

NDA 20-564

FOOD AND DRUG ADMINISTRATION

CENTER FOR DRUG EVALUATION AND RESEARCH

DIVISION OF ANTI-VIRAL DRUG PRODUCTS
(HFD-530)

FINDING OF NO SIGNIFICANT IMPACT

NDA 20-564

REDACTIONS MADE BY APPLICANT

3TC

(lamivudine)

Tablets

The National Environmental Policy Act of 1969 (NEPA) requires all Federal agencies to assess the environmental impact of their actions. FDA is required under NEPA to consider the environmental impact of approving certain drug product applications as an integral part of its regulatory process.

The Food and Drug Administration, Center for Drug Evaluation and Research has carefully considered the potential environmental impact of this action and has concluded that this action will not have a significant effect on the quality of the human environment and that an environmental impact statement therefore will not be prepared.

In support of their new drug application for **3TC Tablets**, **Glaxo Wellcome Inc.** has conducted a number of environmental studies and prepared an environmental assessment in accordance with 21 CFR 25.31a(a) (attached) which evaluates the potential environmental impacts of the manufacture, use and disposal of the product.

Lamivudine is a synthetic drug that is administered as an oral tablet in the treatment HIV infection. The drug substance manufacturing operations will take place at Glaxo Operations (UK) Limited, Montrose, Scotland and Ulverston, England and the drug product will be manufactured and packaged at Glaxo Wellcome Inc, Zebulon, NC. The finished drug product will be used in hospitals, clinics and by patients in their homes.

Lamivudine may enter the environment from excretion by patients, as emissions from manufacturing sites or from disposal of pharmaceutical wastes. Chemical and physical test results indicate that the majority of the drug substance will most likely be restricted to the aquatic environment. No rapid environmental depletion mechanism has been identified.

As lamivudine is expected to persist in the aquatic environment for some time, the toxicity of the material to organisms was characterized. Acute static toxicity studies in water fleas (*Daphnia magna*) and microbial inhibition testing indicate that the drug substance would not be toxic to organisms at the maximum expected environmental concentration

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Disposal of the drug may result from out of specification lots, discarding of unused or expired product, and user disposal of empty or partly used product and packaging. The manufacturer will dispose of waste drug substance and drug product at a licensed incineration facility. At U.S. hospitals and clinics, empty or partially empty packages will be disposed according to hospital/clinic procedures. From home use, empty or partially empty containers will typically be disposed of by a community's solid waste management system which may include landfills, incineration and recycling, while minimal quantities of unused drug may be disposed of in the sewer system.

The Center for Drug Evaluation and Research has concluded that the product can be manufactured, used and disposed of without any expected adverse environmental effects. Precautions taken at the sites of manufacture of the bulk product and its final formulation are expected to minimize occupational exposures and environmental release. Adverse effects are not anticipated upon endangered or threatened species or upon property listed in or eligible for listing in the National Register of Historic Places.

6/22/05 _____
DATE PREPARED BY
Nancy B. Sager
Environmental Scientist
Center for Drug Evaluation and Research

6/23/05 _____
DATE CONCURRED
Robert A. Jerussi, Ph.D.
Associate Director for Chemistry
Center for Drug Evaluation and Research

Attachment: Environmental Assessment

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APPENDIX 6

FREEDOM OF INFORMATION (FOI) RELEASABLE ENVIRONMENTAL ASSESSMENT

1.0 DATE:

May 10, 1995

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2.0 APPLICANT:

Glaxo Wellcome Inc.

3.0 ADDRESS:

Five Moore Drive
Research Triangle Park, NC 27709

4.0 DESCRIPTION OF THE PROPOSED ACTION

4.1 Description of Requested Approval

Glaxo Wellcome Inc. is requesting approval to formulate, package, and market 3TC™ (lamivudine) Tablets for the treatment of HIV infection in symptomatic and asymptomatic children and adults. Each dose of 3TC™ (lamivudine) Tablets will contain 150 mg of lamivudine drug substance which will be compressed into tablets. The marketed product will be dispensed only on order of a licensed physician.

4.2 Need for the Action

Lamivudine is a nucleoside analog which is a potent reverse transcriptase inhibitor that will be administered orally for the treatment of HIV infection. Lamivudine has a rapid oral absorption profile with peak concentrations observed from 0.5 to 1.5 hours after administration. Absolute bioavailability is approximately 85 % in adults and 65% in children in well controlled bioavailability studies. The prolonged intracellular half-life of 10-11 hours of the active moiety, lamivudine triphosphate allows for twice a day dosing.

Lamivudine, therefore, is a novel agent that may provide a significant improvement over existing treatments for HIV infection.

4.3 Locations where Products will be Produced

The drug substance, lamivudine, will be manufactured in bulk by Glaxo Operations (UK) Limited in Montrose, Scotland. One intermediate compound (lamivudine salicylate), necessary for production of lamivudine at the Montrose facility, will be manufactured at the Glaxo Operations (UK) Limited facility in Ulverston, England. The drug product (3TC™ (lamivudine) Tablets) will be manufactured and packaged at Glaxo Wellcome Inc. in Zebulon, North Carolina.

Drug Substance Manufacturing

Glaxo Operations (UK) Ltd
North Lonsdale Road
Ulverston
Cumbria LA12 9DR
United Kingdom

The Glaxo Operations Limited Ulverston facility is located on the northwest coast of England on the shore of Morecambe Bay. Ulverston is a small town (population 12,000) approximately 10 miles from the major industrial center of Barrow-in-Furness. The surrounding area is mainly residential and commercial, with a small amount of industry. Local industries include agriculture, fishing, and light engineering. The Glaxo Operations facility is located on the south side of the Furness peninsula, on the site of a former iron works. The River Leven Estuary runs along the south side of the site to enter Morecambe Bay. The topography in the vicinity of the facility ranges from low lying to moderately hilly. Local weather is typical of a temperate, maritime climate. The Ulverston facility covers approximately 60 acres and contains manufacturing departments, offices, storage areas, laboratories, and workshops, and several small ancillary buildings.

Glaxo Operations (UK) Ltd
10 Cobden Street
Montrose
Angus DD10 SE13
Scotland, United Kingdom

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Glaxo Operations Limited's Montrose facility is located in the town of Montrose, a small town in northeast Scotland between the cities of Aberdeen and Dundee. Other industries in the town include agriculture, fishing and oil field supply services. The facility is located adjacent to the North Sea at the mouth of the South Esk River. The site covers 45 acres and is approximately one mile due east of the Montrose Basin. The site is bounded to the east by the local beach and the North Sea, to the south by the estuary of the South Esk river and to the north by residential, commercial and industrial properties.

Drug Product Manufacturing and Packaging

Glaxo Wellcome Inc.
1011 North Arendell Avenue
Zebulon, North Carolina 27597

Glaxo Wellcome Inc.'s Zebulon, North Carolina facility is located about 25 miles east of Raleigh, North Carolina. The Town covers two square miles and has an approximate population of 2889. Other industries which are located in Zebulon include textile mills, metal finishers and a plastics manufacturer. The site has a total of 224 acres. The Zebulon facility employs approximately 750 people.

4.4 Sites of Product Use

The drug product (3TC™ (lamivudine) Tablets) will be dispensed by prescription in pharmacies and used in private residences, hospitals and clinics, throughout the United States.

4.5 Sites of Disposal

Product that is introduced into the patient will be excreted in the urine and feces and distributed into wastewater treatment systems throughout the United States.

Returned product disposal will occur at high-temperature commercial incinerator facilities that are permitted to dispose of such wastes by appropriate local, state and federal regulatory agencies. Currently, disposal of return product is contracted to:

Chambers Medical Technologies, Inc.
100 Nix Street
Hampton, South Carolina 29924

Chambers Medical Technologies, Inc. holds permit number 1280-0021, issued on July 29, 1986 by the South Carolina Department of Health and Environmental Control (DHEC). The permit has an expiration date of March 31, 1991. DHEC confirms that Chambers Medical Technologies, Inc. applied for a permit renewal as required and is operating under the existing permit until DHEC issues a new permit.

An alternative solid waste incineration facility under consideration for the disposal of returned product is:

BFI - Medical Waste Systems
1168 Porter Avenue
Haw River, North Carolina 27258

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BFI - Medical Waste Systems holds air permit number 5896R7 issued June 18, 1994 by the North Carolina Department of Environment, Health and Natural Resources (DEHNR). The permit has an expiration date of July 1, 1996.

Rejected drug substance and drug product produced at manufacturing sites is disposed of via high temperature incineration either on site or at off-site facilities approved by the respective governments for this purpose. Information on incineration facilities used to destroy rejects can be found in Section 6.

5.0 IDENTIFICATION OF CHEMICAL SUBSTANCES

The chemical substances that are the subject of the proposed action can be divided into five categories: (1) drug substance, (2) drug substance impurities and degradants, (3) drug product excipients, (4) drug substance and drug product manufacturing waste products, and (5) packaging materials and package disposal waste products. Information on the chemical substances identified in each of the categories is discussed in Sections 5.1 through 5.5.

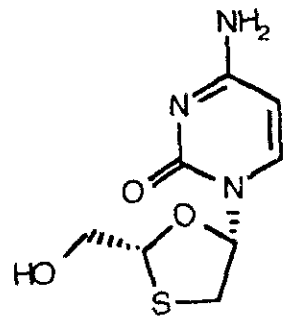
5.1 Drug Substance Information

Approved Names Lamivudine

Chemical Name (-)cis-5-(4-Amino-1,2-dihydro-2-oxo-1-pyrimidinyl)-1,3-oxathiolane-2-methanol

Code Name GR109714X

Structural Formula



REDACTIONS MADE BY APPLICANT

Molecular Formula $C_8H_{11}N_3O_3S$

Molecular Weight 229

CAS Number 134678-17-4

5.2 Drug Substance Impurities and Degradants

A confidential list of drug substance impurities and degradants has been provided to FDA.

5.3 Drug Product Excipients

A confidential list of drug product excipients has been provided to FDA.

5.4 Manufacturing Waste Products

Confidential information on drug substance and drug product manufacturing wastes has been provided to FDA.

5.5 Packaging Materials

The following materials will be used in packaging of the drug substance:

polyethylene bags
polyethylene kegs

These packaging materials will enter the waste stream subsequent to manufacture of the drug product with the exception of the polyethylene kegs which will be recycled.

The following materials will be used in packaging of the drug product:

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Cartons
Caps
Coilers
HDPE bottles
Paper labels
Corrugated RSC cases
Package inserts

These packaging materials will enter the waste stream as a result of product use, and when rejected or expired materials are returned. Information on chemical names, CAS numbers and chemical structures is not available for these widely used commercial packaging materials.

6.0 INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

The drug substance and other substances associated with its manufacture can potentially enter the environment from four main sources: (1) the sites associated with the manufacture of the drug substance, lamivudine, or its intermediates; (2) the sites associated with the manufacture and packaging of the drug product; (3) the sites of use by patients; and (4) waste disposal sites for discarded or rejected product and packaging materials. Sections 6.1 through 6.5 discuss potential emissions from each of these sources.

6.1 Introduction of Substances from Drug Substance Production

The substances expected to be emitted, emission controls, and compliance with relevant environmental and occupational laws for the drug substance manufacturing sites are discussed below.

6.1.1 Substances Expected to be Emitted

The requested approval could potentially result in emissions to the environment from sites of production of starting materials, reagents and excipients used in the manufacture of the drug substance and the drug product. A confidential list of these materials has been supplied to the FDA.

6.1.2 Controls on Emissions

Ulverston

The pH adjusted process effluent from the lamivudine salicylate process is transferred by pipeline to a tidal storage tank which collects other process effluent streams from the site. Discharges of blended effluent are made into the River Leven Estuary for a restricted time at ebb tide.

Seal liquors from vacuum pumps used in the lamivudine salicylate process discharge to the Ainslie Creek system and are collected and transferred by pipeline through a screening system to tidal storage tanks for discharge to the River Leven Estuary for a restricted time at ebb tide.

Gaseous emissions from the lamivudine salicylate process are controlled by wet scrubbing systems. The vent and scrubber exhausts are monitored on a routine basis for acid and alkaline gases and volatile organic compounds, as appropriate.

An on-site incinerator is used to dispose of waste solvent and aqueous streams generated during the preparation of lamivudine salicylate and other products manufactured at the Ulverston facility.

There are no on-site facilities for the incineration of solid waste. Solid waste designated as special waste (process samples or materials contaminated with intermediates or flammable

solvents and not suitable for landfill) is disposed of by incineration at licensed merchant incineration contractors. Some liquid waste streams also may be disposed of by incineration off-site at licensed merchant incineration contractors. It is currently proposed to use the following facilities for this service:

Cleanaway Ltd
Bridges Road
Ellesmere Port, South Wirral
Cheshire
L65 4EQ

Rechem International Ltd
Pontyvelin Industrial Estate
New Road
Pontypool
Gwent
NP4 5DQ

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The authorization for the Cleanaway facility, issued by Her Majesty's Inspectorate of Pollution (HMIP) on 23 August 1993, valid for four years, is AG8233. The authorization for the Rechem facility, issued by HMIP on 16 July 1993, valid for four years, is AG7946.

Some packaging materials, personal protective equipment, and other general industrial waste are landfilled. The address of the facility currently used is:

Bennet Bank
Park Farm
Dalton-in-Furness
Cumbria
UK

The facility is owned and operated by Caird Environmental Ltd.

Montrose

Aqueous effluent generated in the lamivudine production areas is collected in either of two wooden vats. The contents are individually recirculated, brought into the required pH range with sodium hydroxide or hydrochloric acid, sampled and tested.

Pretreated wastewater is discharged to the tidal storage tank which collects all other process effluent streams from the site. Discharges of blended effluent are made into the estuary of the River South Esk one hour after high water to ensure maximum dilution and dispersion. There is no release of process wastewater into the storm water drainage system.

Triethylamine is used in the lamivudine manufacturing process. Any releases are extracted using ventilation, the exhaust of which is scrubbed with hydrochloric acid before emission to atmosphere.

All air emissions from the product finishing suite pass through prefilters and HEPA filters.

Two general purpose incinerators on the site are used to dispose of waste solvent and aqueous streams produced during the manufacture of lamivudine.

There are no on-site facilities for the disposal of solid wastes. Filter bags, HEPA filters, filter elements used in the process and / or contaminated with trace amounts of lamivudine and its intermediates are bulked for disposal by high temperature incineration at a licensed off-site facility.

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The Baldovie Incineration Plant
Forties Road
Baldovie Industrial Estate
Dundee
DD4 ONS
Scotland

Non chemical wastes (eg used fiberboard kegs, general refuse) are also incinerated. The authorization number for this facility, issued by Her Majesty's Industrial Pollution Inspectorate (HMIPI), is IPC/068/1993. The authorization is valid until 30 September 1997.

6.1.3 Regulatory Controls and Compliance

This Section contains discussions of environmental regulatory requirements associated with the production of lamivudine drug substance and compliance with the requirements. Summaries of wastewater, air, solid waste and occupational requirements are included on the following pages.

Ulverston

Table 1 contains a list of environmental regulations applicable to Glaxo Operations Ulverston manufacturing site.

Table 1. Overview of Environmental and Occupational Laws Applicable to the Glaxo Operations (UK) Ltd., Ulverston Facility

WASTEWATER DISCHARGES	Water Act (1989) Control of Pollution Act (1974) Environmental Protection Act (1990)
AIR EMISSIONS	Health and Safety at Work Act (1974) - Health and Safety (Emissions into the Atmosphere) Regulations (1983) - Health and Safety (Emissions into the Atmosphere) Amendment Regulations (1989) Environmental Protection Act (1990)
COLLECTED WASTE	Control of Pollution Act (1974) - Control of Pollution (Special Waste) Regulations (1980) - Control of Pollution (Special Waste) Amendment Regulations (1988) Environmental Protection Act (1990)
OCCUPATIONAL	Health and Safety at Work Act (1974) - Control of Substances Hazardous to Health Regulations (1988)

Discharges of effluent from the site to the River Leven Estuary are controlled by the Integrated Pollution Control authorization for the cephalosporins process granted by HMIP.

Lamivudine salicylate is manufactured in the Development Plant, which is authorized by HMIP.

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Disposal of solid and liquid wastes from the site is controlled by the local authorities under the terms of the Control of Pollution Act (1974) and the Environmental Protection Act (1990). Under the legislation, the transporter and waste disposer are required to hold a relevant license and to operate within the conditions of the license, with all parties involved in the transport and disposal chain demonstrating Duty of Care.

General industrial waste from the site is bulked for disposal and transported by Registered Waste Carriers to the local landfill site (Bennet Bank) which is operated by Caird Environmental Ltd. Each consignment of waste is accompanied by a relevant Duty of Care Transfer Document. Solid and liquid wastes designated as Special Waste are accompanied by a relevant Duty of Care Transfer Document and Section 17 Note, and are carried by Registered Waste Carriers for incineration at licensed merchant incineration contractors.

Emissions to atmosphere are controlled by the Environmental Protection Act 1990. In addition, emissions in the workplace are controlled by the Health and Safety at Work Act (1974) and the subordinate Control of Substances Hazardous to Health Regulations (1988). These are enforced by the Health and Safety Executive. Occupational emissions are assessed to ensure that exposure to substances hazardous to health are controlled and comply at least with the provisions of the Health and Safety at Work Act (1974) and its supporting regulations.

Montrose

Table 2 contains a list of environmental regulations applicable to Glaxo Operations Montrose manufacturing site.

Table 2. Overview of Environmental and Occupational Laws Applicable to the Glaxo Operations (UK) Ltd., Montrose Facility

WASTEWATER DISCHARGES	Water Act (1989) Control of Pollution Act (1974) Environmental Protection Act (1990)
AIR EMISSIONS	Health and Safety at Work Act (1974) Environmental Protection Act (1990)
COLLECTED WASTE	Control of Pollution Act (1974) - Control of Pollution (Special Waste) Regulations (1980) - Control of Pollution (Special Waste) Amendment Regulations (1988) Environmental Protection Act (1990)
OCCUPATIONAL	Health and Safety at Work Act (1974) - Control of Substances Hazardous to Health Regulations (1988)

Up until the implementation of the Environmental Protection Act (1990) in Scotland in April, 1992, legislation controlled pollution to each medium separately (i.e., emissions to atmosphere, discharges to controlled waters and disposal of waste to licensed disposal sites). Part I of the Act and its subsequent regulations include the concept of Integrated Pollution

Control (IPC) and is being implemented in a phased manner to replace the existing registration procedure.

The process for the manufacture of lamivudine has been authorized under the terms of the Environmental Protection Act (1990) since October 1994.

Discharges of effluent to the River South Esk comes under the jurisdiction of the local Tay River Purification Board (TRPB). The Water Act (1989), Control of Pollution Act (1974), and the Environmental Protection Act (1990) allow the discharge of effluent under the conditions of a consent, which specifies limits on the quantity and quality of the effluent.

Details of the consent and monitoring results are held on a public register held at the offices of HMIPI in Edinburgh. Copies are also retained by TRPB in their offices in nearby Arbroath.

Disposal of solid wastes from the Montrose site is controlled by the local authorities under the Control of Pollution Act 1974 and, subsequently, the Control of Pollution (Special Waste) Regulations 1980, the Control of Pollution (Special Waste) Amendment Regulations 1988 and the Environmental Protection Act 1990. Under the legislation, the transporter and waste disposer are required to hold a relevant license and to operate their practices within the conditions of the license with all parties involved in the transport and disposal chain demonstrating a Duty of Care.

Solid waste from the site is collected and transported to the local municipal waste incinerator in Dundee, which is operated by the local authority. Control of the transport and disposal operations is exercised by Angus District Council and Dundee City Council. Each consignment of waste is notified to the authority and annual returns are presented to the local council officers.

Emissions to atmosphere are controlled by the Environmental Protection Act 1990. In addition, emissions in the workplace are controlled by the Health and Safety at Work Act (1974) and the subordinate Control of Substances Hazardous to Health Regulations (1988). These are enforced by the Health and Safety Executive. Occupational emissions are assessed to ensure that exposure to substances hazardous to health are controlled and comply at least with the provisions of the Health and Safety at Work Act (1974) and its supporting regulations.

6.1.4 Effect of Requested Approval on Compliance

The manufacture of lamivudine and its intermediates was undertaken at the Montrose and Ulverston facilities in campaigns during 1994. The processes will be operated to ensure compliance with the Integrated Pollution Control authorizations for the facilities. Monitoring data collected during the manufacture of lamivudine salicylate and lamivudine show that the facilities are in compliance with requirements. Therefore, the requested approval of 3TC™ (lamivudine) 150 mg Tablets is not anticipated to have any impact on the compliance status of the facilities.

6.2 Introduction of Substances from Drug Product Manufacturing and Packaging

The substances expected to be emitted, emission controls, and compliance with relevant environmental and occupational laws associated with the production of 3TC™ (lamivudine) Tablets at the Glaxo Wellcome Inc. Zebulon facility are discussed below.

REDACTIONS MADE BY APPLICANT

6.2.1 Substances Expected to be Emitted

The requested approval could potentially result in emissions to the environment from sites of production of starting materials, reagents and excipients used in the manufacture of the drug substance and the drug product. A confidential list of these materials has been supplied to the FDA.

6.2.2 Controls on Emissions

There are no controls on wastewater emissions from the Zebulon facility. All wastewater is discharged to the City sanitary sewer, where it is treated to meet State standards prior to discharge to surface waters.

All air emissions from manufacturing equipment pass first through a dust collector and then through a bank of HEPA filters. The control equipment is 99.97% effective at removing particulate emissions.

Incineration of rejected and returned product residue from cleaning vessels, and spent HEPA filters is performed by a company under contract with Glaxo Wellcome Inc. Chambers Medical Technologies, Inc. operates an incinerator in Hampton, South Carolina. An alternative contract waste incinerator, currently under consideration, is operated by BFI-Medical Waste Systems at Haw River, North Carolina (see Section 4.5).

6.2.3 Regulatory Controls and Compliance

Emissions of substances into the environment from the Zebulon production facility from all media (air, water and solid waste) are controlled by either the United States Environmental Protection Agency (EPA) Regulations or by more restrictive North Carolina Department of Environment, Health and Natural Resources (NCDEHNR) regulations.

Occupational emissions are controlled by either the United States Occupational Safety and Health Administration (OSHA) or the North Carolina Department of Labor. In some cases regulations are specifically applied to the facility via a permit. In other cases, compliance with only the general regulations is required.

Wastewater discharges from Glaxo Wellcome Inc.'s Zebulon production facility are regulated under the Clean Water Act. The wastewater is discharged to the Zebulon sanitary sewer system. Two specific sets of regulations apply: the Pretreatment Regulations (40 CFR Part 403 et seq.) and the Effluent Guideline for Pharmaceutical Manufacturers (40 CFR 439 et seq.).

Requirements specific to Glaxo Wellcome Inc.'s facility have been placed into a site-specific pretreatment permit issued by the Town of Zebulon.

Air emissions at the facility are regulated under the Clean Air Act. In North Carolina the Clean Air Act requirements for manufacturing facilities are implemented through State regulations 15A NCAC 2D et seq. and 15A NCAC 2Q. The requirements specific to Glaxo Wellcome Inc.'s facility have been placed in an air quality permit issued by NCDEHNR.

Storage and disposal of hazardous waste is regulated under 40 CFR part 260 et. seq. The North Carolina Division of Solid Waste implements the hazardous waste program for the State.

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Any hazardous waste, all rejected and returned drug product as well as most packaging materials are transported off-site and disposed by a contract waste handling company via incineration.

The following waste handling company is currently contracted to manage solid and hazardous waste from Glaxo Wellcome Inc.'s Zebulon facility:

Rollins Chempak
1 Rollins Plaza
Wilmington, Delaware 19803

Glaxo Wellcome Inc. may ship hazardous waste to a Rollins incineration facility in Baton Rouge, Louisiana. The incinerator operates under permit number LAD010395127 issued by the Louisiana Department of Environmental Quality, Hazardous Waste Division.

Hazardous waste may be incinerated at the Glaxo Wellcome Inc. facility in Research Triangle Park, North Carolina under permit number NCD065655599.

There are no specific permit requirements for the generation and disposal of solid waste.

Occupational emissions are regulated under the Occupational Safety and Health Act. The specific requirements which cover occupational emissions can be found in 29 CFR Part 1910 Subpart Z (Toxic and Hazardous Substances). In the case of occupational emissions, compliance with the general regulations is required and no specific permits are issued.

6.2.4 Effect of Requested Approval on Compliance

It is not anticipated that the manufacture of 3TC™ (lamivudine) Tablets will have any detrimental impact on the current compliance status of the Zebulon site. As discussed above, air emissions; types and amounts of collected wastes; and occupational emissions are not specifically regulated at Glaxo Wellcome Inc.'s Zebulon facility. Therefore, only wastewater emissions could potentially impact compliance.

6.3 Statement of Compliance

By signing this Environmental Assessment report, Glaxo Wellcome Inc. states that it is in compliance, or on an enforceable schedule to be in compliance, with all environmental laws and regulations applicable to the production of lamivudine drug substance and 3TC™ (lamivudine) Tablets at its Ulverston, Montrose and Zebulon manufacturing facilities.

6.4 Introductions from Product Use

Except for the drug substance, lamivudine, all components of 3TC™ (lamivudine) Tablets are food or pharmaceutical grade substances that are not specifically regulated under any environmental legislation or regulations, and are discharged into the environment from a wide range of sources. Therefore, only lamivudine is considered in this discussion.

Administered lamivudine and its metabolites will enter the environment primarily through wastewater treatment facilities. The Maximum Expected Emitted Concentration (MEEC) of lamivudine from product use is estimated to be 1.5 mg/l . This estimate is based on an estimated 200 liters per capita daily wastewater discharge (EU Guidelines) and the following worst case scenario:

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1. all the people discharging to a wastewater treatment system are being treated with the maximum recommended 300 milligram daily dose of lamivudine, and

2. 100% of the drug substance passes though the patient into the wastewater.

Using this scenario provides an MEEC estimate which is independent of market volume. The MEEC was calculated as follows:

$$\frac{\text{Daily Application}}{\text{Daily Water Usage}} = \text{MEEC}$$

$$\frac{\text{300 milligrams}}{\text{200 liters}} = 1.5 \text{ mg/l}$$

6.5 Introductions from Product Disposal

It is estimated that there will be no emission to the environment from product disposal. All product in the United States that is returned is disposed of by high-temperature incineration at an off site facility operated by a contract waste disposal firm. All of the drug substance, excipients, and packaging materials are destroyed in the incineration process.

The contractor used to transport and dispose of returned pharmaceuticals is:

Chambers Medical Technologies of South Carolina, Inc.
100 Nix Street
Hampton, SC 29924

The contractor holds permit number 1280-0021, issued by the South Carolina Department of Health and Environmental Control (DHEC). The permit was issued on July 29, 1986 and expired on March 31, 1991. However, the contractor has applied for their permit renewal as required and is operating under the existing permit until DHEC issues a new permit.

An alternative solid waste incineration facility under consideration for the disposal of returned product is:

BFI - Medical Waste Systems
1168 Porter Avenue
Haw River, North Carolina 27258

BFI - Medical Waste Systems holds air permit number 5896R7 issued June 18, 1994 by the North Carolina Department of Environment, Health and Natural Resources (DEHNR). The permit has ar expiration date of July 1, 1996.

7.0 FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

For 3TC™ (lamivudine) Tablets production and use, information presented in Section 6 of this Environmental Assessment report indicates that only the fate of lamivudine need be considered. Other compounds potentially emitted in production of the drug substance and in the production or use of the drug product are introduced into the environment from a wide variety of sources other than those associated with the proposed action. The amounts of these compounds expected to enter the environment as a result of approval of the proposed action are negligible by comparison, and are not expected to result in any adverse environmental

effects. Furthermore, the manufacturing facilities all are in compliance with applicable environmental or occupational health and safety regulations.

The major route of drug substance emission into the environment is excretion following product use and subsequent release into wastewater collection and treatment systems. As discussed in Section 6 of this Environmental Assessment report, all manufacturing losses will be disposed of using procedures in compliance with the applicable national environmental laws and regulations. All returned and rejected drug product will be disposed of via high-temperature incineration. This process destroys all drug substance and excipients prior to emission. Therefore, the environmental fate of drug substance emitted as a result of losses from manufacturing processes or disposal is not considered in this section of the Environmental Assessment report.

7.1 Metabolism

When administered to humans, the principal route of excretion of lamivudine is the urine. Available data indicate that the drug is, to a great extent, excreted unchanged. The only metabolite identified in the urine following repeated oral administration is the trans-sulfoxide, accounting for about 5% of the administered dose. This metabolite is more polar than the parent compound. Thus, lamivudine metabolites are not expected to be emitted in large amounts, relative to the amounts of lamivudine, as a result of usage by humans, and any metabolites that are excreted are not expected to be more environmentally persistent than lamivudine. Accordingly, these compounds are not considered significant for the purposes of this environmental assessment and will not be further evaluated.

7.2 Fate Studies

The fate and effects of chemical substances in the environment are predominately determined by their physical, chemical, and biological characteristics. To determine the environmental fate and effects of lamivudine, several laboratory studies were carried out in accordance with guidelines provided in the Food and Drug Administration (FDA) Environmental Assessment Technical Assistance Handbook. The results of these studies are summarized in Table 3.

As noted above, the major route of drug substance emission into the environment is via excretion in the urine and feces following product use and subsequent release into wastewater collection and treatment systems. Lamivudine was found to be hydrolytically stable over all pH ranges tested; thus, hydrolysis cannot be considered an important removal process. Based on consideration of the Pharmaceutical Manufacturers Association/Food and Drug Administration (PMA/FDA) Environmental Assessment Technical Test Matrix, the results of the minimum data base fate tests indicate that lamivudine will localize primarily into the aquatic environmental compartment:

- the water solubility of this substance is much greater than 10^{-5} Molar,
- the log octanol/water partition coefficient is much less than 2, and
- the vapor pressure is much less than 10^{-7} Torr.

Transport of lamivudine into the terrestrial and atmospheric compartments is expected to be negligible by comparison.

7.3 Fate in Aquatic Ecosystems

A major determinant of the fate of lamivudine in the aquatic compartment is its rate of degradation. Thus, a determination of its aerobic biodegradation in water was carried out in accordance with the FDA Environmental Assessment Technical Handbook, 3.11. The study

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report has been submitted to FDA. The results of this test indicated less than 1% degradation to CO_2 occurred over the 28-day test period. Thus, lamivudine does not meet the current FDA criteria for ready biodegradability (i.e., half-life less than approximately 8 hours for aerobic biodegradation). According to the PMA/FDA Guidelines for preparing environmental assessments, the relatively low octanol/water partition coefficient, indicates that lamivudine is unlikely to bioaccumulate in the tissues of aquatic organisms.

For the aquatic compartment, the worst case estimate of the drug substance's environmental concentration from product use would be equal to the MEEC (1.5 mg/l). This worst case estimate assumes no removal through the wastewater treatment process and discharge into a zero flow stream.

Table 3. Summary of Environmental Fate and Effects Studies Conducted on Lamivudine

Study Name	Results			
hydrolysis rate	hydrolytically stable over all pH ranges.			
vapor pressure	3.4E-8Torr at 25°C (by extrapolation)			
UV/visible spectra at pH 7	peak	molar absorption coefficient	wavelength	absorbance
	1	8680	271	379
	2 (shoulder)	7480	227	326
octanol/water partition coefficient	Log10 K_{ow} at			
	pH 5	pH 7	pH 9	
	-1.86	-1.44	-1.17	
dissociation constant	pK_a =4.26at pH range 3.4-5.2			
water solubility at 20C	pH 5	pH 7	pH 9	
	135 g/l	77.2 g/l	84.4 g/l	
soil sorption/desorption	soil type	pH	Ko_C	
	clay loam	6.5	32.0	
	sandy loam	6.1	30.2	
	sandy silt loam	4.5	108	
biodegradation in soil	15 to 24% mineralization to CO_2 in 64days			
biodegradation in water	1% mineralization to CO_2 in 28 days			
microbial growth inhibition	Inhibitory Concentration > 1000 mg/l Lamivudine (5 species studied)			
acute toxicity to daphnids	EC_{50} = >1000 mg/l Lamivudine			

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8.0 ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES

8.1 Environmental Hazard Assessment

No published studies evaluating the potential environmental toxicity of lamivudine were identified. Therefore, two studies were carried out to evaluate the acute effects of lamivudine on potential environmental receptors: (1) the Microbial Growth Inhibition Study, and (2) a test of acute toxicity to *Daphnia magna*. The results of these studies are summarized in Table 3. These tests were determined to be most appropriate for evaluating the effects of lamivudine in the environment. Emissions to the environment are expected to occur primarily following use of the drug product and would result in release to wastewater treatment plants and, ultimately, to surface water.

The Microbial Growth Inhibition Study determined the toxicity to *Pseudomonas aeruginosa, Azotobacter beijerinckii, Trichoderma harzianum, Aspergillus niger* and *Nostoc commune* microorganisms, typical of those found in soils. The test was carried out according to FDA Technical Assistance Document 4.02. under conditions sufficient to satisfy the requirements of the FDA Good Laboratory Practice Regulations (21 CFR 58). No inhibition of microbial growth was observed under the conditions of the test, the Minimum Inhibitory Concentration was >1000 mg/l.

The acute toxicity to *Daphnia magna* was evaluated according to procedures identified in FDA Technical Assistance Document 4.08. The test determines a median effect concentration (EC_{50}), defined as the concentration resulting in 50% immobilization of the *Daphnia* in the specified time period. The *Daphnia* acute aquatic toxicity study identified both the 24-hour and 48-hour EC_{50}s to be >1000 mg/L lamivudine. The no observed effect concentration at 48 hours was also >1000 mg/L lamivudine.

8.2 Evaluation of Environmental Effects

Small amounts of lamivudine may be excreted by individuals using 3TC™ (lamivudine) Tablets which ultimately may enter the aquatic environment through wastewater treatment plants. Based upon the estimated maximum release of lamivudine to the aquatic compartment and the assumptions set forth in Section 6, a Maximum Expected Emitted Concentration (MEEC) of 1.5 mg/L for the aquatic compartment was calculated for this environmental pathway. This MEEC is less than 1/100 of the EC_{50} for microbial inhibition and the EC_{50} for acute *Daphnia* toxicity. Under 21 CFR 25.15(b), the estimated maximum concentration of lamivudine in the aquatic compartment would be considered non toxic because the MEEC is less than the no observed effect concentrations and less than 1/100 of the median effect concentrations (median lethal concentrations were not identified) determined in environmental effects testing.

Based on consideration of the information presented above, lamivudine, at maximum expected environmental concentrations, is not expected to adversely affect sensitive environmental receptors in the aquatic environmental compartment.

No adverse environmental effects are expected to occur as a result of emissions associated with 3TC™ (lamivudine) Tablets manufacturing processes. Emissions of all substances are within regulatory limits or are collected and disposed of using appropriate, approved procedures.

incinerated at approved facilities capable of destroying the drug substance, excipients, and packaging materials.

9.0 USE OF RESOURCES AND ENERGY

The raw materials used in the production of lamivudine and 3TC™ (lamivudine) Tablets, including the substances used as excipients in the final dosage form, are readily available. The production of this drug product will not cause significant depletion of any natural resources, including energy, minerals/chemicals, and land.

Energy use estimates expected to occur as a result of approval of the requested action are based upon the estimated percentage of total facility usage for the manufacture of 3TC™ (lamivudine) Tablets or its chemical precursors. A review of the manufacturing processes considered in this environmental assessment indicates that the energy resources required to produce 3TC™ (lamivudine) Tablets are in a range which is considered normal for production and distribution of a pharmaceutical product.

9.1 Energy and Land Use

For all manufacturing sites considered in this assessment, the proposed action will be performed within existing facilities and with the present work force. No additional buildings, equipment, landscaping, or construction will be necessary. Therefore, approval of the request to manufacture 3TC™ (lamivudine) Tablets will not affect existing land use in Ulverston, Montrose or Zebulon.

The total annual energy for the manufacture and packaging of 3TC™ (lamivudine) Tablets across all manufacturing sites has been calculated and adjusted to show only the amounts needed to support the fifth year production estimate for the requested approval. The total annual use of electricity, natural gas and fuel oil is expected to be 8797×10^3 kilowatt hours, 4×10^7 kilowatt hours and 1.1×10^6 liters respectively.

9.2 Water Use

Total water use associated with the manufacture of 3TC™ (lamivudine) Tablets is estimated to be 88.5×10^3 cubic meters. This estimate is based manufacturing site water use information adjusted to show only the water needed to support fifth year forecasts for the requested approval.

9.3 Effects on Endangered or Threatened Species

The requested approval is not expected to affect rare, endangered, or threatened species. Effects associated with obtaining raw materials are not a concern because manufacture of the drug substance and drug product do not involve any biological or natural extractions. Effects from loss of habitat will not occur because all manufacturing will be done at existing facilities. Adverse effects on endangered species or local ecosystems from manufacturing emissions are not expected to occur because emission levels are minimal and well below any levels that might cause toxicity (see Section 6 of this environmental assessment report). It is also unlikely that emissions after use will have any adverse environmental effects because resulting environmental concentrations are expected to be minimal and significantly below any observed toxicity levels.

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9.4 Effects on Property Listed in the National Register of Historic Places

The production, use, and disposal of substances associated with the requested approval will have no effect on property listed or eligible for listing in the National Register of Historic Places.

10.0 MITIGATION MEASURES

For all sites, it is projected that no additional structural controls will be needed in order to comply with applicable environmental regulations and permits. However, many non-structural environmental controls which are implemented at the facilities as standard procedures will have the effect of being mitigation measures for the proposed action. Furthermore, standard emergency response procedures will have the effect of being mitigation measures for the proposed action.

10.1 Spill Prevention Control and Countermeasures

Glaxo Wellcome Inc. facilities have emergency procedures designed to prevent or minimize the environmental effects of any potential release of hazardous materials from any of the production units. Summaries of site procedures are outlined in information provided to the FDA.

10.2 Waste Minimization

Glaxo Wellcome Inc. actively pursues opportunities to minimize waste generated at manufacturing facilities. Additional information on the specific initiatives undertaken to minimize waste at each facility has been provided to the FDA.

10.3 Solid and Hazardous Waste Management Procedures

All waste disposal contractors are subjected to audits by Glaxo Wellcome Inc. or a nominated consultant to ensure that they comply, at a minimum, with the legal standards. These audits consist of site visits as well as full review of the contractors' policies and licenses.

10.4 Chemical Hygiene Procedures

Glaxo Wellcome Inc. uses engineering controls to reduce or eliminate chemical exposure in the workplace wherever such controls are technically and economically feasible. When engineering controls prove to be not feasible or provide insufficient protection, the use of personal protective equipment is required. Prior to the start up of new equipment and/or processes, acceptance tests are carried out on the control measures installed to prevent employee exposure.

Prior to production involving a new drug substance which could potentially expose employees to a chemical which has no established exposure limit, a committee develops an internal corporate occupational exposure limit (OEL). The committee members include Medical, Safety and Industrial Hygiene professionals, as well as other individuals knowledgeable about the chemical in question and its effects.

10.5 Emission Controls

In general, areas where releases are likely to contain pollutants are vented to appropriate scrubbers. Scrubbers are monitored on a regular basis. HEPA and bag filtration are used in appropriate manufacturing areas.

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11.0 ALTERNATIVES TO THE PROPOSED ACTION

The only alternative action is no action. The alternative would deny a safe and effective drug to some segments of the public that could benefit from its use. The alternative action is not justified because no adverse environmental effects have been identified in this environmental assessment and none are expected to be associated with the production, use, and disposal of this product.

12.0 LIST OF PREPARERS

REDACTIONS MADE BY APPLICANT

Alan R. Beckham

-Environmental Engineer, Glaxo Inc., 1994 - present
-Environmental Scientist, Glaxochem Ltd, 1987 - 1994
-Scientific Officer, Glaxo Operations (UK) Ltd, 1981 - 1987
- Bachelor of Science in Microbiology
University of Newcastle upon Tyne, 1980

13.0 CERTIFICATION

The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of Glaxo Wellcome Inc.

Thomas F. Cecich — Date: 5/15/95

FAC

Vice President, Safety & Environmental Affairs
Glaxo Wellcome Inc.
Five Moore Drive
Research Triangle Park, NC 27709

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14.0 REFERENCES

Council Directive 93/39/EEC
Draft Guidelines on Environmental Risk Assessment.

Council On Environmental Quality, " Regulations On Implementing National Environmental Policy Act Procedures," Federal Register, Vol. 43, November 29, 1978, p. 55990.

Pharmaceutical Manufacturers Association, "Interim Guidance To The Pharmaceutical Industry For Environmental Assessment Compliance Requirements For The FDA v7," Seminar on Environmental Assessments, Rockville, Md., July 29-30, 1991.

Physicians' Desk Reference, 47th Edition. 1993. Medical Economics Data. Montvale, NJ.

U.S. FDA, "Environmental Assessment Technical Assistance Handbook, U.S. FDA, March 1987 NTIS PB87-175345.

U.S. FDA, "National Environmental Policy Act; Policies and Procedures; Final Rule," Federal Register, Vol. 50, April 26, 1985

15.0 APPENDICES

Appendix 1 Substance Information Sheet

REDACTIONS MADE BY APPLICANT

ATTACHMENT 1

SUBSTANCE INFORMATION SHEET

MATERIAL SAFETY DATA SHEET

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GR109714X
RESEARCH COMPOUND

Glaxo Inc.
Five Moore Drive
Research Triangle Park, NC 27709

Revision Date: 09/23/94

Emergency Contact:
Environmental Safety
(919) 248-2100
(919) 248-2700 (24 hour contact)

SECTION I -- General Information

Chemical Name:	(-)cis-5-(4-Amino-1,2-dihydro-2-oxo-1-pyrimidinyl)-1,3-oxathiolane-2-methanol
Chemical Family:	Therapeutic agent; anti-retrovirus
Agent Name/Synonyms:	GR109714X; 3TC
Molecular Formula:	$C_8H_{11}N_3O_3S$

SECTION II -- Hazardous Ingredients / Identity Information

Hazardous Components	%	Glaxo Limits	OSHA Limits	ACGIH Limits	Other limits (source)
GR109714X	100.00	35 MCG/M3 (8 HR TWA) (OEL)	Not established (PEL)	Not established (TLV)	Not established (NIOSH Limit)

SECTION III -- Physical / Chemical Characteristics

Boiling Point:	Not Applicable (solid)
Vapor Density (air = 1):	Not Applicable (solid)
Specific Gravity (H_2O = 1):	Not Applicable (solid)
Melting Point:	178 - 182 degrees C
Solubility:	70 mg/ml water at 20 degrees C

SECTION IV -- Fire & Explosion Hazard Data

Flash Point (test method):	131 degrees F (TCC)
LEL:	Unknown
UEL:	Unknown

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SECTION IV -- Fire & Explosion Hazard Data (Continued)

Extinguishing Media: Water Spray, Foam, Carbon Dioxide (CO2), Multi-purpose Dry Chemical.

Special Fire Fighting Procedures: The ignition characteristics of the material have not been determined. There is a potential risk of explosion in any operation involving small particles, especially when a dust cloud is produced in an oxygen containing environment.

SECTION VI -- Health Hazard Data

Glaxo Occupational Exposure Limits

Glaxo Estimated Safe Working Level: 0.035 mg/m3 eight-hour time-weighted average (TWA).

Pharmacologic Activity

GR109714X is a potent and selective inhibitor of Human Immunodeficiency Virus 1 (HIV-1) and Human Immunodeficiency Virus 2 (HIV-2) replication.
No information is available regarding potential physiologic effects of this compound.

Signs and Symptoms of Occupational Exposure

No data is available regarding occupational exposure to this compound.

Occupational Health Hazards

GR109714X is a research compound about which limited pharmacologic and toxicologic information is known. Therefore it should be handled in a manner which avoids exposure by any route.

Medical Conditions Aggravated by Exposure

Unknown.

Toxicity Data

GR109714X is a research compound for which limited toxicologic information is available. Toxicology testing for mutagenicity (change in genetic material), carcinogenicity (cancer), and teratogenicity (birth defects) is incomplete. GR109714X was weakly mutagenic at high doses in one of the standard tests for mutagenesis. GR109714X was clastogenic (caused chromosome damage) in a test using human white blood cells but not in other tests. In tests on bone marrow, no significant toxic effects were seen against the cells that generate red and white blood cells.

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SECTION VI -- Health Hazard Data (Continued)

Emergency and First Aid Procedures

Eyes:	Flush thoroughly with large amounts of water. Obtain medical attention.
Skin:	Remove contaminated clothing. Wash all affected areas thoroughly with soap and water. Obtain medical attention.
Inhalation:	Remove to fresh air. If breathing is difficult or ceases, give oxygen or cardiopulmonary resuscitation. Obtain medical attention.
Ingestion:	Rinse mouth with water. Obtain medical attention.

SECTION VII – Precautions for Safe Handling and Use

Spill and Leak Procedures:	Full protective equipment including respirator, gloves, eye protection, and protective clothing should be worn where there is potential for skin exposure or risk of inhalation. Collect spillage by carefully sweeping or by vacuuming with HEPA filtered vacuum and place in labelled, sealed container for reuse or disposal. Wash area with water and detergent to remove the last traces of spillage.
Waste Disposal Methods:	Small quantities may be discharged down the drain with copious quantities of water. However, for final disposal, incinerate within applicable federal, state and local regulations.
Handling and Storage Precautions:	Small quantities of the compound, up to a few grams, should be handled within a containment facility. Small quantities in solution may be handled on open benches. When handled in larger quantities, either during the final stages of synthesis or purification & isolation or during Pharmaceutic Development, manipulations must be carried out in a working area where access is restricted to those whose presence is essential. All equipment must either be totally enclosed or provided with local ventilation designed to capture & contain dust at the point of generation. Such facilities should ensure HEPA filtration of their exhaust before discharge to the atmosphere. Store between 20 and 30 degrees C in a well-sealed container and protect from light. Allow material to reach room temperature before exposing it to air.

SECTION VIII – Control Measures

Ventilation:	Provide local exhaust ventilation at the source of dust generation.
Respiratory Protection:	Respiratory protective equipment may be used to provide additional protection. The respirator should be certified by NIOSH for dusts with a PEL < 0.05 mg/m3. A powered air purifying or air supplied respirator that provides full head covering should be used.

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SECTION VIII -- Control Measures (Continued)

Eye Protection:	Suitable eye protection should be used to prevent dust contact.
Clothing:	All skin contact should be avoided through use of proper protective clothing.
Gloves:	Protective gloves should be worn at all times to avoid skin contact.
Work Practices:	Special care should be taken to ensure that contaminated clothing and protective equipment are properly cleaned after use.
Hygienic Practices:	Wash hands or potentially exposed skin before leaving the work area.

***** End of MSDS *****

NDA 20564

NDA

20596

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-564
NDA 20-596

NOV 17 1995

Glaxo Wellcome Inc.
Attn: David M. Cocchetto, Ph.D.
Director, Regulatory Affairs
Five Moore Drive
Research Triangle Park
North Carolina, 27709

Dear Dr. Cocchetto:

Please refer to your June 30, 1995, New Drug Applications (NDA) submitted pursuant to section 505 (b) of the Federal Food, Drug, and Cosmetic Act for Epivir™ (lamivudine) Tablets, and Epivir™ (lamivudine) Oral Solution.

We acknowledge receipt of your amendments dated:

July 13, 1995	August 21, 1995	October 11, 1995(2)
July 19, 1995	August 25, 1995	October 12, 1995
July 20, 1995	August 30, 1995	October 13, 1995(2)
July 27, 1995	September 6, 1995	October 20, 1995(3)
August 7, 1995	September 8, 1995	October 31, 1995(2)
August 8, 1995(2)	September 12, 1995	November 2, 1995
August 10, 1995(2)	September 15, 1995	November 10, 1995
August 15, 1995	September 27, 1995	November 16, 1995
August 16, 1995	September 28, 1995	November 17, 1995
August 17, 1995(2)	October 2, 1995	

Epivir™ (lamivudine) Tablets, and Epivir™ (lamivudine) Oral Solution are indicated in combination with Retrovir® (zidovudine) for the treatment of HIV infection when therapy is warranted based on clinical and/or immunological evidence of disease progression

We have completed our review of these applications and have concluded that adequate information has been presented to demonstrate that the drug product is safe and effective for use as recommended in the final draft labeling submitted November 17, 1995. Accordingly, this application is approved effective on the date of this letter.

We acknowledge your commitment to comply with the conditions of Accelerated Approval as stated in your November 16, 1995 letter. Additionally, we acknowledge your commitment to conduct the phase 4 studies listed in the above referenced letter.

The final printed label (FPL) must be identical to the November 17, 1995 draft labeling. Marketing the product with FPL that is not identical to this draft labeling may render the product misbranded and an unapproved new drug.

Please submit 20 copies of the FPL as soon as available. Please individually mount ten of the copies on heavy-weight paper or similar material. For administrative purposes, this submission should be designated "FINAL PRINTED LABELING for approved NDA 20-564/20-569." Approval of this labeling is not required before it is used.

Should additional information relating to the safety and effectiveness of the drug become available, revision of that labeling may be required.

Validation of the regulatory methods has not been completed. At the present time, it is the policy of the Center not to withhold approval because the methods are being validated. Nevertheless, we expect your continued cooperation to resolve any deficiencies that may occur.

Please submit one market package of the drug when it is available.

Under section 736(a) (1) (B) (ii) of the Prescription Drug User Fee Act of 1992, this letter triggers the remaining 50% of the fee assessed for these applications. You will receive an invoice for the amount due within the next month. Payment will be due within 30 days of the date of the invoice.

We remind you that you must comply with the requirements for an approved NDA set forth under 21 CFR 314.80 and 314.81.

If you have any questions, please contact, Deborah L. Kallgren, Consumer Safety Officer, 301-443-9553.

Sincerely yours,

David W. Feigal, Jr., M.D., M.P.H.
Director
Division of Antiviral Drug Products
Office of Drug Evaluation II
Center for Drug Evaluation and Research

Draft of November 16, 1995

PRODUCT INFORMATION

Epivir™ Tablets (lamivudine tablets)

Epivir™ Oral Solution (lamivudine oral solution)

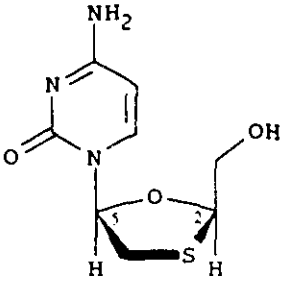
WARNING:

Epivir™ (lamivudine) is indicated for use in combination with Retrovir® (zidovudine) for the treatment of human immunodeficiency virus (HIV) infection when antiretroviral therapy is warranted based on clinical and/or immunological evidence of disease progression. This indication is based on analyses of surrogate endpoints. At present, there are no results from controlled clinical trials evaluating the effect of therapy with Epivir plus Retrovir on the clinical progression of HIV infection, such as the incidence of opportunistic infections or survival.

Patients receiving Epivir plus Retrovir may continue to develop opportunistic infections and other complications of HIV infection, and therefore should remain under close observation by physicians experienced in the treatment of patients with HIV-associated diseases.

DESCRIPTION:

Epivir™ (formerly known as 3TC) is the brand name for lamivudine, a synthetic nucleoside analogue with activity against HIV. The chemical name of lamivudine is (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one. Lamivudine is the (-)enantiomer of a dideoxy analogue of cytidine. Lamivudine has also been referred to as (-)2',3'-dideoxy, 3'-thiacytidine. It has a molecular formula of $C_8H_{11}N_3O_3S$ and a molecular weight of 229.3. It has the following structural formula:



Lamivudine is a white to off-white crystalline solid with a solubility of approximately 70 mg/mL in water at 20°C.

Epivir™ Tablets are for oral administration. Each tablet contains 150 mg of lamivudine and the inactive ingredients magnesium stearate, microcrystalline cellulose, and sodium starch glycolate. Opadry YS-1-7706-G White is the coloring agent in the tablet coating.

Epivir™ Oral Solution is for oral administration. One milliliter (1 mL) of Epivir Oral Solution contains 10 mg of lamivudine (10 mg/mL) in an aqueous solution and the inactive ingredients artificial strawberry and banana flavors, citric acid (anhydrous), edetate disodium, ethanol (6% v/v), methylparaben, propylene glycol, propylparaben, and sucrose.

CLINICAL PHARMACOLOGY:

Mechanism of Action: Lamivudine is a synthetic nucleoside analogue. *In vitro* studies have shown that, intracellularly, lamivudine is phosphorylated to its active 5′-triphosphate metabolite (L-TP), which has an intracellular half-life of 10.5 to 15.5 hours. The principal mode of action of L-TP is inhibition of HIV reverse transcription via viral DNA chain termination. L-TP also inhibits the RNA- and DNA-dependent DNA polymerase activities of reverse transcriptase (RT). L-TP is a weak inhibitor of mammalian α-, β-, and γ-DNA polymerases.

Microbiology: ***Antiviral Activity In Vitro:*** The relationship between *in vitro* susceptibility of HIV to lamivudine and the inhibition of HIV replication in humans has not been established. *In vitro* activity of lamivudine against HIV-1 was assessed in a number of cell lines (including monocytes and fresh human peripheral blood lymphocytes) using standard susceptibility assays. IC_{50} values (50% inhibitory concentrations) were in the range of 2 nM to 15 μM. Lamivudine had anti–HIV-1 activity in all acute virus-cell infections tested. In HIV-1–infected MT-4 cells, lamivudine in combination with zidovudine had synergistic antiretroviral activity. Synergistic activity of lamivudine/zidovudine was also shown in a variable-ratio study.

Drug Resistance: Lamivudine-resistant isolates of HIV-1 have been selected *in vitro*. The resistant isolates showed reduced susceptibility to lamivudine and genotypic analysis showed that the resistance was due to specific substitution mutations in the HIV-1 reverse transcriptase at codon 184 from methionine to either isoleucine or valine. HIV-1 strains resistant to both lamivudine and zidovudine have been isolated.

Susceptibility of clinical isolates to lamivudine and zidovudine was monitored in controlled clinical trials. In patients receiving lamivudine monotherapy or combination therapy with lamivudine plus zidovudine, HIV-1 isolates from most patients became phenotypically and genotypically resistant to lamivudine within 12 weeks. In some patients harboring zidovudine-resistant virus, phenotypic sensitivity to zidovudine by 12 weeks of treatment was restored. Combination therapy with lamivudine plus zidovudine delayed the emergence of mutations conferring resistance to zidovudine.

Pharmacokinetics in Adults: The pharmacokinetic properties of lamivudine have been studied in asymptomatic, HIV-infected adult patients after administration of single intravenous (IV) doses ranging from 0.25 to 8 mg/kg, as well as single and multiple (b.i.d. regimen) oral doses ranging from 0.25 to 10 mg/kg.

Absorption and Bioavailability: Lamivudine was rapidly absorbed after oral administration in HIV-infected patients. Absolute bioavailability in 12 adult patients was

86% ± 16% (mean ± S.D.) for the tablet and 87% ± 13% for the oral solution. After oral administration of 2 mg/kg twice a day to nine adults with HIV, the peak serum lamivudine concentration (C_{max}) was 1.5 ± 0.5 µg/mL (mean ± S.D.). The area under the plasma concentration versus time curve (AUC) and C_{max} increased in proportion to oral dose over the range from 0.25 to 10 mg/kg.

An investigational 25 mg dosage form of lamivudine was administered orally to 12 asymptomatic, HIV-infected patients on two occasions, once in the fasted state and once with food (1,099 kcal; 75 grams fat, 34 grams protein, 72 grams carbohydrate). Absorption of lamivudine was slower in the fed state (T_{max}: 3.2 ± 1.3 hours) compared with the fasted state (T_{max}: 0.9 ± 0.3 hours); C_{max} in the fed state was 40% ± 23% (mean ± S.D.) lower than in the fasted state. There was no significant difference in systemic exposure (AUC_∞) in the fed and fasted states; therefore, Epivir™ Tablets and Oral Solution may be administered with or without food.

The accumulation ratio of lamuvidine in HIV-positive asymptomatic adults with normal renal function was 1.50 following 15 days of oral administration of 2 mg/kg b.i.d.

Distribution: The apparent volume of distribution after IV administration of lamivudine to 20 patients was 1.3 ± 0.4 L/kg, suggesting that lamivudine distributes into extravascular spaces. Volume of distribution was independent of dose and did not correlate with body weight.

Binding of lamivudine to human plasma proteins is low (<36%). *In vitro* studies showed that, over the concentration range of 0.1 to 100 µg/mL, the amount of lamivudine associated with erythrocytes ranged from 53% to 57% and was independent of concentration.

Metabolism: Metabolism of lamivudine is a minor route of elimination. In man, the only known metabolite of lamivudine is the trans-sulfoxide metabolite. Within 12 hours after a single oral dose of lamivudine in six HIV-infected adults, 5.2% ± 1.4% (mean ± S.D.) of the dose was excreted as the trans-sulfoxide metabolite in the urine. Serum concentrations of this metabolite have not been determined.

Elimination: The majority of lamivudine is eliminated unchanged in urine. In 20 patients given a single IV dose, renal clearance was 0.22 ± 0.06 L/hr*kg (mean ± S.D.) representing 71% ± 16% (mean ± S.D.) of total clearance of lamivudine.

In most single-dose studies in HIV-infected patients with serum sampling for 24 hours after dosing, the observed mean elimination half-life ($T_{½}$) ranged from 5 to 7 hours. Total clearance was 0.37 ± 0.05 L/hr*kg (mean ± S.D.). Oral clearance and elimination half-life were independent of dose and body weight over an oral dosing range from 0.25 to 10 mg/kg.

Special Populations: ***Adults With Impaired Renal Function:*** The pharmacokinetic properties of lamivudine have been determined in a small group of HIV-infected adults with impaired renal function, as summarized in Table 1.

Table 1: Pharmacokinetic Parameters (Mean ± S.D.) After a Single 300-mg Oral Dose of Lamivudine in Three Groups of Adults With Varying Degrees of Renal Function (CrCl>60 mL/min, CrCl = 10-30 mL/min, and CrCl<10 mL/min)

Number of subjects	6	4	6
Creatinine clearance criterion	>60 mL/min	10-30 mL/min	<10 mL/min
Creatinine clearance (mL/min)	111 ± 14	28 ± 8	6 ± 2
C_{max} (µg/mL)	2.6 ± 0.5	3.6 ± 0.8	5.8 ± 1.2
AUC∞ (µg·h/mL)	11.0 ± 1.7	48.0 ± 19	157 ± 74
Cl/F (mL/min)	464 ± 76	114 ± 34	36 ± 11

Exposure (AUC∞), C_{max}, and *half-life* increased with diminishing renal function (as expressed by creatinine clearance). Apparent total oral clearance (Cl/F) of lamivudine decreased as creatinine clearance decreased. T_{max} was not significantly affected by renal function. Based on these observations, it is recommended that the dosage of lamivudine be modified in patients with renal impairment (see DOSAGE AND ADMINISTRATION). The effects of renal impairment on lamivudine pharmacokinetics in pediatric patients are not known.

Pediatric Patients: For pharmacokinetic properties of lamivudine in pediatric patients, see PRECAUTIONS: Pediatric Use.

Geriatric Patients: Lamivudine pharmacokinetics have not been specifically studied in patients over 65 years of age.

Gender: *The pharmacokinetics of lamivudine with respect to gender have not been evaluated.*

Race: *The pharmacokinetics of lamivudine with respect to race have not been evaluated.*

Drug Interactions: Lamivudine and zidovudine were coadministered to 12 asymptomatic HIV-positive adult patients in a single-center, open-label, randomized, crossover study. No significant differences were observed in AUC∞ or total clearance for lamivudine or zidovudine when the two drugs were administered together. Coadministration of lamivudine with zidovudine resulted in an increase of 39% ± 62% (mean ± S.D.) in C_{max} of zidovudine.

Lamivudine and trimethoprim/sulfamethoxazole (TMP/SMX) were coadministered to 14 HIV-positive patients in a single-center, open-label, randomized, crossover study. Each patient received treatment with a single 300-mg dose of lamivudine and TMP 160 mg/SMX 800 mg once a day for 5 days with concomitant administration of lamivudine 300 mg with the fifth dose in a crossover design. Coadministration of TMP/SMX with lamivudine resulted in an increase of 44% ± 23% (mean ± S.D.) in lamivudine AUC∞, a decrease of 29% ± 13% in lamivudine oral clearance, and a decrease of 30% ± 36% in lamivudine renal clearance. The pharmacokinetic properties of TMP and SMX were not altered by coadministration with lamivudine.

INDICATIONS AND USAGE:

Epivir™ in combination with Retrovir® (zidovudine) is indicated for the treatment of HIV infection when therapy is warranted based on clinical and/or immunological evidence of disease progression. This indication is based on analyses of surrogate endpoints. At present, there are no results from controlled trials evaluating the effect of Epivir plus Retrovir on clinical progression of HIV infection, such as the incidence of opportunistic infections or survival.

Description of Clinical Studies:

Adults Without Prior Antiretroviral Therapy: Two studies were conducted in patients who received up to 4 weeks of prior antiretroviral therapy. A3001 was a randomized, double-blind study comparing Epivir 150 mg b.i.d. plus Retrovir 200 mg t.i.d.; Epivir 300 mg b.i.d. plus Retrovir; Epivir 300 mg b.i.d.; and Retrovir. 366 adults enrolled with the following demographics: male (87%), Caucasian (61%), median age of 34 years, asymptomatic HIV infection (80%), and baseline CD4 cell counts of 200 to 500 cells/mm^3 (median = 352 cells/mm^3). B3001 was a randomized, double-blind study comparing Epivir 300 mg b.i.d. plus Retrovir 200 mg t.i.d. versus Retrovir. 129 adults enrolled with the following demographics: male (74%), Caucasian (82%), median age of 33 years, asymptomatic HIV infection (64%), and baseline CD4 cell counts of 100 to 400 cells/mm^3 (median = 260 cells/mm^3). Mean changes in CD4 counts through 24 weeks of treatment for studies A3001 and B3001 are summarized in Figures 1 and 2, respectively.

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Figure 1: Mean Absolute CD4 Change (cells/mm^3) From Baseline in Study A3001

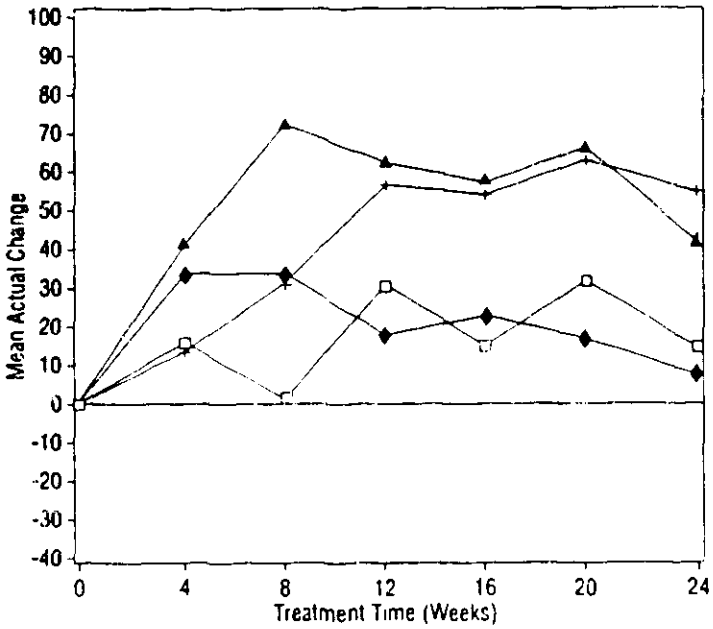

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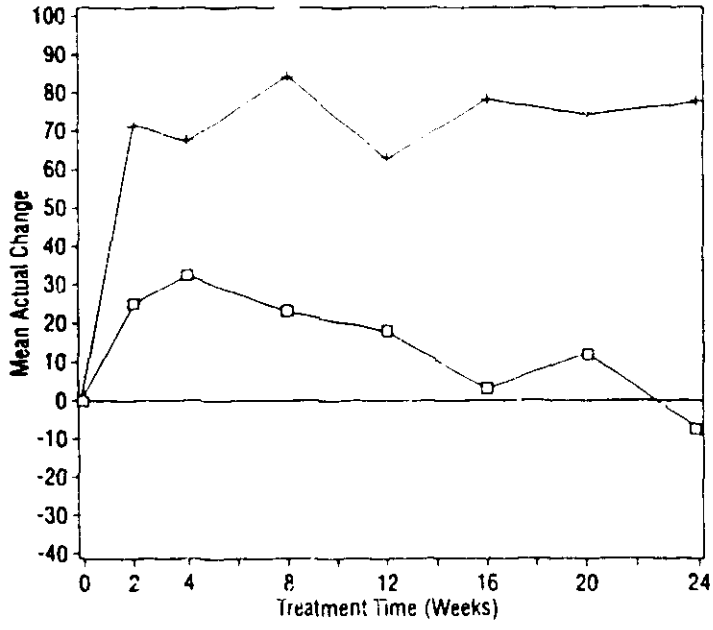
Figure 2: Mean Absolute CD4 Change (cells/mm^3) From Baseline in Study B3001



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Adults With Prior Zidovudine Therapy: Two studies were conducted in patients who received at least 24 weeks of prior zidovudine therapy. A3002 was a randomized, double-blind study comparing Epivir 150 mg b.i.d. plus Retrovir 200 mg t.i.d.; Epivir 300 mg b.i.d. plus Retrovir; and Retrovir plus zalcitabine 0.75 mg t.i.d. 254 adults enrolled with the following demographics: male (83%), Caucasian (63%), median age of 37 years, asymptomatic HIV infection (58%), median duration of prior zidovudine use of 24 months, and baseline CD4 cell counts of 100 to 300 cells/mm^3 (median = 211 cells/mm^3). B3002 was a randomized, double-blind study comparing Epivir 150 mg b.i.d. plus Retrovir, Epivir 300 mg b.i.d. plus Retrovir, and Retrovir. 223 adults enrolled with the following demographics: male (83%), Caucasian (96%), median age of 36 years, asymptomatic HIV infection (53%), median duration of prior zidovudine use of 23 months, and baseline CD4 cell counts of 100 to 400 cells/mm^3 (median = 241 cells/mm^3). Mean changes in CD4 counts through 24 weeks of treatment in studies A3002 and B3002 are summarized in Figures 3 and 4, respectively.

Figure 3: Mean Absolute CD4 Change (cells/mm^3) From Baseline in Study A3002

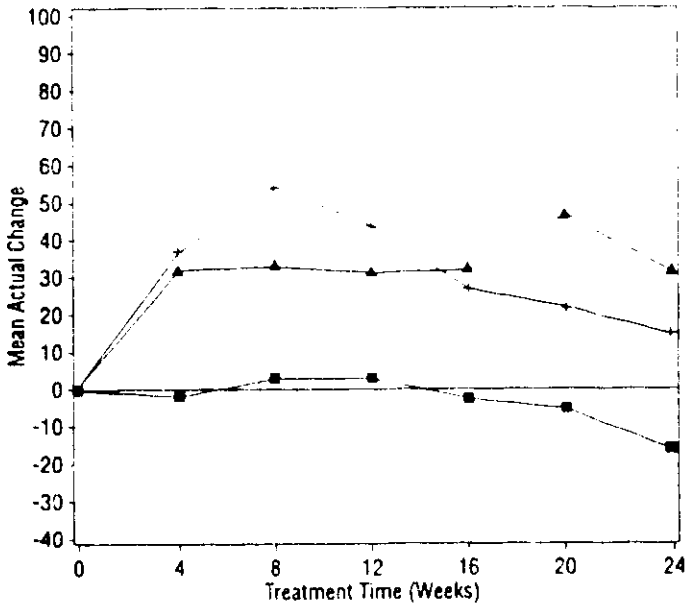

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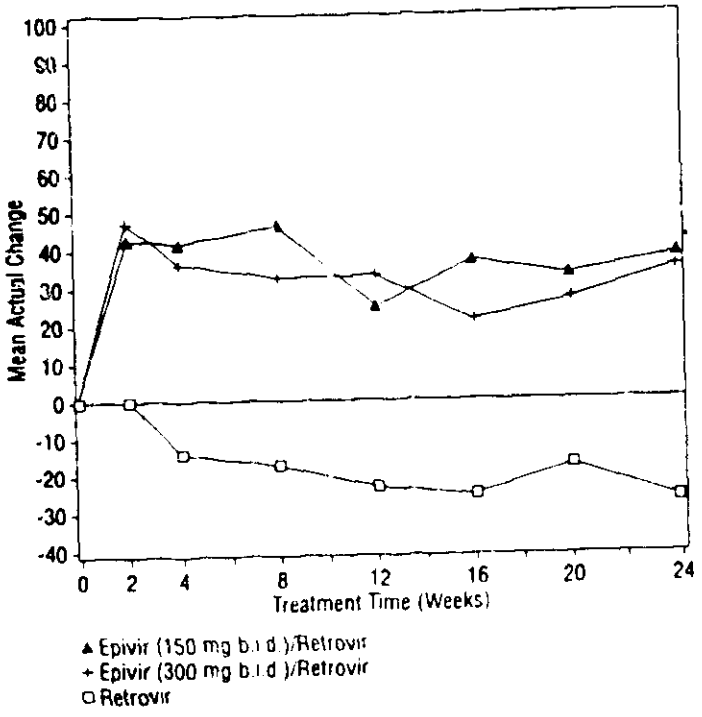
Figure 4: Mean Absolute CD4 Change (cells/mm^3) From Baseline in Study B3002



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214 HIV RNA: Mean changes from baseline HIV RNA are summarized in Table 2.

215
216

Table 2: Mean Changes in log 10 HIV RNA From Baseline in Studies A3001 and A3002 at 24 Weeks of Treatment

217
218

	Mean (± S.D.) Changes in log 10 HIV RNA from Baseline*				
	Epivir 150 mg b.i.d. + Retrovir	Retrovir	Epivir 300 mg b.i.d.	Epivir 300 mg b.i.d. + Retrovir	Retrovir + Zalcitabine
Study A3001 (antiretroviral-naive adults)	-0.9 ± 0.8	-0.3 ± 0.8	-0.4 ± 0.8	-1.0 ± 0.8	not applicable
Study A3002 (antiretroviral-experienced adults)	-0.7 ± 0.8	not applicable	not applicable	-0.7 ± 0.8	-0.7 ± 0.8

219 ***THE CLINICAL SIGNIFICANCE OF CHANGES IN HIV RNA DURING THERAPY IS UNKNOWN.**

220

CONTRAINDICATIONS:

Epivir™ Tablets and Oral Solution are contraindicated in patients with previously demonstrated clinically significant hypersensitivity to any of the components of the products.

WARNINGS:

PANCREATITIS IN PEDIATRIC PATIENTS: IN PEDIATRIC PATIENTS WITH A HISTORY OF PANCREATITIS OR OTHER SIGNIFICANT RISK FACTORS FOR THE DEVELOPMENT OF PANCREATITIS, THE COMBINATION OF EPIVIR™ AND RETROVIR® (ZIDOVUDINE) SHOULD BE USED WITH EXTREME CAUTION AND ONLY IF THERE IS NO SATISFACTORY ALTERNATIVE THERAPY. TREATMENT WITH EPIVIR SHOULD BE STOPPED IMMEDIATELY IF CLINICAL SIGNS, SYMPTOMS, OR LABORATORY ABNORMALITIES SUGGESTIVE OF PANCREATITIS OCCUR (SEE ADVERSE REACTIONS).

The complete prescribing information for *Retrovir should be consulted before* combination therapy with Epivir and Retrovir is initiated.

PRECAUTIONS:

Patients With Impaired Renal Function: Reduction of the dosage of Epivir™ is recommended for patients with impaired renal function (see CLINICAL PHARMACOLOGY and DOSAGE AND ADMINISTRATION).

Information for Patients: Epivir is not a cure for HIV infection and patients may continue to experience illnesses associated with HIV infection, including opportunistic infections. Treatment with Epivir has not been shown to reduce the frequency of such illnesses and patients should remain under the care of a physician when using Epivir. Patients should be advised that the use of Epivir has not been shown to reduce the risk of transmission of HIV to others through sexual contact or blood contamination.

Patients should be advised that the long-term effects of Epivir are unknown at this time.

Epivir Tablets and Oral Solution are for oral ingestion only.

Patients should be advised of the importance of taking Epivir exactly as it is prescribed.

Parents or guardians should be advised to monitor pediatric patients for signs and symptoms of pancreatitis.

Drug Interaction: TMP 160 mg/SMX 800 mg once daily has been shown to increase lamivudine exposure (AUC). The effect of higher doses of TMP/SMX *on lamivudine* pharmacokinetics has not been investigated (see CLINICAL PHARMACOLOGY).

Carcinogenesis, Mutagenesis, and Impairment of Fertility: Long-term carcinogenicity studies of lamivudine in animals have not yet been completed. Lamivudine was not active in a microbial mutagenicity screen or an *in vitro* cell transformation assay, but showed weak *in vitro* mutagenic activity in a cytogenetic assay using cultured human lymphocytes and in the mouse lymphoma assay. However, lamivudine showed no evidence of *in vivo* genotoxic activity in the rat at oral doses of up to 2,000 mg/kg (approximately 69 times the

recommended human dose based on body surface area comparisons). In a study of reproductive performance, lamivudine, administered to rats at doses up to 130 times the usual adult dose based on body surface area comparisons, revealed no evidence of impaired fertility and no effect on the survival, growth, and development to weaning of the offspring.

Pregnancy: ***Pregnancy Category C:*** Reproduction studies have been performed in rats and rabbits at orally administered doses up to approximately 130 and 60 times, respectively, the usual adult dose and have revealed no evidence of harm to the fetus due to lamivudine. Some evidence of early embryolethality was seen in the rabbit at doses similar to those produced by the usual adult dose and higher, but there was no indication of this effect in the rat at orally administered doses up to 130 times the usual adult dose. Studies in pregnant rats and rabbits showed that lamivudine is transferred to the fetus through the placenta. There are no adequate and well-controlled studies in pregnant women. Because animal reproductive toxicity studies are not always predictive of human response, lamivudine should be used during pregnancy only if the potential benefits outweigh the risks.

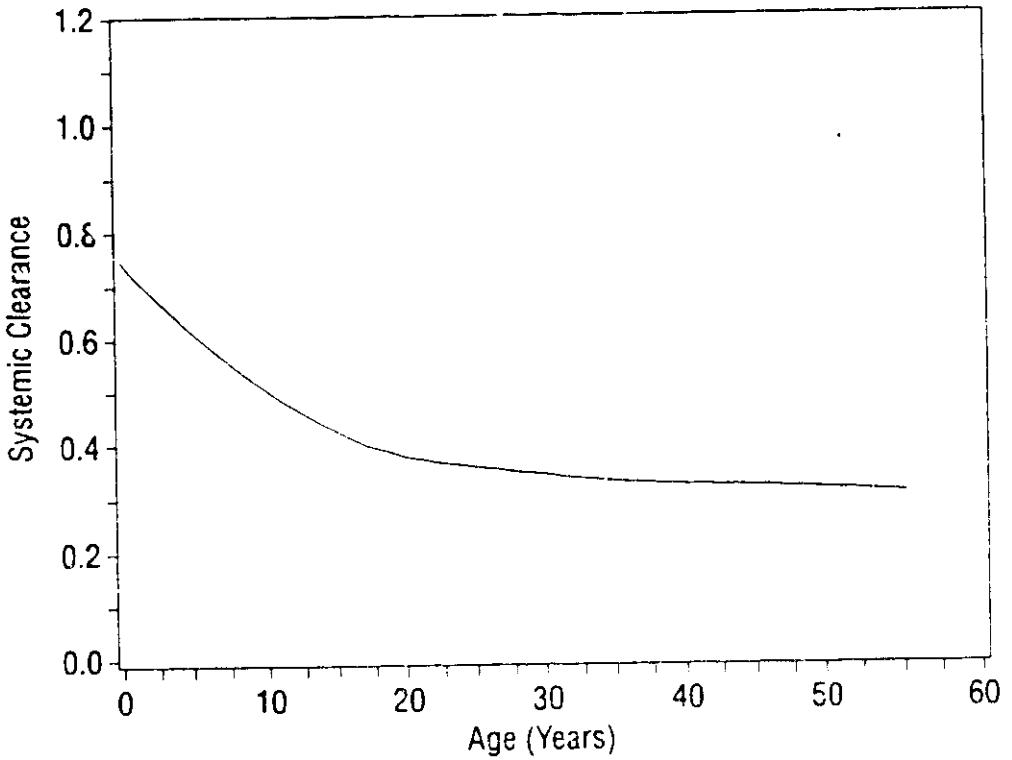
Antiretroviral Pregnancy Registry: To monitor maternal-fetal outcomes of pregnant women exposed to Epivir, an Antiretroviral Pregnancy Registry has been established. Physicians are encouraged to register patients by calling (800) 722-9292, ext.58465.

Nursing Mothers: A study in which lactating rats were administered 45 mg/kg of lamivudine showed that lamivudine concentrations in milk were slightly greater than those in plasma. Although it is not known if lamivudine is excreted in human milk, there is the potential for adverse effects from lamivudine in nursing infants. Mothers should be instructed to discontinue nursing if they are receiving lamivudine. This instruction is consistent with the Centers for Disease Control recommendation that HIV-infected mothers not breast feed their infants to avoid risking postnatal transmission of HIV infection.

Pediatric Use: THERE ARE NO DATA ON THE USE OF EPIVIR IN COMBINATION WITH RETROVIR IN PEDIATRIC PATIENTS.

Lamivudine monotherapy was studied in one open-label, uncontrolled trial (study A2002) in 97 pediatric patients with the following demographics: male (56%), Caucasian (57%), median age of 7.7 years (range: 0.4 to 17.3 years), symptomatic HIV (84%), median duration of prior antiretroviral therapy (148 weeks), and median baseline CD4 of 132 cells/mm^3. Pharmacokinetic properties of lamivudine were assessed in a subset of 57 patients (age range: 4.8 months to 16 years, weight range: 5 to 66 kg) after oral and IV administration of 1, 2, 4, 8, 12, and 20 mg/kg per day. In the 9 infants and children receiving 8 mg/kg per day (the usual recommended pediatric dose), absolute bioavailability was 66% ± 26% (mean ± S.D.), which is less than the 86% ± 16% (mean ± S.D.) observed in adolescents and adults. The mechanism for the diminished absolute bioavailability of lamivudine in infants and children is unknown.

303 Systemic clearance decreased with increasing age in pediatric patients, as shown in
304 Figure 5.
305
306 **Figure 5: Systemic Clearance (L/hr•kg) of Lamivudine in Relation to Age**
307



308
309
310 After oral administration of 8 mg/kg per day of lamivudine to 11 pediatric patients
311 ranging from 4 months to 14 years of age, C_{max} was 1.1 ± 0.6 µg/mL and half-life was
312 2.0 ± 0.6 hours. (In adults with similar blood sampling, the half-life was 3.7 ± 1 hours.)
313 Total exposure to lamivudine, as reflected by mean AUC values, was comparable between
314 pediatric patients receiving an 8 mg/kg/day dose and adults receiving a 4 mg/kg/day dose.
315 Distribution of lamivudine into cerebrospinal fluid (CSF) was assessed in 38 pediatric
316 patients after multiple oral dosing with lamivudine. CSF samples were collected between 2
317 and 4 hours postdose. At the dose of 8 mg/kg/day, CSF lamivudine concentrations in eight
318 patients ranged from 5.6% to 30.9% (mean ± S.D. of 14.2% ± 7.9%) of the concentration
319 in a simultaneous serum sample, with CSF lamivudine concentrations ranging from 0.04 to
320 0.3 µg/mL.
321 See INDICATIONS AND USAGE: Description of Clinical Studies, WARNINGS,
322 ADVERSE REACTIONS, and DOSAGE AND ADMINISTRATION sections.
323
324 **ADVERSE REACTIONS:**
325 **Adults:** Selected clinical adverse events with a ≥5% frequency during therapy with Epivir™
326 150 mg b.i.d. plus Retrovir® (zidovudine) 200 mg t.i.d. compared with zidovudine are listed
327 in Table 3.

328
329 **Table 3: Selected Clinical Adverse Events (≥5% Frequency)**
330 **in Four Controlled Clinical Trials (A3001, A3002, B3001, B3002)**
331

Adverse Event	Epivir 150 mg b.i.d. plus Retrovir (n=251)	Retrovir (n=230)
Body as a whole		
Headache	35%	27%
Malaise and fatigue	27%	23%
Fever or chills	10%	12%
Digestive		
Nausea	33%	29%
Diarrhea	18%	22%
Nausea and vomiting	13%	12%
Anorexia and/or decreased appetite	10%	7%
Abdominal pain	9%	11%
Abdominal cramps	6%	3%
Dyspepsia	5%	5%
Nervous		
Neuropathy	12%	10%
Dizziness	10%	4%
Insomnia & other sleep disorders	11%	7%
Depressive disorders	9%	4%
Respiratory		
Nasal signs & symptoms	20%	11%
Cough	18%	13%
Skin & appendages		
Skin rashes	9%	6%
Musculoskeletal		
Musculoskeletal pain	12%	10%
Myalgia	8%	6%
Arthralgia	5%	5%

332
333 Pancreatitis was observed in 3 of the 656 adult patients (<0.5%) who received Epivir in
334 controlled clinical trials.
335 Selected laboratory abnormalities observed during therapy are listed in Table 4.
336

337 **Table 4: Frequencies of Selected Laboratory Abnormalities**
338 **Among Adults in Four Controlled Clinical Trials (A3001, A3002, B3001, B3002)***
339

Test (Abnormal Level)	Epivir 150 mg b.i.d. Plus Retrovir % (n)	Retrovir % (n)
Neutropenia (ANC<750/mm^3)	7.2% (237)	5.4% (222)
Anemia (Hgb<8.0 g/dL)	2.9% (241)	1.8% (218)
Thrombocytopenia (platelets<50,000/mm^3)	0.4% (240)	1.3% (223)
ALT (>5.0 x ULN)	3.7% (241)	3.6% (224)
AST (>5.0 x ULN)	1.7% (241)	1.8% (223)
Bilirubin (>2.5 ULN)	0.8% (241)	0.4% (220)
Amylase (>2.0 ULN)	4.2% (72)	1.5% (133)

340 ULN=Upper limit of normal ANC=absolute neutrophil count
341 n=Number of patients assessed
342
343 * Frequencies of these laboratory abnormalities were higher in patients
344 with mild laboratory abnormalities at baseline.
345
346 **Pediatric Patients:** Limited information on the incidence of adverse events in children
347 receiving lamivudine monotherapy is available from one open-label, uncontrolled study
348 (see PRECAUTIONS: Pediatric Use section for description of study A2002). **Of 97**
349 **pediatric patients, 14 patients (14%) developed pancreatitis while receiving**
350 **monotherapy with Epivir. In a second ongoing study in 47 pediatric patients (age**
351 **range: 3 months to 18 years) enrolled in an open-label evaluation of**
352 **Epivir/didanosine, Epivir/Retrovir, and Epivir/Retrovir/didanosine, 7 patients (15%)**
353 **developed pancreatitis (see WARNINGS).**
354 Paresthesias and peripheral neuropathies were reported in 13 patients (13%) in study
355 A2002 and resulted in treatment discontinuation in 3 patients.
356 Selected laboratory abnormalities during lamivudine therapy in children are listed in
357 Table 5.
358

Table 5: Frequencies of Selected Laboratory Abnormalities in an Uncontrolled Phase I/II Clinical Trial of Epivir in 97 Pediatric Patients

Test (Abnormal Level)	Patients With Normal Baselines % (n)	Patients With Abnormal Baselines % (n)
Neutropenia (ANC<750/mm^3)	22% (55)	45% (33)
Anemia (Hgb<8.0 g/dL)	2% (50)	24% (46)
Thrombocytopenia (platelets<40,000/mm^3)	0% (68)	25% (12)
ALT (>5.0 x ULN)	4% (51)	29% (42)
AST (>5.0 x ULN)	0% (29)	19% (57)
Amylase (>2.0 ULN)	3% (69)	23% (13)

ULN=Upper limit of normal ANC=absolute neutrophil count
n=Number of patients assessed

OVERDOSAGE:

There is no known antidote for Epivir™. One case of an adult ingesting 6 g of Epivir was reported; there were no clinical signs or symptoms noted and hematologic tests remained normal. It is not known whether lamivudine can be removed by peritoneal dialysis or hemodialysis.

DOSAGE AND ADMINISTRATION:

Adults and Adolescents (12 to 16 years): The recommended oral dose of Epivir™ for adults and adolescents is 150 mg twice daily administered in combination with Retrovir® (zidovudine). *The complete prescribing information for Retrovir should be consulted for* information on its dosage and administration.

For adults with low body weights (less than 50 kg or 110 lbs), the recommended oral dose of Epivir is 2 mg/kg twice daily administered in combination with Retrovir. No data are available to support a dosage recommendation for adolescents with low body weight (less than 50 kg).

Pediatric Patients: The recommended oral dose of Epivir for pediatric patients 3 months to up to 12 years of age is 4 mg/kg twice daily (up to a maximum of 150 mg twice a day) administered in combination with Retrovir. The complete prescribing information for Retrovir should be consulted for information on its dosage and administration.

Dose Adjustment: It is recommended that doses of Epivir be adjusted in accordance with renal function in patients older than age 16 years (see Table 6). (See CLINICAL PHARMACOLOGY section.)

Table 6: Adjustment of Dosage of Epivir in Accordance With Creatinine Clearance

Creatinine Clearance (mL/min)	Recommended Dosage of Epivir
≥ 50	150 mg twice daily
30-49	150 mg once daily
15-29	150 mg first dose, then 100 mg once daily
5-14	150 mg first dose, then 50 mg once daily
< 5	50 mg first dose, then 25 mg once daily

Insufficient data are available to recommend a dosage of Epivir™ in patients undergoing dialysis.

HOW SUPPLIED: Epivir™ Tablets, 150 mg, are white, modified diamond-shaped, film-coated tablets imprinted with "150" on one side and "GXCJ7" on the reverse side. They are available in bottles of 60 tablets (NDC 0173-0470-01) with child-resistant closures. **Store between 2° and 30°C (36° and 86°F) in tightly closed bottles.**

Epivir™ Oral Solution, a clear, colorless to pale yellow, strawberry-banana flavored, liquid, contains 10 mg of lamivudine in each 1 mL in plastic bottles of 240 mL (NDC 0173-0471-00) with child-resistant closures. This product does not require reconstitution. **Store between 2° and 25°C (36° and 77°F) in tightly closed bottles.**

GlaxoWellcome

Glaxo Wellcome Inc.
Research Triangle Park, NC 27709

Manufactured under agreement from **BioChem Pharma Inc.**
275 Armand Frappier Blvd.
Laval, Quebec, Canada H7V 4A7

Epivir™ Oral Solution Manufactured in England

U.S. Patent 5,047,407

November 1995 RL-224 *(code no.)*

TIME SENSITIVE PATENT INFORMATION
Patent Information for
3TC™ (LAMIVUDINE) TABLETS
NDA #20-564

The following submission is made in accordance with the Notice appearing at page 30309 of the Federal Register dated June 8, 1995, Volume 60, No. 110.

By action of the Uruguay Round Agreements Act, Public Law 103-465, which was signed by the President on December 8, 1994, the original expiration date of the following patent listed in NDA #20-564 is changed as follows:

US 5,047,407 to February 8, 2009

The undersigned declares that the above-listed patent covers the formulation, composition, and/or method of use of **3TC™ (LAMIVUDINE) TABLETS**. A New Drug Application for this product has been submitted under Section 505 of the Federal Food, Drug and Cosmetic Act.

Date: June 9, 1995

David J. Levy
Attorney for Applicant
Registered Patent Attorney
Registration No. 27,655

TIME SENSITIVE PATENT INFORMATION
Patent Information for
3TC™ (LAMIVUDINE) ORAL SOLUTION
NDA #20-596

The following submission is made in accordance with the Notice appearing at page 30309 of the Federal Register dated June 8, 1995, Volume 60, No. 110.

By action of the Uruguay Round Agreements Act, Public Law 103-465, which was signed by the President on December 8, 1994, the original expiration date of the following patent listed in NDA #20-596 is changed as follows:

US 5,047,407 to February 8, 2009

The undersigned declares that the above-listed patent covers the formulation, composition, and/or method of use of **3TC™ (LAMIVUDINE) ORAL SOLUTION**. A New Drug Application for this product has been submitted under Section 505 of the Federal Food, Drug and Cosmetic Act.

Date: June 9, 1995

David J. Levy
Attorney for Applicant
Registered Patent Attorney
Registration No. 27,655

EXCLUSIVITY SUMMARY

NDA: 20-564 SUPPLEMENT: _____

Trade Name: EPIVIR™ Generic Name: lamivudine (TABLET)

Applicant Name: Glaxo Wellcome Inc. HFD #: 530

Approval Date: _____
(If Known)

PART I: IS AN EXCLUSIVITY DETERMINATION NEEDED?

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete PARTS II and III of this Exclusivity Summary only if you answer "yes" to one or more of the following question about the submission.

   a. Is it an original NDA?

   YES /X/ NO /__/

   b. Is it an effectiveness supplement?

   YES /__/ NO /X/

   If yes, what type? (SE1, SE2, etc.): _____

   c. Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "no.")

   YES /X/ NO /__/

   If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

   _____

   _____

If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

_____

_____

d. Did the applicant request exclusivity?

YES /X_/ NO /___/

If the answer to (d) is "yes," how many years of exclusivity did the applicant request?

_____

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. Has a product with the same active ingredient(s), dosage form, strength, route of administration, and dosing schedule, previously been approved by FDA for the same use?

YES /___/ NO /X_/

If yes, NDA #_____ Drug Name _____

IF THE ANSWER TO QUESTION 2 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

3. Is this drug product or indication a DESI upgrade?

YES /___/ NO /X_/

IF THE ANSWER TO QUESTION 3 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8 (even if a study was required for the upgrade).

PART II: FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES

(Answer either #1 or #2 as appropriate)

1. Single active ingredient product

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES /___/ NO /X_/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA# _____ _____

NDA# _____ _____

NDA# _____ _____

2. Combination product

If the product contains more than one active moiety (as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one never-before-approved active moiety and one previously approved active moiety, answer "yes." (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved.)

YES /___/ NO /___/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA(s).

a.	_____	_____
b.	_____	_____
NDA#	_____	_____

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8. IF "YES" GO TO PART III.

PART III: THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant." This section should be completed only if the answer to PART II, Question 1 or 2 was "yes."

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies.) If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes," then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

   YES /___/ NO /___/

IF "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying on that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

a. In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /___/ NO /___/

If "no," state the basis for your conclusion that a clinical trial is not necessary for approval AND GO DIRECTLY TO SIGNATURE BLOCK ON PAGE 8:

_____

_____

b Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /___/ NO /___/

(1) If the answer to 2(b) is "yes," do you personally know of any reason to disagree with the applicant's conclusion? If not applicable, answer NO.

YES /___/ NO /___/

If yes, explain:

_____

_____

_____

(2) If the answer to 2(b) is "no," are you aware of published studies not conducted or sponsored by the applicant or other publicly available data that could independently demonstrate the safety and effectiveness of this drug product?

YES /___/ NO /___/

If yes, explain _____

_____

_____

(c) If the answers to (b)(1) and (b)(2) were both "no," identify the clinical investigations submitted in the application that are essential to the approval

_____

_____

Studies comparing two products with the same ingredient(s) are considered to be bioavailability studies for the purpose of this section

3 In addition to being essential, investigations must be "new" to support exclusivity The agency interprets "new clinical investigation" to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously approved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.

a. For each investigation identified as "essential to the approval," has the investigation been relied on by the agency to demonstrate the effectiveness of a previously approved drug product? (If the investigation was relied on only to support the safety of a previously approved drug, answer "no ")

Investigation #1 YES /___/ NO /___/

Investigation #2 YES /___/ NO /___/

If you have answered "yes" for one or more investigations, identify each such investigation and the NDA in which each was relied upon.

_____ _____

_____ _____

b For each investigation identified as "essential to the approval", does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1 YES /___/ NO /___/

Investigation #2 YES /___/ NO /___/

If you have answered "yes" for one or more investigation, identify the NDA in which a similar investigation was relied on:

_____ _____

_____ _____

c. If the answers to 3(a) and 3(b) are no, identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

_____ _____

_____ _____

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with the Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

a. For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on the FDA 1571 as the sponsor?

Investigation #1

IND # _____ YES /___/ NO /___/

Explain _____

_____ _____

Investigation #2

IND # _____ YES /___/ NO /___/

Explain _____

_____ _____

b. For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study?

Investigation #1:

IND # _____ YES /___/ NO /___/

Explain: _____

_____

Investigation #2:

IND # _____ YES /___/ NO /___/

Explain: _____

_____

c. Notwithstanding an answer of "yes" to (a) or (b), are there other reasons to believe that the applicant should not be credited with having "conducted or sponsored" the study? (Purchased studies may not be used as the basis for exclusivity However, if all rights to the drug are purchased (not just studies on the drug), the applicant may be considered to have sponsored or conducted the studies sponsored or conducted by its predecessor in interest.)

YES /___/ NO /___/

If yes, explain: _____

_____

_____ _____

Signature Date

Title:

David W. Feigal Jr. 11-17-95

Signature of Officer Date

Division Director

EXCLUSIVITY SUMMARY

NDA: 20-596 SUPPLEMENT: _____

Trade Name: EPIVIR™ Generic Name: lamivudine

Applicant Name: Glaxo Wellcome Inc. HFD #: 530

Approval Date: _____
(If Known)

PART I: IS AN EXCLUSIVITY DETERMINATION NEEDED?

1. An exclusivity determination will be made for all original applications, but only for certain supplements. Complete PARTS II and III of this Exclusivity Summary only if you answer "yes" to one or more of the following question about the submission.

   a. Is it an original NDA?

   YES /X/ NO /__/

   b. Is it an effectiveness supplement?

   YES /__/ NO /X/

   If yes, what type? (SE1, SE2, etc.): _____

   c. Did it require the review of clinical data other than to support a safety claim or change in labeling related to safety? (If it required review only of bioavailability or bioequivalence data, answer "no.")

   YES /X/ NO /__/

   If your answer is "no" because you believe the study is a bioavailability study and, therefore, not eligible for exclusivity, EXPLAIN why it is a bioavailability study, including your reasons for disagreeing with any arguments made by the applicant that the study was not simply a bioavailability study.

   _____

   _____

If it is a supplement requiring the review of clinical data but it is not an effectiveness supplement, describe the change or claim that is supported by the clinical data:

_____

_____

d. Did the applicant request exclusivity?

YES /X_/ NO /___/

If the answer to (d) is "yes," how many years of exclusivity did the applicant request?

_____

IF YOU HAVE ANSWERED "NO" TO ALL OF THE ABOVE QUESTIONS, GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. Has a product with the same active ingredient(s), dosage form, strength, route of administration, and dosing schedule, previously been approved by FDA for the same use?

YES /___/ NO /X_/

If yes, NDA #_____ Drug Name _____

IF THE ANSWER TO QUESTION 2 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

3. Is this drug product or indication a DESI upgrade?

YES /___/ NO /X_/

IF THE ANSWER TO QUESTION 3 IS "YES," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8 (even if a study was required for the upgrade).

PART II: FIVE-YEAR EXCLUSIVITY FOR NEW CHEMICAL ENTITIES

(Answer either #1 or #2 as appropriate)

1. Single active ingredient product

Has FDA previously approved under section 505 of the Act any drug product containing the same active moiety as the drug under consideration? Answer "yes" if the active moiety (including other esterified forms, salts, complexes, chelates or clathrates) has been previously approved, but this particular form of the active moiety, e.g., this particular ester or salt (including salts with hydrogen or coordination bonding) or other non-covalent derivative (such as a complex, chelate, or clathrate) has not been approved. Answer "no" if the compound requires metabolic conversion (other than deesterification of an esterified form of the drug) to produce an already approved active moiety.

YES /___/ NO /X__/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA# _____ _____

NDA# _____ _____

NDA# _____ _____

2. Combination product

If the product contains more than one active moiety (as defined in Part II, #1), has FDA previously approved an application under section 505 containing any one of the active moieties in the drug product? If, for example, the combination contains one never-before-approved active moiety and one previously approved active moiety, answer "yes." (An active moiety that is marketed under an OTC monograph, but that was never approved under an NDA, is considered not previously approved.)

YES /___/ NO /___/

If "yes," identify the approved drug product(s) containing the active moiety, and, if known, the NDA #(s).

NDA# _____ _____

NDA# _____ _____

NDA# _____ _____

IF THE ANSWER TO QUESTION 1 OR 2 UNDER PART II IS "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8. IF "YES" GO TO PART III.

PART III: THREE-YEAR EXCLUSIVITY FOR NDA'S AND SUPPLEMENTS

To qualify for three years of exclusivity, an application or supplement must contain "reports of new clinical investigations (other than bioavailability studies) essential to the approval of the application and conducted or sponsored by the applicant." This section should be completed only if the answer to PART II, Question 1 or 2 was "yes."

1. Does the application contain reports of clinical investigations? (The Agency interprets "clinical investigations" to mean investigations conducted on humans other than bioavailability studies.) If the application contains clinical investigations only by virtue of a right of reference to clinical investigations in another application, answer "yes," then skip to question 3(a). If the answer to 3(a) is "yes" for any investigation referred to in another application, do not complete remainder of summary for that investigation.

   YES /___/ NO /___/

IF "NO," GO DIRECTLY TO THE SIGNATURE BLOCKS ON PAGE 8.

2. A clinical investigation is "essential to the approval" if the Agency could not have approved the application or supplement without relying on that investigation. Thus, the investigation is not essential to the approval if 1) no clinical investigation is necessary to support the supplement or application in light of previously approved applications (i.e., information other than clinical trials, such as bioavailability data, would be sufficient to provide a basis for approval as an ANDA or 505(b)(2) application because of what is already known about a previously approved product), or 2) there are published reports of studies (other than those conducted or sponsored by the applicant) or other publicly available data that independently would have been sufficient to support approval of the application, without reference to the clinical investigation submitted in the application.

a. In light of previously approved applications, is a clinical investigation (either conducted by the applicant or available from some other source, including the published literature) necessary to support approval of the application or supplement?

YES /___/ NO /___/

If "no," state the basis for your conclusion that a clinical trial is not necessary for approval AND GO DIRECTLY TO SIGNATURE BLOCK ON PAGE 8:

_____

_____

b. Did the applicant submit a list of published studies relevant to the safety and effectiveness of this drug product and a statement that the publicly available data would not independently support approval of the application?

YES /___/ NO /___/

(1) If the answer to 2(b) is "yes," do you personally know of any reason to disagree with the applicant's conclusion? If not applicable, answer NO

YES /___/ NO /___/

If yes, explain:

_____

_____

_____

(2) If the answer to 2(b) is "no," are you aware of published studies not conducted or sponsored by the applicant or other publicly available data that could independently demonstrate the safety and effectiveness of this drug product?

YES /___/ NO /___/

If yes, explain: _____

_____

_____

(c) If the answers to (b)(1) and (b)(2) were both "no," identify the clinical investigations submitted in the application that are essential to the approval.

_____

_____

Studies comparing two products with the same ingredient(s) are considered to be bioavailability studies for the purpose of this section.

3. In addition to being essential, investigations must be "new" to support exclusivity. The agency interprets "new clinical investigation" to mean an investigation that 1) has not been relied on by the agency to demonstrate the effectiveness of a previously approved drug for any indication and 2) does not duplicate the results of another investigation that was relied on by the agency to demonstrate the effectiveness of a previously approved drug product, i.e., does not redemonstrate something the agency considers to have been demonstrated in an already approved application.

a. For each investigation identified as "essential to the approval," has the investigation been relied on by the agency to demonstrate the effectiveness of a previously approved drug product? (If the investigation was relied on only to support the safety of a previously approved drug, answer "no.")

Investigation #1: YES /___/ NO /___/

Investigation #2: YES /___/ NO /___/

If you have answered "yes" for one or more investigations, identify each such investigation and the NDA in which each was relied upon.

_____ _____

_____ _____

b. For each investigation identified as "essential to the approval", does the investigation duplicate the results of another investigation that was relied on by the agency to support the effectiveness of a previously approved drug product?

Investigation #1: YES /___/ NO /___/

Investigation #2: YES /___/ NO /___/

If you have answered "yes" for one or more investigation, identify the NDA in which a similar investigation was relied on:

_____ _____

_____ _____

c. If the answers to 3(a) and 3(b) are no, identify each "new" investigation in the application or supplement that is essential to the approval (i.e., the investigations listed in #2(c), less any that are not "new"):

_____ _____

_____ _____

4. To be eligible for exclusivity, a new investigation that is essential to approval must also have been conducted or sponsored by the applicant. An investigation was "conducted or sponsored by" the applicant if, before or during the conduct of the investigation, 1) the applicant was the sponsor of the IND named in the form FDA 1571 filed with the Agency, or 2) the applicant (or its predecessor in interest) provided substantial support for the study. Ordinarily, substantial support will mean providing 50 percent or more of the cost of the study.

a. For each investigation identified in response to question 3(c): if the investigation was carried out under an IND, was the applicant identified on the FDA 1571 as the sponsor?

Investigation #1:

IND # _____ YES /___/ NO /___/

Explain: _____

_____

Investigation #2:

IND # _____ YES /___/ NO /___/

Explain: _____

_____

b. For each investigation not carried out under an IND or for which the applicant was not identified as the sponsor, did the applicant certify that it or the applicant's predecessor in interest provided substantial support for the study?

Investigation #1:

IND # _____ YES /___/ NO /___/

Explain: _____

_____

Investigation #2:

IND # _____ YES /___/ NO /___/

Explain: _____

_____

c. Notwithstanding an answer of "yes" to (a) or (b), are there other reasons to believe that the applicant should not be credited with having "conducted or sponsored" the study? (Purchased studies may not be used as the basis for exclusivity. However, if all rights to the drug are purchased (not just studies on the drug), the applicant may be considered to have sponsored or conducted the studies sponsored or conducted by its predecessor in interest.)

YES /___/ NO /___/

If yes, explain: _____

_____

_____ _____

Signature Date

Title:

David W Kettl 11-17-95

Signature of Officer Date

Division Director

3TC™ (lamivudine) Tablets
NDA 20-564

Debarment Certification:

In accordance with the certification provision of the Generic Drug Enforcement Act of 1992 as outlined in correspondence dated July 29, 1992 from Daniel L. Michels, Office of Compliance at FDA, Glaxo hereby certifies that to the best of its knowledge and belief, it did not use in any capacity the services of any person debarred under section 306 (a) or (b) of the Generic Drug Enforcement Act of 1992 in connection with this application.

David R. Savello, Ph.D.
Vice President, Regulatory Affairs

6/19/95
Date

3TC™ (lamivudine) Oral Solution
NDA 20-596

Debarment Certification:

In accordance with the certification provision of the Generic Drug Enforcement Act of 1992 as outlined in correspondence dated July 29, 1992 from Daniel L. Michels, Office of Compliance at FDA, Glaxo hereby certifies that to the best of its knowledge and belief, it did not use in any capacity the services of any person debarred under section 306 (a) or (b) of the Generic Drug Enforcement Act of 1992 in connection with this application.

David R. Savello, Ph.D.
Vice President, Regulatory Affairs

6/23/95
Date

M E M O R A N D U M

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
Center for Drug Evaluation and Research

DATE: November 16, 1995

FROM: Rachel E. Behrman, M.D. M.P.H.
Team Leader, Division of Antiviral Drug Products

SUBJECT: NDA 20-564; NDA 20-596

TO: David W. Feigal, Jr. M.D. M.P.H.
Director, Division of Antiviral Drug Products
Acting Director, Office of Drug Evaluation IV

On behalf of the lamivudine review team, I am pleased to recommend that lamivudine be approved in combination with zidovudine for the treatment of HIV infection when therapy is warranted based on clinical and/or immunologic evidence of disease progression. Safety and efficacy of lamivudine in this setting is supported by data from 4 randomized, blinded controlled studies that demonstrated superiority of the lamivudine/zidovudine combination to approved therapies as measured by changes in cd4 cell counts with therapy. Similarly, the lamivudine/zidovudine combination produced more pronounced declines in plasma HIV RNA when compared to approved therapies.

There are no outstanding regulatory issues, either clinical or preclinical, that must be resolved before the agency can take a regulatory action on these NDAs. The letter dated November 16, 1995 from Dr. Palmer of Glaxo-Welcome summarizes both the conditions of accelerated approval and the goals for phase IV study. There are, however, several noteworthy issues that warrant explicit discussion.

SCOPE

Data supporting the safety and efficacy of lamivudine in combination with zidovudine are limited to results from studies evaluating surrogate endpoints, primarily changes in cd4 cell counts with therapy. Therefore, this application falls under the scope of subpart H, accelerated approval, and must demonstrate that lamivudine in combination with zidovudine offers meaningful therapeutic benefit over existing therapies.

This application has challenged us to consider accelerated approval in a new manner. In the past this division has recommended accelerated approval only for those segments of the population who had no satisfactory alternatives because they were either intolerant of existing therapies or because their disease continued to progress despite receiving existing therapies.

The regulations clearly envision the circumstance under which data from surrogate endpoint trials are sufficiently compelling to permit an accelerated approval for those patients who do

have satisfactory alternatives, but for whom the new therapy may well be an improved option over existing therapies. This is the case with lamivudine in combination with zidovudine. It is not simply that the surrogate endpoint responses are superior when compared to approved therapies, which they are. The results are replicated in four randomized, controlled and blinded studies. The results appear to be consistent for patients who have had prolonged therapy with other antiretroviral agents and for those who have never been treated for their HIV infection. The results from cd4 analyses are supported by limited data on changes in plasma HIV RNA. Furthermore, the surrogate effect is unusually durable. For the first time, changes in cd4 cell counts are sustained above baseline values at the 24 week evaluation. In addition to this strikingly robust surrogate effect, it is clear that the combination of lamivudine and zidovudine has a tolerable toxicity profile.

Therefore, it is justified to take this regulatory action which will, for the first time, include patients who have never before received antiretroviral therapy. As with past actions, the indication is intended to convey the potential scope of use, and its limitations, without providing rigid criteria that may inadvertently limit access to therapies.

PEDIATRICS

The applicant has submitted sufficient data on the pharmacokinetics in children to permit selection of a dose for all age ranges except underweight adolescents. The safety profile in children is limited and there is essentially no information on the safety or activity of lamivudine in combination with zidovudine. The application, in collaboration with the has launched a large, randomized, controlled trial in children. In the interim, the applicant has committed to rigorous reporting of pancreatitis in children, the most troubling adverse event noted in early studies.

CONFIRMATORY CLINICAL TRIALS

The review team has longstanding reservations about both the design and pace of the confirmatory program that are documented in the regulatory record and in the records of the advisory committee meetings. In short, the adult trial may have difficultly establishing a difference between lamivudine in combination with zidovudine and the comparators primarily because of the multiple and complex possible treatment regimens. The pediatric trial is more straightforward in design and is likely to be the primary source of efficacy data. However, it is recruiting slowly. In addition, despite our comfort that demonstration of efficacy in children is sufficient to confirm efficacy in adults, it is unclear how information about endpoints that are unique to children can be applied to adults. The applicant has committed to address these concerns.

ACKNOWLEDGEMENT

The review team (Dr. H. Jolson, Dr. P. Flyer, Dr. B. Davitt, Dr. S. Miller, Dr. P. Verma, Dr. N. Battula, Dr. K. Reynolds, Dr. G. Chikami and the project manager, Ms. D. Kallgren) is to be congratulated on their outstanding performance that ensured a timely completion of this project with the highest standards of review.

DRUG STUDIES IN PEDIATRIC PATIENTS
(To be completed for all NME's recommended for approval)

NDA # 20-564 Trade (generic) names EPIVIR™ (lamivudine) Tablets
20-596 EPIVIR™ (lamivudine) Oral Solution

Check any of the following that apply and explain, as necessary, on the next page:

___ 1. A proposed claim in the draft labeling is directed toward a specific pediatric illness. The application contains adequate and well-controlled studies in pediatric patients to support that claim.

___ 2. The draft labeling includes pediatric dosing information that is not based on adequate and well-controlled studies in children. The application contains a request under 21 CFR 210.58 or 314.126(C) for waiver of the requirement at 21 CFR 201.57(f) for A&WC studies in children.

___ a. The application contains data showing that the course of the disease and the effects of the drug are sufficiently similar in adults and children to permit extrapolation of the data from adults to children. The waiver request should be granted and a statement to that effect is included in the action letter.

___ b. The information included in the application does not adequately support the waiver request. The request should not be granted and a statement to that effect is included in the action letter. (Complete #3 or #4 below as appropriate).

___ 3. Pediatric studies (e.g., dose-finding, pharmacokinetic, adverse reaction, adequate and well-controlled for safety and efficacy) should be done after approval. The drug product has some potential for use in children, but there is no reason to expect early widespread pediatric use (because, for example, alternative drugs are available or the condition is uncommon in children.

___ a. The applicant has committed to doing such studies as will be required.

___ (1) Studies are ongoing.
___ (2) Protocols have been submitted and approved.
___ (3) Protocols have been submitted and are under review.
___ (4) If no protocol has been submitted, on the next page explain the status of discussions.

___ b. If the sonsor is not willing to do pediatric studies, attach copies of FDA's written request that such studies be done and of the sponsor's written response to that request

__ 4. Pediatric studies do not need to be encouraged because the drug product has little potential for use in children.

_X_ 5. If none of the above apply, explain.

Explain, as necessary, the foregoing items: As per the final rule (21CFR 201.57(f) the pediatric usage statement is supported by pnarmacokinetics and safety data in children and results of clinical trial results in adults.

Signature of Preparer

11/16/95
Date

cc: Orig NDA
HFD-530/Div File
NDA Action Package

GlaxoWellcome

November 15, 1995

David W. Feigal, Jr., M.D., M.P.H.
Director, Division of Antiviral Drug Products
HFD-530, NLRC, 200
Food and Drug Administration
Attention: Document Control Room
5516 Nicholson Lane
Kensington, MD 20895

RE: NDA 20-564; Epivir™ Tablets (lamivudine tablets);
NDA 20-596; Epivir™ Oral Solution (lamivudine oral solution);
Commitments Regarding Accelerated Approval of Lamivudine

Dear Dr. Feigal:

In accordance with 21 CFR 314.500 et seq., we hereby commit to the following conditions of accelerated approval:

1. Within three to six months of completion of studies 3007 and 300, respectively, (where "completion" is defined as the time when all participants stop randomized, blinded study medication), Glaxo Wellcome will provide FDA with a study report of key analyses of effectiveness and safety, along with corresponding data sets. In advance of the completion of each study, Glaxo Wellcome will seek FDA agreement on the specific efficacy and safety analyses to be conducted.

2. Glaxo Wellcome will submit quarterly updates (beginning in first quarter, 1996) on the progress of studies 3007 and 300 to FDA. These quarterly updates will include total numbers of deaths, clinical endpoints, lost-to-follow-up, and study medication discontinuations. Since these studies are blinded, the quarterly updates will not be broken down by treatment group. Safety reporting will continue under the usual good clinical practice requirements.

3. Major amendments of the design of 3007 and 300 will be submitted to, and discussed with, the FDA prior to enactment.

Glaxo Wellcome Research and Development

Greenford Road
Greenford
Middlesex
UB6 0HE
UK

Telephone
+44 (0)181 [illegible] 3434

4. We agree to amend protocol 3007 (adult clinical endpoint study) in the following respects:
   a. Expand the enrollment of patients without a history of antiretroviral therapy.
   b. Increase the power of the study to improve the likelihood of detecting a clinically meaningful treatment effect.
   c. Address the viability of the zidovudine monotherapy arm in light of results from recent studies (e.g., 175 and Delta) and prospectively redefine the study hypothesis and primary analysis.

5. We agree to amend protoco 300 in the following respects:
   a. Submit all reports of diagnosed pancreatitis to FDA within 15 days of occurrence. We also agree to collaborate with to reported all suspected cases of pancreatitis within 15 days of occurrence. Finally, we agree to provide quarterly submission to FDA of the total numbers of diagnosed and suspected pancreatitis cases by blinded treatment groups in 300.
   b. Collaborate with the to obtain lamivudine blood levels in all pediatric patients diagnosed with pancreatitis as soon as pancreatitis is first suspected.
   c. Develop a proposal for initiating a case-control study with the data generated from the monitoring of drug levels.

6. All pediatric promotional material for Epivir Tablets and Epivir Oral Solution will:
   1) prominently include the disclaimer that "There are no data on the use of Epivir in combination with Retrovir in pediatric patients."
   2) prominently include the warning about pediatric pancreatitis.
   3) not promote lamivudine in combination with zidovudine as initial therapy in children.

7. We agree to comply with the accelerated approval withdrawal procedures described in 21 CFR 314.530 if neither protocol 300 nor protocol 3007 provides verification of clinical benefit and if FDA so requests.

   We also understand that if lamivudine receives traditional approval in the future, the package insert will then reflect the content and composition of our confirmatory clinical trials. Therefore, we understand that the indication subject to traditional approval might, in fact, be more restrictive than the indication for accelerated approval.

8. We hereby commit that, if the results from recent studies evaluating the efficacy of other antiretroviral agents necessitate revising the package insert for zidovudine, we will revise the affected sections of the package insert for lamivudine and submit a Supplemental Application to reflect any clinically relevant information within 30 days of FDA's request.

We also state here our commitments to various Phase IV activities in support of Epivir Tablets and Epivir Oral Solution.

1. We acknowledge the following Phase IV commitments, as noted by the medical officers, and state our intent to pursue these obligations:
   a. Develop or collaborate with others in a program that monitors the potential for development of resistance to antiretroviral nucleoside analogues (other than zidovudine) following lamivudine therapy.
   b. Develop or collaborate with others in surrogate endpoint trials in treatment-naive patients that compare therapy with lamivudine in combination with zidovudine to other clinically relevant combinations of antiretroviral agents.

2. We agree to complete the ongoing rodent carcinogenicity studies with lamivudine and report the results in a timely manner to FDA.

3. We acknowledge the following biopharmaceutics Phase IV commitments and state our intent to pursue these obligations:
   a. Develop a program to investigate lamivudine pharmacokinetics in pediatric and adolescent patients to provide additional data on dose optimization.
   b. Develop a program to investigate the relationship, if any, between systemic exposure to lamivudine and development of pancreatitis in pediatric patients.
   c. Develop a program to investigate the effects of renal insufficiency on lamivudine pharmacokinetics in pediatric patients to provide data for dosing recommendations.
   d. Develop a program to investigate lamivudine pharmacokinetics in patients with advanced HIV disease.
   e. Develop a program to investigate the effects of gender and ethnicity on the pharmacokinetic properties of lamivudine in patients with HIV infection.
   f. Develop a program to investigate the relationship between surrogate markers of lamivudine efficacy and serum concentrations of lamivudine and other antiretroviral drugs.

g. Develop a program to investigate (to the extent that currently available technologies allow) the relationship between serum lamivudine concentrations and intracellular lamivudine triphosphate concentrations in patients dosed with lamivudine.

h. Perform and report a new mass balance study to more fully characterize the disposition of lamivudine in man. (We acknowledge that FDA specifically requested that an study be conducted using radiolabeled lamivudine in patients. However, we believe that alternative methods may be suitable, while avoiding the need to administer a radiolabeled drug to humans. Therefore, we hereby commit to discuss and agree with FDA personnel on the specific methodology to be used for arriving at acceptable information on the disposition of lamivudine in man).

In regard to these biopharmaceutics commitments, we also gratefully acknowledge the offer from FDA's scientific reviewers to review and discuss any proposed protocols prior to implementation. We fully intend to take advantage of this offer.

We believe these commitments will meet your needs as you proceed toward accelerated approval of NDA 20-564 and NDA 20-596. This submission is provided in duplicate. A desk copy has been transmitted via FAX to Debbie Kallgren. Please contact David Cocchetto at (919)-990-5127 for any matters regarding these applications. Thank you.

Sincerely,

James B.D. Palmer, M.D.
Senior Vice President and Director, Group Medical Operations
Glaxo Wellcome Inc.

NDA 20,564
NDA 20,596

DRAFT

Date NDA submitted: June 30, 1995
Date NDA received: July 3, 1995
Date assigned: July 5, 1995
Review completed: November X, 1995

Medical Officer's Review
(Original NDA for NME)

Sponsor: Glaxo Wellcome Inc.
Five Moore Drive
Research Triangle Park, NC 27709

Drug:
Generic: lamivudine (3TC)
Trade: Epivir™
Chemical: (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one

Route: oral

Dosage form: tablet (NDA 20,564)
solution (NDA 20-596)

Strength: 150 mg (tablet)
10 mg/mL (solution)

Proposed indication: treatment of HIV infection

Related INDs:

Related documents: *Major amendments* received: August 30, 1995 (Expanded Access update); October 31, 1995 (Four month safety update).

Minutes of meetings dated: January 11, 1995 (closed session of Advisory Committee); February 13, 1995 (pre-NDA); August 2, 1995 (post-submission); October 6, 1995 (labeling); November 6, 1995 (Advisory Committee).

Medical reviews dated: April 28, 1995 3007); May 30, 1995 (300)

TABLE OF CONTENTS

1. Resume

In support of safety and efficacy of lamivudine in combination with zidovudine in adults, the applicant has submitted the results of four adequate and well controlled surrogate endpoint studies and safety experience from the expanded access program. In support of use in pediatrics, the applicant has submitted safety and pharmacokinetic experience from two open-label , uncontrolled studies in children.

The four controlled adult trials provide adequate evidence that lamivudine when given in combination with zidovudine results in an increase in CD4 cell count through 24 weeks of approximately 50 cells in antiretroviral naive and 30 cells in antiretroviral experienced adults. These CD4 changes were significantly greater than those observed when zidovudine (in naive adults) and zidovudine or zidovudine plus ddC (in experienced adults) were used. The results of the North American trials additionally suggest that this effect may be sustained in some individuals beyond 24 weeks however interpretation of data beyond 24 weeks is limited due to substantial loss to follow-up. Analyses of changes in plasma viremia were consistent with CD4 effects and provided supportive evidence of efficacy.

Lamivudine in combination with zidovudine was generally well tolerated. The frequencies of most clinical and laboratory adverse events were similar between combination therapy and zidovudine monotherapy recipients. The most concerning adverse event was the occurrence of pancreatitis is 15% of children enrolled in two open-label studies. Other notable pediatric safety issues included the occurrences of neuropathy and neutropenia. Although the utility of the expanded access data was limited due to incomplete and passive data collection, the collective experience from controlled trials and expanded access provides a reasonable measure of reassurance about the types and severity of toxicity that may be encountered with lamivudine post-marketing.

Based on these changes in surrogate endpoints, the applicant has request accelerated approval for lamivudine in combination with zidovudine with an indication for patients in whom antiretroviral therapy is warranted based on clinical and/or immunological evidence of disease progression. The broad nature of the proposed indication represents a departure from previous accelerated approval applications, which have intended therapy for populations with progressive disease or those intolerant of all other available therapy. Because data in this application is supportive of clinical benefit over existing therapies, this application and its proposed indication are approvable.

2. Regulatory history

The first IND for lamivudine was submitted on June 21, 1991. Following this, the adult phase I/II program and the pediatric program for lamivudine were initiated in 1991 and 1992, respectively. Controlled clinical trials with surrogate marker endpoints in adults were initiated in May 1993. The expanded access program was initiated in October 1993. A closed session meeting with the Antiviral Drugs Advisory Committee was held on January 11, 1995

to discuss the contents of the NDA and the design of the clinical endpoint studies, and a pre-NDA meeting was held on February 13, 1995. Clinical endpoint studies in children (300) and adults (3007) began recruitment during the second and first quarters of 1995, respectively.

Lamivudine is not approved in any international market.

3. Summary of NDA clinical section

The clinical section of this application includes the study reports of four randomized controlled clinical trials in HIV-infected adults, two uncontrolled trials in children and the expanded access program.

Adult trials (see Table 1)

The four controlled clinical trials enrolled a total of 495 antiretroviral naive and 479 experienced adults; 656 adults overall were randomized to receive lamivudine (LAM) alone or in combination with zidovudine (ZDV). The primary objectives of the studies were to evaluate the effect of lamivudine therapy in combination with zidovudine on the 24 week average difference from baseline (DAVGT) for both CD4 cell count and HIV RNA by PCR (see Attachment 1 for comments from the biometrics reviewer). Secondary measures of efficacy included disease progression or death; changes in other surrogate markers (CD4 percent, neopterin, p24, and β2 microglobulin); and the effect on quality of life parameters. In all studies, patients remained blinded to therapy for 24 weeks. Following the initial 24 week treatment period, patients enrolled in the two North American studies were given the option of receiving continued blinded therapy. Surrogate marker analyses were conducted when the last patient randomized completed 24 weeks of treatment. In contrast, in the two European studies patients who completed 24 weeks of therapy were given the option of receiving open-label lamivudine until the last patient randomized completed 24 weeks

Table 1. Summary of lamivudine (LAM) controlled clinical trials in adults

Study, Location	No. of patients	Treatment Groups	Prior ZDV (range of medians)	CD4 range cells/mm³ (median)	Primary Endpoints
3001 26 North American sites	366	LAM 300mg bid ZDV 200 mg tid LAM 150 mg bid/ZDV 200 mg tid LAM 300 mg bid/ZDV 200 mg tid	< 4 weeks	200-500 (352)	CD4 HIV RNA PCR
3001 14 UK and European sites	129	ZDV 200 mg tid LAM 300 mg bid/ZDV 200 mg tid	< 4 weeks	100-400 (260)	CD4 HIV RNA PCR (subset)
3002 21 North American sites	254	ZDV 200 mg tid/ddC 0.75 mg tid LAM 150 mg bid/ZDV 200 mg tid LAM 300 mg bid/ZDV 200 mg tid	> 24 weeks (72-98 wks)	100-300 (211)	CD4 HIV RNA PCR
3002 32 UK and European sites	223	ZDV 200 mg tid LAM 150 mg bid/ZDV 200 mg tid LAM 300 mg bid/ZDV 200 mg tid	> 24 weeks (83-92 wks)	100-400 (241)	CD4 HIV RNA PCR (subset)

Pediatric trials

At the time of the NDA submission, a total of 144 pediatric patients (range 3 months to 18 years for eligibility) had been enrolled in two ongoing open-label studies of: (1) lamivudine monotherapy (2002, n=97) and (2) lamivudine in combination with other nucleoside analogues (2005, n=47). In 2005, the 47 children were either randomized (n=7) or assigned (n=40) to receive one of five possible combinations of lamivudine, ZDV and ddI. Treatment assignments were based on prior history of antiretroviral use/tolerance and disease progression. Of the 47 children, nine received lamivudine in combination with zidovudine.

Expanded access program

The expanded access program has enrolled 35,267 patients as of October 4, 1995. Although the program has been open since October 1993, the majority of patients have enrolled since November 1994. The North American program enrolls adults and children with progressive, symptomatic HIV disease and CD4 counts ≤ 300 cell/mm3 who are unable to participate in controlled trials because of disease progression or because of intolerance or unresponsiveness to other therapies. Patients are randomized to receive lamivudine at a dose of either 150 mg or 300 mg twice daily (or 8 mg/kg/day for children < 12 years of age). The European program uses similar eligibility criteria but all adults receive the 300 mg twice daily dose of lamivudine. Approximately equal proportions of patients have received the two lamivudine doses.

Demographic and complete clinical adverse event information is available only for patients for whom case report forms have been returned (n=10,685 as of July 12, 1995). Among this subset of 10,685, the vast majority are adults[1] and 95% are male. In addition, spontaneous telephone reports of serious adverse events and deaths have been tabulated.

[1]Fewer than 20 children have been enrolled in the Expanded Access Program.

4.0 Safety and efficacy in antiretroviral-naive adults

4.1 Clinical trial 3001

Design

This study was a randomized, double-blind, multicenter study at 26 North American sites to evaluate the efficacy, safety, quality of life and pharmacokinetics of lamivudine monotherapy, zidovudine, and low and high dose lamivudine/zidovudine combination therapy in HIV-1 infected patients $\geq$ 12 years of age. Eligible patients were ZDV naive ($\leq$ 4 weeks) with CD4 counts ranging from 200 to 500 cells/mm^3. Exclusion criteria included specified laboratory abnormalities, prior ZDV-toxicity resulting in drug discontinuation, previous antiretroviral therapy other than ZDV lasting less than four weeks, and others. The primary surrogate marker endpoints were CD4 cell count and $\log_{10}$ HIV-1 RNA by PCR. Changes in both markers were summarized as average changes from baseline using the DAVGT metric. Secondary surrogate endpoints included CD4 percent, β_2- microglobulin, neopterin, and ICD p24 antigen. Quality of life was assessed using the MOS-HIV Health Status Questionnaire as determined by changes in scores between baseline and the last observed patient questionnaire.

This study was initiated in June 1993 and the study report reflects data collected through October 28, 1994. Surrogate marker analyses were conducted when the last patient randomized completed 24 weeks, however patients were given the option of continuing blinded therapy until the last patient completed 48 weeks of therapy. Once the blinded period was completed, patients were offered the option of receiving open-label lamivudine in combination with zidovudine. Study visits occurred at weeks 2 and 4, and every 4 weeks thereafter until either week 52 or withdrawal from the study.

Study Population

A total of 366 patients were assigned to receive study drug. Across the four treatment groups, the median age was 34 years and the majority of patients were male (87%, range: 83-92%) and Caucasian (61%, range: 59-64%). The median baseline CD4 count ranged from 332-372 cells/mm^3, at least two-thirds of patients in each group had asymptomatic HIV-infection and 15% of the overall population had received prior ZDV therapy. There were no significant differences between treatment groups with respect to demographics, HIV-risk factors, stage of disease or prior ZDV-experience.

> **Medical reviewer's comment: The treatment groups appeared to be well-balanced at baseline with regard to the important demographic and disease characteristics.**

Withdrawals and compliance

Of the 366 randomized patients, the 275 patients (75%) who completed 24 weeks on study drug were evenly distributed between treatment groups. The reasons for discontinuation in the 91 patients included: 29 patients with an adverse event, 41 patients who failed to return and 21 patients with other reasons (such as patient choice, investigator discretion and non-compliance). In the majority of cases, patients who had discontinued drug were also lost to

CD4 follow-up.

Medical reviewer's comments:

1. Of the four controlled trials, 3001 had the highest rate of loss to follow-up. The reason for the higher rate is unclear and larger sites with particularly high rates of withdrawals were chosen for audit by

2. The issue of whether loss to follow-up was differential between treatment groups and may have biased the treatment effect was addressed by Dr. Flyer. His analyses suggest that loss to follow-up did not significantly affect the magnitude of the treatment effect between groups. However, in all treatment groups, those who had less than 24 weeks of follow-up had lower average CD4 changes from baseline, suggesting that patients who failed to experience an initial increase in CD4 count may have been more likely to discontinue drug than those in whom an increase was achieved. This latter conclusion is supported by the observation that rates of drug discontinuation appeared to be higher within the first four weeks of the study when compared to the rest of the study period.

3. Reasons for drug discontinuation appeared to be evenly distributed between treatment groups except for a slight predominance of adverse events reported in the ZDV group.

Efficacy analyses

All efficacy results refer to the intent-to-treat population, which included all randomized patients with confirmed HIV-1 infection, regardless of whether or not drug was actually taken.

There was a statistically significantly greater CD4 increase from baseline over 24 weeks in both ZDV+LAM combination therapy groups compared to the ZDV group (Table 2). Similarly, a statistically significant decrease in log10 HIV RNA over 24 weeks was noted in all three lamivudine-containing groups compared to the ZDV group. In addition, log10 HIV RNA changes were significantly greater in the both LAM combination groups compared to the LAM monotherapy group but there were no significant differences in log10 HIV RNA changes between the two combination groups.

Changes in HIV RNA from baseline were expressed for both the entire study population and for the subset of patients with at least 20,000 copies/ml at randomization as follows: (1) mean change for $\log_{10}$ HIV RNA, (2) median percent change from baseline and (3) median actual change. Examination of graphical displays of changes during the first 24 weeks indicates overall consistency of results despite different scales. In all three methods, the greatest suppression of mean HIV RNA levels occurred within 4 weeks of initiation of therapy; following this time levels of HIV RNA rose and reached a plateau by eight weeks. Mean HIV RNA levels did not rise above baseline in any of the four treatment groups.

Table 2. Summary of 1) AVGT Time-weighted 24-Week Analysis of Surrogate Markers - 3001

Change from baseline	ZDV	LAM (300 mg)	ZDV+LAM (150 mg)	ZDV+LAM (300mg)
Total randomized	93	87	92	94
CD4 cell count				
n	89	84	86	89
Mean	14.94	20.42	49.12	35.07
p-value*	-	0.313	0.001**	0.044
Log10 HIV RNA				
n	86	76	83	92
Mean	-0.32	-0.59	-1.02	-1.07
p-value*	-	<0.001**	<0.001**	<0.001**

*p-value for comparison to ZDV
**indicates statistical significance at 0.05 after adjustment using Bonferroni method.

Twenty-four week analyses of changes in secondary surrogate endpoints demonstrated statistically significant differences in lamivudine combination groups relative to the ZDV monotherapy group for the following markers: percent CD4 cell count (both combination groups), ICD p24 antigen (ZDV+LAM 150 mg group), and β_2 microglobulin and neopterin (ZDV+LAM 300 mg group).

No effect on patient weight or quality of life was demonstrated between treatment groups.

Safety

All safety results refer to the as-treated population, which included all patients with confirmed HIV-1 infection who were randomized, excluding data collected post 30 days permanent study drug discontinuation.

The two doses of lamivudine in combination with zidovudine were generally well tolerated and the overall frequencies and types of adverse events were similar between all four treatment groups (range 92-98%). Three patients died during the study period and the cause of death in these patients did not appear to be study drug related. Serious adverse events were reported in 37 patients (10%) and were distributed evenly between treatment groups. The majority of serious events appeared to be related to manifestations of HIV disease or to trauma. Adverse events led to withdrawal in 33 patients (9%). Withdrawal due to adverse events was highest in the ZDV groups (12%) compared to the other treatment groups (8-9%).

Gastrointestinal symptoms, including nausea and diarrhea were the most frequently reported adverse events overall. Neuropathy occurred in from 7 to 20% of all patients and occurred significantly more frequently in the two monotherapy groups than in either of the combination therapy groups. Other adverse events appeared to be reported in similar frequencies between treatment groups.

Declines from baseline values of hemoglobin, hematocrit and RBC count were observed more frequently in all three ZDV-containing groups than in the LAM monotherapy group. Two patients (one each from the ZDV and the ZDV+LAM 150mg groups) were withdrawn due to anemia. Declines in WBC and neutrophil counts were observed in from 22-33% and from 27-43% of all patients, respectively, and were most frequently observed in patients receiving ZDV+LAM 300 mg. Two patients (one each from the ZDV and the ZDV+LAM 300mg groups) were withdrawn due to neutropenia.

4.2 Clinical tria. 3001

Design

This study was a randomized, double-blind, multicenter study at 14 European sites to evaluate the efficacy and safety of 24 weeks of lamivudine 300mg b.i.d. in combination with zidovudine 200mg t.i.d. compared to zidovudine alone in HIV-1 infected patients $\geq$ 18 years of age. Eligible patients were ZDV naive ($\leq$ 4 weeks) with CD4 counts ranging from 100 to 400 cells/mm^3. Exclusion criteria included specified laboratory abnormalities, prior ZDV-toxicity resulting in drug discontinuation, and any prior anti-HIV therapy other than ZDV or ZDV lasting >four weeks, and others. The primary surrogate marker endpoints were CD4 cell count and $\log_{10}$ HIV-1 RNA by PCR (both analyzed as average changes from baseline using the DAVGT metric). Viral load measurements, including HIV RNA, were performed on a *nonrandom subset of patients*. Secondary surrogate endpoints included CD4 percent, β_2-microglobulin, neopterin, and ICD p24 antigen.

This study was conducted between June 4, 1993 and April 27, 1994. Surrogate marker analysis was conducted when the last patient randomized completed 24 weeks. After completing 24 weeks of blinded therapy, patients were offered open label lamivudine until all patients completed the 24 week blinded treatment period. Study visits occurred at weeks 2 and 4, and every 4 weeks thereafter until either week 24 or withdrawal from the study.

Study Population

A total of 129 patients were assigned to receive study drug. The median ages were 35 years and 32 years in the monotherapy and combination therapy groups, respectively and the majority of patients were male (75% and 72%, respectively) and Caucasian (80% and 85%, respectively). Seven patients (11%) in the monotherapy group had received prior ZDV therapy compared to none in the combination group (p=0.006). Median CD4 count was 248 and 263 cells/mm^3, respectively, in the two groups. Sixty four percent of the enrolled patients were asymptomatic and 9% were classified as having AIDS on entry. There were no significant differences between treatment groups with respect to demographics, HIV-risk factors or stage of disease.

Medical reviewer's comment: With the exception of prior ZDV exposure, the treatment groups appeared to be well-balanced at baseline with regard to the important demographic and disease characteristics.

Withdrawals and compliance

Overall, 88% of randomized patients completed the 24 week treatment period, with no difference in the rate of withdrawal between groups. Of the 16 patients (12%) who were withdrawn from the study, seven withdrew due to an adverse event, five patients failed to return, and four others were withdrawn due to their or their investigator's discretion or due to noncompliance. Of the remaining 113 patients who completed the 24 week randomized period, 110 (96%) entered the open label phase.

Medical reviewer's comment: The overall rate of withdrawal from study was considerably lower in this study than in the North American ZDV-naive study (NUCA3001). The reason for improved retention does not appear to be due to protocol-specified differences and likely reflects cultural differences and the availability of other treatment options.

Efficacy analyses

In both treatment groups, an increase in mean CD4 change from baseline was observed by the two week post-treatment visit. At each treatment visit, mean CD4 changes were greater in the combination group compared to the ZDV group. In the combination group, the mean CD4 change was sustained from weeks two (70.8 cells) through week 24 (77.5 cells) however in the zidovudine group the mean CD4 changes declined after week four (33.6 cells) and was below baseline at week 24 (-9.1 cells). There was a statically significant difference in mean 24 week DAVGT between the treatment groups (Table 3) in favor of combination therapy.

HIV-1 RNA PCR was performed for a subset of patients (enrolled in French centers, n=66) using the Tedder method[2]. Of these 66 patients, 51 had detectable levels of virus at baseline. Mean change from baseline in $\log_{10}$ HIV RNA was greatest for both treatment groups within two weeks after treatment initiation (-0.44 and -0.97 for the monotherapy and combination therapy groups, respectively). In the monotherapy group, mean HIV RNA levels approached baseline levels by weeks 8-12. In the combination group, the mean change in HIV RNA was greater than in the monotherapy group and remained below baseline through week 24 (-0.31). There was a statistically significant difference in mean 24 week DAVGT HIV RNA between the treatment groups in favor of combination therapy.

Table 3. Summary of DAVGT Time-weighted 24-Week Analysis of Surrogate Markers-NUCB3001

Change from baseline	ZDV	ZDV+LAM (300mg)
Total randomized	65	64
CD4 cell count		
n	64	64
Mean	15.44	70.13
p-value*	-	<0.001**
Log10 HIV RNA (Tedder method)		
n	33	32
Mean	-0.15	-0.59
p-value*	-	0.007**

[2]For all other patients, samples were assayed using the Roche Technique at the laboratory of Dr. Brendan Larder. DAVGT results were submitted as an amendment to the NDA and are included in Table 3.

Log10 HIV RNA (Breden Larder method)		
n	31	31
Mean	-0.48	-1.22
P-value*	-	<0.001**

***p-value for comparison to ZDV**

****indicates statistical significance at 0.05 after adjustment using Bonferroni method.**

Analyses of changes in secondary surrogate marker endpoints (including rises in percent CD4, declines in cellular viremia in a subset of 48 patients, declines in p24 antigen, and declines in beta-2-microglobulin and neopterin) supported the primary analyses. There was no evidence of a difference between treatment groups in clinical parameters, which included changes in weight, changes in Karnovsky score, or the occurrence of CDC class B and C illnesses during the 24 week analysis period.

Safety

All safety results refer to the as-treated population, which included all randomized patients with confirmed HIV-1 infection, excluding data collected post 30 days permanent study drug discontinuation.

Lamivudine in combination with zidovudine was generally well tolerated and the overall frequencies and types of adverse events were similar between treatment groups. The incidence of reporting any adverse event was similar across the two treatment groups (95% and 85% in the monotherapy and combination therapy groups, respectively). The majority of adverse events were attributed to the patients' underlying disease. Gastrointestinal symptoms, including nausea and diarrhea were the most frequently reported adverse events overall.

No deaths were reported during the conduct of this study. Seven of the nine patients who reported serious adverse events received lamivudine combination therapy. Five patients (8%) receiving ZDV compared with nine patients (14%) receiving combination treatment experienced at least one grade III or IV clinical event during the study. Anemia was the most frequently reported grade III or IV toxicity. The frequency of grades III or IV laboratory toxicities was similar between the ZDV and combination groups (20% vs. 26%).

Adverse events led to withdrawal in seven patients (5.4%). Withdrawal due to adverse events was higher in the combination group (8%) than in the monotherapy group (3%). Dose reduction was frequently necessary in both groups; 39% and 49% of the monotherapy and combination therapy groups, respectively, required at least one dose reduction during the 24 week period.

4.3 Reviewer's assessment of safety and efficacy in antiretroviral-naive adults

Trials A3001 and B3001 support the safety and efficacy of lamivudine in combination with zidovudine as a treatment for HIV-infection in antiretroviral naive patients.

Evidence of efficacy is primarily derived from the 24 week analyses of CD4 data, which indicates that on average patients receiving combination therapy experienced a higher and more sustained CD4 response than ZDV-monotherapy treated patients. These results suggest that the CD4 response with combination therapy may be sustained beyond 24 weeks. However, evaluation of the treatment effect beyond 24 weeks in problematic because (1) only 54% of the population was still being followed at 52 weeks in 3001 and (2) patients in 3001 were unblinded after 24 weeks.

The two studies together indicate that higher doses of lamivudine (300 mg b.i.d) in combination with zidovudine does not offer an advantage over the lower dose regimen of lamivudine 150 mg b.i.d. in combination with zidovudine. Additionally, in a single study lamivudine monotherapy was not superior to zidovudine and was not as efficacious as lamivudine in combination with zidovudine.

Data on changes in HIV RNA by PCR provide supportive evidence that lamivudine's antiviral effect in naive patients is greater when given in combination with ZDV. The peak effect on HIV RNA occurred within weeks of treatment initiation and appeared to lessen and plateau by 8 weeks. Due to limitations in our understanding of changes in plasma viremia, the clinical importance of modest differences in response are unknown. Specifically, while changes in the HIV RNA levels between combination and ZDV treatment groups were statistically significant, it is not known whether the magnitude of this difference is indicative of a clinical benefit.

Because use of zidovudine is associated with several well-established adverse events and because the study populations were at risk for development of HIV-associated events, characterization of lamivudine's safety profile is problematic. Despite this limitation, the proposed combination appeared to be generally well tolerated and the types and frequencies of adverse events did not differ markedly from patients treated with ZDV alone. Further, there were no clinically significant differences in the frequencies of adverse events reported between the two doses of lamivudine.

In conclusion, lamivudine in combination with zidovudine appears to be a reasonable initial therapy for treatment naive patients based on its sustained effect on surrogate endpoints and its lack of dose-limiting toxicity beyond that experienced with zidovudine. There are several qualifications to this statement, however. First and most importantly, combination therapy's impact on clinical parameters (progression and death) has not been demonstrated. Secondly, the safety of longterm use of lamivudine has not been established. Third, there is no data that compares the surrogate marker response of lamivudine in combination with zidovudine in naive patients with other treatment regimens of potential importance (such as ddI, ZDV + ddI and ZDV + ddC). The first two qualifications have been addressed in the current package insert and through the ongoing clinical confirmatory program. The issue of comparisons to other regimens with proven clinical benefit in naive patients will be addressed as soon as results of these trials are

reviewed.

5.0 Safety and efficacy in antiretroviral-experienced adults

5.1 Clinical tria. 3002

Design

This study was a randomized, ddC double-blind (ZDV open label), multicenter study at 21 North American sites to evaluate the efficacy, safety, quality of life and pharmacokinetics of low and high dose lamivudine/zidovudine combination therapy with ddC/ZDV combination therapy in HIV-1 infected patients $\geq$ 18 years of age. Eligible patients were ZDV experienced ($\geq$ 24 weeks) with CD4 counts ranging from 100 to 300 cells/mm^3. Exclusion criteria included specified laboratory abnormalities and prior ZDV-toxicity resulting in drug discontinuation and others. The primary surrogate marker endpoints were CD4 cell count and $\log_{10}$ HIV-1 RNA by PCR (both analyzed as average changes from baseline using the DAVGT metric). Secondary surrogate endpoints included CD4 percent, β_2- microglobulin, neopterin, and ICD p24 antigen. Quality of life was assessed using the MOS-HIV Health Status Questionnaire and changes in scores between baseline and the last observed patient questionnaire were determined.

This study was initiated in June 1993 and its study report reflects data collected through November 9, 1994. The evaluation period was 24 weeks for purposes of surrogate marker analyses, however patients were given the option of continuing to receive blinded therapy until the last patient completed the 24 week period. Once the blinded period was completed, patients were offered the option of receiving open-label lamivudine and zidovudine. Interim study visits occurred at weeks 2 and 4, and every 4 weeks thereafter until either week 52 or withdrawal from the study.

Study population

A total of 254 patients were assigned to receive study drug: 86 patients were randomized to the ZDV/ddC group, 84 patients to the ZDV/LAM 150 mg group and 84 patients to the ZDV/LAM 300 mg group Across the three treatment groups, the median age was 37 years and the majority of patients were male (83%) and Caucasian (63%). The median baseline CD4 count ranged from 206-221 cells/mm^3 and 58% of patients overall had asymptomatic HIV-infection. Median durations of prior antiretroviral therapy ranged from 510 to 689 days across treatment groups. There were no significant differences between treatment groups with respect to demographics, HIV-risk factors, stage of disease or prior ZDV-experience.

> **Medical reviewer's comment: The treatment groups appeared to be well-balanced at baseline with regard to the important demographic and disease characteristics.**

Withdrawals and compliance

A total of 57 (22%) patients discontinued study drug within the 24-week analysis period. Rates of drug discontinuation were 17%, 22.6% and 27.4%, respectively in the ZDV+ddC, ZDV+LAM150mg and the ZDV+LAM300mg groups, respectively. Within the first two weeks after randomization, 17 patients (7%) discontinued study drug. Of the 57 patients who

discontinued study drug, the majority were also lost to surrogate marker result follow-up.

The primary reason for drug discontinuation was adverse events, which occurred in 10.5%, 9.5% and 15.5% of the treatment groups, respectively.

> **Medical reviewer's comment: The high rate of withdrawal is similar to that observed in the other North American study 3001). In addition to study withdrawal there is a high frequency of missing efficacy data. Across the three treatment groups 35%-44% of patients had one or more missing CD4 values. For any given week, there was highest frequency of missing data for the week 24 visit, wherein 17%-27% of patients had a missing value.**

Efficacy analyses

Increases in mean CD4 change from baseline were evident in both lamivudine combination treatment groups beginning at the first two week post-treatment visit. The rise in mean CD4 counts at eight weeks was 32.5 and 54.2 cells for the lamivudine 150 mg and 300 mg combination groups, respectively. By 24 weeks, the mean rise from baseline was 30.9 and 15.4 cells, respectively. In comparison, mean CD4 counts remained at slightly above or slightly below the baseline for patients in the ZDV+ddC group throughout the 24 week period. There was a statically significant difference in mean 24 week DAVGT between the treatment groups (Table 4) in favor of the two lamivudine combination groups.

Mean change from randomization in $\log_{10}$ HIV RNA was greatest for both lamivudine treatment groups within two weeks after treatment initiation (-0.1.38 and -1.43 for the 150 mg and 300 mg lamivudine combination groups, respectively) and levels of HIV RNA increase thereafter but remained below baseline throughout the treatment period. In the ddC combination group, mean HIV RNA levels were lowest within 2-4 weeks following randomization and thereafter closely approximately levels seen in patients treated with lamivudine. There was a statistically significant difference in mean 24 week DAVGT HIV RNA between the higher dose lamivudine group and the ddC group, only.

> **Medical reviewer's comment: Although there was a statistically significant difference in DAVGT HIV RNA between the higher dose lamivudine and the ddC groups, this difference appears to have been due to differences during the first eight weeks only. Overall, levels of HIV RNA with time for each of the three treatment groups were similar except for the previously noted early differences.**

Table 4. Summary of DAVGT Time-weighted 24-Week Analysis of Surrogate Markers - NUCA3002

Change from baseline	ZDV+ddC	ZDV+LAM (150 mg)	ZDV+LAM (300mg)
Total randomized	86	84	84

CD4 cell count (cells/mm^3)			
n	**81**	**81**	**80**
Mean	**-2.01**	**32.47**	**34.58**
p-value*	**-**	**<0.001****	**<0.001****
Log10 HIV RNA (copies/mL)			
n	**80**	**80**	**75**
Mean	**-0.62**	**-0.78**	**-0.86**
p-value*	**-**	**0.016****	**0.003****

***p-value for comparison to ZDV+ddC**
****indicates statistical significance at 0.05 after adjustment using Bonferroni method**

In analyses of secondary endpoints, there was a statistically significant difference in %CD4 count between the ZDV+LAM150mg and ZDV+ddC groups, and in β_2-microglobulin and neopterin levels between the ZDV+LAM300 mg and ZDV+ddC groups. There were no differences between treatment groups in Karnovsky scores or weight change.

Safety

More than 95% of patients in each of the three treatment groups experienced adverse events. No patients died within the study period. A total of 47 patients (19%) experienced serious adverse events. The proportion of patients who experienced a serious adverse event was highest in the ZDV+LAM300mg group (25%) compared to the ZDV+LAM150mg group (13%) and the ZDV+ddC group (17%). There were no statistically significant differences across groups in the number of patients experiences serious adverse events in any body system category.

A total of 42 patients (17%) were withdrawn due to an adverse event. The proportion of patients who withdrew due to an adverse event was highest in the ZDV+LAM300mg group (21%) compared to the ZDV+LAM150mg group (11%) and the ZDV+ddC group (17%). Neurological events accounted for 15 withdrawals with neuropathy been the reason most frequently in the ddC combination group.

5.2 Clinical trial '3002

Design

This study was a randomized, lamivudine double-blind (ZDV open label), multicenter study at 32 European sites to evaluate the efficacy and safety of low and high dose lamivudine/zidovudine combination therapy compared with ZDV monotherapy in HIV-1 infected patients $\geq$ 18 years of age. Eligible patients were ZDV experienced ($\geq$ 24 weeks) with CD4 counts ranging from 100 to 400 cells/mm^3. Exclusion criteria included specified laboratory abnormalities, prior ZDV-toxicity resulting in drug discontinuation, > 4 weeks or ddI therapy, prior anti-HIV therapy with agents other than ZDV or ddI, and others. The primary surrogate marker endpoints were CD4 cell count changes and changes in $\log_{10}$ HIV-1 RNA by PCR (for a subset of patients). For both surrogate markers, changes were summarized using the DAVGT metric. Secondary surrogate endpoints included CD4 percent, β_2- microglobulin, neopterin, and ICD p24 antigen.

This study was initiated in August 3, 1993 and its study report reflects data collected through September 13, 1994. Surrogate marker analyses were conducted when the last patient randomized completed 24 weeks of blinded treatment. After 24 weeks, patients were offered open-label lamivudine and zidovudine until the last patient completed the 24 week period. Study visits occurred at weeks 2 and 4, and every 4 weeks thereafter until either week 52 or withdrawal from the study.

Study population

A total of 223 patients were assigned to receive study drug: 73 patients were randomized to the ZDV group, 75 patients to the ZDV/LAM 150 mg group and 75 patients to the ZDV/LAM 300 mg group. Across the three treatment groups, the median age ranged from 33.8 to 36.5 years and the majority of patients were male (77-88%) and Caucasian (>95%). The median baseline CD4 count ranged from 247-253 cells/mm^3 and 46.6-57.3% of patients overall had asymptomatic HIV-infection. Median durations of prior antiretroviral therapy ranged from 617-748 days across treatment groups. There were no significant differences between treatment groups with respect to demographics, HIV-risk factors, stage of disease or prior ZDV-experience.

- **Medical reviewer's comment: The treatment groups appeared to be well-balanc at baseline with regard to the important demographic and disease characteristics.**

Withdrawals and compliance

Completion of 24 weeks on study drug was lowest in the ZDV group (81%) and highest in the two combination groups (93% and 89% in the 3TC 150 mg and 300 mg groups respectively). Reasons for discontinuation included adverse events, failure to return and CD4 decline and were each reported more frequently in the ZDV group. In general, patients who discontinued study drug were also lost to CD4 analysis in each of the three groups.

Efficacy analyses

In the ZDV group, no increase in mean CD4 was demonstrated throughout the 24 week treatment period. In comparison, a mean increase in CD4 was found in both combination groups, which was greatest at week two (45.8 and 47.5 cells for the 150 mg and 300 mg groups, respectively) and which declined slightly and remained above baseline throughout the treatment period. As shown in table 5, the DAVGT 24-week analysis of CD4 was significantly higher in both combination groups than in the ZDV group.

HIV RNA levels were determined for a subset of patients using three different methods.

Table 5. Summary of DAVGT Time-weighted 24-Week Analysis of Surrogate Markers 3002

Change from baseline	ZDV	ZDV+LAM (150 mg)	ZDV+LAM (300mg)
Total randomized	73	75	75
CD4 cell count (cells/mm^3)			
n	72	75	72
Mean	-16.87	35.28	32.56
p-value*	-	<0.001**	<0.001**
Log10 HIV RNA (copies/mL) Tedder assay			
n	12	8	8
Mean	-0.02	-0.25	-0.30
p-value*	-	0.56	0.23
Log10 HIV RNA (copies/mL) Brenden-Larder assay			
n	16	16	15
Mean	0.07	0.71	-0.60
p-value*	-	<0.001**	<0.001**
Log10 HIV RNA (copies/mL) NASBA assay			
n	8	12	12
Mean	-0.12	-0.59	-0.81
p-value*	-	0.002**	0.007**

**p-value for comparison to ZDV group

** indicates statistical significance at 0.05 after adjustment using Bonferroni method.

Safety analyses

Death

Serious adverse events

Withdrawals due to adverse events

All adverse events

Laboratory abnormalities

5.3 Reviewer's assessment of safety and efficacy in antiretroviral-experienced adults

6. Uncontrolled studies in pediatric patients

There is no information from controlled clinical trials on the use of lamivudine in combination with zidovudine in children. Limited information on the safety of lamivudine in pediatric patients is derived from two ongoing, open-label studies that have enrolled a total of 144 pediatric patients. Of the 144 pediatric patients, 97 have been enrolled in a study of lamivudine monotherapy 2002) and 47 have been enrolled in a study of combination therapy 2005). Of the 47, only nine have been treated with lamivudine in combination with zidovudine.

2002

The objective of this ongoing open-label, uncontrolled study is to determine safety, preliminary activity and the pharmacokinetic profile of lamivudine monotherapy in 97 HIV-infected children aged from 3 months to 17 years. The study was initiated on April 7, 1992 and the study report includes data collected through November 1, 1994. The study enrolled 18 antiretroviral-naive (arm A), 71 antiretroviral experienced (arm B) and eight "compassionate" treatment (arm C) patients. Eligibility was determined as follows: asymptomatic or mildly symptomatic HIV-positive children and an age-corrected CD4 count rendering them at risk for an opportunistic infection (arm A); intolerance or refractory to prior antiretroviral therapy and P-2 class symptomatic HIV infection and/or an age-corrected CD4 count below the age-corrected specified values (arm B); active opportunistic infection (arm C). Treatment consisted of a single intravenous dose of lamivudine, followed by oral lamivudine dosed initially at 1.0 mg/kg/day and escalated to 4, 12 and 20 mg/kg/day in subsequent dose groups. Starting in October 1992, patients at the lower dose had the option to receive an intermediate dose of 8mg/kg/day. After 24 weeks of treatment, patients receiving the 20 mg/kg/day dose had a dose reduction to 8 mg/kg/day. In addition to pharmacokinetic analyses of lamivudine concentrations in the blood, urine and CSF, the following efficacy parameters were assessed: CD4, serum p24 antigen, neopterin, β2-microglobulin, HIV RNA by PCR, weight, growth and neurocognitive function.

2005

2005 is an open-label trial to evaluate pharmacokinetic interactions, toxicity and tolerability of varying combinations of lamivudine, ZDV and ddI. The study is conducted at two sites and enrolls children age 3 months to 18 years. Children who had received antiretroviral therapy for less than 6 weeks were randomized to one of two triple-drug regimens (n=7); those who had failed antiretroviral therapy due to intolerance or progression (n=40) were assigned to receive one of three possible combinations of lamivudine, ZDV and ddI. Treatment assignments were based on prior history of antiretroviral use/tolerance and disease progression. Of the 47 children, nine received lamivudine in combination with zidovudine.

Medical Reviewer's Comment: The pediatric studies contained in this application were reviewed primarily as a source of safety data because the studies were uncontrolled and because very few children (n=9) received lamivudine in combination with zidovudine. Because of the advanced nature of HIV disease in the

patient populations and because of the uncontrolled study designs, interpretation of safety data from these studies is also problematic.

Safety

Pancreatitis in children receiving lamivudine was the most noteworthy safety issue in these studies. Pancreatitis was reported in 14/97 (14%) and 7/44 (15%) of children enrolled in 2002 an 2005, respectively (see appendix 1 for a further discussion of features of these cases). The 21 patients who developed pancreatitis ranged in age from 2 to 17 years (with a median age of 12 years, which is slightly higher than the median age for either of the two studies). Duration of exposure prior to pancreatitis was highly variable and ranged from 8 to 96 weeks.

All of the children had advanced HIV disease and several had concurrent HIV-associated illnesses such as MAC bacteremia or cryptosporidiosis and many of the children, in particular the adolescents, had lower than ideal body weights. Although prior history is not available for all patients, it is notable that a prior history of pancreatitis was reported in four patients. In addition, concomitant medications may have been an additional risk factors in others, particularly for those who received pentamidine, ddI, or ZDV+ddI.

Other notable adverse experiences in 2002 included peripheral neuropathies and/or paresthesia in 13 patients (13%) and neutropenia in eleven patients (11%). The majority of these cases occurred while patients were receiving the 8mg/kg/day dose. Neuropathy was the reason for withdrawal in three patients and neutropenia resulted in withdrawal in three and dosing interruption in two patients. The frequencies of other adverse events that have been previously observed with other nucleoside analogues are as follows: elevated liver function tests (15%) and anemia (26%). For each adverse event, there was no apparent relationship with lamivudine dose.

Medical reviewer's comment: The incidence of pancreatitis in children (15%) was notably higher than in adult controlled trials (<0.5%). The reason for this difference is not known however differences in baseline disease severity may explain this observation in part. Because most of the children had advanced HIV disease and other risk factors for pancreatitis (including concomitant medications), no definitive conclusions can be reached about lamivudine's association with pancreatitis. However, the high frequency of pancreatitis is disturbing and warrants further investigator to define the safety profile of lamivudine use in children. In addition, the package insert will contain a warning that reflects this information and that urges extreme caution when administering lamivudine in combination with zidovudine to children with a history or other risk factors for pancreatitis.

7. **Expanded Access**

The expanded access program includes three open-label trials in the U.S. and Canada 3004), Europe 3004) and the United Kingdom 3003). In 3004, children and adults with progressive, symptomatic HIV disease and CD4 counts ≤300 cells/mm^3 who were unable to participate in controlled trials because of disease progression or because of intolerance or unresponsiveness to other therapies were randomized to lamivudine at a dose of either 150 mg or 300 mg twice daily (or 8mg/kg/day for children < 12 years of age). In 3004 and 3003, children and adults with the same entry criteria as in NUCA3004 were given twice-daily doses of lamivudine 300 mg (or 8mg/kg/day for children < 12 years of age).

The original NDA submission included information on death and other serious adverse events on 8,815 patients enrolled in the expanded access program as of January 10, 1995. In an amendment dated August 30, 1995, updated information on the following rates was included: 1) death and other serious adverse events on 23,622 persons through June 15, 1995, 2) non-serious adverse events on 10,685 persons through July 12, 1995, and 3) clinical laboratory abnormalities on 7,328 persons through April 1, 1995.

The numbers of persons on whom there is information for each of the above types of adverse events varies depending on how the data are collected. Investigators were instructed to report deaths or serious adverse events immediately by phone and therefore, the denominator for these rates is the entire expanded access population as of the cut-off date. Information on non-serious adverse events is abstracted when case report forms are submitted. however the frequency of case report form submission is variable between investigators. Similarly, information about the rates of laboratory abnormalities is abstracted when laboratory CRFs are submitted.

Among the 23,622 subjects enrolled as of June 15, 1995, 9235 and 14,387 subjects received lamivudine 150 mg and 300 mg, respectively. In the subset of patients for whom case reports have been submitted (n=10,685), the mean age was 40.9 years and the majority were male (95%). Less than one percent of subjects were under 12 years of age. Ethnicity and CD4 cell count were not reported.

In spontaneous telephone reports, death was reported in 2% and 3% of the lamivudine 150mg and 300mg groups, respectively, and withdrawal due to serious adverse events was reported in 1% of each group. None of the deaths were judged by the investigator to be attributable to lamivudine. Serious adverse events were reported for 3% and 4% of the lamivudine 150mg and 300mg treatment groups, respectively. The types of serious adverse events that were reported were generally similar to those reported in controlled clinical trials of lamivudine. The majority of events appeared to be related to complications of advanced HIV-infection and to have occurred in low frequencies. A few notable serious adverse events include the following: blood and lymphatic abnormalities (predominately decreased WBC and anemia) were reported in 151 patients (<1%); serious pancreatitis was reported in 48 subjects (0.2%).

Non-serious adverse event CRFs were submitted in 10,685 patients (42%). The mean baseline CD4 was 94.9 cells/mm3. At least one non-serious adverse event was reported in 33% and 40% of the lamivudine 150mg and 300mg groups, respectively. Most of the adverse events appeared to be related to complications of advanced HIV-infection and to have occurred in low frequencies. A few notable exceptions include the following: neuropathy reported in 6% of each group and blood and lymphatic abnormalities reported in 6% of each group (including predominately decreased WBC and anemia). Non-serious pancreatitis, which was observed in 11% of lamivudine-treated children, was reported in 64 (<1%) of lamivudine-recipients in the expanded access programs.

Medical Reviewer's Comment: While the expanded access program was successful in providing drug to a large number of patients in need for therapy, the value of the data generated is limited in several important respects.
1) First is the issue of length of exposure. Of the total enrollment of 35,267 as of October 4, 1995, a figure which was reported to FDA subsequent to the safety update, the majority have been enrolled since Dec. 1994. In fact between February 1 through September 30, 1995, over 24,000 of the total 35,267 were enrolled. Therefore, the majority of experience in the Expanded Access program represents shorter term use of lamivudine. This is an important limitation when considering lamivudine in combination with zidovudine as initial therapy for patients at early stages of disease.
2) Second is the issue of under-reporting. Interpretation of the Expanded Access data is limited by certain aspects of how the data are collected. Demographic information is not available for the majority of enrollees because receipt of drug was not dependent on return of the baseline demographic form. Further, adverse event and withdrawal case report forms have not been returned for the majority of enrollees and information on serious adverse events and deaths is obtained from investigator-initiated telephone reports. The completeness of this passive method of data collection for serious events has not been verified by audit. Therefore, as one would expect from data collected in this manner, the rates of serious adverse events are substantially lower in the expanded access program than in the controlled trials.

- **Because the types of adverse events were similar in the adult controlled trials and in the expanded access program, it can be reasonably concluded that lamivudine's safety profile during shorter term administration in adults has been reasonably well characterized pre-marketing. However, as noted, the expanded access program does not contribute reliable information about: the rates of these events in adults, the safety of longer-term lamivudine exposure in adults, nor about the safety of lamivudine use in children.**

8. Reviewer's assessment

In support of safety and efficacy of lamivudine in combination with zidovudine in adults, the applicant has submitted the results of four adequate and well controlled surrogate endpoint studies and safety experience from the expanded access program. In support of use in pediatrics, the applicant has submitted safety and pharmacokinetic experience from two open-label , uncontrolled studies in children.

The four controlled adult trials provide adequate evidence that lamivudine when given in combination with zidovudine results in an increase in CD4 cell count through 24 weeks of approximately 50 cells in antiretroviral naive and 30 cells in antiretroviral experienced adults. The results of the North American trials suggest that this effect may be sustained in some individuals beyond 24 weeks however data beyond 24 weeks is less compelling because of substantial loss to follow-up after 24 weeks.

Analyses of changes in plasma viremia provided supportive evidence of efficacy in three respects. First, consistent patterns in mean changes in CD4 and HIV RNA levels provide reassurance that the observed mean CD4 changes are due to an antiviral effect. Second, the HIV RNA analyses suggest that the antiviral effect of lamivudine in combination with zidovudine is sustained over the 24 week treatment period. Third, Dr. Flyer's exploratory analyses suggest that most individuals who have a CD4 response can expect a decrease in plasma viremia, but with the following two qualifications: concordance of responses was not universal and the magnitude of CD4 response did not predict the magnitude of the HIV RNA response.

Lamivudine in combination with zidovudine was generally well tolerated. The frequencies of most clinical and laboratory adverse events were similar between combination therapy and zidovudine monotherapy recipients. The most concerning adverse event was the occurrence of pancreatitis is 15% of children enrolled in two open-label studies. Other notable safety issues include the occurrences of neuropathy and neutropenia. Notwithstanding the limitations of the expanded access data, together the collective experience from controlled trials and expanded access provides a reasonable measure of reassurance about the types and severity of toxicity that may be encountered with lamivudine post-marketing.

Based on these changes in surrogate endpoints, the applicant has request accelerated approval for lamivudine in combination with zidovudine with an indication for patients in whom antiretroviral therapy is warranted based on clinical and/or immunological evidence of disease progression. Because of the broad nature of the proposed indication for lamivudine in combination with zidovudine, this application represents a departure from previous accelerated approval applications, which have intended therapy for populations with progressive disease or those intolerant of all other available therapy. The scope of the regulations extends accelerated approval to those drugs and products used in the treatment of serious or life-threatening diseases, where the products provide meaningful therapeutic

advantage over existing treatment. Examples of "meaningful therapeutic benefit over existing therapies" include the ability to treat unresponsive or intolerant patients or improved response compared to available therapy.

The data in this application is supportive of clinical benefit over existing therapies in the following respects. First, there are consistent findings between each of the 2 studies conducted in similar patient populations. In each of the studies the mean increase in CD4 was greater in lamivudine combination recipients than in control recipients. Also, the changes in CD4 count with combination therapy were replicated at both the 150 mg bid and 300 mg bid lamivudine doses. Second is the issue of durability of response. The four studies suggest that there is a sustained CD4 response to at least 24 weeks. Third, it is reassuring that CD4 response did not appear to be limited to any one subgroup of patients, such as those with a particular baseline CD4 count. Rather, as suggested by Dr. Flyer's analysis, it appears that within the CD4 range studied, a similar CD4 response was achieved regardless of baseline CD4. Fourth, the magnitude of surrogate marker change from baseline was greater in naive than in experienced patients.

Data in this application is insufficient to answer several important questions. Available comparative efficacy information in adults is limited. For treatment naive adults with CD4 > 100 cells, the application contains data comparing zidovudine and LAM in combination to zidovudine alone, and for experienced adults, there are data comparing the combination both to ZDV and ZDV+ddC. Therefore, there is no comparative efficacy data for agents that may be likely to demonstrate clinical benefit, such as ddI , ZDV+ddI and ZDV+ddC in naive patients and ddI and d4T in experienced patients. In the time since these trials were designed, our approach to antiretroviral therapy has evolved. As more choices of antiretroviral agents become available and as our perception of "optimal" therapy evolves, we will be increasingly unable to rank antiretroviral drugs based on data collected during the typical course and timeframe of premarketing drug development. Comparisons to historical data are problematic because of underlying differences in patient populations that may influence the degree of response.

9. Recommendations for regulatory action

This application is approvable. The draft final label and commitments for accelerated approval and phase IV are provided in Appendices 2 and 3, respectively.

DRAFT

Heidi M. Jolson, M.D., M.P.H.
Medical Officer, DAVDP

Concurrences:

Appendix 1. Characteristics of pediatric patients with pancreatitis

Appendix 1. Characteristics of pediatric patients with lamivudine-associated pancreatitis

Patient # Age/Sex Weight	Lamivudine exposure		Prior history of pancreatitis	Prior use of anti-retrovirals	Outcome	Other
	Dose (mg/kg/day)	Duration				
Study NUCA 2002						

Patient # Age/Sex Weight	Lamivudine exposure		Prior history of pancreatitis	Prior use of anti-retrovirals	Outcome	Other
	Dose (mg/kg/day)	Duration				

Patient # Age/Sex Weight	Lamivudine exposure		Prior history of pancreatitis	Prior use of anti-retrovirals	Outcome	Other
	Dose (mg/kg/day)	Duration				

Appendix 2. Draft final lamivudine label

Appendix 3. Accelerated Approval and Phase IV Commitments

NDA 20-564

Medical Officer's Review

"Analysis of the Clinical Significance of Surrogate Marker Changes in the Zidovudine Study"

To support the use of plasma HIV RNA as a surrogate endpoint, the applicant in collaboration with the conducted a retrospective analysis of the 298 study to determine the degree to which changes in plasma HIV RNA could explain the treatment effect that was observed in this study. From the analyses conducted it was concluded that a decline in plasma HIV RNA was a useful marker for antiretroviral drug efficacy and that it predicted from 59% to 104% of the observed treatment effect, depending on the measure of change in HIV RNA that was used in the analysis. Because the analyses were conducted retrospectively, two important factors limit the conclusions that may be drawn for the analyses. These include the relevance of the clinical endpoint that was used as the treatment effect and the integrity of the samples that were used for the HIV RNA determinations. After a brief review of the overall study, these issues will be discussed in more detail.

The 298 Study

298 was a clinical trial comparing early versus late zidovudine for the treatment of patients with symptomatic HIV infection. The design of the trial is shown in Table 1.

Table 1 298

Sponsor	
Location	US
Dates	Part I - January 1987 to January 1991 Part II - January 1991 to January 1994
Design	Randomized, double blind, comparison of early versus late ZDV
Population	Veteran eligible for care at : AIDS-related complex as defined by Walter Reed stages 2-5 (HIV positive, CD4 200 to 500, no history of an OI), subjects with prior AZT therapy excluded.
Stratification	Center and CD4 (200-299 or 300-500)
Dose	zidovudine 250 mg q4h
Primary endpoint	Time to progression from ARC to AIDS or death

Treatment	Early AZT	Late AZT
N	168	170
Stratum 1 (CD4 200-299)	49 (29%)	45 (27%)
Stratum 2 (CD4 300-500)	121 (71%)	123 (73%)
Lost to follow up	9 (5%)	6 (4%)

Treatment Discontinuation	Subjects were to receive open-label AZT if their CD4 cell count dropped below 200 on two consecutive determinations at least six weeks apart
Virology - PBMC co-culture	Baseline (two determinations), at 1, 4, 8, 12 months and every 4 months thereafter

The primary endpoint as defined in the protocol was time to progression to AIDS or death. As shown in Table 2, there was not a statistically significant difference in the primary endpoint between the two treatment groups nor was there a statistically significant difference when death alone was compared between the two treatment groups. If progression to AIDS (censoring on death) was compared, there was a statistically significant difference between the treatment groups. The events

included in this endpoint are listed in Table 2.

Table 2 - Clinical End Points*

	Early	Late	p value	Relative Risk [95% CI]
N	168	170		
AIDS or death	38	48	0.25	1.29 [0.84, 1.97]
Deaths	23	20	0.48	0.81
AIDS related	13	12		[0.44, 1.59]
Non-AIDS related				
HIV progression	6	8		
no progression	4	0		-
Progression to AIDS	28	48	0.02	1.75
KS	6	7		[1.10, 2.80]
Lymphoma	1	6		
PCP	11	15		
Other OI	8	14		
Dementia	0	6		
Wasting	2	0		

*Source N Engl J Med 1992;326:437-43

Validation Analyses for Plasma HIV RNA Changes in 298

Description of the Methodology

To validate the use of plasma HIV RNA as measured by RT-PCR, the method proposed by Freedman (Freedman, 1992; Machado, et.al., 1992) was used. The approach is based on the use of Cox proportional hazard models to determine the ability of the surrogate marker to "explain" the observed treatment effect. This procedure consists of five Cox proportional hazard models.

Model 1	Baseline surrogate marker effects
Model 2	Treatment effect (termed as the unadjusted treatment effect) and baseline surrogate marker effects
Model 3	Surrogate marker effect (based on the metric) and baseline surrogate marker effects
Model 4	Treatment effect (termed as the adjusted treatment effect), surrogate marker effect (based on the metric), and baseline surrogate marker effects
Model 5	Model 4 plus surrogate marker by treatment interaction

In the absence of a significant interaction in Model 5, the coefficients for the unadjusted (Model 2) and adjusted (Model 4) treatment effects are compared to determine the ability of the surrogate marker to explain the observed treatment effect. The result of the comparison is expressed as a percentage of the treatment effect and is calculated as:

percentage of treatment effect explained =
[(Model 2 coefficient-Model 4 coefficient)/Model 2 coefficient]X100

The application of this methodology requires the observation of a clear treatment effect and the ability to appropriately measure changes in the surrogate endpoint. In the analyses presented in the report, the clinical endpoint was progression to AIDS within 365 days of the end of Part I of the study and the measure of change in plasma HIV RNA was the mean change from baseline over 6 months. Using these parameters, change in plasma HIV RNA explained 104% of the observed treatment effect. The conclusions that may be drawn from the analyses are affected by limitations of both the clinical endpoint chosen for the analyses and measurement of plasma HIV RNA that are the result of the retrospective nature of the study. These issues will be discussed in more detail.

Clinical Endpoint - Treatment Effect

Adequate samples for HIV RNA RT-PCR were available for only 270 of the subjects in the study. Because this represented only a subset of the subjects enrolled in the study, the data for these subjects were analyzed to determine if there was a significant treatment effect for this subset. The number of clinical endpoints that occurred in the subjects included in the validation analysis is shown in Table 6. These numbers are based on an intent-to-treat analysis over the total seven years of follow-up. For the primary endpoint of progression to AIDS or death the difference between the treatment groups was not statistically significant. Neither was there a statistically significant difference between the treatment groups for the endpoints of death alone or progression to AIDS (censoring death).

Table 6 - Clinical Endpoints Over Parts I and II

	Early N=129	Late N=141	p value*
AIDS Death	68	74	0.59
Death	53	59	0.78
AIDS	58	73	0.09
ARC	76	98	0.09
ARC AIDS	90	116	0.02
ARC AIDS Death	95	118	0.04

*p-value from log rank test, stratified by baseline CD4
From Table A of the analysis report.

Because of the lack of a significant difference for any of the endpoints examined for the validation subset, the data for these subjects were reanalyzed, censoring their follow-up at 6 month intervals following the end of Part I of the study. The log rank test stratified by baseline CD4 cell count was used for these analyses. The relative risk for AIDS in these 270 subjects at various times is shown in Table 7.

Table 7 - Relative Risk (Early/Late) and p value*

	6 Months	12 Months	18 Months	24 Months	30 Months
AIDS	0.73, 0.17	0.62, 0.03	0.65, 0.04	0.71, 0.08	0.70, 0.06
Death	1.20, 0.43	1.15, 0.85	1.06, 0.85	0.97, 0.87	0.93, 0.73
AIDS Death	0.89, 0.55	0.81, 0.25	0.87, 0.42	0.87, 0.41	0.85, 0.30
ARC	0.81, 0.22	0.81, 0.20	0.80, 0.17	0.80, 0.16	0.77, 0.10
ARC AIDS	0.74, 0.06	0.73, 0.04	0.72, 0.03	0.73, 0.03	0.71, 0.02
ARC AIDS Death	0.79, 0.14	0.77, 0.08	0.77, 0.08	0.78, 0.08	0.76, 0.06

*From Table B of the analysis report

The first time point at which a significant difference in the progression to AIDS was 12 months after Part I. Censoring the data at that point resulted in a hazard ration of 0.62 (p=0.03). Based on these analyses, the endpoint of AIDS at 365 days after the end of part I of the study was selected for use in the validation of HIV RNA as a surrogate endpoint.

Table 8 shows the number of events for each of the analyzed clinical endpoints censoring the data at 365 days after the end of Part I of the study.

Table 8 - Clinical Endpoints Part I - 365 days

	Early N=129	Late N=141	Relative Risk (early/late)	p value*
AIDS	34	57	0.62	0.03
Death	32	32	1.15	0.85
AIDS Death	46	58	0.81	0.25
ARC	66	84	0.81	0.2
ARC AIDS	74	101	0.73	0.04
ARC AIDS Death	80	102	0.77	0.08

*p-value from log rank test, stratified by baseline CD4. From Table C of the analysis report

The clinical endpoint that was selected for use in the analyses has two important characteristics: 1) it does not include death, 2) the follow-up period for the endpoint was censored at a point when there was a significant difference in the two treatment groups. These characteristics limit the clinical relevance of the analysis based on this endpoint.

Measurement of HIV RNA - Integrity of the Plasma Samples

The samples of plasma and serum used in the determination of HIV RNA were stored in two locations, Baltimore, Maryland and Durham, North Carolina. Storage conditions were different at each site. Samples at the Baltimore site were stored at -20°C and those at the Durham site were stored at -70°C. Ten pairs of samples which had been separated at the time of collection and sent to both the Baltimore and Durham sites were assayed for HIV RNA and the copy numbers were compared to examine the effect of the different storage conditions on the samples. As shown in Table 9, the mean percent decrease in the HIV RNA copy number in the samples stored at Baltimore was 71.4% and the range was 45.9% to 96.3%. These data indicate that there was significant degradation of the RNA in the samples that were stored at the Baltimore site.

Table 9 - Comparison of HIV RNA Copy Number in Paired Samples

	HIV RNA (copies/50microliter)		
	Durham	Baltimore	% Change
Sample 1	6632	507	92.4
Sample 2	9842	2934	70.2
Sample 3	844	457	45.9
Sample 4	248	850	65.8
Sample 5	2037	953	53.2
Sample 6	1155	135	88.3
Sample 7	512	218	57.4
Sample 8	7108	1007	85.8
Sample 9	4641	1916	58.7
Sample 10	4449	166	96.3
Mean % Change			71.4%
Range			(45.9 to 96.3)

The 6-month mean change from baseline was used as the measure of HIV RNA response. Subjects were included if they had at least one early follow-up sample. The mean change from baseline was calculated as the mean of the $\log_{10}$ HIV RNA copy number measurements obtained over the first six months post-randomization, minus the baseline measurements. The results are shown in Table 10. The six month baseline values for the subjects in the early treatment group

showed a decline from the baseline value. The values for the subjects in the late treatment group showed a slight increase in value. For the early treatment group the six month mean change in log plasma HIV RNA was -0.65 for those samples stored at Baltimore and -0.36 for the samples stored at Durham. For the late treatment group, the mean change in log plasma HIV RNA was 0.13 for the samples stored at Baltimore and 0.20 for the samples stored at Durham. For both treatment groups, the six month mean HIV RNA values for those samples stored at Baltimore was less than the mean values for the samples stored at Durham. The six month mean change in log plasma HIV RNA was also different within each treatment group when compared across the storage sites. This difference is likely to be due to degradation of RNA in the samples stored at the Baltimore site.

Table 10 - Baseline and Six Month Mean Values for HIV RNA

	Early Treatment		Late Treatment	
	Baltimore	Durham	Baltimore	Durham
	n=89	n=40	n=109	n=32
Baseline RNA				
Mean	2.62	3.01	2.48	2.94
STD	1.02	0.87	1.01	0.68
Range	0 to 4.79	0 to 4.09	0 to 4.55	1.21 to 3.99
6 month HIV RNA values				
Mean	1.97	2.65	2.61	3.14
STD	0.99	0.88	0.89	0.65
Range	0 to 4.35	0 to 3.83	0 to 4.47	1.78 to 4.15
6 month change in HIV RNA				
Mean	-0.65	-0.36	0.13	0.20
STD	0.93	0.59	0.76	0.32
Range	-3.20 to 1.99	-1.60 to 1.98	-1.85 to 2.38	-0.50 to 0.81

These data show that there was significant degradation in the samples that were stored at the Baltimore site. To adjust for this, the analyses in the study report were stratified by the storage location of the samples. However, since 73% of the samples were stored at the Baltimore site this problem adds additional uncertainty to the analyses.

Method of Measurement of the Surrogate

The initial design of the validation study specified that the primary analyses were to be based on the use of the six month mean change from baseline as the measure of change in plasma HIV RNA. Analyses were also performed which used a defined magnitude of the change in plasma HIV RNA as a categorical response variable. The results of these analyses are shown in Table 11. The percent of the treatment effect explained when the magnitude of the drop in plasma HIV RNA was used as the measure of change ranged from 42% to 60%. This differs from the results obtained when the six month mean change was used as the measure of change in plasma HIV RNA. It is apparent from this comparison that the method chosen for the measurement of the change in the surrogate may affect the results of the analysis. It is not clear which of these two particular approaches is more appropriate when considering the pathophysiology of the disease process. A potentially useful measure of a surrogate, in this case plasma HIV RNA, could be defined either on the basis of some pathophysiologic principle (e.g.,

complete suppression of viral replication) or on the basis of a purely empiric observation derived from exploratory analyses (e.g., the relationship of an observed mean change in the surrogate over a specified time period and clinical outcome). In either case, confirmation of the usefulness of the measure should come from prospective studies.

Table 11 - Peak Decrease in Plasma HIV RNA

	Late Therapy (N=139)		Early Therapy (N=124)		% Treatment Effect Explained
HIV RNA	# (%) with change	# (%) with AIDS	# (%) with change	# (%) with AIDS	
3X decrease	17 (12%)	6 (35%)	73 (59%)	14 (19%)	60%
4X decrease	15 (11%)	6 (40%)	62 (50%)	11 (18%)	59%
5X decrease	12 (9%)	5 (42%)	56 (45%)	9 (16%)	59%
7X decrease	9 (6%)	3 (33%)	46 (37%)	6 (13%)	58%
10X decrease	7 (5%)	1 (14%)	29 (23%)	3 (10%)	42%

Conclusions

While the data available from this retrospective analysis of the 298 study provide information on the relationship of changes in plasma HIV RNA and the clinical outcomes that were observed in this study, the limitations that have been discussed do not allow firm conclusions to be drawn about the utility of changes in plasma HIV RNA as a surrogate endpoint. Prospective studies to corroborate the observations from this study are required to establish the relationship of changes in plasma HIV RNA and clinical outcome.

Gary K. Chikami, M.D.
Medical Officer, HFD-530

Concurrences:
HFD-530/SMO/RBehrman
HFD-530/DivDir/DFeigal

NOV 15 1995

Statistical Review and Evaluation

NDA#: 20-564 (tablet) & 20-596 (oral)

APPLICANT: Glaxo Wellcome Inc.

NAME OF DRUG: 3TC (lamivudine)

INDICATION: In combination with Retrovir® for the treatment of adult and pediatric patients with human immunodeficiency virus (HIV) infection

DOCUMENTS REVIEWED: Volumes: 3.40, 3.54, 3.66, 3.67, 3.97, August 10, 1995 response to FDA request, September 15, 1995 response to FDA request

MEDICAL INPUT: H. Jolson (HFD-530)

A. Background

The applicant is seeking an indication for the combination of 150 mg. lamivudine (BID) and ZDV under the accelerated approval regulations. Four controlled clinical trials have been submitted to support this indication. Separate clinical trials are underway to confirm clinical benefit. The primary focus of this review will be the combination with ZDV at the 150 mg. lamivudine dose.

The applicant and the FDA held a preNDA meeting (February 13, 1995) during which the summary statistic (DAVGT) to be used in the statistical analyses to be presented in the NDA was discussed. This summary statistic will be described after the designs are summarized in the following section.

The primary endpoint is the change in CD4 from baseline over the first twenty-four weeks after randomization. The designation of CD4 as the primary endpoint was discussed at the postsubmission meeting between the applicant and the FDA (August 2, 1995). HIV RNA was discussed as being of great interest, but the applicant and FDA agreed that it would be viewed as supportive.

1) Summary of the Designs

Four controlled clinical trials were submitted to support the applicant's request for an indication. Two studies were conducted in the United States and Canada 3001 and 3002) and two studies were conducted in Europe , 3001 and 3002). The studies were conducted either in ZDV naiv 3001 and , 3001) or in ZDV experienced subjects 3002 and 3002). The remainder of this section describes each of these studies with respect to the aspects of each study which have a major impact upon the statistical evaluation of the study results.

3001

Title: A Randomized, Double-Blind Multicente Trial to Compare the Safety and Efficacy of 3TC™ Monotherapy Versus Zidovudine (ZDV) Monotherapy versus 3TC™ Administered Concurrently with

ZDV in the Treatment of HIV-1 Infected Patients Who are ZDV Naive (≤4 Weeks) with CD4 Cell Counts of 200-500/mm^3

Patient Population

This randomized, double-blind, multicenter study was designed to enroll patients at least 12 years of age with 4 or fewer weeks of prior ZDV use and CD4 cell counts from 200 to 500 cells/mm^3. Three-hundred and twenty subjects were to be enrolled in 10-15 centers in the US, its territories and Canada.

Study Treatments

Subjects were randomized equally (80 per arm) to one of the following four treatment arms: (1) ZDV 200 mg, (2) 3TC 300 mg, (3) ZDV 200 mg + 3TC 150 mg, or (4) ZDV 200 mg + 3TC 300 mg. Treatment was to last for at least 32 weeks with patients allowed to continue treatment through 52 weeks. Randomization was conducted within center.

Endpoints and Schedule of Evaluation

The protocol listed a number of laboratory parameters as endpoints: CD4 count, percent CD4, HIV RNA PCR, p24 antigenemia, β_2-microglobulin and neopterin. No particular laboratory parameter was described as primary.

In addition to having a screening CD4 (at least 14 days prior to randomization), subjects were to be evaluated with respect to CD4 at baseline (two measurements: at least 72 hours prior to randomization and at randomization) and at weeks 2 and 4 and then every 4 weeks through week 32. RNA PCR was conducted following a similar schedule, but with no screening value and only one measure at baseline.

Statistical Analysis

Each treatment arm involving 3TC was to be compared to ZDV as the control. For each parameter, NAUC was to be used as the primary summary measure of change with adjustments for the following: baseline value, AIDS defining condition at baseline and rate of change in CD4 prior to randomization. A protocol amendment specified that Dunnett's method for multiple comparisons would be used to produce an overall (two-sided) .05 level of significance. The covariate adjustment for rate of change in CD4 prior to randomization was deleted from the revised protocol. The amended protocol also specified that average CD4 adjusting for baseline CD4 would be used rather NAUC as the primary summary measure. The day of randomization was used to define day 1.

The amended protocol specified that treatment by center interaction would be investigated to determine if centers may be pooled. A qualitative interaction at the .1 level of significance was specified. Centers were not to be pooled if such an interaction were found at the time of analysis. No interim statistical analyses were planned.

NUCA3002

Title: A Randomized 3TC™, ddC Double-Blind (ZDV Open-Labeled) Multicenter Trial to Evaluate the Safety and Efficacy of 3TC™ (low dose) Administered Concurrently with Zidovudine (ZDV) Versus 3TC™ (high dose) Administered Concurrently With ZDV Versus Dideoxycytidine (ddC) Administered Concurrently With ZDV in the Treatment of HIV-1 Infected ZDV-Experienced (>24 Weeks) Patients with CD4 Cell counts of 100-300/mm^3

Patient Population

Subjects were to be at least 18 years of age, have HIV-1 infection, had prior use of ZDV for at least 24 weeks and have a CD4 count of 100-300/mm^3. Two-hundred and twenty-five subjects were to be assigned equally to the treatment arms within 10-15 centers within the United States and Canada.

Study Treatments

Subjects were randomized equally (75 per arm) to one of the following three treatment arms (1) ZDV 200mg + ddC .75 mg, (2) ZDV 200 mg + 3TC 150 mg or (3) ZDV 200 mg + 3TC 300. Treatment was to be administered for 32 weeks with the option of continuation through week 52. Randomization was conducted within center.

Endpoints and Schedule of Evaluation

The endpoints for this study are change from baseline in the following: CD4 count, HIV RNA PCR, development of resistance, p24 antigenemia, β_2-microglobulin and neopterin. The protocol was amended (amendment 5) to specify that CD4, HIV RNA PCR and β_2-microglobulin were the primary endpoints.

CD4 measurements were to be made twice before treatment and once every 4 weeks for the duration of the study. RNA PCR measurements were to be made at randomization, week 2, week 4 and then every 4 weeks. The protocol was amended (amendment 4) to limit the period of surrogate marker analysis to 24 weeks. This amendment also stressed that CD4 measurements should be made through week 52 regardless of treatment status.

Statistical Analysis

The two treatment arms involving 3TC in combination with ZDV were to be compared to the combination of ddC plus ZDV as the control. NAUC was to be used as the primary summary measure for change from baseline with adjustments for the following: baseline value, AIDS defining condition at baseline and rate of change in CD4 prior to randomization. The protocol was amended (amendment 5) to specify that only baseline value would be used as a covariate in the primary analysis which would be based upon the 24 week average. In this analysis, treatment by center interactions would be tested and retained in the analysis if they were found to be significant at the .1 level. Centers associated with the interaction were to be pooled in the analysis. No interim statistical analysis was called for in the protocol. The protocol was amended (amendment 5) to include the use of Dunnett's multiple comparison procedure.

NUCB3001

Title: A Randomised, Controlled 3TC Double-Blinded Trial to Compare the Safety and Efficacy of 3TC in Combination with Zidovudine (ZDV) versus Zidovudine Monotherapy in Treating HIV-1 Infected Patients Who Are Zidovudine Therapy Naive with a CD4 Cell Count Between 100-400 Cells/mm^3

Patient Population

Subjects were to be at least 18 years of age and have a screening CD4 of between 100 and 400 (within 14 days of randomization). One-hundred and thirty subjects were to be enrolled in approximately 10 European sites.

Study Treatments

Randomization in this study was to either (1) the combination of 3TC (300 mg bid) and ZDV (200 mg tid) or (2) ZDV (200 mg tid) alone. Sixty-five subjects were to be randomized to each arm for at least 24 weeks of treatment. Randomization was conducted within center. This was expected to yield 50 subjects per study arm with endpoint measurements. The protocol was modified to allow subjects to continue on open labeled therapy after 24 weeks on study. Additionally, subjects with a new AIDS defining event or 50% drop in CD4 were offered open label 3TC to allow combination with ZDV.

Endpoints and Schedule of Evaluation

CD4 was to be assessed at screening and then two additional times preceding the administration of medication (72 hours prior to randomization and then immediately prior to the administration of medication). CD4 was measured after randomization two weeks and then every 4 weeks starting at week four.

The original protocol specified that RNA PCR will be a secondary endpoint for this study. This measurement was to be made twice at baseline and then at week 2, 4, 8, 12 and 24.

Statistical Analysis

The original protocol specifies the primary endpoint as change from baseline in CD4. Normalized area under the curve (NAUC) was specified in the original protocol as the primary summary statistic. The following covariates were specified in the protocol: baseline CD4, diagnosis with an AIDS defining event prior to randomization and baseline HIV-1 p24 antigenemia. The protocol was modified to include all of the following as primary endpoints: CD4 count, CD4%, p24 antigen, β-2 microglobulin and neopterin. The protocol was again modified to specify CD4 count as the primary endpoint. The covariates to included in the analysis were listed in the modified protocol as diagnosis with prior CDC defined AIDS event (categories A, B or C) and baseline CD4. Centers were to be pooled for the purpose of analysis by country and possibly by geographic region as well.

Two interim analyses were planned. An O'Brien-Fleming stopping rule was specified in the original protocol. This was later modified to not formally review this trial for efficacy. Instead, the modified protocol specified that should "totally unexpected findings be observed in this analysis, statistical

inference will be based on a criterion for significance of $p<.0001$. the use of this approach to the interim analysis ensures that no adjustment of P-values in the final analysis will be necessary."

3002

Title: A Randomised, Controlled Lamivudine (3TC) Double-blinded Trial to Compare the Safety and Efficacy of Zidovudine (ZDV) Monotherapy *versus* Lamivudine Plus ZDV *versus* Zidovudine Monotherapy in Combination in Treating HIV-1 Infected Patients Who Are Experienced with a CD4 Cell Count Between 100-400 Cells/mm^3 [V69]

Patient Population

Subjects were to be at least 18 years of age, with at least 24 weeks of prior use of ZDV and have a screening CD4 of between 100 and 400 (within 14 days of randomization). Two-hundred twenty-five subjects were to be enrolled using 20 European sites.

Study Treatments

Subjects were randomized equally (75 per arm) to one of the following three treatment arms: (1) ZDV 600mg/day, (2) ZDV 600mg/day + 3TC 150 mg/day, or (3) ZDV 600 mg/day + 3TC 300 mg/day. Subjects were to receive study drug for at least 24 weeks.

Endpoints and Schedule of Evaluation

The original protocol specified that a number of endpoints were to be examined for their change from baseline: CD4 count, HIV RNA PCR, cellular viremia, β_2-microglobulin, neopterin, p24 antigen, viral resistance and time to new AIDS defining event. CD4 measurements were to be made twice prior to treatment administration (in addition to the screening value) and then at weeks 2, 4 and every 4 weeks for the remainder of the study. HIV RNA PCR measurements followed the same schedule starting with a measurement at the time immediately prior to treatment administration. Follow-up for CD4 was to be obtained for all subjects regardless of treatment status.

Statistical Analysis

NAUC was to be used as the primary summary measure for change from baseline with adjustments for the following: baseline CD4, AIDS defining condition at baseline and baseline HIV-1 p24 antigenemia. The protocol was amended (#5) to use the following as covariates: baseline for the surrogate being modeled and AIDS defining condition at baseline.

An interim statistical analysis was described in the original protocol. This analysis was deleted from the protocol by a protocol amendment (#1).

2) Statistical Methods Used in the NDA

All four controlled trials were to use DAVGT to summarize both CD4 and RNA PCR data. This summary statistic was calculated using the following formula:

$$\text{DAVGT} = \frac{\text{AUC}}{\text{time}} - \text{baseline}.$$

AUC (area under the curve) was calculated based upon the trapezoidal rule using the actual time elapsed between measurements. It should be noted that the time period between baseline and the first postbaseline CD4 measurement was excluded from the calculation of the AUC. "Time" in the above formula is the amount of time between the first postbaseline measurement and the last postbaseline measurement. An exception to using the actual time of measurement occurred when multiple CD4 measurements were available within "windows" defined by the number of days (week) since randomization: 3-23 (2), 24-41 (4), 42-69 (8), 70-97 (12), 98-125 (16), 126-153 (20) and 154-181 (24). The multiple values were averaged and this average was treated as if it was measured on the average day of the component measurements. These averages were used to replace the observed data values. Data values up through week 26 were used in the calculation for the 24 week DAVGT.

Wilcoxon's test stratified by investigator was used by the applicant for conducting pairwise tests between the control treatment arm and the experimental treatment arms. A Bonferroni adjustment was specified to adjust for multiple comparisons. Additionally, Van Elteren's test was used to make an overall comparison for those studies with three or more treatment arms. The results of this test were not used in the evaluation of the pairwise treatment differences.

B. Applicant's Results

3001

This study was conducted in 26 outpatient clinics beginning on June 10, 1993. The NDA analyses are based upon data collected through October 28, 1994. Three-hundred and sixty-six subjects are included in the data analysis (340 had been specified in the protocol). Enrollment varied from 1 to 44 subjects per investigator.

The subject population was largely male (87%) with a median age of approximately 34 years. Whites made up the majority of subjects (59%) with the largest remaining group (24%) made up of Hispanics.

The following table summarizes the number of subjects with incomplete treatment duration and with incomplete CD4 follow-up by treatment arm for the first 24 weeks after randomization (the categories presented are not mutually exclusive). The NDA lists the reasons for treatment discontinuation prior to 24 weeks. The reasons for discontinuation were comparable over the treatment arms (text table p 66) with most discontinuations listed as either due to adverse events (32%) or failure to return (44%).

CD4 Follow-up and Duration of Treatment (n)

		ZDV n=93	LAM 300 n=87	ZDV+ LAM 150 n=92	ZDV+ LAM 300 n=94
CD4 Follow-up Status	< 2 values[1]	8	7	9	9
	<24 weeks[2]	21	18	19	23
Treatment Duration	<24 weeks[3]	25	19	23	24

Source: [1]Tables 27, 28, 29, 30, V.40
[2]Table 5 V. 40.
[3]Text table p 66, V. 40.

Pairwise stratified Wilcoxon tests were used to compare each treatment arm to ZDV. The Bonferroni procedure was used to adjust for the three multiple comparisons versus ZDV; tests were conducted at the .017 level for an overall .05 level test.

The results for CD4 and HIV RNA are summarized below by treatment group using the 24 week DAVGT. HIV RNA was presented in terms of log base 10. Only subjects with at least 2 post-randomization values were included by the applicant in these analyses. The means for these parameters as well as the results for the pairwise comparisons relative to ZDV are presented in the follow table. All three comparisons to control were statistically significant (taking into account multiple comparisons). The combination of ZDV + LAM 150 produced the greatest response relative to control. The point estimate of the relative benefit of this combination is 38 cells.

CD4 and HIV RNA Change from Baseline
24 week DAVGT

Variable	statistic	ZDV	LAM 300	ZDV+ LAM 150	ZDV+ LAM 300
CD4	mean (n, s.e.)	17 (85, 9)	24 (80, 7)	55 (83, 11)	45 (85, 9)
	p-value*		<.001	.002	.015
RNA PCR - log	mean (n, s.e.)	-.31 (86, .03)	-.59 (77, .04)	-1.12 (80, .07)	-1.15 (88, .07)
	p-value*		<.001	<.001	<.001

Source: Tables 33 and 34, V. 40
*Wilcoxon Test for comparisons to ZDV

The study report presents analyses by age (<34 versus >=34), gender and race. In the study report, the applicant reported findings similar to those found for the overall study population. It was noted that non-Caucasians showed smaller effects relative to control for both CD4 and HIV RNA.

In order to explore the impact of incomplete follow-up on the overall findings, the applicant presented analyses characterizing the treatment effect for subjects with 24 weeks of treatment versus those subjects with less than 24 weeks of treatment. The applicant has concluded that completers and non-completers are comparable at baseline. DAVGT was less for non-completers than for completers across treatment arms with the ZDV group exhibiting the largest difference between completers and noncompleters. This result led to a smaller difference between the lamivudine treatment arms relative to ZDV for

subjects with at least 24 weeks of treatment in comparison to subjects with less than 24 weeks of treatment.

DAVGT - CD4
Mean (s.e.)

Variable		ZDV	LAM 300	ZDV+ LAM 150	ZDV+ LAM 300
Completers	n	68	68	69	70
	mean	27 (11)	25 (8)	58 (13)	49 (10)
Non-Completers	n	17	12	14	15
	mean	-27 (13)	14 (21)	42 (17)	26 (16)

Source: Tables 27, 28, 29, 30 V 40.

3002

This study was conducted in 21 outpatient clinics beginning on June 21, 1993. The NDA analyses are based upon data collected through November 9, 1994. Two-hundred and fifty-four subjects are included in the data analysis (225 were specified in the protocol). Enrollment varied from 1 to 30 subjects per investigator.

The subject population was largely male (83%) with a median age of approximately 37 years. Whites made up the majority of subjects (63%) with the largest remaining group (20%) made up of Hispanics.

The following table summarizes the number of subjects with incomplete treatment duration and with incomplete CD4 follow-up by treatment arm for the first 24 weeks after randomization (the categories presented are not mutually exclusive). The NDA lists the reasons for treatment discontinuation prior to 24 weeks. The reasons for discontinuation were comparable over the treatment arms (text table p 70) with most discontinuations listed as either due to adverse events (53%), failure to return (14%) or patient's choice (16%).

CD4 Follow-up and Duration of Treatment (n)

		ZDV+DDC n=86	ZDV+ LAM 150 n=84	ZDV+ LAM 300 n=84
CD4 Follow-up Status	< 2 values[1]	7	7	4
	<24 weeks[2]	12	18	15
Treatment Duration	<24 weeks[3]	15	19	23

Source: [1]Tables 26, 27, 28, V. 54
[2]Table 5, V. 54.
[3]Table 4 V. 54.

Pairwise stratified Wilcoxon tests were used to compare each treatment arm to ZDV+DDC. The Bonferroni procedure was used to adjust for the two multiple comparisons versus control: tests were conducted at the .025 level for an overall .05 level test.

The results for CD4 and RNA PCR are summarized below by treatment group using the 24 week DAVGT. Only subjects with at least 2 post-randomization values are included in this analysis. The results for the pairwise comparisons relative to ZDV+ddC are presented. The point estimate of the relative advantage of ZDV + LAM 150 to the control arm is 40 cells and is significantly different from 0 for CD4. The treatment effect for ZDV+LAM 150 versus control with respect to HIV RNA fails to meet the Bonferroni criterion for significance.

CD4 and HIV RNA Change from Baseline
24 week DAVGT

Variable	statistic	ZDV+ddC	ZDV+ LAM 150	ZDV+ LAM 300
CD4	mean (n, s.e.)	-2 (79, 6)	38 (77, 9)	39 (80, 7)
	p-value*		.001	.001
RNA PCR - log	mean (n, s.e.)	-.66 (77, .06)	-.80 (78, .07)	-.91 (74, .06)
	p-value*		<.139	<.010

Source: Tables 31 and 32, V. 54.
*Wilcoxon Test for comparison to ZDV+ddC

The study report presents analyses by age (<37 versus >=37), gender and race. The applicant reported findings for each of these subgroup analyses similar to those found for the overall study population.

In order to explore the impact of incomplete follow-up on the overall findings, the applicant presented analyses characterizing the treatment effect for subjects with 24 weeks of treatment versus those subjects with less than 24 weeks of treatment. The applicant has concluded that completers and non-completers are comparable at baseline, but also commented that the treatment groups showed a different pattern in terms of the comparison between completers and noncompleters. In particular, the ZDV+ LAM150 arms had comparable response between groups formed on the basis of completion status while the other two groups had a lower response for non-completers. The following table displays the DAVGT by treatment group and treatment status

DAVGT - CD4
Mean (s.e.)

Variable		ZDV+DDC	ZDV+ LAM 150	ZDV+ LAM 300
Completers	n	70	65	61
	mean	0 (6)	39 (10)	44 (8)
Non-Completers	n	9	19	23
	mean	-19 (23)	35 (18)	23 (12)

Source: Tables 26, 27, 28 V. 54.

3001

This study was conducted in 14 sites in France, Spain, Germany, Belgium and the United Kingdom beginning on June 4, 1993. The NDA analyses are based upon data collected through April 27, 1994. One-hundred and twenty-nine subjects were enrolled and randomized (100 specified in the protocol). Enrollment varied from 2 to 24 subjects per investigator.

The subject population was largely male (approximately 75%) with a median age of approximately 35 years for the ZDV monotherapy arm and 32 years for combination therapy. Whites made up the majority of subjects (80% and 85% for ZDV monotherapy and combination therapy, respectively).

The following table summarizes the number of subjects with incomplete treatment duration and with incomplete CD4 follow-up by treatment arm for the first 24 weeks after randomization (the categories presented are not mutually exclusive). The NDA lists the reasons for treatment discontinuation prior to 24 weeks. The reasons for discontinuation were comparable over the treatment arms (table 5) with most discontinuations listed as either due to adverse events (44%) or failure to return (31%).

CD4 Follow-up and Duration of Treatment (n)

		ZDV n=64	ZDV+LAM n=65
CD4 Follow-up Status	< 2 values[1]	1	1
	<24 weeks[2]	6	6
Treatment Duration	<24 weeks[3]	7	9

Source: [1]Table 21, V. 66
[2]Table 6 V. 66.
[3]Table 5, V. 66.

A stratified Wilcoxon test, with stratification by investigator, was used to compare the combination arm to ZDV for both CD4 and RNA PCR. Tests were conducted at the .05 level.

The results for CD4 and RNA PCR are summarized below by treatment group using the 24 week DAVGT. Only subjects with at least 2 post-randomization values are included in the analysis. Additionally, only the RNA PCR values from the French sites are included. The combination arm showed a significant improvement over the ZDV arm for both CD4 and HIV RNA

CD4 and HIV RNA Change from Baseline
24 week DAVGT

Variable	statistic	ZDV	ZDV+LAM
CD4	mean (n, s.e.)	18 (63, 7)	75 (64, 8)
	p-value*		<.001
RNA PCR - log	mean (n, s.e.)	03, .11)	-.60 (32, .12)
	p-value*		.008

Source: Table 21, V. 66
*Wilcoxon Test

The study report does not describe the results by age, gender or race.

In order to explore the impact of incomplete follow-up on the overall findings, the applicant presented analyses characterizing the treatment effect for subjects with 24 weeks of treatment versus those subjects with less than 24 weeks of treatment. The applicant noted that completers and non-completers differ at baseline with noncompleters having a lower CD4 count at baseline. The means by completion status were not presented by treatment.

3002

This study was conducted in 32 sites in France, Spain, Germany, Holland, Denmark and the United Kingdom beginning on August 3, 1993. The NDA analyses are based upon data collected through September 13, 1994. *Two-hundred and twenty-three subjects enrolled and randomized* (225 specified in the protocol). Enrollment varied from 1 to 19 subjects per investigator

The subject population was largely male (88% for the ZDV monotherapy arm, 85% for ZDV+LAM150 and 77% for the ZDV+LAM300) with a median age of approximately 35 years. Whites made up the majority of subjects (at least 95% in all three groups).

The following table summarizes the number of subjects with incomplete treatment duration with incomplete CD4 follow-up by treatment arm for the first 24 weeks after randomization (the categories presented are not mutually exclusive). The NDA lists the reasons for treatment discontinuation prior to 24 weeks. The reasons for discontinuation were comparable over the treatment arms (table 5) with most discontinuations listed as due to adverse events (44%), failure to return (15%) or patient's choice (22%).

CD4 Follow-up and Duration of Treatment (n)

		ZDV n=73	ZDV+ LAM 150 n=75	ZDV+ LAM 300 n=75
CD4 Follow-up Status	< 2 values[1]	2	1	3
	<24 weeks[2]	11	5	8
Treatment Duration	<24 weeks[3]	14	5	8

Source: [1]Table 6 V. 69.
[2]Table 5, V. 69
[3]Table 21, V 66

As discussed previously, DAVGT was used to summarize both the CD4 and RNA PCR data. Pairwise stratified Wilcoxon tests were used to compare each treatment arm to ZDV. Tests were conducted at the .025 level.

The results for CD4 and RNA PCR are summarized below by treatment. It can be seen that the p-values were smaller than .025 for each comparison. Only subjects with at least 2 post-randomization values are included in the analysis. Additionally, only the RNA PCR values from the Spanish sites are included.

CD4 and HIV RNA Change from Baseline
24 week DAVGT

Variable	statistic	ZDV n=73	ZDV+ LAM 150 n=75	ZDV+ LAM 300 n=75
CD4	mean (n, s.e.)	-18 (71, 6)	38 (74, 6)	32 (72, 6)
	p-value*		<.001	<.001
RNA PCR - log	mean (n, s.e.)	-.02 (12, .12)	-.26 (8, .17)	-.32 (8, .13)
	p-value*		<.001	<.001

Source: Tables 21, 22 V. 69
*Wilcoxon Test for comparison to ZDV

The study report does not describe the results by age, gender or race.

The applicant presented summary data (Table 19, V. 69) characterizing the treatment effect for subjects with 24 weeks of treatment versus those subjects with less than 24 weeks of treatment. The completers and non-completers appear to differ at baseline with noncompleters having a lower CD4 count at baseline. The means by completion status were not presented by treatment.

C. Statistical Reviewer's Comments

1) Primary Analyses of DAVGT

The applicant submitted two set of revised analyses after the initial submission, in which DAVGT was based upon subjects with 2 or more postbaseline measurements. The first supplemental submission (August 10, 1995) contained data sets and DAVGT recalculated based upon all subjects with 1 or more postbaseline measurements. During the review of the initial submission and the August 10 revision, a number of issues arose regarding the calculation of DAVGT. DAVGT was again revised, and analyses and data sets were resubmitted (September 15, 1995). The second revised DAVGT was the primary basis for the FDA statistical review of efficacy. The remainder of this section will address the following: a) the statistical issues leading to the revision of DAVGT and b) revised analyses based upon the submission of September 15.

a) Calculation of DAVGT

In the first set of data files submitted as part of the first supplemental submission (August 10), a number of dates for CD4 and HIV RNA values were missing for th studies. In response to an FDA question, the applicant clarified that when a blood sample was collected on an unscheduled visit a computed visit date based upon session number was used. This computed value was omitted from the data files. The applicant stated that an actual visit date was later found to be available on the laboratory form and that this date could have been used rather than the computed date. This date was used to recalculate DAVGT and was included in the data files submitted on September 15.

The data files provided by the applicant as part of the August 10 submission could not be used to reproduce the mean DAVGT for CD4 and HIV RNA provided in tables as part of the same submission. In response to a question from FDA, the applicant responded that for subjects with only a single value the values in the tables were based upon the simple difference between baseline and the first measurement. In contrast, DAVGT in the data file was based upon the AUC-based formula which is one-half of the simple difference for those subjects with only a single postbaseline measurement. When this situation was discussed with the applicant, the applicant indicated that AUC based approach is more appropriate and would resubmit tables based upon this approach.

Another issue involved subjects with multiple measurements (either for CD4 or HIV RNA) in the "windows" defined in the study report around each scheduled visit. If two or more values occurred in specified time periods corresponding to the scheduled visit, these values and times were averaged prior to the calculation of DAVGT. This type of averaging was not part of the algorithm described in the NDA. The NDA formula for AUC involved no averaging based upon the timing of measurements within "windows". When this discrepancy was brought to the applicant's attention, the applicant indicated that using the actual data values and dates is preferable and a modified DAVGT was calculated accordingly.

In the calculation of the AUC, the time period between baseline and the first postbaseline measurement was treated as the number of days between the average of the baseline days and the first visit. Since the number of days for the first period should be the time between randomization and the first postbaseline visit, this makes the contribution of the first period too large in the calculation of DAVGT. When this was brought to the applicant's attention, the applicant proposed calculating DAVGT based upon the difference between the first visit and the day of randomization. The applicant later modified this approach to base the time period upon the difference between the first day of treatment and the first study visit. This was done to allow the baseline to include the last measurement obtained prior to the initiation of therapy. In some cases this was one day after randomization.

b) Revised Analyses

The revised calculations and data files were provided in the submission of September 15, 1995. The following table contains the summary statistics for DAVGT for CD4 based upon the revisions described above (taken from the submission of 9/15/95) using all subjects with at least one postbaseline CD4 value within the first 24 weeks. The point estimates and corresponding p-values were virtually unchanged from those provided previously by the applicant. The p-values presented in this table were based upon Wilcoxon's test using ranks. This procedure ranks subjects within center and then combines the rank statistic over centers. As such, the mean has been used as a descriptive statistic only and was not used for statistical testing. The mean in this table has been calculated by combining centers and then calculating the mean for each arm as a whole. Means and standard errors based upon the same strategy used for the Wilcoxon test (using the original data instead of ranks) will be presented later in this section.

Revised DAVGT for CD4 and Tests of Significance

Study		control	Difference Relative to Control		
			LAM 300	ZDV+ LAM150	ZDV+ LAM300
3001	mean	15	5	34	20
	p-value		.313	.001	.044
3002	mean	-2	*	34	37
	p-value		*	<.001	<.001
3001	mean	15	*	*	55
	p-value		*	*	<.001
'3002	mean	-17	*	52	49
	p-value		*	<.001	<.001

*treatment not assigned

The following table contains the revised analyses for log HIV RNA for studies 3001 and 3002. 3001 the treatment effects are somewhat reduced relative to those contained in the NDA, but the p-values are still well below the Bonferroni criterion for significance. For 3002 the treatment effects were slightly larger, but the p-value for the comparison of ZDV+LAM150 versus control now meets the Bonferroni criterion for significance which was not met in the original submission.

Revised DAVGT or log HIV RNA and Tests of Significance

Study		control	Difference Relative to Control		
			LAM 300	ZDV+ LAM150	ZDV+ LAM300
3001	mean	-.32	-.27	-.70	-.75
	p-value		<.001	<.001	<.001
3002	mean	-.62		-.16	-.24
	p-value			.016	.003

*treatment not assigned

As discussed above, the applicant performed stratified Wilcoxon tests which were based upon ranking subjects within each center and then calculating a p-value based upon the permutation variance found by conditioning on the observed sample size allocated to each treatment. The means and standard errors provided by the applicant have been estimated by pooling all centers and then calculating the simple means and standard errors. This is not consistent with the weighting scheme utilized in the Wilcoxon procedure which weights centers based upon the harmonic mean of the sample sizes within each center. Similarly, the standard errors presented by the applicant do not adequately reflect the design of the clinical trials.

The following table contains CD4 means and standard errors for the treatment comparisons calculated from the data files provided by the applicant. These standard errors have been calculated using the same procedure used by the applicant to calculate the Wilcoxon tests, but using the actual CD4 values rather than ranks as was done with the Wilcoxon tests. The procedure is known as the extended Cochran-Mantel-Haenszel procedure (Koch, G. Carr, G., Amara, I., Stokes, M. and Uryniak, T. in Statistical

Methodology in the Pharmaceutical Sciences edited by Berry, D., Marcel-Dekker, 1990. The means differ somewhat from the means in the above table because different weights are used to average over centers. Also, this analysis only included subjects from centers with both treatments represented in the pairwise comparison.

Means Differences and Standard Errors for CD4
Extended Cochran-Mantel-Haenszel Procedure

Study		Difference Relative to Control		
		LAM 300	ZDV+ LAM150	ZDV+ LAM300
3001	mean	-3	-34	-21
	Std. Error	11	13	11
3002	mean	*	-33	-34
	Std. Error	*	10	8
3001	mean	*	*	-54
	Std. Error	*	*	12
3002	mean	*	-55	-50
	Std. Error	*	10	10

*treatment not assigned

In summary, the revised analyses confirm the conclusions reached by the applicant in the original NDA submission; lamivudine in combination with ZDV was found (relative to control) to produce a statistically significant increase in CD4 as well as a statistically significant decrease in log HIV RNA. The estimated increase in CD4 (relative to control) was approximately 30-40 cells for the North American studies and 50-60 cells for the European studies. Relative to control, the treatment effects for ZDV-naive subjects and ZDV-experienced subjects were comparable.

2) Degree of Follow-up

One of the primary concerns in reviewing these studies is the degree of follow-up with respect to CD4. The applicant's description of the impact of follow-up focused upon completion of 24 weeks of treatment. Additional analyses were conducted by FDA to investigate the impact of less than complete follow-up. These analyses were conducted using the revised data sets (9/15/95). Two issues have been of primary concern: 1) the proportion of subjects with measurements through week 24 and 2) the intensity of follow-up for this period of time.

The following table summarizes the number of subjects with CD4 values in the interval around week 24 (22-26). The percent of subjects missing this measurement varies from 17% to 26%.

No CD4 Value at Week 24

Study	control	LAM 300	ZDV+ LAM150	ZDV+ LAM300
3001	27% (25/93)	23% (20/87)	26% (24/92)	27% (25/94)
3002	19% (16/86)	*	27% (23/84)	19% (16/84)
3001	17% (11/64)	*	*	17% (11/65)
3002	19% (14/73)	*	17% (13/75)	20% (15/75)

Source: Figure 3, V. 40; Figure 3, V. 54; Figure 1, V. 66; Figure 1, V. 69.
*treatment not assigned

This table does not distinguish between subjects withdrawing prior to week 24 and subjects simply missing a week 24 value. It is possible that subjects withdrawing without a week 24 measurement may have a different treatment effect than subjects simply missing their 24 week measurement. The table on the following page shows subjects classified by their follow-up status respect to CD4. For the studies, subjects were categorized based upon whether or CD4 data after week 24 was present. For the studies, subjects do not have visit data after week 24 and the classification was based upon whether or not a subject missing the week 24 visit had withdrawn from the study prior to week 24.

As was discussed above, 3001 has the worst overall follow-up at week 24. For this study, it can be seen that roughly one-third of the subjects missing the week 24 measurement were still being followed past week 24. For the 3002 study, approximately half the subjects missing a week 24 CD4 were being followed for CD4 after week 24. Similarly for the studies, approximately one-half the subjects missing their CD4 value at week 24 were still being followed. It does not appear that the rates of follow-up differ between the treatment arms.

CD4 Follow-up by Status at Week 24
Number of Subjects

Study	Last CD4 postbaseline	control	LAM300	ZDV+ LAM150	ZDV+ LAM300
3001	none	4	3	6	5
	<24	14	11	10	16
	24	68	67	68	69
	not 24, >24	7	6	8	4
	Total	93	87	92	94
3002	none	4	*	3	4
	<24	3	*	7	5
	24	70	*	61	68
	not 24, >24	9	*	13	7
	Total	86	*	84	84
3001	none		*	*	1
	<24-discont.	6	*	*	6
	<24-cont.	5	*	*	4
	24	53	*	*	54
	Total	64	*	*	65
3002	none	1	*	*	3
	<24-discont.	10	*	6	6
	<24-cont.	3	*	7	6
	24	59	*	62	60
	Total	73	*	75	75

*treatment not assigned

As mentioned previously, the most problematic subjects may be those without any CD4 measurements after baseline and those subjects who discontinued CD4 measurements prior to week 24 due to study withdrawal (these groups are labeled none, <24 and <24-discont. in the above table). It is therefore of interest to see if these groups of subjects differ with respect to CD4 at baseline or their change in CD4. The table on the following page summarizes these comparisons.

For all four studies, there appears to be no consistent relationship between average baseline CD4 and follow-up status. This suggests that the degree of follow-up may not be strongly related to the patient's condition at baseline.

Another concern raised by the degree of missing data, is the extent to which the treatment comparisons have been distorted by the missing data. 3001 is of special concern due to the degree of missing data. For this study, when the comparison between ZDV+LAM150 and control in terms of DAVGT is examined, the difference is largest for subjects with partial follow-up (49=35-(-14)) and smallest for subjects with follow-up after week 24 but lacking a week 24 measurement (10=43-33). The relative difference for subjects with a 24 week measurement lies between the means for these two subgroups (32=50-18).

Mean CD4 at Baseline and Mean DAVGT for CD4 by Follow-up Status

Study	CD4	Follow-up	control	LAM300	ZDV+ LAM150	ZDV+ LAM300
3001	Baseline	none	302	421	412	381
		partial	334	350	341	402
		full (no 24)	406	275	290	395
		full (with 24)	350	341	374	360
	DAVGT	partial	-14	5	35	-11
		full (no 24)	33	68	43	39
		full (with 24)	18	19	50	45
		Total	14	20	48	35
3002	Baseline	none	224	*	219	171
		partial	251	*	228	171
		full (no 24)	196	*	206	208
		full (with 24)	232	*	212	206
	DAVGT	partial	9	*	12	26
		full (no 24)	-8	*	19	48
		full (with 24)	-2	*	37	34
		Total	-2	*	32	35
3001	Baseline	none		*	*	158
		<24-discont.	233	*	*	180
		<24-cont.	279	*	*	423
		24	264	*	*	282
		Total	263	*	*	280
	DAVGT	<24-discont.	9	*	*	50
		<24-cont.	31	*	*	28
		24	15	*	*	76
		Total	15	*	*	70
3002	Baseline	none	351	*	*	182
		<24-discont.	200	*	201	221
		<24-cont.	357	*	293	234
		24	249	*	254	251
		Total	248	*	254	246
	DAVGT	<24-discont.	-15	*	-7	40
		<24-cont.	-60	*	22	36
		24	-15	*	41	32
		Total	-17	*	35	33

*treatment not assigned

Since 3001 has also been conducted with naive subjects (this study used a combination of LAM300, which has been found to be comparable to LAM150), it is of interest to see if the same pattern appears for this study as was seen with 3001. The overall difference in this study is 55 cells (70-55). Numerically, the difference is smallest (-3=28-31) for subjects continuing on study, but lacking a week 24 measurement. The mean difference for subjects with a week 24 measurement (61=76-15) is very similar to the overall difference while the mean difference is 41 (50-9) for subjects who discontinued with partial follow-up. The numbers of patients with no CD4 at week 24 is so small that statistical comparisons are difficult.

The numerical comparisons suggest that the overall treatment effect is consistent over the subgroups formed by degree of follow-up and this supports the conclusion that ZDV+LAM150 is effective relative to control with respect to CD4. The following table shows the mean difference relative to control for ZDV+LAM150 in terms of mean change from baseline CD4 by study week as well as the number of subjects available at each time point. The difference between the control arm and ZDV+LAM150 is consistently between 30 and 40 for the studies and tends to exceed 50 for 3002. This consistency over studies suggests that the difference in DAVGT between ZDV+LAM150 and control is relatively unaffected by the particular time of measurement and that missing CD4 measurements may have relatively little impact upon the overall estimate of treatment effect.

ZDV+LAM150 vs. Control
Difference in Mean Change from Baseline

	3001		3002		3002	
Week	n_c/n_e *	Diff.	n_c/n_e	Diff.	n_c/n_e	Diff.
4	87/84	24	71/75	32	65/70	57
8	82/75	69	74/72	31	65/74	62
12	80/77	34	77/71	30	65/68	47
16	73/71	43	74/69	33	63/70	46
20	71/71	36	72/68	53	59/67	52
24	68/68	28	70/61	47	59/62	65
DAVG		34		34		52

*n_c=sample size for the combination arm and n_c=sample size for the control arm

3) Durability of Response

The NDA contains graphs showing the mean CD4 versus time. These graphs suggest that ZDV in combination with LAM150 may maintain CD4 above baseline for a longer period of time than seen in previous studies of treatments for HIV infection. In previous studies of anti-retroviral therapy there has generally been seen a relatively rapid rise in CD4 (8-12 weeks) followed by a slow decline to baseline. This pattern has been seen in studies involving subjects with extensive prior ZDV use. The following table summarizes this information by presenting the proportion of subjects exceeding baseline at week 24. This table suggests that a higher proportion of subjects, relative to control, exceed baseline for combination therapy involving lamivudine. The difference is fairly small for the naive subjects studied in 3001, but is more pronounced and consistent in the other studies.

Proportion of Subjects Exceeding Baseline at 24 Weeks

Study	control	LAM 300	ZDV+ LAM150	ZDV+ LAM300
3001	.55	.56	.61	.68
3002	.36		.69	.63
3001	.45			.86
3002	.20		.71	.80

The individual data values used to create this table are displayed in the following graph for 3001 for subjects assigned to the ZDV arm and ZDV+LAM150.

CD4 Values by Day of Visit
NUCA 3001

These examinations of the treatment effect over time suggest that combination therapy based upon ZDV plus lamivudine 150 may provide a longer lasting increase in CD4 than has been seen in previous antiretroviral therapy.

4) Subject Level CD4 and HIV RNA Response

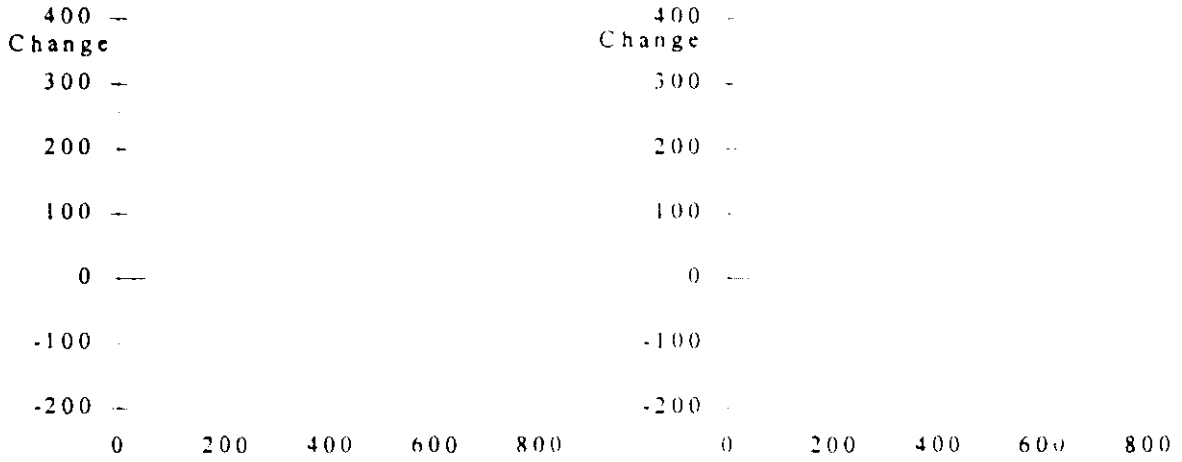
Variability in CD4 Response and Relationship to Baseline CD4

As discussed above, the mean CD4 increase for ZDV+LAM150 relative to control was approximately 30-50 cells across the studies. The data underlying these mean changes possess considerable variability and the treatment groups have considerable overlap. The DAVGT for each subject as well as each subject's baseline CD4 are shown in the following graphs produced from 3001. It can be seen

that there is considerable variability in response, with the change in CD4 varying from a decrease of 200 to an increase of 400 for all four treatments. There appears to be slightly increased variability for higher values of baseline CD4, in particular for monotherapy.

With monotherapy, there appears to be little relationship between the baseline value and change over time. For combination therapy, DAVGT tends to be greater than zero for all values of CD4 with a noticeable smaller change for higher values of baseline CD4. Linear regression analysis finds that approximately 10% of the variability in the change can be accounted for by baseline CD4 for combination therapy. Similar relationships were seen in the other studies.

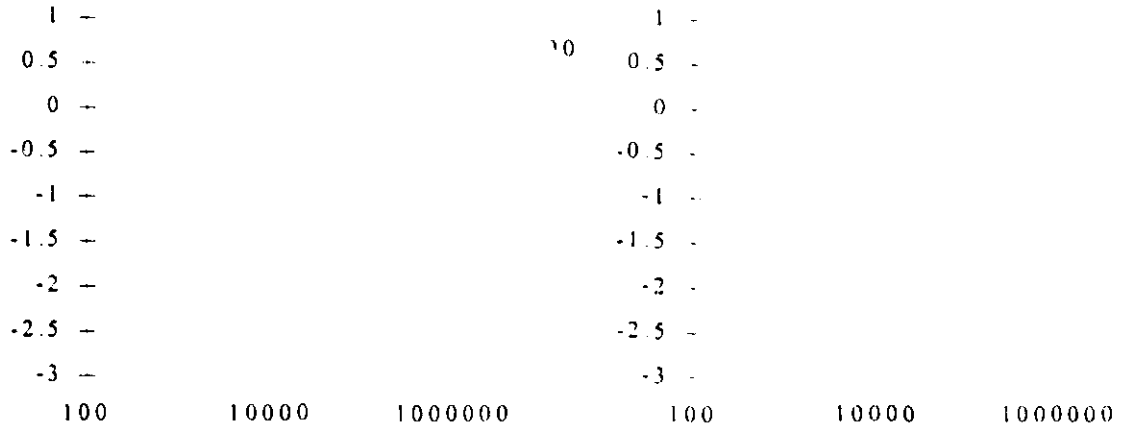
Change in CD4 versus Baseline CD4
NUCA 3001



Relationship Between HIV RNA Response and Baseline HIV RNA

A somewhat different pattern is seen for the log change in HIV RNA. These values are plotted for study 3001 in the graphs below. The limit of detection for this assay is 200 copies. Since a log change corresponds to a 90% drop in copy number, there is a limit to the amount of drop which can be seen on the log scale. Relatively few subjects approach the limit of detection for monotherapy, but this boundary is quite noticeable for combination therapy for subjects with lower copy numbers at baseline.

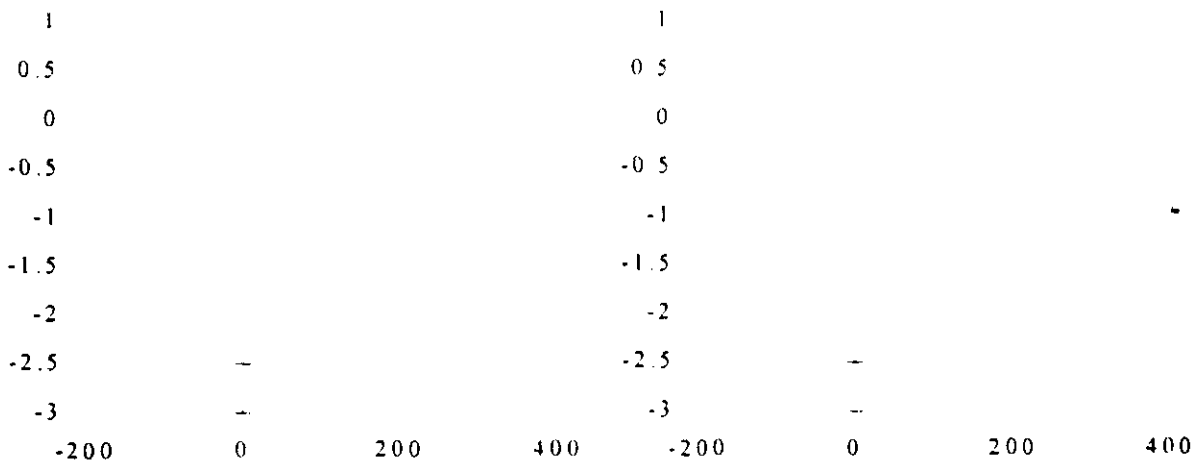
Change in Log HIV RNA versus Baseline HIV RNA
NUCA 3001



Relationship Between the Change in CD4 and the Change in HIV RNA

The applicant's analyses indicate that the conclusions based on the comparison between treatments are comparable for both CD4 and HIV RNA. This does not directly show that there is a consistent response with respect to CD4 and HIV RNA. To further investigate this issue, the change in log HIV RNA and the change in CD4 have been plotted against one another in the graphs on the next page for study 3001. 3002 shows a similar pattern.) It can be seen that for all four treatment arms most subjects have a decrease in log HIV RNA. This is not the case for CD4 for which a large number of subjects have both increases and decreases. Subjects receiving the combination therapy arms tend to have responses in which log HIV RNA decreases while CD4 increases. It can be seen that the relationship for combination arms is relatively weak with those subjects with the biggest decrease in HIV RNA not necessarily having a corresponding increase in CD4 . For monotherapy subjects, there is no clear relationship between HIV RNA and CD4. This analysis suggests that benefit for a given subject may not be equivalently described by changes in CD4 and HIV RNA.

Change in Log HIV RNA versus Change in CD4
NUCA 3001



In summary, the subject level data show a great deal of variability in response. The average differences among the treatments are relatively small relative to the background variability in response. It was also seen that for combination therapy the CD4 response is weakly related to both baseline CD4 and HIV RNA response. Little relationship was seen for monotherapy between CD4 response and baseline CD4 and HIV RNA.

5) Subgroup Analyses

The applicant conducted subgroup analyses based upon race, gender and age for the studies. The gender and age analyses do not suggest that the treatment benefit varies by age or gender. The analysis of race presented by the applicant suggests that Black subjects in 3001 assigned to ZDV+LAM150 may have a smaller CD4 cell response relative to control (ZDV) than White subjects. The applicant's analyses suggest that the higher CD4 values at baseline for Blacks may have contributed to this result. Blacks with higher CD4 values at baseline had the least favorable response to combination therapy. The applicant concluded that in the absence of a biological mechanism the observed racial difference is likely to be an artifact of imbalances in patient characteristics.

The mean and standard errors for the racial groups provided by the applicant (V. 97) show an approximately 100 cell difference between Blacks and Whites (-25 and 75 for DAVG, respectively) for subjects assigned to ZDV+LAM150. The corresponding comparison is approximately 25 for subjects assigned to ZDV (-1 and 24, respectively). This translates into roughly a 75 cell relative treatment difference (100-25) with an approximate standard error of 40 cells. This standard error indicates that the treatment differential between Blacks and Whites has not been very precisely estimated.

The applicant's analysis of subgroups is suggestive of a difference in treatment response between Blacks and Whites. Though these studies have not been powered to detect a difference, these studies raise the concern that there may in fact be such a difference.

D. Statistical Reviewer's Conclusions

The four studies used by the applicant in support of their requested indication under the accelerated approval regulations are of similar design. All four studies are double-blind, randomized trials of relatively short duration providing 24 weeks of CD4 data. Additionally, HIV RNA data was available for all subjects in the North American studies. The major limitation of these studies was the relatively high number of subjects missing surrogate marker data for each of the scheduled visits. Analyses conducted by the applicant and the FDA suggest that the impact of this missing data is minimal.

The analyses provided by the applicant and confirmed by the FDA indicate that a statistically significant increase in CD4 as well as a significant decrease in HIV RNA were associated with the use of lamivudine in combination with ZDV. There was no apparent improved response using 300 mg of lamivudine in combination with ZDV in comparison to 150 mg of lamivudine in combination with ZDV.

The analyses provided by the applicant describe a relatively large treatment differential between Blacks and Whites. The applicant should further investigate this issue.

The analyses conducted by FDA suggest that there is not good agreement between CD4 and HIV RNA at the patient level, though the overall conclusions are comparable for these measures. This suggests that the impact of treatment may be evaluated differently for the same subject depending upon the measure chosen.

The increase in CD4 for ZDV+LAM150 relative to control was almost identical for ZDV naive and ZDV experienced subjects. In the North American studies 3001 and 3002) the mean increase in CD4 (relative to control) was 34 cells for both ZDV naive and ZDV-experienced subjects. In the European studies, the relative increase was approximately 50 cells (only ZDV+LAM300 was used in 3001). The standard errors of the mean differences ranged between 10 and 13 cells over the four studies.

Paul Flyer, Ph.D.
Mathematical Statistician

DRAFT

BIOPHARMACEUTICS/PHARMACOKINETICS REVIEW

NDA: 20-564 (tablets)
20-596 (oral solution)

SUBMISSION DATE: 6/30/95

GENERIC NAME: Lamivudine

BRAND NAME: Epivir™

FORMULATION: Oral tablets, 150 mg
Oral solution, 10 mg/mL

APPLICANT: Glaxo-Wellcome

TYPE OF SUBMISSION: Original application
Type 1P

REVIEWERS: Barbara Davit, Ph D
Kellie Reynolds, Pharm.D.

REVIEW DATE:

SYNOPSIS: The applicant has satisfactorily addressed the following

- Pharmacokinetics of lamivudine after single and multiple doses,
- Pharmacokinetics of lamivudine in pediatric patients,
- Absolute bioavailability of lamivudine after oral administration,
- Dose-proportionality of single doses of lamivudine administered orally to pediatric patients over the range of 0.5 to 10 mg/kg,
- Dose-proportionality of single and multiple doses of lamivudine administered orally to adult patients over the range of 0.25 to 10 mg/kg,
- Pharmacokinetics of lamivudine in renally impaired patients,
- Potential drug interactions between lamivudine, zidovudine, didanosine, and TMP/SMX,
- Effect of food on lamivudine absorption and exposure,
- Bioequivalence between the clinical and commercial formulations

No gender study was submitted to the NDA. There were 7/97 female patients enrolled in Phase I/II Study . 2001. The number of female patients was too limited to permit statistical evaluation. All other pharmacokinetic studies in adult patients were conducted using male patients.

No metabolism study was submitted to the NDA. The applicant determined that excretion is primarily renal, with approximately 70% of the dose excreted unchanged and about 5% of the dose excreted as lamivudine sulfoxide. Approximately 25% of a lamivudine dose is unaccounted for.

No attempt was made to link pharmacokinetics and pharmacodynamics of lamivudine.

This review consists of the summary of studies reviewed under Section 6 of the NDA. Individual study reports are on file in the Division of Pharmaceutical Evaluation III.

PHASE IV COMMITMENTS: The applicant should conduct studies to investigate

- Lamivudine pharmacokinetics in pediatric and adolescent patients to provide additional data for dose optimization,
- The relationship between lamivudine systemic exposure and the development of pancreatitis in pediatric patients;
- Effects of renal insufficiency on lamivudine pharmacokinetics in pediatric patients to provide data for dosing recommendations,
- Lamivudine pharmacokinetics in patients with advanced HIV disease;
- The effects of gender on lamivudine pharmacokinetics,
- The effects of ethnicity on lamivudine pharmacokinetics,
- The relationship between markers of lamivudine efficacy and serum concentrations of lamivudine and other antiretroviral drugs.
- The relationship between serum lamivudine concentrations and intracellular lamivudine triphosphate concentrations in patients dosed with lamivudine;
- ADME of radiolabeled lamivudine in patients

RECOMMENDATION: It is recommended that NDA 20-564 and NDA 20-596 be approved. This reviewer concluded that Section 6 of this NDA contained adequate human pharmacokinetic, bioavailability and bioequivalence data to permit a knowledgeable judgement about whether to approve this application

This reviewer recommends doses of: (1) 150 mg b.i.d. for patients over the age of 12, (2) 4.0 mg/kg b.i.d. for patients under the age of 12; (3) 2.0 mg/kg b.i.d. for patients older than age 12 with body weight less than 50 kg

TABLE OF CONTENTS:

INTRODUCTION: The applicant is seeking approval of 150 mg lamivudine tablets and 10 mg/mL oral solution (Epivir™). This NDA application is to support the approval of Epivir™ tablets and oral solution for the treatment of HIV-infection in combination with Retrovir®. The proposed regimen for adults and adolescents (12 years and older) is 150 mg twice daily administered in combination with Retrovir®. The proposed oral dose of Epivir™ in pediatric patients (3 months to 12 years of age) is 4 mg/kg twice daily (up to a maximum of 150 mg b.i.d.) administered in combination with Retrovir®. Section 6 of this NDA, the Human Pharmacokinetics and Bioavailability Section, consisted of reports of 13 in vivo and 4 in vitro studies.

CHEMISTRY:

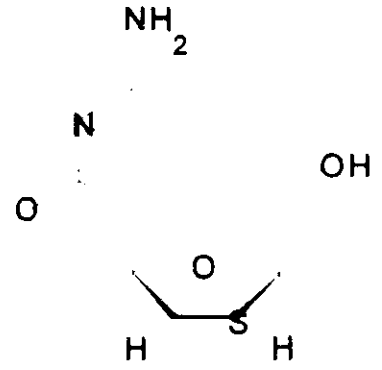
Chemical Name (CAS): (2R-cis)-4-amino-1-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]2(1H)-pyrimidinone

IUPAC Name: 4-amino-1-(2R-hydroxymethyl-[1,3]oxathiolan-5S-yl)-1H-pyrimidin-2-one

Molecular Formula: $C_8H_{11}N_3O_3S$

Molecular Weight: 229.3

Structure:



pH and pK_a Values: The pH of a 1% w/v solution in water is approximately 6.9. The pK_a determined by UV is 4.30 (protonation of NH_2).

Partition Coefficient The log of the partition coefficient between n-octanol and water measured by HPLC at pH 7.4 is -0.7 ± 0.2.

Melting Point:

Specific Rotation:

Stereochemistry: Lamivudine has two stereogenic centers and hence is one of four stereoisomers. Lamivudine is the stereoisomer with the 2R,5S configuration and is levorotatory (-). No racemization of lamivudine to its enantiomer has been observed.

Crystal forms: Lamivudine may exist as either of two pseudopolymorphs (Form I or Form II). Form I is a partial hydrate (0.2 moles water) which melts at approximately 135°C. The thermodynamically stable Form II is nonsolvated and melts at approximately 176°C. The synthesis of lamivudine is controlled to afford only Form II. Form I generally crystallizes when a significant proportion of water is present. All batches of drug substance are examined by infrared spectroscopy and confirmed by comparison with the spectrum of a Form II reference standard. Stability studies of samples stored for up to 24 months at 30°C/50% RH demonstrate that there is no conversion into the less thermodynamically stable Form I in the solid state.

FORMULATION: Manufacture, packaging, quality control of the drug substance lamivudine will be performed by Glaxo Operations UK Ltd., UK. Stage 4 will be performed by Glaxo Operations UK Ltd., Montrose, UK. Manufacture, packaging, quality control, and stability testing of Epivir™ tablets will be performed in accordance with current GMP at Glaxo Wellcome Inc., Zebulon, NC. Manufacture, packaging, quality control, and stability testing of Epivir™ oral solution will be performed in accordance with current GMP at Glaxo Pharmaceuticals UK Ltd., Liverpool, UK.

The composition of the to-be-marketed 150 mg tablets is:

Composition of Tablet Core		
Component	Theoretical mg/tablet	% w/w
lamivudine	150.00	
Microcrystalline Cellulose NF		
Sodium Starch Glycolate NF		
Magnesium Stearate NF		
Total Weight		
Composition of Coating Suspension		
Component	Theoretical mg/tablet	% w/w
Opadry® YS-1-7706-G White		
Purified Water, USP		

The composition of the to-be-marketed Epivir™ oral solution is:

Component	Nominal concentration, mg/mL
Lamivudine	
Sucrose NF	
Methylparaben NF	
Propylparaben NF	
Artificial Strawberry Flavor	
Artificial Banana Flavor	
Citric Acid (Anhydrous)	
Propylene Glycol USP	
Edetate Disodium USP	
Alcohol USP	
Purified Water USP	

Table 1A presents the formulation of all capsules used in clinical trials, and Table 1B presents the formulation of all tablets used in clinical trials (see attachments).

INDICATIONS AND USAGE: Epivir™ is indicated for the treatment of HIV-infection in

combination with Retrovir®.

DOSAGE AND ADMINISTRATION: The proposed regimen for adults and adolescents (12 years and older) is 150 mg twice daily administered in combination with Retrovir® The proposed oral dose of Epivir™ in pediatric patients (3 months to 12 years of age) is 4 mg/kg twice daily (up to a maximum of 150 mg b.i.d.) administered in combination with Retrovir®. Dose reduction is recommended in patients with renal impairment.

RENAL FUNCTION (CrCl, mL/min)	DOSING REGIMEN
≥ 50	150 mg twice daily
30-49	150 mg once daily
15-29	150 mg first dose, then 100 mg once daily
5-14	150 mg first dose, then 50 mg once daily
< 5	50 mg first dose, then 25 mg once daily

LAMIVUDINE PHARMACOKINETICS:

Absorption: The oral absorption properties of lamivudine have been studied in *in vitro* models and in humans. An *in vitro* model study using Caco-2 human intestinal cells incubated with lamivudine suggested that lamivudine has high permeability and is absorbed by a passive diffusion process (Study /92/028). The apparent permeability of lamivudine was 3.2×10^{-7} cm/sec. All formulations tested in clinical trials, tablet, capsule, and oral solution, showed rapid oral absorption with peak concentrations achieved in 0.8 to 1.5 hours. The estimated absorption half-life (harmonic mean) was determined to be 0.133 hr (about 8 minutes) by fitting a two compartment model to data from 10 HIV-positive patients (Study 1001).

Bioavailability: Absolute bioavailability values were comparable for lamivudine 100 mg formulations of an oral solution, capsule, and tablet administered to 12 HIV-positive male patients in a crossover study (Study 1003). Respective mean values ± S.D. were 87 ± 13, 88 ± 16, and 86 ± 17%. Relative to the oral solution, bioavailability was 100 and 98%, respectively, for the capsule and tablet formulations. Mean serum lamivudine concentrations versus time were plotted for the oral solution, capsule, tablet, and IV solution (Figure 1).

Compared with adult values, absolute bioavailability was lower in HIV-positive pediatric patients (n = 52, age 5 months to 16 years), with an arithmetic mean ± S.D. of 69 ± 28% (Study 2002). Absolute bioavailability data showed wide variability in pediatric patients, but mean values were similar for the three formulations used: capsule (n = 25), 1 mg/mL oral solution (n = 11), and 10 mg/mL oral solution (n = 16). Respective arithmetic mean ± S.D. values for the capsule, 1 mg/mL oral solution, and 10 mg/mL oral solution were 74 ± 31, 65 ± 22, and 64 ± 28%. Individual values of F ranged from 19 to 115%. This reviewer plotted absolute bioavailability versus age, using data from Studies 2002, 1001 and 1003.

(Figure 2A, attached). The applicant generated a similar plot, and fit a LOESS curve to the data to show the trend (Figure 2B, attached). As shown in Figures 1A and 1B, absolute bioavailability was lower in pediatric patients than in adults.

Relationship of absorption to dissolution: A polyexponential model was fit to data from Study 1003 using (Manuscript submitted to *J. Clin. Pharm.* for publication). The intercepts and exponents thus generated were used to deconvuolute the rate input function from the oral solution, tablet, and capsule serum concentration data using PCDCON. Plots of the amount absorbed versus time and input rate versus time were obtained from PCDCON (Figures 3A and 3B, attached). However, a number of negative input rates were obtained and were excluded from the input rate versus time plots. The applicant concluded that differences are due primarily to variability in absorption rather than differences due to formulation. The absolute bioavailability of lamivudine as measured by the maximum % of dose absorbed was consistent with the calculated values based on the traditional ratio of AUC_{ORAL}/AUC_{IV} (Figure 3A). Inspection of the superimposed input rate versus time plots (Figure 3B) showed similar profiles for the solution, capsule, and tablet. The parameters MAT and MDT were calculated. The parameter mean absorption time (MAT) was defined as the lamivudine MRT following administration of solution minus the lamivudine MRT following IV dosing. The parameter MDT was defined as the lamivudine MRT following administration of the oral solid minus the MRT following administration of the oral solution. MDT values for both the capsule and tablet were virtually 0, suggesting that dissolution from the capsule and tablet is very rapid and that absorption of lamivudine is due to intrinsic absorption properties rather than to dissolution.

	F, %			T_{max}, hr			MAT	MDT	
	solution	capsule	tablet	solution	capsule	tablet	solution	capsule	tablet
mean	87	88	86	0.9	0.88	0.9	1.32	0.03	-0.11
S.D.	13	16	17	0.23	0.25	0.41	1.35	1.02	0.78
% C.V.	15	19	19	25	29	45	103	3716	-693
median	87	90	90	0.75	0.75	0.75	1.45	-0.05	-0.12
min	67	61	53	0.75	0.5	0.5	-2.52	-1.65	-1.86
max	101	108	105	1.5	1.5	1.5	2.72	1.89	1.11
* in vivo* mean absorption time, MAT = MRT (solution) - MRT (IV)									
in vivo mean dissolution time, MDT = MRT (oral solid) - MRT (solution)									

Effect of food: The effect of food on lamivudine pharmacokinetics in 12 HIV-positive male patients was investigated (Study 1001). Lamivudine was administered either following an overnight fast (with the fast broken 4 hours post-dosing), or immediately after a standard meal (2 scrambled eggs, 2 slices bacon, 4 oz hash browns, 2 pieces of toast with butter, 8 oz whole milk). The dose administered was 50 mg, given as two 25 mg capsules. The meal provided 1267 kcal,

of which 60% of the total kcal were provided as fat

The administration of food with lamivudine significantly decreased C_{max} (40±23%) and delayed T_{max} by 2.28±1.22 hr. There was no significant effect on systemic exposure

Treatment	C_{max} (ng/mL)	T_{max} (hr)	$AUC_{0-\infty}$ (ng*hr/mL)	$T_{1/2}$ (hr)	f_e (% dose)	CL_R (mL/min)
Fasting	513±215	0.88±0.25	1677±427	3.3±0.3	69.7±10	345±69
Fed	273±56	3.15±1.27	1548±249	3.0±0.2	68.6±11	374±54

The 25 mg capsule used in the food effect study was an investigational formulation. The composition of the 25 mg capsule formulation differed markedly from that of the to-be-marketed 150 mg tablet formulation:

Capsule formulation, Batch No. F91/105B, used in food effect study NUCA1001							
Strength	Fill wt.	Lamivudine		Microcrystalline Cellulose		Magnesium Stearate	
		Quantity	%	Quantity	%	Quantity	%
25 mg	310 mg	25 mg	9.0	254 mg	90.5	1.4 mg	0.5

To-be-marketed tablet formulation, Batch No. A94B107, used in BE Study NUCA1004								
Compress Wt	Lamivudine		Na Starch Glycollate		Microcrystalline Cellulose		Magnesium Stearate	
	Quantity	%	Quantity	%	Quantity	%	Quantity	%
300 mg	150 mg	50.00	9 mg	3.00	139	46.25	2.25	0.75

Distribution: Results of clinical pharmacokinetic studies showed that lamivudine is widely distributed throughout the body with modest penetration into the central nervous system. The mean ± S.D. volume of distribution ($V_{D_{area}}$) was 1.3 ± 0.42 L/kg in 20 asymptomatic HIV-positive male patients (Study 1001), suggesting that lamivudine distributes into extravascular spaces. In HIV-positive pediatric patients (Study 2002), V_{ss} values were slightly greater than those of adults in children age 2 and younger, and comparable to adult values in children older than age 2-3. A plot of V_{ss} versus age using data from adult and pediatric patients was generated by the applicant and is attached (Figure 4). Volume of distribution was independent of dose.

Binding of lamivudine to plasma proteins was low (< 36%). In an *in vitro* study using plasma and red cells from a single healthy human volunteer (Study GDM/91/010), the percentage of lamivudine bound to plasma proteins was 36% at a concentration of 0.1 µg/mL. Percentage binding was less than 10% at concentrations of 1, 10, and 100 µg/mL. At lamivudine

concentrations from 0.01 to 100 µg/mL, the percentage of lamivudine in whole blood associated with red cells was moderate (53-57%) and whole blood and plasma lamivudine concentrations were similar, suggesting that lamivudine does not concentrate in red cells. Protein binding would be expected to be low at the proposed dosing regimens. The lower concentrations used in these in vitro studies are comparable to C_{max} values observed at the proposed adult and pediatric dose in clinical trials (about 1.6-1.9 µg/mL) and to C_{ss} anticipated at the proposed adult dosing regimen (0.6 µg/mL).

Cerebrospinal fluid (CSF) samples and simultaneous serum samples were obtained in two studies at approximately 2-4 hours post-dosing. In 6 HIV-positive adult patients with ARC receiving lamivudine at doses from 4 to 10 mg/kg b.i.d. (Study 2001), the CSF/serum ratio ranged from 4 to 8%. In 38 HIV-positive pediatric patients (Study 2002), the lamivudine CSF/serum ratios averaged about 10% in patients receiving from 2.0 to 10.0 mg/kg b.i.d. and about 20% in patients receiving 0.5 or 1.0 mg/kg b.i.d. In 10 pediatric patients receiving the proposed dose of 8 mg/kg/day, the concentration of lamivudine in the CSF was 124.7 ± 90.2 ng/mL, representing 14.2 ± 7.9% of serum concentrations. CSF concentrations increased in proportion to dose.

Elimination: Following administration of a single IV dose ranging from 0.25 to 10.0 mg/kg, clearance in 20 HIV-positive asymptomatic adults averaged 0.32 ± 0.052 L/hr*kg (Study 1001). Renal clearance averaged 0.23 ± 0.014 L/hr*kg. Renal clearance averaged 71 ± 16% of systemic clearance. In pediatric patients (Study 2002), total clearance (normalized to body weight) decreased with increasing age, approaching adult values between ages 10-12. A plot of total clearance versus age was generated by this reviewer using data from Studies 2002, 1001, and 1003 (Figure 5A, attached). The applicant generated a similar plot and fit a LOESS curve to the data to show trends (Figure 5B, attached). In pediatric patients, the percentage of total clearance represented by renal clearance was similar across all ages, and averaged 60 ± 28%.

Metabolism: The only metabolite of lamivudine identified was the trans-sulfoxide metabolite. The metabolite was measured in urine but not in serum. Lamivudine was found to be excreted primarily unchanged in urine within 24 hours of dosing.

The intracellular metabolism of lamivudine to the presumed active moiety lamivudine triphosphate was described using HIV-infected and mock-infected PHA-stimulated human peripheral blood lymphocytes (PBLs) incubated with ^{3}H-lamivudine. ^{3}H-labeled lamivudine monophosphate, diphosphate, and triphosphate were identified in both HIV-infected and uninfected PBLs. The formation of ^{3}H-labeled phosphorylated lamivudine intracellular intermediates increased linearly at lamivudine concentrations up to 10 µM (Study GVR/93/013). The rate of formation of phosphorylated lamivudine intermediates was slower at higher concentrations. The concentration of 10 µM is equivalent to 2290 ng/mL, which is comparable to the range of C_{max} values (1600-1900 ng/mL) observed in patients receiving multiple dose regimens which approximated the proposed dose of 150 mg b.i.d. The intracellular $T_{1/2}$ of the 5'-triphosphate metabolite of ^{3}H-

labeled lamivudine ranged from 10.5 to 15.5 hours (Study GVR/91/005). The initial dosing interval of 12 hours in Phase I/Ii trials was selected based on this finding.

Excretion: The major route of elimination of lamivudine is by excretion of unchanged drug in the urine. In 20 asymptomatic HIV-positive males (Study 1001), 76.6 ± 11.5% of the dose was excreted in the urine following a single IV dose ranging from 1.0 to 8.0 mg/kg (24 hour collection). In 59 HIV-positive adult patients with ARC receiving oral lamivudine from 0.25 to 10.0 mg/kg b.i.d. for 15 days (Study 2001), the mean ± S.D. percentage of lamivudine excreted unchanged in urine (f_e) was 69.7% (12 hour collection). Following a single oral dose of lamivudine ranging from 0.25 to 10.0 mg/kg, f_e was 51.6 ± 23.4% (n=71), and an additional 4.91 ± 1.31% (f_e^M) of the administration of the administered lamivudine dose could be accounted for in the urine as the trans-sulfoxide metabolite of lamivudine (n=12, 12 hour collection). No ADME study using ^{14}C-labeled lamivudine was conducted in humans, so the fate of the remainder of the dose is not known.

Renal clearance of lamivudine is greater than the glomerular filtration rate, suggesting that an active secretory process or ionic trapping is involved. Studies performed in the isolated perfused rat kidney model (Study No. UCP/93/012, Vol. 1.37, submitted to Section 2, Nonclinical Pharmacology and Toxicology, of the NDA) showed that probenecid had no effect on but trimethoprim inhibited renal elimination of lamivudine, suggesting that a significant extent of lamivudine elimination occurs through the organic cation transport system.

Dose-proportionality: Dose-proportionality of lamivudine C_{max} and AUC was investigated in single and multiple dose studies in adult HIV-positive patients. Study 1001 employed a crossover design to investigate single-dose pharmacokinetics of IV and orally-administered lamivudine at doses ranging from 0.25 to 8.0 mg/kg. C_{max} and $AUC_{0-\infty}$ increased in proportion to doses following IV administration. However, $AUC_{0-\infty}$ did not increase in proportion to dose in patients receiving the same doses orally. Mean dose-normalized values of $AUC_{0-\infty}$ were higher at the doses of 0.25 and 1.0 mg/kg than at the doses of 2.0, 4.0, and 8.0 mg/kg. It is possible that the lack of dose-proportionality was due to differences in formulations, as the 0.25 and 1.0 mg/kg groups received 2.5 and 10 mg capsules, whereas the 2.0, 4.0, and 8.0 mg/kg groups also received 100 mg capsules. S.D. values were not reported by the applicant.

Route		Study 1001, single dose				
		0.25	1.0	2.0	4.0	8.0
Intravenous	$AUC_{0-\infty}$, ng*hr/mL	751.7	3311.3	6707.4	12962.2	22790.7
	C_{max}, ng/mL	386	1615	3301	5620	10560
Oral	$AUC_{0-\infty}$, ng*hr/mL	695.8	3176.8	4574.5	9225.8	17953.9
	C_{max}, ng/mL	227	1263	1725	2646	5815

In Studies 2001 and 2001, lamivudine was given orally b.i.d. for 15 days at doses

ranging from 0.25 to 10.0 mg/kg. Sampling was to 12 hours and 8 hours in Studies 2001 2001, respectively. In both studies, AUC_{0-t} and C_{max} increased in proportion to dose in patients given this regimen for 15 days.

Study 2001, Day 15, oral lamivudine given b.i.d						
Dose Level, mg/kg	0.25	0.5	1.0	2.0	4.0	6.0	10.0
N	10	9	12	9	8	10	12
AUC_{0-12hr} ng*hr/mL	671±160	1507±408	2679±902	7821±2105	10185±3101	15720±5643	29109±9055
C_{max}, ng/mL	178±49	467±211	663±335	1914±493	2722±1211	4216±2367	7777±2414

Study 2001, Day 15, oral lamivudine given b.i.d						
Dose level, mg/kg	0.25	0.5	1.0	2.0	4.0	6.0	10.0
N	10	11	7	6	12	8	5
AUC_{0-8hr} ng*hr/mL	694±198	1231±615	3637±1731	4944±1744	9783±2858	15071±3819	23511±6898
C_{max}, ng/mL	247±83	375±175	1029±336	1685±801	3065±1339	3885±952	7368±3003

Plots showing median serum lamivudine concentrations versus time at the oral doses administered in Study 2001 are attached (Figures 6A and 6B). In HIV-positive adults with ARC (Study 2001), the parameters T_{max}, λ_z, $T_{1/2}$, CL_R, f_e, Clo, and V_{SS}/F were dose-independent at doses ranging from 0.25 to 10 mg/kg b.i.d.

	T_{max}, hr	λ_z, hr-1	$T_{1/2}$, hr	CL_R, L/hr	fe	CL_o, L/hr	V_{SS}/F, L
Day 1 (N)	1.21±0.58 (87)	0.246±0.046 (87)	2.94±0.69 (87)	18.0±7.3 (71)	51.6±23.4 (71)	35.2±13.8 (87)	147±63 (87)
Day 15 (N)	1.27±0.53 (70)	0.201±0.049 (70)	3.68±1.01 (70)	18.0±8.5 (59)	69.7±30.1 (59)	27.7±8.5 (70)	106±41 (70)

No studies evaluated dose-proportionality using a fixed dose, although 150 mg and 300 mg fixed doses were studied in pivotal trials 3001 and 3001.

Steady-state Pharmacokinetics: It appeared that steady-state was reached by 15 days of dosing at the proposed regimen. In Study 2001, the accumulation ratio (R) was 1.48 obtained following 15 days of a b.i.d. regimen. This value agreed well with R of 1.3 calculated using λ_z of 0.116 hr-1, assuming that the clinically relevant half-life is 6 hr (see Discussion section of review). No other assessments of steady-state were performed by the applicant.

Bioequivalence Studies: For data analysis in bioequivalence studies, the applicant performed statistical analyses of ln-transformed C_{max} and $AUC_{0-\infty}$ values using SAS PROC GLM. Bioequivalence for ln-transformed C_{max} and $AUC_{0-\infty}$ was performed using the 90% confidence interval using the two one-sided test. Bioequivalence studies were conducted to compare: (1) the to-be-marketed 150 mg tablet formulation with the 300 mg tablet formulation used in pivotal clinical trials; (2) the 300 mg and 75 mg tablet formulations used in pivotal clinical trials with a 100 mg tablet formulation; (3) the 100 mg tablet formulation with an oral solution providing 100 mg lamivudine. The 100 mg tablet formulation was also used to determine absolute bioavailability.

Bioequivalence of capsule and tablet with oral solution: In 12 HIV-positive male patients (Study 1003), the 100 mg capsule (Batch No. F91/155B) and 100 mg tablet (Batch No. CS-3TC10006) formulations were bioequivalent with 100 mL of a 1 mg/mL oral solution (Batch No. CS-3TC10004).

	$AUC_{0-\infty}$, ng*hr/mL			C_{max}, ng/mL		
Comparison	Ratio of means	90% C.I.	P Value	Ratio of means	90% C.I.	P Value
Capsule:Solution	1.00	0.99-1.01	0.8	1.00	0.98-1.02	0.5
Tablet:Solution	0.98	0.98-1.00	0.7	0.98	0.98-1.01	0.7

Bioequivalence of tablet with capsule formulation: This reviewer also evaluated the bioequivalence of the tablet versus the capsule formulation. The 100 mg tablet was bioequivalent to the 100 mg capsule.

	$AUC_{0-\infty}$, ng*hr/mL			C_{max}, ng/mL		
Comparison	Ratio of means	90% C.I.	P Value	Ratio of means	90% C.I.	P Value
Tablet:Capsule	0.97	0.92-1.06	0.6	0.92	0.82-1.06	0.4

Bioequivalence of tablet formulations used in pivotal clinical trials: In 24 HIV-positive asymptomatic male patients (Study 1006), four lamivudine 75 mg tablets (Batch No. A93B7) and one lamivudine 300 mg tablet (Batch No. A93B6) were bioequivalent to three lamivudine 100 mg tablets (Batch No. A92B31). The formulation of the 100 mg tablets, Batch No. A92B31 was the same as that of the 100 mg tablets used in Bioequivalence Study No. 1003, Batch No. CS-3TC10006. The 75 and 300 mg capsule formulations (Batches A93B7 and A93B6, respectively) were used in pivotal clinical trials 3002 and 3002

Comparison	$AUC_{0-\infty}$, ng*hr/mL			C_{max}, ng/mL		
	Ratio of means	90% C I	P Value	Ratio of means	90% C I	P Value
4 x 75 mg tab: 3 x 1	1 04	0 96-1 13	0 459	1 03	0 90-1 19	0 683
1 x 300 mg tab: 3 x 1	1 01	0 93-1 10	0 783	1 08	0 94-1 24	0 348

Bioequivalence of four 75 mg tablets with one 300 mg tablet: This reviewer also evaluated the bioequivalence of four 75 mg tablets compared with one 300 mg tablet. Four 75 mg tablets were bioequivalent to one 300 mg tablet:

Comparison	$AUC_{0-\infty}$, ng*hr/mL			C_{max}, ng/mL		
	Ratio of means	90% C I	P Value	Ratio of means	90% C I	P Value
4 x 75 mg tab: 1 x 300 mg tab	1 02	0 96-1 11	**	0 96	0 84-1 08	**
** not calculated						

Bioequivalence of commercial with clinical trial formulations: The to-be-marketed 150 mg tablet formulation (Batch No. A94B107) was bioequivalent with the 300 mg tablet (A93B38) in 24 healthy male patients (Study 1004). The batch size of the 150 mg tablets used in Study 1004 was 400 kg, representing 100% of a full-scale batch. The 300 mg tablets investigated in Bioequivalence Studies 1006 and 1004 were from batches used in pivotal clinical trials 3(3002.

parameter	treatment	geometric mean	point estimate	90% C I	P value
$AUC_{0-\infty}$ ng*hr/mL	2 x 150 mg 300 mg	10478 10555	0 99	0 96-1 03	0 733
C_{max} ng/mL	2 x 150 mg 300 mg	3397 3279	1 04	0 96-1 11	0 406

Figures 7A and 7B compare serum concentration-time plots for the 150 mg to-be-marketed tablet and the 300 mg tablet used in the pivotal clinical trials.

Special Populations:

Gender: The effects of gender on the pharmacokinetic parameters $CL_{/F}$, $T_{1/2}$, and V_{ss}/F were evaluated by stratifying the data obtained in Study 2002. There appeared to be no gender effects on these parameters. No statistical tests were performed because of the small number of women enrolled (7 women out of 87 patients). Adult females were not enrolled in any of the other pharmacokinetic studies submitted to this NDA.

Pediatric Patients: Two Studies, 2002 and 2005, characterized pharmacokinetics

in pediatric patients. Study 2002 investigated absolute bioavailability, dose-proportionality, steady-state pharmacokinetics, and CSF penetration in patients ranging in age from 5 months to 16 years. For the oral dosing part of the study, patients received either a 1 mg/mL solution, a 10 mg/mL solution, or capsules. Absolute bioavailability in pediatric patients was discussed above. CL, CL_0, V_{SS}, and V_{SS}/F (per unit body weight) decreased with increasing age and body weight. The relationships appeared to be nonlinear. Plots of these parameters versus age, body weight and surface area are attached (Figures 4, 5A and 5B, 8A and 8B, 9A through 9D, 10A and 10B, 11). Although data are quite variable, it appears that CL approached adult values in children beginning at ages 10-12 and that V_{SS} approached adult values in children beginning at ages 2-3. $AUC_{0-\infty}$ and C_{max} (n = 58) increased in proportion to single IV doses ranging from 1.0 to 20.0 mg/kg.

Dose, mg/kg	1.0	2.0	4.0	8.0	12.0	20.0
N	5	9	12	12	9	11
$AUC_{0-\infty}$ ng*hr/mL	1627 ± 959	1796 ± 425	4462 ± 2850	8525 ± 3735	11392 ± 4845	18628 ± 5354
C_{max} ng/mL	707 ± 289	1246 ± 346	2443 ± 1562	4241 ± 2713	5525 ± 2993	8741 ± 3261

$AUC_{0-\infty}$ and C_{max} (n = 60) increased in proportion to single oral doses ranging from 1.0 to 20.0 mg/kg.

Dose, mg/kg	1.0	2.0	4.0	8.0	12.0	20.0
N	7	9	10	12	9	13
$AUC_{0-\infty}$ ng*hr/mL	645 ± 249	1547 ± 530	2726 ± 1405	5056 ± 2286	7941 ± 4700	14264 ± 7095
C_{max} ng/mL	211 ± 86	465 ± 190	711 ± 365	1153 ± 558	2292 ± 1448	4410 ± 1852
T_{max} hours	1.21 ± 0.64	1.17 ± 0.56	1.95 ± 1.21	2.04 ± 1.34	1.44 ± 0.53	1.42 ± 0.91

Plots showing median serum lamivudine concentrations versus time at the IV and oral doses administered in Study 2002 are attached (Figures 12A and 12B).

Renal clearance values were similar in orally (n=34) and IV dosed (n=25) patients. In both IV and orally dosed pediatric patients, renal clearance (expressed as L/kg) decreased with increasing age and approached adult values beginning in patients of ages 10-12.

Serum concentrations of lamivudine were determined at random times on Day 4, and pre- and post-dosing during Weeks 4 and 12 in selected patients. It appeared that steady state had been reached by Week 4 of dosing, as C_{min} values at Weeks 4 and 12 were comparable.

Pancreatitis was reported in 14 patients (14%) in Study 2002. All 14 cases were classified as serious adverse events and 13 cases were considered possibly drug related. Of the 14 cases, 13 received 8 mg/kg/day and one patient received 2 mg/kg/day. Pancreatitis occurred from 12-62 weeks of treatment. Due to limited data, it was not possible to establish a relationship between lamivudine pharmacokinetics and pancreatitis. Pharmacokinetic data were available for only 4 of these patients.

Renal Impairment: The effects of decreased renal function on the pharmacokinetics of lamivudine were investigated in 16 HIV-infected patien 1004). Patients were grouped as follows.

GROUP	N	ENTRY CRITERIA	CrCl (mL/min) mean±SD (range)
Group I Normal renal function	6	CrCl>60 mL/min	111±14 (92-129)
Group II moderate renal impairment	4	CrCl 10-29 mL/min	28±8 (18-36)
Group III end stage renal impairment	6	CrCl<10 mL/min	6±2 (4-9)

Note: CrCl values were determined using the Cockcroft and Gault equation

All patients were administered a single 300 mg lamivudine capsule. Blood and urine samples for the determination of lamivudine concentrations were collected over 48 hours. Five of six patients in group III were anuric. Lamivudine pharmacokinetic parameters are summarized (arithmetic mean±SD) below for the three groups

PARAMETER	GRP I (CrCl>60 mL/min)	GRP II (CrCl 10-30 mL/min)	GRP III (CrCl<10 mL/min)
$AUC_{0-\infty}$ (ng*hr/mL)	11008±1740	47984±18840	156905±74037
C_{max} (ng/mL)	2555±456	3593±768	5777±1182
T_{max} (hr)	1.33±0.86	1.89±1.43	2.21±1.31
CL/F (mL/min)	464.2±75.8	114.0±34.2	36.0±10.8
Ae_{ss} (mg)	219±23	115±32	10 (one patient)
CL_R (mL/min)	342.0±51.9	47.5±23.3	2.0 (one patient)

Compared with patients in Group I (CrCl > 60 mL/min), in patients with moderate renal impairment, mean $AUC_{0-\infty}$ was 336% greater, C_{max} was 41% greater, CL/F was 75% lower, and CL_R was 86% lower. Compared with patients in Group I, in patients with severe renal impairment, mean AUC was 1325% greater, C_{max} was 126% greater, and CL/F was 92% greater. CL_R was only determined for one patient with severe renal impairment.

A linear relationship between CrCl (units of mL/min) and CL/F was described by the equation

$$CL/F(mL/min) = (4.02 * CrCl) + 12.5 \qquad (R^2=0.94)$$

A lamivudine dose reduction scheme was determined by the applicant using CL/F values predicted

using the above equation. Dose reductions were determined such that the predicted total daily exposure to lamivudine would not be lower than that predicted for a patient with normal renal function (CrCl=100 mL/min) administered 150 mg BID

RENAL FUNCTION (CrCl, mL/min)	DOSING REGIMEN
>50	150 mg twice daily
30-49	150 mg once daily
15-29	150 mg first dose, then 100 mg once daily
5-14	150 mg first dose, then 50 mg once daily
5	50 mg first dose, then 25 mg once daily

The applicant simulated serum concentration-time profiles in "normal" subjects and in one subject with severe impairment (Figure 13, attached). The reviewer calculated the predicted daily lamivudine exposure (24 hour AUC) at steady state for patients with varying degrees of renal function. The predicted value assumes that patients were administered lamivudine according to the applicant's dose reduction scheme. AUC(0-τ)ss was estimated as Dose/(CL/F)

CrCl (mL/min)	DOSING REGIMEN	PREDICTED 24 HR AUCss (ng*h/mL)
100	150 mg BID	12063
50	150 mg BID	23419
49	150 mg QD	11934
30	150 mg QD	18783
29	100 mg QD	12912
15	100 mg QD	22894
14	50 mg QD	12116
5	50 mg QD	25562
4	25 mg QD	16579
0	25 mg QD	33333

Using the dose reduction scheme proposed by the applicant, the predicted daily lamivudine exposure at steady-state ranges from approximately the same AUC as in patients with normal renal function to approximately twice the AUC as in patients with normal renal function. Patients with CrCl values approaching 0 mL/min are an exception, the predicted AUC is almost three times the value predicted for patients with normal renal function. However, since the most severely renally impaired group will be on dialysis, AUC values will be less than the predicted values above

The applicant included a loading dose for patients with CrCl<30 mL/min as a part of the dose reduction scheme. They state that current data on reduction in viral load suggest that the

inhibition of viral replication by lamivudine may occur relatively rapidly with corresponding early declines in HIV RNA, thus supporting the need for adequate exposure to lamivudine via the initial dose. The applicant has not provided data to support the assertion that a specific exposure to lamivudine correlates with an early decline in HIV RNA. However, it is recognized by the reviewer that patients with moderate or severe renal impairment (mean elimination $T_{1/2}$ 14.3 and 21.6 hours, respectively) may not reach steady state conditions for up to one week. It is not possible to rule out a benefit of reaching concentrations similar to those at steady state steady state early in therapy. Thus, the reviewer supports the applicant's proposal to include a loading dose of lamivudine for patients with CrCl<30 mL/min. However, it is important that patients understand the maintenance regimen.

Based on findings from Study 1004, the dose reduction schedule proposed by the applicant for patients with renal impairment is appropriate.

Hepatic impairment: No studies were conducted to assess effect of hepatic impairment on lamivudine pharmacokinetics.

Drug interaction studies:

Zidovudine: The effects of zidovudine on lamivudine pharmacokinetics were investigated in 12 HIV-positive male and female patients 1005). Using a 4-way crossover design, each patient received the following four treatments: (A) single 200 mg zidovudine dose; (B) single 300 mg lamivudine dose; (C) 300 mg lamivudine BID for 5 doses, 200 mg zidovudine administered with the 5th dose; and (D) 200 mg zidovudine TID for 9 doses, 300 mg lamivudine administered with the 7th dose. There was no significant change in lamivudine AUC or C_{max} when lamivudine was administered concomitantly with zidovudine vs when lamivudine was administered alone.

PARAMETER	LAMIVUDINE	
	monotherapy	with zidovudine
$AUC_{0-\infty}$ (ng*h/mL)	10900±1702	11069±1361
C_{max} (ng/mL)	3191±589	3303±552
T_{max} (h)	1.11±0.52	0.93±0.35
$T_{1/2}$ (h)	6.71±1.54	6.54±0.84
CL/F_{oral} (L/h)	27.83±3.33	27.29±3.31
CL_R (L/h)	17.25±7.32	18.10±8.73
f_e (%)	0.612±0.236	0.658±0.326

Coadministration with lamivudine resulted in an increase (39 ± 62%) in zidovudine C_{max}. There was no significant change in zidovudine AUC when zidovudine was administered concomitantly with lamivudine vs. when zidovudine was administered alone. The $T_{1/2}$ for zidovudine glucuronide was significantly reduced (26%) when zidovudine was coadministered with lamivudine, however, there was no significant change in zidovudine glucuronide AUC, C_{max}, or

the AUC zidovudine/AUC zidovudine glucuronide ratio values.

TMP/SMX: The effects of TMP/SMX on lamivudine pharmacokinetics were investigated in 14 HIV-positive male and female patients using a 2-way crossover design 1007) Each patient received two treatments: (A) a single 300 mg dose of lamivudine, (B) one TMP/SMX DS (160/800 mg) QD for 5 days, 300 mg dose of lamivudine coadministered with the dose on day 5.

Coadministration of a single 300 mg dose of lamivudine with TMP/SMX (160/800 mg)for 5 days altered the pharmacokinetics of lamivudine, resulting in a 45±23% increase in $AUC_{0-\infty}$, a 29±13% decrease in CL/F and a 30±36% decrease in CL_R. The applicant suggested that these changes in lamivudine pharmacokinetics are due to inhibition of renal tubular secretion by TMP.

PARAMETER	LAMIVUDINE	
	monotherapy	with TMP/SMX
AUC(ng*h/mL)	10513±2989	15245±5753
C_{max} (ng/mL)	3105±1391	3246±1280
T_{max} (h)	1.34±0.70	1.38±0.73
V_{SS}/F (L)	191±190	146±100
$T_{1/2}$ (h)	7.30±0.93	6.26±0.74
λ_z (h^{-1})	0.097±0.013	0.112±0.012
CL/F (L/h)	32.32±18.41	22.57±11.19
CL_R (L/h)	17.16±3.88	11.03±2.49
f_e (%)	60.10±22.63	52.87±13.35

The pharmacokinetic properties of TMP and SMX were not altered by coadministration with lamivudine.

Zidovudine and didanosine: Potential interactions of lamivudine, zidovudine and didanosine were investigated in 37 patients (age 2 to 19 years) enrolled in an ongoing study 2005). All patients received lamivudine at 4 mg/kg/day in addition to: (1) zidovudine, 180 mg/m^2 and didanosine, 135 mg/m^2; (2) zidovudine, 90 mg/m^2 and didanosine, 135 mg/m^2; (3) zidovudine, 180 mg/m^2; (4) didanosine, 135 mg/m^2. Since this study used limited blood sampling up to 6 hr following dosing to determine a pharmacokinetic profile, stepwise regression analysis of data from Study 2002 was used to derive the following formula, which was used to calculate lamivudine AUC values: Lamivudine AUC = 2.51*C_2 + 6.46*C_6 + 0.973*dose (mg/kg), where C_2 = lamivudine concentration at 2 hours and C_6 = lamivudine concentration at 6 hours. Similar regression equations were used to calculate the AUC values for zidovudine and didanosine. Neither zidovudine nor didanosine had any effect on lamivudine AUC values. Lamivudine administration had no effect on AUC values of either zidovudine or didanosine. Lamivudine AUC values (normalized to an exact dose of 4 mg/kg/day) were

	Day 2 lamivudine alone	Day 3 combination dosing	Day 2 and Day 3 combined	Week 4 combination dosing
Mean	5615	5401	5509	6518
S.D.	2368	2774	2564	4743
N	37	37	74	34

The applicant also determined lamivudine AUC_{0-6hr} values using the trapezoidal rule. The applicant performed a regression analysis of AUC_{0-6hr} versus AUC of lamivudine, and concluded that the values were well correlated. The linearly fitted line was described as:

$$AUC_{0-6hr} = 0.69 * AUC + 3.65, R^2 = 0.79$$

Pancreatitis occurred in 7 patients from Study 2005

In vitro studies of drug-drug interactions: The effect of zidovudine on lamivudine phosphorylation was also investigated in PBLs *in vitro* in Study GVR/93/013 (previously described). Concentrations of zidovudine added to incubations of PBLs at concentrations ranging from 5 to 50 µM did not substantially affect the phosphorylation of lamivudine. The effects of TMP, SMX, ddI, ddC, AZT, acyclovir, probenecid, ranitidine, and cimetidine on lamivudine transport across intestinal epithelium were studied in the Caco-2 cell model in Study UCP/92/028 (previously described). (Drugs concentrations were 10 mM (ddI, ddC, ranitidine, and cimetidine) and 1.6 mM for TMP, 6.7 mM for SMX, 1.9 mM for AZT, 5.9 mM for probenecid, and 2 mM for lamivudine). None of these drugs affected lamivudine transport.

DISSOLUTION: The following dissolution specification and method are proposed by the applicant for lamivudine tablets.

Q value of at 30 minutes

Dosage form:
Apparatus type:
Temperature:
Stirrer speed:
Media:
Sampling time:
Wavelength:

This reviewer recommended that the dissolution specification be changed to: Q at 15 minutes. Lamivudine is considered a highly soluble, highly permeable drug. Under SUPAC interim guidance, this is the appropriate dissolution specification (using media of 0.1 N HCl) for a drug with these solubility and permeability properties. This reviewer communicated these concerns to the company via teleconference on 11/9/95. The company responded via telefax

(11/14/95) that it was neither reasonable nor appropriate to tighten this dissolution specification to Q = at 15 minutes. Their response included a reference to an article summarizing a presentation at a recent AAPS workshop (Skelly et al., 1993, *Pharm. Res.* 10:313). The article, which was coauthored by a number of agency staff, recommended that specifications for highly soluble, highly permeable drugs be: Q at 30 minutes in media of 0.1 N HCl. At the present time, the recommendations presented at the workshop and published in *Pharm. Res.* will be used in setting specifications. Therefore, this reviewer concurs with the applicant that the specifications be: Q at 30 minutes. The rationale for using deionized water rather than 0.1 N HCl is presented below. Dissolution data (Table 2) and profiles (Figures 14A, 14B and 14C) are attached.

Dissolution data were provided for the to-be-marketed Epivir™ formulation, 150 mg tablets, Batch No. A94B107 (Figures 14A, 14B, and 14C). This is the same batch of Epivir™ tablets used in Bioequivalence Study No. 1004, which compared the to-be-marketed 150 mg tablet formulation with the 300 mg tablet formulation used in pivotal clinical Trials 3001 '3001.

LABEL: The proposed label is attached, as well as initial and follow-up labeling comments. Labeling discussions are ongoing.

DISCUSSION: The lamivudine-TMP/SMX interaction study, used the *Pneumocystis Carinii Pneumonia* (PCP) prophylactic regimen of TMP/SMX, which is one TMP/SMX DS (160/800 mg) QD for 5 days. On day 5, a 300 mg dose of lamivudine was coadministered with TMP/SMX. Lamivudine exposure was increased under these conditions. The effects of higher regimens of TMP/SMX, such as those used in treating PCP, are not known.

There were discrepancies in the lamivudine elimination half-life values reported in various studies. The discrepancies are most likely due to differences in sampling times. The half-life ranged from

5-7 hours in studies with blood sampling to 24 hours following dosing. In studies with sampling to 8-12 hours, the half-life ranged from 2-3 hours. In a study with blood sampling to 48 hours, a mean half-life of 11.9 hours was observed. The applicant suggested that: (1) the longer half-life is a terminal half-life describing the elimination of drug from a slowly equilibrating tissue compartment and constituting only a small percentage of the total AUC, and (2) the 5-7 hour estimates of half-life best describe the clinically relevant half-life describing the vast majority of the AUC and accounting for accumulation upon repeat dosing. The reviewer concurs with the conclusion that a half-life of 5-7 hours represents the clinically relevant half-life, and observed that the accumulation ratio (R) of 1.48 obtained following 15 days of a b.i.d. regimen agreed well with R of 1.3 calculated using λ_z of 0.116 hr-1 (assuming a $T_{1/2}$ of 6 hr).

The 25 mg capsule used in the food effect study was an investigational formulation, which differed in composition from the to-be-marketed formulations. The applicant argued that lamivudine commercial tablet formulation would demonstrate similar properties to those observed for the capsule formulation following administration with food. The following reasons were given to support this argument: (1) food decreased the rate but not extent of absorption, suggesting that the mechanism was intrinsic changes in lamivudine absorption (primarily due to alterations in gastric emptying) rather than an alteration in dissolution; (2) the mean dissolution time (MDT) for the tablet and capsule was approximately equal to 0, suggesting that dissolution is not a significant factor in the absorption of lamivudine from solid oral formulations. It should be noted that both the tablet and capsule were investigational formulations and the to-be-marketed formulation was not investigated in the study of MDT. However, the Office of Clinical Pharmacology and Biopharmaceutics concurred with the applicant's position, based on the conclusion that, since lamivudine is a highly soluble, highly permeable drug, formulation changes would not significantly affect the drug's dissolution in the G.I. tract.

The applicant stated that since lamivudine activity is dependent on systemic availability rather than absorption rate, the results of the food effect study suggest that lamivudine may be given in either the fasting or fed state. The applicant based this statement on the hypothesis that lamivudine activity is related to intracellular lamivudine triphosphate concentrations, which would be driven by steady-state rather than peak levels. However, no *in vivo* studies investigating the relationship between serum lamivudine concentrations and intracellular lamivudine concentrations were conducted. The Division of Pharmaceutical Evaluation III requested that this relationship be investigated as a Phase IV commitment.

The applicant used a limited sampling strategy to determine lamivudine, zidovudine, and didanosine $AUC_{0-\infty}$ values in the pediatric combination (lamivudine-zidovudine-didanosine) study 2005). Although serum lamivudine concentrations were determined at 0.5, 1, 2, 3, 4 and 6 hours and lamivudine AUC_{0-6hr} values were calculated using the trapezoidal rule, these values were not reported or statistically analyzed. The applicant stated that this was to report AUC values consistently for lamivudine, didanosine, and zidovudine. Although the applicant reported a linear relationship between the limited sampling AUC and $AUC_{0-\infty}$ ($R^2 = 0.79$) using data from the pediatric monotherapy study (in which full pharmacokinetic profiles were generated), a formal

validation of the method used to calculate AUC by limited sampling has not been submitted to the agency.

The applicant concluded that pediatric patients require a higher dose to achieve the same exposures as adult patients, and proposed a dose of 4 mg/kg b.i.d. in pediatric patients. However, it should be noted that absolute bioavailability data in pediatric patients showed wide variability, with individual values ranging from 19 to 122%. Systemic and oral clearance data were also highly variable in pediatric patients, and appeared to approach adult values by age 10-12. Thus, it seems likely that a percentage of patients receiving the proposed 8.0 mg/kg/day dose may receive higher systemic exposures than adults receiving the 300 mg/day fixed dose (providing approximately 4.0 mg/kg/day to a 70 kg adult). However, it is also possible that a number of pediatric patients would receive sub-optimal exposure if the dose were less than 8.0 mg/kg/day, and it was observed that mean AUC values in pediatric patients receiving 8 mg/kg/day were comparable to values from adults receiving 4 mg/kg/day. The mean Day 1 $AUC_{0-\infty}$ value was 5056 ± 2286 (range 1349 to 8333 ng*hr/mL) in 12 pediatric patients receiving 8 mg/kg/day and 5375 ± 1552 (range 2596 to 8467 ng*hr/mL) in 9 adult patients receiving 4 mg/kg/day. There are adequate data supporting safety from clinical trials in adult patients receiving 300 mg b.i.d. (providing approximately 8 mg/kg/day to a 70 kg adult). Thus, based on the data available to date, this reviewer concluded that 8.0 mg/kg/day is the most appropriate dose for patients under the age of 12.

Pancreatitis was associated with lamivudine treatment in pediatric patients enrolled in Studies 2002 and 2005. In study 2002, pancreatitis was reported in 14 patients (14%). All 14 cases were classified as serious adverse events and 12 cases were considered possibly drug related. Pharmacokinetic data were available for only 4 of these patients. AUC values in these patients did not differ from the mean AUC values in their respective dose groups.

The following table presents available pharmacokinetic and demographic data from patients in Study 2002 who developed pancreatitis:

Patient No	Age (yrs)	Sex	body wt (kg)	mg bid	dose/day (mg/kg)	Weeks on study drug	AUC_{PO} (ng*hr/mL)	CL_O (L/hr*kg)	F (%)	Treatment
										d

** Data not available as of this writing.

There was nothing remarkable about either AUC_{0-} or CL values in the four patients for which pharmacokinetic data were available. In Study 2002, the mean ± S.D. AUC_{0-} for all 10 patients receiving 2 mg/kg/day orally was 1547 ± 530 ng*hr/mL. The mean ± S.D. AUC_{0-} for all 12 patients receiving 8 mg/kg/day orally was 5056 ± 2286 ng*hr/mL. However, pharmacokinetic parameters were obtained on Day 2 of this study, and pancreatitis did not occur in these patients until many weeks or over a year of therapy had transpired. Thus, the pharmacokinetic evaluations on Day 2 may not be representative of lamivudine pharmacokinetic profiles at the time at which pancreatitis occurred.

In study 2005, pancreatitis was reported in 7 patients. All 7 cases were classified as serious adverse events and considered possibly drug related. Pharmacokinetic data was available for only 4 of these patients:

Patient No.	Age (yrs)	Sex	body wt (kg)	mg bid	dose/day (mg/kg)	Weeks on study drug	AUCd2	AUCd3	AUCw4

The week 4 AUC values in patients were markedly higher than the mean AUC value for all 31 patients in this study (5056 ± 2286 ng*hr/mL). The reason for the high exposures in these patients is not known. Both patients had low body weights.

Thus, pancreatitis has occurred in approximately 15% of all pediatric patients participating in clinical trials to date. Of the patients with pancreatitis, 52% were adolescents, and 90% of these had low body weight (less than 50 kg). Although it is not possible to establish a relationship between systemic exposure and pancreatitis at this time, it was observed body weights in some patients older than age 12 were so low (two patients between the ages of 12-13 in Study 2002 had body weights of about 20 kg) that these patients could receive a total daily dose as high as 15 mg/kg/day at the proposed regimen of 150 mg fixed dose b.i.d. This reviewer concluded that it is not appropriate to administer the fixed dose 150 mg b.i.d. regimen to patients older than age 12 with low body weight.

It was recommended to the applicant via teleconference (11/9/95) that patients 12 and older with body weight < 50 kg receive a dose of 2 mg/kg b.i.d. The applicant responded that data are inadequate to conclude that this is an efficacious dose for this population. The applicant hypothesized that since development is delayed in this group, lamivudine bioavailability and clearance would be comparable to that of patients younger than age 12. Thus, this group of patients would require 8 mg/kg/day to receive the same exposures as adults and adolescents of normal body weight. For this reason, the applicant submitted a package insert stating that no dosage recommendation could be proposed for adolescents with low body weight (< 50 kg)

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23 pages
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ATTACHMENT 1

ANNOTATED LABEL

Draft of November 15, 1995

PRODUCT INFORMATION

Epivir™ Tablets (lamivudine tablets)

Epivir™ Oral Solution (lamivudine oral solution)

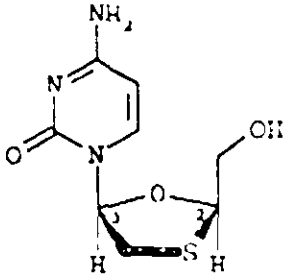
WARNING:

Epivir™ (lamivudine) is indicated for use in combination with Retrovir® (zidovudine) for the treatment of human immunodeficiency virus (HIV) infection when antiretroviral therapy is warranted based on clinical and/or immunological evidence of disease progression. This indication is based on analyses of surrogate endpoints. At present, there are no results from controlled clinical trials evaluating the effect of therapy with Epivir plus Retrovir on the clinical progression of HIV infection, such as the incidence of opportunistic infections or survival.

Patients receiving Epivir plus Retrovir may continue to develop opportunistic infections and other complications of HIV infection, and therefore should remain under close observation by physicians experienced in the treatment of patients with HIV-associated diseases.

DESCRIPTION:

Epivir™ (formerly known as 3TC) is the brand name for lamivudine, a synthetic nucleoside analogue with activity against HIV. The chemical name of lamivudine is (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one. Lamivudine is the (-)enantiomer of a dideoxy analogue of cytidine. Lamivudine has also been referred to as (-)2',3'-dideoxy, 3'-thiacytidine. It has a molecular formula of $C_8H_{11}N_3O_3S$ and a molecular weight of 229.3. It has the following structural formula



Lamivudine is a white to off-white crystalline solid with a solubility of approximately 70 mg/mL in water at 20°C.

Epivir™ Tablets are for oral administration. Each tablet contains 150 mg of lamivudine and the inactive ingredients magnesium stearate, microcrystalline cellulose, and sodium starch glycolate. Opadry YS-1-7706-G White is the coloring agent in the tablet coating.

Epivir™ Oral Solution is for oral administration. One milliliter (1 mL) of Epivir Oral Solution contains 10 mg of lamivudine (10 mg/mL) in an aqueous solution and the inactive ingredients artificial strawberry and banana flavors, citric acid (anhydrous), edetate disodium, ethanol (6% v/v), methylparaben, propylene glycol, propylparaben, and sucrose.

CLINICAL PHARMACOLOGY:

Mechanism of Action: Lamivudine is a synthetic nucleoside analogue. *In vitro* studies have shown that, intracellularly, lamivudine is phosphorylated to its active 5'-triphosphate metabolite (L-TP), which has an intracellular half-life of 10.5 to 15.5 hours. The principal mode of action of L-TP is inhibition of HIV reverse transcription via viral DNA chain termination. L-TP also inhibits the RNA- and DNA-dependent DNA polymerase activities of reverse transcriptase (RT). L-TP is a weak inhibitor of mammalian α-, β-, and γ-DNA polymerases.

Microbiology: ***Antiviral Activity In Vitro:*** The relationship between *in vitro* susceptibility of HIV to lamivudine and the inhibition of HIV replication in humans has not been established. *In vitro* activity of lamivudine against HIV-1 was assessed in a number of cell lines (including monocytes and fresh human peripheral blood lymphocytes) using standard susceptibility assays. IC_{50} values (50% inhibitory concentrations) were in the range of 2 nM to 15 μM. Lamivudine had anti–HIV-1 activity in all acute virus-cell infections tested. In HIV-1–infected MT-4 cells, lamivudine in combination with zidovudine had synergistic antiretroviral activity. Synergistic activity of lamivudine/zidovudine was also shown in a variable-ratio study.

Drug Resistance: Lamivudine-resistant isolates of HIV-1 have been selected *in vitro*. The resistant isolates showed reduced susceptibility to lamivudine and genotypic analysis showed that the resistance was due to specific substitution mutations in the HIV-1 reverse transcriptase at codon 184 from methionine to either isoleucine or valine. HIV-1 strains resistant to both lamivudine and zidovudine have been isolated.

Susceptibility of clinical isolates to lamivudine and zidovudine was monitored in controlled clinical trials. In patients receiving lamivudine monotherapy and combination therapy with lamivudine plus zidovudine, HIV-1 isolates from most patients became phenotypically and genotypically resistant to lamivudine within 12 weeks. In some patients harboring zidovudine-resistant virus, phenotypic sensitivity to zidovudine by 12 weeks of treatment was restored. Combination therapy with lamivudine plus zidovudine delayed the emergence of mutations conferring resistance to zidovudine.

Pharmacokinetics in Adults: The pharmacokinetic properties of lamivudine have been studied in asymptomatic, HIV-infected adult patients after administration of single intravenous (IV) doses ranging from 0.25 to 8 mg/kg, as well as single and multiple (b.i.d. regimen) oral doses ranging from 0.25 to 10 mg/kg.

Absorption and Bioavailability: Lamivudine was rapidly absorbed after oral administration in HIV-infected patients. Absolute bioavailability in 12 adult patients was

86% ± 16% (mean ± S.D.) for the tablet and 87% ± 13% for the oral solution. After oral administration of 2 mg/kg twice a day to nine adults with HIV, the peak serum lamivudine concentration (C_{max}) was 1.5 ± 0.5 µg/mL (mean ± S.D.). The area under the plasma concentration versus time curve (AUC) and C_{max} increased in proportion to oral dose over the range from 0.25 to 10 mg/kg.

An investigational 25 mg dosage form of lamivudine was administered orally to 12 asymptomatic, HIV-infected patients on two occasions, once in the fasted state and once with food (1,099 kcal, 75 grams fat, 34 grams protein, 72 grams carbohydrate). Absorption of lamivudine was slower in the fed state (T_{max}: 3.2 ± 1.3 hours) compared with the fasted state (T_{max}: 0.9 ± 0.3 hours); C_{max} in the fed state was 40% ± 23% (mean ± S.D.) lower than in the fasted state. There was no significant difference in systemic exposure (AUC_∞) in the fed and fasted states; therefore, Epivir™ Tablets and Oral Solution may be administered with or without food.

The accumulation ratio of lamuvidine in HIV-positive asymptomatic adults with normal renal function was 1.50 following 15 days of oral administration of 2 mg/kg b.i.d.

Distribution: The apparent volume of distribution after IV administration of lamivudine to 20 patients was 1.3 ± 0.4 L/kg, suggesting that lamivudine distributes into extravascular spaces. Volume of distribution was independent of dose and did not correlate with body weight.

Binding of lamivudine to human plasma proteins is low (<36%). *In vitro* studies showed that, over the concentration range of 0.1 to 100 µg/mL, the amount of lamivudine associated with erythrocytes ranged from 53% to 57% and was independent of concentration.

Metabolism: Metabolism of lamivudine is a minor route of elimination. In man, the only known metabolite of lamivudine is the trans-sulfoxide metabolite. Within 12 hours after a single oral dose of lamivudine in six HIV-infected adults, 5.2% ± 1.4% (mean ± S.D.) of the dose was excreted as the trans-sulfoxide metabolite in the urine. Serum concentrations of this metabolite have not been determined.

Elimination: The majority of lamivudine is eliminated unchanged in urine. In 20 patients given a single IV dose, renal clearance was 0.22 ± 0.06 L/hr*kg (mean ± S.D.) representing 71% + 16% (mean ± S.D.) of total clearance of lamivudine.

In most single-dose studies in HIV-infected patients with serum sampling for 24 hours after dosing, the observed mean elimination half-life ($T_{½}$) ranged from 5 to 7 hours. Total clearance was 0.37 ± 0.05 L/hr*kg (mean ± S.D.). Total clearance and elimination half-life were independent of dose and body weight over an oral dosing range from 0.25 to 10 mg/kg.

Special Populations: ***Adults With Impaired Renal Function:*** The pharmacokinetic properties of lamivudine have been determined in a small group of HIV-infected adults with impaired renal function, as summarized in Table 1.

Table 1: Pharmacokinetic Parameters (Mean ± S.D.) After a Single 300-mg Oral Dose of Lamivudine in Three Groups of Adults With Varying Degrees of Renal Function (CrCl>60 mL/min, CrCl = 10-30 mL/min, and CrCl<10 mL/min)

Number of subjects	6	4	6
Creatinine clearance criterion	>60 mL/min	10-30 mL/min	<10 mL/min
Creatinine clearance (mL/min)	111 ± 14	28 ± 8	6 ± 2
C_{max} (µg/mL)	2.6 ± 0.5	3.6 ± 0.8	5.8 ± 1.2
AUC_∞ (µg•h/mL)	11.0 ± 1.7	48.0 ± 19	157 ± 74
Cl/F (mL/min)	464 ± 76	114 ± 34	36 ± 11

Exposure (AUC_∞), C_{max}, and half-life increased with diminishing renal function (as expressed by creatinine clearance). Apparent total oral clearance (Cl/F) of lamivudine decreased as creatinine clearance decreased. T_{max} was not significantly affected by renal function. Based on these observations, it is recommended that the dosage of lamivudine be modified in patients with renal impairment (see DOSAGE AND ADMINISTRATION). The effects of renal impairment on lamivudine pharmacokinetics in pediatric patients are not known.

Pediatric Patients: For pharmacokinetic properties of lamivudine in pediatric patients, see PRECAUTIONS: Pediatric Use.

Geriatric Patients: Lamivudine pharmacokinetics have not been specifically studied in patients over 65 years of age.

Gender: The pharmacokinetics of lamivudine with respect to gender have not been evaluated.

Race: The pharmacokinetics of lamivudine with respect to race have not been evaluated.

Drug Interactions: Lamivudine and zidovudine were coadministered to 12 asymptomatic HIV-positive adult patients in a single-center, open-label, randomized, crossover study. No significant differences were observed in AUC_∞ or total clearance for lamivudine or zidovudine when the two drugs were administered together. Coadministration of lamivudine with zidovudine resulted in an increase of 39% ± 62% (mean ± S.D.) in C_{max} of zidovudine.

Lamivudine and trimethoprim/sulfamethoxazole (TMP/SMX) were coadministered to 14 HIV-positive patients in a single-center, open-label, randomized, crossover study. Each patient received treatment with a single 300-mg dose of lamivudine and TMP 160 mg/SMX 800 mg once a day for 5 days with concomitant administration of lamivudine 300 mg with the fifth dose in a crossover design. Coadministration of TMP/SMX with lamivudine resulted in an increase of 44% ± 23% (mean ± S.D.) in lamivudine AUC_∞, a decrease of 29% ± 13% in lamivudine oral clearance, and a decrease of 30% ± 36% in lamivudine renal clearance. The pharmacokinetic properties of TMP and SMX were not altered by coadministration with lamivudine

INDICATIONS AND USAGE:

Epivir™ in combination with Retrovir® (zidovudine) is indicated for the treatment of HIV infection when therapy is warranted based on clinical and/or immunological evidence of disease progression. This indication is based on analyses of surrogate endpoints. At present, there are no results from controlled trials evaluating the effect of Epivir plus Retrovir on clinical progression of HIV infection, such as the incidence of opportunistic infections or survival.

Description of Clinical Studies:

Adults Without Prior Antiretroviral Therapy: Two studies were conducted in patients who received up to 4 weeks of prior antiretroviral therapy. A3001 was a randomized, double-blind study comparing Epivir 150 mg b.i.d. plus Retrovir 200 mg t.i.d.; Epivir 300 mg b.i.d. plus Retrovir; Epivir 300 mg b.i.d.; and Retrovir. 366 adults enrolled with the following demographics: male (87%), Caucasian (61%), median age of 34 years, asymptomatic HIV infection (80%), and baseline CD4 cell counts of 200 to 500 cells/mm^3 (median = 352 cells/mm^3). B3001 was a randomized, double-blind study comparing Epivir 300 mg b.i.d. plus Retrovir 200 mg t.i.d. versus Retrovir. 129 adults enrolled with the following demographics: male (74%), Caucasian (82%), median age of 33 years, asymptomatic HIV infection (64%), and baseline CD4 cell counts of 100 to 400 cells/mm^3 (median = 260 cells/mm^3). Mean changes in CD4 counts through 24 weeks of treatment for studies A3001 and B3001 are summarized in Figures 1 and 2, respectively.

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Figure 1: Mean Absolute CD4 Change (cells/mm³) From Baseline in Study A3001

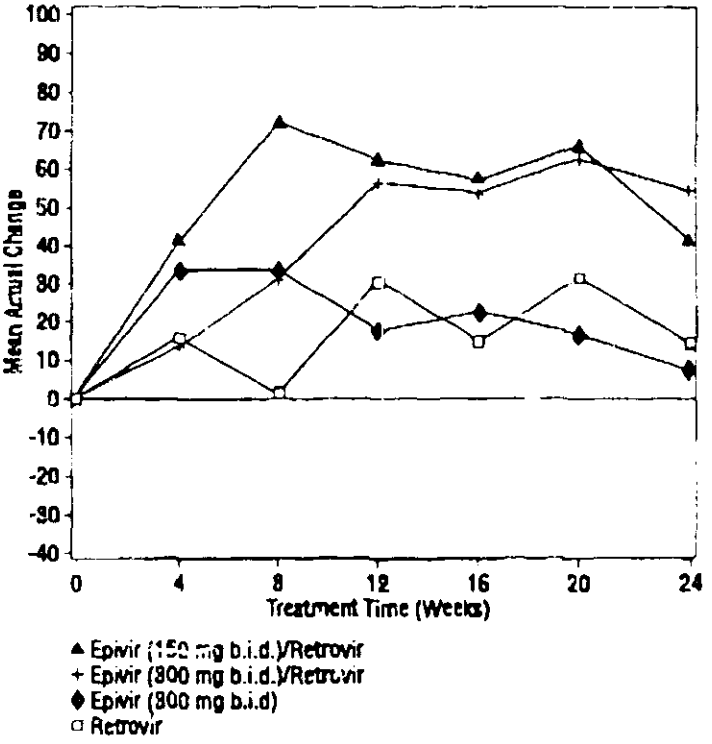

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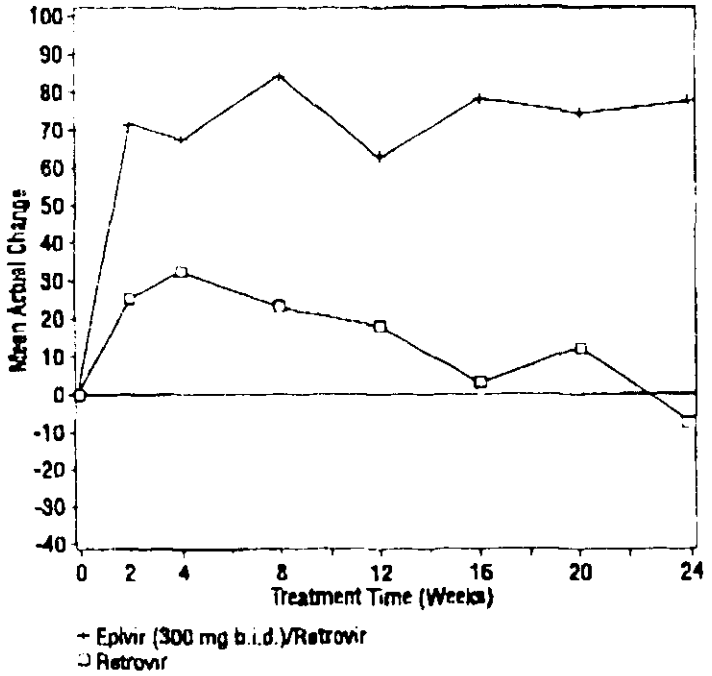
Figure 2: Mean Absolute CD4 Change (cells/mm³) From Baseline in Study B3001



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Adults With Prior Zidovudine Therapy: Two studies were conducted in patients who received at least 24 weeks of prior zidovudine therapy. A3002 was a randomized, double-blind study comparing Epivir 150 mg b.i.d. plus Retrovir 200 mg t.i.d.; Epivir 300 mg b.i.d. plus Retrovir; and Retrovir plus zalcitabine 0.75 mg t.i.d. 254 adults enrolled with the following demographics: male (83%), Caucasian (63%), median age of 37 years, asymptomatic HIV infection (58%), median duration of prior zidovudine use of 24 months, and baseline CD4 cell counts of 100 to 300 cells/mm^3 (median = 211 cells/mm^3). B3002 was a randomized, double-blind study comparing Epivir 150 mg b.i.d. plus Retrovir, Epivir 300 mg b.i.d. plus Retrovir, and Retrovir. 223 adults enrolled with the following demographics: male (83%), Caucasian (58%), median age of 36 years, asymptomatic HIV infection (53%), median duration of prior zidovudine use of 23 months, and baseline CD4 cell counts of 100 to 400 cells/mm^3 (median = 241 cells/mm^3). Mean changes in CD4 counts through 24 weeks of treatment in studies A3002 and B3002 are summarized in Figures 3 and 4, respectively.

Figure 3: Mean Absolute CD4 Change (cells/mm^3) From Baseline in Study A3002

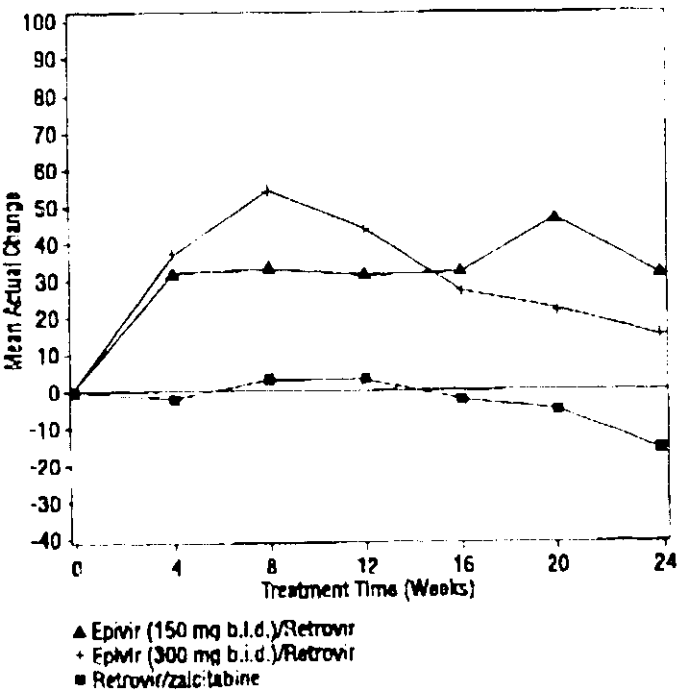
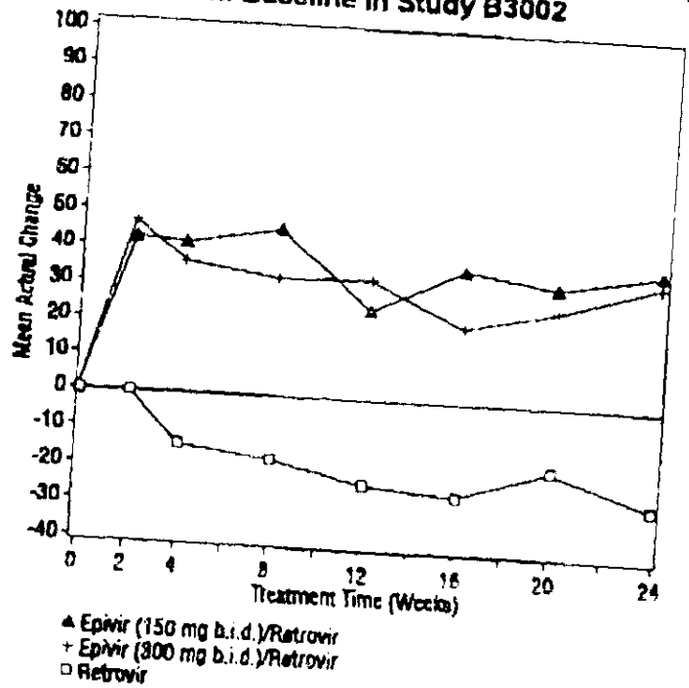


Figure 4: Mean Absolute CD4 Change (cells/mm³) From Baseline in Study B3002



HIV RNA: Mean changes from baseline HIV RNA are summarized in Table 2.

Table 2: Mean Changes in log 10 HIV RNA From Baseline in Studies A3001 and A3002 at 24 Weeks of Treatment

	Mean (± S.D.) Changes in log 10 HIV RNA from Baseline*				
	Epivir 150 mg b.i.d. + Retrovir	Retrovir	Epivir 300 mg b.i.d.	Epivir 300 mg b.i.d. + Retrovir	Retrovir + Zalcitabine
Study A3001 (antiretroviral-naive adults)	-0.9 ± 0.8	-0.3 ± 0.8	-0.4 ± 0.8	-1.0 ± 0.8	
Study A3002 (antiretroviral-experienced adults)	-0.7 ± 0.8			-0.7 ± 0.8	-0.7 ± 0.8

*THE CLINICAL SIGNIFICANCE OF CHANGES IN HIV RNA DURING THERAPY IS UNKNOWN.

CONTRAINDICATIONS:

Epivir™ Tablets and Oral Solution are contraindicated in patients with previously demonstrated clinically significant hypersensitivity to any of the components of the products.

WARNINGS:

PANCREATITIS IN PEDIATRIC PATIENTS: IN PEDIATRIC PATIENTS WITH A HISTORY OF PANCREATITIS OR OTHER SIGNIFICANT RISK FACTORS FOR THE DEVELOPMENT OF PANCREATITIS, THE COMBINATION OF EPIVIR™ AND RETROVIR® (ZIDOVUDINE) SHOULD BE USED WITH EXTREME CAUTION AND ONLY IF THERE IS NO SATISFACTORY ALTERNATIVE THERAPY. TREATMENT WITH EPIVIR SHOULD BE STOPPED IMMEDIATELY IF CLINICAL SIGNS, SYMPTOMS, OR LABORATORY ABNORMALITIES SUGGESTIVE OF PANCREATITIS OCCUR (SEE ADVERSE REACTIONS).

The complete prescribing information for Retrovir should be consulted before combination therapy with Epivir and Retrovir is initiated.

PRECAUTIONS:

Patients With Impaired Renal Function: Reduction of the dosage of Epivir™ is recommended for patients with impaired renal function (see CLINICAL PHARMACOLOGY and DOSAGE AND ADMINISTRATION).

Information for Patients: Epivir is not a cure for HIV infection and patients may continue to experience illnesses associated with HIV infection, including opportunistic infections. Treatment with Epivir has not been shown to reduce the frequency of such illnesses and patients should remain under the care of a physician when using Epivir. Patients should be advised that the use of Epivir has not been shown to reduce the risk of transmission of HIV to others through sexual contact or blood contamination.

Patients should be advised that the long-term effects of Epivir are unknown at this time.

Epivir Tablets and Oral Solution are for oral ingestion only.

Patients should be advised of the importance of taking Epivir exactly as it is prescribed.

Parents or guardians should be advised to monitor pediatric patients for signs and symptoms of pancreatitis.

Drug Interaction: TMP 160 mg/SMX 800 mg once daily has been shown to increase lamivudine exposure (AUC). The effect of higher doses of TMP/SMX on lamivudine pharmacokinetics has not been investigated (see CLINICAL PHARMACOLOGY).

Carcinogenesis, Mutagenesis, and Impairment of Fertility: Long-term carcinogenicity studies of lamivudine in animals have not yet been completed. Lamivudine was not active in a microbial mutagenicity screen or an *in vitro* cell transformation assay, but showed weak *in vitro* mutagenic activity in a cytogenetic assay using cultured human lymphocytes and in the mouse lymphoma assay. However, lamivudine showed no evidence of *in vivo* genotoxic activity in the rat at oral doses of up to 2,000 mg/kg (approximately 65 times the

recommended human dose based on body surface area comparisons). In a study of reproductive performance, lamivudine, administered to rats at doses up to 130 times the usual adult dose based on body surface area comparisons, revealed no evidence of impaired fertility and no effect on the survival, growth, and development to weaning of the offspring.

Pregnancy: ***Pregnancy Category C:*** Reproduction studies have been performed in rats and rabbits at orally administered doses up to approximately 130 and 60 times, respectively, the usual adult dose and have revealed no evidence of harm to the fetus due to lamivudine. Some evidence of early embryolethality was seen in the rabbit at doses similar to those produced by the usual adult dose and higher, but there was no indication of this effect in the rat at orally administered doses up to 130 times the usual adult dose. Studies in pregnant rats and rabbits showed that lamivudine is transferred to the fetus through the placenta. There are no adequate and well-controlled studies in pregnant women. Because animal reproductive toxicity studies are not always predictive of human response, lamivudine should be used during pregnancy only if the potential benefits outweigh the risks.

Antiretroviral Pregnancy Registry: To monitor maternal-fetal outcomes of pregnant women exposed to Epivir, an Antiretroviral Pregnancy Registry has been established. Physicians are encouraged to register patients by calling (800) 722-9292, ext.58465.

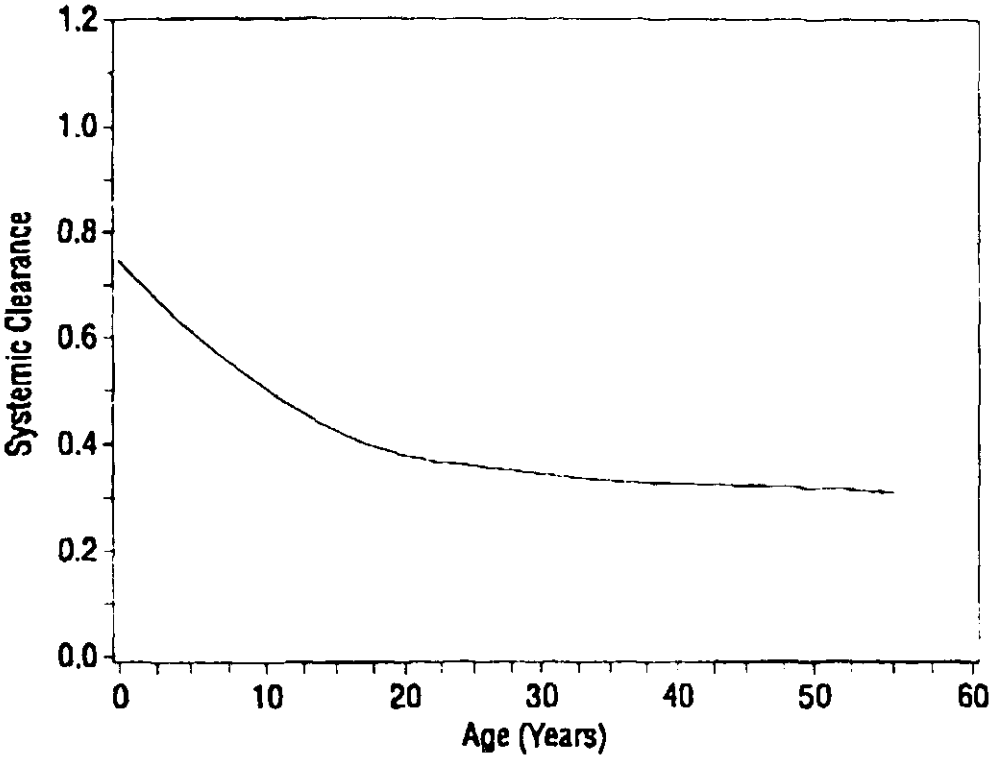
Nursing Mothers: A study in which lactating rats were administered 45 mg/kg of lamivudine showed that lamivudine concentrations in milk were slightly greater than those in plasma. Although it is not known if lamivudine is excreted in human milk, there is the potential for adverse effects from lamivudine in nursing infants. Mothers should be instructed to discontinue nursing if they are receiving lamivudine. This instruction is consistent with the Centers for Disease Control recommendation that HIV-infected mothers not breast feed their infants to avoid risking postnatal transmission of HIV infection.

Pediatric Use: THERE ARE NO DATA ON THE USE OF EPIVIR IN COMBINATION WITH RETROVIR IN PEDIATRIC PATIENTS.

Lamivudine monotherapy was studied in one open-label, uncontrolled trial (study A2002) in 97 pediatric patients with the following demographics: male (56%), Caucasian (57%), median age of 7.7 years (range: 0.4 to 17.3 years), symptomatic HIV (84%), median duration of prior antiretroviral therapy (148 weeks), and median baseline CD4 of 132 cells/mm^3. Pharmacokinetic properties of lamivudine were assessed in a subset of 57 patients (age range: 4.8 months to 16 years, weight range: 5 to 66 kg) after oral and IV administration of 1, 2, 4, 8, 12, and 20 mg/kg per day. In the 9 infants and children receiving 8 mg/kg per day (the usual recommended pediatric dose), absolute bioavailability was 66% ± 26% (mean ± S.D.), which is less than the 86% ± 16% (mean ± S.D.) observed in adolescents and adults. The mechanism for the diminished absolute bioavailability of lamivudine in infants and children is unknown.

Systemic clearance decreased with increasing age in pediatric patients, as shown in Figure 5.

Figure 5: Systemic Clearance (L/hr•kg) of Lamivudine in Relation to Age



After oral administration of 8 mg/kg per day of lamivudine to 11 pediatric patients ranging from 4 months to 14 years of age, C_{max} was 1.1 ± 0.6 µg/mL and half-life was 2.0 ± 0.6 hours. (In adults with similar blood sampling, the half-life was 3.7 ± 1 hours.) Total exposure to lamivudine, as reflected by mean AUC values, was comparable between pediatric patients receiving an 8 mg/kg/day dose and adults receiving a 4 mg/kg/day dose.

Distribution of lamivudine into cerebrospinal fluid (CSF) was assessed in 38 pediatric patients after multiple oral dosing with lamivudine. CSF samples were collected between 2 and 4 hours postdose. At the dose of 8 mg/kg/day, CSF lamivudine concentrations in eight patients ranged from 5.8% to 30.9% (mean ± S.D. of 14.2% ± 7.9%) of the concentration in a simultaneous serum sample, with CSF lamivudine concentrations ranging from 0.04 to 0.3 µg/mL.

See INDICATIONS AND USAGE: Description of Clinical Studies, WARNINGS, ADVERSE REACTIONS, and DOSAGE AND ADMINISTRATION sections.

ADVERSE REACTIONS:

Adults: Selected clinical adverse events with a ≥5% frequency during therapy with Epivir™ 150 mg b.i.d. plus Retrovir® (zidovudine) 200 mg t.i.d. compared with zidovudine are listed in Table 3.

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Table 3: Selected Clinical Adverse Events (≥5% Frequency) in Four Controlled Clinical Trials (A3001, A3002, B3001, B3002)

Adverse Event	Epivir 150 mg b.i.d. plus Retrovir (n=251)	Retrovir (n=230)
Body as a whole		
Headache	35%	27%
Malaise and fatigue	27%	23%
Fever or chills	10%	12%
Digestive		
Nausea	33%	29%
Diarrhea	18%	22%
Nausea and vomiting	13%	12%
Anorexia and/or decreased appetite	10%	7%
Abdominal pain	9%	11%
Abdominal cramps	6%	3%
Dyspepsia	5%	5%
Nervous		
Neuropathy	12%	10%
Dizziness	10%	4%
Insomnia & other sleep disorders	11%	7%
Depressive disorders	9%	4%
Respiratory		
Nasal signs & symptoms	20%	11%
Cough	18%	13%
Skin & appendages		
Skin rashes	9%	8%
Musculoskeletal		
Musculoskeletal pain	12%	10%
Myalgia	8%	8%
Arthralgia	5%	5%

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Pancreatitis was observed in 3 of the 656 adult patients (<0.5%) who received Epivir in controlled clinical trials. Selected grade 3/4 laboratory abnormalities during therapy are listed in Table 4.

337 **Table 4: Frequencies of Selected Grade 3/4 Laboratory Abnormalities**
338 **Among Adults in Four Controlled Clinical Trials (A3001, A3002, B3001, B3002)**
339

Test (Abnormal Level)	Epivir 150 mg b.i.d. Plus Retrovir % (n)	Retrovir % (n)
Neutropenia (ANC<750/mm^3)	7.2% (237)	5.4% (222)
Anemia (Hgb<8.0 g/dL)	2.9% (241)	1.8% (218)
Thrombocytopenia (platelets<50,000/mm^3)	0.4% (240)	1.3% (223)
ALT (>5.0 x ULN)	3.7% (241)	3.6% (224)
AST (>5.0 x ULN)	1.7% (241)	1.8% (223)
Bilirubin (>2.5 ULN)	0.8% (241)	0.4% (220)
Amylase (>2.0 ULN)	4.2% (72)	1.5% (133)

340 ULN=Upper limit of normal ANC=absolute neutrophil count
341 n=Number of patients assessed
342
343 Note: Higher frequencies of Grade 3/4 laboratory abnormalities were
344 reported in patients with Grade 1/2 laboratory abnormalities at baseline.
345
346 **Pediatric Patients: Limited information on the incidence of adverse events in children**
347 **receiving lamivudine monotherapy is available from one open-label, uncontrolled**
348 **study (see PRECAUTIONS: Pediatric Use section for description of study A2002). Of**
349 **97 pediatric patients, 14 patients (14%) developed pancreatitis while receiving**
350 **monotherapy with Epivir. In a second ongoing study in 47 pediatric patients (age**
351 **range: 3 months to 18 years) enrolled in an open-label evaluation of**
352 **Epivir/didanosine, Epivir/Retrovir, and Epivir/Retrovir/didanosine, 7 patients (15%)**
353 **developed pancreatitis (see WARNINGS).**
354 Paresthesias and peripheral neuropathies were reported in 13 patients (13%) in study
355 A2002 and resulted in treatment discontinuation in 3 patients.
356 Selected grade 3/4 laboratory abnormalities during lamivudine therapy in children are
357 listed in Table 5.
358

Table 5: Frequencies of Selected Grade 3/4 Laboratory Abnormalities in an Uncontrolled Phase I/II Clinical Trial of Epivir in 97 Pediatric Patients

Test (Abnormal Level)	Patients With Normal Baselines % (n)	Patients With Abnormal Baselines (Grades 1/2) % (n)
Neutropenia (ANC<750/mm^3)	22% (55)	45% (33)
Anemia (Hgb<8.0 g/dL)	2% (50)	24% (46)
Thrombocytopenia (platelets<40,000/mm^3)	0% (68)	25% (12)
ALT (>5.0 x ULN)	4% (51)	29% (42)
AST (>5.0 x ULN)	0% (29)	18% (57)
Amylase (>2.0 ULN)	3% (69)	23% (13)

ULN=Upper limit of normal ANC=absolute neutrophil count
n=Number of patients assessed

OVERDOSAGE:

There is no known antidote for Epivir™. One case in an adult ingesting 8 g of Epivir was reported; there were no clinical signs or symptoms noted and hematologic tests remained normal. It is not known whether lamivudine can be removed by peritoneal dialysis or hemodialysis.

DOSAGE AND ADMINISTRATION:

Adults and Adolescents (12 to 16 years): The recommended oral dose of Epivir™ for adults and adolescents is 150 mg twice daily administered in combination with Retrovir® (zidovudine). The complete prescribing information for Retrovir should be consulted for information on its dosage and administration.

For adults with low body weights (less than 50 kg or 110 lbs), the recommended oral dose of Epivir is 2 mg/kg twice daily administered in combination with Retrovir. No data are available to support a dosage recommendation for adolescents with low body weight (less than 50 kg).

Pediatric Patients: The recommended oral dose of Epivir for pediatric patients 3 months to up to 12 years of age is 4 mg/kg twice daily (up to a maximum of 150 mg twice a day) administered in combination with Retrovir. The complete prescribing information for Retrovir should be consulted for information on its dosage and administration.

Dose Adjustment: it is recommended that doses of Epivir be adjusted in accordance with renal function in patients older than age 16 years (see Table 6). (See CLINICAL PHARMACOLOGY section.)

Table 6: Adjustment of Dosage of Epivir in Accordance With Creatinine Clearance

Creatinine Clearance (mL/min)	Recommended Dosage of Epivir
≥ 50	150 mg twice daily
30-49	150 mg once daily
15-29	150 mg first dose, then 100 mg once daily
5-14	150 mg first dose, then 50 mg once daily
< 5	50 mg first dose, then 25 mg once daily

Insufficient data are available to recommend a dosage of Epivir in patients undergoing dialysis.

HOW SUPPLIED: Epivir™ Tablets, 150 mg, are white, modified diamond-shaped, film-coated tablets imprinted with "150" on one side and "GXCJ7" on the reverse side. They are available in bottles of 60 tablets (NDC 0173-0470-01) with child-resistant closures. **Store between 2° and 30°C (36° and 86°F) in tightly closed bottles.**

Epivir™ Oral Solution, a clear, colorless to pale yellow, strawberry-banana flavored, liquid, contains 10 mg of lamivudine in each 1 mL in plastic bottles of 240 mL (NDC 0173-0471-00) with child-resistant closures. This product does not require reconstitution. **Store between 2° and 25°C (36° and 77°F) in tightly closed bottles.**

GlaxoWellcome

Glaxo Wellcome Inc.
Research Triangle Park, NC 27709

Manufactured under agreement from BioChem Pharma Inc.
275 Armand Frappier Blvd.
Laval, Quebec, Canada H7V 4A7

Epivir™ Oral Solution Manufactured in England

U.S. Patent 5,047,407

November 1995 RL-224 (code no.)

ATTACHMENT 2

BIOPHARMACEUTICS LABELING COMMENTS

Initial Biopharmaceutics Comments for the Epivir™(lamivudine) Label
Changes and line numbers refer to the September 12 submission

1 Line 110 Delete sentence "The tablet and oral solution have comparable absolute bioavailability " This fact is evident based on the preceding sentence

2 Line 111 Delete sentence "After oral administration of 4 mg/kg C_{max} was " The sentence is not relevant because doses of 4 mg/kg will not be given clinically to adults It would be more appropriate to report C_{max} data obtained using a dose which approximates the clinically relevant dose of 150 mg

3 Line 113 The sentence beginning "The area under the plasma concentration versus time " should be changed to indicate whether the doses specified were given orally The range of C_{max} and AUC values should be included and expressed as means ± standard deviations (SD)

4 Line 115 The sentence beginning "Epivir Tablets were administered " should be altered to indicate that the dosage form used in the food effect study was an investigational 25 mg capsule formulation of lamivudine, not the final formulation (Epivir)

5 Line 115 The results of the food effect study should indicate that pharmacokinetic data (fed vs fasted) are available for 12, not 13, patients

6 Line 119 The data presented regarding the reduction in C_{max} due to food should reflect the variability of the results Please report the percent change in C_{max} as the mean ± SD

7 Line 120 Please include the mean ± SD time that T_{max} was prolonged

8 Line 122 Please state the number of patients (N) used in generating volume of distribution data following IV administration

9 Line 124 Change the first sentence to read "Binding of lamivudine to plasma proteins is low (<36%)

10 Line 131 Delete the sentence beginning "Glucuronide conjugation has not "

11 Line 134 Please state the number of patients (N) used to determine the amount of the dose excreted unchanged in the urine within 4 hours after oral administration

12 Lines 135-136 Please include mean ± SD values for total clearance and renal clearance Also please express renal clearance as a fraction of total clearance

13 Line 137 Please indicate that the half-life ranged from 5 to 7 hours in single dose studies in which plasma sampling was carried out to 24 hours

14 Line 138 Change the sentence beginning "In one study" to read "In one study in which

plasma sampling was carried out to 43 hours..." Express half-life data as the mean ± SD.

15. Line 145: The table caption should specify the criteria for the different groups: "...with varying degrees of renal function (CrCl>60 mL/min, CrCl 10-30 mL/min, CrCl<10 mL/min)"

16. Line 147: Make the following changes in table 1:

a. Between "number of subjects" and "creatinine clearance", include the variable 'group" For each group, specify the creatinine clearance criteria.

b. Include AUC values in the table.

c. $T_{1/2}$ values reported in this table were not determined in a manner consistent with $T_{1/2}$ values reported elsewhere in the label. If $T_{1/2}$ values from different studies or groups of patient populations are compared, the manner in which they are determined should be consistent.

17. Line 149: Change sentence to read: "The results show increases in exposure (AUC), C_{max}, and half-life with diminishing renal function (as expressed by creatinine clearance)."

18. Line 153: Change "reduced creatinine clearance" to "renal impairment".

19. Line 154: This entire section discussing pediatric patients should be moved to the Pediatric Use section (Line 405). Thus, Line 154 should read: ***Pediatric Patients:*** For pharmacokinetic properties of lamivudine when administered to pediatric patients, see PEDIATRIC USE.

20. Line 155: The number of pediatric patients in which pharmacokinetics were assessed should be indicated, rather than the total number of pediatric patients in the study.

21. Line 157: In the sentence beginning "The 97 patients...", The number of patients and the age range should only include patients for whom pharmacokinetics were assessed. Patients greater than 16 years old should not be included. (21 CFR 201.57 defines pediatric patients as the age group from birth to 16 years.)

22. Line 158: In the sentence beginning "Absolute bioavailability was...",

a. Indicate the age range of the patients,

b. Indicate any change in bioavailability with age, and

c. Delete the word "comparatively"

23. Line 162: Indicate the age range of the 12 pediatric patients

24. Line 165: Delete the sentence "There were no...range of doses"

25. Lines 170-172:

a. State that the distribution into CSF was assessed after multiple dosing.

b. If possible, state the concentration range of lamivudine in CSF, in addition to the % of serum concentration.

c. Indicate the time when the CSF samples were drawn relative to dosing.

26. Line 175: A drug interaction subsection should be added at the end of the CLINICAL PHARMACOLOGY section. Information from lines 355 to 369 should be moved to this section. For both studies, this section should include a very brief statement describing study design, followed by the pharmacokinetic results. Unless there was reason *a priori* to expect a drug-drug interaction, the phrase "the potential drug-drug interaction" should not be included. The sentence beginning on line 365 "Pharmacokinetic analysis..." should be condensed: "After coadministration with TMP/SMX, lamivudine AUC increased by , oral clearance increased by..., and renal clearance decreased by...". Please express the percent changes in AUC, oral clearance, and renal clearance as the means ± SDs.

27. Line 335: Change sentence "Consideration should be..." to "It is recommended that the dose of Epivir be reduced in patients with impaired renal function".

28. Line 355: As mentioned previously, the bulk of this subsection should be moved to the end of the CLINICAL PHARMACOLOGY section. The Drug Interactions subsection of the PRECAUTIONS section should state only the clinically relevant results of the drug interaction studies and refer back to the CLINICAL PHARMACOLOGY section

29. Line 520: The word "Adult" should precede "Patients"

30. Line 522: The sentence beginning "Doses of Epivir may be..." should be changed to "It is recommended that doses of Epivir be adjusted in accordance with renal function. (see Table 6)"

31. Table 6. Please simplify the first column of table 6. Since units are designated in the table heading, there is no need to repeat them throughout the table

Creatinine clearance (mL/min)
≥ 50
30-49
15-29
5-14
< 5

32. Table 6. Please explain why a loading dose is necessary for the three groups of patients with creatinine clearance values < 30 mL/min.

33. Please add a statement indicating that there are insufficient data to recommend a dosage for patients undergoing hemodialysis.

34. These are initial comments. Additional recommendations may be forwarded to the applicant as the review progresses.

BIOPHARMACEUTICS LABELING COMMENTS
NDA 20-564

The following comments refer to the proposed package insert of 11/2/95.

1. Line 40
CHANGE second sentence to read: "In vitro studies have shown that, intracellularly, lamivudine is phosphorylated to its active 5'-triphosphate metabolite (L-TP), which has an intracellular half-life of 10.5 to 15.5 hours."

2. Line 71
CHANGE sentence to read: "The pharmacokinetic properties of lamivudine have been studied in asymptomatic, HIV-infected adult patients after administration of single intravenous (IV) doses ranging from 0.25 to 8.0 mg/kg and single and multiple (b.i.d. regimen) oral doses ranging from 0.25 to 10.0 mg/kg."

3. Line 73
DELETE last sentence beginning: "Patients receiving..."

4. Line 85
Express the change in C_{max} in the fed state as the mean ± S.D.

5. Line 89
INSERT the following, to immediately follow the Absorption and Bioavailability section: "The accumulation ratio of lamivudine was 1.48 following 15 days of administration at the recommended dosage regimen in HIV-positive asymptomatic adults with normal renal function". **OK**

6. Line 93
DELETE sentence beginning: "The distribution of lamivudine in whole blood..."

7. Line 94
CHANGE sentence to read: "In vitro studies showed that, over the concentration range of 0.1 to 100 µg, mL, the amount of lamivudine associated with erythrocytes ranged from 53 to 57% and was independent of concentration.

8. Line 97
CHANGE sentence to read: " In man, the only known metabolite of lamivudine is the trans-sulfoxide metabolite. Within 12 hours after a single oral dose of lamivudine in 6 patients, 5.20 ± 1.43% (mean ± S.D.) of the dose was excreted as the trans-sulfoxide metabolite in the urine. Serum concentrations of this metabolite have not been determined."

9. Line 105
Please state the mean ± S.D. percentage of lamivudine total clearance represented by renal

clearance.

10. Line 107
CHANGE sentence to read: " In one study with extended blood sampling (through 48 hours), the terminal elimination half-life was 11.9 ± 3.3 hours (mean ± S.D.)."

11. Table 1:
DELETE all half-life values from table.
CHANGE Epivir to lamivudine in the title.

12. Line 131
Insert the following sections, to follow the ***Geriatric Patients*** section:

"***Gender:*** The pharmacokinetics of lamivudine with respect to gender have not been evaluated."
"***Race:*** The pharmacokinetics of lamivudine with respect to race have not been evaluated."

13. Line 135
CHANGE sentence to read: "Coadministration of lamivudine did result in a mean ± S.D.% increase in the zidovudine C_{max}."

14. Lines 137 through 146
CHANGE Epivir to lamivudine throughout this section.

15. Line 236
DELETE sentence beginning: "Patients with impaired renal function..."

16. Line 251
INSERT the following in the PRECAUTIONS section:
"**Drug Interactions:** TMP/SMX DS once daily has been shown to increase lamivudine exposure (AUC). The effect of higher doses of TMP/SMX on lamivudine pharmacokinetics has not been investigated. See CLINICAL PHARMACOLOGY Section."

17. Line 293
INSERT the following, to precede this section: "Systemic clearance decreased with increasing age in pediatric subjects".
Follow this sentence with a plot showing the relationship of systemic clearance to age in pediatric and adult patients.

18. Line 296
DELETE the sentences beginning "There were no significant differences in $T_{1/2}$" and "AUC and C_{max} increased in proportion to. "

19. Line 297
CHANGE sentence to read: "Total exposure to lamivudine, as reflected by mean AUC values, was comparable between pediatric patients receiving an 8 mg/kg/day dose and adults receiving a 4 mg/kg/day dose."

20. Line 302
CHANGE sentence to read: "At the dose of 8 mg/kg/day, CSF lamivudine concentrations in 8 patients were 5.6 % to 30.9 % (mean ± S.D. of 14.2 ± 7.9%) of the concentration in a simultaneous serum sample, with CSF lamivudine concentrations ranging from 0.04 to 0.3 µg/mL.

21. Line 366
INSERT the following: "For adults and adolescents with body weight < 50 kg or 110 pounds, the recommended oral dose of Epivir is 2 mg/kg twice daily administered in combination with Retrovir".

22. Line 371
DELETE sentence beginning: "Adult patients with impaired renal function..."

23. Line 372
DELETE sentence beginning: "In addition, apparent total oral clearance of lamivudine..."

24. Line 373
INSERT the following sentence: "See CLINICAL PHARMACOLOGY section."

25. Line 379
CHANGE sentence to read: "Insufficient data are available to recommend a dosage of lamivudine in patients undergoing dialysis."

BIOPHARMACEUTICS LABELING COMMENTS
NDA 20-564

The following comments refer to the proposed package insert of 11/10/95:

1. Throughout label
CHANGE mcg to µg

2. Line 82
CHANGE 8 mg/kg to 10 mg/kg

3. Line 83
CHANGE 25-mg to 25 mg (remove hyphen)

4. Line 85
INSERT the following, to follow the sentence ending: "...once with food (1267 kcal; A grams fat, B grams protein, C grams carbohydrate).

5. Line 88
CHANGE AUC to AUC_∞

6. Line 90
CHANGE sentence to read: "The accumulation ratio of lamivudine in HIV-positive asymptomatic adults with normal renal function was 1.50 following 15 days of oral administration of 2 mg/kg b.i.d."

7. Line 106
DELETE sentence beginning: "Within 4 hours after a single IV dose in 20 patients..."

8. Lines 101 to 116
CHANGE this section as follows:

Elimination: In most single-dose studies in HIV-infected patients with serum sampling for 24 hours after dosing, the observed mean elimination half-life ($T_{1/2}$) ranged from 5 to 7 hours. Total clearance was 0.37 ± 0.05 L/hr*kg (mean ±S.D.). Total clearance and elimination half-life were independent of dose and body weight over an oral dosing range from 0.25 to 10 mg/kg.

Metabolism: NO CHANGES TO THIS PORTION OF TEXT (lines 101-105).

Excretion: The majority of lamivudine is eliminated unchanged in urine. In 20 patients given a single IV dose, renal clearance was 0.22 ± 0.06 L/hr*kg (mean ± S.D.) representing 71% ± 16% (mean ± S.D.) of total clearance of lamivudine.

9. Line 122, Table 1
DELETE "Tablets" from title

10. Line 127
CHANGE sentence to read: "Exposure (AUC∞), C_{max}, and half-life increased with diminishing renal function (as expressed by creatinine clearance)."

11. Line 132
INSERT the following sentence, to precede the Pediatric Patients section: "The effects of renal impairment on lamivudine pharmacokinetics in pediatric patients are not known."

12. Line 141
Change sentence to read: "Lamivudine and zidovudine were coadministered to 12 adymptomatic HIV-positive adult patients in a single-center, open-label, randomized, crossover study."

13. Line 143
CHANGE AUC to AUC∞

14. Line 145
CHANGE sentence to read: "Coadministration of lamivudine with zidovudine resulted in an increase of 39% ± 62% (mean ± S.D.) in C_{max} of zidovudine."

15. Line 147
CHANGE sentence to read: "Lamivudine and trimethoprim/sulfamethoxazole (TMP/SMX) were coadministered to 14 HIV-positive patients in a single-center, open-label, randomized, crossover study "

16. Line 151
CHANGE this sentence to read: "Coadministration of TMP/SMX with lamivudine resulted in an increase of 44% ± 23% (mean ± S.D.) in lamivudine AUC∞, a decrease of 29 ± 13% in lamivudine oral clearance, and a decrease of 30 ± 36% in lamivudine renal clearance."

17. Line 168
Please be consistent in use of either semicolons or commas following each treatment.

18. Line 329
CHANGE Retrovir® dosage: "...plus Retrovir® (zidovudine) 200 mg t.i.d. compared with..."

19. Line 387
CHANGE sentence to read: " It is recommended that doses of Epivir be adjusted in accordance with renal function in patients older than age 16"

20. Line 391
Table 6, first column (Creatinine Clearance)
CHANGE 5 to <5

21 Line 393
CHANGE lamivudine to Epivir™

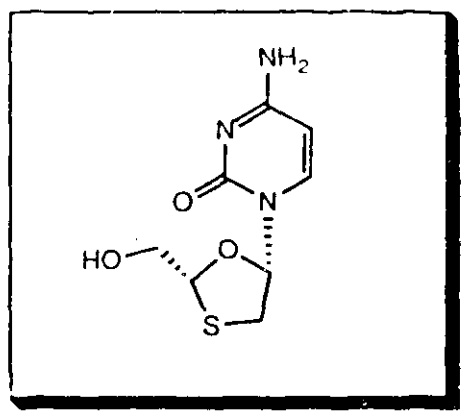
MAR 20 1995

PHARMACOLOGIST'S REVIEW

NDA 20-564
Date Submitted: December 22, 1994
Date Assigned: December 23, 1994
Date Completed: February 17, 1995
Assigned Reviewer: Pritam S. Verma, Ph.D.

SPONSOR: Glaxo Research Institute, Inc.
Five Moore Drive
Research Triangle Park, NC 27709

DRUG: Lamivudine



Chemical Names:
A: (2R-cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5yl)-(1H)-pyrimidin-2-one
B: (2R-cis)-4-amino-1-[2-hydroxy methyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone
Code Name: GR109714X
Other Name: 3TC
Molecular Formula: $C_8H_{11}N_3O_3S$
Molecular Weight: 229.3

pKa: 4.30 (protonation of NH_2)
Solubility: In water at 20°C about 70 mg/ml
Description: White to off-white crystalline solid

FORMULATION:

RELATED IND: IND

INDICATION: Treatment of HIV infection

INTRODUCTION

GR109714X (3TC; Lamivudine), the (-) enantiomer of 4-amino-1-[2-hydroxymethyl)-1, 3-oxathiolan-5-yl]-(1H)-2-pyrimidincne is a novel dideoxynucleoside analog developed as a potential treatment for individuals infected with HIV. Early research and development was carried out using the racemate, GR103665X. However, Lamivudine was found to have a more favorable cellular toxicity profile with an equivalent antiviral activity when compared with the racemate. This NDA is for Lamivudine as an antiretroviral inhibitor in combination with Retrovir or as monotherapy. Presently, the sponsor has submitted a Pre-NDA Submission of the NONCLINICAL PHARMACOLOGY AND TOXICOLOGY TECHNICAL SECTION of this NDA.

BACKGROUND

Lamivudine has been shown to be metabolized intracellularly to its 5'-triphosphate which has a half-life of 12 to 15 hours. It is proposed that, because of the long intracellular half-life, twice daily dosing in humans will allow a constant level of the triphosphate to be maintained intracellularly. The triphosphate has been shown to inhibit HIV-reverse transcriptase and act as a chain terminator upon incorporation into DNA. It is surmised that Lamivudine has a common mechanism of action with AZT, ddC and ddI in that Lamivudine is phosphorylated to its 5'-triphosphate derivative which inhibits reverse transcriptase enzyme by competing with the natural nucleotide triphosphates for binding and/or acts as an alternative substrates for reverse transcriptase leading to termination of the viral DNA chain.

For the viewpoint of safety, conceptually, a DNA chain terminator should show little or no effect on mammalian enzymes at the concentrations that inhibit the viral enzyme within cells. In this context, this compound is found to be a weak inhibitor of mammalian DNA polymerases: α, ß and γ. DNA polymerase α is though to be involved in semiconservative DNA replication; polymerase ß is involved in DNA repair; and γ polymerase is ultimately responsible for the normal functioning of mitochondria. Mitochondrial damage has been linked to peripheral neuropathy. Thus, Lamivudine may have the potential to cause peripheral neuropathy in man, although it is speculated by the sponsor that the potential is less than the other dideoxynucleoside analogues which are currently in clinical use.

NON-CLINICAL TOXICOLOGY

Toxicity Studies Summary: The following studies marked with an asterisk were conducted in accordance with the FDA Good Laboratory Practices Regulations.

I. Acute Toxicity Studies

1. **Lamivudine: Acute Oral Toxicity Study in Mice, Batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., June 1991, (M12712-WPT/91/098)***

2. **Lamivudine: Pilot Acute Intravenous Toxicity Study in Mice, Batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., October, 1991, (M12704-WPT/91/087)**

3. **Lamivudine: Acute Intravenous Toxicity Study in Mice, Batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., November, 1991, (M12708-WPT/91/110)***

4. **Lamivudine: Pilot Acute Intravenous Toxicity Study in Rats, Batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., October, 1991, (R12705-WPT/91/088)**

5. **Lamivudine: Acute Intravenous Toxicity Study in Rats, Batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., November, 1991, (R12709-WPT/91/111)***

II. Subchronic/Chronic/Carcinogenicity Studies

1. **Lamivudine: Pilot oral 30 day study of hemato-toxicity to mice, Batch No. C1758/233/1, Glaxo Group Research Ltd., Hertfordshire, England, April 26, 1993, (WPT/92/200/Study No. M13366)**

2. **Lamivudine and AZT: 36 day oral study of hemato-toxicity to mice, Batch No. C1817/126/1, Glaxo Group Research Ltd., Hertfordshire, England, April 26, 1993, (WPT/92/419/Study No. M13367)***

3. **Lamivudine: One month oral study to determine the effects, when give in combination with interferon (alpha), on the hematology of mice, Batch No. UFP0001, Glaxo Group Research Ltd., Hertfordshire, England, August 10, 1993, (WPT/92/572/Study No. M20009)***

4. Lamivudine: Palatability in the diet of mice, Batch No. C1817/116/1, Glaxo Group Research Ltd., Hertfordshire, England, June 21, 1993, (WPT/92/322/Study No. M13284)*

5. Lamivudine: 13 week oral (dietary) to mice preliminary to an oncogenicity study, Batch No. UFJ0012, Glaxo Group Research Ltd., Hertfordshire, England, March 23, 1994, (WPT/93/196/Study No. M13285)*

6. Lamivudine: A Pilot Five Day Oral Toxicity Study in AHA Rats, Batch # C1021/179/1 and C1021/181/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., April, 1991, (R12636-WPT/91/091)

7. Lamivudine: One Month Oral Toxicity Study in Rats, Batch # 1021/181/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., June, 1991, (R12637-WPT/91/104)*

8. Transmission electron microscopy of skeletal muscle from rats after 1 month oral treatment with Lamivudine, Batch No. UFJ0012, Glaxo Group Research Ltd., Hertfordshire, England, June 21, 1993, (WPT/93/185/Study No. R12637)*

9. Lamivudine: One month oral toxicity study in the rat - supplementary histopathology report, Glaxo Group Research Ltd., Hertfordshire, England, February 1994, (WPT/93/563-Study # R12637)*

10. Lamivudine: Thirteen Week Oral Toxicity Study in Rats, Batches # C1803/128/1 and C1758/226/1, Glaxo Group Research Ltd, Direzione Ricerche di Tossicologia, Glaxo S.p.a., Verona, Italy, January, 1992, (R12749-WPT/91/435)*

11. Lamivudine: Six month oral toxicity study in the rat, Batch No. C1817/126/1, Glaxo Group Research Ltd., Hertfordshire, England, April 8, 1993, (WPT/93/361/Study No. R13470)*

12. Lamivudine: Palatability in the diet of rats, Batch No. C1817/126/1, Glaxo Group Research Ltd., Hertfordshire, England, June 21, 1993, (WPT/92/323/Study No. R13287)*

13. Lamivudine: 13 week oral (dietary) rats preliminary to an oncogenicity study, Batch No. UFP 0004, Glaxo Group Research Ltd., Hertfordshire, England, December 23, 1993, (WPT/93/197/Study No. R13288)*

14. Lamivudine: Two Week Oral Toxicity Study in Dogs, Batch # C1803/152/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., November, 1991, (D12750-WPT/91/222)*

15. Lamivudine: Thirteen Week Oral Toxicity Study in Dogs, Lots # C1013/111/1, C1817/86/1 and C1817/83/1, June, 1992, Direzione Ricerche di Tossicologia, Glaxo S.p.a., Verona, Italy, (D12873-WPT/92/132)*

16. Supplementary report to: Lamivudine: Thirteen Week Oral Toxicity Study in Dogs, Lots # C1013/111/1, C1817/86/1 and C1817/83/1, June, 1992, Direzione Ricerche di Tossicologia, Glaxo S.p.a., Verona, Italy, (VTX/94/037/D12873)*

17. Lamivudine: 52 weeks oral (gavage) toxicity study of dogs, Batch No. UFJ 008, Glaxo Group Research Ltd., Hertfordshire, England, December 2, 1993 (WPT/92/407/Study No. D13604)*

18. Lamivudine: Multidose toxicity study - marmosets (seven day intravenous), Batch No. C1450/295/1, January, 1991 (WPT/90/416-Study No. P12148)

19. Lamivudine: A 14 Day Intravenous Toxicity Study in Marmosets, Batch # C1803/74/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., June, 1991, (P12678-WPT/91/079)*

III. Special Toxicity Studies

1. Lamivudine: Acute eye irritation test in the rabbit, Batch No. C1817/126/1, Glaxo Group Research Ltd., Hertfordshire, England, February 4, 1993, (WPT/92/209/Study No. L13610)*

2. Lamivudine: Acute dermal irritation test in the rabbit, Batch No. C1013/111/1, Glaxo Group Research Ltd., Hertfordshire, England, February 26, 1992, (WPT/91/325/Study No. L13056)*

3. Lamivudine: Evaluation of contact sensitizing potential in female guinea gigs using a split-adjuvant technique, Batch No. C1758/226/1, Glaxo Group Research Ltd., Hertfordshire, England, June 25, 1993, (WPT/92/207/Study No. 13034)*

4. Lamivudine: Dose finding study for anaphylaxis test in guinea pigs, Tsukuba Research Labs, Nippon Glaxo, Japan, June 7, 1994, Batch No. UFJ0012, (NTX/94/001/Study # 932001)

5. Lamivudine: Acute systemic anaphylaxis test in guinea pigs, Tsukuba Research Labs, Nippon Glaxo, Japan, June, 1994, Batch No. UFJ0012, (NTX/94/002/Study # 200661)*

IV. Reproductive and Developmental Toxicity Studies

1. Lamivudine: Effects of oral administration upon the reproductive performance of a parental dosed generation (F_0) of AHA rats and upon the peri- and post-natal development of the resulting two successive, untreated generations (F_1 and F_2), Batch No. UFP 0002, Glaxo Group Research Ltd., Hertfordshire, England, June 9, 1994, (WPT/93/210/Study No. R13738)

2. Lamivudine: Embryotoxicity and Teratogenicity Study in Rats, Batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., October, 1991, (R12768-WPT/91/196)

3. Lamivudine: Embryotoxicity and Teratogenicity Study in Rats, Batch # C1758/226/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., not dated, (R12899-WPT/91/305)*

4. Lamivudine: A further investigation into the effects of twice daily oral administration on pregnant AHA rats and their progeny in utero, (Report of findings following external and visceral or skeletal fetal examination), batch # C1758/233/1, Glaxo Group Research Ltd, Ware, Hertfordshire, England, 11 December, 1991, (WPT/91/443/R13161)*

5. Lamivudine: Embryotoxicity and teratogenicity study - rabbits. December, 1991, lot # C1803/152/1 and C1578/226/1, Glaxo Group Research Ltd., UK. (Study No. WPT/91/333/Study # L12895)

6. Lamivudine: A Second Preliminary Embryotoxicity and Teratogenicity Study in Rabbits, Lot # C1803/152/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., December, 1991, (L13030-WPT/91/334)

7. Lamivudine: Segment II Embryotoxicity and Teratogenicity Study in Rabbits, Lot # C1758/233/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., August, 1992, (L13069-WPT/92/014)*

8. Lamivudine: A further study to assess the effects of oral administration on pregnant Dutch rabbits and their offspring, Batch No. C1758/233/1, Glaxo Group Research Ltd., Hertfordshire, England, August 3, 1993, (WPT/92/184/Study No. L13280)*

9. Lamivudine: A preliminary study to assess the effects of oral administration to pregnant and lactating AHA rats and of direct oral administration to their juvenile offspring, Batch No. C1817/116/1, Glaxo Group Research Ltd., Hertfordshire, England, October 5, 1993, (WPT/92/404/Study No. R13609)*

10. Lamivudine: The effects of oral administration to pregnant and lactating AHA rats and of direct oral administration to their juvenile offspring, Batch No. UFP0001, Glaxo Group Research Ltd., Hertfordshire, England, March 11, 1993, (WPT/93/165/Study No. R13739)*

V. Mutagenicity Studies

1. Lamivudine: Genetic toxicity study - microbial mutagenicity screen, Batch # C1034/185/1, Glaxo Group Research Ltd., Hertfordshire, England, September, 1990 (WPT/90/238)*

2. Lamivudine: Microbial Mutagenicity Screen, Batches # C1460/129/4, B1916/47/2, C1619/18/3 and B1916/52/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., October, 1990, (U12455-WPT/90/240)

3. Lamivudine: Microbial mutagenicity screen, Batch No. UFP0003, Glaxo Group Research Ltd., Hertfordshire, England, March 15, 1994, (WPT/93/246/Study No. V14001)*

4. Lamivudine: Genetic toxicity study - mouse L5178Y cell line mutagenicity assay, Batch # C1034/185/1, Glaxo Group Research Ltd., Hertfordshire, England, June, 1991 (Report # WPT/90/271)

5. Lamivudine: An Assessment of Mutagenic Potential in the Mouse Lymphoma TK Locus Assay, Batch # 90G05B26, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., May, 1991, (V12638-WPT/91/018)*

6. **Lamivudine: Genetic Toxicity Study in BALB/c 3T3 Mouse Embryo Cell Assay, Lot # C1758/229/1, June, 1992, (M13156-UTX/92/018/Study # M13156)***

7. **Lamivudine: Pharmaceutical data to support study M1315618. Genetic Toxicity Study in BALB/c 3T3 Mouse Embryo Cell Assay, Lot # C1758/229/1, June, 1992, (WPT/94/296)**

8. **Lamivudine: Cytogenetic Evaluation Using Cultured Human Lymphocytes, Batch # 1916/47/2 and 1916/52/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., April, 1991, (Y12629/Y12850-WPT/90/393)**

9. **Lamivudine: Metaphase analysis in human lymphocytes in vitro, Batch No. UFP0003, Glaxo Group Research Ltd., Hertfordshire, England, July 29, 1993, (WPT/93/120/Study No. V14104)***

10. **Lamivudine: Genetic toxicity study - rat micronucleus test, Lot # 1916/52/1, Glaxo Group Research Ltd., Hertfordshire, England, June 1991, (WPT/90/275)**

11. **Lamivudine: Lack of induction of metaphase chromosome damage in Sprague Dawley CD rat bone marrow cells following oral administration, Batch No. UFP0003, Glaxo Group Research Ltd., Hertfordshire, England, April 30, 1993, (WPT/93/195/Study No. R14091)***

12. **Lamivudine: Genetic toxicity study - rat unscheduled DNA synthesis, Batch # c1034/200/1, Glaxo Group Research Ltd., Hertfordshire, England, June 1991 (WPT/90/408/Study No. R12395)***

13. **Lamivudine: Lack of activity in male rat liver unscheduled DNA synthesis (UDS) assay following oral administration, Batch No. C1953/195/4, Glaxo Group Research Ltd., Hertfordshire, England, March 25, 1994, (WPT/93/536/Study No. R20104)***

Toxicity Studies Review:

I. Acute Toxicity Studies

1. Acute Oral Toxicity Study in Mice, Batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., June 1991, (M12712-WPT/91/098)*

A group of 10 male and 10 female B6C3F1 mice were fed by gavage Lamivudine in acetate buffer, pH 3.7, at doses of 2 g/kg two

times, four hour apart. A group of 5 males and 5 females served as vehicle controls. The animals were observed for signs of ill health throughout the study and individual body weights were recorded at days 1, 3, 8 and 15. On day 3, five males and five females from each treatment group were killed. The lungs, hearts, stomachs, kidneys, livers and small intestines of each animal were preserved in buffered formalin and examined microscopically. The remainder of the animals were killed on day 15 and similarly necropsied.

An increase in sexual behavior was recorded in all the treated males following both doses with frequent mountings for ten min after the first and twenty min following the second dose. All the treated animals exhibited an increase in general activity for a period of up to two hr after dosing. There were no other pathological findings. The maximum non-lethal dose was therefore greater than 2 g/kg bid.

2. Pilot Acute Intravenous Toxicity Study in Mice, Batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., October, 1991, (M12704-WPT/91/087)

Two male and two female B6C3F1 mice were administered Lamivudine in acetate buffer, pH 3.7, intravenously at a dose of 2 g/kg. The animals were watched for adverse signs for the first six hr after dosing and twice daily for the remainder of the study. Body weights were recorded on the day of dosing and on days 3 and 8 after dosing. All animals were killed on day 8 and subjected to a macroscopic examination.

There were no deaths seen during the study. The only adverse, recorded effects were seen during the first 30 min after dosing. These included walking on tiptoe gate, closed eyes and swept back ears as well as flicking movements of the tail. Thus, a lethal dose of Lamivudine in mice is greater than 2 g/kg when the compound is administered intravenously.

3. Acute Intravenous Toxicity Study in Mice, Batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., November, 1991, (M12708-WPT/91/110)*

Ten male and ten female B6C3F1 mice were administered Lamivudine in acetate buffer, pH 3.7, intravenously at a dose of 2 g/kg. Five male and five female control animals were administered vehicle alone by the same route. The animals were watched for adverse signs for the first six hr after dosing and twice daily for the remainder of the study. Body weights were recorded on the day of dosing and on days 3, 8 and 15 of the study. Half the treated animals were killed on day 3 of the study. The remainder, including the controls, were killed on day 15. A macroscopic examination was carried out on all animals in the study on the

day of their death. Samples of the lungs, hearts, injection sites, kidneys and livers were examined microscopically.

One female control animal convulsed immediately after dosing. The animal exhibited subdued posture and subdued behavior for ten min. One male control was prostrate for 30 min after dosing. This animal exhibited subdued behavior, half closed eyes and hunched posture for the first two hr after which he appeared normal. One male and a female died immediately after dosing. The male was convulsive prior to death. Both animals had congested lungs at necropsy but the sponsor did not consider this anomaly to be sufficient to cause death. No cause of death was given. One other male convulsed immediately after dosing but appeared normal by 30 minutes. Other signs including rapid breathing, hunched posture, walking on tiptoed gait and half closed eyes were noted among both dosed and control animals. All animals appeared normal within two hr. Other than in the two animals which died, there were no drug related abnormalities recorded either macroscopically or microscopically during the study.

In conclusion, Lamivudine at a single dose of 2000 mg/kg was lethal to 10% of mice. There were no reported histological anomalies considered to be related to treatment with the drug in the surviving animals.

4. Pilot Acute Intravenous Toxicity Study in Rats, Batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., October, 1991, (R12705-WPT/91/088)

Two male and two female AHA rats were administered Lamivudine in acetate buffer, pH 3.7, intravenously at a dose of 2 g/kg. The animals were watched for adverse signs for the first six hr after dosing and twice daily for the remainder of the study. Body weights were recorded on the day of dosing and on days 3 and 8 after dosing. All animals were killed on day 8 and subjected to a macroscopic examination.

There were no deaths seen during the study. After dosing, the animals exhibited severe signs of distress for up to 1.5 hr. These included prostration, rapid breathing, reddened extremities, hunched posture, loss of body tone and disinclination to move. No adverse effects of the drug were noted otherwise throughout the study.

5. Acute Intravenous Toxicity Study in Rats, Batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., November, 1991, (R12709-WPT/91/111)*

Ten male and ten female AHA rats were administered Lamivudine in acetate buffer, pH 3.7, intravenously at a dose of 2 g/kg. Five male and five female control animals were administered vehicle

alone by the same route. The animals were watched for adverse signs for the first six hr after dosing and twice daily for the remainder of the study. Body weights were recorded on the day of dosing and on days 3, 8 and 15 of the study. Half the treated animals were killed on day 3 of the study. The remainder, including the controls, were killed on day 15. A macroscopic examination was carried out on all animals in the study on the day of their death. Samples of the lungs, heart, injection site, kidneys and liver were examined microscopically.

One control male, one control female and one drug treated male died shortly after dosing. The three animals exhibited shallow or labored respiration with gasping immediately after dosing. The female convulsed shortly before dying. The only microscopic anomalies seen in these animals were blood in the urinary bladder of the female and a mottled liver in the male which had been dosed with the drug.

Comments: Dosing intravenously with acetate buffer, pH 3.7, is obviously a life threatening operation. Administration of the buffer probably contributed to the deaths seen in the single dose mouse study reviewed above. After dosing, many animals exhibited severe signs of distress for up to 2 hr. These included prostration, rapid breathing, hunched posture and disinclination to move. Among the animals that were necropsied at 3 and 15 days, the only macroscopic anomaly was a black area on the liver of a control female at day 15 while a few of the dosed animals had evidence of a reddened thymus at day 3. Microscopic examination of the site of injection of the controls as well as the dosed animals showed that the vehicle was slightly irritating.

In general, intravenous administration of 2 g/kg of Lamivudine in a single dose was well tolerated in AHA rats.

II. Subchronic/Chronic/Carcinogenicity Studies

1. Lamivudine: Pilot oral 30 day study of hemato-toxicity to mice, Batch No. C1758/233/1, Glaxo Group Research Ltd., Herffordshire, England, April 26, 1993, (WPT/92/200/Study No. M13366)*

Groups of male and female mice (age: 7-8 weeks; strain: B6C3F1; 5 animals/sex/group) were administered lamivudine as a solution twice daily via oral gavage at dosage levels of 0 (vehicle control), 250, 500, 1000 or 2000 mg/kg/day for 30 consecutive days. Results: males (1000 or 2000 mg/kg/day) demonstrated slightly higher values of packed cell volumes, hemoglobin concentrations and red blood cell counts than control values; a similar effect was not noted for the females. Mean corpuscular hemoglobin concentrations for males (2000 mg/kg/day) were marginally lower than those of controls.

Comments: In this study, dosages of 500 and 2000 mg/kg/day may be considered NOELs for males and females, respectively.

2. Lamivudine and AZT: Hemato-toxicity to mice by repeated oral administration for 36 days, Batch Numbers: Lamivudine-C1817/126/1, AZT-2N0075, Glaxo Group Research Ltd, Hertfordshire, England, 10 September 1992, (WPT/92/419/GXO433/921460)

Groups of male and female mice (age: 42 days; weight: within 3 g; 10 animals/sex/group) were administered orally the test materials (Lamivudine and AZT) at dosage levels of 180 mg/kg/day Lamivudine + 150 mg/kg/day AZT; 600 mg/kg/day lamivudine + 150 mg/kg/day AZT; 2000 mg/kg/day Lamivudine + 150 mg/kg/day AZT; 2000 mg/kg/day Lamivudine; 150 mg/kg/day AZT; or control vehicle twice daily for a period of 36-37 days. The objective of the study was to assess the hemato-toxicity of Lamivudine, both in combination and in comparison with AZT, when given to mice by repeated oral administration. Mortality: one unscheduled death from an intubation accident. Clinical signs: darkening of the tail was noted for all groups receiving AZT, either in combination with Lamivudine or alone. Body weights: there was no treatment-related effect. Hematology: mice of either sex receiving Lamivudine and AZT in combination showed an anaemic response which was evidenced by reduced packed cell volumes, hemoglobin concentrations, red cell counts and reticulocyte counts. Absolute indices indicated increased individual cellular volume and hemoglobin content. Inclusion bodies (Heinz bodies) were noted at all combination-treated dosages. Platelet counts were increased for all combination-treated groups. A slight decrease in total white cell counts, associated with decreased neutrophil counts, was noted for males (2000 mg/kg/day Lamivudine + 150 mg/kg/day AZT). Male groups receiving Lamivudine or AZT alone demonstrated significantly reduced neutrophil counts. Biochemistry: all combination-treated groups showed a tendency towards increased inorganic phosphorus and chloride levels; the effect on chloride was not dosage-dependent. Cholesterol levels for mice of either sex receiving 600 or 2000 mg/kg/day Lamivudine + 150 mg/kg/day AZT were marginally higher than those of controls. Marginally increased cholesterol levels were also noted for males receiving Lamivudine or AZT alone. Bone marrow examination: mice of either sex receiving Lamivudine and AZT in combination showed higher total myeloid counts; the effect was not dosage-dependent. For males receiving the high dosage level, a marginally reduced erythroid fraction and increased myeloid to erythroid ratio was noted. Counts of bone marrow cell types other than myeloid or erythroid tended to be reduced for all treatment groups receiving combination dosages or AZT alone. Organ weights: spleen weights for mice of either sex receiving 600 or 2000 mg/kg/day Lamivudine + 150 mg/kg/day AZT were marginally higher than those of controls. Microscopic pathology: no treatment-related effects were noted.

Comments: All groups receiving Lamivudine and AZT in combination demonstrated mild anemia at all dosage levels. This effect may be associated with an increase in the myeloid fraction in the bone marrow and the increased spleen weights for mice receiving the mid and high dosages. These effects also occurred in the group receiving AZT alone. With regard to Lamivudine, females receiving 2000 mg/kg/day Lamivudine alone were marginally anemic; however, there was no effect on the bone marrow or the spleen weights. Thus, the results suggest that Lamivudine has a lower hemato-toxic potential compare to AZT. However, a combination of Lamivudine and AZT would increase the hemato-toxic potential of AZT.

3. Lamivudine: One month oral study to determine the effects, when give in combination with interferon (alpha), on the hematology of mice, Batch No. UFP0001, Glaxo Group Research Ltd., Hertfordshire, England, August 10, 1993, (WPT/92/572/Study No. M20009)

Groups of male and female mice (age: 7-8 weeks; strain: B6C3F1; 12 animals/sex/group) were administered Lamivudine as a solution (Baxter's Water For Irrigation) twice daily via oral gavage at dosage levels of 0 (vehicle control), 180, 600 or 2000 mg/kg/day for 30 days. For the last 14 days, Lamivudine was given in combination with 10,000 units daily of interferon (alpha). Appropriate control groups were included in the study. The objective of the study was to assess the hemato-toxicity of Lamivudine in combination with interferon (alpha). Results: a slight, but statistically significant, increase was seen in mean cell volume, mean cell hemoglobin and spleen weight in the 2000 mg/kg/day group.

Comments: In the combination study, interferon (alpha) did not enhance the hemato-toxic potential of Lamivudine.

4. Lamivudine: Palatability in the diet of mice, Batch No. C1817/116/1, Glaxo Group Research Ltd., Hertfordshire, England, June 21, 1993, (WPT/92/322/Study No. M13284)*

Groups of male and female mice (age: 7-8 weeks; strain: B6C3F1; 10 animals/sex/group) received Lamivudine in their diet at daily dose levels of 0 (control), 90/180 (dosage level increased at the end of one week 90 to 180, low), 425/600 (dosage level increased at the end of one week 425 to 600, mid), 1000 or 2000 (high) mg/kg/day for 21 days. All mice were housed singly. Blood samples were collected on the last day of treatment. Results: weight gain was significantly reduced (4-9%) in both males and females (high). Absorption: mean plasma concentration (low) was 0.5 and 1.3 µg/ml in male and female animals, respectively. In conclusion, mice may be given Lamivudine in their diet

5. Lamivudine: 13 week oral (dietary) to mice preliminary to an oncogenicity study, Batch No. UFJ0012, Glaxo Group Research Ltd., Hertfordshire, England, March 23, 1994, (WPT/93/196/Study No. M13285)*

Groups of male and female mice (age: 6 weeks; strain: B6C3F1; 12 animals/sex/group) received Lamivudine in their diet at daily dose levels of 0 (control), 2000 (low), 3000 (mid), 4000 (high) mg/kg/day for 13 weeks. Blood samples were collected on the last day of treatment. Results: weight gain was significantly reduced (10-14%) in both males and females at all dose levels. Hematology showed slight reduction of erythrocyte parameters and also lymphocyte counts at all dose levels. Histologically, the treatment-related change was a slight reduction of fat deposits in the liver at all dose levels. Absorption: at the end of the treatment, absorption studies showed AUCs of 196 (low) and 448 μg*hr/ml (high), with little differences between the sexes.

Comments: A NOEL dose could not be determined in the study. The sponsor has selected a 2000 mg/kg/day for the subsequent carcinogenicity study as the potential maximum tolerated dosage level.

6. Lamivudine: Pilot Five Day Oral Toxicity Study in Rats, Batch # C1021/179/1 and C1021/181/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., April, 1991, (R12636-WPT/91/091)

Five male and five female AHA rats (weight: 152.7 - 235.3 g for males and 149.1 - 203.9 g for females; age: 5 - 7 weeks; strain: Wistar/Sprague-Dawley derived with Wistar characteristics) were administered by gavage Lamivudine in acetate buffer, pH 3.7, twice daily at 12 hr intervals at doses of 0 (vehicle control), 1500 or 2000 mg/kg/day. These groups were designated as the main study. For an absorption study, two separate groups (4 animals/sex/group) were gavaged with two doses of drug (1500 or 2000 mg/kg/day) for 5 days twice-a-day (10 doses). The animals were observed daily for signs of ill health and their body weights recorded at day 1 and 6. All animals were treated for 5 days and killed on day 6. Blood samples were drawn for various determinations. The main study animals were submitted to necropsy at which time six organs were weighed and samples of ten organs or tissues were examined microscopically for histological lesions.

In the high dose animals, an increase in monocyte counts and an increase in the plasma alkaline phosphatase and calcium levels were found together with a slight increase in the inorganic phosphate levels for both sexes. There was no treatment effect at the lower dose.

Table 1 shows that the C_{max} and C_{min} levels observed throughout this study. C_{min} levels indicate that there was continual systemic exposure to drug.

Table 1
Plasma Levels of Lamivudine in Rats Following Oral Administration of the Drug Twice-a-Day For Five Days

Dose #	Absorption Parameters (μg/ml)	Male		Female	
		Doses of Lamivudine			
		1.5 g/kg	2.0 g/kg	1.5 g/kg	2.0 g/kg
1	C_{max} C_{min}	98.9 3.2	167.0 3.1	81.8 2.7	107.0 6.9
2	C_{max} C_{min}	92.2 3.7	140.0 4.5	77.2 5.7	128.0 5.1
3	C_{max} C_{min}	92.7 2.8	172.0 5.8	90.0 3.6	133.0 8.3
5	C_{max} C_{min}	71.5 2.5	154.0 4.3	96.5 4.3	146.0 6.8
7	C_{max} C_{min}	91.9 2.2	128.0 4.4	84.3 2.5	110.0 6.2
9	C_{max} C_{min}	79.2 2.4	149.0 4.5	95.3 3.3	98.7 7.1
10	C_{max} C_{min}	90.6 6.1	119.0 14.4	102.0 3.3	103.0 13.6

Comments: The NOEL was 1500 mg/kg/day in this study. In both males and females, there is an evidence [elevated C_{min} at dose # 10]*for accumulation of drug in plasma.

7. Lamivudine: One Month Oral Toxicity Study in Rats, Batch # 1021/181/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., June, 1991, (R12637-WPT/91/104)

Twenty male and twenty female AHA rats per group were fed by gavage with 0 (vehicle), 45, 300 or 2000 mg/kg of Lamivudine in acetate buffer, pH 3.7. The animals were dosed twice daily for 35 to 37 days. They were observed daily for signs of ill health and reaction to treatment. Body weights and food consumption were recorded weekly throughout the study. The eyes of all the animals were examined prior to dosing and on days 35 and 59 and a hearing

test was administered pretreatment and on day 35. Blood was collected for hematology and chemistry evaluations. Samples for urinalysis were collected from individually caged animals. On day 36, 37, 38 and the day after the final dose, 12 animals of each sex were killed. Twelve tissues were weighed while these and another 30 were prepared for histological examination. Six animals per group were held until day 62 or 63, after which they were killed and subjected to necropsy. Blood samples were taken for pharmacokinetic studies. Each animal was bled only once on each occasion.

At the high dose, the males showed a slight lowering of hemoglobin and red blood cell counts with a decreased hematocrit at day 13 and a lower red blood cell count at day 28. At day 28, the males also had a slightly higher mean cell volume, with an increased monocyte and basophil count, and a decrease in the prothrombin time. The mean cell hemoglobin was increased in the females at day 13 and 28. The serum urea in males and serum total protein and albumin in females were raised at day 14 and 28. There were slight elevations in alkaline phosphatase and inorganic phosphate levels in the males and slight reductions in alanine aminotransferase and creatinine in the females at day 28. Urinary proteins were increased in both males and females at both day 16 and 30. Slight reductions in relative organ weights were noted in the females in lung, liver, brain and spleen.

Very few anomalies were seen in the intermediate dose animals. In the males, the mean cell volume was slightly higher than controls and there was an increase in the monocyte count on day 28. The females had a slight decrease in alanine aminotransferase and creatinine at day 28. At the end of the recovery period, all hematological and chemistry values had returned to normal in both the high as well as in the intermediate level.

Results of absorption studies are summarized in Table 2. The values for males and females have been pooled. The compound was well absorbed following administration at all doses with peak plasma concentrations occurring at one hour after dosing. C_{max} and AUC were found to increase with dose in a linear manner and the values obtained were similar on day 1 and 35.

Table 2
Absorption of Lamivudine in the Rat*

Dose (mg/kg/day)	Day 1		Day 35	
	C_{max} (µg/ml)	AUC (µg*hr/ml)	C_{max} (µg/ml)	AUC (µg*hr/ml)
45	3.3	11.7	4.1	22.7
300	22.6	86.5	30.0	119.0
2000	93.1	415.0	117.0	590.0

* Blood samples were pooled for both males and females in a group

Comments: The sponsor concluded that the NOEL in the study was 300 mg/kg twice a day. However, there were slight effects at this level. The true NOEL was 90 mg/kg/day. There is an indication at day 35 that there is an accumulation [elevated AUCs at all 3 doses] of this agent in plasma.

8. Transmission electron microscopy of skeletal muscle from rats after 1 month oral treatment with lamivudine, Batch No. UFJ0012, Glaxo Group Research Ltd., Hertfordshire, England, June 21, 1993, (WPT/93/185/Study No. R12637)*

Two groups of rats (3 animals/sex/group) received Lamivudine via gavage twice daily at dose levels of 0 (control) or 2000 mg/kg/day (high) for 35 or 36 days. The purpose of the study was to study whether Lamivudine treatment resulted in degenerative changes in muscle mitochondria or changes in other components of skeletal muscle fibers similar to that was seen with the AZT treatment. Transmission electron microscopy (TEM) was performed on quadriceps muscle of rats following the treatment. Results: TEM indicated no changes in the ultrastructure of mitochondria, myofibrils, sarcoplasmic reticulum and T-tubules. Conclusion: the Lamivudine treatment did not cause ultrastructural changes in quadriceps muscle fibers in rats.

9. Lamivudine: One month oral toxicity study in the rat - supplementary histopathology report, Glaxo Group Research Ltd., Hertfordshire, England, February 1994, (WPT/93/563-Study # R12637)*

This report provided additional histopathological information for study R12637, a one month oral toxicity study of Lamivudine in the rat (WPT/91/104). The caecum from all animals and the liver from control and high dosage group animals were examined by light microscope at the end of the treatment period. The additional histopathology was performed because of some treatment related findings noted in the caecum in a subsequent six month rat study

(WPT/93/361) and because of serious adverse hepatic toxicity leading to death in some cases in patients with another compound in this class, fialuridine (FIAU). Results: there was no histopathological findings in any tissue that were considered to result from treatment with Lamivudine and therefore the conclusions in the original report (WPT/91/104) remain unaffected.

10. Lamivudine: Thirteen Week Oral Toxicity Study in Rats, Batches # C1803/128/1 and C1758/226/1, Glaxo Group Research Ltd, Direzione Ricerche di Tossicologia, Glaxo S.p.a., Verona, Italy, January, 1992, (R12749-WPT/91/435)*

Twenty male and twenty female AHA rats were administered Lamivudine in acetate buffer, pH 3.7, by gavage, twice daily (12 hours apart) at doses of 0 (vehicle control), 90, 600 or 4000 mg/kg/day for 91 days. All animals were observed for clinical signs at least 5 times daily. Individual body weights were recorded twice pretreatment, twice a week during the first 4 weeks of dosing, then once a week. Food consumption was determined weekly. The eyes were examined by indirect ophthalmoscopy once during pretreatment, at week 13 of the study and at week 17 during the recovery period. Blood was taken for hematological studies and for clinical chemistry evaluations at weeks 5, 9, 13 and 17; urinalysis studies were also carried out during the same weeks. Twelve of each group of animals were killed at week 14 and submitted to necropsy. The remaining eight animals were allowed to recover for four weeks and were killed at week 18 for necropsy. Eleven organs were weighed from each animal and 45 tissues and organs were examined histologically from the control and high dose animals as well as for animals dying during the test period.

Two high dose animals died during the study, a male at day 59 and a female at day 71. The male was killed as a result of the deterioration of his general condition and the female was found dead in her cage during the second dosing period.

At 13 weeks, both males and females had reduced red blood cell counts with a reduction in the total iron binding capacity, increased γ-globulins and increased urine specific gravities. The males had slight increases in body weight, alkaline phosphatase, urine potassium, chloride and protein and a reduction in urine volume and pH as well as a reduction in liver weights. The females had an increase in aspartate and alanine aminotransferase and blood glucose and a decrease in amylase, chloride and cholesterol levels.

The only histopathological anomalies were seen in the kidneys of the males. At the high dose, dilated tubules were seen at the high dose and in one animal in the intermediate dose group.

There was an increased incidence in renal pelvic dilation in the high dose males killed at the end of dosing. All the kidneys at all doses were examined.

Comments: The NOAEL in this study was 600 mg/kg/day. This is equivalent to a human dose of approximately 85 mg/kg/day based on relative body surface areas for a 70 kg human.

11. Lamivudine: Six month oral toxicity study in the rat, Batch No. C1817/126/1, Glaxo Group Research Ltd., Hertfordshire, England, April 8, 1993, (WPT/93/361/Study No. R13470)

Groups of AHA rats (weight: 147-354 g; age: 7-8 weeks; 20-32 animals/sex/group) received Lamivudine (solution) twice daily via gavage at dosage levels of 0 (vehicle control), 90 (low), 425 (mid) or 2000 mg/kg/day (high) for a period of 198 days after which animals were killed. Only in control and high dosages, 11 animals/sex/group were maintained untreated from 199 to day 268 as recovery groups. Blood samples were collected on days 1, 38, 100 and 177. Mortality: twenty animals (control: 9; low: 5; mid: 3; high: 3) were killed for humane reasons or found dead during the study. Clinical signs that were described prior to their deaths appeared to show no relationship to dosage and comprised one or more of noisy and/or labored breathing, subdued behavior, piloerection, hunched posture, coldness to touch, thin appearance, hair loss, convulsions and red discharge on or around the mouth and snout. Clinical observations: hair loss and or scab formation on various parts of the body, noisy breathing (generally characterized by rattling, croaking or whistling) and red staining and/or discharge from, on or around one or more of the snout, eyes, forepaws, mouth and perineum were noted (high). No abnormal clinical observations were noted during the recovery period. Hematology: reductions in erythrocyte and reticulocyte counts, and increases in mean cell hemoglobin, mean cell volume and reticulocyte count were noted on day 30 (high). Mean cell hemoglobin concentration (high) was statistically significantly increased on day 188. The changes in erythrocyte count, mean cell volume and mean cell hemoglobin were still apparent following a recovery period on day 224 but returned to normal by day 255. Clinical chemistry: slight but statistically significant increased in mean serum ALT and AST were seen on day 188 (high). Statistically significant reductions in serum cholesterol and triglyceride throughout the treatment period and in bilirubin on days 90 and 188 were noted for both sexes (high). The change in triglycerides was still apparent on day 224 following a period of recovery but had returned to normal by day 255. Urine analysis: animals showed a slight but statistically significant increase in urine potassium, chloride and specific gravity compared to controls during the treatment period generally from day 92 (high); the changes were reversed during the recovery period. Absorption studies: Lamivudine concentration versus time profiles

on days 1, 38, 100 and 177 indicated that the majority of animals from all dosage groups were continually exposed to the test compound throughout the study. Peak plasma concentrations of Lamivudine were similar after the first and second doses and occurred generally by approximately 1 to 2 hr after dosing. Organ Weights: statistically significant reductions in liver weights compared to control were noted at the end of the treatment (high); the change was reversed at the end of the recovery period. Pathology: at the end of the treatment period a slight or moderate diffuse mucosal hyperplasia, often associated with a degree of inflammatory cell infiltration was noted in the caecum of seven males and eight females (high). Diffuse subepithelial eosinophilic material was also recorded in the caecum for five males at this dosage. The mucosal hyperplasia and inflammatory cell infiltration showed evidence of regression but subepithelial eosinophilic material was still present in three males and one female (high) at the end of the recovery period.

Comments: Lamivudine was well tolerated by rats at oral dosages up to 2000 mg/kg/day bid administered over a period of 6 months and there were no findings that would preclude further controlled clinical evaluation of the test compound in man. The dosage of 425 mg/kg/day bid may be considered a NOEL in the study.

12. Lamivudine: Palatability in the diet of rats, Batch No. C187/126/1, Glaxo Group Research Ltd., Hertfordshire, England, June 21, 1993, (WPT/92/323/Study No. R13287)*

Groups of rats (age: 27-29 days; strain: HanIbm WIST; 5 animals/sex/group) received Lamivudine in their diet at daily dose levels of 0 (control), 180, 600, 1000 or 2000 mg/kg/day for 21 days. Results: the test compound was adequately absorbed. Dietary intake was unaffected. There were no treatment related effects. Conclusion: the diet was palatable at all dosage levels.

13. Lamivudine: 13 week oral (dietary) rats preliminary to an oncogenicity study, Batch No. UFP 0004, Glaxo Group Research Ltd., Hertfordshire, England, December 23, 1993, (WPT/93/197/Study No. R13288)*

Groups of male and female rats (age: 6 weeks; strain: HanIbm WIST; weight: 115-187 g; 12 animals/sex/group) received Lamivudine in their diet at daily dose levels of 1000 or 2000 for male rats and doses of 1500 or 3000 mg/kg/day for female rats for 13 weeks. Blood samples were collected on the last day of treatment. Results: there was a transient body weight retardation for females (high) and for males receiving either of the doses, with consequent impact on food utilization efficiency. Absorption: at the end of the treatment, absorption studies showed AUCs of 328 and 356 μg*hr/ml in males, and 342 and 749 μg*hr/ml in females. Conclusion: doses of 1000 mg/kg/day in the

males and 1500 mg/kg/day in the females may be considered NOELs, respectively. The sponsor has not decided the dosage levels for the subsequent carcinogenicity study.

14. Lamivudine: Two Week Oral Toxicity Study in Dogs, Batch # C1803/152/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., November, 1991, (D12750-WPT/91/222)*

Three male and female beagle dogs (weight: 7 - 8.5 kg for males and 5.6 - 8.4 kg for females; age: 3 - 6 months) were dosed by gavage for two weeks with 1500 mg/kg/day of Lamivudine dissolved in acetate buffer, pH 3.7. Dosing was carried out twice a day 12 hours apart. An additional male and female were dosed with vehicle as controls. All animals were observed daily for adverse clinical signs while their food consumption was recorded daily and their body weights were recorded predose and on days 1, 4, 8, 11 and on day 15, the day of necropsy. Blood was obtained for hematological, clinical chemistry and drug level evaluations Urine was collected for examination. On day 15, the day after the final dose, the animals were killed and subjected to necropsy. The animals were exsanguinated and a full macroscopic examination was carried out. Thirteen organs were weighed and 38 tissues or organs were subjected to histological examination.

There were no deaths in the study. Loose feces and isolated cases of emesis were seen in both treated and control animals. There were otherwise, no treatment related adverse clinical observations. Slight reductions in hemoglobin, hematocrit and red blood cell counts as well as moderate reductions in leukocyte and neutrophil counts were seen in both males and females at the end of dosing. A slight increase of aspartate and alanine aminotransferase was seen in both males and females at the end of dosing. Treated females had slightly smaller thymuses and slightly larger ovaries compared to the control animal and treated males had slightly smaller prostates. On microscopic examination, the greatest effect of the drug was to the liver of both treated males and females. The lesions, which ranged from very slight to very severe, included the presence of mixed inflammatory foci, centrilobular mixed inflammatory cell infiltrate, centrilobular coarse vacuolation and focal necrosis (in one female). In general the liver lesions were more severe than slight. Slight thymic atrophy was seen in a number of animals.

The mean plasma levels for males and females on day 1 and 14 are presented in Table 3 and pharmacokinetic parameters calculated for the mean plasma level-time data are depicted in Table 4. The parameters indicated that repeated administration of drug for 14 days at this dose level caused a decrease in plasma clearance in both males and females dogs, leading to increased in plasma AUCs and hence drug exposure.

Table 3
Mean Plasma Levels of Lamivudine in Beagle Dogs Receiving Repeated Oral Administration of at 1500 mg/kg/day BID

Time (hr)	Mean Plasma Levels (μg/ml)			
	Male		Female	
	Day 1	Day 14	Day 1	Day 14
0	0	8.8	0	31.2
1	151.7	156.3	174.3	159.0
2	191.3	218.3	178.0	238.7
3	145.3	176.7	137.3	262.0
4	89.6	153.0	89.9	232.7
6	25.5	100.6	23.5	115.5
8	6.2	48.3	5.7	74.4
12	0.8	18.8	0.6	49.6

Table 4
Pharmacokinetic Parameters of Lamivudine in Beagle Dog

Pharmacokinetic Parameters	Male		Female	
	Day 1	Day 14	Day 1	Day 14
AUC (μg*hr/ml)	694	1169	690	1578
Clearance (l/hr/kg)	2.2	1.2	2.2	0.8
Volume of Distribution (l/kg)	3.7	4.5	3.5	4.2

Comments: In view of the fact that livers of all 6 treated dogs in this study are moderately/severely affected (mixed inflammatory foci, centrilobular mixed inflammatory cell infiltrate or centrilobular coarse vacuolation) and that focal liver necrosis is present in one treated female, coupled with elevated ALT and AST, taken together suggest that liver is a target organ for this compound in dogs. The thymic atrophy seen may be a treatment-related toxicity.

Repeated administration of Lamivudine bid for 14 days caused a reduction in plasma clearance of this agent in both male and female dogs leading to an increase in systemic drug exposure.

15. Lamivudine: Thirteen Week Oral Toxicity Study in Dogs, Lots # C1013/111/1, C1817/86/1 and C1817/83/1, June, 1992, Direzione Ricerche di Tossicologia, Glaxo S.p.a., Verona, Italy, (D12873-WPT/92/132)

Six male and 6 female beagles per group were fed Lamivudine in acetate buffer, pH 3.7, at dose of 0 (vehicle), 90, 520 or 3000 mg/kg/day for 13 weeks. All solutions were administered twice daily (at equal doses) at approximately 12 hr intervals by esophageal catheter. All animals were observed at least 4 times daily during the dosing period and 2 times daily during the recovery period. Body weights were recorded 5 times prior to dosing on the day of the first dose and weekly thereafter. Ophthalmoscopy was carried out 3 times prior to dosing and at the end of the dosing or the recovery period. Electrocardiography studies were carried out 2 times prior to dosing and during weeks 4, 12 and 16 (during the recovery period). A panel of neurological reflexes and reactions were carried out 2 times prior to dosing and during weeks 4, 12 and 16 (during the recovery period). Blood was collected once prior to dosing and during weeks 5, 13 and 17 (during the recovery period) for hematology and clinical chemistry measurements. Urine for analysis was collected overnight to coincide with the collection of blood. The day after the final dose, 4 male dogs per group and all the remaining female dogs were killed. The remaining 4 males were allowed to continue without dosing for an additional 4 weeks (recovery). At death, a full macroscopic examination was carried out on all animals. Bone marrow smears were prepared for examination and 12 organs were weighed. Samples of 41 organs and tissues from all groups were prepared for histological (microscopic) examination.

There were five mortalities during the study. Two of the fatalities were caused by dosing accidents during the first week of the study. The two animals were replaced. Three females dosed at 3000 mg/kg/day died, one on day 32, the second on day 42 and the final animal on day 48.

The first female which died (day 32), ate no food from day 26. At week 5, this dog had elevated alanine transaminase (5 times normal) and lactic dehydrogenase levels and a slight increase in lymphocytes. There was a 4-fold increase in the urinary sodium and a slight increase in urinary potassium. At necropsy, the liver was markedly yellowish with severe diffuse fatty changes confirmed with Oil Red O (ORO) staining and centrilobular congestion. The stomach was distended and filled with a large amount of transparent mucous but no evidence of microscopic findings. The animal also had slight congestion of the renal parenchyma but no evidence of microscopic abnormalities. There was severe congestion of intestinal mucosae with multifocal mucosal ulcerations. This was accompanied by multifocal

mononuclear-cell infiltrates in the small intestine and focal hemorrhage in the large intestine. There was edema and hemorrhage in the lungs with severe bronchopneumonia. The animal showed thickening of the pericardium with diffuse hemorrhage of the endocardium and focal hemorrhage of the pericardium as well as pale coloration of the heart. The animal had moderate lymphoid depletion of the spleen with diffuse congestion, focal hemorrhage, multifocal germinal center depletion and diffuse erythrophagocytosis of the lymph nodes. Death was attributed to severe congestive cardiopulmonary failure with a superimposed bacterial and fungal infection.

A second female was killed on day 42 due to the deterioration of its general condition. From day 32, all food (including canned food) was left uneaten. From day 31, the animal showed subdued behavior and salivated during dosing on a number of occasions. At week 5, there was a slight increase in the alanine transaminase and lactate dehydrogenase levels and a decrease in the urinary chloride level. At necropsy there was a severe discoloration of the liver with diffuse ORO positive staining. The lungs were slightly reddened and were slightly congested. The spleen had a slight lymphoid depletion and was slightly reduced in size. The stomach had multifocal small eroded areas with some superficial edema. The intestines were slightly congested with moderate edema in the large intestine. The kidneys were diffusely congested with diffuse tubular cell vacuolation. The cells of the bone marrow were severely depleted.

A third female showed subdued behavior and reduced appetite from day 38. Canned meat was fed to the animal from day 42 without appreciable results. The animal was killed on day 48 because of the deterioration of its general condition. At week 5, there was a slight increase in red blood cell counts and hemoglobin values and a slight decrease in lymphocytes. There were increases in alanine and aspartate transaminase levels as well as lactate dehydrogenase and blood urea nitrogen levels. The animal showed decreases in the blood glucose and α-amylase levels. There was an increase in urinary potassium and a decrease in urinary chloride. At necropsy, the lungs were moderately distended with reddening, severe congestion, edema and a localized inflammatory cellular infiltrate. There was emphysema in one lobe. The liver was yellow colored and friable with severe fatty changes (ORO positive). The spleen was reduced in size with slight lymphoid depletion. The stomach had multifocal erosions with marked biliary reflux. The small intestines had moderate diffuse congestion. The kidneys showed yellow discoloration with diffuse tubular-cell vacuolation and eosinophil deposition. The animal had focal ulceration of the tongue and a pituitary cyst. The bone marrow was severely depleted of cells.

The most common clinical sign during the study was salivation and

difficulty with gavage insertion in the high dose animals. Body weight and food consumption decreases were seen only in the 3 females which died or were killed during the study. There was a decrease in red blood cells at the intermediate and high dose in males at both 5 and 13 weeks and in the high dose females at 13 weeks. At the high dose, there was an increase in the mean corpuscular volume in the males at 5 weeks and in the females at 13 weeks. At 13 weeks, the increase was seen in the males at the intermediate as well as the high dose. At the high dose, there was an increase in the mean corpuscular hemoglobin in the males and females at 5 weeks and in the females at 13 weeks. At 13 weeks, the increase was seen in the males at the intermediate as well as the high dose. At 13 weeks, there was a slight increase in the activated partial thromboplastin time at the intermediate and the high dose in males. At the high dose, there was a decrease in total white blood cells in the females at 5 and 13 weeks and in the males at 13 weeks. At weeks 5 and 13, there was an increase in the total protein in the males at both the intermediate and high dose and in the females at the high dose. At 13 weeks, there was a decrease in the levels of α-1 globulin at the intermediate and high dose in the males and an increase in ß- and γ-globulin in both the males and females at the high dose. At the high dose, the specific gravity of the urine was increased in the males and females at both 5 and 13 weeks and the volume was decreased at 13 weeks. At 13 weeks, the urinary chlorine levels were increased at the intermediate and high dose and the potassium levels were increased at the high dose in both the males and females; urinary proteins were increased at the high dose in the females. The kidney weight was increased at the high dose in the males. Drug related changes were seen in the liver and thymus of the high dose animals only. The liver lesions were centrilobular lipid deposition and a diffuse depletion of cellular glycogen (confirmed by periodic acid Schiff stain). A moderate atrophy of the thymus was observed in 2 male high dose animals.

Comments: The dose at which there were no adverse effects in this study was 90 mg/kg/day. This is equivalent to a human dose of approximately 50 mg/kg/day based on relative body surface areas.

16. Supplementary report to: Thirteen Week Oral Toxicity Study in Dogs, Lots # C1013/111/1, C1817/86/1 and C1817/83/1, June, 1992, Direzione Ricerche di Tossicologia, Glaxo S.p.a., Verona, Italy, (VTX/94/037/D12873)*

This report contained supplementary information to Verona report number VTX/92/011/WPT/92/132 which described the findings of a 13 week oral toxicity study of Lamivudine in dogs. The microscopic examination of the caecum, which was included in the original protocol design, was performed on all dogs following detection of caecal changes in the 6 month rat study (WPT/93/361). Results:

no microscopic changes related to Lamivudine administration were observed in the caecum of all animals killed following the treatment or recovery periods. The conclusion drawn in the main report was considered valid.

17. Lamivudine: 52 weeks oral (gavage) toxicity study of dogs, Batch No. UFJ 008, Glaxo Group Research Ltd., Hertfordshire, England, September 1994, (WPT/92/407/Study No. D13604)

Groups of male and female beagle dogs (weight: 7.8-11.8 kg; age: 23-27 weeks; 4 animals/group) received Lamivudine by oral gavage at dose levels of 0 (vehicle), 45 (low), 260 (mid) or 1500 (♂), 1000 (♀) mg/kg/bid (high) for a period of 52 weeks. On completion of the 52-week dosing period, the control and high dose groups (2 animal/sex/group) were maintained for an 8-week recovery period. Clinical signs: treatment-related increased incidence of liquid feces was recorded in high dose animals. One male experienced a single incident of liquid feces during the recovery period. Hematology: lower RBC counts, associated with increased mean cell volumes, were noted in male and female animals (mid and high) throughout the treatment period. Packed cell volumes and hemoglobin concentrations were reduced in females (high) in week 5 and decreased packed cell volumes and increased mean corpuscular hemoglobin concentrations were noted for male and female animals at all doses in week 51. These trends demonstrated reversibility by week 8 of the recovery period. A significant decease in white cell count (associated with decreases in neutrophils and lymphocytes) for high dose animals was noted during the treatment period; in week 4 of the recovery period, a degree of improvement was seen in the parameter. Biochemistry: increased total protein levels, associated with increased globulin levels, were noted for males (high) when compared to the controls. Increased activity of GPT and GOT were recorded for male animals at all dose levels. Slightly elevated GOT levels remained apparent during the recovery period for males (high). Increased vitamin B_{12} levels were noted for animals in all treated groups and increased folate levels were recorded in mid and high dose animals. During the recovery period, the vitamin B_{12} levels reverted to similarity with the controls. Urinalysis: a slight increase in specific gravity was noted for male and female animals (high) during the treatment period. At week 4 of recovery, the specific gravity was comparable to the controls. Organ weights: thymus weight were lower in terminally killed male dogs (high). Slightly elevated spleen weights were recorded in recovery killed female dogs (high). Histopathology: an increase incidence of splenic hemosiderosis was found in 2/4 terminal male and 3/4 terminal female dogs (high). Liver: subcapsular fibrosis at the median cleft (one terminal high dose and one recovery high dose males) and focal portal fibrosis (one high dose recovery male) were noted. Absorption studies: AUC data for the doses are

presented in Table 5 and clearly show increased exposure on week 52 relative to day 1 for all three dose groups.

Table 5
The AUC Values of Lamivudine Following 12-Month Oral Administration to Dogs

Dose mg/kg/bid	Male			Female		
	Day 1 1st Dose	Week 52 1st Dose	Week 52 2nd Dose	Day 1 1st Dose	Week 52 1st Dose	Week 52 2nd Dose
45	43	94	130	39	55	71
260	185	426	588	172	259	319
1500 (♂) 1000 (♀)	435	948	1610	417	872	1170

Comments: Lamivudine was well tolerated by dogs. Since an effect on red blood cell parameters was noted at all dosage levels, a NOEL could not be identified in the study. The results of absorption studies indicate that repeated administration of Lamivudine at all dosages leads to increased systemic exposure of both male and female dogs.

18. Lamivudine: Multidose toxicity study - marmosets (seven day intravenous), batch C1450/295/1, Report # WPT/90/416 January, 1991

One male and one female marmoset were administered the racemate GR103665X (±) in 0.9% sodium chloride solution intravenously at a dose of 100 mg/kg/day for four days, followed by a dose of 300 mg/kg/day for three days and finally at a dose of 300 mg/kg twice a day (with a four hour interval between doses). This was designated group A. A second pair of marmosets received a dose of 300 mg/kg twice a day for seven days (group B). The animals were killed on the day after their final dose. Monitoring was daily for the duration of the study and body weights were determined at intervals throughout. Blood was drawn predose and immediately prior to necropsy for hematology and clinical chemical studies. Urine samples were collected once on days 8/9 for group A and on days 4/5 for group B. Ten organs and tissues were examined for histological lesions. Blood was drawn from both groups for plasma drug concentrations. Groups A and B were bled predose, and at 30 minutes and four hours after dosing on the first day of treatment. Animals in group A were bled 30 minutes and 4 hours after each occasion that the dose increased as well as 30 minutes and four hours after the first dose and 30 minutes after the second dose on day eight. Animals in group B were bled 30

minutes, four hours and 24 hours after dosing on day seven.

Lesions were seen around the dosing site on all animals. These were described as mild bruising to surface sores. Animals from both groups had a reduction in hemoglobin, hematocrit and erythrocyte counts and a slight reduction in plasma albumin and total protein. There was a slight increase in bilirubin, urea and creatinine phosphokinase in both groups. Blood and protein was detected in the urine of animals in both groups. The animals in group B also had a markedly decreased platelet count and an increased leucocyte count. Plasma samples taken four hours after dosing showed a large reduction in drug concentration while comparison of samples taken 30 minutes after the first and second dose on a single day showed no indication of accumulation. The plasma concentrations are shown in Table 6.

Table 6.

Plasma Concentrations of GR103665X (±) in Marmosets

Day A	Dose mg/kg		GR103665X (±) Concentration (µg/ml) Group A		
			30 min (dose 1)	4 hr (dose 1)	30 min (dose 2)
1	150	♂			
	150	♀			
5	300	♂			
	300	♀			
12	300 bid	♂			
	300 bid	♀			

Day B	Dose mg/kg		GR103665X (±) Concentration (µg/ml) Group B			
			Predose	30 min	4 hr	24 hr
1	300 bid	♂				
	300 bid	♀				
7	300 bid	♂				
	300 bid	♀				

\- No sample taken
ND indicates no compound detected

19. A 14 Day Intravenous Toxicity Study in Marmosets, Batch # C1803/74/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., June, 1991, (P12678-WPT/91/079)*

Two groups of male common marmosets (weight: 273 - 328 g; age: 12 - 18 months; 4/group) were administered either vehicle or Lamivudine (450 mg/kg/day bid for first 4 days and 600 mg/kg/day bid for next 10 days) intravenously in 0.9% sterile sodium chloride solution for 14 days. The primary purpose of the study was to determine the effect of the drug on the heart. The animals were examined at least twice a day for signs of distress while body weights were recorded pre-treatment and on days 1, 5, 8, 12 and the day of necropsy. An electrocardiographic examination was carried out on days 1 and 10. Blood was drawn for hematological and clinical chemistry evaluations predose and on days 9 and 15. On the day after dosing, the animals were killed and submitted to necropsy. The hearts were weighed and samples from 40 tissues or organs examined for histological anomalies.

Bruises, abrasions and scarring was found in all animals at the site of injection. There were slight decreases in the red blood cell counts, hematocrit and hemoglobin concentrations in the treated animals at day 15. There were slight rises in the serum bilirubin and potassium concentrations at the same time point. No electrocardiograph effects were seen that could be related to treatment. Since there was only a single test article group, a NOEL was not seen in the study.

Comments: The various electrocardiograph studies reported in this IND were examined by Dr. Kuei Meng Wu. He agreed that no adverse effects were reported throughout the studies.

III. Special Toxicity Studies

1. Lamivudine: Acute eye irritation test in the rabbit, Batch No. C1817/126/1, Glaxo Group Research Ltd., Hertfordshire, England, February 4, 1993, (WPT/92,209/Study No. L13610)*

Two male and one female New Zealand White rabbits (weight: 2.55-2.91; age: 12-16 weeks) received 10 mg of the test material into the conjunctival sac of the right eye. The eyelids were then held together for approximately one second. Assessment of ocular damage/irritation was made approximately 1, 3, 6, 4, 48 and 72 hr after the treatment. Results: no ocular reactions were noted in any treated eye during the study.

2. Lamivudine: Acute dermal irritation test in the rabbit, Batch No. C1013/111/1, Glaxo Group Research Ltd., Hertfordshire, England, February 26, 1992, (WPT/91/325/Study No. L13056)*

Two male and one female New Zealand White rabbits (weight: 2.24-

2.99; age: 12-16 weeks) were clipped free of fur from the dorsal/flank area. A quantity of 0.5 g of the test material, moistened with 0.5 ml of distilled water, was introduced under a 2.5 cm * 2.5 cm gauze patch and placed in position on the shorn skin. The patch was secured in position with a strip of surgical adhesive tape. Four hr after the application, patches and any residual test material were removed by gentle swabbing with cotton wool soaked in distilled water. The test sites were examined for evidence of primary irritation at 1, 24 and 72 hr after removal of the patches. Results: very slight erythema was noted at one treated skin on 1 hr after patch removal. All treated skin sites appeared normal 24 hr and 72 hr after the treatment. Conclusion: Lamivudine was non-irritant to rabbit skin under the conditions of the study. No corrosive effects were noted.

3. Lamivudine: Evaluation of contact sensitizing potential in female guinea gigs using a split-adjuvant technique, Batch No. C1758/226/1, Glaxo Group Research Ltd., Hertfordshire, England, June 25, 1993, (WPT/92/207/Study No. 13034)*

Female Dunkin and Hartley derived guinea pigs (weight: approximately 300 g; age: 6 weeks; 10 animal/sex/group) were tested for potential contact sensitivity by repeated dermal application of the test material as 10, 5, 2.5 or 1% w/v solution (initial), together with intradermal administration of Freund's Complete Adjuvant (challenge). Initial irritancy titration: all concentrations of the test material produced negligible irritancy. The maximal concentration (10% w/v) of the test material was selected for the challenge procedure. Challenge: none of the treated animals showed a positive reaction with a 10% w/v solution of the test material. Conclusion: the contact sensitizing potential of Lamivudine was considered to be very low in the study.

4. Lamivudine: Dose finding study for anaphylaxis test in guinea pigs, Tsukuba Research Labs, Nippon Glaxo, Japan, June 7, 1994, Batch No. UFJ0012, (NTX/94/001/Study # 932001)

Groups of male guinea pigs (3/group) were administered Lamivudine at dose levels of 0.01, 0.1, 1.0 or 10 mg/animal via ip, sc or im routes to determine the doses of the test compound for sensitization and elicitation in an active systemic anaphylaxis test. Results: no deaths occurred and no abnormalities were observed due to Lamivudine in any animals. 10 mg/animal was considered to be appropriate for administration of Lamivudine for the sensitization and elicitation systemic anaphylaxis test.

5. Lamivudine: Acute systemic anaphylaxis test in guinea pigs, Tsukuba Research Labs, Nippon Glaxo, Japan, June, 1994, Batch No. UFJ0012, (NTX/94/002/Study # 200661)*

Groups of male guinea pigs (5/group) were administered Lamivudine at a dose level of 10 mg/ml/animal via ip or sc routes 3 times/week for 3 weeks to investigate the antigenicity of Lamivudine. Results: no systemic anaphylaxis reaction was observed with any group treated with Lamivudine alone or mixture of Lamivudine and protein for sensitization and challenge. Conclusion: Lamivudine has a low possibility of causing allergic adverse reactions in clinical use since Lamivudine did not show any immunogenicity under the experimental conditions.

IV. Reproductive and Developmental Toxicity Studies

1. Lamivudine: Effects of oral administration upon the reproductive performance of a parental dosed generation (F_0) of AHA rats and upon the peri- and post-natal development of the resulting two successive, untreated generations (F_1 and F_2), Batch No. UFP 0002, Glaxo Group Research Ltd., Hertfordshire, England, June 9, 1994, (WPT/93/210/Study No. R13738)

Groups of parental (F_0) generation of AHA rats (24 animals/group) were administered Lamivudine via oral gavage at dosages of 0 (control) 90 (low), 450 (mid) or 2000 mg/kg/bid (high) throughout gametogenesis, pairing, pregnancy and lactation. Fetal development and offspring growth, development and maturation (F_1) and development of subsequent F_2 generation were assessed. **Results F_0:** Mortality: ten male and eleven female rats died [due to gavage accidents] during the dosing period. Body weight and food consumption: statistically significant increases in bodyweight gain and food consumption were noted in high dose animals. Males (mid) showed increased bodyweight gain. Absorption data: 2 hr after dosing on one occasion in the week prior to paring, mean plasma levels for male rats were 6.3, 34.4 and 89.0 and for females were 9.9, 37.5 and 133 µg/ml in the low, mid and high dose groups, respectively. F_1 offsprings: survival, growth and development of the offsprings to weaning were unaffected. F_1 generation: bodyweight gain of males and females (high) was reduced before mating. Mean number of vertical movements and vertical activity (rearing) were reduced for males (high). An increase in gestation length (high) was noted. Post mortem examination revealed significant increase ($p<0.05$) in prostate weight in the high dose males. F_2 offsprings: survival, growth and development of the offsprings were unaffected.

Comments: Changes seen in the study do not contra-indicate the proposed clinical use of Lamivudine. A dosage of 450 mg/kg/bid may be considered a NOEL.

2. Lamivudine: Embryotoxicity and Teratogenicity Study in Rats, Batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., October, 1991, (R12768-WPT/91/196)

In this preliminary study, five pregnant female AHA rats per group were administered Lamivudine in acetate buffer, pH 3.7, by gavage twice a day at 0 (vehicle control), 45, 500 or 2000 mg/kg/day. Dosing was carried out on days 7 through 16 inclusively. The animals were observed daily for adverse effects of the drug and body weights were recorded throughout the study. On days 7 and 16 of pregnancy, blood was collected from two rats in each group at 1 hour after the first dose for the determination of drug plasma levels. On day 21 of pregnancy, all the surviving rats were killed, a post mortem examination was carried out and all abnormal tissues were preserved for histological examination. The ovaries and uterus, together with its contents, were removed and the position in the uterus of each live fetus together with early and late implantation deaths were recorded. Live fetuses were examined for gross external abnormalities, weighed and killed. One half of the fetuses were dissected for examination of their viscera. They were then fixed and stained with Alizarin Red S for skeletal examination. The remaining fetuses were fixed in Bouin's fluid prior to a visceral examination and sex determination. Three fetuses from the control, one from the low dose, three from the intermediate dose and one from the high dose groups were retained in Bouin's fluid and examined histologically after visceral examination.

No deaths occurred during the study and the drug was without clinical effect on the dams. Drug levels determined one hour after the first dose showed linearity between the two lowest dose levels but not between the intermediate and highest dose level. The mean body weights of the animals administered the highest dose were marginally increased over those of the other three groups. Macroscopic examination of the fetuses at term showed four major abnormalities in the 45 mg/kg/day group. Three of the abnormalities occurred in litter mates from one dam. One fetus had unilateral microphthalmia and internal hydrocephalus, the diaphragm of a second was partially non-muscularized while retro-esophageal aorta was found in a third. Umbilical hernia was recorded in another fetus from a second litter. No anomalies were recorded in the other groups. The incidence of skeletal anomalies was similar among groups. The only other adverse effect was the appearance of a liquid effusion in the pericardial sac of the animals which had been fixed in Bouin's fluid.

Comments: The sponsor considered the effusion to be a possible artefact since it was seen only in the fixed fetuses and not in those examined immediately after death. The conclusion of the study was that dosages up to 2000 mg/kg/day would be suitable for a full organogenesis study in AHA rat.

3. Embryotoxicity and Teratogenicity Study in Rats, Batch # C1758/226/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., not dated, (R12899-WPT/91/305/Draft Report)*

Groups of pregnant AHA rats (weight: 160 - 230 g; age: 8 weeks; 36 animals/group) were administered Lamivudine in acetate buffer, pH 3.6, twice a day, twelve hr apart, at doses of 0 (vehicle control), 45, 300 and 2000 mg/kg. Dosing took place between days 7 and 16 of pregnancy. Twenty-four animals from each group were killed at term while the remaining 12 were allowed to litter. The animals were observed for clinical signs while their body weights and food consumption were recorded throughout. In the litters killed at term, the number of corpora lutea on each ovary, the position of each fetus and whether there occurred early or late embryonic/fetal death, fetal weights or external abnormalities of the fetuses were recorded. The live fetuses were killed and half were examined for visceral abnormalities, fixed and examined for skeletal abnormalities. The mothers were killed and subjected to a postmortem examination. In the litters allowed to wean, the litter size and number of animals of each sex were recorded while the pups were observed for clinical abnormalities and their body weights were recorded at days 2, 4, 8, 12, 16 and 20 after birth. The pups were killed at 21 days post birth and their hearts and lungs examined and preserved in Bouins solution for further histological examination. The pups were subjected to a postmortem examination and discarded.

An additional four pregnant females per group were treated with drug in an identical manner to those animals described above. Blood samples were taken from these animals at 2 and 24 hours after treatment on the first and last day of dosing for the estimation of plasma levels of the drug. Two hr following the administration of doses of 45, 300 or 2000 mg/kg/day to the additional four pregnant females on day seven of gestation (the day of the first dose), the plasma concentrations were 3.5, 15.2 and 66.6 µg/ml respectively. Although the dose response is not linear, the high dose produces a plasma concentration four times greater than the next lower. Plasma concentrations determined at two hr after the last dose indicated that there is little accumulation of the drug with time.

There was a slight increase in body weight gain at the high dose between days 12 and 16 of pregnancy. Absolute body weights were not statistically effected throughout the study. There was no effect of the drug on either the gestation length or index. There was an increase in the incidence of renal pelvic cavitation seen in the intermediate and high dose groups of females killed at term (incidences of 2, 1, 4 and 8 in the control, low, intermediate and high doses, respectively). Only one high dose female in the group allowed to litter was found to have pelvic cavitation in one kidney. No other treatment related anomalies

were noted. There was no effect of dosing on the number of implantations or the number of pre- or post-implantation losses nor was gestation length affected by treatment. The number of live fetuses, fetal sex ratio, and fetal body weight were all unaffected by treatment. There was no effect of treatment on the viscera or on the incidence of skeletal findings. In all cases, the body weights of the affected fetuses were consistently higher than those which did not exhibit the effusion. In this study, this effusion was seen in control animals as well as treated. The incidence was 0.6% in the control animals and 1.0, 2.4 and 0.6% in the low, intermediate and high dose groups respectively. The sponsor considers the dark colored effusion to be a post mortem fixation artefact related to the larger size of the affected fetuses and the associated delay in fixation of the tissues. The general condition as well as the number of offspring born, the survival, the offspring sex ratio, the birth index and the body weights of the F1 offspring were all unaffected by treatment. On necropsy, other than the syndrome discussed below, no abnormalities were observed that could be related to treatment.

Four fetuses from the intermediate dose group in which the mother was killed at term had a syndrome which included ablepharia (also called cryptophthalmos, a developmental anomaly in which the skin is continuous over the eyeballs without any indication of the formation of eyelids), kinked tail, protruding tongue, and renal hypoplasia. Skeletal examination revealed degrees of curving, shortening and/of thickening of the radius or tibia. The same syndrome was noted in one of the F1 pups from the low dose group (found dead at day two after delivery) in the groups allowed to live until weaning. The results are presented in Table 1.

Table 1
Teratogenicity of Lamivudine in Rats

Dose (mg/kg/day bid)	No. of Affected		No. of Affected	
	Fetuses	Litters	Pups	Litters
Control	0/330	0/23	0/160	0/12
45	0/311	0/24	1/157	1/11
300	4/339	1/24	0/154	0/12
2000	0/336	0/24	0/147	0/12

Comments: In this study, a litter containing pups with renal hypoplasia, ablepharia and protruding tongue is recorded. Although the syndrome is seen in only two litters in the present study, because of the rare nature of the defects and the

similarity of defects seen in teratogenicity studies with a closely related compound (ddC) in a second species (mice), one must conclude that the spectrum of major malformations are a result of treatment with Lamivudine. The sponsor is aware of this and indicates that further studies will be carried out to assess the teratogenic potential of this drug.

4. Lamivudine: A further investigation into the effects of twice daily oral administration on pregnant AHA rats and their progeny in utero (Report of findings following external and visceral or skeletal fetal examination), batch # C1758/233/1, Glaxo Group Research Ltd, Ware, Hertfordshire, England, 11 December, 1991, (WPT/91/443/R13161)*

Three groups of mated female AHA rats {strain: Wistar/Sprague Dawley derived with Wistar characteristics; age: 7 weeks; weight: 220 g; 30 animals/group (50 animals/control); day 1 of pregnancy = day of mating} were administered Lamivudine, twice daily approximately 12 hr apart, by oral gavage at a standard dosage volume of 10 ml/kg (in acetate buffer, pH 3.5-3.7) during gestation days 7 to 16 at dose levels of 0 (vehicle control), 45 or 300 mg/kg/day. All mated females were killed by inhalation of carbon dioxide on day 21 of pregnancy, and ovaries and uterus were removed from each dam for examination. All live fetuses were killed by ip injection of sodium pentobarbital. A total of 1435 fetuses were examined and major abnormalities were recorded in 8 of these specimens (4 in controls, 2 in each of the 45 and 300 mg/kg groups). Abnormal digits, comprising malpositioning, fusion and absence unilaterally, (1 fetus, 1 dam in 300 mg/kg); internal hydrocephalus (1 fetus, 1 dam in control); microphthalmia (1 fetus in each dam in control and 300 mg/kg); retro-oesophageal/tracheal aorta (2 fetuses, 2 dams in control, and 2 fetuses, 2 dams in 45 mg/kg) were recorded. External/Visceral Findings: there was considered to be no effect of treatment of Lamivudine on either of the findings recorded. Skeletal Findings: incidence of non-ossification of the hyoid bone was increased compared to the control group at 45 and 300 mg/kg, the incidence being 5.2, 9.9 ($p<0.05$) and 7.4 in control, 45 and 300 mg/kg, respectively. The incidence of supernumery rib formation, bilateral, was statistically increased at 300 mg/kg only. Variants: there were statistically significant increased incidences of poor ossification of the 6th sternebra at 300 mg/kg, the incidence being 74.6, 71.4 and 80.9% in control, 45 and 300 mg/kg.

Comments: Abnormal digit formation was found in one fetus from 300 mg/kg bid treated group. Because of its isolated occurrence, it is unlikely that the digit defect is associated with treatment. Digit defects, in association with other defects in various animal species, have been recorded with nucleoside analogues, particularly with ddC (a drug very similar in

structure to Lamivudine) treatment (Teratology 42: 131-136, 1990).

5. Lamivudine: Embryotoxicity and teratogenicity study - rabbits. Study No. WPT/91/333, December, 1991, drug lots C1803/152/1 and C1578/226/1.

Five pregnant Dutch rabbits per group were administered Lamivudine in sodium acetate buffer pH 5.7 by gavage twice daily (12 hours apart) at doses of 0 (vehicle control), 250, 1000 and 2000 mg/kg/day between days 8 and 20 of pregnancy inclusively. These animals were designated as the dose groups. They were examined at least once daily for clinical signs. The animals were weighed on day 1 of pregnancy, on days 4, 6, 8-20 and every other day until the termination of the study. On day 7, all animals were bled for the determination of progesterone levels. All surviving animals were killed on day 30 for the examination of their uterine contents. The number of corpora lutea on each ovary, the number of implantation sites, the position of each fetus and early or late embryonic/fetal deaths and the fetal weights were recorded. Each live fetus was examined for external abnormalities. They were killed, dissected and examined. They were examined for skeletal abnormalities after staining with Alizarin Red S. Any external, visceral or skeletal abnormalities were recorded. All the fetal tissue was retained in buffered 4% formaldehyde. An additional 2 pregnant females per group were treated with Lamivudine identically for the estimation of plasma drug levels. This group of animals was designated as the absorption group. The animals were examined at least once daily for clinical signs. The animals were weighed on day 1 of pregnancy, on days 4, 6 and from 8 to 20. Blood samples were collected from each of the animals in the absorption group on day 8 of pregnancy at 0.5, 1, 2, 4, 8 and 12 hours after the first dose, on day 9 predose and 2 hours after dosing and on day 20 predose and 0.5, 1, 2, 4, 8, 12 and 24 hours after the first dose (the only dose given). On day 21, the absorption groups were killed.

One low dose and one high dose animal were killed due to dosing accidents. One intermediate dose animal aborted on day 30. On postmortem examination, the animal was found to have a pale liver and lungs with liquified cecal contents. One high dose animal aborted on day 23 and one was found dead on day 20 but no postmortem abnormalities were found. One high dose animal in the absorption group was killed on day 18 due to its poor condition.

Upon initiation of dosing, there was a decrease in body weight in all the animals. Except in the lose dose animals, the body weight was lower at the end of the study than at the beginning of dosing. In the low dose animals, the body weight at the end of the study was approximately equal to the starting body weight.

At postmortem examination, several animals at all doses had few or no fecal pellets in the lower intestine. The incidence of pre-implantation loss was increased in all treatment groups. The percentages were 3.6 in the controls and 39.1, 31.4 and 29.4 in the low, intermediate and high dose groups respectively. Post-implantation loss was unaffected by treatment although this observation may have been compromised by the fact that one control litter had a 100% incidence of intra-uterine deaths. Compared with historical values, the mean fetal body weights from the dams in the vehicle control group were markedly reduced. The mean fetal weight in the low and intermediate groups were higher than those of the controls (and barely within historical limits). The high dose fetal weights were markedly reduced compared to those in the concurrent controls. A total of 64 fetuses were examined, 25 of which had major abnormalities. The number of fetuses examined (with the number of major abnormalities in parenthesis) was 19 (7), 12 (2), 22 (10) and 11 (11) in the control, 250, 1000 and 2000 mg/kg groups respectively. There was delayed skeletal development in the fetuses from the two high dose groups. A syndrome of abnormalities in the group treated with 2000 mg/kg/day was recorded. The syndrome consisted of subcutaneous edema in the neck and torso region with forelimb arthrogyropsis (persistent flexure or contracture), incomplete formation of the abdominal muscles, lateral cerebral ventricle dilation, non-eruption of upper incisors, abnormally bulbous heart, shortening or thickening of the ribs and curving or thickening of the long bones. Four of the eleven fetuses had palate cleft or other palatine defects.

Comment: The sponsors state that while an involvement of Lamivudine in the fetal findings cannot be excluded, the poor maternal tolerance of the pH 3.7 acetate buffer may also be a contributing factor. The sponsor further states that a preliminary oral organogenesis study was carried out using sterile water (see below).

The pharmacokinetic data reported in this study were very sketchy. A significant increase in plasma levels was observed between the first and last occasion of administration while there was an increase in the plasma levels as a function of dose. However, the variation among animals was too great and the quantity of data too sparse to say much more.

6. A Preliminary Embryotoxicity and Teratogenicity Study in Rabbits, Lot # C1803/152/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., December, 1991, (L13030-WPT/91/334)

In this preliminary study, five pregnant Dutch rabbits per group were administered Lamivudine in water by gavage twice daily (12 hr apart) at doses of 0 (venicle control), 500 and 1000 mg/kg/day bid between days 8 and 20 of pregnancy inclusively. The animals

were examined at least once daily for clinical signs. The animals were weighed on day 1 of pregnancy, on days 4, 6, 8 - 20 and every other day until the termination of the study. On day 7, all animals were bled for the determination of progesterone levels, while on days 8 and 20 of pregnancy, blood was collected from two animals in each group at 1, 2, 4 or 8 hr after dosing for the determination of drug plasma levels. All surviving animals were killed on day 30 for the examination of their uterine contents. The number of corpora lutea on each ovary, the number of implantation sites, the position of each fetus and early or late embryonic/fetal deaths and the fetal weights were recorded. Each live fetus was examined for external abnormalities. They were killed, dissected and examined. They were examined for skeletal abnormalities after staining with Alizarin Red S. Any external, visceral or skeletal abnormalities were recorded. All the fetal tissue was retained in buffered 4% formaldehyde.

No animals died during the study. One control animal had what appeared to have blood in the cage on two occasions. On postmortem it was found to have only implantation scars in utero and was considered to have aborted. Several low dose animals had reduced feces and one had colored urine on days 23 - 25. All the high dose animals had loose feces, reduced intake and were thin. Several had colored urine (orange) and soiled coats. One animal was found not to be pregnant. Two high dose animal were found to have aborted on postmortem examination. One high dose animal had a viable litter and one was seen to have delivered her litter early (day 27). The body weight gain was similar in the control and low dose group. The high dose group body weight was only based on one animal since the other four had either aborted, delivered early or were not pregnant. The one animal which had a viable litter lost weight rapidly from the time of dosing until the end of the study. The food consumption of the high dose group was dramatically reduced by day 10 of the study and remained low throughout. At postmortem, one low dose animal was found to have extensive dark areas on the surface of the kidney. Pre-implantation loss was higher in the low dose group than in the controls (43.8 to 14.3%). The mean fetal weight in the high dose group was markedly reduced (however, only one litter survived to term) compared to those in the low dose and control groups. The only fetal effect considered to be related to treatment was a delay in skeletal development at both dosage groups.

The plasma levels at day 8 and 20 for the high and low doses are shown Table 2.

Table 2
Plasma Levels of Lamivudine in Pregnant Rabbits Receiving Repeated Oral Administration of Lamivudine

Sample Time (hr)	Dose bid (mg/kg/day)			
	500		1000	
	Day 8	Day 20	Day 8	Day 20
	Plasma Levels of Lamivudine (µg/ml)			
1				
2				
4				
8				

It can be seen from Table 2 that the drug accumulates over time and that the plasma concentration increases with increasing dose.

Comments: The sponsor concluded that oral dosages up to 500 mg/kg/day formulated in sterile water would be suitable for twice daily administration in a full organogenesis study in the Dutch rabbit.

7. Segment II Embryotoxicity and Teratogenicity Study in Rabbits, Lot # C1758/233/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., August, 1992, (L13069-WPT/92/014)*

Groups of pregnant Dutch rabbits (weight: 2.1 - 2.3 kg; age: 16 weeks; 16 animals/group) were administered by gavage Lamivudine in water at doses of 0 (vehicle control), 45, 150 or 500 mg/kg/day bid, at 12 hr intervals, between days 8 to 20 of pregnancy inclusively. All the animals were examined daily before and after treatment for signs of ill health or clinical signs. Body weights were recorded on days 1, 4, 6, 8 - 20 inclusive and every other day thereafter until day 30. Food and water consumption was recorded daily. The surviving animals were killed on day 30 and examined for the number of corpora lutea and number of implantation sites, the position of each fetus and early or late embryonic or fetal death and fetal weight. Each live fetus was examined for external abnormalities. Visceral examination was carried out after dissection after which the cranial caps were removed, fixed and stained with the eviscerated carcasses in Alizarin Red S for skeletal examination. Approximately 50% of the heads were removed, fixed in Bouin's fluid and examined by serial section. On day 8 and day 20, blood was taken from each of two animals per group per time point at 2, 4, 8 and 12 hr after the first dose for the determination of drug plasma concentrations.

The animals dosed at 500 mg/kg/day bid lost weight compared to the other groups throughout the course of the study. Between day 1 and day 30, the control, low and intermediate dose animals gained between 5.6% (control) and 9.9% (intermediate dose) of their starting body weight but the high dose animals lost 0.5%. This was reflected in a lower food and water consumption for the high dose animals throughout the course of the study. The high dose animals also had an increased incidence of clinical signs compared to the other groups. The clinical signs included reduced or loose feces, orange urine, soiled coats with watery salivation and vocalizing. There was significant evidence of a drug-related trend in pre-implantation losses in all the dosed groups. The incidence was 16.8, 52.1, 32.1 and 41.0% in the control, low, intermediate and high dose groups, respectively (p-value for trend <0.01). Three females (2 intermediate and one high dose) showed complete resorption of the whole litter and 9 females (1 control, 2 low, 2 intermediate and 4 high dose animals) showed no evidence of pregnancy at day 30 despite positive progesterone assays suggestive of pregnancy. These findings indicate that treatment with Lamivudine may have had an effect at early post-implantation (which would have been described as pre-implantation losses). A summary of caesarian data is provided in Table VII.

A significant reduction of the number of live fetuses was seen at term. The incidence was 5.9, 3.8, 4.4 and 4.7 live fetuses per dam in the control, low, intermediate and high dose groups (p-value for trend <0.05). Major defects were recorded in 11 fetuses, 1 each in the control and high dose group, 3 in the low dose group and 6 in the intermediate dose group. The defect in the control group animal was an abnormal closure of the coronal suture. This same defect was found in the 6 animals of the intermediate dose group (in 3 litters). The low dose defects were an interrupted aortic arch in one fetus, sternebral fusion in a second and one animal with multiple defects (hydranencephalus, severely domed skull, olfactory bulb cavities, cleft palate and areas of non-fusion of premaxilla to palatal shelves). At the high dose, there was one animal with multiple defects (hydrocephalus, fusion of the upper jaw incisors, partial formation of the nasal turbinates, cardiac atrial and ventricular septal defects, persistent truncus arteriosus, severe tail shortening and marked fusion between the sternebrae). Skeletal examination showed a reduction in epiphyseal ossification at the high dose. There were no significant findings during external and visceral examinations.

Results of absorption studies are summarized in Table 3. The data. Plasma levels of this compound increased with dose and sustained systemic exposure of the rabbits was achieved. However, the results showed that there was a significant increase in the plasma levels (3.5 - 5 times) of Lamivudine between the first and last administration at all three doses.

Table 3
Summary of Caesarian Data

Effects on Pregnancies	Control Group	Low Dose Group	Mid Dose Group	High Dose Group
No. of Pregnancies	16	14	15	15
No. of Abortions	0	1	1	3
No. with Resorptions	0	0	2	1
No. of Females with Litters	15	12	11	9
No. of Viable fetuses/Litters	5.9	3.8	4.4	4.7
Pre-implantation Loss (%)	16.8	52.1	32.1	41
Post-implantation Loss (%) (Including Total Resorptions)	5.3	2.2	15.8	10.6
Post-implantation loss (%) (Excluding Total Resorptions)	5.3	2.2	12.7	8.7

Table 4
Mean Plasma Levels of Lamivudine in Pregnant Rabbits After Repeated Oral Administration

Dose (mg/kg/day) bid	Time of Sample (hr)	Plasma Lamivudine Levels (µg/ml)	
		Day 8	Day 20
45	2 4 8 12		
150	2 4 8 12		
500	2 4 8 12		

Comments: An increased pre-implantation loss and a consequent decreased in mean number of viable fetuses per litter is seen in all treated groups suggesting a possible early embryolethal effect of Lamivudine in rabbits. The sponsor is planning to conduct a further organogenesis study to determine the reproducibility of this effect and to determine the no effect level for this observation. The incidence of affected fetuses in control group in this study is 1.1%. The fact that no abnormal

suture closures are seen in the high dose animals does complicate the interpretation of the data as to whether the drug is teratogenic. At the high dose, one animal shows abnormal skeletal development (a reduction in epiphyseal ossification and increased incidence of unilateral supernumerary rib) It is possible that the abnormality is related to maternal effects (body weight loss and reduction in food consumption) at this dose level. The higher occurrence abnormalities in the intermediate dose group may have been an incidental finding not related to drug administration.

8. Lamivudine: A further study to assess the effects of oral administration on pregnant Dutch rabbits and their offspring, Batch No. C1758/233/1, Glaxo Group Research Ltd., Hertfordshire, England, August 3, 1993, (WPT/92/184/Study No. L13280)*

Groups of pregnant rabbits (17 animals/group) were administered Lamivudine via oral gavage twice daily on days 8-20 of pregnancy at dosages equivalent to 0 (control), 7.5 (low), 20 (mid) or 45 mg/kg/day (high). There was no significant effect of treatment on maternal body weight gain, food consumption and water consumption. There were no significant abnormal findings at necropsy of females on day 30 of pregnancy. General condition: clinical observations included reduced and loose feces, colored urine and hair loss and were similar for all groups with the exception of slightly increased severity and frequency of reduced feces (high). Absorption studies: plasma levels increased with dose, but sustained systemic exposure of the rabbits to Lamivudine was achieved only in high dose group of animals. Uterine examination: pre-implantation loss was slightly increased (mid and high). The increase was considered treatment-related; though it did not achieve statistically significance. Post-implantation loss was slightly increased (mid and high). Fetal body weight: was slightly increased (mid and high) when compared to the control group. Fetal examination: there was considered to be a possible treatment-related effect on the incidence of skeletal defects, specifically the induction of supernumerary ribs at all dose levels (3.7, 14.1, 17.5 and 13% in the control, low, mid and high, respectively) employed.

Comments: The incidence of skeletal defects (supernumerary ribs) in the fetuses had also been observed in a pervious study (WPT/92/014). The sponsor has cited two references from the scientific literature that the occurrence of supernumerary ribs in the fetuses has been shown in some species to be indicative of maternal toxicity or stress and it appears to be a temporary state, reversible postnatally, and so has no permanent structural effect on fetal skeleton. Regardless, there appeared to be a possible treatment-related effect [maternal toxicity or stress] that may have caused the skeletal defects.

9. Lamivudine: A preliminary study to assess the effects of oral administration to pregnant and lactating AHA rats and of direct oral administration to their juvenile offspring, Batch No. C1817/116/1, Glaxo Group Research Ltd., Hertfordshire, England, October 5, 1993, (WPT/92/404/Study No. R13609)*

Groups of pregnant rats (12 animals/group) were administered Lamivudine via oral gavage twice daily from day 17 of pregnancy until litter day 21 at dosages equivalent to 0 (control) or 2000 mg/kg/day (high) and offsprings were dosed orally once daily at levels of 0 (control), 500 (low), 1000 (mid) or 2000 mg/kg/day (high) from day 3 to litter day 28. The purpose of this preliminary study was to provide information to determine suitable oral dosages of Lamivudine for administration in a full Segment III study. Conclusion: this study will not be reviewed in detail. The sponsor has concluded that dosage levels of 2000 mg/kg/day bid (dams) and 2000 mg/kg/day once daily (offsprings) should be used as a high dose level in the main Segment III study.

10. Lamivudine: The effects of oral administration to pregnant and lactating AHA rats and of direct oral administration to their juvenile offspring, Batch No. UFP0001, Glaxo Group Research Ltd., Hertfordshire, England, March 11, 1993, (WPT/93/165/Study No. R13739)

Groups of pregnant AHA rats (15 animals/group) were administered Lamivudine via oral gavage twice daily from day 17 of pregnancy until litter day 21 at dosages equivalent to 0 (control) 90 (low), 450 (mid) or 2000 mg/kg/day (high) and their offsprings were dosed orally once daily at levels of 0 (control), 90 (low), 450 (mid) or 2000 mg/kg/day (high) from litter day 3 to litter day 16 22 or 43. The purpose of this study was to assess the effects of oral administration of Lamivudine on pregnant and lactating rats and their offsprings. Absorption data: plasma concentrations achieved in the dams were similar to results obtained in adult female rats receiving similar dosages in the six month toxicity study (WPT33/361). Plasma Lamivudine contentrations at 2 hr post dose in juveniles rats increased with increasing dose and were approximately three times greater than those in adults receiving an equivalent dose. General observations: food consumption during late pregnancy was reduced (high), clinical observations (swollen/reddened anus/rectum) were noted during the lactation period with associated inflammatory changes of the anus (high). These changes consisted of chronic inflammation, hyperplasia and erosion or ulceration of the squamous epithelium of the anorectal junction and sebaceous gland hyperplasia. Examination of the cecum of dams (high) revealed a minimal or slight diffuse epithelial hyperplasia. Offsprings (high) showed a pattern of clinical observations and pathological changes similar to the dams. An increased incidence of urination

upon handling was observed in some offsprings (mid or high). Compared to controls, statistically significant decreased in absolute mean spleen weight were observed in male and female offsprings (mid or high); no abnormal pathology was observed. A reduction in testes weight and dilation of the seminiferous tubules were noted in offsprings (high). A dose related delay in incisor eruption (male and female offsprings at high) and precocious eye opening were recorded at all treatment groups in male offsprings and at mid and high doses in the females. Clinical chemistry: a slight increase in mean serum urea concentration was seen in offsprings (high). Statistically significant decreases were also seen in mean serum bilirubin concentration in female offsprings (low and high).

Comments: Decrease in testes weight and seminiferous tubule dilation in offsprings was attributed to the treatment (high). A dosage level of 450 mg/kg/day bid may be considered no toxicological effect level for the dams. Toxicity was seen at all dose levels for male offsprings.

V. Mutagenicity Studies

1. Lamivudine: Genetic toxicity study - microbial mutagenicity screen Report # WPT/90/238 September, 1990

Four preliminary microbiological screens were used to determine the genetic toxicity of the racemate GR103665X (+) (batch C1034/185/1). The screens were the Ames test using S. typhimurium strains TA98, 100 1535 and 1537, a yeast gene conversion assay using S. cerevisiae, an E. coli mutagenicity test using strains WP2(pKM101) and WP2uvrA(pKM101), and a liquid pre-incubation Ames test using S. typhimurium strains TA98, 100, 1535 and 1537. All tests but the fourth were carried out in the presence and absence of an S-9 mixture from phenobarbitone/ß-naphthoflavone induced rat liver. The other was carried out only in the presence of the S-9 mixture.

GR103665X (±) was mutagenic in the Ames test at doses of 5000 µg per plate. The compound was active in strains TA100 and TA1535 and only in the presence of an S-9 activating system.

2. Microbial Mutagenicity Screen, Batches # C1460/129/4, B1916/47/2, C1619/18/3 and B1916/52/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., October, 1990, (U12455-WPT/90/240)

This mutagenicity screen tested GR109712X (+) and Lamivudine in the Ames test against S. typhimurium strain TA1535 in the presence and absence of activation with S-9 mixture from phenobarbitone/ß-naphthoflavone induced rat liver. It was found that the (+) enantiomer GR109712X was mutagenic at doses of 3000, 4000 and 5000 µg per plate in the presence of S-9 activation. The

enantiomer Lamivudine was not active at any dose tested in the presence or absence of S-9 activation.

3. Lamivudine: Microbial mutagenicity screen, Batch No. UFP0003, Glaxo Group Research Ltd., Hertfordshire, England, March 15, 1994, (WPT/93/246/Study No. V14001)*

Lamivudine was tested in both Ames and liquid pre-incubation (Yahagi) assays at test concentrations up to 5000 µg/plate (free base) using Salmonella typhimurium TA1535, TA1537, TA98 and TA100. The fluctuation tests were carried out up to a maximum concentration of 1000 µg/ml (free base) using Escherichia coli WP2 (pKM101) and WP2 uvrA (pKM101). All tests except Yahagi were carried out in the absence and presence of a rat liver S9-mix that had been derived from the livers of rats pre-treated with phenobarbitone/ß-naphthoflavone. Results: Lamivudine was not detectably mutagenic towards any of the bacterial tester strains used in the study.

4. Lamivudine: Genetic toxicity study - mouse L5178Y cell line mutagenicity assay Report # WPT/90/271 June, 1991

The racemate GR103665X (±) (batch C1034/185/1) was tested for mutagenicity in the mouse L5178Y cell line mutagenicity test. The compound was not mutagenic in the presence of rat liver aroclor 1254 induced S-9 at doses as high as 5000 µg/ml. However, in the absence of S-9, the compound induced mutations in this cell line at all doses tested between 1000 and 2500 µg/ml (the highest dose tested in the absence of S-9). At the highest doses, increases in small colonies relative to controls indicated that the activity could probably be related to a clastogenic effect.

5. An Assessment of Mutagenic Potential in the Mouse Lymphoma TK Locus Assay, Batch # 90G03B26, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., May, 1991, (V12638-WPT/91/018)*

The compounds GR103665X, GR109712X and Lamivudine were tested at doses of 0, 100, 1000, 1500, 2000 and 2500 µg/ml in the absence of S-9 activation for mutagenicity in the mouse L5178Y cell line mutagenicity test. GR109712X induced increases in the mean mutational frequency of 3.6 to 5.3 times the control between concentrations of 1000 to 2500 µg/ml. Lamivudine was not mutagenic at doses up to 1500 µg/ml. At doses of 2000 and 2500 µg/ml, it induced an increase in mutational frequency of 2.0 and 2.9 times the control value. Again, at the highest doses, increases in small colonies relative to controls indicated that the activity could probably be related to a clastogenic effect.

Comments: Although Lamivudine is mutagenic in this test system, it is less mutagenic than its enantiomer GR109712X.

6. Genetic Toxicity Study in BALB/c 3T3 Mouse Embryo Cell Assay, Lot # C1758/229/1, June, 1992,
(M13156-UTX/92/018/Draft Report)

In a BALB/c 3T3 cell transformation assay, Lamivudine was found to be negative at concentrations up to 320 µg/ml in the absence of an Aroclor 1254-induced rat liver S-9 preparation. In the presence of the activating system, the test compound was negative at concentrations as high as 5000 µg/ml.

7. Pharmaceutical data to support study M1315618. Genetic Toxicity Study in BALB/c 3T3 Mouse Embryo Cell Assay, Lot # C1758/229/1, June, 1992,
(M13156-UTX/92/018)

In a BALB/c 3T3 cell transformation assay, Lamivudine was found to be negative at concentrations up to 320 µg/ml in the absence of an Aroclor 1254-induced rat liver S-9 preparation. In the presence of the activating system, the test compound was negative at concentrations as high as 5000 µg/ml.

8. Cytogenetic Evaluation Using Cultured Human Lymphocytes, Batch # 1916/47/2 and 1916/52/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., April, 1991, (Y12629/Y12850-WPT/90/393)

The compounds: GR109712X and Lamivudine were evaluated for cytotoxicity using cultured human lymphocytes in the absence of S-9 activation. Both compounds induced a dose related and statistically significant increase in aberrant cells at doses of 100 and 300 µg/ml and therefore they are clastogenic. GR109712X was clastogenic at doses of 30 µg/ml whereas Lamivudine was not. Thus, Lamivudine is somewhat less clastogenic than the other enantiomer although both are genotoxic.

9. Lamivudine: Metaphase analysis in human lymphocytes in vitro, Batch No. UFP0003, Glaxo Group Research Ltd., Hertfordshire, England, July 29, 1993, (WPT/93/120/Study No. V14104)*

In vitro, human lymphocytes were treated with Lamivudine at dose levels of 30, 100 or 300 µg/ml in the absence or presence of S9 mix (derived from rat liver homogenates) to evaluate chromosome aberration potential of the test compound. Results: Lamivudine (300 µg/ml) demonstrated a statistically significant increase in the frequency of cells with aberrations both in the presence and absence of metabolic activation (S9-mix). Greatest activity was seen in the absence of metabolizing enzymes. No clastogenic effects were seen at 30 or 100 µg/ml. Conclusion: Lamivudine was found to be a direct acting clastogen to human lymphocytes in vitro.

10. Lamivudine: Genetic toxicity study - rat micronucleus test, Lot # 1916/52/1, Glaxo Group Research Ltd., Hertfordshire, England, June 1991, (WPT/90/275)

Fourteen male AHA rats per group were administered the racemate GR103665X (±) in 0.9% sterile saline by intravenous injection at doses of 0 (vehicle control), 75, 150 and 300 mg/kg at time 0 and 4 hours. At 24 and 48 hours, seven animals of each group were killed and their bone marrow cells examined for detectable chromosome damage in the form of micronuclei. A group of five animals were administered a single dose of cyclophosphamide at 20 mg/kg and examined at 24 hours as a positive control. GR103665X (±) showed no evidence of clastogenic activity, nor did it induce cytotoxic or cytostatic effects on erythroblast formation after intravenous administration.

11. Lamivudine: Lack of induction of metaphase chromosome damage in Sprague Dawley CD rat bone marrow cells following oral administration, Batch No. UFP0003, Glaxo Group Research Ltd., Hertfordshire, England, April 30, 1993, (WPT/93/195/Study No. R14091)*

Groups of rats (5 animals/sex/group) were given a single oral dose of Lamivudine at 0 (vehicle control), 0 (cyclophosphamide 10 mg/kg, positive control), 500, 1000 or 2000 mg/kg to assess the clastogenic effects in bone marrow cells. Animals were killed at 6, 24 or 28 hr after the administration and bone marrow cells were scored for the presence of chromosome aberrations. Results: there was no evidence of an increase in the incidence of chromosome aberrations in animals treated with Lamivudine when compared to the vehicle control group. The positive control material produced a marked increase in the frequency of chromosome aberrations. Conclusion: Lamivudine was considered to be non-clastogenic under the conditions of the study.

12. Lamivudine: Genetic toxicity study - rat unscheduled DNA synthesis, Batch # c1034/200/1, Glaxo Group Research Ltd., Hertfordshire, England, June 1991 (WPT/90/408/Study No. R12395)*

Three male AHA rats per group were administered two intravenous injections, four hours apart, of the racemate GR103665X (±) (batch C1034/200/1) in 0.9% sterile saline at doses of 0 (vehicle control), 150 or 300 mg/kg/dose. Two or 12 hours after the second dose, the animals were killed, hepatocytes were isolated and incubated in media containing tritiated thymidine. Autoradiographs were prepared and exposed for 14 days in the cold. They were developed, fixed, stained and read for the number of cells in repair as well as for the mean net number of grains (the number of grains in the nucleus - the number of grains in the cytoplasm). A positive control at a single dose by gavage of 75 mg/kg of 2-acetylaminofluorine was administered to two

animals which were examined after 12 hours. The studies were repeated two times. The racemate GR103665X (±) did not induce unscheduled DNA synthesis at total doses of 300 or 600 mg/kg in either of the two studies.

Comments: The sponsor has indicated that three month toxicity studies are underway in rats and dogs. The results of these studies should be submitted in the support of the IND without delay.

13. Lamivudine: Lack of activity in male rat liver unscheduled DNA synthesis (UDS) assay following oral administration, Batch No. C1953/195/4, Glaxo Group Research Ltd., Hertfordshire, England, March 25, 1994, (WPT/93/536/Study No. R20104)*

Groups of male rats (5 animals/group) were given a single oral dose of Lamivudine at 0 (vehicle control), 0 (2-acetylaminofluorene 75 mg/kg, positive control), 500, 1000 or 2000 mg/kg to assess unscheduled DNA synthesis (UDS) in the hepatocytes. Animals were killed at 2 and 12 hr after the administration and hepatocytes were isolated from all animals and incubated in media containing radiolabelled thymidine. Autoradiographs were prepared and subsequently analyzed for induction of UDS. Results: The positive control material gave the expected positive response inducing marked levels of UDS. Lamivudine did not induce UDS in the hepatocytes of male rats. Conclusion: under the conditions of the study approximately 2 and 12 hr after the oral administration, Lamivudine did not induce UDS in the hepatocytes of male rats.

NON-CLINICAL PHARMACOKINETICS AND TOXICOKINETICS

Summary of Absorption, Distribution, Metabolism and Excretion (ADME) Studies:

1. **Bioanalytical support data for Lamivudine and AZT: Effect on hematology of mice (GDM/93/009/Study No. M13367)**

2. **Bioanalytical support data for Lamivudine: One month oral study to determine the effects when given in combination with interferon (alpha); on the hematology of mice (GDM/94/014/Study No. M20009)**

3. **Bioanalytical support data for Lamivudine: Palatability in the diet of mice (GDM/93/010/Study No. M13284)**

4. **Bioanalytical support data for Lamivudine: 90 days oral (dietary) toxicity for mice preliminary to an oncogenicity study (GDM/93/075/Study No. M13285)**

5. The metabolism of Lamivudine in mouse, rat, dog and man (GDM/94/123)

6. Radioactivity balance study and metabolic profile of radioactivity in urine following a single oral or a single intravenous dose of [^{3}H]-Lamivudine (45 mg/kg) in the mouse (GDM/92/045/Study No. 92/DM/45)

7. Pharmacokinetics of a Single Oral or Intravenous Radioactive Dose in Rats, Batch # C1282/75/4, Glaxo Group Research Ltd, Greenford, Middlesex, U.K., April, 1991, (GDM/91/015)

8. The pharmacokinetics of Lamivudine and total radioactivity following a single oral or single intravenous dose of [^{3}H]-Lamivudine (45 mg/kg) in the female AHA rat, Study No. GDM/91/77, March, 1992, Glaxo Group Research Ltd., Greenford, Middlesex, United Kingdom, Drug lot C1013/75/1 (non-radioactive drug), C1282/75/4 (radioactive drug).

9. Pharmacokinetics of a Single Intravenous Dose in Rats, Batch # C1404/194/1, Glaxo Group Research Ltd, Greenford, Middlesex, U.K., May, 1991, (GDM/91/021)

10. Pharmacokinetics of Single Oral Doses in Rats, Batch # C1021/179/1, Glaxo Group Research Ltd, Greenford, Middlesex, U.K., February, 1991, (GDM/91/003)

11. Bioanalytical support data for Lamivudine: Metaphase analysis in the rat bone marrow in vivo (GDM/93/037/Study No. R14091)

12. Lamivudine: Pharmacokinetic study in the juvenile AHA rat, batch # C1758/233/1, Glaxo Group Research Ltd, Ware, Hertfordshire, England, 1 April, 1992, (WPT/92/190/R13355)

13. Bioanalytical support data for Lamivudine: A pilot 5 day oral toxicity study in AHA rats (GDM/91/012/Study No. R12636)

14. Plasma levels of Lamivudine monitored during a one month oral toxicity study in the AHA rat (GDM/91/020/Study No. R12637)

15. Bioanalytical support data for Lamivudine: Effects of oral administration upon the reproductive performance of a parental dosed generation (F_0) of AHA rats and upon the peri- and post-natal development of the resulting two successive, untreated generations (F_1 and F_2), Batch No. UFP 0002, Glaxo Group Research Ltd.,

Hertfordshire, England, June 9, 1994, (GDM/94/059/WPT/92/210/Study No. R13738)

16. Bioanalytical support data for a 13 week oral toxicity study of Lamivudine in the AHA rat, Study No. GDM/91/089, February, 1992, Glaxo Group Research Ltd., Greenford, Middlesex, United Kingdom, Drug lot C1013/75/1.

17. Bioanalytical support data for Lamivudine: Six month oral toxicity study in the AHA rat (GDM/93/024/Study No. R13470)

18. Bioanalytical support data for Lamivudine: A preliminary study to assess the effects of oral administration to pregnant and lactating AHA rats and of direct oral administration to their juvenile offspring (GDM/93/031/Study No. R13609)

19. Bioanalytical support data for Lamivudine: Effect of oral administration to pregnant and lactating AHA rats and effect of direct oral administration to their juvenile offspring (GDM93/038/Study No. R13739)

20. Bioanalytical support data for Lamivudine: Palatability in the diet of rats (GDM/92/055/Study No. R13287)

21. Bioanalytical support data for Lamivudine: 90 days oral (dietary) toxicity to rats preliminary to an oncogenicity study (GDM/93/076/Study No. R13288)

22. Bioanalytical support data for GR109714x: effects of oral administration upon pregnant AHA rats. Study No. GDM/91/083, January, 1992, drug lot C1013/75/1.

23. In Vitro Red Blood Cell Binding and Plasma Protein Binding of Radiolabelled Lamivudine in Rat, Dog and Man, Batch # C1284/257/2, Glaxo Group Research Ltd, Greenford, Middlesex, U.K., February, 1991, (GDM/91/010)

24. Whole body autoradiography - rats Report # GDM/90/040 January, 1991

25. Excretion balance study (day 19) and placental transfer of radioactivity (day 12 and day 19) in the pregnant female AHA rat after oral administration (45 mg/kg) of [^{3}H]-Lamivudine. Report No. GDM/91/050, January, 1992, non-radioactive drug lot C1013/75/1, radioactive drug lot C1282/75/4.

26. Lamivudine: A study of the milk transference following oral administration (45 mg/kg) to the rat (GDM/92/017/Study No. 92/DM/17)

27. The renal disposition of Lamivudine in the isolated perfused rat kidney (UCP/93/012)

28. To investigate the interaction of Lamivudine with rat and human hepatic microsomal cytochrome P450's utilizing testosterone as probe substrate (GDM/93/028/Study No. 93/DM/028)

29. The expression of Cytochrome P450 isoenzymes in hepatic microsomes from male and female rats after oral administration of Lamivudine in a 90 day toxicity study (GDM/94/125/Study No. R13288)

30. Radioactivity Balance Study of a Single Oral or Intravenous Radiolabelled Dose in Rats, Batch # C1284/275/2, Glaxo Group Research Ltd, Greenford, Middlesex, U.K., March, 1991, (GDM/91/014)

31. To investigate the excretion balance and metabolic profile of radioactivity in urine following a single oral dose of [^{3}H]-Lamivudine (45 mg/kg) in the Han Wistar rat (GDM/94/076/Study No. 94/DM/76)

32. Radioactivity Excretion Balance and Metabolic Profile in Urine After One Month Oral Toxicity Study of an Oral Dose in Rats, Batch # C1282/75/4, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., April, 1991, (GDM91/017)

33. The Pharmacokinetics of Lamivudine and Total Radioactivity on Day 20 of Pregnancy in the Female Dutch Rabbit After Oral Administration of [^{3}H]-Lamivudine, Lot # C1758/226/1, C1282/75/4, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., March, 1992, (GDM/91/058)

34. Bioanalytical Support Data for Lamivudine: A Study to Assess the Effects of Oral Administration on Pregnant Dutch Rabbits and Their Progeny, Lot # C1404/194/1, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., March, 1992, (GDM/92/008)

35. Bioanalytical support data for Lamivudine: A further study to assess the effects of oral administration on pregnant Dutch rabbits, batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, England, 3 June, 1992, (GDM/92/035/L13280)*

36. Excretion Balance and Placental Transfer of Radioactivity in the Pregnant Female Dutch Rabbit After Oral Administration of [^{3}H]-Lamivudine, Lot # C1758226/1, C1282/75/4, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., March, 1992, (GDM/91/059)

37. Pharmacokinetics and Excretion of a Single Oral or Intravenous Dose in Dogs, Batch # C1284/275/2, Glaxo Group Research Ltd, Greenford, Middlesex, U.K., May, 1991, (GDM/91/008)

38. An Investigation of the Pharmacokinetics and Total Radioactivity and the Excretion Balance of Radioactivity in the Female Beagle Dog Following the Administration of [^{3}H]-Lamivudine (30 mg/kg) By the Oral and Intravenous Routes, Lot # C1758/233/1, C1282/75/4, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., March, 1992, (GDM/91/080)

39. Determination of the Pharmacokinetics of Lamivudine in the Dog Following a Single Oral Dose, Lot # C1013/75/1, C1803/125/1, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., January, 1992, (GDM/91/036)

40. Bioanalytical support data for Lamivudine in the dog following a single dose ranging rom 250 to 2000 mg/kg (GDM/94/161)

41. Two Week Oral Toxicity Study in Dogs, Batch # C1803/152/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., November, 1991, (D12750-WPT/91/222)

42. Bioanalytical support data for Lamivudine: A 13-week oral toxicity study in beagle dogs, batch # C1013/75/1, Glaxo Group Research Ltd, Greenford, Middlesex, England, 23 April, 1992, (GDM/92/034/D12873)

43. Bioanalytical support data for Lamivudine: A 52-week oral toxicity study in beagle dogs (GDM/94/081/Study No. D13604)

44. Transport mechanism of Lamivudine and drug interactions in Caco-2 cells (UCP/92/028)

45. Investigation into the potential for interaction between midazolam and Lamivudine with the human liver Cytochrome P450 isoenzyme CYP3A (GDM/94/124/Study No. L18/94/014)

Review of Absorption, Distribution, Metabolism and Excretion (ADME) Studies:

1. Bioanalytical support data for Lamivudine and AZT: Effect on hematology of mice (GDM/93/009/Study No. M13367)

Plasma levels of Lamivudine and AZT were monitored at the end of a 36 day oral toxicity study in mice (WPT/92/419). Male and female mice were divided into six groups receiving twice daily oral doses of Lamivudine amounting to 0, 180, 600 or 2000 mg/kg/day with co-administration of AZT at 150 mg/kg/day or 0 or 2000 mg/kg/day without co-administration of AZT. On the last day of treatment, blood samples were taken from two animals per sex per group at 0, 1, 2, 4 and 8 hr post dose. Plasma derived from these samples was assayed for Lamivudine and AZT using HPLC following protein precipitation. Results: plasma level data indicated that the oral co-administration of Lamivudine and AZT at the dose levels had no effect on the pharmacokinetics of either Lamivudine or AZT in the mouse.

2. Bioanalytical support data for Lamivudine: One month oral study to determine the effects when given in combination with interferon (alpha); on the hematology of mice (GDM/94/014/Study No. M20009)

Plasma levels of Lamivudine were monitored at the end of a 30 day oral toxicity study in mice (WPT/92/572). Male and female mice were divided into six groups receiving twice daily oral doses of Lamivudine amounting to 0, 180, 600 or 2000 mg/kg/day with co-administration of interferon (alpha) at 10,000 units daily or 0 or 2000 mg/kg/day without co-administration of interferon (alpha). On the last day of treatment, blood samples were taken from two animals per sex per group at 0, 1, 2, 4 and 8 hr post dose. Plasma derived from these samples was assayed for Lamivudine using an HPLC method. Results: plasma level data indicated that the oral co-administration of Lamivudine and interferon (alpha) at the dose levels had no effect on the pharmacokinetics of Lamivudine in the mouse.

3. Bioanalytical support data for Lamivudine: Palatability in the diet of mice (GDM/93/010/Study No. M13284)

Plasma levels of Lamivudine were monitored during the course of a palatability study (WPT/92/322) in which male and female mice received dietary dosage of Lamivudine at levels of 0, 180, 600, 1000 or 2000 mg/kg/day for 21 days. Blood samples were taken from three animals/sex/group at 0, 2, 6, 8, 12, 16 and 20 hr post dose on the last day of treatment. Plasma was assayed for Lamivudine using an HPLC method. Results: AUC calculated for the 2000 mg/kg/day group (170 μg*hr/ml) are similar to 202 μg*hr/ml achieved after gavage administration (Study No. M13367) in mice.

Conclusion: the study suggest that systemic exposure of the animals to Lamivudine was approximately equivalent for the two methods of oral administration (dietary and gavage).

4. Bioanalytical support data for Lamivudine: 90 days oral (dietary) toxicity for mice preliminary to an oncogenicity study (GDM/93/075/Study No. M13285)

In a preliminary study prior to the full oncogenicity study, plasma levels of Lamivudine were monitored during the course of a 13 weeks oral (dietary) study (WPT/93/196) in which male and female mice received Lamivudine at dose levels of 0, 2000, 3000 or 4000 mg/kg/day. Blood samples were taken from 2 animals/sex/group on one occasion during week 12 at 4, 8, 12, 16, 20 and 24 hr. Plasma was assayed for Lamivudine using an HPLC method. Results: AUCs calculated over 24 hr increased with increasing dose level. This increase in AUC was proportional to the dose in both male and female mice. The AUCs were comparable to those obtained from gavage administration at equivalent dose levels (Study No. M13367) in mice.

5. The metabolism of Lamivudine in mouse, rat, dog and man (GDM/94/123)

Mouse: ^{3}H-Lamivudine (45 mg/kg) was administered by oral and iv routes to male and female on separate dosing occasions. Results: unchanged drug accounted for approximately 90% of the urinary radioactivity following both oral and iv administration of ^{3}H-Lamivudine to the mouse. The remainder of the urinary radioactivity was accounted for by two unidentified metabolites present in approximately equal portions, for both dose routes. The chromatographic retention time of one of these metabolites corresponded to that of GI138870X, the trans-sulfoxide of Lamivudine.

Rat: ^{3}H-Lamivudine (45 mg/kg) was administered by oral and iv routes to male and female on separate dosing occasions. Results: unchanged drug accounted for approximately 96% of the urinary radioactivity following both oral and iv administration of ^{3}H-Lamivudine to the mouse. The remainder of the urinary radioactivity was accounted for by two unidentified metabolites neither of which accounted more than 5% of the urinary radioactivity. NMR analysis identified one of these metabolites as GI138870X, the trans-sulfoxide of Lamivudine.

Dog: following both oral and iv administration of ^{3}H-Lamivudine (45 mg/kg) to dogs, chromatographic analysis of the urine revealed the presence of two major metabolite peaks (designated MET 1 and MET 2) in addition to unchanged drug. In total these metabolite peaks accounted for 57% of the urinary radioactivity

following oral administration (MET 1 = 20% and MET 2 = 37%), and 41% following oral iv administration (MET 1 = 14% and MET 2 = 27%).

Man: following oral administration of ^{3}H-Lamivudine (45 mg/kg) to man, up to 85% of the dose was found to be excreted unchanged in the urine. Subsequent capillary electrophoretic analysis of urine samples collected over a single oral dose interval of 12 hr from patient taking part in Study 2001 revealed that in addition to parent drug, a peak was present with a migration time which corresponded to that of standard GI138870X, the trans-sulfoxide of Lamivudine. The total amount of sulfoxide excreted by each patient in this study expressed as percentage of the total dose, accounted for about 5% of the administered Lamivudine.

6. Radioactivity balance study and metabolic profile of radioactivity in urine following a single oral or a single intravenous dose of [^{3}H]-Lamivudine (45 mg/kg) in the mouse (GDM/92/045/Study No. 92/DM/45)

Male and female mice received a single oral or an intravenous dose of [^{3}H]-Lamivudine (45 mg/kg) to determine the excretion of radioactivity and the metabolic profile of radioactivity in urine. Urine and feces were collected for up to 48 hr after dosing. Results: the mean total recovery of radioactivity over the 48 hr sample collection period following the iv administration was about 68% (range 50.9-97.9%). Urinary excretion accounted for about 55% (range: 37.6-79.6) of the nominally administered dose, the majority in the first 24 hr after dosing. Fecal excretion accounted for about 5% (range: 0.5-16.3%) of the administered dose.

The mean total recovery of radioactivity over the 48 hr sample collection period following the oral administration was about 89% (range: 83.3-97.9%). Urinary excretion accounted for about 54% (range: 36-81.3%) of the administered radioactivity, the majority in the first 24 hr after dosing. Fecal excretion accounted for about 29% (range: 12.6-41.3%) of the administered dose.

Metabolic profiling of the 0-24 hr urine samples indicated that the metabolic profiles following iv and oral administrations of the labelled compound were qualitatively similar. Unchanged Lamivudine accounted for approximately 90% of the urinary radioactivity for both routes of administration. The remainder of the urinary radioactivity was accounted for by two metabolites (Met 1 and Met 2) in approximately equal proportions, for both dose routes. The chromatographic retention time of one of these metabolites corresponded to that of GI38870X, the trans-sulfoxide of Lamivudine.

7. Pharmacokinetics of a Single Oral or Intravenous Radioactive Dose in Rats, Batch # C1282/75/4, Glaxo Group Research Ltd, Greenford, Middlesex, U.K., April, 1991, (GDM/91/015)

Three male AHA rats per time point were fed orally by gavage with ^{3}H-Lamivudine in distilled water at 45 mg/kg. At predose, 20 and 40 min and at 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 and 24 hr, a group was bled for the determination of plasma radioactivity and concentration of drug. A second group of 45 rats were dosed intravenously with the same formulation at the same dose. Three rats were bled at 0, 5, 10, 20, and 40 min and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hr for the same determinations as above.

The plasma concentrations of Lamivudine and total radioactivity, expressed as equivalents of Lamivudine were in very close agreement throughout all the measurements indicating that there is little systemic exposure to metabolites at the dose used in the study. After intravenous administration, the drug exhibited biexponential elimination with half-lives of approximately 20 min and 1.5 hr. The bioavailability calculated from the areas under the concentration curves was about 60%. The pharmacokinetic data are summarized in Table 1.

Table 1
Mean Pharmacokinetic Parameters of Lamivudine After Single Dose in the Rat

Parameters	Route of Administration & Dose	
	Oral (45 mg/kg)	Intravenous (45 mg/kg)
C_{max} (µg/ml)	4.04	83.4
T_{max} (hr)	1.5	na
AUC, (µg*hr/ml)	18.8	31.7
Cl (ml/min)	na	4
$T_{1/2}$ (min)	na	20
$T_{1/2}$ (min)	2.3	1.58
Vd (ml)	na	542

na = not applicable

Comments: The apparent volume of distribution of the terminal phase was about 540 ml suggesting that the distribution of drug

was greater than could be attributed to distribution in total body water in rat. The serum clearance of the drug was calculated to be 4 ml/min after iv administration of which renal clearance accounted for about 3.3 ml/min. This value is higher than the maximum GFR for drug indicating the importance of active tubular secretion in the renal elimination of the compound in rat.

8. The pharmacokinetics of Lamivudine and total radioactivity following a single oral or single intravenous dose of [^{3}H]-Lamivudine (45 mg/kg) in the female AHA rat, Study No. GDM/91/77, March, 1992, Glaxo Group Research Ltd., Greenford, Middlesex, United Kingdom, Drug lot C1013/75/1 (non-radioactive drug), C1282/75/4 (radioactive drug).

Forty-five female AHA rats were dosed, by gavage, with [^{3}H]-Lamivudine in water at a dose of 45 mg/kg. Blood was taken from groups of 3 rats at times of 0 (undosed animals), 20 and 40 minutes, 1, 1.5, 2.5, 3, 4, 5, 6, 8, 10, 12 and 24 hours after dosing for the determination of the concentration of the drug in the plasma. A second group of 45 rats were dosed intravenously with [^{3}H]-Lamivudine in water at a dose of 45 mg/kg. Blood was taken from 3 animals at times of 5, 10, 20 and 40 minutes and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 hours after dosing for the determination of the concentration of the drug in the plasma. The pharmacokinetic parameters are shown in Table 2.

Table 2

Pharmacokinetics of Lamivudine in Female Rats at 45 mg/kg

Parameter	Oral	Intravenous
Cmax (μg/ml)	4.50 ± 0.95	72.7 ± 15.8
Tmax (hr)	1.5[a]	-
AUC_{0-24} (μg*hr/ml)	17.6	25.5

a. Calculated using nominal bleeding times.

For the intravenous dose, the clearance was 4.5 ml/min, the T½ α was 24 minutes, the T½ ß was 1.2 hours and the volume of distribution was 449 ml. The bioavailability of the orally administered dose was 69%. Following dosing, there was close agreement between the number of equivalents of radioactivity and the number of equivalents of unchanged drug in the plasma as determined by HPLC (indicating little metabolism).

Comments: That most of the radioactivity was in the form of the parent drug is not surprising. In an earlier study in rats, about 90% of an intravenous dose of radioactive Lamivudine was found to be excreted in the urine. Of this, approximately 96% of the radioactivity was found to be parent drug.

9. Pharmacokinetics of a Single Intravenous Dose in Rats, Batch # C1404/194/1, Glaxo Group Research Ltd, Greenford, Middlesex, U.K., May, 1991, (GDM/91/021)

Five male AHA rats were administered Lamivudine at 2000 mg/kg in 0.02 M acetate buffer, pH 3.5, by intravenous injection. At 5, 10, 20, 40 min and at 1, 1.5, 2, 3, 4, 6, 8, 12 and 24 hr, the animals were bled from the tail vein for the determination of plasma concentrations of drug. The compound was eliminated biexponentially with an initial $T_{1/2}$ of 40 min and a terminal $T_{1/2}$ of 6.5 hr. Other pharmacokinetic parameters are shown in Table 3.

Table 3
Mean Pharmacokinetic Parameters of Single I.V. Dose of Lamivudine (2000 mg/kg) in the Rat

Parameters (Mean)				
AUC, (μg*hr/ml)	$t_{1/2\alpha}$ (hr)	$t_{1/2\beta}$ (hr)	Clearance (ml/min)	Vd (ml)
3645	0.70	6.5	1.9	1068

Comments: After intravenous dosing of the drug at 45 mg/kg (previous study), plasma clearance was about 4.0 ml/min compared to the value of about 2.0 ml/min found in this study. The large apparent volume of distribution of Lamivudine suggests that the compound may be distributed extravascularily in rats.

10. Pharmacokinetics of Single Oral Doses in Rats, Batch # C1091/179/1, Glaxo Group Research Ltd, Greenford, Middlesex, U.K., February, 1991, (GDM/91/003)

Groups of AHA rats (3/group) were administered Lamivudine by gavage in acetate buffer, pH 4.4, at doses of 600, 800, 1000, 1200 and 1500 mg/kg. A separate sample was administered in a 1% solution of hydroxypropyl methylcellulose at a dose of 2000 mg/kg. Blood was taken by tail bleeding at 20 and 40 min and at 1, 2, 3, 4, 6, 8, 10, 12, 16, 20 and 24 hr for the determination of plasma concentrations of the drug. The mean pharmacokinetic parameters are given in Table 4.

Table 4
Mean Pha[illegible]c Parameters of Single Doses of Lamivudine in the Rat

Parameters	Dose (mg/kg)					
	600	800	1000	1200	1500	2000
C_{max} (μg/ml)	41.2	52.0	58.3	62.5	82.7	107
T_{max} (hr)	2.0	1.0	2.0	2.0	2.0	2.0
AUC (μg*hr/ml)	227	324	379	416	557	648
$T_{1/2}$ (hr)	3.7	3.9	3.5	4.0	4.6	4.6

The observed T_{max} was 2 hr for all but the 800 mg/kg dose. The plasma half-life increased from 3.7 hr at 600 mg/kg to 4.6 hr at 1500 and 2000 mg/kg doses. A plot of AUC and C_{max} against doses indicated that a linear relationship existed for both parameters (plot not shown).

Comments: First, the linear relationship that exists between AUCs and doses when Lamivudine is administered to rats between 600 and 2000 mg/kg indicates that neither the absorption nor the elimination processes are saturated at these doses. The linearity also indicates that there was no significant difference between the 2000 mg/kg dose formulated as a suspension and the other doses which were solutions. Secondly, the increases in plasma half-lives (3.7 - 4.6 hr) suggest that, at higher doses for this compound, the drug absorption would continue for a prolonged period; under these circumstance, the plasma half-life derived will reflect the rate constant for both absorption and elimination rather than that for elimination alone.

11.. Bioanalytical support data for Lamivudine: Metaphase analysis in the rat bone marrow in vivo (GDM/93/037/Study No. R14091)

Plasma levels of Lamivudine were measured in rats as part of a chromosome aberration study (WPT/93/195). Rats were allocated into five dose groups receiving either cyclophosphamide (positive control), vehicle control or Lamivudine at dose levels of 500, 1000 and 2000 mg/kg by oral administration. Plasma was assayed using an HPLC method. Results: all plasma samples taken two hr after dosing (500, 1000 or 2000 mg/kg) contained detectable levels of Lamivudine confirming exposure of the animals to the test compound.

12. Lamivudine: Pharmacokinetic study in the juvenile AHA rat, batch # C1758/233/1, Glaxo Group Research Ltd, Ware, Hertfordshire, England, 1 April, 1992, (WPT/92/190/R13355)

Two groups of juvenile AHA rats (strain: Wistar/Sprague Dawley derived with Wistar characteristics; age: 16 days; 21 animals/group; source: six females were mated by males, and were allowed to litter. On litter day 16, litters were randomly allocated to one of the two treatment groups) were administered a single dose of 45 or 2000 mg/kg Lamivudine, by oral gavage at a standard dose volume of 10 ml/kg (in acetate buffer, pH 3.7). Blood samples were taken by exsanguination under isoflurane anaesthesia, three pups in each treatment group were assigned to each of the following time points: predose, 1, 2, 4, 8, 12 and 24 hr post dose, and assayed by a validated HPLC-UV assay method to determine plasma Lamivudine concentrations. This study was designed to provide information on the pharmacokinetic profile of oral administration of drug in juvenile rats. General condition: no reaction to the treatment was recorded. Absorption of Lamivudine was adequately demonstrated in both dose groups (Table 5, 6 and 7).

Table 5
Mean Plasma Levels of Lamivudine in Juvenile Rats Following a Single Oral Dose of Lamivudine

Time (hr)	Mean Plasma Levels of Lamivudine (µg/ml) Dose (mg/kg)	
Predose		
1		
2		
4		
8		
12		
24		

nd: not detectable by the analytical method.

Table 6
Mean Plasma AUCs of Lamivudine in Juvenile and Adult Rats Following a Single Oral Dose of Lamivudine

Age	Mean Plasma AUCs of Lamivudine (µg*hr/ml)
Juvenile	
Adult	

Table 7
Comparison of Juvenile and Adult Rats C_{max} Values (µg/ml) Following a Single Oral Dose of Lamivudine

Age	Mean Plasma C_{max} values of Lamivudine (µg/ml)	
	Dose (mg/kg)	
Juvenile		
Adult		

Comments: The mean plasma AUC and C_{max} values obtained at both dose levels in juvenile rats are at least double of those calculated from adult rats (previous single dose studies). Thus, the data indicate that systemic exposure and intensity to Lamivudine of a juvenile rat is appreciably greater than that of an adult rat receiving an equivalent dose. The major differences between the adult and juvenile plasma level-time curves appear to occur during the early part of the profile, which would suggest that the differences are due to changes in absorption and distribution rather than clearance of the compound.

13. Bioanalytical support data for Lamivudine: A pilot 5 day oral toxicity study in AHA rats (GDM/91/012/Study No. R12636)

Two groups (4 rats/sex/group) were administered Lamivudine via gavage twice daily at 12 hr intervals at doses of 1500 or 2000 mg/kg/day for 5 days (10 doses). Plasma samples were taken at 2 and 12 hr after dosing. Table 8 shows the C_{max} and C_{min} levels observed throughout this study. C_{min} levels indicate that there was continual systemic exposure, with the lowest observed plasma level above 2 µg/ml, at both dose levels, and showed little

evidence of accumulation.

Table 8
Plasma Levels of Lamivudine in Rats Following Oral Administration of the Drug Twice-a-Day For Five Days

Dose #	Absorption Parameters (µg/ml)	Male		Female	
		Doses of Lamivudine			
		1.5 g/kg	2.0 g/kg	1.5 g/kg	2.0 g/kg
1	C_{max} C_{min}	98.9 3.2	167.0 3.1	81.8 2.7	107.0 6.9
2	C_{max} C_{min}	92.2 3.7	140.0 4.5	77.2 5.7	128.0 5.1
3	C_{max} C_{min}	92.7 2.8	122.0 6.8	90.0 3.6	133.0 8.3
5	C_{max} C_{min}	71.5 2.5	194.0 4.3	96.5 4.3	146.0 6.8
7	C_{max} C_{min}	91.9 2.2	128.0 4.4	84.3 2.5	110.0 6.2
9	C_{max} C_{min}	79.2 2.4	149.0 4.5	95.3 3.3	98.7 7.1
10	C_{max} C_{min}	90.6 6.1	119.0 14.4	102.0 3.3	103.0 12.6

14. Plasma levels of Lamivudine monitored during a one month oral toxicity study in the AHA rat (GDM/91/020/Study No. R12637)

Groups of male and female rats were administered Lamivudine via oral gavage at dose levels of 45, 300 or 2000 mg/kg/day bid for a period of one month. Plasma levels of Lamivudine were monitored on days 1, 7, 21 and 35. Results: pharmacokinetic parameters are summarized in Table 9. The values for males and females were pooled. The compound was well absorbed following administration at all doses with peak plasma concentrations occurring at one hour after dosing. C_{max} and AUCs were found to increase with dose in a linear manner.

Table 9
Pharmacokinetic parameters of Lamivudine in rats* receiving oral dosing of Lamivudine for a month

Dose (mg/kg/day)	Day 1		Day 35	
	C_{max} (µg/ml)	AUC (µg*hr/ml)	C_{max} (µg/ml)	AUC (µg*hr/ml)
45	3.3	11.7	4.1	22.7
300	22.6	86.5	30.0	119.0
2000	93.1	415.0	117.0	590.0

* Blood samples were pooled for both males and females in a group

Comments: There is an indication at day 35 that there is an accumulation [elevated AUCs at all 3 doses] of this agent in plasma.

15. Bioanalytical support data for Lamivudine: Effects of oral administration upon the reproductive performance of a parental dosed generation (F_0) of AHA rats and upon the peri- and post-natal development of the resulting two successive, untreated generations (F_1 and F_2), Batch No. UFP 0002, Glaxo Group Research Ltd., Hertfordshire, England, June 9, 1994, (GDM/94/059/WPT/93/210/Study No. R13738)

Groups of male and female rats were administered Lamivudine via oral gavage at dosages of 0 (control) 90 (low), 450 (mid) or 2000 mg/kg/bid (high) for 10 weeks prior to pairing in the case of the males, and 3 weeks prior to pairing for the females. Results Absorption data: 2 hr after dosing on one occasion in the week prior to paring, mean plasma levels for male rats were 6.3, 34.4 and 89.0 and for females were 9.9, 37.5 and 133 µg/ml in the low, mid and high dose groups, respectively.

Comments: Plasma levels in both the male and female rats increased with increasing dose and were in close agreement with those seen at 2 hr post-dose in previous studies using the same or similar dose level of drug (GDM/93/024/GDM/93/038).

16. Bioanalytical support data for a 13 week oral toxicity study of Lamivudine in the AHA rat, Study No. GDM/91/089, February, 1992, Glaxo Group Research Ltd., Greenford, Middlesex, United Kingdom, Drug lot C1013/75/1.

Thirty-eight male and 38 female AHA rats per group were administered Lamivudine in water, by gavage in two doses, 12 hours apart for 92 consecutive days. Blood was taken from each of 2 males and 2 females per time point for the determination of

plasma levels of the parent drug according to the following schedule:

Day 1	1st dose - predose, 1, 2, 4, 8 and 12 hours post 2nd dose - 1, 2, 4, 8 and 12 hours post
Day 38	1st dose - predose, 2 and 12 hours post
Day 91	2nd dose - 16, 24 hours post
Day 92	1st dose - 1, 2, 4, 8 and 12 hours post.

Comments: Unfortunately, the 1st dose at the 45 mg/kg level was administered at a concentration 30% higher than it should have been. This alters only one set of results and does not compromise the conclusions (see Table 10).

The results of the study after the first dose on day 1 and the dose on day 92 are shown in Table 10. The AUC_{0-12} for the second dose on day 1 was not calculated. In general, the plasma concentrations were somewhat higher after the second dose.

Table 10

AUC_{0-12} (μg*hr/ml) for Rats Dosed With Lamivudine

Dose mg/kg bid	Males		Females	
	Day 1	Day 92	Day 1	Day 92
45	19.4[a]	18.0	16.3[b]	23.7
300	84.9	127	60.9	162
2000	441	519	530	860

a. This dose was actually 60 mg/kg. Assuming that, at low doses, the exposure is linear, this value should have been approximately 14.9 μg*hr/ml.

b. This dose was actually 60 mg/kg. Assuming that, at low doses, the exposure is linear, this value should have been approximately 12.5 μg*hr/ml.

The plasma levels increase with dose. At day 92, they are higher than at the beginning of the study with the difference being more marked in the female than the male. Although the results are not shown in Table 2, Lamivudine was detected at all time points examined including days 38 and 92 (the limit of detection was 0.1 μg/ml).

17. Bioanalytical support data for Lamivudine: Six month oral toxicity study in the AHA rat (GDM/93/024/Study No. R13470)

Plasma levels of Lamivudine were measured during the course of a 6 month oral toxicity study (WPT/93/361) in which rats received Lamivudine at oral dose levels of 0, 90, 425 or 2000 mg/kg/day bid. Blood samples were taken from two animals/sex/group at various times after dosing. Plasma was assayed using an HPLC method. Results: plasma AUC values calculated for days 1 (137 µg*hr/ml for males at 425 mg/kg/day bid) and day 177 (127 µg*hr/ml for males at 425 mg/kg/day bid) for each dose level did not show any consistent increase or decrease in exposure over the 6 month dosing period except for female rats in the high dose group where an increase was observed. All plasma samples taken after dosing from rats in the 90, 425 and 2000 mg/kg/day bid dose groups contained detectable levels of Lamivudine, with the exception of three samples taken 12 post dose from animals (90 mg/kg/day bid). These data indicated continual exposure of these animals to the test compound.

18. Bioanalytical support data for Lamivudine: A preliminary study to assess the effects of oral administration to pregnant and lactating AHA rats and of direct oral administration to their juvenile offspring (GDM/93/031/Study No. R13609)

Plasma levels of Lamivudine were measured during the course of a preliminary toxicity study (WPT/92/404). Based on the conclusions of this preliminary study, the sponsor has conducted a following definitive toxicity study; hence, this study will not be reviewed.

19. Bioanalytical support data for Lamivudine: Effect of oral administration to pregnant and lactating AHA rats and effect of direct oral administration to their juvenile offspring (GDM93/038/Study No. R13739)

Groups of pregnant AHA rats were administered Lamivudine via oral gavage twice daily from day 17 of pregnancy until litter day 21 at dosages equivalent to 0 (control) 90 (low), 450 (mid) or 2000 mg/kg/day (high) and their offsprings were dosed orally once daily at levels of 0 (control), 90 (low), 450 (mid) or 2000 mg/kg/day (high) from litter day 3 to litter day 16, 22 or 43. Plasma levels of Lamivudine were measured in dams on litter day 12 and in male and female juveniles on litter day 16. Plasma was assayed for Lamivudine using an HPLC method. Results: plasma levels of Lamivudine in adult and juvenile rats are shown in Table 11. The drug levels in adult and juvenile rats increased with increasing dose, but the amounts in juvenile rats were approximately three times greater than those in adults receiving an equivalent dose. There was no apparent difference in plasma levels between male and female juvenile rats.

Table 11
Mean Plasma Levels of Lamivudine Two Hours Post Dose in Adult and Juvenile Rats Receiving Repeat Oral Administration of Lamivudine

Generation	Sex	Litter Day	Mean Plasma Levels (µg/ml)		
			Low	Mid	High
Adult	Female	12			
Juvenile	Male	16			
Juvenile	Female	16			

20. Bioanalytical support data for Lamivudine: Palatability in the diet of rats (GDM/92/055/Study No. R13287)

Plasma levels of Lamivudine were measured during the course of a palatability study (WPT/92/323) in which male and female rats received dietary doses of Lamivudine for 21 days. The dose levels were equivalent to 0, 180, 600, 1000 or 2000 mg/kg/day. Blood samples were taken from three animals/sex/group receiving 180 and 2000 mg/kg/day at 2, 4, 6, 8, 12, 16 and 20 hr post dose on the last day of treatment. Plasma was assayed by an HPLC method for Lamivudine. Results: AUCs appeared to have proportional relationship between the two dose levels investigated during the study. AUC for the 2000 mg/kg/day group (487 µg*hr/ml) was comparable to those (441 µg*hr/ml) calculated in a previous study after administration of an equivalent dose by gavage (Study No. R12749). This suggested that the systemic exposure of the animals to Lamivudine is equivalent for the two methods of oral administration.

21. Bioanalytical support data for Lamivudine: 90 days oral (dietary) toxicity to rats preliminary to an oncogenicity study (GDM/93/076/Study No. R13288)

In a preliminary study prior to the full carcinogenicity study (WPT/93/198), plasma levels of Lamivudine were measured during the course of the 90 day study in which rats received Lamivudine by dietary administration at dose levels equivalent to 0, 1000 or 2000 mg/kg/day for males and 0, 1500 or 3000 mg/kg/day for females. Blood samples were taken from two animals/sex/group on one occasion during week 11 at 4, 8, 12, 16, 20 and 24 hr. Plasma was assayed using an HPLC method. Results: AUCs increased with increasing dose. The increase in AUC was proportional to the increasing dose in the females but not in the males In the females, AUCs were comparable (dietary: 342 µg*hr/ml at 1500 mg/kg/day) to those calculated in Study No. R12749 after administration of Lamivudine by gavage (379 µg*hr/ml). A similar

comparison of AUCs in the male suggested that systemic exposure in the male receiving 1000 mg/kg/day was greater than expected.

22. Bioanalytical support data for GR109714x: effects of oral administration upon pregnant AHA rats. Study No. GDM/91/083, January, 1992, drug lot C1013/75/1.

Thirty-six pregnant AHA rats per group were administered Lamivudine in acetate buffer, pH 3.6, twice a day by gavage, twelve hours apart, at doses of 0 (vehicle control), 45, 300 and 2000 mg/kg. Dosing took place between days 7 and 16 of pregnancy. An additional four pregnant females per group were treated with drug in an identical manner to those animals described above. Blood samples were taken from these animals at 2 and 24 hours after treatment on the first and last day of dosing for the estimation of plasma levels of the drug.

The plasma levels found on days 7 and 16, two hours after the first dose are shown in Figure 1. The figure shows that there were only slight differences in the plasma levels upon multiple dosing of the drug. This is in contrast with studies carried out in rabbits, in which the drug was seen to accumulate with time. It also shows that the plasma levels as a function of the dose approached linearity. Limited data were reported when the plasma levels were determined 12 hours after dosing. At the low dose, very few of the samples contained detectable drug.

Figure 1
Plasma Levels of Lamivudine in Pregnant Rats

23. In Vitro Red Blood Cell Binding and Plasma Protein Binding of Radiolabelled GR109714 in Rat, Dog and Man, Batch # C1284/257/2, Glaxo Group Research Ltd, Greenford, Middlesex, U.K., February, 1991, (GDM/91/010)

Blood was obtained from healthy AHA rats, beagle dogs and humans. All the donors were male. Five ml aliquots of freshly collected heparinized whole blood were placed into a water bath for ten min. A 50 µl aliquot of ^{3}H-Lamivudine in distilled water was added to give final concentrations of 100, 10, 1 and 0.1 µg/ml. The mixtures were incubated for 30 minutes at 37°C. The percentage of Lamivudine associated with the cellular fraction (F) was calculated according to the equation, $F = [1-((C_p/C_b) X (1-H))] X 100\%$ where C_p is the radioactivity in the plasma, C_b is the blood radioactivity and H is the final hematocrit value.

Fresh plasma was placed into dialysis bags and aliquots of the radiolabelled Lamivudine was placed into the plasma to give concentrations of either 100, 10, 1 or 0.1 µg/ml. The dialysis bag was placed into a test tube containing buffered saline and incubated overnight. Protein binding was calculated according to the equation percentage = T-F/T X 100% where T is the concentration of radiolabelled drug in plasma at equilibrium, F = 0.948B, B is the concentration of radiolabel in the saline at equilibrium and 0.948 is the ratio of plasma water content to buffer water content. The results of the two studies are presented in Table 12.

Table 12
Binding of Lamivudine to Red Blood Cells or Plasma Proteins (Rat, Dog and Human)

Type of Blood	Concentration of Lamivudine (µg/ml)	Percentage in Red Blood Cells	Percent Binding to Plasma Proteins
Rat	100	46.8	< 10
	10	44.3	< 10
	1	46.5	16
	0.1	41.0	49
Dog	100	39.8	< 10
	10	42.7	< 10
	1	42.5	12
	0.1	46.6	42
Human	100	56.1	< 10
	10	54.3	< 10
	1	53.5	< 10
	0.1	54.7	36

The plasma protein binding was dependent on the concentration range of the drug and the extent of binding was similar in all species. At the lowest concentration studied (0.1 µg/ml), the protein binding increased to 36% in man, 42% in dog and 49% in rat.

Comments: First, the extent of association of the drug with red blood cells was of similar magnitude in rat, dog and human. Second, the binding of drug to plasma proteins was concentration dependent.

24. Whole body autoradiography - rats Report # GDM/90/040 January, 1991

Eight male and eight female AHA rats and one male RH rats were fed ^{3}H labeled racemate GR103665X (±) (batch 21290) in water by gavage at a dose of 30 mg/kg. At 0.25, 0.5, 1, 2, 4, 8, 24 and 96 hours, one male and one female AHA rat was killed and subjected to autoradiography. The RH rat was autoradiographed after eight hours of dosing

Fifteen minutes after dosing, radioactivity was found in the stomach, small intestine, kidney, bladder with lesser amounts seen in the liver. A low level of radioactivity could be seen throughout the body. Over the first hour, the levels of radioactivity generally increased in the tissues with the highest level being in the kidney and bladder. A trace of radioactivity was seen in the brain and the tissue concentrations were generally higher than those in the blood. After one hour the tissue radioactivity declined. After eight hours, only the kidney and large intestine had appreciable radioactivity with a trace in the testes. By 24 hours, radioactivity could be seen only in the lower gastrointestinal tract. By 96 hours, no radioactivity could be detected in the animals. In general, the male and female AHA rats showed similar patterns of labeling. After eight hours, the only significant labeling in the RH rat was in the kidney. By 24 hours, no radioactivity could be detected in any tissue. The sponsors were specifically looking for binding of radioactivity to melanin in this animal.

The study showed that the drug was rapidly distributed throughout the body and that the major route of elimination was renal.

25. Excretion balance study (day 19) and placental transfer of radioactivity (day 12 and day 19) in the pregnant female AHA rat after oral administration (45 mg/kg) of [^{3}H]-Lamivudine. Report No. GDM/91/050, January, 1992, non-radioactive drug lot C1013/75/1, radioactive drug lot C1282/75/4.

Two pregnant AHA rats were dosed by gavage with 45 mg/kg of [^{3}H]-

Lamivudine in distilled water on day 12 of pregnancy. One hour after dosing, the rats were killed and prepared for whole body autoradiography. On day 19 of pregnancy, five additional animals were dosed by gavage with 45 mg/kg of [^{3}H]-Lamivudine and placed in metabolism cages. Urine and feces were collected at 24 hour intervals for two days. The animals were killed, their bodies macerated in alkali and the radioactivity of the carcasses was determined. The cages were washed to collect residual radioactivity. An additional 3 pregnant animals were dosed by gavage with 45 mg/kg of [^{3}H]-Lamivudine in distilled water on day 19 of pregnancy. At 1, 4 and 24 hours after dosing, one rat per time point was killed and prepared for whole body autoradiography.

On day 12, the radiolabeled material was widely distributed throughout the maternal tissues with the exception of the central nervous system. The levels in the maternal blood and the placenta were significantly higher than in the fetus. On day 19 of pregnancy, at the 1 hour time point, the distribution of material in the maternal tissues was similar to that seen on day 12. The concentration of material in the placenta was slightly higher than in the maternal blood. The concentration in the fetus was approximately half that of the placenta and did not seem to accumulate in any fetal organ. By 4 hours post-dose, the level of radioactivity had decreased in the maternal tissues and had risen slightly in the fetus. All radioactivity had been cleared by 24 hours.

Urinary excretion of radioactivity accounted for about 55% of the administered dose. Most (about 96% of the excreted material) was excreted during the first 24 hours. Excretion in the feces accounted for about 50% of the administered dose. Approximately 96% of the material in the urine and feces was unchanged drug. Two minor but unidentified, metabolites were seen on HPLC. Less than 3.5% of the radioactivity was recovered from the cage washes or the carcass. The total recovery of radioactivity over a 48 hour period was about 108%.

26. Lamivudine: A study of the milk transference following oral administration (45 mg/kg) to the rat (GDM/92/017/Study No. 92/DM/17)

Plasma and milk concentrations of Lamivudine were determined following a single oral administration (45 mg/kg) of Lamivudine to lactating female rats. Blood and milk samples were collected at various time points up to 24 hr post-dose. Plasma and milk samples were assayed for Lamivudine using an HPLC method. Results: peak plasma concentrations of Lamivudine were observed in the 0.5 hr samples with a mean plasma concentration of 11.1 µg/ml. Lamivudine levels in plasma then decreased steadily to a

concentration of 0.3 μg/ml after 8 hr. Peak concentrations of Lamivudine in milk of about 14 μg/ml were found at 4 hr post-dose, falling to 4.8 μg/ml after 8 hr. Lamivudine was not detected in either milk or plasma after 24 hr with the exception of one plasma sample in which the concentration was 0.9 μg/ml.

Comments: Lamivudine was absorbed into the systemic circulation of the dams and then Lamivudine was transferred into the maternal milk. From this study it may be concluded that during the Segment III pre- and post-natal reproductive toxicity studies for orally administered Lamivudine suckling pups would be exposed to the drug.

27. The renal disposition of Lamivudine in the isolated perfused rat kidney (UCP/93/012)

Kidneys were isolated from male Sprague-Dawley rats and perfused to screen for potential drug interactions between Lamivudine and several other clinical relevant drugs likely to be concomitantly administered to AIDS patients. Eight drug interactions were studied along with Lamivudine alone at a concentration of 500 ng/ml. Lamivudine was added to the recirculating perfusate alone or with relevant concentration of either AZT (1 μg/ml) ddC (2 μg/ml), ddI (1 μg/ml), probenecid (50 μg/ml), trimethoprim (4 μg/ml), ranitidine (400 μg/ml), sulfa-methoxazole (80 μg/ml) or cimetidine (2 μg/ml). Viability was assessed in all perfusions by monitoring glomerular filtration rate and fractional reabsorption of glucose and sodium. Renal clearance and excretion ratio (ER) were determined to assess the effect of concomitant drug administration on Lamivudine disposition in the perfused kidney. Results: renal clearance and ER of Lamivudine were 3.06 ml/min and 3.67, respectively. Renal clearance and ER were significantly reduced to 1.25 ml/min and 1.43, respectively, by trimethoprim. A slight but significant reduction in ER was observed by co-administration of ddC with Lamivudine. No other significant drug interactions were observed.

Comments: These results suggest that drug interactions between Lamivudine and trimethoprim, and to a lesser extent ddC, warrant further clinical study.

28. To investigate the interaction of Lamivudine with rat and human hepatic microsomal cytochrome P450's utilizing testosterone as probe substrate (GDM/93/028/Study No. 93/DM/028)

The potential interaction between Lamivudine and various hepatic cytochrome P450s (rat: normal, phenobarbitone and ß-naphthoflavone; man: normal hepatic microsomes) involved in the metabolism of testosterone was investigated in vitro using microsomes prepared from male AHA rats and man. ^{14}C-testosterone

was incubated in the presence of Lamivudine, cimetidine and ketoconazole. Cimetidine and ketoconazole (1-500 μM for rat and 10-100 μM for human) were included as reference inhibitors of CYP2C11 and CYP3A, respectively. HPLC radiochromatograms of the microsomal extracts were examined to ascertain inhibition of testosterone metabolites. Results: inhibition of the expected testosterone metabolites was demonstrated by the references. Lamivudine (1-500 μM) in the normal rat microsome inhibited the formation of 6ß, 2α, 16α-hydroxy testosterone and androstenedione. In the induced microsomes, Lamivudine (100 and 500 μM) inhibited the formation of 6α, 7α, 16α, 16ß and at 100 μM only 2α-hydroxy testosterone. In the two human microsomal samples studied, Lamivudine (1-500 μM for one sample and 100 μM for the other) inhibited the formation of 6ß-hydroxy testosterone by 50%.

Comments: From these results, there are implications of Lamivudine interactions with cytochrome P450 in rat and man.

29. The expression of Cytochrome P450 isoenzymes in hepatic microsomes from male and female rats after oral administration of Lamivudine in a 90 day toxicity study (GDM/94/125/Study No. R13288)

Hepatic microsomes were prepared from liver samples obtained from male and female rats which had received Lamivudine by oral (dietary) administration for 90 days at dose levels of 0, 1000 or 2000 mg/kg/day for males and 0, 1500 or 3000 mg/kg/day for females (Study No. R13288). The purpose of this study was to determine the effect of Lamivudine on the expression of cytochrome P450 isozyme (CYP) in male and female rat hepatic microsomes. Results: the expression of CYP1A2, CYP2E1, CYP3A1 and CYP4A appeared to be unaffected by the administration of Lamivudine in both male and female rats at the specified doses. Hepatic CYP2B2 was below the limit of quantification in all samples. Hepatic CYP1A1 was not detected in any of the samples.

Comments: The findings of this study suggested that Lamivudine might not interfere in the hepatic clearance of other substances which might be administered concomitantly.

30. Radioactivity Balance Study of a Single Oral or Intravenous Radiolabelled Dose in Rats, Batch # C1284/275/2, Glaxo Group Research Ltd, Greenford, Middlesex, U.K., March, 1991, (GDM/91/014)

Four male and four female AHA rats were fed ^{3}H-Lamivudine in water by gavage at a dose of 45 mg/kg. A second group of three males and females were administered the same drug intravenously. Urine and feces were collected at 24 hr intervals for 48 hr. The amount of radioactivity in the urine, feces, carcass and cage

washings was determined. The total recovery of radioactivity after either route of administration was about 96%. After an oral dose, the amount of radioactivity in the urine was 61% after 48 hr (59% after 24 hr). The total recovery in the feces was 34%. When the route of administration was intravenous, the majority (87%) of the excreted dose was in the urine after 24 hr. By 48 hr, the total had reached 90%. Fecal excretion accounted for only

5% of the dose. Chromatographic analysis showed that the majority, approximately 96 to 98 % of the excreted doses were due to unchanged drug. The bioavailability calculated from the urinary recovery was approximately 68%.

31. To investigate the excretion balance and metabolic profile of radioactivity in urine following a single oral dose of [^{3}H]-Lamivudine (45 mg/kg) in the Han Wistar rat (GDM/94/076/Study No. 94/DM/76)

Four male rats were orally dosed by gavage with a single dose of ^{3}H-Lamivudine (45 mg/kg) to determine the excretion and metabolic profile of radioactivity in urine. Urine and feces were collected for up to 48 hr after dosing. Results: the mean total recovery of radioactivity over the 48 hr sample collection period following administration of the dose was about 93% (range 84.6-102.5%). Urinary excretion accounted for about 57% of the administered radioactivity, the majority in the first 24 hr after dosing. Fecal excretion accounted for about 35% (range 28-47.5%) of the administered dose. Metabolic profiling of the 0-24 hr urine samples revealed that unchanged drug accounted for approximately 92% of the urinary radioactivity. The remainder of the urinary radioactivity was accounted for by two metabolites, Met 1 (2%) and Met 2 (6%) of the urinary radioactivity. The chromatographic retention time of Met 2 corresponded to that of GI138870X, the trans-sulfoxide of Lamivudine.

32. Radioactivity Excretion Balance and Metabolic Profile in Urine After One Month Oral Toxicity Study of an Oral Dose in Rats, Batch # C1282/75/4, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., April, 1991, (GDM91/017)

Two male and two female AHA rats per group were administered Lamivudine in acetate buffer, pH 3.7, by gavage at doses of 0 (vehicle control, the vehicle control was dosed with 45 mg/kg of the drug spiked with radioactivity at day 36), 45, 300 and 2000 mg/kg/day twice daily for 35 days. On day 36, the animals were dosed with the same concentration of unlabeled compound which had been spiked with ^{3}H-Lamivudine. The 24 hr urine and feces samples were collected and the radioactivity determined. The percentages of radioactivity found in the samples are shown in Table 13.

Table 13
Excretion of Lamivudine in the Rat at 24 Hours

Dose (mg/kg)	Percent Excretion		
	Urine	Feces	Total
45 (Control)	40.3	47.8	88.1
45	37.5	49.6	87.1
300	38.4	49.8	88.1
2000	30.0	35.0	65.0

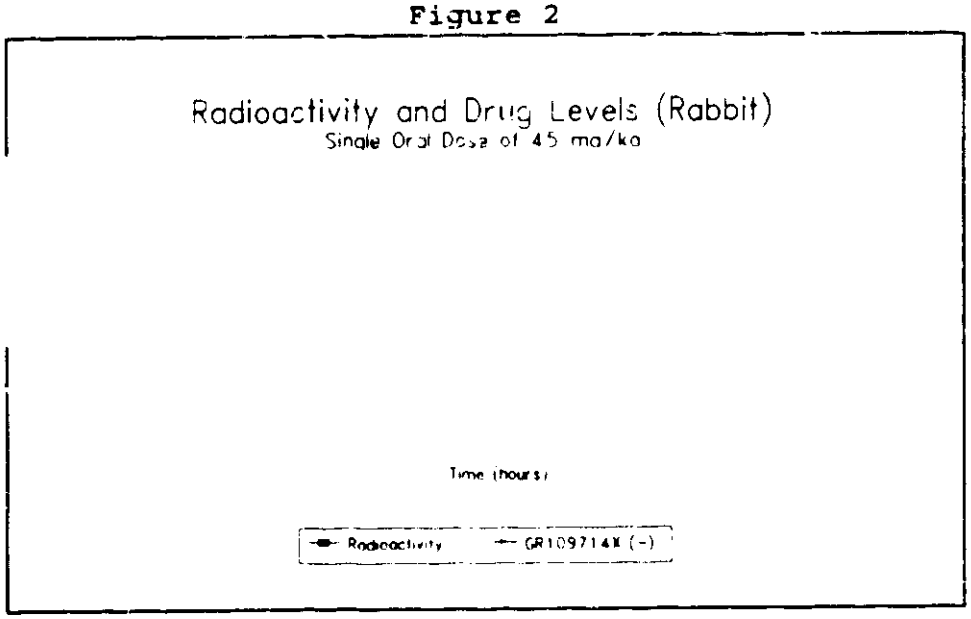
Analysis of the urine samples with HPLC indicate that most (approximately 94%) of the radioactivity is due to parent drug (Figure not shown).

Comments: There are no differences in the drug recovery in urine and feces or the metabolic profiles of radioactivity in urine of rats receiving drug. The data suggest that the extent of absorption is independent of dose (up to 300 mg/kg).

33. The Pharmacokinetics of Lamivudine and Total Radioactivity on Day 20 of Pregnancy in the Female Dutch Rabbit After Oral Administration of [^{3}H]-Lamivudine, Lot # C1758/226/1, C1282/75/4, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., March, 1992, (GDM/91/058)

Four pregnant Dutch rabbits were dosed orally by gavage with [^{3}H]-Lamivudine in water at 45 mg/kg. The animals were bled at 20 and 40 min and at 1, 2, 4, 6, 8, 10, 12, 24, 28, 32 and 48 hr after dosing for the determination of radioactivity and concentration of the parent drug in the plasma. The mean concentrations of each are shown in Figure 2.

Figure 2



In this study, the parent drug accounted for approximately 69% of the radioactivity. After 12 hr, a majority of the radioactivity in the plasma was due to in vivo tritium exchange. This was shown by measuring the radioactivity after the samples were dried.

Comments: One often expects some exchange with water when metabolism experiments are carried out using tritiated drug. Unless there was a significant exchange, the conclusions would not change to any great extent. In this experiment, the amount of exchange was small. There was a great deal of variability among the animals. The C_{max} ranged between 8.5 and 23.3 μg/ml (with a T_{max} between 0.35 and 1.07 hr) while the AUC_{0-48} ranged from 20.1 to 76 μg*hr/ml. The half-life ranged between 2.7 and 5.1 hr.

34. Bioanalytical Support Data for Lamivudine: A Study to Assess the Effects of Oral Administration on Pregnant Dutch Rabbits and Their Progeny, Lot # C1404/194/1, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., March, 1992, (GDM/92/008)

Two pregnant Dutch rabbits per group were administered Lamivudine in water by gavage, at doses of 0 (vehicle control), 45, 150 or 500 mg/kg twice daily from day 8 to day 20 of pregnancy inclusive. Blood was drawn from each animal at 2, 4, 8, and 12 hr on days 8 and 20 for the determination of plasma drug levels.

The mean plasma drug levels are presented in the following Table 14. The results showed that there was an increase in the plasma levels between the first and last administration at all three

doses. Plasma levels increased with increasing dose and continual exposure was achieved at all dose levels.

Table 14
Mean Plasma levels of Lamivudine in Pregnant Rabbits After Repeated Oral Administration[a]

Dose (mg/kg) bid	Time of Sample (hr)	Plasma Levels of Lamivudine (µg/ml)	
		Day 8	Day 20
45	2 4 8 12		
150	2 4 8 12		
500	2 4 8 12		

a: Dosing was from day 8 to day 20 of pregnancy inclusive.

35. Bioanalytical support data for Lamivudine: A further study to assess the effects of oral administration on pregnant Dutch rabbits, batch # C1013/75/1, Glaxo Group Research Ltd, Ware, Hertfordshire, England, 3 June, 1992, (GDM/92/035/L13280)*

Three groups of pregnant rabbits {strain: Dutch; 2 animals/group (4 animals/high dose); day 1 of pregnancy = day of mating} were administered Lamivudine, twice daily approximately 12 hr apart, by oral gavage at a standard dosage volume of 10 ml/kg (in acetate buffer, pH 3.5-3.7) during gestation days 8 to 20 at dosage levels of 7.5 (low dose), 20 (mid dose) or 45 mg/kg/day (high dose). Blood samples were collected via the marginal ear vein at 2, 4, 8 and 12 hr after the first dose on day 8 and 20 of pregnancy, and assayed by a validated HPLC-UV assay method to determine plasma Lamivudine concentrations. This study was designed to form part of a toxicology evaluation for the compound. Results are presented in Table 15 and 16. There was a significant increase in the plasma levels of Lamivudine between the first (day 8) and last (day 20) administration at all three dose levels. Plasma levels increased with dose and continual systemic exposure of the rabbit to drug for 12 hours was achieved only in high dose animals on day 8.

Table 15
Mean Plasma Levels of Lamivudine in Pregnant Dutch Rabbits After Repeated Oral Administration

Dose (mg/kg) (bid)	Time (hr)	Plasma Lamivudine (µg/ml)	
		Day 8	Day 20
7.5	2 4 8 12		
20	2 4 8 12		
45	2 4 8 12		

nd: not detectable by the analytical method

Table 16
Plasma AUCs of Lamivudine in Pregnant Dutch Rabbits After Repeated Oral Administration

Dose (mg/kg) (bid)	Plasma Lamivudine AUC (µg*hr/ml)	
	Day 8	Day 20
7.5		
20		
45		

Comments: Repeated administration of Lamivudine in pregnant rabbits can lead to accumulation of the drug in the animals.

36. Excretion Balance and Placental Transfer of Radioactivity in the Pregnant Female Dutch Rabbit After Oral Administration of [^{3}H]-Lamivudine, Lot # C1758226/1, C1282/75/4, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., March, 1992, (GDM/91/059)

Three pregnant Dutch rabbits were dosed orally by gavage with [^{3}H]-Lamivudine in water at 42.2 mg/kg. At 1, 4 and 24 hr after dosing, the animals were killed and a sample of amniotic fluid

was collected from each and analyzed by scintillation counting. Two fetuses from each dam were prepared for whole body autoradiography.

No images were found after exposure of film for 12 weeks. The level of radioactivity in the fetuses was below the limit of detection (17 ng equivalents/mg of tissue). Amniotic fluid had very low levels of radioactivity. No more than 0.003% of the administered radioactivity reached the fetuses.

In an additional study, four pregnant Dutch rabbits were dosed orally by gavage with [^{3}H]-Lamivudine in water at 45 mg/kg on day 20 of pregnancy. Urine and feces were collected at 24 hr intervals for 2 days. The animals were killed and radioactivity was determined in selected tissues. The metabolic cages were washed and residual radioactivity was determined.

At the end of this portion of the study, it was found that only 2 of the rabbits were pregnant. There was no difference in the route of excretion between pregnant and non-pregnant animals. The total recovery of radioactivity over the 48 hr collection period was approximately 67%. Urinary excretion accounted for 50% of the dose, the majority being excreted during the first 24 hr. Of this, approximately 80% was unchanged parent. Two polar metabolites (accounting for 7 and 16% of the urinary dose respectively) were found but not identified. Fecal excretion was variable and accounted for between 3 and 30% of the dose. Of this, 95% was unchanged drug. About 4.5% of the dose was recovered in the cage washes. The metabolic products were similar in the pregnant and non-pregnant animals. The level of radioactivity remaining in the tissues was very low except in the gastrointestinal tract, indicating that there was little retention of compound. Levels in the fetuses of the pregnant animals showed less than 0.007% of the total radioactivity.

Comments: There seems to be a little effect of pregnancy on the metabolism and excretion of the drug.

37. Pharmacokinetics and Excretion of a Single Oral or Intravenous Dose in Dogs, Batch # C1284/275/2, Glaxo Group Research Ltd, Greenford, Middlesex, U.K., May, 1991, (GDM/91/008)

Four male beagles were dosed with ^{3}H-Lamivudine in water at 30 mg/kg by intravenous infusion. After an unspecified recovery time, the same animals were dosed with the same compound by oral administration. Blood was drawn at various times after dosing (up to 96 hr) for the determination of the drug in the plasma. Urine and feces samples were collected for the determination of radioactivity every 24 hr for 96 hr. The pharmacokinetic parameters are summarized in Table 17.

Table 17
Mean Pharmacokinetic Parameters of Lamivudine in the Dog

Dose (mg/kg) & Route	AUC, (µg*hr/ml)	C_{max} (µg/ml)	T_{max} (hr)	$T_{\frac{1}{2}}$ (hr)	Cl (l/hr)
30, oral	61.6	26.6	0.67	1.6	5.6
30, iv	74.4	-	-	1.7	5.2

The pharmacokinetic parameters showed a close agreement between the oral and intravenous routes of administration. The volume of distribution calculated for this compound ranged from 10.1 to 15.5 liters (mean = 12.5 liters or 0.87 l/kg; rat vd = 2.92 l/kg) which is equivalent to the volume occupied by total body water in dog confirming the low distribution into tissues which would be expected from a polar compound such as this. The plasma clearance was about 5.4 l/hr, indicating a relatively rapid clearance. This was reflected by the elimination half-life (1.65 hr).

Following the intravenous dose, 81.7% of the radioactivity was found in the urine and 1% in the feces after 24 hr. By 96 hr, the totals were 86.7 and 1.8% respectively. Following the oral dose, 89.5% of the radioactivity was found in the urine and 1.5% in the feces after 24 hr. By 96 hr, the totals were 97.3 and 2.0% respectively. The compound was extensively metabolized in the dog. Two major metabolites were found in the urine. These accounted for 57% of the equivalent radioactivity after oral and 41% after intravenous administration. The bioavailability in this study was approximately 83%.

Comments: No differences in the mechanism of clearance of Lamivudine were noted between the two routes of administration. The drug accounted for only 39% of the urinary recovered radiolabel after oral and 48% and after intravenous administrations. The remaining radiolabel was shown to consist of two metabolite regions on HPLC.

If the GFR of the dog is taken to be approximately 3.2 l/hr, the maximum rate at which drug could be cleared by filtration can be derived by GFR x fu, where fu is the fraction of drug unbound in the plasma. Plasma protein binding studies have shown Lamivudine to be approximately 10% protein bound in dog plasma at the concentrations detected after administration at 30 mg/kg. This means that clearance by glomerular filtration could occur no faster than 0.9 x 3.2 = 2.9 l/hr which when compared to the overall mean value for renal clearance of 2.3 l/hr suggest that tubular secretion does not play a significant role in the elimination of Lamivudine in dogs.

38. An Investigation of the Pharmacokinetics and Total Radioactivity and the Excretion Balance of Radioactivity in the Female Beagle Dog Following the Administration of [^{3}H]-Lamivudine (30 mg/kg) By the Oral and Intravenous Routes, Lot # C1758/233/1, C1282/75/4, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., March, 1992, (GDM/91/080)

Four female beagles were administered either a single intravenous or a single oral (by gavage) dose of [^{3}H]-Lamivudine in water at a dose of 30 mg/kg. They were placed in metabolism cages for 96 hr. After intravenous dosing, blood was collected from each animal at 5, 10, 20 and 40 min and 1, 1.5, 2, 3, 4, 6, 8, 24, 48, 72 and 96 hr for the determination of radioactivity and parent drug levels. After oral dosing, blood was drawn at 20 and 40 min and at 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 24, 48, 72 and 96 hr again for the determination of radioactivity and parent drug levels. Urine and feces were collected at 24 hr intervals over 96 hr for the determination of radioactivity and parent drug levels. The cages were washed and the amount of residual radioactivity determined. The pharmacokinetic parameters which were calculated using a computer program called SIPHAR are shown in Table 18.

Table 18
Pharmacokinetics of Lamivudine in Female Dogs After Administration of 30 mg/kg

Route	C_{max} (µg/ml)	T_{max} (hr)	$AUC_{0-\infty}$ (µg*hr/ml)	$T_{\frac{1}{2}}$ (hr)
Oral	15.4	1.25	40.1	1.3
Intravenous	-	-	58.6	1.7

After intravenous administration, the plasma clearance was 5.7 l/hr, the renal clearance was 1.5 l/hr and the volume of distribution was 13.4 liters. The bioavailability was 67%. The mean concentration of radioactivity and parent drug after oral and intravenous administration are shown in Figures 3 and 4.

It is obvious that Lamivudine is metabolized to a great extent in the female dog. Excretion occurs mainly into the urine where approximately 90% of the dose (oral or intravenous administration) appeared within the 96 hours of the study. Of the material appearing in the urine, only 27% was parent drug after oral and 37% as parent after intravenous administration. Only 2 to 4% of the radioactivity appeared in the feces.

Figure 3

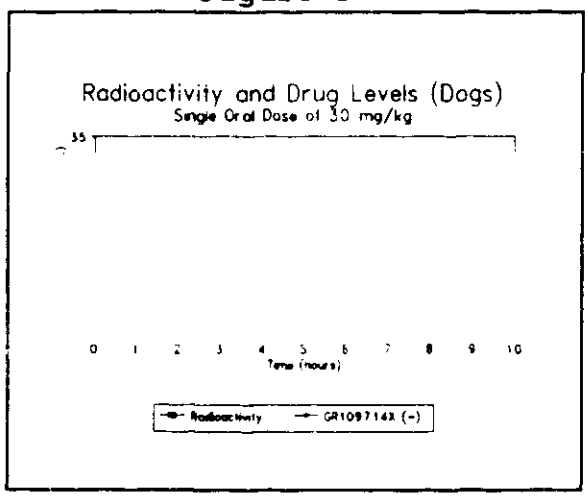
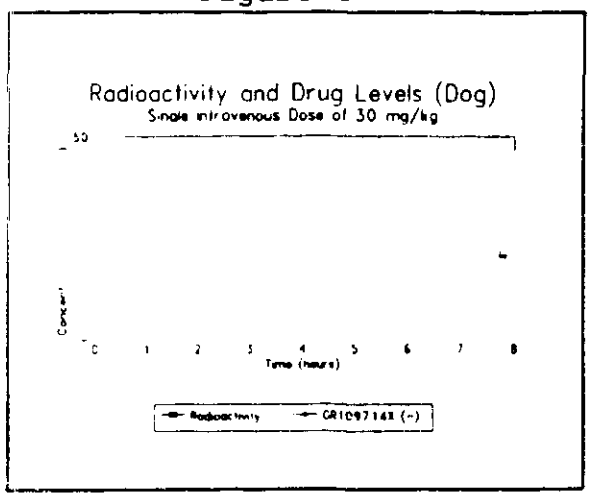


Figure 4



Comments: The pharmacokinetics of the plasma levels of parent drug is very similar in the female rat and the female dog after intravenous administration. The bioavailability is 69% in the rat and 67% in the dog. In the dog, the clearance is 5.7 l/hr and in the rat it is 0.27 l/hr. If one examines the clearance as a function of body surface area, one finds that for the dog, the clearance is 1.0 ml/hr*cm² and for the rat it is 0.83 ml/hr*cm². If one examines the clearance as a function of body weight, the value for the dog is 0.48 l/hr*kg and for the rat, 1.35 l/hr*kg. Obviously, interspecies scaling for this parameter (clearance) is best estimated on the basis of body surface area, not surprising for a drug eliminated mainly in the urine. The volume of distribution is 13.4 liters for the dog and 0.449 liters for the rat. This gives a ratio of 29.8 for the value in the dog divided by that in the rat. The ratio of surface areas for the dog and the rat is 17.7 and that of body weights is 60, indicating that the volume of distribution should best be described by some combination of the two for interspecies scaling. However, Lamivudine is metabolized extensively in the dog but not in the rat.

39. Determination of the Pharmacokinetics of Lamivudine in the Dog Following a Single Oral Dose, Lot # C1013/75/1, C1803/125/1, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., January, 1992, (GDM/91/036)

Three male beagle dogs per group, were administered a single dose of Lamivudine in acetate buffer, pH 3.5, by gavage, at doses of

250, 1000, 1500 or 2000 mg/kg. Blood was taken at 20, 40 min and at 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 24, 26, 28, and 30 hr after dosing for the determination of plasma levels of the drug. A compilation of the pharmacokinetic parameters are given in Table 19. Examination of the table indicates that in order to achieve maximum exposure in the dog, a single dose of approximately 1500 mg/kg is sufficient. Beyond that, little increases in systemic exposure will be achieved.

Table 19
Pharmacokinetic Parameters of Lamivudine in the Dog

Dose (mg/kg)	C_{max} (µg/ml)	T_{max} (hr)	AUC (µg*hr/ml)	$T_{\frac{1}{2}}$ (hr)
500	140	2.5	749	2.6
1000	224	2.0	1370	1.7
1500	302	2.5	1961	2.8
2000	267	1.7	1353	2.1

40. Bioanalytical support data for Lamivudine in the dog following a single dose ranging rom 250 to 2000 mg/kg (GDM/94/161)

Two male conscious beagle dogs with exteriorized carotid artery loops were administered a single oral dose of Lamivudine via oral gavage at dose levels of 0 (vehicle control), 100 (low), 300 (mid) or 600 mg/kg (high) on separate study days with a minimum interval of 7 days (Study No. S20599). Blood samples were taken after the dosing; plasma derived was assayed for Lamivudine. Results: all plasma samples taken after dosing from the dogs contained detectable levels of drug. The Cmax levels ranged from 28.6 to 54.6 µg/ml for the low dose, from 85.0 to 124 µg/ml for the mid dose and from 157 to 199 µg/ml for the high dose. The AUC values were 85 to 170, 349 to 607 and 564 to 959 µg*hr/ml for the low, mid and high dose groups, respectively. The plasma level data for dog K65 showed a proportional increase in AUC with increasing dose. Data from the other dog (K67) demonstrated a non-linear increase in AUC with increasing dose.

Comments: Plasma data from one dog demonstrated a non-linear increase in AUC values with increasing dose indicating an apparent saturation of clearance at the higher dose levels.

41. Two Week Oral Toxicity Study in Dogs, Batch # C1803/152/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., November, 1991, (D12750-WPT/91/222)*

Three male and female beagle dogs (weight: 7 - 8.5 kg for males and 5.6 - 8.4 kg for females; age: 3 - 6 months) were dosed by gavage for two weeks with 1500 mg/kg/day of Lamivudine dissolved in acetate buffer, pH 3.7. Dosing was carried out twice a day 12 hours apart. An additional male and female were dosed with vehicle as controls. All animals were observed daily for adverse clinical signs while their food consumption was recorded daily and their body weights were recorded predose and on days 1, 4, 8, 11 and on day 15, the day of necropsy. Blood was obtained for hematological, clinical chemistry and drug level evaluations Urine was collected for examination. On day 15, the day after the final dose, the animals were killed and subjected to necropsy. The animals were exsanguinated and a full macroscopic examination was carried out. Thirteen organs were weighed and 38 tissues or organs were subjected to histological examination.

There were no deaths in the study. Loose feces and isolated cases of emesis were seen in both treated and control animals. There were otherwise, no treatment related adverse clinical observations. Slight reductions in hemoglobin, hematocrit and red blood cell counts as well as moderate reductions in leukocyte and neutrophil counts were seen in both males and females at the end of dosing. A slight increase of aspartate and alanine aminotransferase was seen in both males and females at the end of dosing. Treated females had slightly smaller thymuses and slightly larger ovaries compared to the control animal and treated males had slightly smaller prostates. On microscopic examination, the greatest effect of the drug was to the liver of both treated males and females. The lesions, which ranged from very slight to very severe, included the presence of mixed inflammatory foci, centrilobular mixed inflammatory cell infiltrate, centrilobular coarse vacuolation and focal necrosis (in one female). In general the liver lesions were more severe than slight. Slight thymic atrophy was seen in a number of animals.

The mean plasma levels for males and females on day 1 and 14 are presented in Table 20 and pharmacokinetic parameters calculated for the mean plasma level-time data are depicted in Table 21. The parameters indicated that repeated administration of drug for 14 days at this dose level caused a decrease in plasma clearance in both males and females dogs, leading to increased in plasma AUCs and hence drug exposure.

Table 20
Mean Plasma Levels of Lamivudine in Beagle Dogs Receiving Repeated Oral Administration of at 1500 mg/kg/day BID

Time (hr)	Mean Plasma Levels (µg/ml)			
	Male		Female	
	Day 1	Day 14	Day 1	Day 14
0				
1				
2				
3				
4				
6				
8				
12				

Table 21
Pharmacokinetic Parameters of Lamivudine in Beagle Dog

Pharmacokinetic Parameters	Male		Female	
	Day 1	Day 14	Day 1	Day 14
AUC (µg*hr/ml)	694	1169	690	1578
Clearance (l/hr/kg)	2.2	1.2	2.2	0.8
Volume of Distribution (l/kg)	3.7	4.5	3.5	4.2

Comments: In view of the fact that livers of all 6 treated dogs in this study are moderately/severely affected (mixed inflammatory foci, centrilobular mixed intlammatory cell infiltrate or centrilobular coarse vacuolation) and that focal liver necrosis is present in one treated female, coupled with elevated ALT and AST, taken together suggest that liver is a target organ for this compound in dogs. The thymic atrophy seen may be a treatment-related toxicity.

Repeated administration of Lamivudine bid for 14 days caused a reduction in plasma clearance of this agent in both male and female dogs leading to an increase in systemic drug exposure.

42. Bioanalytical support data for Lamivudine: A 13-week oral toxicity study in beagle dogs, batch # C1013/75/1, Glaxo Group Research Ltd, Greenford, Middlesex, England, 23 April, 1992, (GDM/92/034/D12873)*

Four groups of dogs (strain: beagle; 6 animals/sex/group) were administered Lamivudine, twice daily approximately 12 hr apart, by oral gavage at a standard dosage volume of 10 ml/kg (in acetate buffer, pH 3.5-3.7) at dosage levels of 0 (control), 45 (low dose), 260 (mid dose) or 1500 mg/kg/day (high dose) for 91-96 days. Blood samples were collected at various time points on day 1, day 14, day 35, day 42, day 46, day 48 and day 91 of the study; urine collections were made over a 12 hr period after the first dose on day 89. All the samples were analyzed by a validated HPLC-UV assay method to determine Lamivudine concentrations in plasma and urine. This study was designed to form part of a toxicology evaluation for the compound. The mean plasma levels of Lamivudine in samples from the three treatment groups on day 1 and day 91 are presented in Tables 22, 23 and 24. The results showed adequate absorption of drug in both male and female dogs. In both male and females, plasma AUCs increased on day 91 compared to the ones on day 1 (Table 25). Mean percentage urinary recovery of Lamivudine in 0-12 hr collections taken from animals in the three treatment groups after the first dose on day 89 are presented in Table 26. These data showed that there was no significant differences between males and females or between treatment groups in the urinary recovery of uncharged drug.

Table 22
Mean Plasma Levels of Lamivudine in Beagle Dogs Receiving Repeated Oral Administration of 45 mg/kg Twice Daily

Time (hr)	Mean Plasma Lamivudine Concentrations (μg/ml) at 45 mg/kg					
	Male			Female		
	Day 1	Day 91 1 Dose	Day 91 2 Dose	Day 1	Day 91 1 Dose	Day 91 2 Dose
Pre						
1						
2						
4						
6						
8						
12						

Table 23
Mean Plasma Levels of Lamivudine in Beagle Dogs Receiving Repeated Oral Administration of 260 mg/kg Twice Daily

Time (hr)	Mean Plasma Lamivudine Concentrations (μg/ml) at 260 mg/kg					
	Male			Female		
	Day 1	Day 91 1 Dose	Day 91 2 Dose	Day 1	Day 91 1 Dose	Day 91 2 Dose
Pre						
1						
2						
4						
6						
8						
12						

Table 24
Mean Plasma Levels of Lamivudine in Beagle Dogs Receiving Repeated Oral Administration of 1500 mg/kg Twice Daily

Time (hr)	Mean Plasma Lamivudine Concentrations (μg/ml) at 1500 mg/kg					
	Male			Female		
	Day 1	Day 91 1 Dose	Day 91 2 Dose	Day 1	Day 91 1 Dose	Day 91 2 Dose
Pre						
1						
2						
4						
6						
8						
12						

Table 25
Mean Plasma AUCs in Beagle Dogs Receiving Repeated Oral Administration of Lamivudine Twice Daily

Dose (mg/kg) (bid)	Plasma Lamivudine AUCs (μg*hr/ml)					
	Male			Female		
	Day 1	Day 91 1 Dose	Day 91 2 Dose	Day 1	Day 91 1 Dose	Day 91 2 Dose
45	38	57	50	50	75	52
260	257	387	349	182	346	293
1500	1084	1652	2162	565	1512	2139

Table 26
Mean Percentage Urinary Recovery of Lamivudine on Day 89 in 0-12 Hr Collections From Dogs After Receiving Repeated Oral Administration of Lamivudine Twice Daily

Dose (mg/kg) (bid)	Percentage Recovery of Lamivudine in Urine	
	Male	Female
45	23.4	20.6
260	20.4	33.0
1500	16.6	23.5

Comments: There are some apparent differences in plasma levels between day 1 and day 91 and between dose 1 and dose 2 on day 91 in the three treatment groups. At all three dose levels, the T_{max} appears to be later after the second dose on day 91 than after the first dose on either day 1 or day 91, which could suggest that the rate of absorption or the rate of plasma clearance was slower after the second dose of the day relative to that of the first. Secondly, increased AUCs on day 91 compared to day 1 suggest that repeated administration of Lamivudine at the dose levels leads to accumulation of drug in dogs.

43. Bioanalytical support data for Lamivudine: A 52-week oral toxicity study in beagle dogs (GDM/94/081/Study No. D13604)

Groups of male and female beagle dogs (weight: 7.8-11.8 kg; age: 23-27 weeks; 4 animals/group) received Lamivudine by oral gavage at dose levels of 0 (vehicle), 45 (low), 260 (mid) or 1500 (♂), 1000 (♀) mg/kg/bid (high) for a period of 52 weeks (Study No. D13604). Plasma levels were monitored on day 1 and weeks 6, 26 and 52. Results: the mean plasma levels of Lamivudine in samples

taken from the three treatment groups on weeks 6 and 26 of the study are presented in Table 27. AUC data for the doses are presented in Table 28 and clearly show increased exposure on week 52 relative to day 1 for all three dose groups.

Table 27
Mean Plasma Levels of Lamivudine in Dogs

Dose mg/kg/bid	Nominal Time (hr)	Male		Female	
		Lamivudine (µg/ml)			
		WK 6	WK 26	WK 6	WK 26
45	0				
	2				
260	0				
	2				
1500 ♂ 1000 ♀	0				
	2				

Table 28
The AUC Values of Lamivudine Following 12-Month Oral Administration to Dogs

Dose mg/kg/bid	Male			Female		
	Day 1 1st Dose	Week 52 1st Dose	Week 52 2nd Dose	Day 1 1st Dose	Week 52 1st Dose	Week 52 2nd Dose
45						
260						
1500 (♂) 1000 (♀)						

Comments: At all dose levels the plasma AUC values calculated for the first and second doses on week 52 of the study are higher than those calculated for the first dose on day 1. This observation suggested that repeated administration of Lamivudine at these dose levels leads to increased systemic exposure of both male and female dogs.

44. Transport mechanism of Lamivudine and drug interactions in Caco-2 cells (UCP/92/028)

Transport and cellular uptake of Lamivudine at concentrations of

0.02, 0.2, 2.0, 20 and 200 μM across Caco-2 cell monolayers were studied to investigate the interaction of Lamivudine with drugs which may be used concurrently with Lamivudine clinically. The effect of trimethoprim (1.6 mM), sulfamethoxazole (6.7 mM), ddI (2 and 10 mM), ddC (2 and 10 mM), AZT (1.9 and 2 mM), acyclovir (6.7 mM), probenecid (5.9 mM), ranitidine (10 mM) and cimetidine (10 mM) on Lamivudine (2 mM) was studied. Results: the transport rate was linearly related to the concentration of Lamivudine from 0.02 to 200 mM, suggesting a passive diffusion process. None of the compounds tested significantly changed the transport of Lamivudine in Caco-2 cells.

45. Investigation into the potential for interaction between midazolam and Lamivudine with the human liver Cytochrome P450 isoenzyme CYP3A (GDM/94/124/Study No. L18/94/014)

Midazolam is a specific probe substrate which is hydrolyzed at the C-1 and C-4 positions specifically by human liver cytochrome P450 isoenzyme CYP3A. Human liver microsomes were pooled and incubated with Lamivudine (1 to 500 μM) in the presence of midazolam. The aim of this study was to investigate the findings of an interaction study carried out using testosterone as a probe substrate, which indicated that might be a possible interaction of Lamivudine with the human liver cytochrome P450 isozyme, CYP3A (GDM/93/028). Ketoconazole (0.1 mM), which specifically inhibits CYP3A, was used as a positive control. An incubation of midazolam with human liver microsomes in the absence of NADPH was included (negative control) to demonstrate that the metabolism of midazolam was P450 mediated. Results: the HPLC analysis of the incubates showed that, in the absence of inhibitors, midazolam was metabolized to 1-hydroxy and 4-hydroxy midazolam. There was a negligible turnover of midazolam when incubated with human liver microsomes in the absence of NADPH, indicating that the metabolism of midazolam was P450 mediated. Incubation of midazolam in the presence of ketoconazole greatly reduced the formation of the hydroxy metabolites. The incubations containing Lamivudine showed no overall drop in the formation of the hydroxy metabolites produced when compared to the midazolam control. Conclusion: these data showed that there was no interaction of CYP3A with Lamivudine.

PHARMACOLOGY STUDIES

I. Ancillary Pharmacology Studies:

1. **Overt central and peripheral pharmacodynamic effects following acute oral administration of Lamivudine in the conscious rat and dog, Batch # C1803/84/1, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., June, 1991, (WBA/91/005)***

2. The effects of intravenously administered Lamivudine on the cardiovascular and respiratory systems of anesthetized cats, Batch # C1603/84/1, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., June, 1991, (WBA/91/003)*

3. The effects of acute oral administration of Lamivudine on cardiovascular function and respiration rate in conscious beagle dogs, June, 1994 (WPT/94/218)*

4. Drug interaction study: effects of orally administered Lamivudine on the duration of pentobarbitone-induced loss of righting reflex (sleeping time) in mice (WBA/90/039)

II. Cytotoxicity and Mechanism of Action Studies

1. Cytotoxicity tests in uninfected primary and established lymphocytes and monocyte/macrophages, Batch # C1758/244/1, June 1991, (GVR/91/006)

2. Cytotoxicity and therapeutic index in human peripheral blood lymphocytes, Batch # C1460/141/1, June 1994, (GVR/94/017)

3. Effect on human T-cell activation in tro, Batch # C1013/75/1, June 1994, (GVR/93/032)

4. Myelotoxicity studies on human bone marrow progenitor cells: a final report on a study by

, Batch # C1055/185/1, December 1991, (GVR/91/030)

5. Metabolism in uninfected and HIV-1 infected peripheral blood lymphocyte cells in vitro, Batch # C1460/141/1, June 1991, (GVR/_1/005)

6. Concentration-dependence and the effect on AZT on the phosphorylation of Lamivudine in human peripheral blood lymphocytes in vitro, Batch # C1291/38/1, June 1994, (GVR/93/013)

7. Studies with human platelet phosphorylase, Batch # C1055/185/1, June 1991, (GVR/91/003)

8. Effects of Lamivudine 5'-triphosphate on the HIV-1 reverse transcriptase, Batch # C1460/209/2, June 1991, (GVR/91/008)

9. **Effect on Lamivudine triphosphate on the DNA-dependent DNA polymerase activity of HIV-1 reverse transcriptase, Batch # C1460/209/2, December 1991, (GVR/91/026)**

10. **Chain termination studies with Lamivudine triphosphate, Batch # C1460/209/2, June 1991, (GVR/91/009)**

11. **Steady state kinetic studies confirm that Lamivudine 5'-triphosphate exerts its anti-HIV activity primarily by chain termination, Batch # C1460/209/2, December 1992, (GVR/92/013)**

12. **The incorporation of Lamivudine 5'-monophosphate into DNA by HIV-1 reverse transcriptase and human DNA polymerase, Batch # C1653/21/1, 1994, (GVR/94/007)**

13. **Effect of Lamivudine on deoxynucleotide pools in cultured U937 cells, Batch # C1013/75/1, December 1991, (GVR/91/027)**

14. **Effect on mitochondrial DNA content, Batch # C1013/75/1, June 1991, (GVR/91/017)**

15. **Effect of Lamivudine triphosphate on mammalian DNA polymerases, Batch # C1460/209/2, June 1991, (GVR/91/010)**

Pharmacology Studies Reviews:

I. Ancillary Pharmacology Studies:

1. Overt central and peripheral pharmacodynamic effects following acute oral administration of Lamivudine in the conscious rat and dog, Batch # C1803/84/1, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., June, 1991, (WBA/91/005)*

Three male RH rats per dose were treated by gavage with Lamivudine in acetate buffer, pH 3.7, at doses of 100, 300 or 600 mg/kg. Three control rats per group were dosed with vehicle. The animals were placed in clear chambers and observed for changes in behavior, skeletal muscle tone, reflexes and overt gastrointestinal, neurological and autonomic effects by comparison with control animals using an extensive check list. The animals were observed for the first 30 min and at 1, 2 and 4 hr after dosing. They were examined every day for 7 days.

The only adverse effects noted in the animals were slight diarrhea in all the animals at 100 mg/kg and one of the animals at 600 mg/kg.

Groups of two male and two female beagle dogs were treated by gavage with Lamivudine in acetate buffer, pH 3.7, at doses of 0 (vehicle control), 300 or 600 mg/kg. They were observed for changes in behavior, skeletal muscle tone, reflexes and overt gastrointestinal, neurological and autonomic effects using an extensive check list. Observations were carried out continuously for the first hr and at 2, 3 and 5 hr after dosing. Recordings of heart rate, body temperature and respiration rate were carried out at predose and at 1, 3, 5 and 24 hr post-dose. The animals were kept for 7 days for observation.

The only recorded adverse effect was vomiting in the male dog at 600 mg/kg at approximately 15 min after dosing.

2. The effects of intravenously administered Lamivudine on the cardiovascular and respiratory systems of anesthetized cats, Batch # C1803/84/1, Glaxo Group Research Ltd., Greenford, Middlesex, U.K., June, 1991, (WBA/91/003)*

One male and one female BGF cat were anesthetized, cannulated for the infusion of test material and set up for the recording of arterial blood pressure, heart rate, tracheal inflation pressure and EKG. At 45 minute intervals, each was intravenously infused with 0 (vehicle), 10, 30 and 100 mg/kg of Lamivudine in 0.9% saline. Measurements were made throughout the study. Arterial blood samples were taken prior to dosing and 30 minutes after the infusion of each new dose for the determination of pH, pCO_2 and pO_2.

No adverse effects were seen in the study.

Comments: Dr. K.M. Wu, a reviewing pharmacologist in the DAVDP, who has expertise in the area, examined this study as well all other studies in this submission which dealt with measurements of heart function. In all cases, his conclusions concurred with those of the sponsor as to the interpretation of the test results.

In separate studies, the racemate GR103665X (±) administered by gavage to conscious beagle dogs at 100 or 300 mg/kg had no effect on blood pressure, heart and respiration rates and electrocardiographic examinations. The general well being and behavior of the animals was not adversely affected.

The effect of the racemate GR103665X (±) at oral doses of 100, 300 and 600 mg/kg in mice had no effect on the pentobarbitone induced sleeping time.

The racemate GR103665X (±) administered by intravenous injection to anesthetized cats had no adverse effect on the heart rate or

arterial blood pressure nor any adverse effect on electrocardiograms. The drug had no adverse effect on tracheal inflation pressure or blood values of pH, pCO_2 or pO_2.

The racemate GR103665X (±) administered by gavage to rats or dogs at doses of 10, 30 or 100 mg/kg induced minimal adverse events. One rat out of three in the two high dose groups had slight diarrhea. No adverse effects were seen in dogs.

3. The effects of acute oral administration of Lamivudine on cardiovascular function and respiration rate in conscious beagle dogs, June, 1994 (WPT/94/218)*

Two male conscious beagle dogs with exteriorized carotid artery loops were administered a single oral dose of Lamivudine via oral gavage at dose levels of 0 (vehicle control), 100 (low), 300 (mid) or 600 mg/kg (high) on separate study days with a minimum interval of 7 days to investigate the acute cardiovascular, respiratory and electrocardiographic effects. Results: there were no effects on gross behavior or clinical state after any dose of Lamivudine in either dog. There were no significant effects on arterial blood pressure, heart rate or ECG rhythm or intervals. All plasma samples taken after dosing with Lamivudine showed detectable levels of drug indicating exposure to the test compound. The Cmax values were 54.6, 85.0 and 124.0 μg/ml for the low, mid and high dose groups, respectively. Conclusion: the lack of adverse effects in the dog of oral doses of Lamivudine up to 150-fold higher than that required for pharmacological activity or the anticipated acute clinical dose (4 mg/kg bid) supports the clinical use of this compound.

4. Drug interaction study: effects of orally administered Lamivudine on the duration of pentobarbitone-induced loss of righting reflex (sleeping time) in mice (WBA/90/035)

Groups of male mice (10/group) were administered Lamivudine at dose levels of 0 (vehicle control or positive control: cimetidine 150 mg/kg), 100 (low), 300 (mid) or 600 mg/kg (high) via oral gavage to study the effect of the test compound on the duration of righting reflex loss (sleeping time) induced by pentobarbitone. One hr after the dosing, each mouse received pentobarbitone (40 mg/kg; iv) to induce loss of righting reflex. Results: Lamivudine did not affect the duration of pentobarbitone-induced loss of righting reflex (sleeping time) in mice. Cimetidine (positive control; known to interact with hepatic drug metabolizing enzymes) significantly prolonged sleeping time. Conclusion: these findings indicate that Lamivudine was unlikely to interact with drug metabolizing enzymes in the liver.

II. Cytotoxicity and Mechanism of Action Studies

1. Cytotoxicity tests in uninfected primary and established lymphocytes and monocytes/macrophages, Batch # C1758/244/1, June 1991, (GVR/91/006)

In initial cell proliferation studies with several lymphocyte cell lines and one monocyte/macrophage cell line, the cytotoxicity of Lamivudine was studied in comparison with the (+) enantiomer, GR109712X and, the racemic mixture, GR103665X. Results: these studies showed that Lamivudine was not cytotoxic to most of the cell lines tested up to 440 μM concentration. In contrast, GR109712X and GR103665X were cytotoxic; ID50% [value represents the concentrations of compound required to inhibit cell proliferation or thymidine uptake by 50%] were 13-394 μM and 4-153 μM, respectively. Conclusion: the cytotoxicity exhibited by the racemic mixture, GR103665X, resided in the (+) enantiomer.

In more extensive studies in vitro, the cytotoxicity of Lamivudine relative to AZT, ddC and ddI was investigated. Results: in general, Lamivudine was the least cytotoxic of the four compounds, followed closely by ddI. The most cytotoxic compound was ddC.

2. Cytotoxicity of Lamivudine, AZT, ddC and ddI in human peripheral blood lymphocytes, Batch # C1460/141/1, June 1994, (GVR/94/017)

In in vitro experiments, the cytotoxicity of Lamivudine to human PBLs relative to AZT, ddC and ddI was determined by studying thymidine uptake in the PBLs. Results: the overall trend with the PBLs is that Lamivudine and ddI were the least inhibitory, with ID50% values in the range of >437-2838 μM and >423-2371 μM, respectively. AZT was the most consistently toxic inhibitor of the thymidine uptake in PBLs with ID50% values in the range of 34-94 μM; ddC was more cytotoxic than AZT to cells from some donors but much less toxic to others, producing ID50% values of 6-2464 μM.

3. Effect on human T-cell activation in vitro, Batch # C1013/75/1, June 1994, (GVR/93/032)

The effect of Lamivudine, AZT and ara-AMP on the uptake of thymidine in human T-cells after activation by phytohaemagglutinin (PHA) or anti-CD3 antiserum was studied. Results: both PHA and anti-CD3 antiserum activated normal human T-cells. In both cases, inhibition of thymidine uptake was greater with AZT (ID50% = 6.7-13.5 μM) than ara-AMP (ID50% = 60.5 μM) and greater with ara-AMP than with Lamivudine (ID50% = 436 μM). Conclusion: there was no evidence for immunosuppression by Lamivudine in the system.

Comments: This type of assay did not distinguish between compound-related cytotoxicity and immunosuppression at the cellular level. Thus, the mode of action of AZT or ara-AMP in inhibiting the thymidine uptake by T-cells was not clear.

4. Myelotoxicity studies on human bone marrow progenitor cells: a final report on a study by C1055/185/1, December 1991, (GVR/91/030)

In in vitro comparative studies with AZT, ddC, ddI, ara-C, 3FT, and d4T, Lamivudine was tested for activity against several hemopoietic progenitor cells. Results: Lamivudine was found to have an ID50% >100 μM against pluripotent progenitor cells, erythroid progenitor cell, early and late granulocyte-macrophage progenitor cells, stromal progenitor cells, hemopoietic supportive stroma, non-adherent hemopoietic cells and stromal fibroblasts. Lamivudine was at least 10-10000 times less cytotoxic (depending on the cell type) than AZT and ddC in these studies. Lamivudine had no marked effect on GM-CSF production by bone marrow stromal cells at 1 mM concentrations under conditions where AZT stimulated GM-CSF production at 10 μM.

5. Metabolism in uninfected and HIV-1 infected peripheral blood lymphocyte cells in vitro, Batch # C1460/141/1, June 1991, (GVR/91/005)

Intracellular metabolism of [^{3}H]-Lamivudine was examined in HIV-infected and mock-infected phytohemagglutinin-stimulated PBLs in vitro. Results: Lamivudine triphosphate accumulated equally in HIV-infected and mock-infected PBLs, and reached 40% more of the total intracellular metabolites after 4 hr. The rate of decay of Lamivudine triphosphate [measured as t½] in HIV-infected and mock-infected PBLs ranged from 10.5-15.5 hr.

Comments: The results indicated that metabolism of Lamivudine to its 5'-triphosphate was not dependent on viral infection of cells; and hence, good intracellular levels were detectable in both infected and uninfected cells.

6. Concentration-dependence and the effect on AZT on the phosphorylation of Lamivudine in human peripheral blood lymphocytes in vitro, Batch # C1291/38/1, June 1994, (GVR/93/013)

The phosphorylation of [^{3}H]-Lamivudine was examined in both HIV-infected and mock-infected phytohemagglutinin-stimulated PBLs at different extracellular concentrations of up to 500 μM in vitro. Results: the formation of intracellular metabolites was dependent on the extracellular concentration of the test compound. The amount of Lamivudine triphosphate formed was linear up to 10 μM

concentration after 4 hr of incubation. Combination studies with 10 μM Lamivudine in the presence of AZT showed that AZT had no substantial effect on the phosphorylation of Lamivudine.

7. Studies with human platelet phosphorylase, Batch # C1055/185/1, June 1991, (GVR/91/003)

Lamivudine was incubated with human platelets to study the catabolism of the test compound. Results: no deamination product or base was detected in the platelet rich suspensions at various incubation periods. The natural substrate for thymidine phosphorylase, thymidine and deoxyuridine, were found to be phosphorolysed to their corresponding bases after the incubation in platelet rich suspensions. Conclusion: under these conditions, Lamivudine was shown to be resistant to both deamination and phosphorolysis by human platelet pyrimidine nucleoside phosphorylase.

8. Effects of Lamivudine 5'-triphosphate on the HIV-1 reverse transcriptase, Batch # C1460/209/2, June 1991, (GVR/91/008)

The ability of Lamivudine 5'-triphosphate to inhibit HIV-1 reverse transcriptase in a novel heteropolymer assay system [using either a bacteriophage MS2 RNA template with a specific oligo-deoxynucleotide as primer of specific homopolymer template/primers] was examined. The reaction conditions were optimized for the template and the kinetic constants were determined [data not shown]. A comparison of inhibition kinetics was made with ddC 5'-triphosphate (ddCTP), ddA 5'-triphosphate (ddATP) and AZT 5'-triphosphate (AZTTP) Results: Lamivudine 5'-triphosphate inhibited HIV reverse transcriptase with an apparent Ki (Kiapp) of 8-18 μM dependent on the template/primer system utilized and in a competitive manner with respect to dCTP for binding to the enzyme. The Kiapp for ddCTP, ddATP and AZTTP were 0.32-2.8, 0.08-0.1 and 0.01-0.05 μM, respectively. Conclusion: Lamivudine 5'-triphosphate was a competitive inhibitor of the HIV-1 reverse transcriptase and Lamivudine might, in part, inhibit HIV replication by direct competition for the binding sites of the enzyme.

9. Effect on Lamivudine triphosphate on the DNA-dependent DNA polymerase activity of HIV-1 reverse transcriptase, Batch # C1460/209/2, December 1991, (GVR/91/026)

The effect of Lamivudine 5'-triphosphate was investigated against both the DNA-dependent DNA polymerase and RNA-dependent DNA polymerase activities of HIV-1 reverse transcriptase. Results: for Lamivudine 5'-triphosphate, at [dCTP] = Km, the average value for IC50 (the concentration giving 50% inhibition) was 23.4 μM. The average IC50 values for ddATP, ddCTP and AZTTP were 0.4, 1.44 and 0.48 μM, respectively. Conclusion: Lamivudine triphosphate

was equally active against both the DNA-dependent DNA polymerase and RNA-dependent DNA polymerase activities of HIV-1 reverse transcriptase. It was less potent as an inhibitor towards these activities than ddATP, ddCTP or AZTTP.

10. Chain termination studies with Lamivudine triphosphate, Batch # C1460/209/2, June 1991, (GVR/91/009)

The ability of Lamivudine 5'-triphosphate to serve as a substrate for reverse transcriptase was studied using a modification of Sanger dideoxy-DNA sequencing method. Results: Lamivudine 5'-triphosphate terminated transcription by HIV-1 reverse transcriptase at positions identical to those where ddCTP terminated. Conclusion: the results suggested that Lamivudine 5'-monophosphate was incorporated into the newly synthesized DNA and terminated transcription at that point. Lamivudine might, in part, inhibit HIV replication by activity at the triphosphate level and by chain termination of reverse transcription.

11. Steady state kinetic studies confirm that Lamivudine 5'-triphosphate exerts its anti-HIV activity primarily by chain termination, Batch # C1460/209/2, December 1992, (GVR/92/013)

In study-state kinetic studies using a primer extension/chain termination assay, it was determined whether HIV-1 reverse transcriptase catalyzed the incorporation of Lamivudine 5'-monophosphate (using Lamivudine 5'-triphosphate as the substrate) into a DNA primer annealed to a RNA template. Results: the efficiency of Lamivudine 5'-triphosphate as substrate for HIV-1 reverse transcriptase was less by greater than 2 orders of magnitude for the enzyme when compared with AZTTP. Conclusion: the study provided evidence that Lamivudine exerted a major proportion of its antiviral effect by chain termination of the elongated viral genome and not as a competitive inhibitor.

12. The incorporation of Lamivudine 5'-monophosphate into DNA by HIV-1 reverse transcriptase and human DNA polymerase, Batch # C1653/21/1, 1994, (GVR/94/007)

The effect of Lamivudine 5'-triphosphate on HIV-1 reverse transcriptase (RNA dependent DNA polymerase activity) and human DNA polymerase gama was investigated. Results: Lamivudine 5'-triphosphate was approximately 33-fold less effective as a substrate for DNA dependent DNA polymerase than RNA dependent DNA polymerase activities. Lamivudine 5'-triphosphate was a substrate for human DNA polymerase gama (DNA dependent DNA polymerase activity). The product of this reaction (with Lamivudine 5'-monophosphate incorporated at the 3' end) was also a substrate for the 3'-5' exonuclease of DNA polymerase gamma.

Comments: The results indicated that although Lamivudine 5'-triphosphate was a substrate for the DNA dependent DNA polymerase activity of human DNA polymerase gamma, the product of this reaction was also a substrate for the 3'-5' exonuclease activity and therefore may explain the lack of mitochondrial toxicity exhibited by Lamivudine (GVR/91/017).

13. Effect of Lamivudine on deoxynucleotide pools in cultured U937 cells, Batch # C1013/75/1, December 1991, (GVR/91/027)

The effect of Lamivudine upon deoxynucleotide pools in U937 cells (a human monocyte-like cell line) was investigated. Results: Lamivudine had no effect upon the level of any the four natural deoxynucleoside triphosphates an any concentration of the test compound. In contrast, 200 μM AZT reduced dGTP levels and increased dCTP levels. Conclusion: this study provided no indication that Lamivudine interfered with normal deoxynucleotide metabolism.

14. Effect on mitochondrial DNA content, Batch # C1013/75/1, June 1991, (GVR/91/017)

This study compared the effects of Lamivudine, AZT, ddC and ddI upon mitochondrial DNA synthesis in CEM cells obtained from ATCC, Rockville, MD. Results: relative to nuclear DNA content, Lamivudine had no effect upon mitochondrial DNA up to 500 μM concentration for up to 8 days. In contrast, AZT, ddC and to a lesser extent, ddI, all reduced mitochondrial DNA content. ddC had the most potent effect upon levels of mitochondrial DNA which fell by approximately 50% after 4 days at concentrations greater than 50 μM.

15. Effect of Lamivudine triphosphate on mammalian DNA polymerases, Batch # C1460/209/2, June 1991, (GVR/91/010)

The effect of Lamivudine 5'-triphosphate on DNA polymerases: α, ß and gamma from HeLa Ohio cells was investigated. At [dCTP)s = Km, IC50 values (the concentration giving 50% inhibition) were determined. Results: the test compound produced mean IC50 values of 175, 24.8 and 43.8 μM for α, ß and gamma polymerases, respectively. In contrast, ddCTP and ddATP are potent inhibitors of DNA polymerases gamma (IC50 values = 0.027-0.07 μM) and ß (IC50 values are between 0.5 and 0.8 μM). Conclusion: Lamivudine triphosphate was significantly less inhibitory towards DNA polymerases gamma and ß than ddATP or ddCTP. It was, however, more inhibitory to these enzymes than AZTTP. None of the tested compounds was potent inhibitor of α polymerase.

CONCLUSIONS

There are no nonclinical pharmacology and toxicology issues which would preclude the approval of this Pre-NDA. The sponsor submitted protocols, which have been approved by the ECAC, to initiate the two-year carcinogenicity studies in mice and rats.

With the submission of the NDA, the doses used in the animal toxicity studies will be equated to the doses in humans. The issue of labelling will be addressed when the NDA is submitted and the review of the labelling appended to this review.

Pritam S. Verma, Ph.D.
Reviewing Pharmacologist

PHARMACOLOGIST'S REVIEW

NDA 20-596
Date Submitted: July 3, 1995
Date Assigned: July 7, 1995
Date Completed: July 31, 1995
Assigned Reviewer: Pritam S. Verma, Ph.D.

SPONSOR: Glaxo Wellcome, Inc.
Five Moore Drive
Research Triangle Park, NC 27709

DRUG: Lamivudine

Chemical Names:

A: (2R-cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5yl)-(1H)-pyrimidin-2-one

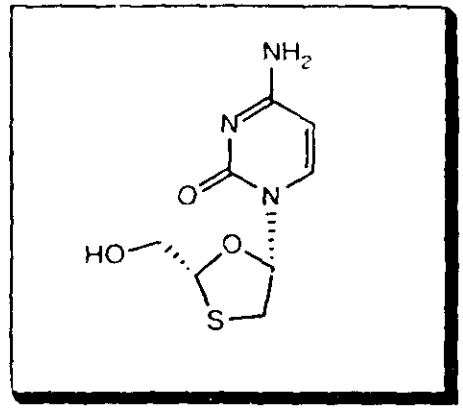
B: (2R-cis)-4-amino-1-[2-hydroxy methyl)-1,3-oxathiolan-5-yl]-2(1H)-pyrimidinone

Code Name: GR109714X

Other Name: 3TC™

Molecular Formula: $C_8H_{11}N_3O_3S$

Molecular Weight: 229.3



pKa: 4.30 (protonation of NH_2)

Solubility: In water at 20°C about 70 mg/ml

Description: White to off-white crystalline solid

FORMULATIONs: 3TC™ Tablets are for oral administration. Each tablet contains 150 mg of lamivudine and the inactive ingredients microcrystalline cellulose, sodium starch glycolate and magnesium stearate. Opadry YS-1-7706-G White is the coloring agent in the tablet coating.

3TC™ Oral Solution is for oral administration. One milliliter (1 ml) of the solution contains 10 mg of lamivudine (10 mg/ml) in an aqueous solution and the inactive ingredients artificial strawberry and banana flavors, citric acid (anhydrous), edetate disodium, ethanol (6% v/v), methylparaben, propylene glycol, propylparaben and sucrose.

RELATED INDs and NDAs: IND nd
NDA 20-564

INDICATION: Treatment of HIV infection in selected patients

INTRODUCTION

GR109714X (3TC™; Lamivudine), the (-) enantiomer of 4-amino-1-[2-hydroxymethyl)-1, 3-oxathiolan-5-yl]-(1H)-2-pyrimidinone is a novel dideoxynucleoside analog developed as a potential treatment for individuals infected with HIV. Early research and development was carried out using the racemate, GR103665X. However, Lamivudine was found to have a more favorable cellular toxicity profile with an equivalent antiviral activity when compared with the racemate. The sugar ring of lamivudine is novel in that it contains a sulfur at the 3' position as a second heteroatom. The sponsor has submitted NDA 20-596 which describes the development of 3TC Tablets and 3TC Oral Solution for the treatment of selected patients with HIV infection. The NONCLINICAL PHARMACOLOGY AND TOXICOLOGY TECHNICAL SECTION of the NDA has been reviewed previously and is incorporated here as an appendix # 1 by reference from NDA 20-564. Presently, the sponsor has submitted four additional reports of toxicology studies in NDA 20-564. These additional studies were specifically designed and conducted to characterize the toxicity profile of novel degradation products of lamivudine that were recently observed in the aqueous solution product (IND Serial No. 179).

BACKGROUND

Lamivudine has been shown to be metabolized intracellularly to its 5'-triphosphate which has a half-life of approximately 10.5 to 15.5 hours. It is proposed that, because of the long intracellular half-life, twice daily dosing in humans will allow a constant level of the triphosphate to be maintained intracellularly. The triphosphate has been shown to inhibit HIV-reverse transcriptase and act as a chain terminator upon incorporation into DNA. It is surmised that Lamivudine has a common mechanism of action with AZT, ddC and ddI in that Lamivudine is phosphorylated to its 5'-triphosphate derivative which inhibits reverse transcriptase by competing with the natural nucleotide triphosphates for binding and/or acts as an alternative substrate for reverse transcriptase leading to termination of the viral DNA chain.

For the viewpoint of safety, conceptually, a DNA chain terminator should show little or no effect on mammalian enzymes at the

concentrations that inhibit the viral enzyme within cells. In this context, this compound is found to be a weak inhibitor of mammalian DNA polymerases: α, ß and γ. DNA polymerase α is though to be involved in semiconservative DNA replication; polymerase ß is involved in DNA repair; and γ polymerase is ultimately responsible for the normal functioning of mitochondria. Mitochondrial damage has been linked to peripheral neuropathy. Thus, Lamivudine may have the potential to cause peripheral neuropathy in man, although it is speculated by the sponsor that the potential is less than the other dideoxynucleoside analogues which are currently in clinical use.

NON-CLINICAL TOXICOLOGY

Toxicity Studies Summary: The following studies were conducted in accordance with the FDA Good Laboratory Practices Regulations.

1. **Lamivudine (spiked with degradation products GI166910X and GI229457X): Acute Oral Toxicity Study in the B6C3F1 Mouse, Batch # UFP1017, C2551/258/4 and U4669/3/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., May 15, 1995, (M20635/WPT/94/264)**

2. **Lamivudine: 29 Day Oral Toxicity Study in the Rat to Assess the Effect of Degradation Products GI166910X and GI229457X), Batch # UFP1020, C2514/284/1 and U4681/38/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., May 23, 1995, (R20744/WPT/95/072)**

3. **Lamivudine: Mammalian cell mutation test at the thymidine kinase locus in mouse lymphoma L5178Y cells: Comparison of activity of lamivudine alone with a sample of lamivudine spiked with two breakdown products (GI166910X and GI229457X) identified in the 10 mg/ml oral solution for pediatric use, Batch # UFP1017, C2551/258/4 and U4669/3/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., May 16, 1995, (Study No. V20642/WPT/94/399)**

4. **Lamivudine: 29 Day Oral Toxicity Study in the Rat to Assess the Effect of Degradation Products GI166910X and GI229457X): Appendix 1. Toxicological evaluation, (R20744/WPT/95/072)**

Study #4 has been reviewed as a toxicologic evaluation section of Study #2; hence, it will not be reviewed.

Toxicity Studies Review:

1. Lamivudine (spiked with degradation products GI166910X and GI229457X): Acute Oral Toxicity Study in the B6C3F1 Mouse, Batch # UFP1017, C2551/258/4 and U4669/3/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., May 15, 1995, (M20635/WPT/94/264)

Three groups of male and female mice (10 animals/sex/group) received two oral doses [approximately 12 hr apart] of either 2000 mg/kg of lamivudine alone or 2000 mg/kg of lamivudine spiked with 80 mg/kg of GI166910X (4% w/w) and 30 mg/kg of GI229457X (1.5% w/w), or vehicle to assess the acute toxicity of lamivudine together with two of its above mentioned degradation products. Results: there were no deaths. There were no clinical observations related to treatment with either lamivudine alone or lamivudine spiked with the two degradation products. On day 2 and 14, scabs were noted on the abdomen for a few males from all treated groups including controls (day 2 only); these were occasionally associated with hair loss on day 14 only. On day 14, hair loss from the head was present for all control females. These findings were considered to be incidental observations for this strain of mouse. On day 7, body weight was statistically significantly higher ($p=0.05$) for females treated with lamivudine alone compared to females treated with lamivudine spiked with the two degradation products. Body weight for the females treated with lamivudine spiked with the two degradation products was similar to vehicle controls. Histopathology: chronic dermatitis, characterized variously by skin ulceration, epithelial hyperplasia and hyperkeratosis was present in males noted to have scabs at autopsy. The severity of splenic extramedullary hematopoiesis was increased in males killed on day 14 treated with lamivudine alone or spiked with the degradation products. There was a strong correlation between the occurrence of scab formation and increased severity of splenic hematopoiesis. Conclusion: the acute toxicity of lamivudine in the mouse was unaffected when it was administered in combination with two of its degradation products, GI166910X (4% w/w) and GI229457X (1.5% w/w).

2. Lamivudine: 29 Day Oral Toxicity Study in the Rat to Assess the Effect of Degradation Products GI166910X and GI229457X), Batch # UFP1020, C2514/284/1 and U4681/38/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., May 23, 1995, (R20744/WPT/95/072)

Four groups of male and female AHA rats (12-20 animals/sex/group) received via gavage twice daily (approximately 12 hr apart) oral doses of vehicle or lamivudine (spiked with the degradation products of lamivudine: GI166910X and GI229457X) for 29-32 consecutive days to investigate whether the presence of the degradation products affects the toxicity of lamivudine according to a study design and dosages in Table 1. On the last day of the

study, 12 animals/sex/group were killed for post mortem studies; the remaining animals (control and high) were maintained after the treatment period for 30/31 day to study the regression/progression of an abnormalities. Results: one female (control) was found dead on day 21. All other animals survived until schedules autopsy. Clinical Observations: there were no signs that were considered to have arisen from the treatment. Body Weight and Food Consumption: mean body weight of male rats in all treated groups was slightly but significantly decreased ($p=0.05$) compared to the control on day 7 and for males (low) throughout the remainder of the treatment period. Mean food consumption was slightly but statistically significantly higher than control for males (high) between days 7-28 and for females between days 17-21.

Table 1
Study design and dosages

Group Name	Dosage (mg/kg/bid)		
	lamivudine	GI166910X (0.7% w/w)	GI229457X (0.18% w/w)
Control	0	0	0
Low	45	0.32	0.08
Mid	300	2.1	0.54
High	2000	14	3.6

Hematology: statistically significant changes in red blood cell parameters, compared to control, were noted in both sexes (high) and in females (low and mid). These changes comprised reductions in hematocrit (females only), erythrocyte and platelet counts and elevations in mean cell volume, mean cell hemoglobin and mean cell hemoglobin concentrations (female only). They were generally apparent in females by day 21 but with the exception of platelet count were not seen in males until day 43R (following a 14 day recovery period). Changes in erythrocyte count and mean cell hemoglobin in both sexes were still present on day 53R but remained slight and generally showed signs of regression. Clinical Chemistry: a statistically significant elevation in serum triglycerides compared to control was noted in all males (mid and high) on days 9 and 21. The effect persisted in males and appeared for the first time in females (high) on day 43R but was not noted in either sex on day 53R. This finding has not been noted in previous studies with lamivudine and therefore may have been influenced by the presence of the degradation products. Changes in one or more urinary parameters were noted during the

treatment period and consisted of a marginal elevation in urinary pH and, statistically significant (p=0.05) elevation in urinary sodium and potassium and a reduction in urinary total protein. These changes were noted in males (high) on day 24 (with the exception of a marginal elevation in urinary pH in females at this dosage) and were not present during the recovery period. Organ Weights: when using terminal body weight as covariate, statistically significant reductions (p=0.05) compared to control were noted (high) for lung weight (both sexes) and liver and kidney weights (male). Thymus weight was reduced in all treated females. When using initial body weight as covariate, statistically significant reductions compared to controls were noted for kidney weight in all males treated group, thymus weight in all female treated groups and heart weight in males (high). At the end of the recovery period compared to the control animals, liver weight was reduced in males (high) when initial body weight was used as the covariate. Toxicokinetic Evaluation: is summarized in Table 2. Plasma level data from both male and female treated animals showed an approximately proportional increase in Cmax and AUC.

Table 2
Mean values (in terms of lamivudine) of toxicokinetic parameters

Lamivudine Parameters	Dosage of lamivudine (mg/kg/bid)					
	45		300		2000	
	Day 1	Day 28	Day 0	Day 28	Day 0	Day 28
♂: Tmax (hr)	1	2	1	2	2	2
Cmax (µg/ml)	2.6	2.3	24.2	17.6	90.9	88.3
AUC (µg*hr/ml)	9	14	93	88	524	669
♀: Tmax (hr)	2	1	2	2	1	1
Cmax (µg/ml)	3	3.6	24.4	19.8	80	86.2
AUC (µg*hr/ml)	11	17	112	118	570	684

Gross Pathology: one male (high) showed caecal distension with fluid contents. Histopathology: treatment-related, very slight, diffuse mucosal hyperplasia of the caecum characterized by variable degrees of hyperplastic glands with reduced numbers of mucous cells and increased mucosal height with basophilia and nuclear crowding, was present at the end of the treatment period in 10/12 males and 5/12 females (high). The other microscopic finding comprised very slight focal erosion of the gastric antrum and was present in a few animals from both treated and control

groups. This finding was not considered treatment-related. In the recovery group, there were no treatment related microscopic findings noted in the caecum or stomach.

Comments: The presence of the degradation products GI169910X (0.7) and GI229457X (0.18%) appeared to alter the toxicity profiles of lamivudine slightly. Those not previously associated with lamivudine treatment included increased serum triglycerides and changes in urinary pH and protein but these were not associated with any apparent histological findings. The sponsor concluded that the maximum no (toxicological) effect level in the study was 300 mg/kg twice a day. However, considerable changes were noted at this level. A dosage of 45 mg/kg/bid may be considered a NOAEL. On the basis of body surface area conversion, and equivalent dose of lamivudine in humans would be 6.42 mg/kg/bid.

3. Lamivudine: Mammalian cell mutation test at the thymidine kinase locus in mouse lymphoma L5178Y cells: Comparison of activity of lamivudine alone with a sample of lamivudine spiked with two breakdown products (GI166910X and GI229457X) identified in the 10 mg/ml oral solution for pediatric use, Batch # UFP1017, C2551/258/4 and U4669/3/1, Glaxo Group Research Ltd, Ware, Hertfordshire, U.K., May 16, 1995, (Study No. V20642/WPT/94/399)

Both lamivudine alone and lamivudine spiked with its degradation products: GI166910X (4% w/w) and GI229457X (1.5% w/w) were tested at doses of 0, 1000, 1500, 2000 or 2500 µg/ml in the absence of S-9 activation in the mouse L5178Y cell line mutagenicity test. Results: both lamivudine and lamivudine-spiked were weakly mutagenic at the TK locus of mouse lymphoma cells in the absence of rat liver S9-mix. There was no significant difference in mutant frequency between the two regimens. Additionally, there was no evidence that the lamivudine-spiked increased the cytotoxicity of the test material. Conclusion: these data indicated that the presence of the two breakdown products [GI166910X (4% w/w) and GI229457X (1.5% w/w)] in a lamivudine sample did not exacerbate the toxicity or mutagenicity of the test material.

DRAFT LABELLING AND LABELS

A suggested redraft of the label is given in Appendix # 7.

APPENDICES

Seven appendices are attached. These are listed below.

1. **Review of NDA # 20-564 dated 3/16/95.**
2. **Summary of animal toxicity studies.**
3. **Summary of animal pharmacokinetic studies.**
4. **Summary of human pharmacokinetic studies.**
5. **Comparison of animal doses with human doses.**
6. **Comparison of rat, dog and human ADME parameters.**
7. **A suggested redraft of the label.**

CONCLUSIONS

Acute toxicity studies: lamivudine has a low acute toxicity via the oral or iv routes in both mice and rats. The acute oral or iv administration of lamivudine (4000 mg/kg) was adequately tolerated by both mice and rats and was not associated with any target organ toxicity.

Chronic toxicity studies: lamivudine was adequately tolerated in the rat at doses up to 2000 mg/kg/bid for 6 months. Treatment-related effects included minor hematological (mainly red blood cell parameters), clinical chemistry and urinalysis changes, and mucosal hyperplasia of the caecum. A dose of 450 mg/kg/day was identified as a NOEL. Based on the drug exposure comparisons, a dose of 16.9 mg/kg/day would be considered an equivalent dose in humans. In dogs, doses of 1500/kg/bid in males and 1000 mg/kg/bid in females for a period of 12 months were adequately tolerated. Treatment-related changes included reductions in red cell counts at all dose levels, associated with increased MCV and MCH, and reductions in total leucocyte, neutrophil and lymphocyte counts in high dose animals, but with no effect on bone marrow cytology. A dose of 45 mg/kg/day was identified as a NOEL. Based on the drug exposure comparisons, a dose of 9.5 mg/kg/day would be considered an equivalent dose in humans.

ADME studies in rat, dog and man: following iv administration to the rat, lamivudine showed a bi-exponential elimination with dominant half-life of about 20 min. Lamivudine was cleared almost entirely by renal elimination in rats. The value for renal clearance (3.3 ml/min) exceeded the GFR in rats and thus tubular secretion must play a significant role in the elimination of lamivudine in this species. Lamivudine was rapidly and extensively absorbed following an oral administration as evidenced by a Tmax of about 1 hr and oral bioavailability of about 60% in the rat and 80% in the dog. The oral bioavailability of lamivudine in man was approximately 80%. Data from studies with radiolabelled lamivudine in dog following an oral or iv

administration showed that lamivudine accounted for 40% of the plasma radiolabelled drug due to the presence of circulating metabolites. Following an oral administration to the dog, approximately 97% of a radiolabelled dose of lamivudine was recovered in the urine The bioavailability of lamivudine in the dog was limited by metabolism rather than absorption. This was in contrast to the rat where about 35% of an oral dose was recovered in feces as unchanged lamivudine suggesting that in this species the bioavailability was limited by incomplete absorption.

Following the oral or iv administration of radiolabelled lamivudine to the rat, the majority of the radiolabelled drug was excreted in the urine. Thus, approximately 90% of the iv dose and 60% of the oral dose was excreted in the urine in the first 24 hr. The unchanged drug accounted for up to 96% of the urinary excreted radioactivity for both the routes of administration. Urinary excretion was also predominant following the oral or iv administration of lamivudine to dogs, with up to 99% of a radiolabelled dose excreted in the urine in the first 24 hr. In contrast to the rat, metabolic clearance played a significant role in the clearance of lamivudine in the dog.

Studies performed in man showed that the renal clearance of unchanged drug was the predominant mechanism of clearance for lamivudine. Approximately 85% of an iv dose and 70% of an oral dose was excreted as unchanged drug in the urine. The trans-sulfoxide metabolite of lamivudine was identified in urine samples from patients following the repeated oral administration, accounting for about 5% of the administered dose. Lamivudine did not undergo extensive first-pass metabolism. The absolute bioavailability of the compound is likely to be same as the amount absorbed. Thus, about 90% of the orally absorbed drug was accounted for in the urine as either parent or the trans-sulfoxide of lamivudine. The remaining portion of the oral dose was likely to be material unabsorbed from the gastrointestinal tract. In conclusion, the excretion and metabolic profile of lamivudine in man was more like that seen in the rat than in dog.

Comparison of doses from the rat and dog toxicity studies to the therapeutic dose in humans: based on the body surface area equivalence and drug exposure values (AUCs), the NOELs from the rat and dog toxicity studies were compared with the human therapeutic dose [approx. 4.0 mg/kg/day; AUC=7.8 μg*kg/ml]. For instance, in the 6-month rat and 12-month dog toxicity studies, the NOELs were 425 mg/kg/bid (AUC = 132 μg*hr/ml) and 45 mg/kg/bid (AUC = 74.5 μg*hr/ml), respectively. Thus on the basis of the drug exposure comparisons from the rat and dog NOELs, equivalent doses in humans would be 16.9 mg/kg/day and 9.5 mg/kg/day, respectively.

There are no nonclinical pharmacology and toxicology issues which

would preclude the approval of this NDA. The sponsor submitted protocols, which have been approved by the ECAC, to initiate the two-year carcinogenicity studies in mice and rats and will be completed as a phase 4 commitment.

Pritam S. Verma, Ph.D.
Reviewing Pharmacologist

Concurrences:

HFD-530/DFreeman 8/21/95
HFD-530/JFarrelly 8/17/95
HFD-530/PVerma 8/16/95

Disk:
HFD-530/JFarrelly

cc

HFD-530/NDA 20-596
HFD 340
HFD-530/PVerma
HFD-530/HJolson
HFD-530/SMiller
HFD-530/NBattula
[redacted]
HFD-345/GJames

Appendix # 1

See review NDA 20-564

Appendix # 2

Summary of animal toxicity studies.

Table 1
Summary of acute/subacute toxicity studies

Species (strain)	Mode of administration	Approximate LD^{50} (mg/kg)	Approx. NOAEL (mg/kg/day)
Mice (B6C3F1) acute	iv	>2000	-
	oral	>2000	-
Rats (AHA) acute	iv	>2000	-
	oral	>2000	-
Mice 13-week	oral	-	600
Rats 5-day	oral	-	3000
Rats 5-week	oral	-	600

Table 2
Summary of subchronic/chronic toxicity studies in rats and dogs

Study	Dose level (mg/kg/bid)	Laboratory findings	Post mortem findings, NOELs & equivalent doses in man
Rat 1 month	45	statistically significant (p=0.05) increases in cholesterol & ALP; reduction in ALT, creatinine, albumin & amylase (high)	statistically significant (p=0.05) relative weight reduction of lung, liver, brain and spleen (high)
	300		
	2000		
Rat 3 month	45	the degree of severity increased compared to 1 month study plus statistically significant changes in urinalysis parameters (high)	microscopic changes consisted of slightly increased incidence of renal tubular dilation in males (high)
	300		
	2000		
Rat 6 month	90	a number of hematological, chemistry & urine parameters showed significant (p=0.05) changes (high)	a diffuse mucosal hyperplasia; diffuse subepithelial eosinophilic material in caecum (high). NOEL= 425 mg/kg/day; Based on AUC = 17 mg/kg/day in humans
	425		
	2000		
Dog 3 month	45	decreased erythrocytes (mid & high); increased ALT, creatinine, CK & AST (mid & high)	absolute kidney weight increased (high); hypoplasia and focal pleural adhesion of the lung (high)
	260		
	1500		
Dog 12 month	45	statistically significant changes occurred in chemistry, hematology & urine parameters (mid & high)	Minimal hemosiderosis was observed in 2/4 (♂ high); focal subcapsular/portal fibrosis in liver of one high dose male. NOEL = 45 mg/kg/day; Base on AUC= 9.5 mg/kg/day in humans
	260		
	1000 (♀)		
	1500 (♂)		

Appendix # 3

Summary of animal pharmacokinetic studies.

Table 1

Mean pharmacokinetic parameters of single iv and oral dose of lamivudine in rats

Parameters	Intravenous dose (45 mg/kg)	Oral dose (45 mg/kg)
AUC, (µg*hr/ml)	25.5	17.6
$t_{\frac{1}{2}\beta}$ (hr)	1.2	-
Plasma clearance (ml/hr)	4.5	-
Renal clearance (ml/hr)**	3.7	-
Volume of distribution (ml)$	449	-
Tmax (hr)	-	1.5
Cmax (µg/ml)	72.7	4.5
F (%)	100	69
Urine recovery (%)***	90	-

**This value was higher than the maximum glomerular filtration rate for lamivudine indicating the importance of active tubular secretion in the elimination of this compound.

***Unchanged drug accounted for approximately 96% of the urinary recovery for both the iv and oral routes of administration.

$The apparent volume of distribution suggested greater distribution than could be attributed to total body water in rats.

Table 2

Mean pharmacokinetic parameters of single oral or iv dose of lamivudine in dog

Parameters	Intravenous dose (30 mg/kg)	Oral dose (30 mg/kg)
AUC, (µg*hr/ml)	74.4	61.6
$t_{\frac{1}{2}\beta}$ (hr)	1.7	1.6
Plasma clearance (l/hr)	5.2	5.6
Renal clearance (l/hr)**	2.5	2.1
Volume of distribution (l)$	12.4	12.6
Tmax (hr)	-	0.67
Cmax (µg/ml)	-	26.6
F (%)	100	83
Urinary recovery (%)	92-102	82-91

**The renal and metabolic clearance are approximately equal importance in dog. The data suggest that tubular secretion does not play a significant role in the elimination of lamivudine.

$The volume of distribution was equivalent to total body water in dogs.

Table 3

Kinetic data from subchronic\chronic toxicity studies in rats and dogs

Dose (mg/kg/day)	Dose level (mg/kg/bid)	C_{max} (µg/ml)		AUC (µg*hr/ml)	
		1st sample	2nd sample	1st sample	2nd sample
Rat 1 month	45	3.3	4.1	13.7	22.7
	300	23.5	30	92.7	119
	2000	111	117	530	590
Rat 3 month	45	5	4.7	17.9	20.9
	300	20	30.9	72.9	145
	2000	90.6	94.6	486	690
Rat 6 month	90	9	11.8	37.5	69.3
	425	38.8	31.7	132	133
	2000	115	113	507	624
Dog 3 month	45	16.2	21.5	43.9	66.2
	260	69	89.8	220	367
	1500	164	331	825	1582
Dog 12 month	45	12.3	22.9	41.2	74.5
	260	53.8	85.9	179	343
	1000 ♀	134	213	417	872
	1500 ♂	149	216	435	948

Approximate Cmax values were either 1 hr or 2 hr post dose.

For the rat 1 month study the first sample was collected after the second dose on day 1 and the second sample is after the second dose during the final week of dosing. For the other studies, the first sample was collected after the first dose on day 1 and the second sample was after the first dose during the final week of dosing.

Appendix # 4

Summary of human pharmacokinetic studies.

Table 1

Mean pharmacokinetic parameter of four different formulations of lamivudine administered as a single 100 mg dose in 14 patients

Treatment	AUC_∞ (µg*h/ml)	Cmax (µg/ml)	Tmax (h)	t½ (h)	F (%)
IV	4.4	1.7	-	8.3	-
Oral solution	3.8	1.0	0.9	8.3	87
Oral capsule	3.8	1.0	0.8	8.4	88
Oral tablet	3.7	1.0	0.9	8.3	86

Table 2

Mean pharmacokinetic parameter of three different regimens of lamivudine oral tablet administered as a single dose in 20 patients

Treatment	AUC_∞ (µg*h/ml)	Cmax (µg/ml)	Tmax (h)	t½ (h)
A: 4*75 mg tablet	12.1	2.9	0.9	5.4
B: 3*100 mg tablet	11.7	2.8	1.2	5.1
C: 1*300 mg tablet	11.8	3.0	0.9	5.3

Table 3

Mean pharmacokinetic parameter of single iv doses of lamivudine in 18 patients.

IV doses (mg/kg/day)	AUC_∞ (µg*h/ml)	Cmax (µg/ml)	t½ (h)	Cl (l/hr/kg)	Vdss (l/kg)
1.0	1.3	0.6	1.4	0.3	0.9
2.0	1.7	1.2	1.6	0.5	1.1
4.0	3.9	2.1	1.9	0.5	1.1
8.0	7.9	3.7	1.6	0.5	1.2
12.0	10.6	4.9	1.5	0.5	1.2
20.0	17.8	8.1	1.4	0.5	1.2

Table 4

Mean pharmacokinetic parameter of single oral doses of lamivudine in 18 patients.

Oral doses (mg/kg/day)	AUC_∞ (µg*h/ml)	Cmax (µg/ml)	t½ (h)	Cl (l/hr/kg)
1.0	0.5	0.1	1.9	0.8
2.0	1.4	0.4	2.1	0.6
4.0	2.3	0.6	2.3	0.8
8.0	4.4	1.0	2.0	0.9
12.0	6.7	1.8	1.9	0.8
20.0	12.7	4.0	1.7	0.8

Table 5

Mean pharmacokinetic parameter of repeat oral doses of lamivudine in 104 patients.

Study days	Day 1			Day 15		
Doses (mg/kg/day)	AUC_∞ (µg*h/ml)	Cmax (µg/ml)	t½ (h)	AUC_∞ (µg*h/ml)	Cmax (µg/ml)	t½ (h)
0.5	0.5	0.1	2.3	0.6	0.2	3.5
1.0	0.9	0.3	2.6	1.1	0.3	3.0
2.0	1.9	0.6	2.2	3.3	0.9	3.3
4.0	3.5	1.1	2.1	4.6	1.4	3.2
8.0	8.8	3.0	2.1	9.4	2.7	2.7
12.0	12.7	3.9	2.4	14.6	3.7	3.5
20.0	22.5	7.2	2.5	22.8	6.8	2.6

Appendix # 5

Comparison of animal doses with the human therapeutic dose.

Table 1

Comparison of kinetic data from subchronic/chronic rat and dog toxicity studies with the human therapeutic dose [approx. 4.0 mg/kg/day; AUC=7.8 μg*kg/ml]

Study	Dose level (mg/kg/bid)	Cmax (μg/ml)	AUC (μg*hr/ml)	NOEL	Body surface area (BSA & AUC) equivalent dose in man
Rat 1 month	45	3.3	22.7	45 mg/kg/bid	BSA = 12.8 mg/kg/day AUC = 3 mg/kg/day
	300	23.5	119		
	2000	111	590		
Rat 3 month	45	5	20.9	300 mg/kg/bid	BSA = 42.8 mg/kg/day AUC = 18.6 mg/kg/day
	300	20	145		
	2000	90.6	690		
Rat 6 month	90	9	69.3	425 mg/kg/bid	BSA = 60.7 mg/kg/day AUC = 17 mg/kg/day
	425	38.8	133		
	2000	115	624		
Dog 3 month	45	16.2	66.2	45 mg/kg/bid	BSA = 45 mg/kg/day AUC = 8.5 mg/kg/day
	260	69	367		
	1500	164	1582		
Dog 12 month	45	12.3	74.5	45 mg/kg/bid	BSA = 45 mg/kg/day AUC = 9.5 mg/kg/day
	260	53.8	343		
	1000 (♀)	134	872		
	1500 (♂)	149	948		

The kinetic data from subchronic/chronic toxicity studies in rats and dogs showed that the proposed therapeutic dose of 300 mg/day (approximately 4 mg/kg/day) produced a mean steady-state Cmax of 1.9 μg/ml. The corresponding mean AUC value at this dose was 7.8 μg*hr/ml. Thus, the clinical exposure was considerably lower than that achieved in the toxicity studies. Based on the body surface area equivalence, the proposed clinical dose is also safe to proceed.

Appendix # 6

Comparison of rat, dog and human ADME parameters.

Table 1
Comparison of rat, dog and human ADME parameters

Parameters	Rat	Dog	Human
Tmax (hr)	1.5	0.67	1.5
$t_{\frac{1}{2}\beta}$ (hr)	oral 2.3 ♂; 1.2 ♀	1.7	5-7
Plasma clearance (l/hr)	4 ml/min	oral, 5.6	20-25
Renal clearance (l/hr)**	3.3 ml/min	oral, 2.1	15-20*
Volume of distribution (l)	0.54	12.4, (3.7 l/kg)	1.02-1.47 (l/kg)
Percent in red blood cells	41-47	40-47	54-56
Protein binding (%)	10-49	10-42	10-36
F (%)	60	80	80
Urinary recovery (%)*	60% of oral dose; 96% unchanged drug	99% of oral dose; 80% unchanged drug	90% of oral dose; 60-70% unchanged
Fecal recovery (%)	35% of oral dose unchanged	3% of oral dose	10?
Metabolites	5% of oral dose; GI138870X & trans-sulfoxide in urine	57% of urinary radioactivity; metabolites(MET1=20%; MET2=37%, trans-sulfoxide+cis-sulfoxide)	5% of dose; trans-sulfoxide metabolite in urine

**It was likely that ionic trapping in the acidic urine or an active secretory process was involved in renal elimination as renal clearance was greater than GFR (1.8 ml/min). Renal clearance was predominant mechanism of elimination in the rat, with active tubular secretion playing a significant role. In the dog renal and metabolic clearance were of approximately equal importance. Thus, the pharmacokinetic behavior of lamivudine in man was more like that seen in the rat than in dogs. Therefore, in the toxicological studies, the rat would be an appropriate species for predicting the safety of lamivudine and its metabolites.

Appendix # 7

A suggested revision of the label.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Long-term carcinogenicity studies of lamivudine in animals are in progress, but have not yet been completed. Lamivudine was not active in a microbial mutagenicity screen or an *in vitro* cell transformation assay, but showed weak *in vitro* mutagenic activity in a cytogenetic assay using cultured human lymphocytes and in the mouse lymphoma assay. ~~However,~~ Lamivudine showed no evidence of *in vivo* genotoxic activity in the rat at oral doses of up to 4,000 mg/kg per day (approximately 150 times the recommended human dose based on body surface area comparisons). ~~Reproduction studies have been performed in rats and rabbits at orally administered doses up to 1,000 and 250 times, respectively, the usual adult dose and have revealed no evidence of impaired fertility or harm to the fetus due to lamivudine.~~ **In a study of reproductive performance, lamivudine, administered to rats at doses up to 150 times the recommended adult dose based on body surface area comparisons, revealed no evidence of impaired fertility and no effect on the survival, growth and development to weaning of the offspring.**

Pregnancy: ***Pregnancy Category ~~B~~ C:*** Reproduction studies have been performed in rats and rabbits at orally administered doses up to ~~1,000 and 250~~ approximately 45 and 2 times, respectively, those determined at the ~~usual~~ recommended adult dose, based on lamivudine AUC measurements. ~~and have revealed no evidence of impaired fertility or harm to the fetus due to lamivudine~~. Some evidence of early embryolethality was seen in the rabbit at ~~doses~~ exposures similar to those produced by ~~of 10 times~~ the ~~usual~~ recommended adult dose and higher, but there was no indication of this effect in the rat at ~~doses~~ exposures up to ~~1,000~~ 45 times those at the ~~usual~~ recommended adult dose. Some delays in development were seen in rats at exposures above 4 times those seen at the recommended adult dose. Studies in pregnant rats and rabbits showed that lamivudine is transferred to the fetus through the placenta. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, ~~this drug~~ lamivudine should be used during pregnancy only if ~~clearly needed~~ the potential benefit justifies the potential risk to the fetus.

OVERDOSAGE: There is no known antidote for 3TC™. One case of acute overdose in an adult ingesting 6 g of 3TC was reported; there were no clinical signs or symptoms noted and hematologic tests remained normal. One other adult patient errantly ingested 3TC 1,200 mg per day plus Retrovir (zidovudine) 1,200 mg per day for approximately 2 weeks; he had a Grade 3 decrease in absolute neutrophil count that resolved upon reduction of doses of 3TC and Retrovir. In Phase I studies, 3TC was administered at doses up to 20 mg/kg per day (i.e., approximately five times the usual recommended dose in adults) without serious consequences. Lamivudine has very low acute toxicity in laboratory animals. The maximum tolerated dose in the mouse and rat is in excess of 4,000 mg/kg per day by the oral route (approximately 75 and 150 times the exposure due to the recommended human dose based on body surface area comparisons ~~1,000 times the usual adult human dose~~). It is not known whether lamivudine can be removed by peritoneal dialysis or hemodialysis.

DIVISION OF ANTIVIRAL DRUG PRODUCTS

Review of Chemistry, Manufacturing and Controls

NDA #: **20-564**

CHEMISTRY REVIEW #: 1 **DATE REVIEWED:** 14-Nov-95

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	23-May-95	24-May-95	25-May-95
Amendment (NC)	8-Aug-95	10-Aug-95	14-Sep-95
Amendment (NC)	10-Aug-95	11-Aug-95	22-Aug-95
Amendment (BC)	8-Sep-95	11-Sep-95	14-Sep-95
Amendment (BC)	28-Sep-95	29-Sep-95	12-Oct-95
Amendment (BC)	2-Oct-95	3-Oct-95	12-Oct-95
Amendment (BL)	12-Oct-95	13-Oct-95	7-Nov-95
Amendment (BC)	13-Oct-95	16-Oct-95	13-Nov-95
Amendment	18-Oct-95	NA	NA
Amendment (BC)	20-Oct-95	23-Oct-95	23-Oct-95
Amendment (NC)	2-Nov-95	3-Nov-95	13-Nov-95
Amendment	13-Nov-95	NA	NA

NAME / ADDRESS OF APPLICANT: Glaxo, Inc.
5 Moore Drive
Research Triangle Park, NC 27709

DRUG PRODUCT NAME

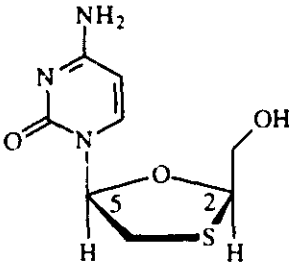
Proprietary: Epivir™ Tablets
Nonproprietary: lamivudine
Code Name/#: GR109714X

PHARMACOLOGICAL CATEGORY: Antiviral
INDICATION: Anti-HIV
DOSAGE FORM/STRENGTH: 150 mg film-coated tablets
ROUTE OF ADMINISTRATION: Oral

CHEMICAL NAME / STRUCTURAL FORMULA:

2(1H)-Pyrimidinone, 4-amino-1-[2-hydroxymethyl-1,3-oxathiolan-5-yl]-(2R-cis)
Registry Number [134678-17-4]

"(-)-3TC"
"(-)-BCH-189"



$C_8H_{11}N_3O_3S$ Formula Weight: 229.26

SUPPORTING DOCUMENTS:

RELATED DOCUMENTS:

Facsimile of 5-Oct-95 (CMC comments on carton/container labels)
Facsimile of 10-Oct-95 (CMC questions regarding drug substance and tablets)
Facsimile of 28-Sep-95 (CMC comments on package insert)
Facsimile of 8-Nov-95 (CMC comments regarding limits and expiry)
11-Aug-95 Teleconference (Stability update and electronic copy of NDA)
17-Oct-95 and 20-Oct-95 Teleconferences (Cameo container)
29-Sep-95 Teleconference (CMC comments on package insert)
Chemistry Reviews of DMF
Chemistry Review of NDA 20-596 (lamivudine oral solution)
Chemistry Reviews of IND

CONSULT REVIEWS:

Reviews of proprietary names by CDER Labeling and Nomenclature Committee:
11-Jul-95, 3TC™
7-Sep-95, Epivir™
Environmental Assessment reviewed by HFD-004.
Product specific inspection of Ulverston UK, Montrose UK, Zebulon NC and Research Triangle Park NC manufacturing and quality control sites.

REMARKS / COMMENTS:

DRUG SUBSTANCE: ***Satisfactory***

Lamivudine is a nucleoside analog which is related to the unnatural enantiomer of deoxycytidine. Intracellularly it is converted to the triphosphate which acts as a chain terminator of HIV reverse transcription. The unusual chirality of lamivudine may be responsible for its low toxicity relative to other nucleoside analogs. In clinical trials, the combination of lamivudine and zidovudine results in delayed acquisition of zidovudine resistance (and reversal of pre-existing zidovudine resistance in some patients).

Lamivudine is a non-hygroscopic white solid with a melting point of It exists in two pseudopolymorphic forms, Form I (partial hydrate) and Form II, both of which have high aqueous solubility (150 and 180 mg/mL at 35°C). Adequate evidence is presented to show that the manufacturing method of the drug substance consistently produces only Form II, and that interconversion does not occur on storage. The proof of structure, including absolute

Reprocessing options for lamivudine and a synthetic intermediate were briefly outlined in the NDA without giving criteria, examples, or details of the procedures. This deficiency was conveyed to the Applicant on 11/8/95. Additional details were then provided in NC 13-Nov-95, and are found satisfactory.

Batch analyses are provided on 14 commercial scale lots of drug substance, either in the original submission or, after request, in the 13-Oct-95 and 13-Nov-95 amendments. Cross references to clinical and stability lots of drug product are also supplied.

Stability studies show no time-dependent changes in appearance, assay or impurity levels. A 48-month retest period had been requested in the NDA, but it is recommended that a retest period of 24 months for bulk lamivudine be granted on the basis of 12 months of primary stability data with 36 months of supportive data.

DRUG PRODUCT: ***Satisfactory***

The drug product is a white, film-coated, modified diamond-shaped tablet, engraved GX CJ7 on one side and 150 on the other. The tablet cores are prepared by ordered blending and direct compression. The excipients are microcrystalline cellulose, sodium starch glycolate and magnesium stearate, and the pigmented film coating is Opadry® YS-1-7706-G. All excipients are USP or NF grade with the exception of the Opadry film coating, which is described in DMF All components have a target proportion listed in the batch formula, with a range of ±10% for the inert ingredients. The excipient ranges are considered acceptable because they do not exceed the Level 1 composition limits defined in the Immediate Release Scale-Up and Post Approval Change (SUPAC-IR) Interim Guidance.

The drug product specifications proposed in the NDA include appearance, identity (HPLC or IR), lamivudine content by HPLC, drug-related impurities by HPLC, dissolution and content uniformity. Based upon release data from 6 commercial scale batches, we recommended that the total impurity limit be lowered and, after consulting a member of the Drug Product Committee, we requested that a specification for water content also be added. The sponsor has agreed to these changes (NC 13-Nov-95). Discussions regarding the dissolution media and sampling time points have been held between Chemistry and Biopharmaceutics representatives in this Division, with input from the Applicant (see Biopharmaceutics review of NDA 20-564 and CMC review of IND . The negotiated dissolution parameters (Q= at 30 min in water at rpm) are acceptable.

The container/closure proposed in the NDA is a round 80-mL HDPE bottle (holding 60 tablets) with a child-resistant closure, a polyester coiler, and a heat-sealed, tamper-evident liner. All packaging components are covered by DMFs, with appropriate letters of authorization, and 12 months of stability data are provided on 4 lots of tablets in the proposed container. The Applicant has amended the NDA (20-Oct-95) to request approval of an additional packaging presentation, a "cameo bottle" also holding 60 tablets, but without the

need for a coiler. The cameo bottle is composed of the NDA container/closure with an additional concentric HDPE insert which reduces the effective internal volume to approximately 30 mL. The cameo bottle is judged to be acceptable on the basis of the equivalent construction materials, the fact that the container/closure seal remains unchanged (verification provided in . , and with the support of 3 months of stability data (room temperature and accelerated) on 3 lots of tablets in the cameo container.

The primary stability data consist of 3 months (1 lot of MRt4 tablets) and 12 months (3 lots of Rt4 tablets) at 30°C/60%RH. The supportive data include 18 months at 30°C/50%RH on 2 lots of tablets made from drug substance. All data are for commercial scale batches (400 kg), manufactured on production equipment, and packaged in the NDA container/closure. No time-dependent changes occur during these studies or under accelerated storage conditions. The sponsor has agreed to an expiry period of 18 months (24 months had been requested in the NDA). We support the Applicant's statement that full stability testing of three post-approval batches is not necessary due to the inclusion of data from four production-like batches in the NDA. At our request, the Applicant agrees that a pre-approval supplement will be submitted prior to discontinuing any time points in the commercial stability protocol.

ENVIRONMENTAL ASSESSMENT: ***Satisfactory***
The EA for this new molecular entity was submitted for consulatative review to HFD-005 on 7-Jul-95. This review is complete, and a FONSI (signed by Nancy Sager and Robert Jerussi) was issued on 28-Aug-95.

METHODS VALIDATION: ***Pending***

LABELING: ***Satisfactory***
The original proprietary name, 3TC, was judged by both the CDER Labeling and Nomenclature Committee (L&NC) and the Division to be a source of potential prescription error. The Applicant's second choice, Epivir, was judged to be acceptable by both the L&NC (with reservations) and the Division. At our request, the product name was modified to "Epivir™ Tablets (lamivudine tablets)" so that the dosage form descriptor is included in both the proprietary and established names. On the basis of the stability data which show no degradation at 30°C and 2°C, we requested that the storage statement be changed from "Store at or below 30°C (86°F) in tightly closed bottles" to read "Store between 2° and 30°C (36° and 86°F) in tightly closed bottles". The package insert, as amended, is acceptable.

ESTABLISHMENT INSPECTION: ***Satisfactory***
In the intended commercial process ( ) for manufacture of the drug substance, the first three steps are carried out at Ulverston, UK. The final step (conversion of the salicylate salt to lamivudine) is carried out at Montrose, UK. Because of a planned shut-down of the Ulverston site in Aug/Sep 1995, all UK inspections were scheduled for Jun/Jul 1995. The Ulverston and Montrose bulk drug sites, as well as the Zebulon NC (tablet manufacture) and Research Triangle Park, NC (NDA stability testing) sites have all been determined to have acceptable Pre-Approval Inspection status, with inspection dates between 14-Jun-95 and 26-Jul-95. A final update request was submitted on 8-Nov-95.

CONCLUSIONS & RECOMMENDATIONS:

The NDA submission and accompanying amendments provide adequate information on the chemistry, manufacturing and controls for Epivir™ Tablets (lamivudine tablets). The product specific inspections of the manufacturing facilities and the Environmental Impact Assessment are acceptable. The NDA, as amended, is therefore **approvable** from the chemistry perspective.

Stephen P. Miller 11/16/95
Stephen P. Miller, Ph.D.
Review Chemist

Concurrence:
HFD-530/DFeigal
HFD-530/CChen CNC 11/16/95

cc:

Orig. NDA 20-564	HFD-530/DFeigal	HFD-530/Pharm
Orig. NDA 20-596	HFD-530/CChen	HFD-102/CSO
HFD-530/Div. File	HFD-530/SMiller	
HFD-830/Div. File	HFD-530/MO	
HFD-830/ESheinin	HFD-530/Micro	File: N 20-564\000CNR01.64b

DIVISION OF ANTIVIRAL DRUG PRODUCTS
Review of Chemistry, Manufacturing and Controls

NDA #: 20-596

CHEMISTRY REVIEW #: 1 **DATE REVIEWED:** 14-Nov-95

SUBMISSION TYPE	DOCUMENT DATE	CDER DATE	ASSIGNED DATE
Original	23-May-95	24-May-95	25-May-95
Amendment (BC)	28-Sep-95	29-Sep-95	12-Oct-95
Amendment (BC)	2-Oct-95	3-Oct-95	12-Oct-95
Amendment (BL)	12-Oct-95	13-Oct-95	7-Nov-95
Amendment (NC)	23-Oct-95	NA	NA
Amendment (NC)	2-Nov-95	3-Nov-95	13-Nov-95
Amendment	13-Nov-95	NA	NA

NAME / ADDRESS OF APPLICANT: Glaxo, Inc.
5 Moore Drive
Research Triangle Park, NC 27709

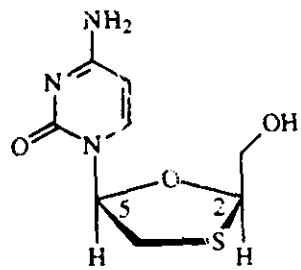
DRUG PRODUCT NAME
Proprietary: EPIVIR™ Oral Solution
Nonproprietary: lamivudine
Code Name/#: GR109714X

PHARMACOLOGICAL CATEGORY: Antiviral
INDICATION: Anti-HIV
DOSAGE FORM/STRENGTH: Oral Solution, 10 mg/mL, 240 mL bottle
ROUTE OF ADMINISTRATION: Oral

CHEMICAL NAME / STRUCTURAL FORMULA:

2(1H)-Pyrimidinone, 4-amino-1-[2-hydroxymethyl-1,3-oxathiolan-5-yl]-(2R-cis)
Registry Number [134678-17-4]

"(-)-3TC"
"(-)-BCH-189"



$C_8H_{11}N_3O_3S$ Formula Weight: 229.26

SUPPORTING DOCUMENTS:
DMF
DMF
DMF
DMF

RELATED DOCUMENTS:
Facsimile of 5-Oct-95 (CMC comments on carton/container labels)
Facsimile of 8-Nov-95 (CMC comments regarding limits and expiry)
17-Oct-95 and 20-Oct-95 Teleconferences (CMC questions on oral solution)
Chemistry Reviews of IND
Chemistry Review of NDA 20-564 (lamivudine 150 mg tablets)

CONSULT REVIEWS:

Trade name reviews by CDER Labeling and Nomenclature Committee.
11-Jul-95, 3TC™
7-Sep-95, Epivir™
Environmental Assessment reviewed by HFD-004.
Product specific inspection of Ulverston UK, Montrose UK, Speke UK, Zebulon NC and Research Triangle Park NC manufacturing and quality control sites.
Microbial tests and specifications reviewed by Carol Vincent of HFD-805.

REMARKS / COMMENTS:

All specifications and controls on the drug substance are identical to those reported in NDA 20-564 for the lamivudine tablets, and are incorporated by reference.

The majority of clinical studies were carried out using oral solution manufactured from lamivudine which was produced by the The pediatric clinical endpoint study 300) will use several lots of oral solution derived fron The proposed commercial product will be manufactured from bulk lamivudine which is synthesized by the

The drug product is a 10 mg/mL solution of lamivudine in a water/ethanol/propylene glycol mixture . Sucrose and artificial flavors (strawberry and banana) are used to mask the bitter taste of the drug substance. Methylparaben and propylparaben in conjunction with the ethanol and sucrose act as preservatives. Citric acid and edetate (EDTA) disodium are used to control the pH to 5.0 - 6.5. The developmental history of the formulation with emphasis on stability and preservative effectiveness has been described in the literature (Ref 1). All components conform to USP specifications with the exception of the artificial flavors which are covered under DMF

The oral solution is packaged in white HDPE bottles which are capped with polypropylene or polypropylene/HDPE child-resistant closures. The portion of the closure liner that contacts the product is composed of biaxially-oriented polyethylene terephthalate film . All components are manufactured from FDA food grade materials which comply with CFR specifications. The bottle and the film meet the specifications for extractables (21 CFR 177.1520 and USP <661>). No monitoring for extractables was carried out in the stability studies, although the samples were stored horizontally.

The drug product specifications include appearance, identity (HPLC and TLC), lamivudine concentration by HPLC, drug-related impurities by HPLC, pH, methylparaben and propylparaben by HPLC, ethanol by GC and microbial limit tests. Negotiations with the sponsor resulted in tightening of limits on four of the drug-related impurities and ethanol (NC 13-Nov-95). In this amendment, the sponsor has also provided the in-house action limits to further define the impurity profile that is typically encountered at release. The microbial limits and antimicrobial preservative effectiveness testing (as clarified in NC 23-Oct-95) were reviewed by Carol Vincent (HFD-805), and judged to be appropriate for this product.

The primary stability data consists of 3 months at 25°C/60%RH on one lot of oral solution and 12 months on three lots of oral solution. Supportive data consists of 18 months at 30°C/50%RH on two lots of product. All lots are production-like. Unlike the behavior of the drug substance and tablet, measurable decomposition of the oral solution occurs on storage. The primary modes of decomposition are hydrolysis and ethanolysis of the exocyclic amine, cleavage of the glycosidic bond, and oxidation to the sulfoxides. An expiry period of 18 months is granted (24 months was requested) on the basis of the 12 months of real time data supported by the 18 months under intermediate accelerated conditions (30°/50%RH). Disparity of the accelerated data from the single lot relative to the three Rt4 lots may be due to anomalously high initial data for the former (NC 23-Oct-95). The sponsor has agreed to place one of the initial commercial lots of oral solution on the full stability protocol in order to provide further evidence of the equivalence of th products.

The sponsor has amended the container and carton labels to include a statement of the alcohol content, and a revised storage condition. The package insert, as amended, is acceptable.

The Ulverston UK and Montrose UK bulk drug manufacture sites were inspected on June 26-30 and July 3-7, respectively, and found to be acceptable. However, the Speke (Liverpool) site of drug product manufacture and QC testing was inspected on June 19-23, and a 483 was issued. Through two meetings between the sponsor and the Office of Compliance, a plan for redesign and validation of purified water system was established, and this site now has acceptable status. The Zebulon, NC plant (secondary site for packaging and labeling) and the Research Triangle Park, NC site (NDA stability testing) have acceptable cGMP status.

The analytical methodology is adequately described including the relevant validation. The Methods Validation package was submitted to the International Operations Branch, HFC-133, on 25-Sep-95. As of 14-Nov-95, validation of the analytical methodology for the oral solution is not yet complete.

The EA review is complete, and a FONSI (signed by Nancy Sager and Robert Jerussi) was issued on 28-Aug-95.

CONCLUSIONS & RECOMMENDATIONS:

The NDA submission and accompanying amendments provide adequate information on the chemistry, manufacturing and controls for Epivir™ Oral Solution (lamivudine oral solution). The Environmental Impact Assessment is complete, and the manufacturing facilities have acceptable cGMP status. The NDA, as amended, is therefore **approvable** from the chemistry perspective.

SM 11/15/95

Stephen P. Miller, Ph.D.
Review Chemist

Concurrence:
HFD-530/DFeigal
HFD-530/CChen

cc:

Orig. NDA 20-596	HFD-530/DFeigal	HFD-530/Pharm
Orig. NDA 20-564	HFD-530/CChen	HFD-102/CSO
HFD-530/Div. File	HFD-530/SMiller	
HFD-830/Div. File	HFD-530/MO	
HFD-830/ESheinin	HFD-530/Micro	File: N 20-596\000CNR01.96b

DRAFT

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

NDA #: 20-564

REVIEWER	: N. Battula
CORRESPONDENCE DATE	: 12-22-94
CDER RECEIPT DATE	: 12-23-94
REVIEW ASSIGN DATE	: 07-06-95
REVIEW COMPLETE DATE	: 11-15-95

SPONSOR: Glaxo Wellcome Inc.
Five Moore Drive
Research Triangle park, NC 27709

SUBMISSION REVIEWED: *Original*

DRUG CATEGORY: Anti-HIV, nucleoside analogue

INDICATION: Treatment of HIV infection in selected patients

DOSAGE FORM: Tablets

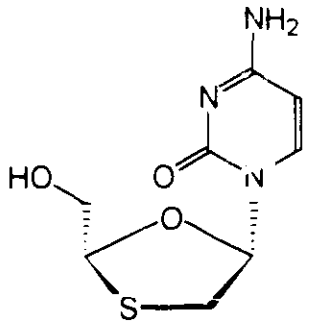
PRODUCT NAMES:

a. PROPRIETARY: Epivir™

b. NONPROPRIETARY: Lamivudine

c. CHEMICAL: (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl)-(1H)-pyrimidin-2-one. Molecular weight = 229.3
Molecular formula = $C_8H_{11}N_3O_3S$

STRUCTURAL FORMULA:



SUPPORTING DOCUMENTS: IND

BACKGROUND: This original New Drug Application for Epivir™ (previously used names, Lamivudine and 3TC™), submitted by Glaxo Wellcome Inc. is proposed for the treatment of HIV infections in selected patients. Epivir™ like Retrovir™ is a nucleoside analogue and both are inhibitors of HIV reverse transcriptase. The applicant is seeking the approval of the FDA for combination treatment of Epivir™ with Retrovir™ under the Accelerated Approval of New Drugs for Serious or Life-Threatening Illnesses. The indication proposed is that Epivir™ in combination with Retrovir™ be approved for the treatment of Retrovir-naive and Retrovir-experienced HIV-infected adult patients with CD_4+ T cell counts ≤500 cells/μl and for pediatric patients who meet the Centers for Disease Control and Prevention guidelines for antiretroviral therapy. The indication requested is based on the results of immunologic and virologic surrogate endpoint responses.

Human immunodeficiency virus, the etiological agent of the acquired immunodeficiency syndrome is an RNA virus that replicates through a DNA intermediate. The DNA copy of the viral RNA (proviral DNA) integrates with the cellular DNA (forming the provirus), thus establishing the viral infection. Transcription of the proviral DNA and translation of the viral transcripts results in the production of the progeny HIV. Thus, the HIV replication cycle can be divided into phases. The pre-integration early phase in which viral DNA synthesis is carried out by HIV reverse transcriptase, an enzyme which comes repackaged in the virion, and the post-integration late phase in which the progeny virions are produced by the combined action of both the host cell and viral coded functions.

All of the currently approved anti-HIV chemotherapeutic agents act by inhibiting HIV reverse transcriptase-directed viral DNA synthesis which is essential for establishing viral infection. As such these drugs can only effect one part of the virus life cycle mediated by viral RT i.e., prevent virus spread by blocking new rounds of infections only. HIV RT inhibitors, however, have no effect on already established infections from which HIV replication can continue. Therefore, nucleoside analogues can reduce the virus load incompletely but fail to effect the virus production from the large reservoir of already infected cells in HIV positive subjects. Chronic treatment with nucleoside analogues which is essential to keep virus load down leads to mechanistic toxicity since theses drugs also inhibit cellular DNA polymerases albeit to different degrees. Therefore, there is a great need for additional drugs that repress viral replication and minimize side effects to the patient population.

An apparently unavoidable consequence of treatment of HIV infections with anti-HIV agents is the development of resistance to the challenging drug due to variants emerging in the presence of

the drug and due to preexisting variants (natural polymorphism). HIV replication is remarkably inaccurate both by virtue of it being an RNA virus and because of the greater infidelity of DNA synthesis mediated by the viral RT. The higher rates of replication errors (10^{-4}) result in the production of progeny virions, of which the genomic RNA of each is molecularly different from each other. Combination of this inherent variability and prolonged treatment with currently available antiviral agents results in the emergence and selection of viruses with reduced susceptibility to the challenging drug. The finite therapeutic effectiveness of the anti-HIV drugs may be due to the emergence of resistance to these drugs. Therefore, novel approaches to seek new therapeutic agents that could stall the emergence of resistance and the resurgence of virus production to provide a greater and prolonged benefit in HIV-infected individuals is awaited.

Epivir is a nucleoside analogue and thus shares some of the structural and mechanistic properties with other nucleoside analogues clinically approved for the treatment of HIV infection but also differs from them in several respects. The shared properties include its prodrug status requiring metabolic activation by cellular enzymes for inhibition of HIV-RT activity. The distinguishable properties include its stereospecificity. It is the (-) enantimer of 2'3'-dideoxycytidine with a structural difference evidenced by presence of a sulfur as a second heteroatom instead of a methylene group at the 3' position in the sugar ring. In addition, orientation of the cytosine base is in the opposite configuration to that in the natural nucleoside cytosine. Unlike nucleoside analogue drugs 3TC induces rapid resistance, at rates similar to the experimental nonnucleoside RT inhibitors, with decreased sensitivity to 3TC; a consequence conventionally considered undesirable. Paradoxically, the rapid induction of 3TC resistance unexpectedly in AZT resistant HIV restores sensitivity to the virus making the combination use of these two drugs (3TC+AZT) a net benefit in terms of decreased virus load and increased CD_4+ T cells in the treated patients. Furthermore, in spite of the 3TC resistant mutation with decreased in vitro sensitivity the virus appears to grow slowly in patients (3002). Recent preliminary evidence also suggests that 3TC resistant virus containing mutation at position 184 of HIV RT seem to replicate with greater fidelity (1,2). A consequence of increased fidelity is a reduction in the rate of emergence of HIV variants.

In the microbiology portion of this NDA review a general summary of the preclinical microbiology is provided. The studies include: (1) the in vitro anti-HIV activities of 3TC and its selectivity in different cell types infected with a variety of laboratory and clinical HIV isolates, (2) antiviral activity in combination with other anti-HIV compounds, (3) drug sensitivity profiles (phenotypic and genotypic resistance) of HIV treated with 3TC in combination with other retrovirals and (4) metabolism and mechanism of action of 3TC

measured, syncytial cell formation in which adjacent cells fuse due to the action of virion gp120 to form giant cells and cytopathic effect (CPE) or cell death. CPE is generally determined by counting the remaining viable cells electronically or by measuring selective dye uptake such as trypan blue or by measuring the metabolism of the cells using the MTT dye assay. The advantage of the MTT assay is that it allows a parallel assessment of the antiviral and cytotoxic effects of test compounds in virus infected cells and in parallel mock-infected cells, respectively.

HIV RNA in cell culture studies and in clinical studies was quantified using the reverse transcription coupled polymerase chain reaction (RT-PCR) developed by the Roche Molecular Systems. Based on its design, the assay appears to be specific, sensitive and reproducible with a 4-5 log unit dynamic range for RNA quantification. The lower detection limit of viral RNA by this assay is 200 copies/ml which corresponds to 100 HIV virions. The major deficiency of the assay is that it measures the physical amount of 142 base viral RNA out of approximately 9200 base long HIV RNA and does not give a clue as to its functionality or dysfunctionality. For example, for same decrease in plasma HIV RNA in response to RT inhibitor drug therapy and protease inhibitor drug therapy, the RNA could be functionally different because of the different mechanism of action of these two classes of drugs. In spite of the draw backs RT-PCR is still the most widely used and accepted assay for quantifying HIV RNA.

The concentrations of the test compound required to reduce each of the virus-induced effects (such as p24, syncytia etc.) by 50% and 90% are referred to as IC_{50} and IC_{90}, respectively. These values were determined graphically from plots of inhibition against concentration of the test compound. Similarly, the cytotoxicity of the test compounds were determined by plotting cytotoxicity against concentration of the test drug. The values are expressed as ID_{50} and ID_{90} (i.e., concentrations of drug producing 50% and 90% cytotoxicity in cell culture, respectively). Therapeutic Index (TI) is a calculated value which represents the ratio of cytotoxicity ID_{50} to antiviral activity IC_{50}.

Antiviral activity and cytotoxicity in established cell lines: Table 1 is a summary of the anti-HIV activity of 3TC determined in a variety of established lymphocyte and monocyte/macrophage cell lines infected with HIV-1 isolates from different geographical locations. In each host cell-virus strain combination a multiplicity of infection (MOI, representing the ratio of units of infectious virus to target cells) of $1x10^{-3}$ infectious doses/cell was employed.

Table 1. Antiviral activity* of 3TC in established cell lines infected with lab isolates of HIV-1

Host cell	Virus strain	Assay endpoint	Antiviral activity (μM) IC_{50}	IC_{90}
MT-4	HIV-1_{RF}	RT	0.004	0.032
JM	HIV-1_{GB8}	Syncytium	0.15	1.6
JM	HIV-1_{GB8}	p24	0.07	0.39
C8166	HIV-1_{RF}	Syncytium	0.10	1.53
C8166	HIV-1_{RF}	p24	0.61	1.77
C8166	HIV-1_{RF}	RT	0.02	0.15
H9	HIV-1_{IIIB}	Syncytium	0.02	0.65
H9	HIV-1_{IIIB}	p24	0.01	0.13
U937	HIV-1_{U455}	syncytium	0.15	1.4
U937	HIV-1_{U455}	p24	0.13	1.1
CEM	HIV-2_{ROD}	Syncytium	0.04	0.13

* The values are averages compiled from 2-4 different experiments.

The data presented in Table 1 indicates that 3TC exerts antiviral activity against a range of HIV-1 strains isolated from different geographical locations. The table also shows that 3TC is active against one strain of HIV-2 tested. The antiviral activities vary depending on the cell type, virus strain and the assay used to measure such activity. The IC_{50} values were in the range of 0.01-0.61 μM and the IC_{90} values were in the range of 0.03-1.77 μM.

Tables 2, 3 and 4 show a summary of the antiviral activity, cytotoxicity and calculated TI of 3TC along with AZT, ddC and ddI. The activities were determined in different host cell/virus strain/end point assays. In all these assays the four nucleoside analogues were run in parallel to minimize variation. The data presented are average values calculated from 3 different experiments.

Table 2. Antiviral activity and therapeutic indices for 3TC, AZT, ddC and ddI in MT-4 cells infected with HIV-1 strain RF

Compound	Antiviral activity IC_{50} (μM)[A]	Cytotoxicity ID_{50} (μM) [B]	TI	Antiviral activity IC_{50} (μM) RT assay	TI
3TC	0.67	2067	3085	0.004	516750
AZT	0.11	<3.7	<34	0.0003	<12333
ddC	0.07	86	1229	0.001	86000
ddI	8.5	833	98	0.68	1225

(A) Antiviral activity and cell viability was determined by MTT assay

(B) Cytotoxicity was measured in mock-infected MT-4 cells

Table 3. Antiviral activity and therapeutic indices for 3TC, AZT, ddC and ddI in C8166 cells infected with HIV-1 strain RF

Com-pound	Cytotoxicity ID_{50} (μM)	Antiviral Activity Syncytial assay	TI	Antiviral activity p24 assay	TI	Antiviral activity RT assay	TI
3TC	8733	0.1	87330	0.61	14316	0.015	582200
AZT	3700	0.023	160870	0.08	46250	<0.003	1110000
ddC	>4625	0.13	32808	0.89	4792	0.027	157962
ddI	7338	2.3	3190	>21	<349	1.35	5436

Antiviral activity is expressed as the micromolar concentration of the test drugs required for 50% inhibition (IC_{50}) of the product of the assay. Cytotoxicity was measured in mock-infected C8166 cells.

Table 4. Antiviral activity in JM cells infected with HIV-1_{GB8}, U937 monocyte/macrophage cell line infected with HIV-1_{U455} and CEM cells infected with HIV-2_{ROD}

Com-pound	Antiviral activity Syncytial assay in JM cells	Antiviral activity p24 assay in JM cells	Antiviral activity Syncytial assay in U937 cells	Antiviral activity Syncytial assay in CEM cells
3TC	0.44	0.13	0.1	0.13
AZT	>374	>374	0.13	0.02
ddC	0.09	0.01	ND	ND
ddI	2.1	1.95	ND	ND

The data in Tables 2, 3 and 4 permit an evaluation of the rank order of antiviral activity of 3TC, AZT, ddC and ddI by comparison of their IC_{50} values. The values varied depending on the host cell-virus strain combination and the assay endpoint. The general conclusion from the data is that 3TC was consistently more active than ddI and consistently less active than AZT but showed activity similar to that of ddC. Comparison of IC_{90} values of nucleoside analogues show a trend similar trend as IC_{50} value. Comparison of TI values show that 3TC exhibits favorable TI indicating that it is more selective than AZT, ddC and ddI.

To shed light on the target of 3TC in HIV and the stage of HIV infection that 3TC exerted its antiviral effects the sponsor investigated two other variables that could potentially influence the effect of 3TC. (1) The effect of varying the multiplicity of infection which would change observed drug effects if the target is virus rather than the cell and (2) the effect of the time of addition of drug to determine if the drug acts at an early stage (pre-integration effects mediated by HIV-RT) or late stage in infection (post integration effects). In these assays two independent and different host cell-virus strain combinations assays were used (C8166/HIV-1_{LAV} /CPE and MT-4/HIV-1_{RF}). In parallel experiments AZT, ddC and ddI were also included for comparison. The racemic mixture of 3TC, hereafter represented as (±)3TC, was used in this set of experiments.

In assays in which varying the multiplicity of infection (MOI) was varied by 64 fold, a consistent increase in IC_{50} (10-1000 fold) with all of the four test compounds was observed indicating a similar mechanism of action of these four nucleoside analogues. The result is consistent with the interpretation that increased MOI increases the expression of virus coded target which in turn is expected to increase the amount of drug required to achieve an IC_{50} concentration of the test compounds and therefore the drug is virus specific. Varying the time of addition of (-) 3TC showed that the drug is effective until 6 hours post-infection and delaying addition resulted in a significant loss of antiviral activity. The results indicate that the antiviral target of 3TC is a function that is required early (before 6 hours) after infection. The effect of time of addition of AZT, ddC and ddI were similar to (-) 3TC. The data are consistent with belief that these four nucleoside analogues are inhibitors are HIV-1 RT.

Antiviral activity of 3TC in chronically infected cells: Acutely infected cells differ from chronically infected cells in that the latter have stably integrated provirus and the progeny HIV is produced from the proviral DNA without the involvement of the viral reverse transcriptase. In acute infection, however, reverse transcription of the HIV RNA genome into DNA and its integration into the host cell genome is required. The expectations of RT inhibitors is that they would have no effect on the virus production from chronically infected cells but interfere with the initiation of new infection.

H9 cells chronically infected with HIV-1_{RF} or HIV-2_{ROD} have stably integrated viral DNA from which infectious progeny virions are produced. The effect of (-) 3TC on the production of both HIV-1 or HIV-2 from chronically infected H9 cells was tested by the addition of drug up to 219 μM (which is >100 times the IC_{90} value in acute infection). Results from these studies showed in less than 20% inhibition of virus yield compared to untreated controls. (The replication of these two viruses has previously been shown to be inhibited by 3TC in acute infections). The result indicates that 3TC while inhibiting virus production from acutely infected cells showed no effect on virus production from chronically infected cells.

In parallel experiments the effect of nucleoside analogues AZT, ddC and ddI on HIV production from chronically infected cells was also investigated. The response of these nucleoside analogues was similar to that of 3TC in that even at 100 times the IC_{50} concentrations there was marginal or no effect on virus yield. The small percent ($<20\%$) inhibition of virus production at high concentrations could be due to reduced cell growth and viability in the infected cultures.

The combined studies on the anti-HIV activities of 3TC indicate that the target of 3TC is an early function required for the establishment of virus infection. 3TC had no effect on virus production from established infections (i.e.,chronically infected cells). These results are consistent with the notion that the target of nucleoside analogues is the viral RT.

Table 5. Anti- HIV-1 activities of 3TC, AZT, ddC and ddI in human PBMC as determined by p24 inhibition assay*.

Comp-ound	Strain 111B		Strain LAV		Strain Mn		Strain RF		Strain U455	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
3TC	0.002	0.031	0.089	0.996	0.019	0.84	0.006	0.111	0.032	0.24
AZT	0.002	0.014	0.002	0.606	0.002	0.041	0.003	0.020	0.002	0.029
ddC	0.003	0.035	0.096	0.97	0.048	0.87	0.009	0.16	0.018	0.21
ddI	0.188	5.45	1.16	11.2	0.376	6.52	0.326	11.7	0.96	11.43

*The antiviral concentrations, IC_{50} and IC_{90}, represent the concentrations (in μM) of compound required to inhibit p24 synthesis by 50% and 90% respectively. The values given are an average from 3 independent assays.

Antiviral activity against HIV-1 and HIV-2 in human PBMC: The antiviral activity of 3TC in PBMC infected with HIV-1 isolates from different geographical locations (HIV-1 strains RF, LAV, MN, U455 and 111_B) was determined. Nucleoside analogues AZT, ddC and ddI were also tested in parallel with 3TC to determine the relative antiviral activity and rank order of these compounds. To minimize the effects of differences in donor cell metabolism, PBMC from one donor were used to assay all four compounds for activity against any one virus strain. The assay endpoint in these studies was the production/inhibition of the viral p24 antigen.

The results presented in Table 5 show that 3TC exerts antiviral activity against a diverse range of HIV-1 isolates in human PBMC cultures of different donors. The IC_{50} values ranged from 0.002-0.11 μM and the IC_{90} values ranged from 0.031-1.0 μM. The rank order of antiviral activity show that 3TC is consistently more active than ddI but less active than AZT. The antiviral activities in PBMC are similar to those found in studies using established cell lines.

In studies designed to determine the cytotoxicity of 3TC to PBMC and macrophages it was found that 3TC in the concentration range of 0.1 - 3 mM was not cytotoxic as determined by trypan blue exclusion and cell survival by MTT assay indicating a therapeutic advantage of greater than 300 fold. At these concentrations cell growth and viability were not affected indicating that these concentrations were neither cytotoxic nor cytostatic.

In comparative cytotoxicity studies (expressed as ID_{50} and ID_{90} in μM) the effect of 3TC relative to three other nucleoside analogues AZT, ddC and ddI on PBMC was determined and the summary results are presented in Table 6. Each row in the table represents the same pool of PBMC in which the effect of the four nucleoside analogues was determined.

The results in Table 6 show that 3TC was consistently less cytotoxic than AZT or ddC and similar to ddI. The cytotoxic effect of 3TC to PBMC was similar to that observed in established cell lines. The therapeutic index calculated in different experiments ranged from 25000-76000 indicating that 3TC monotherapy is unlikely to be cytotoxic to lymphocytes in vivo.

Table 6. PBMC cytotoxicity as determined by [^{3}H]-thymidine uptake

3TC		AZT		ddC		ddI	
ID_{50}*	ID_{90}*	ID_{50}	ID_{90}	ID_{50}	ID_{90}	ID_{50}	ID_{90}
>437	>437	94	318	389	>474	>423	>423
>437	>437	37	187	6	>474	>423	>423
>437	>437	47	281	379	>474	>423	>423
1659	4367	47	262	107	>4739	2371	>4234
2620	>4367	34	195	33	>4739	2286	>4234
2533	6769	90	674	2464	>4739	2032	3599

* The ID_{50} and ID_{90} are concentrations in μM.

Myelotoxicity studies: In vitro studies on bone marrow toxicities have been predictive of in vivo bone marrow toxicity. Previous reports comparing the effects of AZT and ddI on in vitro myelotoxicity have suggested that the former inhibits hematopoietic precursor cell replication and

the latter showed minimal or no myelotoxicity. These in vitro studies correlated well with the known induction of anaemia in AZT-treated patients and minimal or no bone marrow suppression in ddI-treated patients. Thus, to evaluate the potential toxicity of 3TC to the bone marrow in man the sponsor carried out cytotoxicity studies with precursor cells of the hematopoietic lineage and compared the effects of 3TC with the other nucleoside analogues AZT, ddC and ddI.

Results of 3TC myelotoxicity studies indicate that even at concentrations as high as 10^{-4} M there was no toxic effect in colony assays against erythroid, granulocyte/macrophage, pluripotent or stromal progenitor cells from healthy donors. On the other hand in parallel assays both AZT and ddC were found to be much more toxic to these cells. The cytotoxicity profile of ddI however was similar to that of 3TC. These results suggest that 3TC at the proposed clinical dose of 150 mg bid (approximately 4 mg/kg) is unlikely to induce bone marrow toxicity. These in vitro observations are consistent with the lack of significant bone marrow suppression in Phase 1/2 dose escalation studies with 3TC monotherapy. Significant bone marrow toxicity however was observed in NUCA 2001 trial at the high dose of 20 mg/kg/day.

Antiviral effects in combination with other anti-HIV compounds: Treatment with nucleoside analogues requires chronic and continuous treatment to reduce the rate of HIV replication. The use of regimens containing combinations of several anti-HIV agents is increasingly considered to be the most effective use of such agents, since the current drugs provide at best temporary benefit when used alone and in all cases drug-resistant virus can be isolated from treated patients. The use of drug combinations is considered likely to improve efficacy and to reduce the potential for selection of resistant virus. In vitro studies of combination therapy provide a guide to determine the best combination. To select appropriate drug combinations which would reduce development of low cross-resistance, exhibit nonoverlaping cytotoxicities, and demonstrate in vitro synergy, the sponsor tested the combination of 3TC with the nucleoside analogues AZT, ddC and ddI to select the combinations which displayed enhanced drug activity and reduced cytotoxicity.

The antiviral activities of each of the test compounds alone and in combination were tested in MT-4 cells infected with HIV-1 strain RF. The assay endpoint was the measurement of the fraction of cells protected against virus cytopathic effect by the drug. IC_{50} values were calculated from the dose response curves of 3TC in combination with AZT, ddC or ddI. The effect of drug combinations expressed as combination index (CI) was calculated by the median effect principle to indicate whether each combination was synergistic ($CI < 1$), antagonistic ($CI > 1$) or additive ($CI = 1$).

The antiviral effects of the combination of 3TC with each of AZT, ddC and ddI were examined by the MT-4/HIV-1 strain $_{RF}$/ cell viability method and the CI calculated. The antiviral CI values for the combination 3TC with ddC (0.93) or ddI (0.73) were near to unity which reflect the additive activities of these compounds. However the CI value for the combination of 3TC with AZT (0.36) showed synergistic effect. In other studies the combination of 3TC with RO 31-8959 (Roche protease inhibitor) and a non-nucleoside RT inhibitor R82150 also showed synergistic effects. The cytotoxicity of these drug combinations, determined by measuring [3H]-thymidine uptake, showed that the combination of 3TC with AZT was no more cytotoxic than either compound alone indicating that the antiviral synergy is not due to increased toxicity to the infected cells.

As a more direct representation of the clinical situation the in vitro antiviral effects of the combination of 3TC with AZT were analyzed in PBMC infected with four clinical isolates. All of the isolates were from treatment naive patients and were only cultured in human PBMC. The isolates included three of European origin (C0008, C0019 and WP34/29) and a fourth isolate of North American origin (MCK). The results obtained in the combination studies of PBMC infected with clinical isolates were mixed. In the case of isolate MCK the combination appeared synergistic and in the case of isolate WP34/29 the combination was antagonistic; results with the other 2 strains showed that the combination was additive. Thus, the 3TC+AZT combination results in PBMC were different from the synergistic combination effect observed with established cell line MT4 infected with lab isolate HIV-1 strain RF.

Triple drug combination studies: Triple combinations of 3TC with nucleoside RT inhibitors AZT, d4T and ddI and with nonnucleoside RT inhibitor nevirapine were analyzed in 5 sets of 3-drug combinations. The triple combinations include 3TC with d4T and AZT; d4T and nevirapine; AZT and nevirapine; ddI and nevirapine; and AZT and ddI. All of the combinations were tested at a fixed equimolar ratio except that the combination of 3TC+AZT+ddI was evaluated at variable ratios. The host cell-virus strain combination of MT4 cells infected with HIV-1 strain RF was used. The end point used was surviving cells which measured by the MTT assay. Mock-infected and test compound treated cells provided a measure of cytotoxicity of the test compounds and correspondingly treated virus infected cells provided a measure of antiviral activity.

The IC_{50} and IC_{90} concentrations in each of the triple combinations were as good as any single or dual combinations which indicates that there was no antagonism of antiviral activity. In all cases

the combinations were additive to synergistic. The triple combination of 3TC+AZT+ddI was synergistic. Similarly, there was no evidence of increased cytotoxicity with any double or triple combinations.

It is important to note that these studies on the combination effects were based on a series of calculations and extrapolations in complex cellular systems. For example, it is not known if the differences in the intracellular levels of the activation enzymes and half-lives of 5'-phosphorylated derivatives of these nucleosides could contribute to differences in combination effects observed in vitro and in vivo. Within the limits of this study a combination of 3TC with AZT showed an apparent advantage. The conclusions drawn by these studies must be interpreted with caution because of the simple experimental design. The conclusions drawn from the triple combination studies have no bearing on this NDA since the NDA does not propose triple combination therapy in HIV-1 infected individuals.

Activity of 3TC in SCID-hu mouse model: The SCID-hu mouse is a model in which human fetal lymphoid organs (liver, thymus or lymph node) are transplanted into a CB-17 scid/scid mouse which lacks functional T-cells and B-cells. Upon transplantation of stem cells differentiation occurs and human CD_4+ and CD8+ T-cells and IgG appear in the peripheral circulation. HIV replication occurs in the human cells and can be measured by in situ hybridization of sections of the transplanted human tissue using HIV genomic probes or, alternatively, viremia can be measured by RT-PCR.

The SCID-hu mice were infected with HIV-1 strain JR-CSF on the day following initiation of antiviral treatment. Antiviral activity was assessed by the presence or absence of HIV RNA in mouse plasma by PCR. In these mice 3TC was inactive at 500 mg/kg BID. However, 3TC at higher concentrations of 1000 mg/kg and 2000 mg/kg BID cleared plasma viremia in 1/8 mice and 3/8 mice, respectively. In parallel experiments AZT at 100 mg/kg BID suppressed infection in all mice tested. The testing of 3TC effects in SCID-hu mouse was incomplete and therefore no conclusions could be drawn from the study.

Effect of anti-infective agents on anti-HIV activity of 3TC in vitro: Treatment of HIV infected patients generally involves not only anti-HIV drugs but also other anti-infective agents to control opportunistic infections. Therefore, the sponsor evaluated the effect of a range of anti-infective agents on the antiviral activity of 3TC. The drugs studied include the antibiotics: amikacin, azlocillin, ceftazidime, ciprofloxacin, gentamicin, benzylpenicillin, piperacillin, and vancomycin; antivirals: acyclovir, ganciclovir and GR95168X (a Glaxo compound which has anti-viral activity

against human herpes viruses), and anti-fungals: clotrimazole, flucanazole, and ketoconazole along with pentamidine which has activity against *pneumocystis carnii*.

The antiviral activity of the racemic mixture of 3TC in combination with one of the anti-infective agents was investigated using the MT-4/HIV-1 strain RF/MTT assay. The results presented indicate that none of the anti-infective agents tested in vitro in combination with the racemic mixture of 3TC affected the antiviral activity of the compound at concentrations noncytotoxic to the host cells. There is therefore no indication from the in vitro studies that combination therapy with any of these agents studied would interfere with anti-HIV activity of 3TC.

Activity against viruses other than retroviruses: To determine the antiviral specificity of 3TC the sponsor determined the antiviral activity against a variety of viruses other than retroviruses. The viruses tested include the human herpesvirus; HSV-1, HSV-2, VZV, CMV and EBV, the respiratory viruses, rhinoviruses types 2 and 14, influenza viruses A and B, and the respiratory syncytial virus. Antiviral assays were performed in cell lines chosen for susceptibility to the viruses under test. The results suggest that 3TC had no activity against any of the viruses tested in the study even at the highest dose used (438 uM). Additionally, 3TC did not show cytotoxicity up to 438 uM the highest concentration tested in these assays. The lack of activity against viruses unrelated to HIV suggests that 3TC activity is HIV-1 specific.

Activity of 3TC against hepatitis B virus in vitro: Hepatitis viruses utilize reverse transcriptase in the replication of their DNA. The sponsor investigated the effect of 3TC on the replication of hepatitis B virus. It was found that in vitro 3TC inhibited the production of replicative HBV-DNA intermediates and mature virion HBV-DNA. 3TC also decreases duck hepatitis B virus DNA in chronically infected duck hepatocytes with an IC_{50} of 0.44 uM. The sponsor stated that 3TC is also being developed as for treatment of hepatitis B and this indication will be the subject of a separate NDA.

In other studies the sponsor showed that 3TC at 438 um was not active against the protozoan parasite *Entamoeba histolytica*, Gram-positive and Gram-negative bacteria S. *aureus*, *E.coli*, *Enterobacter cloacae* and *P. aeruginosa*, mycobacteria species, *M. avium*, *M. bovis* and *M. tuberculosis* and opportunistic fungal infections including *Candida* species, *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *Pneumocystis carnii*. Based on the results of these in vitro studies the sponsor suggests that 3TC is unlikely to have any effect on these organisms in the clinic.

RESISTANCE STUDIES: Upon administration of any of the clinically available nucleoside analogues and nonnucleoside analogue RT inhibitors, HIV infected individuals develop resistance to these drugs. In vitro experiments have been predictive of this potential for the emergence of resistance. The rate of emergence of resistance appears to differ from drug to drug. To explore the potential for the emergence of resistance the sponsor attempted to select for 3TC-resistant variants in vitro by serial passaging of HIV-1 on permissive cells in the presence of increasing concentrations 3TC.

The host cell/virus strain/end point assay system used for selecting virus with reduced susceptibility to 3TC was C8166/HIV-1_{RF}/ CPE assay. The antiviral assay system used to test for the loss of susceptibility to 3TC was MT4/HIV-1_{RF}/MTT formazan assay. Drug susceptibility profiles of HIV-1 variants were determined by analysis of antiviral activity and cytotoxicity against all tested anti-HIV agents in MTT assay. The compounds tested for cross-resistance to 3TC resistant isolates include the nucleoside analogue RT inhibitors AZT, ddC and ddI, the nonnucleoside analogues L-697,639, R-81250 and nevirapine and the protease inhibitor Ro 31-8959. In addition cross-resistance was also investigated against the (+) enantimer of thiacytidine and the data are presented in Table 7.

Table 7. Drug Sensitivity profile of the 3TC- resistant variant of HIV-1*

Compound	Wild-type HIV-1 RF		3TC-Resistant Variant	
	IC_{50} (μM)	Toxicity (μM)	IC_{50} (μM)	Toxicity(μM)
3TC(-)	0.36	>4.57	>457	457
3TC(+)	2.31	45.7	3.88	45.7
AZT	0.03	>3.7	0.015	>3.7
ddC	0.16	>4.74	0.22	>4.74
ddI	7.34	424	12.3	>424
R82150	0.18	31	0.083	31
Nevirapine	0.095	>38	0.015	>38
L-697,639	0.008	>0.28	0.008	>0.28
Ro-318959	0.035	>1.5	0.03	>1.5

*Data in the table represent average values of 3 experiments

The data in Table 7 show that the 3TC resistant variants remain sensitive to nucleoside analogue RT inhibitors AZT, ddC and ddI; the nonnucleoside analogue RT inhibitors L-697,639, R82150, Nevirapine and the Roche protease inhibitor R-318959. However, (-)3TC resistant variants were sensitive to (+)3TC suggesting the enantiomeric specificity for the selected mutant virus. The data suggests the potential benefit of the use of multi-drug therapy to treat HIV-1 infections.

The sponsor investigated the potential to select in vitro HIV-1 variants resistant to both 3TC and a second RT inhibitor like AZT. Tables 8 and 9 show a summary of the drug sensitivity profiles of HIV-1 variants selected by in vitro passage of AZT-resistant HIV-1 in increasing concentrations of 3TC without and with maintaining AZT pressure. The sponsor stated that no cytotoxicity was found with any of the drugs at the concentrations tested with any of the viruses, so only antiviral data was presented.

Table 8: Drug sensitivity* profile of an HIV-1 variants selected by in vitro passage of AZT-resistant HIV-1 in increasing concentrations of 3TC only.

Virus	Selection pressure	3TC	AZT	ddC	Ro 31-8959
AZT-resistant HIV-1	None	0.7	>3.7	0.12	0.005
AZT-resistant HIV-1	3TC	>437	0.11	0.16	0.005
HIV-1 strain RF	None	1.35	0.12	0.79	0.005

* Values are average of two experiments

The data in Table 8 shows that AZT resistant virus when passaged in increasing concentrations of 3TC only i.e., in the absence of AZT pressure, selected HIV-1 variants with phenotypic resistance to 3TC (>625 x reduction in sensitivity) but with restored sensitivity to AZT (> 40-fold increase in sensitivity). Such phenomenon of suppressed phenotypic AZT resistance due to induction of other mutations in RT have been reported previously. The data in the table also show that the selected variant remained sensitive to ddC and the Roche protease inhibitor.

Table 9: Drug sensitivity* profile of an HIV-1 variant selected by in vitro passage of AZT-resistant HIV-1 in increasing concentrations of 3TC while maintaining AZT pressure

HIV-1 variant	Selection pressure	IC_{50} (μM)					
		3TC	AZT	ddC	ddI	Nevirapine	Ro31-8959
3TC-resistant	None	>437	0.010	0.80	24.3	0.14	0.046
AZT-resistant	None	0.96	>3.7	0.16	9.4	0.09	0.018
AZT-resistant	3TC+AZT	>437	>3.7	3.14	23.4	0.12	0.037
HIV-1 strain RF	None	0.37	0.13	0.14	17.4	0.12	0.046

* Values are an average of 3 experiments

The results in table 9 show that AZT-resistant virus when passaged in the presence of both AZT and 3TC selected variants that are phenotypically resistant to both drugs, > 1000-fold to 3TC compared to the parent virus and there was no change in the susceptibility to AZT i.e., the AZT-resistance was sustained. These double resistant variants remained sensitive to ddI, nevirapine and Roche protease inhibitor with comparable values as the parent. However, the variants showed reduced sensitivity to ddC of 15-24 fold in different experiments.

The sponsor stated that attempts to isolate a virus resistant to both AZT and 3TC by passaging 3TC-resistant virus in increasing concentrations of AZT while maintaining 3TC pressure or passage of the sensitive HIV-1 RF in simultaneously increasing concentrations of 3TC and AZT were unsuccessful. In a recent report (3) not included in the submission, dual resistant virus isolation only from AZT-resistant virus passaged in the presence of constant concentration of AZT and increasing concentration of 3TC was described. The results indicate that there is no evolutionary barrier for the acquisition of resistance to both AZT and 3TC. Consistent with the in vitro findings it was recently reported that dual resistance was acquired in patients treated with both of these drugs.

The relative ease or time taken to achieve the in vitro selection of variants cannot be used as a measure of the in vivo situation. However, these data may be useful in predicting the likelihood of HIV-1 drug resistance in the clinic.

Genotypic characterization of the phenotypically resistant HIV: The nature of mutation elicited in the HIV RT by in vitro selection of drug resistant isolates with both nucleoside analogues and the nonnucleoside analogue RT inhibitors is to a large extent predictive of the mutation pattern observed in the patients during treatment. The nature of the mutation in 3TC resistant isolates in vitro and cross-resistance effects may also be of predictive value in the clinic. In an effort to define the genetic basis of resistance phenotypically 3TC-resistant HIV-1 variants were generated by standard procedures of serial passing of three lab strains of HIV-1 and one primary isolate in successively increasing concentrations of the test drug.

Table 10. Drug sensitivity profiles of HIV isolates selected in vitro.

Isolate	Status	IC_{50} (μM)				Codon at 184
		3TC	AZT	ddI	ddC	
HIV-1 $_{RF}$	Wild-type	0.16	0.056	7.63	0.22	Met
	Selected	>437	0.015	12.3	0.22	Ile
HXB2	Wild-type	0.39	0.02	3.18	0.42	Met
	Selected	>437	0.001	3.82	0.4	Ile
C19*	Wild-type	1.25	ND	ND	ND	Met
	Selected	92	ND	ND	ND	Ile
HIV-1_{IIIB}	Wild-type	0.8	0.04	19	0.45	Met
	Selected	936	0.05	76	2.5	Val

+The results represents the average of at least two experiments each performed in duplicate

* HIV-1 clinical isolate obtained from an antiretroviral naive patient

Results presented in Table 10 show that all preselection HIV isolates were susceptible to 3TC, AZT, ddI and ddC. Most of the clones selected for 3TC resistance showed resistance of more than 100 fold. In order to determine the genetic changes the nucleotide sequence of the RT gene was determined for the three 3TC-resistant strains. In each case there was a single amino acid substitution in the RT gene of 3TC resistant strains at position 184 from methionine to isoleucine

or valine. The isoleucine substituted at 184 conferred greater specific resistance to 3TC and these viruses retained their ddC and ddI sensitivity. Valine substitution at codon 184 conferred reduced sensitivity to ddI and ddC. The valine substitution at 184 of HIV RT confers resistance to 3TC and low level cross resistance to ddI and ddC.

The biological significance of the amino acid change at position 184 was confirmed by construction of recombinant viruses containing isoleucine at position 184 of RT and subsequent testing of their susceptibilities. HXB2 (F1-3/184I) is the 184 mutant derived from the wild type recombinant virus HXB2 (F1-3). H257-6 was an AZT-treated patient isolate containing mutations at positions 41 and 215 and into this genetic background a recombinant mutation at 184 was introduced to generate H257-6/184I. As shown in table 10 recombinant viruses containing 184 isoleucine showed a >100 fold increase in their IC_{50}. In addition introduction of isoleucine substitution at codon 184 into a background of two known AZT-resistant mutations (amino acid positions 41 and 215) in a clinical isolate resorted the susceptibility of this virus to AZT. There was a decline of approximately 50-fold resistance to AZT. This provides further support for the clinical use of AZT and 3TC together in delaying the development of resistance.

Table 11. 3TC sensitivities of recombinant viruses

Recombinant virus	Amino Acid at Position*			IC50% Values(μM)	
	41	215	184	3TC	AZT
HXB2(F1-3)	wt	wt	wt	0.6	ND#
HXB2 (F1-3/184I)	wt	wt	M	>100	ND
H257-6	M	M	wt	4.4	2.8
H257-6/184I	M	M	M	>100	0.05

* Wild-type amino acids are Methionine 41, Threonine 215 and Methionine 184; mutant amino acids are 41 Leucine, 215 Tyrosine and 184 Isoleucine.
# ND, Not done.

MECHANISM OF ACTION: The presumed mechanism of action of nucleoside analogues is that they are initially metabolized to their respective 5'-triphosphates (dNTPS) by cellular nucleoside and nucleotide kinases. Accordingly the prodrug 3TC is converted into the active drug form, 3TCTP. The 3TCTP competes with natural (physiological) nucleoside triphosphates for the nucleotide binding site on the viral RT. This competition is believed to inhibit the rate of viral DNA synthesis (both RNA-directed and DNA-directed DNA polymerase activities of RT) by decreasing the incorporation of the natural deoxyribonucleotides. In addition, the triphosphates of the nucleoside analogues also serve as substrates and become incorporated into the growing DNA chain of the HIV DNA. The incorporated nucleotide of 3TC lacks the 3'-hydroxyl group no phosphodiester bond formation can occur with the next incoming nucleotide; consequently, the DNA chain termination stops. As a result full length proviral DNA synthesis required for integration and establishment of infection is prevented.

Metabolism of 3TC: Upon entering cells nucleoside analogues are subject to biotransformation by cellular enzymes. Therapeutic effects depends on the anabolic metabolism which leads to the formation of their triphosphates and their decay inside cells. These metabolic conversions are host cell and nucleoside analogue dependent. The sponsor investigated the metabolic activation of 3TC in HIV-1 strain RF infected and mock-infected PBMC prepared from different donors. The kinetics of metabolic conversion of radioactive [^{3}H]-3TC in the PBMC was analyzed by HPLC radiochromatography after up to 24 hours of incubation.

The results of the time course of phosphorylation of 3TC show that four components representing the parent nucleoside and its mono, di and triphosphate derivatives were detected in both HIV-1 infected and mock infected cells. The active drug, the 5-triphosphate of 3TC, by 4 hours of exposure represents 40% or more of intracellular phosphates. After 24 hours the triphosphate level dropped to approximately 20%. Although there is variation in the extent of phosphorylation from donor to donor the 3TCTP formation appeared independent of viral infection of the cells.

Studies on the effect of the external concentration of 3TC on the formation of intracellular phosphate derivatives show that the rate of formation of 3TC-TP was linear up to 10 μM and then increased at a slower rate at higher concentrations. The reaction was not saturable at the highest concentration (500 μM 3TC) in the extracellular medium. The intracellular concentration of 3TCTP at 500 μM of 3TC in the extracellular medium was 17 pmol/10^6 PBMC.

The effect of 3TC phosphorylation in uninfected PBMC in drug combination studies was carried out with [^{3}H]-3TC fixed at a concentration of 10 μM and varying concentrations of (5-50 μM) AZT for 4 hours. The results showed that AZT had no substantial effect on the phosphorylation of 3TC in uninfected PBMC indicating that these two drugs can exert antiviral effect independent of each other.

In order to determine the rate of decay of 3TCTP, PBMC were incubated with [^{3}H]-3TC for 4 hours to allow build up of detectable intracellular levels of 3TC-TP. Subsequently, the compound was removed and samples were taken over the following 24 hours to be analyzed by HPLC for 3TCTP. The rates of decay were determined from the peak areas of 3TC-TP and the 50% reduction (half-life) in the peak area determined. The results suggest that the half-life in mock infected cells was 12-15.5 hours and in HIV infected cells was 10.5-13.5 hours. The half-life of 3TC-TP is substantially longer than the half-lives for AZTTP (1 hour), ddC-TP (2.6 hours), d4T-TP (3.5 hours) and similar to that of ddI-TP (12 hours). These in vitro results support the use of 3TC twice daily in the clinical trials.

Catabolic degradation pathway: Nucleoside analogues are also substrates for cellular phosphorolyases which degrade the nucleosides to their bases and sugar. Human platelets are a rich source of these catabolic enzymes. After 93 hours of incubation in platelet enriched medium no detectable degradation of 3TC was detected indicating that it is not (or is a poor) substrate for the deaminases and phosphorolyases.

Effect of 3TC on HIV RT: To define the mechanism of action the sponsor investigated the effect of 3TCTP on the RNA-dependent DNA polymerase activity, Tne DNA-dependant DNA polymerase activity and DNA chain termination during DNA polymerization. In the analysis of RNA-directed DNA synthesis heteropolymeric (MS-2 RNA) and homopolymeric (poly rI. oligo dC) RNA templates were used. In the analysis of DNA-dependant DNA polymerase activity activated calf thymus DNA template was used and in the case of DNA chain termination studies MS2 RNA template hybridized to a complementary deoxy oligonucleotide 5'-CACTCCGAAGTGCGT-3' was used. In all cases purified recombinant HIV-RT was employed in DNA polymerization

Table 12. Effect of deoxyribonucleoside triphosphate on HIV RT activity*

Compound	K_i (μM)		Calf thymus DNA as template I_{50}# at [dNTP]=K_m μM
	MS2 RNA as template	Poly rI as template	
3TCTP	12.4	10.6	23.4
ddATP	0.09	NR	0.4
ddCTP	0.33	1.9	1.4
AZT-TP	0.03	NR	0.48
K_m dATP = 1.0 μM K_m dCTP = 1.7 μM K_m dTTP = 1.1 μM			

* The values are an average of 2 or 3 experiments

I_{50} is the concentration of the compound giving half the uninhibited (control) rate

The results presented in Table 12 show that 3TCTP inhibited the RNA-dependent DNA polymerase activity of RT with an apparent inhibition constant ($K_{i\,app}$) of 10.6-12.4 μM depending on the template used. The $K_{i\,app}$ for DNA dependent DNA-polymerase activity was 23.4 μM. Studies on the kinetics of inhibition suggested that 3TCTP inhibition is competitive with respect to the physiological substrate (dCTP) for binding to the HIV-RT.

In tests designed to determine the DNA chain termination by 3TCTP, the DNA polymerization reaction consisted of MS2 RNA template hybridized to a complementary deoxy oligonucleotide 5'-CACTCCGAAGTGCGT-3' primer, the four deoxyribonucleoside triphosphates, 3TCTP and the HIV reverse transcriptase. The synthetic product was then analyzed on a DNA sequencing gel. Comparison of the primer extension products with ddCTP control showed that 3TCTP monophosphates were incorporated at identical positions to the ddCTP control. The results show that incorporation of 3TC monophosphate caused DNA chain termination thus blocking HIV DNA replication in infected cells.

inhibitory to the 3 DNA polymerases. Polymerase α was least inhibited by any of the four nucleoside analogue substrates. Polymerases β and γ are significantly inhibited by ddATP and ddCTP but 3TCTP is less inhibitory than ddATP and ddCTP. The general conclusion from this report is that 3TCTP has a favorable inhibitory profile on the tested DNA polymerases by being less inhibitory than ddATP and ddCTP.

Table 13. Effect of dideoxyribonucleoside triphosphates on human DNA polymerases

DNA Polymerase	I_{50} (50% inhibitory concentration of the compound in μM)			
	3TC-TP	ddATP	ddCTP	AZTTP
Polymerase α	175	>500	>500	>850
Polymerase β	25	0.75	0.056	>475
Polymerase γ	44	0.068	0.031	147

The K_m values of each of the 4 natural nucleoside triphosphates for DNA polymerases α, β and γ were approximately 3.5 μM, 0.20 μM and 2.5 μM, respectively.
I_{50} = Concentration of the compound giving half the uninhibited control rate.

CONCLUSIONS: 3TC is metabolically optimized to biotransform into the active drug form 3TCTP. At identical input concentrations, 3TC builds higher concentrations of its triphosphate than any of the clinically available nucleoside analogues AZT, ddC, ddI and d4T. This desired effect seem to be achieved by virtue of its stereochemical nature, by being a poor substrate for catabolic/degradative enzymes which otherwise would break it down to its component sugar and base and by being a superior substrate for anabolic/activation enzymes essential for its conversion into the active drug form. In addition, the active form of the drug 3TCTP has a half-life of 10-15 hours which is much longer than the half-lives of AZTTP, ddCTP and d4TTP but some what similar to ddATP. This longer half-life allows BID dosing of 3TC in HIV-infected individuals. The major disadvantage of 3TC, however, is that the concentrations of its triphosphate required to inhibit HIV is greater than the triphosphates of AZT, ddC and d4T but similar to that of ddA.

The antiviral target of 3TC is HIV reverse transcriptase. The data from virologic studies showing inhibition of HIV by 3TC, the data from the multiplicity of infection studies showing that 3TC acts on a target specific to the virus, the time of addition studies showing that 3TC acts early after infection (within the first 6 hours) and lack of effect post 6 hours, and the data showing lack of

effect on virus production in chronically infected cells are consistent with HIV RT being the target of 3TC. Biochemical studies on the kinetics of enzyme inhibition and viral DNA synthesis support that 3TC competes with the physiological substrate for the nucleoside binding site thus slowing the rate of DNA polymerization by HIV RT. Chain termination studies by HIV RT show that 3TCMP was incorporated at sites identical to the natural substrate dCMP and terminate DNA chain from further elongation. The data from the combined virologic and biochemical studies confirm that the target of 3TC is the HIV RT and it inhibits the enzyme by competing with natural nucleoside substrates and terminates the viral DNA synthesis by incorporating into the growing DNA chain.

3TC is an incomplete anti-HIV agent. The consequences of specific HIV RT targeting limits the antiviral effect of 3TC to the early phase (acute infection) of the virus replication cycle with no effect on the late phase (chronic infection). In the continuous presence of 3TC only new rounds of infection and viral spread are inhibited without effect on virus production from proviral DNA of latently infected cells. In addition 3TC which requires activation by phosphorylating enzymes for exerting its effects further limit its antiviral activity to those cells that are endowed with the capacity to phosphorylate 3TC. Terminally differentiated cells such as macrophages with no replication potential either lack or shed the very enzymes required for the activation of 3TC to serve as a drug. These reservoirs of HIV in infected individuals go unchallenged by 3TC.

HIV rapidly develops resistance against 3TC. Cell culture studies show that HIV rapidly adapts to overcome 3TC pressure with loss of susceptibility to the drug (phenotypic resistance). The acquisition of resistance has been attributed to a single amino acid substitution in the viral RT (genotypic resistance) at codon 184 from methionine to isoleucine or valine. Emergence of resistance is an unavoidable consequence of treatment with anti-HIV drugs. The combination of an inherently high mutation rate (approximately $1x10^{-4}$ per replication) and exposure to 3TC permits selection of resistant variants. The codon 184 is in the highly conserved Tyr, Met^{184}, Asp, Asp (YMDD) region which is adjacent to the putative catalytic site of the HIV-RT. What is remarkable of 3TC is the very rapid rate of emergence of resistance and in this regard resembles the rate of emergence of resistance elicited by experimental nonnucleoside RT inhibitors. Paradoxically, the mutation at codon 184 of RT restores AZT sensitivity to AZT resistant HIV. The restoration of sensitivity suggested potential combination therapy for antiviral activity.

In vitro 3TC resistance is predictive of in vivo 3TC resistance. Analysis of viral RNA from 3TC monotherapy showed the emergence of resistance mutation (3.4) as early as 7 days after the initiation of treatment and by 8 weeks all of the 20 patients in the trial had the resistant strain.

Similarly, analysis of blinded clinical RNA samples collected longitudinally from NUCB3001 trial (50 of 129 analyzed, 25 individuals from AZT monotherapy arm and 25 individuals from 3TC+AZT combination arm) showed that the 184 mutation was detected in the combination by 8 weeks in 95% of the patients (5). At week 24 more of the combination group contained wild type at the AZT resistance codons (75% compared with 31% for AZT alone) All samples except one from the combination group contained the 184 mutation and none of the AZT group contained this mutation. The data suggest combination therapy with 3TC+AZT delay the emergence of mutations conferring resistance to zidovudine.

3TC resistance is apparently beneficial. Clinical resistance emerges in >95% of the treated patients as early as 8 weeks in both 3TC monotherapy and combination therapy with AZT. Therapeutic benefit as measured by surrogate marker responses (viral RNA copy number and CD_4+ T cells) is derived in combination therapy of 3TC with AZT up to 24 weeks (up to about 50 weeks in some patients). The mutation at 184 of RT restored AZT sensitivity to AZT resistant virus and also delayed the emergence of AZT resistant mutations thus sustaining the antiviral activity of AZT longer than otherwise. It is interesting to note that in monotherapy with 3TC (n=20) although there was 3TC resistant mutation as early as 1 week the viral RNA and p24 remained below the base line in most patients up to the 12 weeks tested. The benefit may not last beyond approximately one year since co-resistance to both the drugs (with multiple mutations) has been reported (6) with rise in viral RNA to the base line.

PHASE 4 CONSIDERATIONS: In vitro and in vivo studies indicate the emergence of co-resistance to both 3TC and AZT, suggesting no evolutionary barriers for the acquisition of simultaneous resistance to the combination. In future clinical trials including the clinical endpoint trials in longitudinally collected patients samples please examine HIV for phenotypic resistance and viral RNA for genotypic resistance (to both of the drugs) in a subset of patients.

In HIV-infected patients treated with non-nucleoside and nucleoside RT inhibitors including 3TC (3,5,7) drug resistant mutants of HIV emerge. The appearance of resistant mutants in serum is followed by an increase in viral RNA and by a decrease in CD_4+ T cells. Please evaluate the effect of the emergence HIV variants co-resistant to AZT and 3TC on the HIV RNA copies and CD_4 + T cells.

During the course of anti-HIV therapy, in addition to drug resistance in HIV changes in biological phenotype, a switch between non-syncytial inducing (NSI) and syncytial inducing (SI) phenotype has been observed. A recent report (7) described the conversion of NSI to SI during

3TC treatment and the presence of SI was clearly associated with an increased risk of developing 3TC resistance. Please investigate in a sub set of patient population the potential link between NSI/SI viral phenotype and the emergence of resistance.

RECOMMENDATIONS: The microbiology section of the draft label as currently written is acceptable (lines 41-69, copy attached). With respect to microbiology this the NDA is approved.

References:

1. Wainberg M A.et al., Fourth workshop on viral resistance. Annapolis, MD September 10-12.1995, p 35
2. Drosopoulos W C. et al., Fourth workshop on viral resistance. Annapolis, MD September 10-12.1995, p 36
3. Schuurman R. et al. J Infect Dis. 171:1411-1419, 1995
4. Van Leeuwen R. et al. J Infect Dis. 171:1166-1171, 1995
5. Larder B A. et al. Science 229: 696-699, 19955.
6. HIV drug resistance: Fourth International workshop. Sardinia, Italy 6-9 July 1995, p52
7. Wainberg M A.et al., AIDS 9: 351-357, 1995

Narayana Battula

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Microbiologist

CONCURRENCES:

HFD-530/Deputy Dir _____Signature_____Date

HFD-530/SMicro _____Signature_____Date

CC:

HFD-530/Original IND
HFD-530/Division File
HFD-530/MO
HFD-530/Pharm
HFD-530/Chem
HFD-530/SMicro
HFD-530/Review Micro
HFD-530/Kallgren, D.

Draft Label: Microbiology

CLINICAL PHARMACOLOGY:

Mechanism of Action: Lamivudine is a synthetic nucleoside analogue. *In vitro* studies have shown that, intracellularly, lamivudine is phosphorylated to its active 5'-triphosphate metabolite (L-TP), which has an intracellular half-life of 10.5 to 15.5 hours. The principal mode of action of L-TP is inhibition of HIV reverse transcription via viral DNA chain termination. L-TP also inhibits the RNA- and DNA-dependent DNA polymerase activities of reverse transcriptase (RT). L-TP is a weak inhibitor of mammalian α-, β-, and γ-DNA polymerases.

Microbiology: ***Antiviral Activity In Vitro:*** The relationship between *in vitro* susceptibility of HIV to lamivudine and the inhibition of HIV replication in humans has not been established. *In vitro* activity of lamivudine against HIV-1 was assessed in a number of cell lines (including monocytes and fresh human peripheral blood lymphocytes) using standard susceptibility assays. IC_{50} values (50% inhibitory concentrations) were in the range of 2 nM to 15 μM. Lamivudine had anti–HIV-1 activity in all acute virus-cell infections tested. In HIV-1–infected MT-4 cells, lamivudine in combination with zidovudine had synergistic antiretroviral activity. Synergistic activity of lamivudine/zidovudine was also shown in a variable-ratio study.

Drug Resistance: Lamivudine-resistant isolates of HIV-1 have been selected *in vitro*. The resistant isolates showed reduced susceptibility to lamivudine and genotypic analysis showed that the resistance was due to specific substitution mutations in the HIV-1 reverse transcriptase at codon 184 from methionine to either isoleucine or valine. HIV-1 strains resistant to both lamivudine and zidovudine have been isolated.

Susceptibility of clinical isolates to lamivudine and zidovudine was monitored in controlled clinical trials. In patients receiving lamivudine monotherapy and combination therapy with lamivudine plus zidovudine, HIV-1 isolates from most patients became phenotypically and genotypically resistant to lamivudine within 12 weeks. In some patients harboring zidovudine-resistant virus, phenotypic sensitivity to zidovudine by 12 weeks of treatment was restored. Combination therapy with lamivudine plus zidovudine delayed the emergence of mutations conferring resistance to zidovudine.

DRAFT

MICROBIOLOGY REVIEW
DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)

NDA #: 20-596

REVIEWER	: N. Battula
CORRESPONDENCE DATE	: 12-22-94
CDER RECEIPT DATE	: 12-23-94
REVIEW ASSIGN DATE	: 07-06-95
REVIEW COMPLETE DATE	: 11-15-95

SPONSOR: Glaxo Wellcome Inc.
Five Moore Drive
Research Triangle park, NC 27709

SUBMISSION REVIEWED: Original

DRUG CATEGORY: Anti-HIV, nucleoside analogue

INDICATION: Treatment of HIV infection in selected patients

DOSAGE FORM: Oral solution

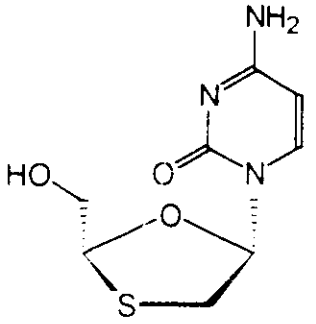
PRODUCT NAMES:

a. PROPRIETARY: Epivir™

b. NONPROPRIETARY: Lamivudine

c. CHEMICAL: (2R,cis)-4-amino-1-(2-hydroxymethyl-1,3-oxathiolan-5-yl-(1H)-pyrimidin-2-one. Molecular weight = 229.3
Molecular formula = $C_8H_{11}N_3O_3S$

STRUCTURAL FORMULA:



SUPPORTING DOCUMENTS: INI

the drug and due to preexisting variants (natural polymorphism). HIV replication is remarkably inaccurate both by virtue of it being an RNA virus and because of the greater infidelity of DNA synthesis mediated by the viral RT. The higher rates of replication errors (10^{-4}) result in the production of progeny virions, of which the genomic RNA of each is molecularly different from each other. Combination of this inherent variability and prolonged treatment with currently available antiviral agents results in the emergence and selection of viruses with reduced susceptibility to the challenging drug. The finite therapeutic effectiveness of the anti-HIV drugs may be due to the emergence of resistance to these drugs. Therefore, novel approaches to seek new therapeutic agents that could stall the emergence of resistance and the resurgence of virus production to provide a greater and prolonged benefit in HIV-infected individuals is awaited.

Epivir is a nucleoside analogue and thus shares some of the structural and mechanistic properties with other nucleoside analogues clinically approved for the treatment of HIV infection but also differs from them in several respects. The shared properties include its prodrug status requiring metabolic activation by cellular enzymes for inhibition of HIV-RT activity. The distinguishable properties include its stereospecificity. It is the (-) enantimer of 2'3'-dideoxycytidine with a structural difference evidenced by presence of a sulfur as a second heteroatom instead of a methylene group at the 3' position in the sugar ring. In addition, orientation of the cytosine base is in the opposite configuration to that in the natural nucleoside cytosine. Unlike nucleoside analogue drugs 3TC induces rapid resistance, at rates similar to the experimental nonnucleoside RT inhibitors, with decreased sensitivity to 3TC; a consequence conventionally considered undesirable. Paradoxically, the rapid induction of 3TC resistance unexpectedly in AZT resistant HIV restores sensitivity to the virus making the combination use of these two drugs (3TC+AZT) a net benefit in terms of decreased virus load and increased CD_4+ T cells in the treated patients. Furthermore, in spite of the 3TC resistant mutation with decreased in vitro sensitivity the virus appears to grow slowly in patients 3002). Recent preliminary evidence also suggests that 3TC resistant virus containing mutation at position 184 of HIV RT seem to replicate with greater fidelity (1,2). A consequence of increased fidelity is a reduction in the rate of emergence of HIV variants.

In the microbiology portion of this NDA review a general summary of the preclinical microbiology is provided. The studies include: (1) the in vitro anti-HIV activities of 3TC and its selectivity in different cell types infected with a variety of laboratory and clinical HIV isolates, (2) antiviral activity in combination with other anti-HIV compounds, (3) drug sensitivity profiles (phenotypic and genotypic resistance) of HIV treated with 3TC in combination with other retrovirals and (4) metabolism and mechanism of action of 3TC

Throughout the microbiology portion of the NDA the sponsor emphasized the properties and potential benefits of 3TC as a monotherapeutic agent. In the evaluation of 3TC it is important to remember that the proposed use of this drug is in combination with Retrovir and therefore the beneficial and detrimental properties of each of these circumstance need consideration.

SUMMARY

Anti-HIV activity of 3TC in vitro: Nucleoside analogues are prodrugs and inside cells they undergo biotransformation by sequential phosphorylation to form the active drugs. The active drug forms of nucleoside analogues correspond to their 5'-triphosphate derivatives and in the case of 3TC it is 3TC 5'-triphosphate. Due to the differential levels of phosphorylating enzymes in different cell types, the extent of phosphorylation and consequently the active drug formation varies. Therefore, the antiviral activities of 3TC can be host cell dependent. In addition, differences between HIV strains at the level of gene sequences coding for the RT, the target of 3TC, could lead to strain differences in antiviral activity. Therefore, to avoid these host cell-virus strain biases and to lend support and perspective to the antiviral activities of 3TC, the sponsor examined the anti-HIV activity of 3TC in different host cell-virus strain combinations to approximate the balance of cells and viruses in vivo.

Set up of antiviral assays: *Materials and Methods:* The choice of established cell lines, primary cells, and viruses used for infecting the host cells depends on the type of viral attribute intended for quantification and tropism of the virus for a particular cell type.

Cells and viruses used: Established CD_4+ T lymphocyte lines, MT-4 cells, C8166 cells, CEM cells, H9 cells, JM cells, CEM cells and the primary cells PBMC were used. Laboratory-passaged lymphotropic HIV strains selected from different geographic locations such as: HIV-1 strain RF, an isolate from Haiti; HIV-1 strain GB8, a strain isolated from the UK; HIV-1 strain 111B (USA); HIV-1 strain U455 a monocyte-macrophage tropic strain isolated from Uganda, HIV-1 strain LAV (France) and HIV-1 strain MN (USA); HIV-1 strain BRU (France) and HIV-2 strain ROD a lymphotropic strain isolated from Senegal, were used in the experiments.

Endpoints and their measures: Standard assay methods were used and they are essentially of two kinds: (1) assays based on the yield of viral components in the culture medium: In this system there are 3 viral components usually measured, virion associated RT activity (radioisotope incorporation measurements), viral p24 (immune assay) and viral RNA (quantitative PCR), and (2) Assays based on cell damage due to infection: generally two effects on infected cells are

measured, syncytial [illegible] in which adjacent cells fuse due to the action of virion gp120 to form giant cel[illegible] c effect (CPE) or cell death. CPE is generally determined by counting the rer[illegible] ells electronically or by measuring selective dye uptake such as trypan blue or b[illegible] e metabolism of the cells using the MTT dye assay. The advantage of the MTT assay [illegible] allows a parallel assessment of the antiviral and cytotoxic effects of test compounds in virus infected cells and in parallel mock-infected cells, respectively.

HIV RNA in cell culture studies and in clinical studies was quantified using the reverse transcription coupled polymerase chain reaction (RT-PCR) developed by the Roche Molecular Systems. Based on its design, the assay appears to be specific, sensitive and reproducible with a 4-5 log unit dynamic range for RNA quantification. The lower detection limit of viral RNA by this assay is 200 copies/ml which corresponds to 100 HIV virions. The major deficiency of the assay is that it measures the physical amount of 142 base viral RNA out of approximately 9200 base long HIV RNA and does not give a clue as to its functionality or dysfunctionality. For example, for same decrease in plasma HIV RNA in response to RT inhibitor drug therapy and protease inhibitor drug therapy, the RNA could be functionally different because of the different mechanism of action of these two classes of drugs. In spite of the draw backs RT-PCR is still the most widely used and accepted assay for quantifying HIV RNA.

The concentrations of the test compound required to reduce each of the virus-induced effects (such as p24, syncytia etc.) by 50% and 90% are referred to as IC_{50} and IC_{90}, respectively. These values were determined graphically from plots of inhibition against concentration of the test compound. Similarly, the cytotoxicity of the test compounds were determined by plotting cytotoxicity against concentration of the test drug. The values are expressed as ID_{50} and ID_{90} (i.e., concentrations of drug producing 50% and 90% cytotoxicity in cell culture, respectively). Therapeutic Index (TI) is a calculated value which represents the ratio of cytotoxicity ID_{50} to antiviral activity IC_{50}.

Antiviral activity and cytotoxicity in established cell lines: Table 1 is a summary of the anti-HIV activity of 3TC determined in a variety of established lymphocyte and monocyte/macrophage cell lines infected with HIV-1 isolates from different geographical locations. In each host cell-virus strain combination a multiplicity of infection (MOI, representing the ratio of units of infectious virus to target cells) of $1x10^{-3}$ infectious doses/cell was employed.

Table 1. Antiviral activity* of 3TC in established cell lines infected with lab isolates of HIV-1

Host cell	Virus strain	Assay endpoint	Antiviral activity (μM) IC_{50}	IC_{90}
MT-4	HIV-1 $_{RF}$	RT	0.004	0.032
JM	HIV-1 $_{GB8}$	Syncytium	0.15	1.6
JM	HIV-1 $_{GB8}$	p24	0.07	0.39
C8166	HIV-1 $_{RF}$	Syncytium	0.10	1.53
C8166	HIV-1 $_{RF}$	p24	0.61	1.77
C8166	HIV-1 $_{RF}$	RT	0.02	0.15
H9	HIV-1 $_{IIIB}$	Syncytium	0.02	0.65
H9	HIV-1 $_{IIIB}$	p24	0.01	0.13
U937	HIV-1 $_{U455}$	syncytium	0.15	1.4
U937	HIV-1 $_{U455}$	p24	0.13	1.1
CEM	HIV-2 $_{ROD}$	Syncytium	0.04	0.13

* The values are averages compiled from 2-4 different experiments.

The data presented in Table 1 indicates that 3TC exerts antiviral activity against a range of HIV-1 strains isolated from different geographical locations. The table also shows that 3TC is active against one strain of HIV-2 tested. The antiviral activities vary depending on the cell type, virus strain and the assay used to measure such activity. The IC_{50} values were in the range of 0.01-0.61 μM and the IC_{90} values were in the range of 0.03-1.77 μM.

Tables 2, 3 and 4 show a summary of the antiviral activity, cytotoxicity and calculated TI of 3TC along with AZT, ddC and ddI. The activities were determined in different host cell/virus strain/end point assays. In all these assays the four nucleoside analogues were run in parallel to minimize variation. The data presented are average values calculated from 3 different experiments.

Table 2. Antiviral activity and therapeutic indices for 3TC, AZT, ddC and ddI in MT-4 cells infected with HIV-1 strain RF

Compound	Antiviral activity IC_{50} (μM)[A]	Cytotoxicity ID_{50} (μM) [B]	TI	Antiviral activity IC_{50} (μM) RT assay	TI
3TC	0.67	2067	3085	0.004	516750
AZT	0.11	< 3.7	< 34	0.0003	< 12333
ddC	0.07	86	1229	0.001	86000
ddI	8.5	833	98	0.68	1225

(A) Antiviral activity and cell viability was determined by MTT assay

(B) Cytotoxicity was measured in mock-infected MT-4 cells

Table 3. Antiviral activity and therapeutic indices for 3TC, AZT, ddC and ddI in C8166 cells infected with HIV-1 strain RF

Com-pound	Cytotoxicity ID_{50} (μM)	Antiviral Activity Syncytial assay	TI	Antiviral activity p24 assay	TI	Antiviral activity RT assay	TI
3TC	8733	0.1	87330	0.61	14316	0.015	582200
AZT	3700	0.023	160870	0.08	46250	< 0.003	1110000
ddC	> 4625	0.13	32808	0.89	4792	0.027	157962
ddI	7338	2.3	3190	> 21	< 349	1.35	5436

Antiviral activity is expressed as the micromolar concentration of the test drugs required for 50% inhibition (IC_{50}) of the product of the assay. Cytotoxicity was measured in mock-infected C8166 cells.

Table 4. Antiviral activity in JM cells infected with HIV-1_{GB8}, U937 monocyte/macrophage cell line infected with HIV-1_{U455} and CEM cells infected with HIV-2_{ROD}

Com-pound	Antiviral activity Syncytial assay in JM cells	Antiviral activity p24 assay in JM cells	Antiviral activity Syncytial assay in U937 cells	Antiviral activity Syncytial assay in CEM cells
3TC	0.44	0.13	0.1	0.13
AZT	>374	>374	0.13	0.02
ddC	0.09	0.01	ND	ND
ddI	2.1	1.95	ND	ND

The data in Tables 2, 3 and 4 permit an evaluation of the rank order of antiviral activity of 3TC, AZT, ddC and ddI by comparison of their IC_{50} values. The values varied depending on the host cell-virus strain combination and the assay endpoint. The general conclusion from the data is that 3TC was consistently more active than ddI and consistently less active than AZT but showed activity similar to that of ddC. Comparison of IC_{90} values of nucleoside analogues show a trend similar trend as IC_{50} value. Comparison of TI values show that 3TC exhibits favorable TI indicating that it is more selective than AZT, ddC and ddI.

To shed light on the target of 3TC in HIV and the stage of HIV infection that 3TC exerted its antiviral effects the sponsor investigated two other variables that could potentially influence the effect of 3TC. (1) The effect of varying the multiplicity of infection which would change observed drug effects if the target is virus rather than the cell and (2) the effect of the time of addition of drug to determine if the drug acts at an early stage (pre-integration effects mediated by HIV-RT) or late stage in infection (post integration effects). In these assays two independent and different host cell-virus strain combinations assays were used (C8166/HIV-1_{LAV} /CPE and MT-4/HIV-1_{RF}). In parallel experiments AZT, ddC and ddI were also included for comparison. The racemic mixture of 3TC, hereafter represented as (±)3TC, was used in this set of experiments.

In assays in which varying the multiplicity of infection (MOI) was varied by 64 fold, a consistent increase in IC_{50} (10-1000 fold) with all of the four test compounds was observed indicating a similar mechanism of action of these four nucleoside analogues. The result is consistent with the interpretation that increased MOI increases the expression of virus coded target which in turn is expected to increase the amount of drug required to achieve an IC_{50} concentration of the test compounds and therefore the drug is virus specific. Varying the time of addition of (-) 3TC showed that the drug is effective until 6 hours post-infection and delaying addition resulted in a significant loss of antiviral activity. The results indicate that the antiviral target of 3TC is a function that is required early (before 6 hours) after infection. The effect of time of addition of AZT, ddC and ddI were similar to (-) 3TC. The data are consistent with belief that these four nucleoside analogues are inhibitors are HIV-1 RT.

Antiviral activity of 3TC in chronically infected cells: Acutely infected cells differ from chronically infected cells in that the latter have stably integrated provirus and the progeny HIV is produced from the proviral DNA without the involvement of the viral reverse transcriptase. In acute infection, however, reverse transcription of the HIV RNA genome into DNA and its integration into the host cell genome is required. The expectations of RT inhibitors is that they would have no effect on the virus production from chronically infected cells but interfere with the initiation of new infection.

H9 cells chronically infected with HIV-1_{RF} or HIV-2_{ROD} have stably integrated viral DNA from which infectious progeny virions are produced. The effect of (-) 3TC on the production of both HIV-1 or HIV-2 from chronically infected H9 cells was tested by the addition of drug up to 219 μM (which is >100 times the IC_{90} value in acute infection). Results from these studies showed in less than 20% inhibition of virus yield compared to untreated controls. (The replication of these two viruses has previously been shown to be inhibited by 3TC in acute infections). The result indicates that 3TC while inhibiting virus production from acutely infected cells showed no effect on virus production from chronically infected cells.

In parallel experiments the effect of nucleoside analogues AZT, ddC and ddI on HIV production from chronically infected cells was also investigated. The response of these nucleoside analogues was similar to that of 3TC in that even at 100 times the IC_{50} concentrations there was marginal or no effect on virus yield. The small percent ($<20\%$) inhibition of virus production at high concentrations could be due to reduced cell growth and viability in the infected cultures.

The combined studies on the anti-HIV activities of 3TC indicate that the target of 3TC is an early function required for the establishment of virus infection. 3TC had no effect on virus production from established infections (i.e.,chronically infected cells). These results are consistent with the notion that the target of nucleoside analogues is the viral R^T.

Table 5. Anti- HIV-1 activities of 3TC, AZT, ddC and ddI in human PBMC as determined by p24 inhibition assay*.

Comp-ound	Strain 111B		Strain LAV		Strain Mn		Strain RF		Strain U455	
	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
3TC	0.002	0.031	0.089	0.996	0.019	0.84	0.006	0.111	0.032	0.24
AZT	0.002	0.014	0.002	0.606	0.002	0.041	0.003	0.020	0.002	0.029
ddC	0.003	0.035	0.096	0.97	0.048	0.87	0.009	0.16	0.018	0.21
ddI	0.188	5.45	1.16	11.2	0.376	6.52	0.326	11.7	0.96	11.43

*The antiviral concentrations, IC_{50} and IC_{90}, represent the concentrations (in μM) of compound required to inhibit p24 synthesis by 50% and 90% respectively. The values given are an average from 3 independent assays.

Antiviral activity against HIV-1 and HIV-2 in human PBMC: The antiviral activity of 3TC in PBMC infected with HIV-1 isolates from different geographical locations (HIV-1 strains RF, LAV^V, MN, U455 and 111_B) was determined. Nucleoside analogues AZT, ddC and ddI were also tested in parallel with 3TC to determine the relative antiviral activity and rank order of these compounds. To minimize the effects of differences in donor cell metabolism, PBMC from one donor were used to assay all four compounds for activity against any one virus strain. The assay endpoint in these studies was the production/inhibition of the viral p24 antigen.

The results presented in Table 5 show that 3TC exerts antiviral activity against a diverse range of HIV-1 isolates in human PBMC cultures of different donors. The IC_{50} values ranged from 0.002-0.11 μM and the IC_{90} values ranged from 0.031-1.0 μM. The rank order of antiviral activity show that 3TC is consistently more active than ddI but less active than AZT. The antiviral activities in PBMC are similar to those found in studies using established cell lines.

In studies designed to determine the cytotoxicity of 3TC to PBMC and macrophages it was found that 3TC in the concentration range of 0.1 - 3 mM was not cytotoxic as determined by trypan blue exclusion and cell survival by MTT assay indicating a therapeutic advantage of greater than 300 fold. At these concentrations cell growth and viability were not affected indicating that these concentrations were neither cytotoxic nor cytostatic.

In comparative cytotoxicity studies (expressed as ID_{50} and ID_{90} in μM) the effect of 3TC relative to three other nucleoside analogues AZT, ddC and ddI on PBMC was determined and the summary results are presented in Table 6. Each row in the table represents the same pool of PBMC in which the effect of the four nucleoside analogues was determined.

The results in Table 6 show that 3TC was consistently less cytotoxic than AZT or ddC and similar to ddI. The cytotoxic effect of 3TC to PBMC was similar to that observed in established cell lines. The therapeutic index calculated in different experiments ranged from 25000-76000 indicating that 3TC monotherapy is unlikely to be cytotoxic to lymphocytes in vivo.

Table 6. PBMC cytotoxicity as determined by [^{3}H]-thymidine uptake

3TC		AZT		ddC		ddI	
ID_{50}*	ID_{90}*	ID_{50}	ID_{90}	ID_{50}	ID_{90}	ID_{50}	ID_{90}
>437	>437	94	318	389	>474	>423	>423
>437	>437	37	187	6	>474	>423	>423
>437	>437	47	281	379	>474	>423	>423
1659	4367	47	262	107	>4739	2371	>4234
2620	>4367	34	195	33	>4739	2286	>4234
2533	6769	90	674	2464	>4739	2032	3599

* The ID_{50} and ID_{90} are concentrations in μM.

Myelotoxicity studies: In vitro studies on bone marrow toxicities have been predictive of in vivo bone marrow toxicity. Previous reports comparing the effects of AZT and ddI on in vitro myelotoxicity have suggested that the former inhibits hematopoietic precursor cell replication and

the latter showed minimal or no myelotoxicity. These in vitro studies correlated well with the known induction of anaemia in AZT-treated patients and minimal or no bone marrow suppression in ddI-treated patients. Thus, to evaluate the potential toxicity of 3TC to the bone marrow in man the sponsor carried out cytotoxicity studies with precursor cells of the hematopoietic lineage and compared the effects of 3TC with the other nucleoside analogues AZT, ddC and ddI.

Results of 3TC myelotoxicity studies indicate that even at concentrations as high as 10^{-4} M there was no toxic effect in colony assays against erythroid, granulocyte/macrophage, pluripotent or stromal progenitor cells from healthy donors. On the other hand in parallel assays both AZT and ddC were found to be much more toxic to these cells. The cytotoxicity profile of ddI however was similar to that of 3TC. These results suggest that 3TC at the proposed clinical dose of 150 mg bid (approximately 4 mg/kg) is unlikely to induce bone marrow toxicity. These in vitro observations are consistent with the lack of significant bone marrow suppression in Phase 1/2 dose escalation studies with 3TC monotherapy. Significant bone marrow toxicity however was observed in NUCA 2001 trial at the high dose of 20 mg/kg/day.

Antiviral effects in combination with other anti-HIV compounds: Treatment with nucleoside analogues requires chronic and continuous treatment to reduce the rate of HIV replication. The use of regimens containing combinations of several anti-HIV agents is increasingly considered to be the most effective use of such agents, since the current drugs provide at best temporary benefit when used alone and in all cases drug-resistant virus can be isolated from treated patients. The use of drug combinations is considered likely to improve efficacy and to reduce the potential for selection of resistant virus. In vitro studies of combination therapy provide a guide to determine the best combination. To select appropriate drug combinations which would reduce development of low cross-resistance, exhibit nonoverlaping cytotoxicities, and demonstrate in vitro synergy, the sponsor tested the combination of 3TC with the nucleoside analogues AZT, ddC and ddi to select the combinations which displayed enhanced drug activity and reduced cytotoxicity.

The antiviral activities of each of the test compounds alone and in combination were tested in MT-4 cells infected with HIV-1 strain RF. The assay endpoint was the measurement of the fraction of cells protected against virus cytopathic effect by the drug. IC_{50} values were calculated from the dose response curves of 3TC in combination with AZT, ddC or ddI. The effect of drug combinations expressed as combination index (CI) was calculated by the median effect principle to indicate whether each combination was synergistic ($CI < 1$), antagonistic ($CI > 1$) or additive ($CI = 1$).

The antiviral effects of the combination of 3TC with each of AZT, ddC and ddI were examined by the MT-4/HIV-1 strain $_{RF}$/ cell viability method and the CI calculated. The antiviral CI values for the combination 3TC with ddC (0.93) or ddI (0.73) were near to unity which reflect the additive activities of these compounds. However the CI value for the combination of 3TC with AZT (0.36) showed synergistic effect. In other studies the combination of 3TC with RO 31-8959 (Roche protease inhibitor) and a non-nucleoside RT inhibitor R82150 also showed synergistic effects. The cytotoxicity of these drug combinations, determined by measuring [3H]-thymidine uptake, showed that the combination of 3TC with AZT was no more cytotoxic than either compound alone indicating that the antiviral synergy is not due to increased toxicity to the infected cells.

As a more direct representation of the clinical situation the in vitro antiviral effects of the combination of 3TC with AZT were analyzed in PBMC infected with four clinical isolates. All of the isolates were from treatment naive patients and were only cultured in human PBMC. The isolates included three of European origin (C0008, C0019 and WP34/29) and a fourth isolate of North American origin (MCK). The results obtained in the combination studies of PBMC infected with clinical isolates were mixed. In the case of isolate MCK the combination appeared synergistic and in the case of isolate WP34/29 the combination was antagonistic; results with the other 2 strains showed that the combination was additive. Thus, the 3TC+AZT combination results in PBMC were different from the synergistic combination effect observed with established cell line MT4 infected with lab isolate HIV-1 strain RF.

Triple drug combination studies: Triple combinations of 3TC with nucleoside RT inhibitors AZT, d4T and ddI and with nonnucleoside RT inhibitor nevirapine were analyzed in 5 sets of 3-drug combinations. The triple combinations include 3TC with d4T and AZT; d4T and nevirapine; AZT and nevirapine; ddI and nevirapine; and AZT and ddI. All of the combinations were tested at a fixed equimolar ratio except that the combination of 3TC+AZT+ddI was evaluated at variable ratios. The host cell-virus strain combination of MT4 cells infected with HIV-1 strain RF was used. The end point used was surviving cells which measured by the MTT assay. Mock-infected and test compound treated cells provided a measure of cytotoxicity of the test compounds and correspondingly treated virus infected cells provided a measure of antiviral activity.

The IC_{50} and IC_{90} concentrations in each of the triple combinations were as good as any single or dual combinations which indicates that there was no antagonism of antiviral activity. In all cases

the combinations were additive to synergistic. The triple combination of 3TC+AZT+ddI was synergistic. Similarly, there was no evidence of increased cytotoxicity with any double or triple combinations.

It is important to note that these studies on the combination effects were based on a series of calculations and extrapolations in complex cellular systems. For example, it is not known if the differences in the intracellular levels of the activation enzymes and half-lives of 5'-phosphorylated derivatives of these nucleosides could contribute to differences in combination effects observed in vitro and in vivo. Within the limits of this study a combination of 3TC with AZT showed an apparent advantage. The conclusions drawn by these studies must be interpreted with caution because of the simple experimental design. The conclusions drawn from the triple combination studies have no bearing on this NDA since the NDA does not propose triple combination therapy in HIV-1 infected individuals.

Activity of 3TC in SCID-hu mouse model: The SCID-hu mouse is a model in which human fetal lymphoid organs (liver, thymus or lymph node) are transplanted into a CB-17 scid/scid mouse which lacks functional T-cells and B-cells. Upon transplantation of stem cells differentiation occurs and human CD_4+ and CD8+ T-cells and IgG appear in the peripheral circulation. HIV replication occurs in the human cells and can be measured by in situ hybridization of sections of the transplanted human tissue using HIV genomic probes or, alternatively, viremia can be measured by RT-PCR.

The SCID-hu mice were infected with HIV-1 strain JR-CSF on the day following initiation of antiviral treatment. Antiviral activity was assessed by the presence or absence of HIV RNA in mouse plasma by PCR. In these mice 3TC was inactive at 500 mg/kg BID. However, 3TC at higher concentrations of 1000 mg/kg and 2000 mg/kg BID cleared plasma viremia in 1/8 mice and 3/8 mice, respectively. In parallel experiments AZT at 100 mg/kg BID suppressed infection in all mice tested The testing of 3TC effects in SCID-hu mouse was incomplete and therefore no conclusions could be drawn from the study.

Effect of anti-infective agents on anti-HIV activity of 3TC in vitro: Treatment of HIV infected patients generally involves not only anti-HIV drugs but also other anti-infective agents to control opportunistic infections. Therefore, the sponsor evaluated the effect of a range of anti-infective agents on the antiviral activity of 3TC. The drugs studied include the antibiotics: amikacin, azlocillin, ceftazidime, ciprofloxacin, gentamicin, benzylpenicillin, piperacillin, and vancomycin; antivirals: acyclovir, ganciclovir and GR95168X (a Glaxo compound which has anti-viral activity

against human herpes viruses), and anti-fungals: clotrimazole, flucanazole, and ketoconazole along with pentamidine which has activity against *pneumocystis carnii*.

The antiviral activity of the racemic mixture of 3TC in combination with one of the anti-infective agents was investigated using the MT-4/HIV-1 strain RF/MTT assay. The results presented indicate that none of the anti-infective agents tested in vitro in combination with the racemic mixture of 3TC affected the antiviral activity of the compound at concentrations noncytotoxic to the host cells. There is therefore no indication from the in vitro studies that combination therapy with any of these agents studied would interfere with anti-HIV activity of 3TC.

Activity against viruses other than retroviruses: To determine the antiviral specificity of 3TC the sponsor determined the antiviral activity against a variety of viruses other than retroviruses. The viruses tested include the human herpesvirus; HSV-1, HSV-2, VZV, CMV and EBV, the respiratory viruses, rhinoviruses types 2 and 14, influenza viruses A and B, and the respiratory syncytial virus. Antiviral assays were performed in cell lines chosen for susceptibility to the viruses under test. The results suggest that 3TC had no activity against any of the viruses tested in the study even at the highest dose used (438 μM). Additionally, 3TC did not show cytotoxicity up to 438 μM the highest concentration tested in these assays. The lack of activity against viruses unrelated to HIV suggests that 3TC activity is HIV-1 specific.

Activity of 3TC against hepatitis B virus in vitro: Hepatitis viruses utilize reverse transcriptase in the replication of their DNA. The sponsor investigated the effect of 3TC on the replication of hepatitis B virus. It was found that in vitro 3TC inhibited the production of replicative HBV-DNA intermediates and mature virion HBV-DNA. 3TC also decreases duck hepatitis B virus DNA in chronically infected duck hepatocytes with an IC_{50} of 0.44 μM. The sponsor stated that 3TC is also being developed as for treatment of hepatitis B and this indication will be the subject of a separate NDA.

In other studies the sponsor showed that 3TC at 438 μm was not active against the protozoan parasite *Entamoeba histolytica*, Gram-positive and Gram-negative bacteria S. *aureus*, *E.coli*, *Enterobacter cloacae* and *P. aeruginosa*, mycobacteria species, *M. avium*, *M. bovis* and *M. tuberculosis* and opportunistic fungal infections including *Candida* species, *Cryptococcus neoformans*, *Aspergillus fumigatus*, and *Pneumocystis carnii*. Based on the results of these in vitro studies the sponsor suggests that 3TC is unlikely to have any effect on these organisms in the clinic.

RESISTANCE STUDIES: Upon administration of any of the clinically available nucleoside analogues and nonnucleoside analogue RT inhibitors, HIV infected individuals develop resistance to these drugs. In vitro experiments have been predictive of this potential for the emergence of resistance. The rate of emergence of resistance appears to differ from drug to drug. To explore the potential for the emergence of resistance the sponsor attempted to select for 3TC-resistant variants in vitro by serial passaging of HIV-1 on permissive cells in the presence of increasing concentrations 3TC.

The host cell/virus strain/end point assay system used for selecting virus with reduced susceptibility to 3TC was C8166/HIV-1_{RF}/ CPE assay. The antiviral assay system used to test for the loss of susceptibility to 3TC was MT4/HIV-1_{RF}/MTT formazan assay. Drug susceptibility profiles of HIV-1 variants were determined by analysis of antiviral activity and cytotoxicity against all tested anti-HIV agents in MTT assay. The compounds tested for cross-resistance to 3TC resistant isolates include the nucleoside analogue RT inhibitors AZT, ddC and ddI, the nonnucleoside analogues L-697,639, R-81250 and nevirapine and the protease inhibitor Ro 31-8959. In addition cross-resistance was also investigated against the (+) enantimer of thiacytidine and the data are presented in Table 7.

Table 7. Drug Sensitivity profile of the 3TC- resistant variant of HIV-1*

Compound	Wild-type HIV-1 RF		3TC-Resistant Variant	
	IC_{50} (μM)	Toxicity (μM)	IC_{50} (μM)	Toxicity(μM)
3TC(-)	0.36	>4.57	>457	457
3TC(+)	2.31	45.7	3.88	45.7
AZT	0.03	>3.7	0.015	>3.7
ddC	0.16	>4.74	0.22	>4.74
ddI	7.34	424	12.3	>424
R82150	0.18	31	0.083	31
Nevirapine	0.095	>38	0.015	>38
L-697,639	0.008	>0.28	0.008	>0.28
Ro-318959	0.035	>1.5	0.03	>1.5

*Data in the table represent average values of 3 experiments

The data in Table 7 show that the 3TC resistant variants remain sensitive to nucleoside analogue RT inhibitors AZT, ddC and ddI; the nonnucleoside analogue RT inhibitors L-697,639, R82150, Nevirapine and the Roche protease inhibitor R-318959. However, (-)3TC resistant variants were sensitive to (+)3TC suggesting the enantiomeric specificity for the selected mutant virus. The data suggests the potential benefit of the use of multi-drug therapy to treat HIV-1 infections.

The sponsor investigated the potential to select in vitro HIV-1 variants resistant to both 3TC and a second RT inhibitor like AZT. Tables 8 and 9 show a summary of the drug sensitivity profiles of HIV-1 variants selected by in vitro passage of AZT-resistant HIV-1 in increasing concentrations of 3TC without and with maintaining AZT pressure. The sponsor stated that no cytotoxicity was found with any of the drugs at the concentrations tested with any of the viruses, so only antiviral data was presented.

Table 8: Drug sensitivity* profile of an HIV-1 variants selected by in vitro passage of AZT-resistant HIV-1 in increasing concentrations of 3TC only.

Virus	Selection pressure	3TC	AZT	ddC	Ro 31-8959
AZT-resistant HIV-1	None	0.7	>3.7	0.12	0.005
AZT-resistant HIV-1	3TC	>437	0.11	0.16	0.005
HIV-1 strain RF	None	1.35	0.12	0.79	0.005

* Values are average of two experiments

The data in Table 8 shows that AZT resistant virus when passaged in increasing concentrations of 3TC only i.e., in the absence of AZT pressure, selected HIV-1 variants with phenotypic resistance to 3TC (>625 x reduction in sensitivity) but with restored sensitivity to AZT (> 40-fold increase in sensitivity). Such phenomenon of suppressed phenotypic AZT resistance due to induction of other mutations in RT have been reported previously. The data in the table also show that the selected variant remained sensitive to ddC and the Roche protease inhibitor.

Table 9: Drug sensitivity* profile of an HIV-1 variant selected by in vitro passage of AZT-resistant HIV-1 in increasing concentrations of 3TC while maintaining AZT pressure

HIV-1 variant	Selection pressure	IC_{50} (μM)					
		3TC	AZT	ddC	ddI	Nevirapine	Ro31-8959
3TC-resistant	None	>437	0.010	0.80	24.3	0.14	0.046
AZT-resistant	None	0.96	>3.7	0.16	9.4	0.09	0.018
AZT-resistant	3TC+AZT	>437	>3.7	3.14	23.4	0.12	0.037
HIV-1 strain RF	None	0.37	0.13	0.14	17.4	0.12	0.046

* Values are an average of 3 experiments

The results in table 9 show that AZT-resistant virus when passaged in the presence of both AZT and 3TC selected variants that are phenotypically resistant to both drugs, > 1000-fold to 3TC compared to the parent virus and there was no change in the susceptibility to AZT i.e., the AZT-resistance was sustained. These double resistant variants remained sensitive to ddI, nevirapine and Roche protease inhibitor with comparable values as the parent. However, the variants showed reduced sensitivity to ddC of 15-24 fold in different experiments.

The sponsor stated that attempts to isolate a virus resistant to both AZT and 3TC by passaging 3TC-resistant virus in increasing concentrations of AZT while maintaining 3TC pressure or passage of the sensitive HIV-1 RF in simultaneously increasing concentrations of 3TC and AZT were unsuccessful. In a recent report (3) not included in the submission, dual resistant virus isolation only from AZT-resistant virus passaged in the presence of constant concentration of AZT and increasing concentration of 3TC was described. The results indicate that there is no evolutionary barrier for the acquisition of resistance to both AZT and 3TC. Consistent with the in vitro findings it was recently reported that dual resistance was acquired in patients treated with both of these drugs.

The relative ease or time taken to achieve the in vitro selection of variants cannot be used as a measure of the in vivo situation. However, these data may be useful in predicting the likelihood of HIV-1 drug resistance in the clinic.

Genotypic characterization of the phenotypically resistant HIV: The nature of mutation elicited in the HIV RT by in vitro selection of drug resistant isolates with both nucleoside analogues and the nonnucleoside analogue RT inhibitors is to a large extent predictive of the mutation pattern observed in the patients during treatment. The nature of the mutation in 3TC resistant isolates in vitro and cross-resistance effects may also be of predictive value in the clinic. In an effort to define the genetic basis of resistance phenotypically 3TC-resistant HIV-1 variants were generated by standard procedures of serial passing of three lab strains of HIV-1 and one primary isolate in successively increasing concentrations of the test drug.

Table 10. Drug sensitivity profiles of HIV isolates selected in vitro.

Isolate	Status	IC_{50} (μM)				Codon at 184
		3TC	AZT	ddI	ddC	
HIV-1 $_{RF}$	Wild-type	0.16	0.056	7.63	0.22	Met
	Selected	>437	0.015	12.3	0.22	Ile
HXB2	Wild-type	0.39	0.02	3.18	0.42	Met
	Selected	>437	0.001	3.82	0.4	Ile
C19*	Wild-type	1.25	ND	ND	ND	Met
	Selected	92	ND	ND	ND	Ile
HIV-1_{IIIB}	Wild-type	0.8	0.04	19	0.45	Met
	Selected	936	0.05	76	2.5	Val

*The results represents the average of at least two experiments each performed in duplicate

* HIV-1 clinical isolate obtained from an antiretroviral naive patient

Results presented in Table 10 show that all preselection HIV isolates were susceptible to 3TC, AZT, ddI and ddC. Most of the clones selected for 3TC resistance showed resistance of more than 100 fold. In order to determine the genetic changes the nucleotide sequence of the RT gene was determined for the three 3TC-resistant strains. In each case there was a single amino acid substitution in the RT gene of 3TC resistant strains at position 184 from methionine to isoleucine

or valine. The isoleucine substituted at 184 conferred greater specific resistance to 3TC and these viruses retained their ddC and ddI sensitivity. Valine substitution at codon 184 conferred reduced sensitivity to ddI and ddC. The valine substitution at 184 of HIV RT confers resistance to 3TC and low level cross resistance to ddI and ddC.

The biological significance of the amino acid change at position 184 was confirmed by construction of recombinant viruses containing isoleucine at position 184 of RT and subsequent testing of their susceptibilities. HXB2 (F1-3/184I) is the 184 mutant derived from the wild type recombinant virus HXB2 (F1-3). H257-6 was an AZT-treated patient isolate containing mutations at positions 41 and 215 and into this genetic background a recombinant mutation at 184 was introduced to generate H257-6/184I. As shown in table 10 recombinant viruses containing 184 isoleucine showed a >100 fold increase in their IC_{50}. In addition introduction of isoleucine substitution at codon 184 into a background of two known AZT-resistant mutations (amino acid positions 41 and 215) in a clinical isolate resorted the susceptibility of this virus to AZT. There was a decline of approximately 50-fold resistance to AZT. This provides further support for the clinical use of AZT and 3TC together in delaying the development of resistance.

Table 11. 3TC sensitivities of recombinant viruses

Recombinant virus	Amino Acid at Position*			IC50% Values(μM)	
	41	215	184	3TC	AZT
HXB2(F1-3)	wt	wt	wt	0.6	ND#
HXB2 (F1-3/184I)	wt	wt	M	>100	ND
H257-6	M	M	wt	4.4	2.8
H257-6/184I	M	M	M	>100	0.05

* Wild-type amino acids are Methionine 41, Threonine 215 and Methionine 184; mutant amino acids are 41 Leucine, 215 Tyrosine and 184 Isoleucine.
# ND, Not done.

MECHANISM OF ACTION: The presumed mechanism of action of nucleoside analogues is that they are initially metabolized to their respective 5'-triphosphates (dNTPS) by cellular nucleoside and nucleotide kinases. Accordingly the prodrug 3TC is converted into the active drug form, 3TCTP. The 3TCTP competes with natural (physiological) nucleoside triphosphates for the nucleotide binding site on the viral RT. This competition is believed to inhibit the rate of viral DNA synthesis (both RNA-directed and DNA-directed DNA polymerase activities of RT) by decreasing the incorporation of the natural deoxyribonucleotides. In addition, the triphosphates of the nucleoside analogues also serve as alternate substrates and become incorporated into the growing DNA chain of the HIV DNA. Since the incorporated nucleotide of 3TC lacks the 3'-hydroxyl group no phosphodiester bond formation can occur with the next incoming nucleotide; consequently, the DNA chain growth stops. As a result full length proviral DNA synthesis required for integration and establishment of infection is prevented.

Metabolism of 3TC: Upon entering cells nucleoside analogues are subject to biotransformation by cellular enzymes. Therapeutic effects depends on the anabolic metabolism which leads to the formation of their triphosphates and their decay inside cells. These metabolic conversions are host cell and nucleoside analogue dependent. The sponsor investigated the metabolic activation of 3TC in HIV-1 strain RF infected and mock-infected PBMC prepared from different donors. The kinetics of metabolic conversion of radioactive [^{3}H]-3TC in the PBMC was analyzed by HPLC radiochromatography after up to 24 hours of incubation.

The results of the time course of phosphorylation of 3TC show that four components representing the parent nucleoside and its mono, di and triphosphate derivatives were detected in both HIV-1 infected and mock infected cells. The active drug, the 5-triphosphate of 3TC, by 4 hours of exposure represents 40% or more of intracellular phosphates. After 24 hours the triphosphate level dropped to approximately 20%. Although there is variation in the extent of phosphorylation from donor to donor the 3TCTP formation appeared independent of viral infection of the cells.

Studies on the effect of the external concentration of 3TC on the formation of intracellular phosphate derivatives show that the rate of formation of 3TC-TP was linear up to 10 μM and then increased at a slower rate at higher concentrations. The reaction was not saturable at the highest concentration (500 μM 3TC) in the extracellular medium. The intracellular concentration of 3TCTP at 500 μM of 3TC in the extracellular medium was 17 pmol/10^6 PBMC.

The effect of 3TC phosphorylation in uninfected PBMC in drug combination studies was carried out with [^{3}H]-3TC fixed at a concentration of 10 μM and varying concentrations of (5-50 μM) AZT for 4 hours. The results showed that AZT had no substantial effect on the phosphorylation of 3TC in uninfected PBMC indicating that these two drugs can exert antiviral effect independent of each other.

In order to determine the rate of decay of 3TCTP, PBMC were incubated with [^{3}H]-3TC for 4 hours to allow build up of detectable intracellular levels of 3TC-TP. Subsequently, the compound was removed and samples were taken over the following 24 hours to be analyzed by HPLC for 3TCTP. The rates of decay were determined from the peak areas of 3TC-TP and the 50% reduction (half-life) in the peak area determined. The results suggest that the half-life in mock infected cells was 12-15.5 hours and in HIV infected cells was 10.5-13.5 hours. The half-life of 3TC-TP is substantially longer than the half-lives for AZTTP (1 hour), ddC-TP (2.6 hours), d4T-TP (3.5 hours) and similar to that of ddI-TP (12 hours). These in vitro results support the use of 3TC twice daily in the clinical trials.

Catabolic degradation pathway: Nucleoside analogues are also substrates for cellular phosphorolyases which degrade the nucleosides to their bases and sugar. Human platelets are a rich source of these catabolic enzymes. After 93 hours of incubation in platelet enriched medium no detectable degradation of 3TC was detected indicating that it is not (or is a poor) substrate for the deaminases and phosphorolyases.

Effect of 3TC on HIV RT: To define the mechanism of action the sponsor investigated the effect of 3TCTP on the RNA-dependent DNA polymerase activity, The DNA-dependant DNA polymerase activity and DNA chain termination during DNA polymerization. In the analysis of RNA-directed DNA synthesis heteropolymeric (MS-2 RNA) and homopolymeric (poly rI. oligo dC) RNA templates were used. In the analysis of DNA-dependant DNA polymerase activity activated calf thymus DNA template was used and in the case of DNA chain termination studies MS2 RNA template hybridized to a complementary deoxy oligonucleotide 5'-CACTCCGAAGTGCGT-3' was used. In all cases purified recombinant HIV-RT was employed in DNA polymerization.

Table 12. Effect of deoxyribonucleoside triphosphate on HIV RT activity*

Compound	K_i (μM)		Calf thymus DNA as template I_{50}# at [dNTP]=K_m μM
	MS2 RNA as template	Poly rI as template	
3TCTP	12.4	10.6	23.4
ddATP	0.09	NR	0.4
ddCTP	0.33	1.9	1.4
AZT-TP	0.03	NR	0.48
K_m dATP = 1.0 μM K_m dCTP = 1.7 μM K_m dTTP = 1.1 μM			

* The values are an average of 2 or 3 experiments

I_{50} is the concentration of the compound giving half the uninhibited (control) rate

The results presented in Table 12 show that 3TCTP inhibited the RNA-dependent DNA polymerase activity of RT with an apparent inhibition constant ($K_{i\,app}$) of 10.6-12.4 μM depending on the template used. The $K_{i\,app}$ for DNA dependent DNA-polymerase activity was 23.4 μM. Studies on the kinetics of inhibition suggested that 3TCTP inhibition is competitive with respect to the physiological substrate (dCTP) for binding to the HIV-RT.

In tests designed to determine the DNA chain termination by 3TCTP, the DNA polymerization reaction consisted of MS2 RNA template hybridized to a complementary deoxy oligonucleotide 5'-CACTCCGAAGTGCGT-3' primer, the four deoxyribonucleoside triphosphates, 3TCTP and the HIV reverse transcriptase. The synthetic product was then analyzed on a DNA sequencing gel. Comparison of the primer extension products with ddCTP control showed that 3TCTP monophosphates were incorporated at identical positions to the ddCTP control. The results show that incorporation of 3TC monophosphate caused DNA chain termination thus blocking HIV DNA replication in infected cells.

It is important to note that all of the assays used in the mechanism of action studies are artificial systems which bear little resemblance to natural template-primer that the HIV-RT would encounter in the infected cells.

Effect of 3TC on deoxyribonucleotide pools in U937 cells: The relative amounts of deoxynucleoside triphosphates in U937 cells (human monocyte-like cell line) were determined after treatment with high concentration (200 μM) of AZT, which is cytotoxic to most cells, while the cells were treated with noncytotoxic concentrations of 3TC. At this cytotoxic concentration it is expected that AZT would alter the levels of dNTPs to a greater degree than 3TC used at non cytotoxic concentrations. The sponsor's claim that 3TC had not interfered with the normal metabolism of deoxyribonucleotides is based on this poorly designed experiment and the conclusion is not supported. Furthermore, these studies were conducted in a single cell line with single time point exposure of 12 hours and the general conclusion regarding all deoxyribonucleotides is inappropriate.

Effect of 3TC on the mitochondrial DNA content: In studies designed to test the potential incorporation of 3TC-TP into the mitochondrial DNA, the sponsor examined the kinetics of incorporation and excision of 3TCMP into the DNA by the mitochondrial DNA polymerase-γ. As expected 3TCTP served as a substrate for the mitochondrial polymerase and was incorporated into the growing DNA chain. The sponsor then examined the ability of the enzyme for its proof reading ability (by virtue of the polymerase associated 3'-5' exonuclease activity) by testing excision of the incorporated 3TCMP. In parallel experiments DNA products with chain terminating ddCMP and the non-chain terminating natural dCMP were also included. The rates of excision by the mitochondrial DNA polymerase-γ in all three cases were similar. Based on this data the sponsor claimed that 3TCMP was excised from the mitochondrial DNA and this reflected in the low mitochondrial cytotoxicity. The results show that in terms of incorporation and excision the DNA polymerase-γ does not distinguish the natural substrate dCMP and the chain terminating analogues ddCMP or 3TC-MP. The evidence does not support the sponsor's conclusion but argues that 3TCMP effect on mitochondrial polymerase is similar to that of ddCMP.

Effect of 3TCTP on human cellular DNA polymerases: The relative inhibitory effect of 3TC-TP on 3 major cellular DNA polymerases was compared with nucleoside analogues AZT, ddC and ddI. Cellular DNA polymerase α, β and γ are believed to play a major role in semi-conservative DNA replication, DNA repair synthesis and mitochondrial DNA synthesis, respectively. The effect of the nucleoside analogues on the DNA polymerases δ and ϵ was not investigated as these enzymes are difficult to purify. The results presented in the Table 13 show AZTTP is the least

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inhibitory to the 3 DNA polymerases. Polymerase α was least inhibited by any of the four nucleoside analogue substrates. Polymerases β and γ are significantly inhibited by ddATP and ddCTP but 3TCTP is less inhibitory than ddATP and ddCTP. The general conclusion from this report is that 3TCTP has a favorable inhibitory profile on the tested DNA polymerases by being less inhibitory than ddATP and ddCTP.

Table 13. Effect of dideoxyribonucleoside triphosphates on human DNA polymerases

DNA Polymerase	I_{50} (50% inhibitory concentration of the compound in μM)			
	3TC-TP	ddATP	ddCTP	AZTTP
Polymerase α	175	>500	>500	>850
Polymerase β	25	0.75	0.056	>475
Polymerase γ	44	0.068	0.031	147

The K_m values of each of the 4 natural nucleoside triphosphates for DNA polymerases α, β and γ were approximately 3.5 μM, 0.20 μM and 2.5 μM, respectively.
I_{50} = Concentration of the compound giving half the uninhibited control rate.

CONCLUSIONS: 3TC is metabolically optimized to biotransform into the active drug form 3TCTP. At identical input concentrations, 3TC builds higher concentrations of its triphosphate than any of the clinically available nucleoside analogues AZT, ddC, ddI and d4T. This desired effect seem to be achieved by virtue of its stereochemical nature, by being a poor substrate for catabolic/degradative enzymes which otherwise would break it down to its component sugar and base and by being a superior substrate for anabolic/activation enzymes essential for its conversion into the active drug form. In addition, the active form of the drug 3TCTP has a half-life of 10-15 hours which is much longer than the half-lives of AZTTP, ddCTP and d4TTP but some what similar to ddATP. This longer half-life allows BID dosing of 3TC in HIV-infected individuals. The major disadvantage of 3TC, however, is that the concentrations of its triphosphate required to inhibit HIV is greater than the triphosphates of AZT, ddC and d4T but similar to that of ddA.

The antiviral target of 3TC is HIV reverse transcriptase. The data from virologic studies showing inhibition of HIV by 3TC, the data from the multiplicity of infection studies showing that 3TC acts on a target specific to the virus, the time of addition studies showing that 3TC acts early after infection (within the first 6 hours) and lack of effect post 6 hours, and the data showing lack of

effect on virus production in chronically infected cells are consistent with HIV RT being the target of 3TC. Biochemical studies on the kinetics of enzyme inhibition and viral DNA synthesis support that 3TC competes with the physiological substrate for the nucleoside binding site thus slowing the rate of DNA polymerization by HIV RT. Chain termination studies by HIV RT show that 3TCMP was incorporated at sites identical to the natural substrate dCMP and terminate DNA chain from further elongation. The data from the combined virologic and biochemical studies confirm that the target of 3TC is the HIV RT and it inhibits the enzyme by competing with natural nucleoside substrates and terminates the viral DNA synthesis by incorporating into the growing DNA chain.

3TC is an incomplete anti-HIV agent. The consequences of specific HIV RT targeting limits the antiviral effect of 3TC to the early phase (acute infection) of the virus replication cycle with no effect on the late phase (chronic infection). In the continuous presence of 3TC only new rounds of infection and viral spread are inhibited without effect on virus production from proviral DNA of latently infected cells. In addition 3TC which requires activation by phosphorylating enzymes for exerting its effects further limit its antiviral activity to those cells that are endowed with the capacity to phosphorylate 3TC. Terminally differentiated cells such as macrophages with no replication potential either lack or shed the very enzymes required for the activation of 3TC to serve as a drug. These reservoirs of HIV in infected individuals go unchallenged by 3TC.

HIV rapidly develops resistance against 3TC. Cell culture studies show that HIV rapidly adapts to overcome 3TC pressure with loss of susceptibility to the drug (phenotypic resistance). The acquisition of resistance has been attributed to a single amino acid substitution in the viral RT (genotypic resistance) at codon 184 from methionine to isoleucine or valine. Emergence of resistance is an unavoidable consequence of treatment with anti-HIV drugs. The combination of an inherently high mutation rate (approximately $1x10^{-4}$ per replication) and exposure to 3TC permits selection of resistant variants. The codon 184 is in the highly conserved Tyr, Met^{184}, Asp, Asp (YMDD) region which is adjacent to the putative catalytic site of the HIV-RT. What is remarkable of 3TC is the very rapid rate of emergence of resistance and in this regard resembles the rate of emergence of resistance elicited by experimental nonnucleoside RT inhibitors. Paradoxically, the mutation at codon 184 of RT restores AZT sensitivity to AZT resistant HIV. The restoration of sensitivity suggested potential combination therapy for antiviral activity.

In vitro 3TC resistance is predictive of in vivo 3TC resistance. Analysis of viral RNA from 3TC monotherapy showed the emergence of resistance mutation (3.4) as early as 7 days after the initiation of treatment and by 8 weeks all of the 20 patients in the trial had the resistant strain.

Similarly, analysis of blinded clinical RNA samples collected longitudinally from '3001 trial (50 of 129 analyzed, 25 individuals from AZT monotherapy arm and 25 individuals from 3TC+AZT combination arm) showed that the 184 mutation was detected in the combination by 8 weeks in 95% of the patients (5). At week 24 more of the combination group contained wild type at the AZT resistance codons (75% compared with 31% for AZT alone) All samples except one from the combination group contained the 184 mutation and none of the AZT group contained this mutation. The data suggest combination therapy with 3TC+AZT delay the emergence of mutations conferring resistance to zidovudine.

3TC resistance is apparently beneficial. Clinical resistance emerges in ≥95% of the treated patients as early as 8 weeks in both 3TC monotherapy and combination therapy with AZT. Therapeutic benefit as measured by surrogate marker responses (viral RNA copy number and CD_4+ T cells) is derived in combination therapy of 3TC with AZT up to 24 weeks (up to about 50 weeks in some patients). The mutation at 184 of RT restored AZT sensitivity to AZT resistant virus and also delayed the emergence of AZT resistant mutations thus sustaining the antiviral activity of AZT longer than otherwise. It is interesting to note that in monotherapy with 3TC (n=20) although there was 3TC resistant mutation as early as 1 week the viral RNA and p24 remained below the base line in most patients up to the 12 weeks tested. The benefit may not last beyond approximately one year since co-resistance to both the drugs (with multiple mutations) has been reported (6) with rise in viral RNA to the base line.

PHASE 4 CONSIDERATIONS: In vitro and in vivo studies indicate the emergence of co-resistance to both 3TC and AZT, suggesting no evolutionary barriers for the acquisition of simultaneous resistance to the combination. In future clinical trials including the clinical endpoint trials in longitudinally collected patients samples please examine HIV for phenotypic resistance and viral RNA for genotypic resistance (to both of the drugs) in a subset of patients.

In HIV-infected patients treated with non-nucleoside and nucleoside RT inhibitors including 3TC (3,5,7) drug resistant mutants of HIV emerge. The appearance of resistant mutants in serum is followed by an increase in viral RNA and by a decrease in CD_4+ T cells. Please evaluate the effect of the emergence HIV variants co-resistant to AZT and 3TC on the HIV RNA copies and CD_4 + T cells.

During the course of anti-HIV therapy, in addition to drug resistance in HIV changes in biological phenotype, a switch between non-syncytial inducing (NSI) and syncytial inducing (SI) phenotype has been observed. A recent report (7) described the conversion of NSI to SI during

3TC treatment and the presence of SI was clearly associated with an increased risk of developing 3TC resistance. Please investigate in a sub set of patient population the potential link between NSI/SI viral phenotype and the emergence of resistance.

RECOMMENDATIONS: The microbiology section of the draft label as currently written is acceptable (lines 41-69, copy attached). With respect to microbiology this the NDA is approved.

References:

1. Wainberg M A.et al., Fourth workshop on viral resistance. Annapolis, MD September 10-12.1995, p 35
2. Drosopoulos W C. et al., Fourth workshop on viral resistance. Annapolis, MD September 10-12.1995, p 36
3. Schuurman R. et al. J Infect Dis. 171:1411-1419, 1995
4. Van Leeuwen R. et al. J Infect Dis. 171:1166-1171, 1995
5. Larder B A. et al. Science 229: 696-699, 19955.
6. HIV drug resistance: Fourth International workshop. Sardinia, Italy 6-9 July 1995, p52
7. Wainberg M A.et al., AIDS 9: 351-357, 1995

Narayana Battula

Narayana Battula, Ph.D.
Microbiologist

CONCURRENCES:

HFD-530/Deputy Dir _____Signature_____Date

HFD-530/SMicro _____Signature_____Date

CC:

HFD-530/Original IND
HFD-530/Division File
HFD-530/MO
HFD-530/Pharm
HFD-530/Chem
HFD-530/SMicro
HFD-530/Review Micro
HFD-530/Kallgren, D.

Draft Label: Microbiology

CLINICAL PHARMACOLOGY:

Mechanism of Action: Lamivudine is a synthetic nucleoside analogue. *In vitro* studies have shown that, intracellularly, lamivudine is phosphorylated to its active 5'-triphosphate metabolite (L-TP), which has an intracellular half-life of 10.5 to 15.5 hours. The principal mode of action of L-TP is inhibition of HIV reverse transcription via viral DNA chain termination. L-TP also inhibits the RNA- and DNA-dependent DNA polymerase activities of reverse transcriptase (RT). L-TP is a weak inhibitor of mammalian α-, β-, and γ-DNA polymerases.

Microbiology: *Antiviral Activity In Vitro:* The relationship between *in vitro* susceptibility of HIV to lamivudine and the inhibition of HIV replication in humans has not been established. *In vitro* activity of lamivudine against HIV-1 was assessed in a number of cell lines (including monocytes and fresh human peripheral blood lymphocytes) using standard susceptibility assays. IC_{50} values (50% inhibitory concentrations) were in the range of 2 nM to 15 µM. Lamivudine had anti–HIV-1 activity in all acute virus-cell infections tested. In HIV-1–infected MT-4 cells, lamivudine in combination with zidovudine had synergistic antiretroviral activity. Synergistic activity of lamivudine/zidovudine was also shown in a variable-ratio study.

Drug Resistance: Lamivudine-resistant isolates of HIV-1 have been selected *in vitro*. The resistant isolates showed reduced susceptibility to lamivudine and genotypic analysis showed that the resistance was due to specific substitution mutations in the HIV-1 reverse transcriptase at codon 184 from methionine to either isoleucine or valine. HIV-1 strains resistant to both lamivudine and zidovudine have been isolated.

Susceptibility of clinical isolates to lamivudine and zidovudine was monitored in controlled clinical trials. In patients receiving lamivudine monotherapy and combination therapy with lamivudine plus zidovudine, HIV-1 isolates from most patients became phenotypically and genotypically resistant to lamivudine within 12 weeks. In some patients harboring zidovudine-resistant virus, phenotypic sensitivity to zidovudine by 12 weeks of treatment was restored. Combination therapy with lamivudine plus zidovudine delayed the emergence of mutations conferring resistance to zidovudine.

OCT 19 1995

NDA 20-564
NDA 20-596

Glaxo Wellcome Incorporated
ATTN: David Cocchetto, Ph.D.
Five Moore Drive
Research Triangle Park, NC 27709

Dear Dr. Cocchetto:

Please refer to your New Drug Applications (NDA) submitted June 30, 1995 under section 505 (b) of the Federal Food, Drug, and Cosmetic Act for Epivir Tablets and Epivir Oral Solution.

We also refer to your submission dated September 12, 1995, which requested comment on replacing the 3TC proprietary name with Epivir . Our Division has no objections to Epivir as the proprietary name. For your information, comments from the CDER Labeling and Nomenclature Committee (L&NC) are provided below:

1) The prefix "Epi-" is closely associated with products that contain epinephrine and could therefore lead to misinterpretation. However, the L&NC recognizes that there exist many trademarked "Epi-" products unrelated to epinephrine (Epiderm, Epifoam, Epilyt).

2) The "-vir" portion of the trademark is an official USAN stem and the L&NC has supported the USAN guideline of discouraging the practice of incorporating USAN syllables into proprietary names. However, the L&NC recognizes that "vir" has been included in numerous anti-viral proprietary names (Foscavir, Retrovir, Zovirax, Famvir).

If you have any further comments or questions please call Deborah L. Kallgren, Consumer Safety Officer, at (301) 443-9553.

Sincerely yours,

10.19.95

David W. Feigal, Jr., M.D., M.P.H.
Director
Division of Antiviral Drug Products
Office of Drug Evaluation II
Center for Drug Evaluation and Research

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
Rockville MD 20857

NDA 20-564
NDA 20-596

AUG 22 1995

TRANSMITTED VIA FACSIMILE

Glaxo Wellcome Inc.
David M. Cocchetto, Ph.D.
Regulatory Affairs
Five Moore Drive
Research Triangle Park, NC 27709

Re: Proposed proprietary name(s) for lamivudine

Dear Dr. Cocchetto:

Please refer to your letter of August 8, 1995, submitted under section 505(b) of the Federal Food, Drug, and Cosmetic Act for 3TC™ (lamivudine) 150 mg Tablets and 3TC™ (lamivudine) 10 mg/mL Oral Solution.

We have completed our review of this correspondence, providing for the trade name research carried out by
The Division concurs with the guidance from the Labeling and Nomenclature Committee that 3TC is not an acceptable proprietary name.

In addition to concerns previously raised by the Labeling and Nomenclature Committee, our Division is concerned that a proprietary name such as '3TC', which begins with a numeral, may cause confusion when orders are given verbally to a pharmacy. This concern was also voiced by approximately 1/3 of the pharmacists in the (Q7 and Q9).

Finally, your argument that two names are less confusing than three is significantly compromised by the proposal that lamivudine indicated for hepatitis B would have a separate trade name. A recent news clip from Reuters highlights the potential for confusion:

Please advise the Agency on how you intend to proceed. In your response please outline your plans for tablet imprinting.

If you would like to discuss this issue further, we would propose a teleconference at your earliest convenience.

Should you have any questions, please contact Debbie Kaligren, Consumer Safety Officer at (301) 443-9553.

Sincerely yours,

8-22-95

David W. Feigal, Jr., M.D., M.P.H.
Director
Division of Antiviral Drug Products
Office of Drug Evaluation II
Center for Drug Evaluation and Research

END

J.H.M. RESEARCH &DEVELOPMENT, INC. 5776 SECOND STREET,N.E. WASH. DC 20011