CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-500

Microbiology Review(s)

Microbiology Review
Division of Antiviral Drug Products (HFD-530)

NDA: 21-500	Serial #: 000	**Reviewer**:	N. Battula
Date submitted:	September 3, 2002	**Date received**:	September 5, 2002
Date assigned:	September 9, 2002	**Date reviewed**:	June 5, 2003

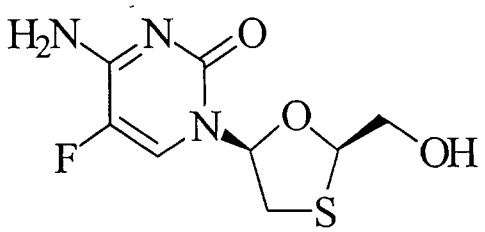
Additional submissions reviewed:

	Supplement #	Date of Correspondence	Date of Receipt
NDA: 21-500	B1	03-25-03	03-26-03
NDA: 21-500	N-000 (C)	04-01-03	04-02-03
NDA: 21-500	BL	04-16-03	04-17-03

Sponsor: Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404

Product name(s):
Non-proprietary: Emitricitabine, FTC
Proprietary: Emtriva™
Chemical: 5-fluoro-1-(2R,5S)-[2-(hydroxymethyl)-[1,3]-oxathiolan-5-yl] cytosine

Structural formula:



Molecular formula: $C_8H_{10}FN_3O_3S$

Molecular weight: 247.24

Dosage form/route of administration: Capsule 200 mg / Oral

Indication: Treatment of HIV-1 infection in adults in combination with other antiretroviral agents

Related documents: IND [redacted]

BACKGROUND: Triangle Pharmaceuticals submitted this original New Drug Application to the Division of Antiviral Drug Products (HFD-530), FDA in support of Coviracil capsules for the treatment of HIV-1 infection. Subsequent to the submission of this application, Triangle Pharmaceuticals was acquired by Gilead Sciences, Inc. of Foster City, California. The applicant, Gilead Sciences, Inc., is seeking an indication for the treatment of HIV-1 infection in combination with other antiretroviral agents. In support of the indication, the applicant submitted 48-week efficacy and safety data from two Phase III clinical studies, FTC-301A and FTC-303. The requested indication is based on the analysis of plasma HIV-1 RNA levels and CD4+ cell counts from controlled studies of Emtriva for 48-week duration in antiretroviral-naïve patients and antiretroviral-treatment-experienced patients who were virologically suppressed on an HIV treatment regimen. The primary focus of this review is the non-clinical and clinical microbiology aspects of emitricitabine. The drug names FTC or emitricitabine are interchangeably used throughout this review.

To date there are 18 drugs approved for the treatment of HIV-1 infection. These 18 drugs fall under 4 different mechanistic classes: 1. nucleoside analogue reverse transcriptase inhibitors (NRTIs)*, 2. non-nucleoside reverse transcriptase inhibitors (NNRTIs), 3. protease inhibitors (PIs), and 4. fusion inhibitors. Emitricitabine is an NRTI. Currently, there are three NNRTIs and seven NRTIs approved for the treatment of HIV infection. Out of the seven NRTIs, four are pyrimidine nucleoside analogues (lamivudine, stavudine, zalcitabine, and zidovudine) and three are purine nucleoside analogues (abacavir, didanosine, and tenofovir). Among the pyrimidine nucleoside analogues, two of them (stavudine and zidovudine) are analogues of the natural (physiological) nucleoside substrate, thymidine, and two of them (lamivudine and zalcitabine) are analogues of the natural nucleoside substrate, cytosine. FTC is also is a nucleoside analogue of the natural (physiological) nucleoside, cytosine.

The current standard of care for the treatment of HIV infection is combination therapy that combines drugs from two or three of the available mechanistic classes. This combination treatment often referred to as highly active antiretroviral therapy (HAART) leads to dramatic decreases in viral load accompanied by a marked decrease in the morbidity and mortality associated with HIV/AIDS. In spite of reduction in viral load due to HAART, the virus is able to develop resistance to all of the approved antiretroviral agents. The inability of the currently available drugs to completely suppress viral replication, the associated toxicity during chronic use, drug intolerance, inadequate adherence and alterations in bioavailability limit the utility of current HAART regimens. As a result, there often is virologic failure with the emergence of virus resistant to one or

* For abbreviations, please see Appendix-1

more of these drugs. Therefore, there is a perennial need for additional new drugs with increased efficacy, decreased toxicity, lower pill burden, and once-a-day dosing.

Continued viral replication in the presence of antiretroviral therapy (ART) leads to additional mutations resulting in a broad class cross-resistance with no drug options for the treatment of HIV. Therefore, key challenges to improving ARV therapy include developing more effective drugs. FTC, the candidate drug of this application, is a pyrimidine nucleoside analogue which acts by inhibiting HIV reverse transcriptase mediated viral DNA synthesis, a prerequisite to the establishment of viral infection. Therefore, FTC, like other HIV RT inhibitors, can affect one part of the virus life-cycle mediated by the viral RT, i.e., inhibit virus spread by blocking new rounds of infection only. HIV RT inhibitors, however, have no effect on the production of virus from an integrated provirus. Therefore, nucleoside analogues can reduce the virus load incompletely by failing to block virus production from the reservoirs of already infected, long-lived cells in HIV positive subjects.

The microbiology portions of the FTC NDA review evaluations include: 1. Antiviral activity in vitro of FTC against laboratory and clinical isolates of HIV, 2. Antiviral activity in vitro in combination with other antiretroviral agents, 3. Cytotoxicity of FTC, 4. Metabolism and mechanism of action of FTC, 5. Emergence of resistance to FTC in HIV grown in cell culture, and 6. Emergence of resistance to FTC in vivo in clinical studies of HIV-infected subjects. Based on the information available in the open literature and the information that the applicant provided, a microbiology portion of the label was generated reflecting the current understanding of the microbiology aspects of FTC. The original microbiology section of the label submitted by the applicant is also included in this review to provide reference for the identification of changes made after review of the NDA.

SUMMARY

In vitro antiviral activity of FTC: The applicant provided a summary of antiviral activity data primarily extracted from published literature along with some studies that they have conducted. The references cited and reports provided include antiviral activity evaluations in a variety of host cell-virus test systems that provided a perspective into the antiviral activity of FTC. The HIV strains used in these studies include the conventionally used laboratory and clinical isolates, and the host cells used include lymphoblastoid cell lines, peripheral blood mononuclear cells (PBMCs), and the MAGI-CCR5 cell line. Results of the studies indicate that emitricitabine displays antiviral activity in these different cell-virus test systems.

Data presented in Table 1 (Vol. 1.3, p. 30) indicates that of the antiviral activity of FTC, represented as the 50% inhibitory concentration (IC_{50}) on HIV-1 strains, varied from 0.001 to 0.50 μM. In these antiviral activity studies, different viral strains and cell types were used. The MOI used varied from 0.001 to 0.1, the length of time of infection varied from 45 minutes to 5 hours, and a variety of assays were used to determine the degree of inhibition of HIV replication. The results thus indicate that the apparent IC_{50} value of emitricitabine was in the range of (0.001 to 0.50 μM) against the laboratory strains of HIV. In other studies, the applicant reported the IC_{50} values of emitricitabine against clinical isolates of HIV-1 (see Table 1) were in the range of 0.62 to 0.64 μM.

Table 1. Antiviral activity of emitricitabine on laboratory strains of HIV-1 and HIV-2

Virus	Cell Type	Assay	IC_{50} (μM)
$HIV\text{-}1_{IIIB}$	CEM	RT	0. 1 [a,b]
	MT-4	Cytoprotection	0.5 [a,b]
	PBMC	RT	0.1[b]
$HIV\text{-}1_{LAI}$	CEM	RT	0.009[a]
	HT4-6C	RT	0.02[a]
	PBMC	P24	0.009[b]
	PBMC	RT	0.001[d]
$HIV\text{-}1_{LAI}$	CEM	Cytoprotection	0.04[c]
	MT-2	Cytoprotection	0.62[c]
	PBMC	P24	0.03[c]
	PBMC	P24	0.0014[c]
$HIV\text{-}2_{ZY}$	MT-4	RT	1.5[a]
	CEM	RT	0.1[a]
$HIV\text{-}2_{ROD2}$	PBMC	P24	0.007[b]

a. HIV-1 infected MT-4, CEM and HT4-6C (2) cells were washed three times in fresh media, resuspended and incubated with various concentrations of emtricitabine for 6 to 14 days. Antiviral activity was determined by measuring the reverse transcriptase activity in the cell supernatant (3).

b. Virus replication (1) was monitored by RT activity in PBMC supernatant and by p24 antigen in CEM cell supernatant. Cytoprotection of MT-4 cells was monitored by propidium diiodide assay.

c. IC_{50} values were determined in MT-2 cells , CEM cells and PBMCs (4). In the determination of antiviral activity in MT-2 and CEM cells,

cells/well) and incubated in the presence of various concentrations of emtricitabine for 4 days. The final volume in each well was 200µl. At the end of the incubation period antiviral activity was determined by analysis of p24 antigen in the culture supernatant.

d. Antiviral activities were determined (5) in activated human PBMCs infected with HIV- 1_{LAI} at an MOI of 0.1. Emitricitabine was added to the cells 45 min after infection and the concentration of RT in the supernatant was determined as described in footnote c.

e. Replication of HIV-1 in MT-4 cells was determined (6) using the MTT assay.

The applicant submitted summary data from published reports that compared the anti-HIV activity of emitricitabine with that of lamivudine. The results in Table 2 (Vol. 3.1, p. 31) show that emitricitabine was more potent (IC_{50} = 0.01 to 0.09 µM) than lamivudine (IC_{50} = 0.07 to 3.2 µM) in these different virus-cell test systems using different assays. However, the applicant has not reported the relative cytotoxicity of these compounds in parallel experiments to reflect the relative therapeutic index of these compounds.

Table 2. Relative antiviral activity of emitricitabine and lamivudine against laboratory strains of HIV-1

HIV-1 strain	Cell line	IC_{50} (µM)		
		Emitricitabine	Lamivudine	Sensitivity Ratio[d]
LAI[a]	PBMC	0.018	0.19	11
IIIB[b]	PBMC	0.01	0.07	7
IIIB[b]	MT-4	0.5	3.2	6
LAI[a]	MT-2	0.3	1.6	5
HXB2[c]	MT-4	0.09	0.24	3
LAI[a]	$CD4^+$HeLa	0.06	0.18	4

a.Emtricitabine was tested for antiviral activity in PBMCs (7) using a p24

(7).

c.Antiviral activity was determined by plaque reduction assay (8) in HT4LacZ-1 cells. The IC_{50} values represent the concentration of compound that inhibited virus plaque formation by 50%. The results represent a mean of at least two replicate assays.

d.Defined as the ratio: IC_{50} (lamivudine)/IC_{50} (emtricitabine).

The effect of MOI, time of addition and protein binding on the antiviral activity of FTC.

1. The effect of MOI on the antiviral activity of emtricitabine was assessed in MAGI-CCR5 (19) and in PBMCs. In MAGI-CCR5 an increase of about 6-fold in virus titer (9) resulted in no change in the IC_{50} value (10). In PBMCs increasing the MOI from 0.01 to 0.1 caused approximately a 10-fold higher IC_{50} value (11). Increasing the MOI above 0.1 produced no further change.

2. In studies on the effect of time of addition, various concentrations of emtricitabine were added to MAGI-CCR5 cells infected with HIV-1_{LAI} (12) and the IC_{50} values determined at -1, 0, + 1, +2, +4, +6, and +8 hours post-infection. Results of the study showed that addition of FTC for up to 6 hours post-infection maximal antiviral activity was maintained. However, adding FTC 8 hours post-infection resulted in a decrease in antiviral activity, consistent with the mechanism of action of NRTI inhibition of HIV.

3. The potential effect of serum proteins and α-1 acidic glycoprotein on the antiviral activity of FTC was evaluated in CEM cells. Cells were infected with HIV-1_{LAI} and maintained in culture in the presence of various concentrations of emtricitabine in standard 10% fetal bovine serum (FBS) containing media, or in modified media where FBS was replaced with 25% human serum albumin, or 25% human serum albumin plus 1 mg/ml alpha-1 acidic glycoprotein (AAG). Neither the medium containing 25% human serum albumin alone or in combination with 1.0 mg/ml AAG affected the antiviral activity of FTC (13), indicating that emtricitabine activity was not affected by binding to serum proteins.

Antiviral activity of FTC in combination with other antiretroviral agents: The applicant submitted in vitro antiretroviral drug combination effect data from their own studies and from published literature. The combination effect was evaluated on the basis of isobologram analysis (14), using the MacSynergy™ II (21) software program and the summary results are presented in Tables 3 (Vol. 1.3, p. 36) and 4 (Vol. 1.3, p. 37). Results of isobologram analysis with two-drug combinations (1, 15, 16, 17, 20) of emitricitabine with each of the approved NNRTIs: delavirdine, efavirenz and nevirapine, with each of the approved NRTIs: abacavir, didanosine, lamivudine, stavudine, zalcitabine, and zidovudine, and with each of the PIs: amprenavir, indinavir, nelfinavir and ritonavir indicated that the combinations were additive to synergistic in antiviral activity. Similarly, isobologram analysis of three-drug combination assays (18) summarized in Table 4 (Vol. 3.1, p. 36) showed additive to synergistic antiviral activity.

Table 3. Antiviral activity of emitricitabine in combination with other antiretroviral agents

Compounds	Class	Cell line	HIV strain	Results
Delavirdine	NNRTI	MT-2	LAI	Additive to synergistic
Efavirenz	NNRTI	MT-2	LAI	Additive to synergistic
Nevirapine	NNRTI	MT-2	LAI	Additive to synergistic
Abacavir	NRTI	MT-2	LAI	Additive to synergistic
Didanosine	NRTI	MT-4	IIIB	Synergistic
Didanosine	NRTI	MT-2	IIIB	Additive
Didanosine	NRTI	MT-2	LAI	Additive to synergistic
Lamivudine	NRTI	MT-2	LAI	Additive to synergistic
Stavudine	NRTI	MT-2	IIIB	Additive
Stavudine	NRTI	MT-2	LAI	Additive to synergistic
Zalcitabine	NRTI	MT-4	IIIB	Synergistic
Zalcitabine	NRTI	MT-2	IIIB	Additive
Zidovudine	NRTI	MT-4	IIIB	Synergistic
Zidovudine	NRTI	MT-2	IIIB	Additive
Zidovudine	NRTI	MT-2	LAI	Additive to synergistic
Amprenavir	PI	MT-4	IIIB	Synergistic
Amprenavir	PI	MT-2	LAI	Additive to synergistic
Indinavir	PI	MT-2	LAI	Additive to synergistic
Nelfinavir	PI	MT-2	LAI	Additive to synergistic
Ritonavir	PI	MT-2	LAI	Additive to synergistic

Table 4. Antiviral activity of emitricitabine and lamivudine in 3-drug combination with nevirapine and stavudine

Compound 1	Compound 2	Nevirapine	Results
FTC	d4T	0	Synergistic
FTC	NVP	0	Synergistic
FTC	d4T	0.01	Synergistic
3TC	d4T	0	Synergistic
3TC	NVP	0	Synergistic
3TC	d4T	0.01	Synergistic

In the 3-drug combination activity studies PBMCs (21) were infected with HIV-1_{LAI} at a MOI of 0.001. The cells were incubated for 4 hours and plated in the following layout:

Compound 1 and Compound 2 combinations:

FTC (0.1 0 µM- 0.39 nM), d4T (0.50 µM -7.8 nM)
3TC (0.25 µM - 0.97 nM), d4T (0.50 µM -7.8 nM)
FTC (0.10 µM - 0.39 nM), d4T (0.50 µM -7.8 nM) with 0.01 µM NVP overlay
3TC (0.25 µM - 0.97 nM), d4T (0.50 µM -7.8 nM) with 0.01 µM NVP overlay
FTC (0.10 µM - 0.39 nM), NVP (0.10 µM -1.56 nM)
3TC (0.25 µM - 1.95 nM), NVP (0.10 µM -1.56 nM)

The cells were incubated with compound for three days and the level of viral replication was monitored using HIV-1 p24 antigen ELISA. Nine plates were tested for each compound combination. Median inhibition values were determined and entered into the MacSynergy ™ II program (22). This program calculates a theoretical additive value for each compound combination based on the values generated by the compounds alone. The theoretical additive values are subtracted from the experimental values generated by each compound combination to give a value of synergy (positive value) or antagonism (negative value). These synergy and/or antagonism values are plotted with their corresponding compound combinations.

Calculating the IC_{50} ratios of each compound in combination with the other compounds or alone generates an isobologram. The results are plotted on a graph. Points falling on this line are considered to be additive. Points lying above the line are considered to be antagonistic. Points lying below this line are considered to be synergistic

Antiviral activity of emitricitabine on clinical isolates of HIV: The applicant referred (2, 4) to the comparative antiviral activity data of emtricitabine and lamivudine on 4 clinical isolates of HIV-1. The results of the antiviral activity of these clinical isolates in PBMCs presented in Table 5 (Vol. 3.1, p. 39), indicate that the IC_{50} value for emitricitabine was in the range of 0.002 to 0.02 µM and is similar to the antiviral activity on laboratory isolates.

Table 5. Inhibition of replication of HIV-1 clinical isolates by emitricitabine*

Virus	IC_{50} µM	
	Emitricitabine	**Lamivudine**
J6[a]	0.002	0.01
2:DR2[a]	0.002	ND[c]
WT-Pre-AZT[b]	0.008	ND
WT-MKC09-day 29[b]	0.02	ND

* Data for a were from reference 2 and data for b were from reference 4 and from references therein. In these studies PBMCs were infected with the clinical isolates at a MOI of 0.01 for 3 to 5 hours at 37°C in culture media. Cells were then plated in a 96-well microtiter plate (1×10^5 cells per 200 µl well) and incubated in the presence of various concentrations of emtricitabine for 4 days. At the end of the incubation period antiviral activity was determined by quantification of p24 antigen in the culture supernatant.

c. ND = Not Determined.

The applicant provided data on the effect of emitricitabine on cell-to-cell transmission of viral infection from HIV-1 infected PBMCs to uninfected PBMCs. In this study, PBMCs from HIV-1 infected patients (23) collected from sixty-one HIV-1 infected individuals drawn between 1986 to 1991 were co-cultured with PBMCs isolated from uninfected donors. The naturally infected PBMCs served as a source of a diversified population of virus not selected by in vitro propagation. At the end of the co-culture period, viral replication was evaluated by HIV-1 p24 ELISA. Antiviral activity expressed as mean IC_{50}, IC_{90}, and IC_{99} values (Table 6, Vol. 3.1, p. 39) show that the IC_{50}, IC_{90} and IC_{99} values were 0.0085, 0.055 and 0.43 µM, respectively. A potency ranking (based on IC_{90} values) indicates that emtricitabine was the most potent compound followed by zalcitabine, lamivudine, zidovudine, TIBO, and didanosine. The applicant attributed the low potency ranking for zidovudine to be the result of inclusion of PBMCs from AZT experienced patients in the co-culture.

Table 6. Relative antiviral activity of RT inhibitors in HIV-1 infected PBMC using a co-culture method[1]

Inhibitor	IC_{50} µM	IC_{90} µM	IC_{99} µM
FTC	0.0085	0.055	0.43
3TC	0.11	0.3	0.85
ddC	0.011	0.074	0.6
ddI	0.76	6.4	65.8
AZT	0.055	0.53	6.4
TIBO R82913	0.17	0.67	2.95

1. Standard sources of clinical samples for HIV-1 infection were established using 61 reference cell specimens obtained from patients infected for 1 to 7 years. The samples were assayed by limiting dilutions. Freshly thawed uninfected stimulated PBMCs were added as targets for the *de novo* infections and subsequent viral spread. To determine antiviral activities, compounds were added to the culture in 5-fold serial dilutions at least 2 hours before co-cultivation. Antiviral compounds were in the media throughout the co-cultivation period. Supernatants were tested for HIV-1 by p24-ELISA after 12 to 14 days in co-culture. The final results are expressed as IC_{50}, IC_{90} and IC_{99} values and were derived from computer generated median effect plot of dose response data.

The applicant evaluated the antiviral activity of emtricitabine on different subtypes of HIV-1 clinical isolates (group M and 0). IC_{50} values were determined in MAGI-CCR5 and PBMCs (7). Within each host cell system, the IC_{50} values were comparable for all of the tested subtypes of HIV-1. Results presented in Table 7 (Vol. 3:1, p. 40) show that emitricitabine was active against group M isolates with an IC_{50} value ranging from 7.0 to 75 nM. In this comparative study emtricitabine appeared more active than didanosine and lamivudine, and had activity comparable to that of zidovudine for all subtypes of HIV-1 tested. Overall the IC_{50} values of HIV-l subtypes was 2- to 5-fold higher in MAGI-CCR5 that in PBMCs.

Table 7. Antiviral activity of NRTIs against HIV-1 group M and group 0 isolates in PBMC and MAGI-CCR5 cells

Isolate	Subtype	Host Cell	IC_{50} µM			
			AZT	3TC	ddI	FTC
Group M						
RW/92/008	A	PBMC[a]	0.008	0.054	0.26	0.012
		MAGI-CCR5[b]	0.085	0.20	3.0	0.055
	C	PBMC	0.035	0.027	0.49	0.017
		MAGI-CCR5	0.033	0.17	0.95	0.032
	D	PBMC	0.003	0.026	0.21	0.007
		MAGI-CCR5	0.035	0.11	1.70	0.030
	E	PBMC	0.039	0.069	0.50	0.028
		MAGI-CCR5	0.080	0.15	1.50	0.065
	F	PBMC	0.003	0.022	0.34	0.009
		MAGI-CCR5	0.045	0.15	1.50	0.050
	G	PBMC	0.008	0.090	0.34	0.030
		MAGI-CCR5	0.150	0.18	2.50	0.075
Group 0						
BCF03	0	PBMC	0.09	0.20	2.20	0.065
		MAGI-CCR5	0.028	2.50	4.75	0.014

a. The antiviral activity of emtricitabine was determined by measuring the concentration of HIV-1 p24 (7)

b. The MAGI assay with chemiluminescent detection (24) was used to determine the activity of emtricitabine against HIV-1 (10, 19). MAGI-CCR5 cells were

In vitro Cytotoxicity studies: The intent of the in vitro cytotoxicity studies was to distinguish if the antiviral activity observed was due to the direct effect of emitricitabine on the virus or an indirect effect due to cytotoxicity of the emitricitabine to the cells. Along with the in vitro antiviral activity studies, the applicant reported on the in vitro cytotoxicity studies to evaluate the potential cytotoxicity of emitricitabine.

Table 8 (Vol. 3.1, p. 63) shows the results of the in vitro cytotoxicity of emitricitabine in human T-lymphocytes (cell lines MT-4, CEM and Molt-4), in B-lymphocytes (IM9), in human PBMC from healthy donors, and in Vero (African green monkey) cells. The results show that emitricitabine like lamivudine was not cytotoxic to any of the tested cell lines at concentrations up to and including 24.7 µg/ml (100 µM). Zalcitabine and zidovudine showed varying degrees of toxicity to the tested cell lines.

Table 8. Cytotoxicity of emitricitabine for lymphocytic and monocytic cells

Cell Line	CC_{50} µM			
	Emitricitabine	**Lamivudine**	**Zalcitabine**	**Zidovudine**
MT4	>100	>100	---	>100
MT4	>200	>100	>100	---
CEM	>100	>100	---	14
CEM	>100	>100	6	---
Molt-4	>100	>100	10	---
IM9	>100	>100	70	---
PBMC	>100	>100	---	>100
Vero	>100	>100	---	28

--- not assayed

The applicant has not clearly stated what cytotoxicity assay(s) was used or the assay methodology applied to the different cells to evaluate the cytotoxicity of emitricitabine. Some of the assays included cell proliferation with cell numbers measured by a hemocytometer, cell viability measuring neutral red dye uptake, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) dye uptake, propidium iodide/DNA incorporation assay. The published references that the applicant cited often referred to other references. The summary data that the applicant provided are limited to grasp a perspective on the cytotoxicity of emitricitabine. However, from a totality of data in the publications on the antiviral activity and cytotoxicity it appears that the antiviral activity of emitricitabine is due to the direct antiviral activity of the compound and not related to the potential indirect cytotoxicity of emitricitabine.

In vitro anti-HBV activity of FTC: The applicant referred to published reports that addressed the in vitro antiviral activity of FTC against hepatitis B virus (HBV). In these reports the anti-HBV effect of FTC on the production of intracellular and extracellular

HBV DNA was determined (39, 40) in stably HBV-transfected human hepatoma cell line HepG2 2.2.15. In one study (39) the FTC IC_{50} values for the intracellular and extracellular HBV DNA was 0.16 ± 0.01 μM and 0.04 ± 0.006 μM, respectively. In another study (40) the IC_{50} value for the extracellular HBV DNA was 0.01 ± 0.005 μM.

The applicant also referred to a published report (41) on the effect of FTC on HBV DNA replication in primary human hepatocytes. Although the IC_{50} value was not calculated, FTC at 2.0 μM completely inhibited the production of intracellular HBV DNA, even when added 24 hours after infection. The IC_{50} value calculated for the inhibition of extracellular virus production was 0.02 μM, a value that is comparable to that determined in HepG2 2.2.15 cells. In both of the cell types used in the determination of anti-HBV activity, FTC was not cytotoxic at concentrations up to 200 μM. These results suggest that FTC has antiviral activity against HBV in vitro.

Mechanism of FTC 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, FTC, inside cells is converted into the active drug form, FTC-TP, by sequential phosphorylations with cellular enzymes. The active form, FTC-TP, competes with natural (physiological) nucleoside triphosphates for the nucleotide-binding site on the viral reverse transcriptase. This competition is believed to inhibit the rate of HIV 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 (in this case FTC-TP) also serves as an alternate substrate thereby incorporated into the growing DNA chain of the HIV DNA. Since the incorporated FTC-MP nucleotide lacks the 3'- hydroxyl group, no phosphodiester bond formation can occur with the next incoming nucleotide; consequently, the DNA chain growth stops. Thus, the full-length viral DNA synthesis that is required for integration and establishment of infection is prevented.

Metabolism of FTC: The applicant referred to a publication designed to investigate the metabolic activation of FTC in HepG2 cells and potential inhibitory effects on hepatitis B virus replication (25, 26). In this study HepG2 cell were incubated for 24 hours with [6-^{3}H]-FTC at concentrations of 0.01, 0.10 and 1.0 µM. FTC and its 5'-mono, 5'-di and 5'-triphosphates were identified by comparison of their retention time on ion-exchange HPLC with those of authentic standards. The time course showed that the nucleotides of FTC were formed rapidly and reached a steady state intracellular concentration by 3 to 6 hours. The intracellular concentration of these metabolites increased in a linear manner indicating that the anabolic pathway was not saturated over the concentration range tested. The concentration of FTC-5'-diphosphate was somewhat higher than the

concentrations of the 5'-mono and 5'-triphosphate derivatives (1, 25, 26, 27). Results of this study are consistent with the metabolic activation of FTC to the active drug form the FTC-5'-triphosphate. The applicant also reported studies (28, 29) that showed the formation of phosphorylated metabolites in the PBMCs of healthy volunteers dosed with FTC. However, the effect some of the other nucleoside analogues that may be used in the combination treatment of HIV infection may have on the phosphorylation of FTC is unknown. The intracellular half-life of FTC-5'-triphosphate in hepG2 2.2.15 and CEM cells ranged from 2 to 5 hours (1, 42). However, the applicant indicated that in PBMCs from healthy volunteers dosed orally with 200 mg of emitricitabine QD, the half-life of FTC-5'-triphosphate was approximately 39 hours (29).

Inhibition of HIV-1 Reverse Transcriptase by the 5'-Triphosphate of FTC: To define the mechanism of action of FTC the applicant examined the effect of FTC-TP on the RNA-dependent DNA polymerase and DNA-dependent DNA polymerase activities of HIV RT using defined synthetic templates. Steady state kinetic experiments with emtricitabine 5'-triphosphate and the natural substrate dCTP showed that the K_m values are 0.013 μM for emtricitabine 5'-triphosphate and 0.071 μM for dCTP for HIV-RT. The K_i values for emtricitabine 5'-triphosphate inhibition of HIV-RT catalyzed RNA-dependent DNA synthesis and DNA-dependent DNA synthesis were calculated to be 0.6 μM and 0.43 μM, respectively. In comparison, the K_i values for lamivudine 5' -triphosphate inhibition of HIV-RT catalyzed RNA-dependent and DNA-dependent DNA synthesis were comparable at 0.97 μM and 0.7 μM, respectively (30). Kinetic constants for the incorporation of the 5'-triphosphates of deoxycytidine, emitricitabine and lamivudine into the RNA/DNA template/primer are presented in Table 9 (Vol. 3.1, p. 48). The data show the binding of the nucleotide substrate to the enzyme-DNA complex form a catalytically competent ternary complex (K_d), the maximum rate of incorporation of the single nucleotide 5'-monophosphate (k_{pol}), and the overall efficiency of incorporation, which is defined as the quotient (k_{pol}/K_d). Kinetics of single nucleotide incorporation (31) of deoxycytidine 5'-triphosphate, lamivudine 5' -triphosphate and emtricitabine 5'-triphosphate opposite a template guanosine in RNA-dependent DNA synthesis catalyzed by HIV-1 RT was determined. Results of the kinetic studies (Table 9, Vol. 3.1, p. 48) show that the overall rate of incorporation of the oxathiolane nucleoside analogues is significantly slower than that observed for the natural substrate dCTP as evidenced by the values of k_{pol}. The K_{pol} value for dCTP ranged from 280 to 700 times greater than the corresponding K_{pol} values for oxathiolane nucleoside analogues. However, the K_d values show that the nucleoside analogue substrates bind much tighter to the active site of the enzyme-DNA complex than does the natural substrate, with the K_d values of the analogues being approximately 6- to 30-fold lower than those of natural substrate. Both the K_d and k_{pol} values suggest that emtricitabine 5'-triphosphate to be a preferred substrate for the enzyme compared to lamivudine 5'-triphosphate. The result of the k_{pol} and K_d

analysis suggests a 10-fold increased efficiency (k_{pol}/k_d) of inhibition by emtricitabine 5'-triphosphate relative to lamivudine 5'-triphosphate. Emtricitabine 5'-triphosphate is incorporated almost an order of magnitude more efficiently than is lamivudine 5'-triphosphate during RNA-dependent DNA synthesis.

Results of the reported steady state kinetic studies are consistent with the proposed mechanism of action of emitricitabine, i.e., the active metabolite of emitricitabine, emitricitabine 5'-triphosphate, inhibits viral DNA synthesis: 1. by competing with the natural substrate, dCTP, and 2. by incorporation into the growing DNA chain and as a consequence terminating DNA chain elongation.

Table 9. Kinetic constants for the incorporation of the 5'-triphosphates of deoxycytidine, emitricitabine and lamivudine into the RNA/DNA template/primer

Compound	Template/Primer	K_{pol} (S^{-1})	K_d (μM)	K_{pol}/K_d (μM/S^{-1})
Deoxycytidine 5'-triphosphate	r44/d23[a] R45/D23[b]	9 ± 2 22.9 ± 0.7	16 ± 5 30 ± 4	- 0.76
Emitricitabine5'-triphosphate	r44/d23[a] R45/D23[b]	0.240 ± 0.02 0.082 ± 0.005	1.7 ±0.3 1.4 ± 0.4	- 0.06
Lamivudine5'-triphosphate	R45/D23[b]	0.033 ± 0.002	5.0 ± 0.8	0.0067

a. Pre-steady state kinetic data (30) were collected using a rapid mixing apparatus and the equations for the calculations are detailed in the reference.
Primer/template r44/d23
5' –GGGGATCCTCTAGAGTCGACCTG-3'
3'-CCCCUAGGAGAUCUCAGCUGGACGUCCGUACGUUCGAACAGAGG- 5'

b. Transient kinetic (31) experiments using rapid quench methodology were performed. The pre-steady state analysis was conducted under conditions in which the duplex concentration was in 3-fold excess relative to the enzyme concentration. The reaction was carried out by mixing a solution containing the preincubated complex of 100.0 nM HIV-1 RT and 300.0 nM [^{32}P] labeled R45/D23 DNA/RNA heteroduplex with a solution of 10.0 mM Mg^{2+} and varying concentrations of dNTP (in the range of 0.5 μM to 500μM). Polymerization reactions were quenched with 0.3 M EDTA at time intervals ranging from 3 ms to 3 min. DNA polymerization products and RNA cleavage products were quantified by sequencing gel analysis.

Primer/template R45/D23:
5' -* GCCTCGCAGCCGTCCAACCAACT-3'
3'-CGGAGCGUCGGCAGGUUGGUUGAGUUGGAGGCUAGGUUACGGCAGG *- '5'

Effects of FTC-TP on Human cellular DNA Polymerases α, β, γ and ε: In order for FTC to be useful in the clinic, its metabolically active form, FTC-TP, should not only be

active against the HIV-RT but should ideally also show little or no effect on cellular DNA polymerases at concentrations which will be attained within cells at the treated doses. The applicant referred to one publication (1) which tested the inhibitory effect of emtricitabine 5'- triphosphate on human HeLa cell DNA polymerases α, β, γ and ε. Activated calf thymus DNA was used as the template for analysis of each enzyme. Under these conditions, emtricitabine 5'-triphosphate was found to be a weak inhibitor of each of the human DNA polymerases when compared to HIV-RT. Apparent K_i values were 6.0 μM for polymerase α, 17.0 μM for polymerase β, 6.0 μM for polymerase γ, and 150 μM for polymerase ε, compared to 0.17 μM for HIV-1 RT (1). These data suggests that emtricitabine 5'-triphosphate is a weak inhibitor of mammalian DNA polymerase α, β, ε and mitochondrial DNA polymerase γ.

In summary, the experimental data that the applicant provided is consistent with the proposed mechanism which states that: emtricitabine, a synthetic nucleoside analog of cytosine, is phosphorylated by cellular enzymes to form emtricitabine 5'-triphosphate. Emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 reverse transcriptase by competing with the natural substrate deoxycytidine 5'-triphosphate and by being incorporated into nascent viral DNA, which results in chain termination. Emtricitabine 5'-triphosphate is a weak inhibitor of mammalian DNA polymerase α, β, ε and mitochondrial DNA polymerase γ.

In vitro resistance studies:

In vitro selection of HIV-1 variants resistant to emitricitabine: Upon administration of any of the clinically available NRTIs, NNRTIs, PIs or fusion inhibitors of HIV-1, the virus in the infected individuals develops resistance to these drugs. In vitro experiments evaluating the emergence of resistance to antiretroviral agents have generally been predictive of the potential for emergence of resistance and help in prospectively following the patient isolates in clinical settings. In support of the evaluations for potential emergence of resistance to emitricitabine, the applicant referred to some of the published reports in the open literature.

The emergence of resistance to emitricitabine was examined by serial passage of the HIV on MT-4 cells in the presence of increasing concentrations of emitricitabine. In these studies the wild type virus, HIV-1_{HXB2}, or the zidovudine mutant virus, HIV-1_{RTMC} (containing D67N, K70R, T215Y, and K219Q mutations), was passaged in MT-4 cells in the presence of increasing concentrations of emitricitabine or lamivudine. Rapid emergence of resistance occurred with both compounds. By the fourth passage of HIV-1_{HXB2} and the second passage of HIV-1_{RTMC}, the IC_{50} values exceeded 50 μM and by passage six the IC_{50} values were in excess of 250 μM. These emitricitabine-induced mutants were highly cross-resistant to lamivudine but showed no cross-resistance to

diadanosine, zidovudine, or the NNRTI nevirapine. Genotypic analysis of the RT gene showed a change at codon 184 of the RT, with methionine replaced by valine. Based on synergy data of the mutant viruses with zidovudine and the lack of cross-resistance, the authors suggested that combination of the oxathiolan analogues (lamivudine and emitricitabine) with zidovudine might slow the emergence of resistance to emtricitabine and/or lamivudine. Indeed, passaging virus in the presence of increasing concentrations of emtricitabine and 50 μM zidovudine was able to delay appreciably, but not prevent, the emergence of emtricitabine resistant virus (8).

In other studies (32) the relative potential for HIV-l to develop resistance to lamivudine and emtricitabine was evaluated by serial passage of the virus in human PBMCs or MT-2 cells in the presence of increasing drug concentrations. Results of this study showed that after two or more cycles of infection, lamivudine-resistant virus emerged. Lamivudine-resistant virus was cross-resistant to emitricitabine but remained sensitive to didanosine and zidovudine. DNA sequence analysis of the RT gene amplified from resistant viruses consistently identified mutations at codon 184 from Met to either Val or Ile.

The applicant reported on the relative antiviral activity evaluations of FTC against a panel of clinical isolates (Tables 10, Vol. 3.1, p. 57 and 11, Vol. 3.1, p. 58). The panel consists of a series of recombinant wild type clinical isolates and recombinant isolates containing anywhere from one to twelve mutations. Consistent with earlier in vitro passaging experiments, a high level of resistance is imparted by introduction of the M184V mutation against any mutation background. Moderate resistance to emtricitabine was seen for the highly mutated isolate (M41L, E44D, D67N, T69D, L74I, K101E, V108I, V118I, Y181C, G190A, L210W, T215Y, in Table 11, Vol.3.1, p. 58) which contains the two mutations associated with moderate resistance to lamivudine (E44D, V118I) (33). In addition, moderate resistance was observed for both emtricitabine and lamivudine with isolates harboring the K65R mutation or a multi-drug resistance (MDR) genotype containing the T69S(SS) insertion as previously described for lamivudine (33, 34, 35).

Table 10. Phenotypic susceptibility of recombinant HIV-1 constructs

Genotype	IC_{50} (μM)		
	Emitricitabine	**Lamivudine**	**Zidovudine**
WT ($HIV\text{-}1_{HXB2}$)[a]	0.058	0.3235	0.0305
K65R, F116Y, Q151M, V106Ia	2.607	3.327	0.696
M41L, D67N, K70R, A98S, Y181C M184V. G190A, L219W, T215Y[a]	>5	>31.25	0.47
V75I, M184V[a]	>125	>31.25	0.028
A62V, T69S(SS), K70R, T215Y[a]	0.893	2.566	0.965
K103N, V108I/V, P225H[a]	0.123	0.618	0.0358
G190A, K238T[a]	<0.122	0.541	<0.05
V106A, G190A[a]	0.292	2.16	>125
$HIV\text{-}1_{IIIB}$[b]	0.01	0.02	0.01
V106A[b]	0.03	0.03	0.014
V106A, F227L[b]	0.003	0.007	0.003
V106A, F227L, K101I, Y181C[b]	0.01	0.03	0.01

a. Clinical isolates of HIV-1 from the VIRCO database (36) were tested against emtricitabine. Recombinant viruses were created in the $HIV\text{-}1_{HXB2}$ backbone as described (37). Viruses are considered phenotypically sensitive to inhibition by a compound if the fold differences are ≤ 4 and phenotypically resistant to inhibition if the fold differences are ≥ 10. Genotypic analysis of viral isolates was performed by dideoxysequencing on an —— system. Fold differences are based on changes from the laboratory strain of $HIV\text{-}1_{HXB2}$ and may not reflect the differences from the patients' baseline (WT) isolate.

b. The data presented (38) are the mean of two to four experiments. The experimental variation was within 25 %. CEM cells were infected with $HIV\text{-}1_{IIIB}$ for 4 days at 37°C in culture media. Cell cultures were examined and syncitia formations were determined by microscopic evaluation. IC_{50} values were defined as the concentration of compound that decreases the number of syncitia by 50%.

Table 11. Phenotypic susceptibility of recombinant HIV-1 generated from clinical Isolates

Genotype	IC_{50} (μM)[a]		
	Emitricitabine	Lamivudine	Zidovudine
WT:LAI	0.619[b]	2.567[b]	0.487[b]
WT (n=16)	0.64[c]	2.998[c]	0.917[c]
L100I	0.595	2.350	0.160
G190A	0.220	0.900	0.205
G333E	1.350	1.265	0.260
M184V	>20.00	>50.00	0.140
K103T	0.330	1.100	0.140
V108I	0.205	0.650	0.135
K103N	0.680	4.000	0.250
K103N, M184V	>20.0	>50.00	0.185
E138K, M184V	>20.00	>50.00	0.13
L74V, K103N	2.34	>2.26	1.87
K103R, Y188C	1.13	1.16	0.72
K103N, Y181C	0.55	1.17	0.29
K70R, L74V, M184V	>20.00	>20.00	0.90
K103T, V106I, M184V	>20.00	>20.00	0.36
K101Q, E138K, K103N	0.55	0.48	0.67
K103N, V108I, M184V	>20.00	>20.00	0.31
T215Y, K103N, L210W	0.73	1.05	>2.00
M41L, K101R, M184V, T215Y	>20.00	>20.00	>2.00
A98S, F116Y, Q151M, T215Y	1.45	0.55	>2.00
T69N, K70R, M184V, K219Q	>20.00	>20.00	1.23
D67N, K70R, M184V, G190A	>20.00	>50.00	0.35
D67N, T69D, K103R, T219Q	2.58	2.92	5.75
A62V, A98S, K101D, K192Q, M184V	>20.00	>50.00	0.35
M41L, D67N, M184V, L210W, T215Y	>20.00	>20.00	>2.00
D67N, K70R, E138A, M184V,T215Y, K219E	>20.00	>20.00	>2.00
M41L, D67N, Y181C, M184V, L210W, T215Y	>25.00	>20.00	>2.00
M41L, D67N, T69D, V108Y, M184V, T215Y	>20.00	>20.00	>2.00
A62V, V75M, K103N, F116Y, Q151M, M184V	>20.00	>20.00	>2.00
D67N, T69D, K70R, K103N, M184V, T215Y, K219Q	>20.00	>20.00	>2.00
M41L, D67N, A98G, K101E, K103N, M184V, G190A, L210W, T215Y, G333E	>20.00	>20.00	>2.00
M41L, E44D, D67N, T69D, L74I, K101E, V108I, V118I, Y181C, G190A, L210W, T215Y	7.27	6.63	1.85

a. IC_{50} values are expressed as the median value for at least 3 replicates.
b. IC_{50} value is the average of at least 10 replicates
c. IC_{50} value is the average of replicates from 16 different recombinants displaying a WT genotype

HIV-1 resistance evaluations in Phase 3 studies: FTC-301A and FTC-303

In support of the application for the clinical use of emitricitabine, the applicant conducted two clinical studies:

1. Study FTC-301A was a randomized (1:1) double blind, multicenter, equivalence trial in 571 ART-naïve patients that evaluated the efficacy and safety of FTC and d4T in combination with ddI and efavirenz. The study duration was 48-week with patients entering the study having plasma HIV-1 RNA levels of >5000 copies/ml. The primary end point of the study was the proportion of patients at week 48 with undetectable plasma HIV-1 RNA (<50 copies/ml).

2. Study FTC-303 was a randomized (2:1) open-label, multicenter equivalence study that evaluated the efficacy and safety of FTC and 3TC in 440 HIV-infected patients who have been on a triple regimen containing 3TC, either d4T or ZDV, and either a PI or an NNRTI prior to study entry. Patients were either switched from 3TC to FTC while continuing on their current background therapy or continued on their current 3TC-containing regimen. Study duration was 48 weeks with patients entering the study having plasma HIV-1 RNA levels of <400 copies/ml. The primary endpoint of the study was the proportion of patients at week 48 with plasma HIV-1 RNA at or below 400 copies/ml.

The primary objective of study FTC-301A was to assess the safety and efficacy of emitricitabine as compared to stavudine when used with in a background regimen containing didanosine and efavirenz.
The stated microbiology-related secondary objectives of the study were:

- To compare the time to virologic failure between the treatment arms
- To compare the change from baseline values in plasma HIV-1 RNA
- To compare between treatment arms the proportion of patients who were virologic failures
- To determine the magnitude of the CD4+ cell count and percent increase above baseline in each arm, and
- To characterize the RT genotype of isolates from patients who were considered virologic failures

The primary objective of study FTC-303 was to establish the equivalence of antiviral activity between emitricitabine and lamivudine by comparing the proportion of

randomized subjects at 48 weeks whose plasma HIV-1 RNA levels remain at or below 400 copies/ml

The stated microbiology-related secondary objectives of the study were:

- To compare the time to virologic failure between the treatment arms
- To compare the proportion of subjects whose plasma HIV-1 RNA levels remain below the limit of quantification
- To determine the magnitude of CD4+ and CD8+ cell count and percent increase above baseline in each treatment arm, and
- Characterize the RT genotype of isolates from subjects who were considered virologic failures

Virologic failure in these studies was defined as either never achieving plasma HIV-1 RNA <400 copies/ml during the 48-wk study or having plasma HIV-1 RNA >400 copies/ml confirmed on two consecutive visits after achieving <400 copies/ml.

HIV-1 RNA was quantified by the [redacted] (<400 copies/ml) and/or the UltraSensitive Procedure (<50 copies/ml)

The microbiology-related baseline characteristic and treatment effects of patients randomized to either treatment groups in studies FTC-301A and FTC-303 are summarized in Table 12. For additional statistical details on the clinical studies FTC-301A and FTC-303, please see the statistical review by Dr. Susan Zhou.

Table 12. Microbiology-related information of patients enrolled in Phase III studies: FTC-301A and FTC-303 (intent-to treat)

Patient Information	FTC-301A		FTC-303	
	d4T arm[1]	FTC arm[2]	3TC arm[3]	FTC arm[4]
Number of patients	285	286	146	294
Mean baseline plasma HIVRNA Log_{10}	4.82	4.83	1.76	1.78
Mean baseline CD4+ cell count	323.8	312.3	533.2	542.5
Change in viral RNA at week 48@	-2.92	-3.08	+0.02	+0.01
Change in CD4+ cell count at week 48@	+133.2	+168.3	+61.0	+29.4
Prior ART in months (mean/median)	Naive	Naive	31.3/23.9	37.3/29.5

@ = No imputations were applied

1. d4T arm in FTC-301A (n=285): FTC placebo (QD) + d4T (40 mg BID if ≥ 60 kg; 30mg BID if <60 kg) + didanosine (400 mg QD if ≥60 kg; 250 mg QD if <60 kg) + efavirenz (600 mg QD)

2. FTC arm in FTC-301A (n=286): FTC (200 mg QD) + d4T placebo (BID) + didanosine (400 mg QD if ≥ 60 kg; 250 mg QD if <60 kg) + efavirenz (600 mg QD)

3. 3TC arm in study FTC-303 (n=146): patient continued on the same lamivudine-containing antiretroviral regimen

4. FTC arm in FTC-303 (n=294): patient's current lamivudine treatment was replaced with emtricitabine while continuing with the same background antiretroviral regimen

The applicant conducted retrospective genotypic resistance evaluations on patients with confirmed virologic failures. DNA sequence analysis of HIV RT gene was performed on baseline and time of failure samples to evaluate the development of genotypic changes associated with the study drug. The applicant supplied summary data on genotyping is presented below.

FTC-301A: Genotypic analysis of virologic failures: Overall 58 patients experienced virologic failures in the study. Paired analysis could be done on 57 patients (16 in the FTC arm and 41 in the d4T arm). Sequence analysis was performed retrospectively on baseline and time of failure samples to evaluate the development of genotypic changes associated with the study drug.

Table 13 (Vol. B1, p. 6) shows the evaluation of 16 baseline isolates obtained from patients with virologic failures in the FTC arm of study 301A (line listing of data for patients was provided separately and not presented here). At baseline, there were no FTC (M184I/V) or ddI (L74V/K65R) associated mutations in this cohort. Six (31.3%) patients entered the study with HIV-1 harboring mutations associated with NRTI or NNRTI resistance. NNRTI-associated mutations were the most prevalent in this group (n=5). Three patients entered the study with the HIV-1 harboring thymidine analogue mutations and three patients had the efavirenz-associated K103N resistance mutation at baseline. Similar findings were observed in the baseline isolates obtained from patients in the d4T subgroup of virologic failures where 13 out of 41(31.7%) patients entered the study with drug-associated mutations in the HIV RT. As in the FTC arm, the NNRTI mutations were the most prevalent (n=9) followed by thymidine analogue mutations (n=6). Five of the NNRTI mutations were K103N.

Table 13. Genotypic profile of patients with VF at baseline in FTC-301A

HIV-RT mutations at baseline	FTC arm (N=16)	D4T arm (N=41)
FTC[1]	0	0
NNRTI[2]	5(31.3%)	9 (21.9%)
Thymidine analogue mutations (D4T)[3]	3(18.8%)	6 (14.6%)
ddI[4]	0	0
WT[5]	10 (62.5%)	28 (68.3%)

[1]M184 I/V
[2]Any change at A98, L100, K101, K103, V106, V108, E138, Y181, Y188?, G190, P225
[3]Any change at M41, D67, K70, L210, T215, K219
[4]Any change at K65, L74
[5]No changes at amino acid positions associated with resistance

Table 14 (Vol. B1, p. 7) shows the genotypic analysis of isolates at the time of virologic failure. Eleven out of sixteen (68.8%) patients in the FTC arm and 35 out of 41 (85.4%) patients in the d4T arm developed new mutations associated with study drug. In the FTC arm, 10 out of 11 patients developed new NNRTI mutations and 6 out of 11 patients developed the M184I/V mutation. One patient also had an amino acid change at position K65N that may be associated with ddI resistance. In the d4T arm, all 35 patients who developed new mutations at the time of VF had developed NNRTI associated mutations, 6 out of 35 developed thymidine analogue associated mutations, and 3 out of 35 developed ddI associated mutations (L74V).

Table 14. Genotypic profile of patients with new mutations at VF in FTC-301A

HIV-RT mutations	FTC arm (N=16)	D4T arm (N=41)
Patient isolates with at least one new mutation at the time of VF[1]	11/16 (68.8%)	35/41 (85.4%)
FTC[2]	6/16 (31.3%)	0
NNRTI[3]	10/16 (62.5%)	35/41 (85.36)
Thymidine analogue mutations (D4T)[4]	0	6/41 (14.63%)
ddI[5]	1/16 (6.3%)	3/41 (7.32%)

[1]Some patients had multiple mutations at time of virologic failure
[2]M184 I/V
[3]Any change at A98, L100, K101, K103, V106, V108, E138, Y181, Y188, G190, P225
[4]Any change at M41, D67, K70, L210, T215, K219
[5]Any change at K65, L74

FTC-303: Overall 34 patients experienced virologic failure, 23 in the FTC arm and 11 in the 3TC arm. Genotypic analysis was attempted retrospectively on the plasma HIV-RNA obtained from all patients who experienced protocol defined virologic failure. Matched comparison could be done for 19 of 23 patient samples from the FTC arm and 4 of 11 samples from the 3TC arm. Due to the low plasma HIV-1 RNA of patients entering the study (<400 copies/ml), complete sequence analysis of baseline isolates was only obtained for 15 of 34 patients who experienced virologic failure in the study. Overall, sequence data around the M184 codon was available at baseline for 23 of the 34 patients who experienced virologic failure.

Table 15 (Vol. B1, p. 11) shows that in the FTC subset, the M184I/V mutation was present in 17/19 (89.5%) isolates at baseline. For the 12 virologic failures for which complete HIV RT sequence is available, 6 out of 12 had the M184V mutation alone, 4 out of 12 had thymidine analogue mutations in addition to the M184V and 3 out of 12 had NNRTI-associated mutations in addition to the M184V. In the 3TC subset, the M184V mutation was present in 3 out of 4 isolates at baseline. For the three patients for whom complete HIV RT sequence is available, one had the M184V alone, one had an NNRTI mutation and one had a thymidine analogue mutation and an NNRTI mutation in addition to the M184V.

Table 15. Genotypic profile of patients with virologic failures at baseline (FTC-303)

HIV-RT mutations at baseline	**FTC (N= 23)**	**3TC (N = 11)**
Unable to obtain PCR product	4	7
Patients with M184 Sequence	19	4
M184V	17/19 (89.5%)	3/4 (75%)
Complete Sequence available	12	3
M184V	11/12 (91.7%)	2/3 (66.7%)
Thymidine analogue mutations [1]	4/12 (33.3%)	2/3 (66.7%)
NNRTI [2]	3/12 (25%)	1/3 (33.3%)

[1] Any change at M41, D67, K70, T215, K219

[2] Any change at A98, L100, K101, K103, V106, V108, E138, Y181, Y188, G190, P225

Genotypic data were available for 33 of the 34 patients at time of virologic failure: 23 out of 23 in the FTC arm and 10 of 11 in the 3TC arm. Two of the patients in the FTC subgroup who were wild type at position M184 at baseline developed the M184V mutation. One patient had developed another NRTI-associated resistance mutation in addition to M184I/V. In the 7 patients for which a partial baseline genotype is available, sequencing of the complete HIV-RT at virologic failure revealed the presence of NRTI and NNRTI mutations.

Genotypic Analysis of Patients with Viral Load of >50 copies/ml in study FTC-303: To evaluate the association between baseline M184 sequence and virologic failure during the study, the applicant attempted to genotype the region around the M184 codon of all patients entering the study with plasma HIV-1 RNA >50 copies/ml at screening or at baseline. Eighty patients in the study had HIV RNA >50 copies/ml at screening or at baseline and genotypic data was obtained for 66 out of 80 (82.5%) patients. Forty-three of the 66 (65%) isolates evaluated contained the M184V/I mutation.

According to the applicant, the results obtained from this analysis demonstrate that patients with HIV-1 RNA > 50 copies/ml who had the M184 I/V mutation at study entry are significantly more likely to develop virologic failure (19/43, 44.2%) during study than patients without M184 I/V at study entry (2/23, 8.7%) (p=0.005). These findings are consistent within both the FTC and 3TC subgroups.

Based on the data on the emergence of resistance in clinical studies the applicant concluded that resistance to FTC was associated with the development of the M184I/V mutation in the HIV RT. The incidence of the M184I/V mutation in treatment-naive patients experiencing virologic failure following combination therapy with FTC was 37.5% (FTC301A). Patients experiencing virologic failure without the M184 I/V mutation remained phenotypically sensitive to inhibition by FTC and all other nucleosides. These results support the genotypic findings and confirm that M184I/V is the only mutation associated with resistance to emitricitabine in these patients.

CONCLUSIONS:

A major portion of the non-clinical data submitted to the microbiology section of this NDA was derived from publications in the open literature. The anti-HIV-1 activity test systems employed to determine the extent of inhibition of HIV-1 replication by FTC included a variety of host cell-virus infection models and different assays to quantify HIV replication. Results of these studies provided a broad perspective into the antiviral activity of the drug. The IC_{50} values in these different studies ranged from 0.0013 to 0.64 μM corresponding to 0.003 to 0.158 μg/ml.

Based on the results from the PK studies (protocol 106, Ref. 28), the applicant stated that "The steady-state trough plasma emitricitabine concentration following 200 mg once daily dose averaged 0.07 μg/ml (0.28 μM), 5-fold higher than the mean in vitro IC_{90} value for the anti-HIV activity of emitricitabine." However, the IC_{50} value for emitricitabine against clinical isolates was approximately 0.15 μg/ml (Table 11)

suggesting that at 200 mg QD the plasma concentration was around the IC_{50} value and may not reach the 5-fold higher than the mean IC_{90} concentration that the applicant indicated. However, the metabolically active form of the drug is FTC-TP and what was measured in the PK study was the prodrug, FTC. The applicant has not provided data on the metabolic optimization of the active drug form and its relation to antiviral activity.

Resistance to FTC seems to emerge rapidly both in vitro after a few passages of the virus in cells and in vivo after a few a weeks of monotherapy. This pattern of the emergence of resistance to the oxathiolan cytosine analogue, FTC, is similar to another approved oxathiolan cytosine analogue, 3TC. Resistance to either of these two drugs is associated with the emergence of the M184I/V mutation in the highly conserved Tyr, Met^{184}, Asp, Asp (YMDD) region that is adjacent to the putative catalytic site of the HIV RT. In the case of 3TC, additional mutations in HIV RT that contribute to the 3TC resistance have also been reported in patients who were on long term therapy with other ARV agents. With respect to FTC, the applicant stated that only the M184I/V mutation is responsible for the resistance. The potential for additional sites of mutation that contribute to FTC resistance is likely in long-term usage of FTC in combination with other ARV agents.

The applicant has not reported studies on the anti-HIV activity of FTC on HIV production from chronically infected cells and on terminally differentiated cells with poor anabolic activities toward nucleosides and their analogues. Based on prior understanding of the mechanism of action of nucleoside analogues on HIV replication, a lack of, or, a poor anti-HIV effect for FTC was expected on these subgroups of cells endowed with the ability to produce the virus in HIV infected individuals. Acutely infected cells differ from chronically infected cells in that the latter have stably integrated proviral DNA from which progeny HIV could be produced without the involvement of viral reverse transcriptase. Therefore, FTC an inhibitor of HIV RT would have no effect on virus production from chronically infected cells but can interfere with the initiation of new infections. Terminally differentiated cells such as macrophages with no replication potential either lack or shed the very enzymes required for the anabolism of FTC to its metabolically active form and as such fail to inhibit HIV production.

RECOMMENDATIONS: The proposed mechanism of inhibition of HIV replication by the nucleoside analogue, FTC, is through inhibition of the HIV reverse transcriptase. Consistent with the mechanism of action, the applicant's studies showed that FTC inhibits HIV replication in vitro. Consistent with the in vitro antiviral activity, the drug product Coviracil, at the prescribed dose of 200-mg QD, inhibited HIV replication in vivo as quantified by reduction of the viral RNA in the plasma of treated patients. Concomitant with the reduction in viral load there were increases in the CD4+ cell

counts. The microbiology section of the draft package as revised below is acceptable. Therefore, with respect to microbiology the drug is recommended for approval.

The applicants version of the microbiology label

MICROBIOLOGY:

Mechanism of Action:

2 pages redacted from this section of the approval package consisted of draft labeling

REFERENCES:

1. Painter, G., M. H. St. Clair, S. Chingm, J. Noblin, L. Wang and P. A. Furman (1995). "524W91". Drugs of the Future 20: 761-765.

2. Schinazi, R. F., A. McMillan, D. Cannon, R. Mathis, R. M. J. Lloyd, A. Peck, J.-P. Sommadossi, M. H. St. Clair, J. E. Wilson, P. A. Furman, G. Painter, W.-B. Choi and D. C. Liotta (1992). "Selective inhibition of human immunodeficiency viruses by racemates and enantiomers of cis-5-fluoro-1-[2-(hydroxymethyl)-1,3 -oxathiolan-5-yl]cytosine". Antimicrob. Agents Chemother. 36: 2423-2431.

3. Averett, D. R. (1989). "Anti-HIV compound assessment by two novel high capacity assays". J. Virol. 23: 263-276.

4. Doc # 462 v-2. Borroto-Esoda, K. (2001). "Antiviral activity of FTC, (2R-cis)-4-amino- 5- fluoro-1-[2-(hydroxymethyl)-1,3 -oxathiolan- 5- yl]- 2(1 H)-pyrimidinone, against HIV -1 ". (p. 1-2). Triangle Pharmaceuticals Inc., Durham, NC, USA.

5. Jeong, L. S., R. F. Schinazi, J. W. Beach, H. O. Kim, S. Nampalli, K. Shanmuganathan, A. J. Alves, A. McMillan, C. K. Chu and R. Mathis (1993). "A symmetric synthesis and biological evaluation of I3-L-(2R,5S)- and a-L-(2R,5R)-1 ,3-oxathiolane-pyrimidine and -purine nucleosides as potential anti-HIV agents". Journal of Medicinal Chemistry 36: 181-195.

6. Gosselin, G., R. F. Schinazi, J.-P. Sommadossi, C. Mathe, M.-C. Bergogne, A.-M. Aubertin, A. Kirn and J.-L. 1mbach (1994). "Anti-human immUnodeficiency virus activities of the I3-L enantiomer of2',3'-dideoxycytidine and its 5-fluoro derivative in vitro". Antimicrob. Agents Chemother. 38: 1292-1297.

7. Doc # 10498-v2. (2001). "Emtricitabine was evaluated *in vitro* for its antiviral activity against various subtypes of HIV -1, groups M and 0, and HIV -2". (p. 1-5). Triangle Pharmaceuticals. Inc., Durham, NC, USA.

8. Tisdale, M., S. Kemp, N. R. Parry and B. Larder (1993). "Rapid in vitro selection of human immunodeficiency virus type 1 resistant to 3'-thiacytidine inhibitors due to a mutation in the YMDD region of reverse transcriptase". Proc. Natl. Acad. Sci. U. S. A. 90: 5653-5656.

9. Kimpton, J. and M. Emerman (1992). "Detection of replication-competent and pseudotyped human immunodeficiency virus with a sensitive cell line on the basis of activation of an integrated beta-galactosidase gene". J. Virol. 66: 2232-2239.

10. Doc # 10518-v2. (2001). "MAGI-LU assay validation 1: Inhibitory effect of FTC on HIV-1_{LAI} viral infection is independent of multiplicity of infection (MOI) of the infecting virus". (p. Triangle Phannaceuticals. Inc., Durham, NC, USA.

11. Doc # 11773. (2001). "Effect of multiplicity of infection on inhibition of HIV-1 replication by FTC". (p. 1-5). Triangle Pharmaceuticals. Inc., Durham, " NC, USA.

12. Doc # 10247. (2000). "DXG, FTC, and AZT: Time of addition". (p.1-3). Triangle Pharmaceuticals. Inc., Durham, NC, USA.

13. Doc # 463. (1997). "The effect of human serum on the antiviral activity of FTC was assessed in CEM cells infected with the LAI strain of HIV- 1". (p 1-2). Triangle Pharmaceuticals. Inc., Durham, NC, USA.

14. Chou, T .-C. and P. Talalay (1984). "Quantitative analysis of dose-effect relationships: The combined effects of multiple drugs or enzyme inhibitors". Advance in Enzvme Regulation 22: 27-55.

15. Bridges, E. G., G. E. Dutschman, E. A. Gullen and Y.-C. Cheng (1996). "Favorable interaction of [3-L(-) nucleoside with clinically approved anti-HIV nucleoside analogues for the treatment of human immunodeficiency virus". Biochem. Pharmacol. 51: 731-736.

16. Doc # 470. (1997). "In vitro synergy studies of FTC in combination with MKC 442, AZT, nelfinavir (NELF), nevirapine (NEV) against HIV". (p. Triangle Pharmaceuticals. Inc. Durham, NC, USA

17. Doc # 10804. (2001). "In vitro synergy studies with FTC and other anti- HIV compounds". (p. 1-13). Triangle Pharmaceuticals. Inc. Durham, NC, USA.

18. Doc # 12207. (2001). "Synergy of emtricitabine and lamivudine in combination with stavudine and nevirapine against HIV". (p. 1-10). Triangle Phannaceuticals Inc., Durham, NC, USA.

19. Doc # 11118. (2001). "MAGI-LU assay validation 4: Inter- and intra-assay reproducibility". (p.1-3). Triangle Pharmaceuticals Inc. Durham, NC, USA.

20. St. Clair, M. H., J. Millard, J. F. Rooney, M. Tisdale, N. R. Parry, B. M. Sadler, M. R. Blum and G. Painter (1996). "In vitro antiviral activity of 141 W94 (vx-478) in combination with other antiretroviral agents". Antiviral Res. 29: 53-56.

21. Doc # 12475-vl. (2001). "Mac Synergy 2 user Manuel". (p. 1-60). Triangle Pharmaceuticals Inc. Durham, NC, USA.

22. (1990). "A three dimensional model to analyze drug-drug interactions". Antiviral Res 14: 181-206

23. Mathez, D., R. F. Schinazi, D. C. Liotta and J. Leibowitch (1993). "Infectious amplification of wild-type human immunodeficiency virus from patients' lymphocytes and modulation by reverse transcriptase inhibitors *in vitro*" Antimicrob. Agents Chemother: 37:2206-2211.

24. Doc # 11419. (2001). "Evaluation of the antiviral activity of emtricitabine against HIV-1 (group M and subtype 0) and HIV-2 using the MAGI-LU assay in cMAGI cells ". (p.1-5). Triangle Pharmaceuticals Inc. Durham, NC, USA.

25. Furman, P. A., M. Davis, D. C. Liotta, M. Parr, L. W. Frick, D. J. Nelson, R. E. Dornsife, J. A. Wurster, L. J. Wilson, J. A. Fyfe, J. V. Tuttle, W. H. Miller, L. Condreay, D. R. Averett, R. F. Schinazi and G. R. Painter (1992). "The anti-hepatitis B virus activities, cytotoxicities, and anabolic profiles of the (-) and (+) enantiomers of cis-5-fluoro-1-[2-(hydroxymethyl)-1 ,3-oxathiolan-5-yl]cytosine". Antimicrob. Agents Chem other. 36: 2686-2692.

26. Parr, M. T., D. R. Averett, K. L. Prus, W. H. Miller and D. J. Nelson (1994). "Intracellular metabolism of (-)-and (+ *)-cis-* 5 -fluoro-l- [2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine in HepG2 derivative 2.2.15 (subclone P5A) cells". Antimicrob. Agents Chemother.38: 1230-1237.

27. Furman, P. A., J. E. Wilson, J. E. Reardon and G. Painter (1995). "The effect of absolute configuration on the anti-HIV and anti-REV activity of nucleoside analogues ". Antivir. Chem. Chemother. 6: 345-355.

28. Doc # 12144vl. (2001). "An evaluation of the absorption, distribution, metabolism and excretion of 14c-labeled emtricitabine (FTC) in healthy male volunteers -protocol number FTC-1 06 ". (p. 1-95). Triangle Pharmaceuticals Inc., Durham, NC, USA.

29. Rousseau, F. S., J. O. Kahn, M. Thompson, D. Mildvan, D. Shepp, J. P. Sommadossi, J. Delehanty, J. N. Simpson, L. H. Wang, J. B. Quinn, C. Wakeford and C. Van der Horst (2001). "Prototype trial design for rapid dose selection of antiretroviral drugs: An example using emtricitabine (Coviracil)". J. Antimicrob. Chemother. 48: 507-13.

30. Wilson, J. E., A. Aulabaugh, B. Caligan, S. McPherson, J. K. Wakefield, S. ,ablonski, C. D. Morrow, J. E. Reardon and P. A. Furman (1996). "Human immunodeficiency virus type-1 reverse transcriptase -Contribution of Met-184 to binding of nucleoside 5'-triphosphate". J. BioI. Chem. 271: 13656-13662.

31. Feng, J. Y., J. Shi, R. F. Schinazi and K. S. Anderson (1999). "Mechanistic studies show that (-)-FTC- TP is a better inhibitor of HIV-1 reverse transcriptase than 3TC-TP". FASEB J. 13: 1-7.

32. Schinazi, R. F., R. M. J. Lloyd, M.-H. Nguyen, D. Cannon, A. McMillan, N. Ilksoy, (8C. K. Chu, D. C. Liotta, H. Z. Bazmi and J. W. Mellors (1993). "Characterization of human immunodeficiency viruses resistant to oxathiolane-cytosine nucleosides". Antimicrob. Agents Chemother. 37: 875-881.

33. Hertogs, K., S. Bloor, V. De Vroey, C. Van Den Eynde, P. Dehertogh, A. Van Cauwenberge, M. Sturmer, T. Alcorn, S. Wegner, M. Van Houtte, V. Miller and B. Larder (2000). "A novel immunodeficiency virus type I reverse transcriptase mutational pattern confers phenotypic lamivudine resistance in the absence of mutation 184 V". Antimicrob. Agents Chemother. 44: 568-573.

34. Gu, Z., R. Fletcher, E. Arts, M. Wainberg and M. Parniak (1994). "The K65R mutant reverse transcriptase ofHIV-l cross-resistant to 2', 3'-dideoxycytidine, 2',3'-dideoxy-3'- thiacytidine, and 2',3'- dideoxyinosine shows reduced sensitivity to specific dideoxynucleoside triphosphate inhibitors in vitro ". J. Biol. Chem. 269: 28118-28122.

35. Gu, Z., Q. Gao, H. Fang, H. Salomon, M. Parniak, E. Goldberg, J. Cameron and M. Wainberg (1994). "Identification of a mutation at codon 65 in the IKKK motif of reverse (transcriptase that encodes human immunodeficiency virus resistance to 2',3'-dideoxycytidine and 2',3'-dideoxy-3'-thiacytidine". Antimicrob. Agents Chemother. 38: 275-281.

36. Doc # 11148. (2001). "Phenotypic evaluation of FTC, DXG and MKC442 on recombinant clinical isolates of HIV-1". (p. 1-8). Triangle Pharmaceuticals Inc. Durham, NC, USA

37. Hertogs, K., M.-P. de Bethune, V. Miller, T. Ivens, P. Schel, A. Van Cauwenberge, C. Van den Eynde, V. van Gerwen, H. Azijn, M. van Houtte, F. Peeters, S. Staszewski, M. Conant, S. Bloor, S. Kemp, B. Larder and R. Pauwels (1998). "A rapid method for simultaneous detection of phenotypic resistance to inhibitors of protease and reverse transcriptase in recombinant human immunodeficiency virus type 1 isolates from patients treated with antiretroviral drugs"; Antimicrob. Agents Chemother. 42: 269-276.

38. Balzarini, J., H. Pelemans, R. Esnoufand E. De Clercq (1998). "A novel mutation (F227L) arises in the reverse transcriptase of human immunodeficiency virus type 1 on dose-escalating treatment of HIV type I-infected cell cultures with the nonnucleoside reverse transcriptase inhibitor thiocarboxanilide UC- 781". AIDS Res. Hum. Retroviruses 14: 255-260.

39. Schinazi, R. F., G. Gosselin, A. Faraj, B. E. Korba, D. C. Liotta, C. K. Chu, C. Mathe, J.-L. Imbach and J.-P. Sommadossi (1994). "Pure nucleoside enantiomers of 13-21,3'-dideoxycytidine analogs are selective inhibitors of hepatitis B virus in vitro". Antimicrob. Agents Chemother. 38: 2172-2174.

40. Furman, P. A., M. Davis, D. C. Liotta, M. Parr, L. W. Frick, D. J. Nelson, R. E. Dornsife, J. A. Wurster, L. J. Wilson, J. A. Fyfe, J. V. Tuttle, W. H. Miller, L. Condreay, D. R. Averett, R. F. Schinazi and G. R. Painter (1992). "The anti-hepatitis B virus activities, cytotoxicities, and anabolic profiles of the (-) and (+) enantiomers of cis-5-fluoro-1-[2-(hydroxymethyl)-1 ,3-oxathiolan-5-yl]cytosine". Antimicrob. Agents Chem other. 36: 2686-2692.

41. Condreay, L. D., J. P. Condreay, R. W. Jansen, M. T. Paffand D. R. Averett (1996). "(-)-cis-5-fluoro-1-[2-(hydroxymethyl)-1 ,3-oxathiolan-5-yl]cytosine (524W91) inhibits hepatitis B virus replication in primary human hepatocytes ". Antimicrob. Agents Chemother. 40: 520-523.

42. Paff, M. T., D. R. Averett, K. L. Prus, W. H. Miller and D. J. Nelson (1994). "Intracellular metabolism of (-)-and (+)-*cis*- 5 -fluoro-l- [2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine in HepG2 derivative 2.2.15 (subclone P5A) cells". Antimicrob. Agents Chemother.38: 1230-1237.

Narayana Battula, Ph.D.

Concurrence:

HFD-530/ Assoc Dir. _____ Date _____

APPENDIX-1

List of Abbreviations

Abbreviation	Description
(-)FTC	emtricitabine, Coviracil
3TC	lamivudine, Epivir
524W91	emtricitabine
AAG	alpha-1 acid glycoprotein
AIDS	acquired immunodeficiency syndrome
AZT	zidovudine, Retrovir
BFU-E	burst forming unit -erythroid precursor
BID	twice a day
CC_{50}	concentration required to reach 50% cytotoxicity
CFU-GM	granulocyte macrophage precursor
CTL	cytotoxic T -lymphocytes
d4T	stavudine, Zerit
ddC	zalcitabine, Hivid
ddI	didanosine, Videx
DNA	deoxyribonucleic acid
EC_{50}	concentration required to produce 50% inhibition
EC_{90}	concentration required to produce 90% inhibition
EDTA	ethylenediaminetetraacetic acid
ELISA	enzyme-linked immunosorbent assay
FCS	fetal calf serum
FTC	emtricitabine
GM-CSF	granulocyte macrophage colony stimulating factor
HBV	hepatitis B virus
HCMV	human cytomegalovirus
HepG2	human hepatoblastoma cell line
HIV-1	human immunodeficiency virus type 1
HIV-RT	human immunodeficiency virus reverse transcriptase
HPLC	high performance liquid chromatography
HS	human serum
HSV	herpes simplex virus

IC_{50}	concentration required to produce 50% inhibition
IC_{90}	concentration required to produce 90% inhibition
K_i	inhibition constant

Abbreviation	Description
K_m	Michaelis constant
MAGI	multinuclear activation of a galactosidase inhibitor
MAGI-LU	multinuclear activation of a galactosidase inhibitor-luminescence assay
MDR	multi-drug resistance mg milligram min minute
MKC	emivirine, Coactinon
MKC-442	emivirine, Coactinon
MOI	multiplicity of infection
MTT	3-[4,5-dimethylthiazol-2-yl}-2,5-diphenyl tetrazolium bromide
NELF	nelfinavir, Viracept
NEV	nevirapine, Viramune
NNRTI	non-nucleoside reverse transcriptase inhibitor
NRTI	nucleoside reverse transcriptase inhibitor
NVP	nevirapine, Viramune
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
PHA	phytohemagglutinin
Pol γ	DNA polymerase γ
QD	once a day
RNA	ribonucleic acid
RT	reverse transcriptase
RTI	reverse transcriptase inhibitor
RT -PCR	reverse transcriptase-polymerase chain reaction
TC_{50}	concentration required to give 50% cytotoxicity
TIBO	compound R82913
TID	three times a day
WT	wildtype
ZDV	zidovudine, Retrovir

APPENDIX-2

AMINO ACIDS – ABBREVIATED NOMENCLATURE

Single letter code	Abbreviation	Amino acid
A	Ala	Alanine
B	Asx	Aspartic acid or Asparagine (unknown)
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
X	---	Unknown
Y	Tyr	Tyrosine
Z	Glx	Glutamic acid or Glutamine (unknown)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Narayana Battula
7/3/03 09:05:34 AM
MICROBIOLOGIST

FTC micro review

Julian O Rear
7/3/03 10:33:30 AM
MICROBIOLOGIST

James Farrelly
7/3/03 12:47:01 PM
PHARMACOLOGIST