CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-481

MICROBIOLOGY REVIEW(S)

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

NDA: 21-481	Serial #: 000	**Reviewer:**	N. Battula
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Additional NDA submissions reviewed:

	Supplement #	**Date of Correspondence**	**Date of Receipt**
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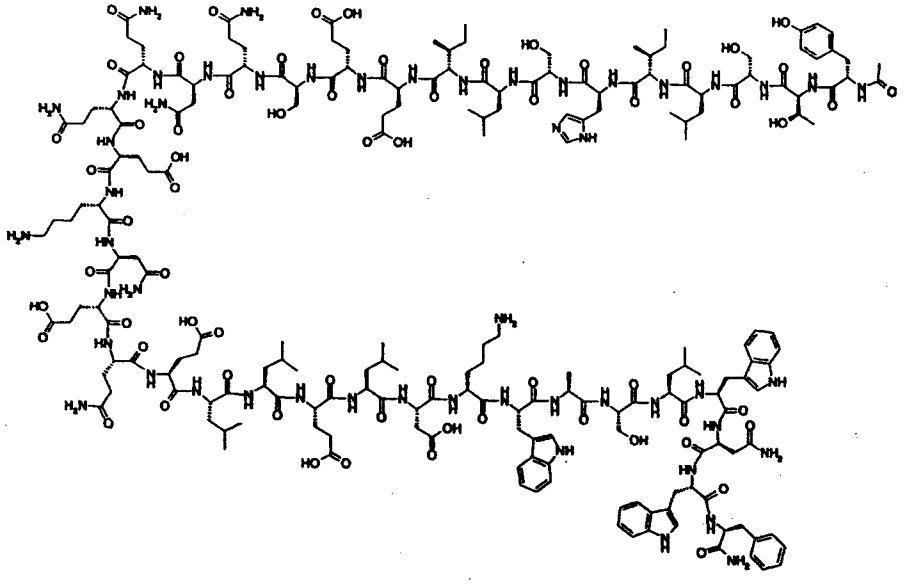
NDA: 21-481	BL	02-21-2003	03-12-2003

Sponsor: Hoffman-LaRoche Inc.
340 Kingsland Street
Nutley, NJ 07110

Product name(s):
Proprietary: Fuzeon™
Non-proprietary: Enfuvirtide or T-20

Amino Acid Sequence: CH_3CO-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-NH_2
Molecular Formula = $C_{204}H_{301}N_{51}O_{64}$
Molecular weight = 4,492 Dalton

Structure:



Dosage form/route of administration: Lyophilized powder for injection: 90 mg bid sc
Indication: Treatment of HIV infection in treatment-experienced patients
Related documents: IND ——

BACKGROUND: This original New Drug Application is submitted by Hoffman-La Roche Inc. in support of Fuzeon for the treatment of HIV-1 infection. The applicant is seeking the indication that Fuzeon in combination with other antiretroviral agents is indicated for the treatment of HIV-1 infection in treatment-experienced patients with evidence of HIV-1 replication despite ongoing antiretroviral therapy. The applicant is requesting approval for Fuzeon under the Accelerated Approval of New Drugs for Serious or Life-threatening Illnesses. In support of these requests, the applicant submitted 24-week efficacy and safety data from two Phase III clinical studies, T20-301 and T20-302. In addition to the Phase III studies, the applicant also submitted supportive data from three Phase II clinical studies: T20-205, T-20-206 and T-20-208. The requested indication is based on analysis of plasma HIV-1 RNA levels and $CD4^+$ cell counts in controlled studies of FUZEON of 24 weeks duration. Subjects enrolled were treatment-experienced adults with evidence of HIV-1 replication despite ongoing antiretroviral therapy. There were no studies of Fuzeon in antiretroviral naïve patients. The primary focus of this review is on the microbiology-related aspects of the Fuzeon NDA.

Until recently, efforts to treat HIV infection have principally concentrated on the development of drugs that disrupt the replication cycle of the virus subsequent to the entry of virus into the host cells. These targets have been the viral enzymes, HIV reverse transcriptase, required for formation of the provirus, and HIV protease, required for particle maturation. As a result of these efforts the drugs available for the treatment of HIV infection consist of three different mechanistic classes: (1) nucleoside reverse transcriptase inhibitors (NRTIs)*, (2) non-nucleoside reverse transcriptase inhibitors (NNRTIs), and (3) protease inhibitors (PIs). The current standard of care for the treatment HIV disease is combination therapy that combines drugs from two or three of these available classes. Combination therapy for the treatment of HIV-1 infection is also referred to as highly active antiretroviral therapy (HAART).

HAART leads to dramatic decreases in viral load accompanied by a marked decrease in the morbidity and mortality associated with HIV/AIDS. Simultaneous with the development of new and effective antiretroviral agents (ARVs) for treatment of HIV infection were significant advances in the testing procedures that quantify the levels of HIV RNA in plasma and evaluate phenotypic and genotypic resistance. Viral load assays allow clinicians to monitor the success of antiviral therapy and phenotypic and genotypic assays guide treatment changes when initial and subsequent treatments fail. In addition, advances in basic sciences revealed that HIV persists in infected individuals for the duration of their life, the virus undergoes rapid replication, mutation and selection. In

* For abbreviations, please see index 1

spite of the reductions in viral load due to HAART, the virus is able to develop resistance to all of the approved antiretroviral agents.

The combined effects of the inability of the currently available drugs to purge latent reservoirs of HIV, limited antiviral potency of existing therapeutic regimens, increased toxicity, while alterations in bioavailability limit the utility of HAART regimens. As a result, there often is virologic failure leading to the emergence of resistant virus to one or more of these drugs. Continued viral replication in the presence of ARVs leads to additional mutations resulting in a broad inter- and intra-class cross-resistance with no drug options for the treatment of HIV. Therefore, key challenges to improving ARV therapy include developing more effective drugs and new drugs that target different stages of the virus life cycle. Enfuvirtide, the candidate drug of this application, targets an early step in the course of HIV infection and replication, i.e., entry of virus into cells.

Figure 1. Potential antiviral targets in HIV-1 life cycle

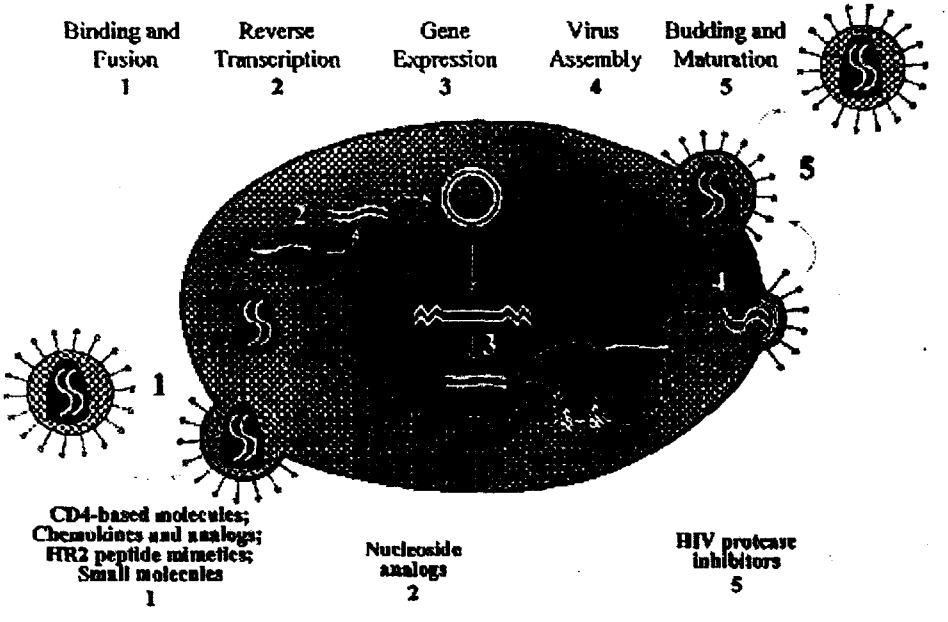
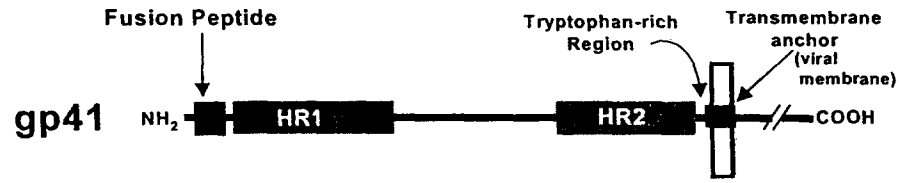


Figure 1 depicts several potential antiviral targets (Vol. 47 page 7) in the HIV life cycle that can be interfered with to block the virus replication. As stated earlier all of the currently approved antiretroviral agents target either the viral reverse transcriptase (at step 2) or the viral protease (at step 5). Enfuvirtide, the candidate drug of this NDA targets an early step of virus infection, i.e., the fusion of the opposing membranes of the virus and cells. Membrane fusion requires the viral envelope glycoproteins.

HIV-1 envelope glycoprotein gp41 is one of two HIV envelope glycoproteins, which is initially synthesized as a precursor protein, gp160 that is cleaved by cellular enzymes to yield the viral surface protein gp120 and the viral transmembrane protein gp41. The gp41 of HIV-1_{HXB2} is 276 amino acids long spanning the viral membrane. The ectodomain contains two functionally important leucine-zipper like heptad repeat regions, the heptad repeat region 1 (HR1) and the heptad repeat region 2 (HR2) encompassing amino acid residues 17-86 and 99-162, respectively (Fig. 2, Vol. 47, page 8, by gp41 numbering system).

Enfuvirtide is a 36-amino acid synthetic peptide composed of natural L-amino acids. The N-terminal amine of the peptide was modified by acetylation and the C-terminus of the peptide was converted to the amide, to protect the peptide from rapid degradation by cellular enzymes. The primary amino acid sequence of enfuvirtide was derived from a naturally occurring motif (Fig. 2), the heptad repeat 2 region (HR2). HR2 represents envelope amino acid residues 643-648 and is found within the envelope transmembrane glycoprotein gp41. T-20 was derived from the amino acid sequence of the human immunodeficiency virus type 1 strain HXB2 (HIV-1_{HXB2}). T-20 is the first drug submitted as an NDA, which specifically inhibits the function of the gp41 of HIV-1. Thus, enfuvirtide represents the first member of a new class of antiretroviral agents.

Figure 2. HIV-1 gp41 amino acid residue numbers and functional regions



	HR1	HR2
env numbering	aa 528 - 597	aa 610 - 673
gp41 numbering	aa 17 - 86	aa 99 - 162

	T20
env numbering	aa 638 - 673
gp41 numbering	aa 127 - 162

Amino acid sequence numbering based on HIV-1 HXB2 Sequence (Los Alamos Sequence Data Base)

(D.M. Lambert, Trimeris, Inc.)

As mentioned above, T-20 was derived from a heptad repeat. Heptad repeats are unique protein motifs with the capacity to form alpha helical coiled-coils that twist around one another to form supercoils. Viral fusion peptides often contain coiled-coil structures, an architecture that controls oligomerization and ability to function as a molecular recognition system (1). High-resolution (2.0 Angstrom) crystallography determinations of gp41 have revealed heterotrimeric coiled-coil structures. These structures are made up of a triple-stranded coiled-coil of α-helices from the leucine zipper-like repeat domain in gp41. In the ectodomain of gp41 the HR1 motif is close to the N-terminal fusion peptide region and the HR2 motif close to the C-terminal region and the two motifs are separated by non-coiled-coil flanking region. In the HIV-1 infection process the HR1 and HR2 motifs play a critical role in the formation of fusion pores and viral entry into the cells. Enfuvirtide is believed to exert antiviral activity through binding to the HR1 domain, thereby inhibiting intramolecular interactions between the HR1 and HR2 domains that are necessary for the fusion of the virus and the host cell membranes

The microbiology portions of the Fuzeon NDA review evaluations include data addressing: the mechanism of enfuvirtide action, antiviral activity and cytotoxicity of

enfuvirtide, emergence of resistance in HIV to enfuvirtide in cell culture, emergence of resistance in HIV to enfuvirtide in vivo in clinical studies and usage of secondary receptor and shifts in their usage during Fuzeon treatment. Based on the information that the applicant provided and from the information available in the open literature, a microbiology portion of the label is generated reflecting the current understanding of the microbiology aspects of enfuvirtide. The original microbiology label submitted by the applicant is also included in this review to provide reference for the identification of changes made after the review of the NDA. In the course of the review process several questions that have not been addressed but essential for understanding the mechanism of action of the drug and mechanism of the drug's failure emerged. The applicant was requested to address these issues under phase 4 considerations.

MECHANISM OF ACTION

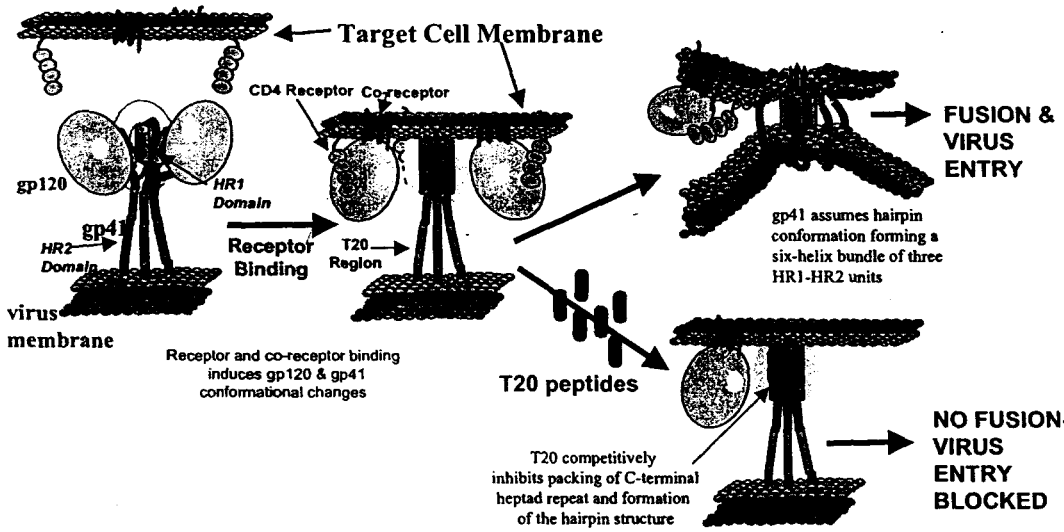
HIV-1 entry into cells is a complex process involving a cascade of events that have not been fully elucidated. The initiation of infection involves the interaction of viral and cellular proteins followed by the fusion of the viral and cellular membranes and entry of viral core into the cell. The viral determinants for entry are the viral envelope glycoproteins, the surface gp120 and the transmembrane gp41. The cellular determinants that permit the viral entry are the cell surface protein CD4, the primary binding site for the viral gp120, and one of several coreceptors, which are chemokine molecules. The viral ligands and the cellular receptors involved in the initiation of infection and viral entry are described briefly.

Figure 3 depicts a cartoon of the viral and cellular determinants (Vol. 47 page 9) believed to be involved in the fusion process. The viral envelope glycoprotein, gp160, is initially synthesized as a polyprotein that is enzymatically cleaved yielding two mature proteins, the surface gp120 and the transmembrane glycoprotein gp41. These envelope proteins assemble on the viral surface as trimeric complexes containing three gp120 molecules, each weakly associated with the other as trimers by non-covalent linkages. HIV infection is initiated by high affinity binding of viral gp120 to the cell surface glycoprotein, CD4, the primary receptor for HIV-1. The gp120-CD4 association induces conformational changes in gp120 that lead to the exposure and/or formation of a binding site(s) for specific chemokine receptors such as CCR5, CXCR4 and CCR3 which serves as obligate coreceptors for virus entry. These interactions with receptors and coreceptors induce additional rearrangements in the helical structures of the gp41 leading to the formation of hydrophobic hairpin structures consisting of six-helix bundles of HR1-HR2 units, pulling the viral and cellular membranes together into close proximity and thus promoting the fusion of the viral and cellular membranes. Enfuvirtide is believed to

mimic the HR2 by competitive association with the native segment and thereby interferes with intramolecular interactions between the HR1 and HR2 domains necessary for the formation of hairpin structures essential for the fusion and entry of HIV-1 into the cells.

In summary, the viral gp120 directs target-cell recognition and determines the viral tropism and the gp41 mediates the fusion between the viral and cellular membranes resulting in the release of viral core into the host cell. Enfuvirtide inhibits the entry of HIV-1 into cells by interfering with gp41 mediated fusion.

Figure 3. Inhibition of HIV-1 entry



(M.K. Delmedico and D.M. Lambert, Trimeris, Inc.)

Antiviral activity studies of enfuvirtide in cell culture

The applicant conducted a number of studies using different host cell-virus combination systems to provide a broad perspective into the anti-HIV activity of enfuvirtide. HIV strains employed in these studies include the conventionally used laboratory and clinical isolates, pretreatment (baseline) isolates from phase 2 and phase 3 studies, clinical and laboratory isolates that have preferential use of cellular coreceptors, and HIV isolates resistant to NRTIs, NNRTIs, and PIs. The host cells used for viral infection in these studies include established cell lines, primary cells and recombinant cell lines that were constructed to contain specific cellular coreceptors. The summary results indicate

enfuvirtide displays anti-HIV activity in these multiple cell-virus test systems used for antiviral activity studies.

The host cell virus systems to evaluate the antiviral activity studies include: (1) cell-cell fusion by transmission of cell-bound virus (from infected cells) to uninfected cells and HIV envelope mediated cell-cell fusion, and (2) transmission of cell-free virus infection of uninfected recipient cells. The second set of studies involves use of different cell types: T-lympoblastoid cell lines, monocyte/macrophage cells, primary peripheral blood mononuclear cells and recombinant cell lines with HIV laboratory and clinical isolates and recombinant constructs of HIV-1.

Inhibition of HIV-mediated cell-cell fusion: Transmission of HIV-1 from certain infected and virus producing cells (i.e., effector cells) to uninfected cells (i.e., target cells) leads to their fusion resulting in the formation of giant fused cells or ——— The ——— can be scored by different techniques including microscopy. In this study the ability of enfuvirtide to inhibit HIV-1 induced cell-cell fusion between CEM cells chronically infected with HIV-1_{LAI} and uninfected MOLT-4 cells was evaluated by a ——— assay.

[ ]

Table 1 shows the relative concentrations of enfuvirtide required for the inhibition of ——— mediated by different HIV isolates. In these studies, the IC_{50} (concentration required for 50% inhibition) ranged from 1.0 to 15.0 ng/ml, and the IC_{90} (concentration required for 90% inhibition) ranged from 2.0 to 51.0 ng/ml. The activity appears to be specific to HIV-1 as the data in Table 1 shows that a more than 4 logs higher concentration of enfuvirtide was needed to inhibit HIV-1 compared to HIV-2. The data further suggests that activity is sequence specific as the inhibition of HIV-1 required much lower concentration of enfuvirtide in comparison to HIV-2. A control 36-amino acid peptide containing a scrambled enfuvirtide sequence showed no cell–cell fusion activity indicating that the inhibition of ——— is sequence specific (4)

Table 1. Inhibition of [redacted] by enfuvirtide.

HIV stain/isolate	Enfuvirtide, ng/ml	
	IC_{50}	IC_{90}
HIV-1_{LAI}	2	6
HIV-1_{MN}	15	51
HIV-1_{RF}	7	24
HIV-1_{SF2}	2	5
HIV-1_{IIIB}	1	2
HIV-1_{G910}	1	2
HIV-2_{NIHZ}	4,600	30,000

Inhibition of HIV envelope mediated cell to cell fusion: In this set of experiments, the applicant reported studies on the effect of enfuvirtide on HIV-1 envelope glycoprotein expressing cell mediated fusion (compared to the virion expressing cells reported above) with uninfected target cells. The inhibitory effect of enfuvirtide was evaluated by a [redacted] assay.

In this study, human 293 T cells (effector cells) transiently expressing various HIV-1 envelope glycoproteins (Table 2) and labeled with a [redacted] probe, [redacted] were cocultured with uninfected human U87 cells (target cells) expressing CD4 and either coreceptor CCR5 or CXCR4 at 37^{0}C for 40 min at various concentrations of enfuvirtide. The fused cells were scored for [redacted] transfer under [redacted] microscopy.

Data presented in Table 2 show that the IC_{90} ranged from 200 to 500 ng/ml in the [redacted] assay. Enfuvirtide was also active against one strain each representing HIV-1 clades E and G. Activity against HIV-2 was 4- to 10-fold lower than HIV-1

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Table 2. Enfuvirtide Inhibition of HIV-induced cell-cell fusion: Comparison of HIV-1 clades and effects of co-receptor usage

Envelope	Clade	Coreceptor		
		CXCR4	CCR5	IC_{90} (ng/ml)
HIV-1				
HXB2	B	+	-	200
SF33	B	+	-	340
SF162	B	-	+	500
JRFL	B	-	+	450
93TH976.17	E	-	+	450
92UG975.10	G	-	+	200
HIV-2				
UC1		-	+	2000
UC2		+	-	2000

Values shown are an average of at least three independent experiments

The results from cell to cell fusion studies (Tables 1 and 2) indicate that enfuvirtide is an inhibitor of HIV-1 spread via cell to cell and infection of naïve cells. In the [redacted] assay, enfuvirtide was more than 4000-fold less potent against HIV-2 in comparison to HIV-1, indicating lack of activity in cell culture and by extrapolation lack of activity against HIV-2 infections in vivo.

Inhibition of cell-free HIV-1 infection of uninfected cells: Antiviral activity of Enfuvirtide against laboratory isolates of HIV: Established cell lines and primary cells (peripheral blood mononuclear cells) were infected with laboratory and clinical isolates of HIV. The effect of various concentrations of enfuvirtide on virus production was determined by measuring reverse transcriptase activity in cell supernatants. The applicant referred to a published paper (2) without providing the data. The data retrieved from the publication and summarized in Tables 3 and 4 indicate that enfuvirtide is a selective inhibitor of HIV-1. Comparison of antiviral activity data in Tables 1 and 2 with Tables 3 and 4 shows that enfuvirtide is a more potent inhibitor of cell-cell fusion than of infection by cell-free virus.

Table 3. Inhibition of infection by laboratory isolates of HIV*

virus	IC_{50} ng/ml	
	Enfuvirtide	DP-116*
$HIV\text{-}1_{LAI}$	90	>40,000
$HIV\text{-}2_{NIHZ}$	26,000	>40,000

*DP-116 is a scrambled 36 aa peptide

Comparison of data in Tables 1, 3 and 4 suggests that blocking infection with cell-free $HIV\text{-}1_{LAI}$ virus requires a 45- to 550-fold higher concentration of enfuvirtide than that required to inhibit cell-cell fusion. Much higher concentrations (290-fold) of enfuvirtide are required to inhibit HIV-2. Control scrambled peptides DP-116 or DP-180 showed no antiviral activity. More than a 100-fold difference in potency was observed against HIV-1 compared with [redacted] and [redacted] assays indicating that the inhibitory activity of enfuvirtide is specific to HIV-1. This publication (2) also stated, without showing data, that enfuvirtide failed to inhibit [redacted] in another retrovirus system, human T-cell leukemia virus type –1.

Table 4. Enfuvirtide inhibition of primary HIV-1 isolate infection of [redacted]*

Inhibitor	IC_{50} ng/ml		
	$HIV\text{-}1_{596}$	$HIV\text{-}1_{598}$	$HIV\text{-}1_{LAI}$
Enfuvirtide	2800	1200	1100
DP-180*	>20,000	>20,000	>20,000

*DP-180 is a scrambled 36 aa peptide, different from DP-116

In the [redacted] based assays, an enfuvirtide concentration of about 12-fold greater concentration (1100 ng/ml) is required to achieve an inhibitory level similar to that of enfuvirtide in an identical assay with CEM cells (90 ng/ml). This difference is attributed to the target cells rather than the virus or the peptides since similar concentrations of enfuvirtide inhibit infection of [redacted] by the prototypic $HIV\text{-}1_{LAI}$ and the two primary isolates $HIV\text{-}1_{596}$ and $HIV\text{-}1_{598}$.

Antiviral activity of enfuvirtide against clinical isolates: The applicant evaluated the antiviral activity of enfuvirtide on the baseline (pre-treatment) clinical HIV-1 isolates in the [redacted] assay. The baseline isolates were expanded by PBMC-co-culture of patient samples from Phase II trials [redacted]-003, T20-205, T20-206 and T20-208 and were tested for sensitivity to enfuvirtide in the [redacted] assay.

MAGI (Multinuclear Activation of a Galactosidase Indicator cell assay): MAGI and cMAGI assays were used to assess the inhibitory activity of enfuvirtide against different isolates of HIV-1. MAGI cells are a recombinant HeLa cell line constructed by introducing a CD4 cDNA and HIV-1-LTR driven β-galactosidase reporter gene with a replication-defective amphotropic retrovirus vector. [redacted]

[redacted]

The applicant employed the [redacted] assay strategy to identify the coreceptor usage in the clinical HIV isolates in phase 3 studies as well as determining the antiviral activity of enfuvirtide against clinical HIV-1 isolates. Several HIV isolates with known coreceptor preference served as positive controls.

Summary data presented in Table 5 show that the baseline (pretreatment) isolates from early clinical studies displayed a wide range of sensitivities to enfuvirtide in the [redacted] assay. The sensitivities to enfuvirtide ranged from an IC_{50} of [redacted] ng/ml.

Table 5. Enfuvirtide susceptibility for baseline isolates in phase 2 trials

Trial	N	Enfuvirtide IC_{50} ng/ml range	Median IC_{50} ng/ml	Geometric mean IC_{50} ng/ml
TRI-003	43	0.5-216	20	23.2
T20-205	55	0.4-137	13	12.4
T20-206	27	2.0-84	20	17.5
T20-208	39	0.4-278	20	19.0

The influence of coreceptor tropism on enfuvirtide sensitivity was determined on 130 clinical HIV-1 isolates obtained at baseline from subjects enrolled in clinical studies T20-

205, T20-206 and T20-208. The data presented in Table 8 shows that the sensitivities ranged quite widely from an IC_{50} value of 0.4 ng/ml to 480 ng/ml with a geometric mean value of 15.8 ng/ml ±0.0569 (sd) (0.089 to 107 nM geometric mean 3.2 nM)

Antiviral activity of against non-clade B HIV-1 isolates: To evaluate the antiviral activity of enfuvirtide against different HIV clades, representative isolates were tested. Enfuvirtide and azidothymidine (AZT) were evaluated in parallel experiments for their ability to inhibit infection of PBMC cultures by HIV-1 isolates representing clades A through G. In this test, the level of virus replication in PBMC culture was determined by ——— assay as the endpoint for calculating the IC_{50} and IC_{90}.

Data presented in Table 6 show that enfuvirtide was active against representative HIV clade isolates. The IC_{50} for enfuvirtide ranged from 4 to 280 nM and for AZT the IC_{50} ranged from ——— nM. These data support that enfuvirtide is broadly active against the genetic diversity represented by HIV-1 clades B through F isolates in cell culture experiments. The IC_{50} values for the clades A and G isolates was similar to failure isolates described below.

Table 6. Antiviral activity of enfuvirtide against different clades of HIV-1

Viral Subtype	Viral Isolate	Enfuvirtide (nM)		AZT (nM)	
		IC_{50}	IC_{90}	IC_{50}	IC_{90}
A	UG273	129	1182	69	700
B	US2	29	98	6	20
C	US268	42	543	3	26
D	SE465	38	82	8	23
E	CM240	4	40	0.5	56
F	BZ126	14	50	11	50
G	HH8793	280	558	35	648

Antiviral activity in macrophages: The ability of enfuvirtide to inhibit the replication of clinical monocyte-macrophage tropic HIV isolates was evaluated in freshly prepared monocyte-macrophages isolated from normal human donors. Infection of cells was performed with monocytotropic HIV-1 strains BaL or ADA and the matched pairs of AZT-sensitive and AZT-resistant virus isolates. Virus replication was determined by ——— assay and was used as the endpoint for calculating the IC_{50}. Enfuvirtide inhibited the replication of BaL and ADA strains of HIV-1 in macrophages with an IC_{50} of approximately 50 ng/ml and 200 ng/ml (11.1 nM and 44.5 nM) respectively.

AZT evaluated in parallel as a positive anti-HIV control compound exhibited an IC_{50} of approximately 20 ng/ml against the BaL and ADA strains of HIV-1 (plots not shown here). Toxicity as evaluated by ——— assay showed no toxicity to the monocyte-macrophages at the highest tested concentration (22260 nM) of enfuvirtide or at the highest tested concentration (1,000 nM) of AZT. These results indicate that enfuvirtide inhibits the replication of monocyte and macrophage tropic HIV-1 strains BaL and ADA at concentrations that are not toxic to monocyte-macrophage cultures.

Antiviral activity against HIV-1 with CXCR4 or CCR5 coreceptor specificity: HIV can use one of many coreceptors (the most commonly used are CCR5, CXCR4 or both) during the binding of virus to target cell. The coreceptor preference determines the tropism of the virus. CCR5 coreceptor preference is associated with non-syncytium induction (NSI) and CXCR4 coreceptor preference is associated with syncytium induction (SI). There is a tendency for the virus with CCR5 coreceptor preference to predominate in the early asymptomatic stages of HIV infection and virus with CXCR4 or dual (X4R5) preference to predominate in late stage infection. The binding of gp120 to different coreceptors might affect the nature or kinetics of conformational changes, which take place in gp120 and gp41 during the binding and fusion process. This in turn may alter the access or affinity of enfuvirtide to its target.

The applicant evaluated the antiviral activity of enfuvirtide against a panel (n=12) of laboratory HIV-1 strains and clinical isolates from NIAID in the ——— assay. Three prototypic laboratory-adapted isolates, IIIB, RF, and DH012, and nine PBMC passaged primary isolates were included in the study. All 12 viruses tested were well characterized in terms of their co-receptor specificity and SI/NSI status. The coreceptor preference (X4, dual, or R5) and syncytia inducing characteristics (SI or NSI) are also shown in the table. Data provided in the Table 7 shows that IC_{50} values ranged from 4 ng/ml (0.89 nM) for HIV-1_{IIIB} to 50.0 ng/ml (11.13 nM) for clinical isolate NIAID 301727, but that there was no clear correlation between IC_{50} or IC_{90} and viral co-receptor specificity. The inhibitory concentrations of enfuvirtide observed in this study are in agreement with inhibitory concentrations reported in this review. There appears to be no apparent correlation of enfuvirtide potency with virus coreceptor preference of syncytium inducing phenotype.

Table 7. Enfuvirtide inhibition of HIV-1 isolates with different coreceptor preference

HIV-1 isolate	Preferred Coreceptor	IC_{50} ng/ml	IC_{90} ng/ml	No. of Expts.
IIIB	CXCR4 (SI)	4	67	9
RF	CXCR4 (SI)	27	379	4
DHO12	R5X4 (dual tropic)	27	253	2
NIAID301593	R5X4 (dual tropic)	14	99	2
NIAID301657	CCR5 (NSI)	8	38	3
NIAID301660	CCR5 (NSI)	12	133	1
NIAID301712	CCR5 (NSI)	9	52	9
NIAID301715	CCR5 (NSI)	20	209	2
NIAID301724	CCR5 (NSI)	13	220	1
NIAID301727	CCR5 (NSI)	50	400	2
NIAID302054	CXCR4 (SI)	9	61	1
NIAID302073	CCR5(NSI)	24	173	3

SI =syncytium inducing X4=CXCR4
NSI= non-syncytium inducing R5= CCR5

The similar degree of antiviral activity of enfuvirtide against virus of CXCR4 or CCR5 co-receptor specificity was supported by further analysis of the 130 pre-treatment clinical isolates from Phase II clinical trials. Isolates were characterized as being CXCR4 or CCR5 tropic based on the ratio of their infectious titer in MAGI cells (which express CXCR4) and cMAGI cells (which express CXCR4 and CCR5 co-receptors). CXCR4 tropism was indicated by an infectious titer ratio on MAGI and cMAGI cells of ≥ 0.1 while CCR5 tropism was indicated by a ratio of ≤ 0.01. Intermediate values (0.01 to 0.1) were classified as dual tropic. Data presented in Table 8 indicates no difference in sensitivity to enfuvirtide between clinical isolates with CXCR4 tropism (n=64) or CCR5 tropism (n=58).

Table 8. Enfuvirtide inhibition of 130 baseline isolates in the ——— assay.

Coreceptor tropism	N	Minimum IC_{50} ng/ml	Maximum IC_{50} ng/ml	Geometric mean IC_{50} ng/ml	Standard Deviation
CCR5	58	0.4	480	15	0.076
CXCR4	64	———	———	18	0.036
Dual	8	———	———	10	0.021

Cytotoxicity of enfuvirtide:

In the course of enfuvirtide antiviral activity studies the applicant conducted parallel experiments with enfuvirtide to evaluate the potential cytotoxic effects of the drug. The intent of the cytotoxicity studies was to distinguish if the antiviral activity observed was due to the drugs direct effect on the virus or an indirect effect due to toxicity of the drug to the cells. The cytotoxicity of enfuvirtide in these studies was assessed by the ——— assay.

In the different cytotoxicity studies reported, the antiviral activities of enfuvirtide were seen at concentrations approximately 10^4 - 10^5 times less than those required to visualize cytotoxic effects in tissue culture. These results indicate that the antiviral activity of enfuvirtide was not an artifact of generalized cytotoxicity.

Antiviral activity against resistant isolates of HIV-1: The applicant evaluated the antiviral activity of enfuvirtide against recombinant virus constructs and primary clinical isolates containing resistance mutations to RT and protease inhibitors. The antiviral activity of enfuvirtide was evaluated against HIV isolates characterized to be resistant to all current antiretroviral agents. These studies involved the use of a ——— assay.

[ ]

[redacted]

Table 9. Summary of Enfuvirtide activity against recombinant viruses containing resistance to RT and PR inhibitors ([redacted] assay)

Recombinant virus (Mutations to class of ARVs)	Isolates tested (N)	IC_{50} nM mean (range)
		Enfuvirtide
None	6	3.8 [redacted]
Single-class	21	2.9 [redacted]
Dual-class	33	2.9 [redacted]
Triple-class	26	3.5 [redacted]

Table 9 shows summary data for 86 recombinant viruses that contained reverse transcriptase and protease regions derived from patient isolates and evaluated for their sensitivity to enfuvirtide in the [redacted] assay. These data (59/86 clade B derived viruses were resistant multiple classes of ARVs with 33 of these resistant to two ARV classes and 26 others resistant to all three ARV classes) suggest that enfuvirtide susceptibility was similar for the recombinant viruses resistant to 0, 1, 2 and 3 classes of ARVs and did not show cross-resistance to enfuvirtide.

Table 10. Susceptibility of primary clinical isolates to RT and PR class of drugs and susceptibility to enfuvirtide

Genotype-based virtual phenotypic resistance		IC_{50} nM Geometric mean
No. of ARV class resistance	Isolates tested (N)	
0	5	4.0
Single	6	3.6
Dual	6	3.3
Triple	18	2.9

To evaluate the antiviral activity of enfuvirtide against RT-resistant and PI- resistant isolates, in the presence of the wild-type Env gene, 35 primary clinical virus isolates (— cocultured virus isolates from patients enrolled in phase II studies) were evaluated for their sensitivity to enfuvirtide in the — assay and the sensitivity to RT and PR inhibitors was inferred with the — assay.

The results presented in Table 10 show that sensitivity of primary virus isolates to enfuvirtide is independent of their resistance to 0, 1, 2 and 3 classes of current ARV drugs. The combined results from Tables 9 and 10 indicate that the antiviral activity of enfuvirtide against both recombinant and primary virus isolates was independent of resistance to RT and PR inhibitors. The lack of cross-resistance is consistent with the different mechanism of action of enfuvirtide.

Antiviral activity in combination with other antiretroviral agents: Combination therapy with antiretroviral agents is the standard for the treatment of HIV infection. It is important therefore to assess the in vitro antiviral activity of enfuvirtide in combination with currently available antiretroviral drug classes. Therefore the applicant tested enfuvirtide paired with the reverse transcriptase inhibitors zidovudine, lamivudine and efavirenz, and the protease inhibitors indinavir and nelfinavir.

[ ]

Zidovudine, lamivudine or indinavir together with enfuvirtide displayed synergistic antiviral action when used to inhibit the infection of CEM cells by HIV-1_{IIIB}. The degree of synergy depended upon both the drug ratios and the endpoint being assessed (e.g. ED_{50} or ED_{95}). At some drug ratios and end points, the combined drug activity was only additive but there was no evidence of antiviral antagonism.

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Table 11. Combination indices from CEM cell-HIV-1_{IIIB} infection by — assay

Combinations enfuvirtide with	Ratio by weight	Relationship (CI*)	Combination index			
			ED_{50}#	ED_{75}	ED_{90}	ED_{95}
Zidovudine	1:1	+++	0.46	0.50	0.58	0.67
	2:1	+++	0.30	0.39	0.52	0.62
	2.5:1	+++	0.59	0.49	0.41	0.37
	10:1	+++	0.96	0.62	0.41	0.32
	20:1	+++	0.55	0.49	0.44	0.42
Combinations enfuvirtide with	**Ratio by weight**	**Relationship**	**Combination index**			
			ED_{50}	ED_{75}	ED_{90}	ED_{95}
Lamivudine	6:1	+++	0.60	0.59	0.58	0.57
	5.3:1	+++	0.63	0.58	0.54	0.47
	3:1	+++	0.65	0.71	0.79	0.84
	2.66:1	+++	0.42	0.37	0.33	0.31
	1.33:1	+++	0.45	0.59	0.81	0.81
	1:1.5	+++	0.50	0.61	0.78	0.94
	1:2.66	+/-	0.98	0.97	0.96	0.96
Combinations enfuvirtide with	**Ratio by weight**	**Relationship**	**Combination index**			
			ED_{50}	ED_{75}	ED_{90}	ED_{95}
Indinavir	4:1	++	0.91	0.81	0.72	0.66
	1:1	+++	0.39	0.49	0.61	0.72
	1:32	+/-	1.11	1.09	1.08	1.07

*+++ CI of 0.3 – 0.7 = synergism; ++CI of 0.7 – 0.85 = moderate synergism; + CI of 0.85 – 0.9= slight synergism; +/- CI of 0.9 – 1.1= additive; $-(-)_n$ CI of >1.1 degrees of antagonism
#ED50, 75, 90 and 95 =drug concentration required to achieve 50%, 75%, 90%, 95% inhibition expressed as effective dose

The Combination Index (CI) value is a quantitative measure of the degree of drug interaction in terms of additive effect as defined by Chou and Talalay (3). The CI values are defined such that values much less than 1.0 are synergistic, values around 1.0 are additive and values much greater than 1.0 are antagonistic. The degree to which CI is below or above 1 defines the degree of synergy/antagonism. Here the relationship is defined by: +++ CI of 0.3 – 0.7 = synergism; ++CI of 0.7 – 0.85 = moderate synergism; + CI of 0.85 – 0.9= slight synergism; +/- CI of 0.9 – 1.1= additive; $-(-)_n$ CI of >1.1 degrees of antagonism

In other experiments the antiviral activity of enfuvirtide in combination with efavirenz or indinavir on the acute infection of MT-4 cells with HIV-1_{HXB2} was evaluated by measuring the RT activity in cell supernatants. The results of these experiments, presented in isobologram format in the submission (Vol. 48 pp214-219), indicate that enfuvirtide exhibited additive to synergistic activity in dual combination with efavirenz and indinavir.

Antiviral activity of close analogues of enfuvirtide: During synthesis and/or catabolism of enfuvirtide in vivo some close analogues of enfuvirtide could potentially be present as minor contaminants. The applicant conducted a comprehensive evaluation of the antiviral activity of close analogues of enfuvirtide. One hundred twenty two impure peptides and research grade enfuvirtide were tested in the MAGI ——— assay (The modified peptide sequences and their IC_{50} values are presented in Volume 48 pages 250 to 254). Enfuvirtide impurity peptides include ——— (n=31); ——— (n=36) ——— (n=33); ——— (n=8) and ——— (n=8). The concentration of peptide which reduces infection by 50% (IC_{50}) was determined for each of these.

The results suggest that in general leucine/isoleucine exchanges had little effect on antiviral potency. All other types of change tended to produce analogues with a marked decrease in antiviral potency where the change was towards the center of the peptide (particularly residues 12 to 24) but only modest decrease in antiviral potency when the change was near the N- or C-terminus. No analogues had significantly better activity than enfuvirtide. The data therefore suggest that in case any of these analogues are produced as minor contaminants of enfuvirtide or are catabolites in vivo, the majority of these would retain some degree of antiviral activity.

HIV resistance to enfuvirtide

Resistance evaluations in vitro by cell culture studies: To evaluate the emergence of enfuvirtide resistance in vitro, CEM-4 cells infected with primary HIV-1 isolate DH012 or with uncloned HIV-1_{IIIB} or with a clone of HIV-1_{NL4-3} were sequentially passaged in increasing concentrations of enfuvirtide. Virus production in the cell supernatant was determined on the basis of reverse transcriptase activity. After five passages of the virus over a 5 to 6 week period, enfuvirtide-resistant virus emerged from the primary HIV isolate or uncloned or cloned virus. The resistant concentration was 10-fold or greater than that required to completely inhibit the parental HIV-1 strains.

To determine the genetic basis of enfuvirtide resistance, the envelope region of resistant viruses was amplified by reverse transcriptase mediated polymerase chain reaction (RT-PCR) and the viral envelope DNA was sequenced to determine mutations in the viral DNA. Sequencing results in each case showed that the genotypic change(s) was limited to the amino-terminal region of the gp41 gene, mapping to the amino acid positions 36-38. The common wild-type amino acid sequence for residues 36-38 is GIV*. The enfuvirtide resistant viruses selected in vitro contained SIM (in HIV-1_{IIIB}); DTV (in HIV-1_{NL4-3} in which wild type is DIV) and GIG in the HIV-1_{DHO12} clinical isolate.

To confirm that these mutations were responsible for conferring resistance to enfuvirtide, recombinant HIV containing mutations in amino acid positions 36-38 were generated by site-directed mutagenesis. The mutations were introduced into an HXB3 background at positions 38-38 (GIV) and subcloned into HIV-1_{NL4-3}. Results from these studies confirmed that the mutations 36S, 36D, 37T and 38M could all contribute to resistance to enfuvirtide. In these studies, when mutations were introduced at 2 amino acid positions there was a more than 10-fold reduction in susceptibility to enfuvirtide and with single mutations there was a less than 10-fold change. The applicant suggested that for clinically significant resistance, these mutations had to be present in pairs. However, evaluation of the resistance data does show that single mutations often showed greater than 10-fold decrease in enfuvirtide susceptibility (see Table 12.)

Enfuvirtide resistance studies conducted during the phase 2 clinical studies suggested that genotypic resistance conferring mutations in gp41 covered a wider region than the amino acids 36 to 38 that conferred resistance in vitro. The genotypic changes observed in these early clinical studies showed mutations in the amino acids 36-45 of gp41.

To confirm that the mutations in the amino acids region 36-45 of gp41 indeed conferred resistance to enfuvirtide, the applicant constructed into HIV-1_{NL4-3}, the recombinant viruses containing the mutations in the amino acids 36-45 and evaluated them for resistance. Based on the wild-type sequence of GIVQQQNNLL (Table 12) changes in the mutant recombinant virus susceptibility were >500 fold for the dual mutations V38E + N42S. In general, the susceptibility changes for single amino acid changes were lower than for double substitutions. The data also shows that some of the single mutations such as with V38A, Q40H and N43D conferred greater than a 10-fold decrease in susceptibility.

* One-letter code and 3 letter abbreviations for amino acids are presented in Appendix 2.

Table 12. Change in susceptibility in the site-directed mutants of viruses of pNL4-3 strain

Gp41 aa 36-45 sequence	Amino acid substitution	Enfuvirtide (IC_{50} µg/ml)	Mean	Fold change from wild-type**
GIVHQQNNLL	Q39H	0.011 0.014	0.0125	1.4
GIAQQQNNLL	V38A	0.160 0.216	0.188	20.9
GIVQQQNDLL	N43D	0.210		23.3
GIVQQQNNML	L44M	0.015 0.026	0.021	2.3
GIVQQQNNLM	L45M	0.017		1.9
GIVQHQNNLL	Q40H	0.256		28.4
GIVQQQTNLL	N42T	0.045		5.0
GIVQQQNKLL	N43K	0.063		7.0
GIVQQQTSLL	N42T, N43S	0.727		80.8
GIVQQQTKLL	N42T, N43K	0.388		43.1
GIAQQQDNLL	V38A, N42D	1.685		187
GIEQQQSNLL	V38E, N42S	7.893 4.419	6.156	684
GIAQQQTNLL	V38A, N42T	1.782		198
SIVQQQNNLL	G36S	0.088		9.8
SIVQQQNNML	G36S, L44M	0.181		20.1
GIVQQQNSLL	N43S	0.067		7.4
GIVQQQDNLL	N42D	0.027		3.0
GIVQQQENLL	N42E	0.015		1.7
GIVQQQSNLL	N42S	0.006		0.7
SIMQQQNNLL	G36S, V38M	0.656 0.413 0.424	0.498	55.3
GIMQQQNNLL	V38M	0.042		4.7
DIMQQQNNLL	(G36D), V38M	0.881		97.9
GIVQQQNNLL* (wild-type)	D36G	0.002 0.013 0.012	0.009	
PNL4-3* (DIVQQQNNLL)		0.091		10.1

* Wild-type pNL4-3 virus has [redacted] at position 36 and the IC_{50} value for this genotype is given at the foot of the table. Wild-type viruses usually have a 36G residue. Thus, all other viruses in this table are based on the 36G genotype the "wild-type" IC_{50} value for which is also provided at the foot of the table

** Fold change was calculated from the mean IC_{50} value for wild-type virus. If the median value is used slightly lower fold change values will be obtained. Thus "worst case" values are given here.

Antiviral activity and resistance evaluations in Phase III clinical studies:

The applicant conducted two similar phase 3 trials, studies T-20-301 and T20-302, which are randomized, open label, multicenter studies of patients receiving the recommended dose of 90 mg of enfuvirtide taken twice-daily by subcutaneous injection with an optimized oral antiretroviral background regimen. Both of these studies are similar in design and represent patients with extensive experience with antiretroviral exposure to all 3 classes of antiretroviral agents. To be eligible for the study enrollment patients were required to have prior exposure to all three classes of ARVs and to be on stable therapy for ≥ 4 weeks prior to study entry. Patients were also required to have documentation of a plasma HIV-1 RNA of [redacted]

Table 13. Baseline microbiology-related characteristics of patients enrolled in Phase III studies

Patient information	Study 301		Study 302	
	T-20+OB	OB	T-20 +OB	OB
No. of patients	326	165	335	169
Mean baseline RNA $\log_{10}$	5.1	5.1	5.1	5.1
Mean baseline CD4 cell count /μl	121.3	108.9	150.6	146.2
Mean PSS* at entry	1.7	1.8	1.4	1.4
Mean GSS** at entry	1.9	1.9	1.6	1.7
Number of prior ARV use mean	12.17		11.96	
Mean duration of use (years)	7.35		7.47	

*PSS = phenotypic sensitivity score, ** GSS = genotypic sensitivity score

The microbiology-related baseline characteristics of patients randomized to either treatment group are presented in Table 13. The treatment groups, enfuvirtide plus optimized background (T-20+OB) and optimized background (OB) as active control, were similar at baseline with respect to mean viral load (T-20+OB and OB, 5.1 $\log_{10}$ copies/ml) and mean CD4 cell counts (T-20+OB, 136.2 cells/μl; OB, 127.6 cells / μl). The treatment groups were also similar with respect to genotypic sensitivity score (GSS) and phenotypic sensitivity score (PSS) at baseline. The patients enrolled in these studies had a mean 7 years of prior ARV treatment, and a mean 12 ARVs were used previously. Pooling of these two studies for virology helps to provide a larger sample of patients and improved precision in the estimation of treatment differences between T-20+OB versus OB alone. The two phase 3 studies were also very similar in terms of study patient population, design, patient selection criteria, study conduct, monitoring and planned

analysis not shown in this review (see clinical review by M. Baylor and statistical review by T. Hammerstrom for details) supports the pooling of studies T-20-301 and T-20-302. The planned duration of these studies is 48 weeks of treatment with an optional 48-week extension.)

The primary objectives of the clinical studies are:

1. To demonstrate that Fuzeon added to an optimized background (OB) regimen provides an additional drop in plasma HIV-1 RNA of at least 0.5 $\log_{10}$ copies/ml compared to the OB regimen alone at week 24, as measured by the difference between the two treatment arms in the mean changes from baseline value in plasma HIV-1 RNA ($\log_{10}$ copies/ml) at week 24.

2. To demonstrate the durability of efficacy of the T-20+OB regimen as measured by the percentage of patients who responded at week 24 and maintained response in each category or better at week 48, with a viral load of:
   a) [redacted],
   b) [redacted], and
   c) 1.0 $\log_{10}$ decrease from baseline but [redacted], or had
   d) Virological failure at week 24, and maintained their response in each category or improved at week 48.

The applicant stated that durability of viral suppression of the T-20+OB regimen would be analyzed at week 48 and reported separately.

Microbiology-related secondary objective of each study was, to evaluate the percentage of patients with a $\geq$1.0 $\log_{10}$ drop in plasma HIV-1 RNA; plasma HIV-1 RNA [redacted], and plasma HIV-1 RNA [redacted] at week 24 and week 48.

The primary efficacy measures for the studies was the change from baseline to week 24 in viral load and CD4 cell count as assessed by the change in $\log_{10}$ transformed plasma HIV-1 RNA levels.

The secondary efficacy parameters for the week 24 analysis included the following:
Percent of patients who meet each of the different definitions of virological response;
Time to virological response;
Time to virological failure;
Change from baseline in CD4 cell counts;

For virological responder analyses, three categories of virological response were defined as follows:

1. HIV-1 RNA [redacted]
2. HIV-1 RNA [redacted], and
3. HIV-1 RNA >1.0 $\log_{10}$ copies/ml decrease from baseline

In order to be qualified as a virological responder in a specific category, a patient had to have two consecutive viral load values that met the criterion for virological failure.

Change in CD4 Cell Counts: CD4 cell counts were assessed at screening, baseline, and every 4 weeks thereafter until week 24. Quantitative analyses of CD4 cell counts were performed by using a [redacted]

Virologic failure: Virologic failure was defined as any one of the following:

1. Plasma HIV-1 RNA level < 0.5 $\log_{10}$ decrease from baseline either on two consecutive measurements (≥14 days apart) or on three consecutive measurements with ≥14 days between the first and third measurements, starting at weeks 6 and 8 or any time after week 8; or

2. Plasma HIV-1 RNA < 1.0 $\log_{10}$ decrease from baseline either on two consecutive measurements (≥14 days apart) or on three consecutive measurements with ≥14 days between the first and the third measurement, starting at weeks 14 and 16 or any time after week 16; or

3. This criterion contained 2 parts, part 3A and part 3B, both of which had to be satisfied for a patient to have met criterion 3 for virological failure.

> 3A) Patient achieved an HIV-1 RNA level of ≥2.0 $\log_{10}$ decrease from baseline either on two consecutive measurements (≥ 14 days apart) or on three consecutive measurements with ≥14 days between the first and the third measurement. If so, then the patient qualified for criterion part 3B.
>
> 3B) Patient met part 3A and had HIV-1 RNA level rebound from the average of the two lowest values (which did not have to be from consecutive measurements) by > 1.0 $\log_{10}$ either on two consecutive measurement ≥14 days apart) or on three consecutive measurements with ≥14 days between the first and the third measurements, starting at weeks 6 and 8 or any time after week 8.

The applicant attempted to perform HIV envelope (Env) susceptibility testing to enfuvirtide and gp41 genotyping for baseline samples from all Fuzeon + OB patients

prior to the start of Fuzeon dosing. Resistance testing for approved antiviral was performed at screening and at the earliest of the following:
Time of virologic failure,
Week 48 of treatment or
Premature discontinuation.

In a similar fashion, resistance testing for patients on treatment was performed at the earliest of the following:
Time to virologic failure,
Week 48 (if the HIV-1 RNA was greater than ——— , or
Premature discontinuation.

In this submission, the applicant provided viral Env susceptibility to enfuvirtide obtained through study week 24. The data also include genotypic changes showing the relationship between the presence of mutations in gp41 at amino acids 36-45 and changes in virus susceptibility to enfuvirtide in patients meeting the criteria for virologic failure. The applicant indicated that a separate 48-week clinical virology report with complete analysis of correlation between phenotypic changes on treatment and genotypic substitutions would be presented when data are available.

Plasma HIV-1 RNA levels were to be assessed at every study visit. Quantitative analysis of plasma HIV-1 RNA was performed using an investigational ——— Assay ———and standard sample preparation (sensitivity range: ——— copies/ml). Any sample analysis which had a HIV-1 RNA level of ——— copies/ml with standard sample preparation was to be repeated using ultrasensitive sample preparation (sensitivity range: ——— copies/ml). If any sample had an HIV-1 RNA level of ——— copies/ml with standard sample preparation, sample analysis was to be repeated using a diluted sample.

In the event that virological failure was suspected, plasma HIV-1 RNA measurement was to be repeated at least 14 days after the initial assessment that indicated failure to confirm virologic failure.

Definition of Genotypic and Phenotypic Sensitivity Scores: The applicant defined the genotypic and phenotypic sensitivity scores by the following algorithm, which is reproduced below.
Genotypic sensitivity score (GSS) is the sum of the genotypic scores and is defined as the total number of drugs in a patient's actual OB regimen to which a patient had genotypic sensitivity as deduced from gene sequence and mutation analysis. For each drug in the

OB regimen, a score of 1 was assigned if the HIV-1 genotype results did not indicate reduced susceptibility for that ARV (GT=1). A score of 0 was assigned if reduced susceptibility was indicated (GT=0). The GT score was assigned to 0 for Tenofovir if a patient had mutation K65R or had 3 or more of thymidine analog-associated resistance mutations (TAM: M41L, D67N, K70R, L210W, T215Y/F or K219Q/E/N) which must include either M41L or L210W. Otherwise the GT score was 1 for Tenofovir. Thus, GSS is the sum of the GT scores for all the drugs in a patient's OB regimen, and the higher the GSS, the more likely the regimen was to be effective.

For example, if a patient is taking 4 ARVs but his/her HIV-1 is sensitive to only 1 of the 4 ARVs, the patient's GSS=1. If the patient's HIV-1 had genotypic sensitivity to all 4 ARVs, then his/her GSS=4, and there are 4 drugs in the regimen likely to be effective against the patient's HIV-1 virus.

Phenotypic sensitivity score (PSS) is the sum of the phenotypic scores and is defined as the total number of drugs in a patient's OB regimen to which a patient had phenotypic sensitivity, derived from testing a patient's virus against ARV drugs *in vitro*. For each drug in the OB regimen, a score of 1 was assigned if phenotypic results indicate sensitivity (PT susceptibility score was 1 or 2 based on the degree of susceptibility from the virologic report), or 0 if the virus was phenotypically resistant (PT=0). Hypersensitive HIV-1 virus was assigned a phenotypic score of 2; however, it was given a value of 1 for the purposes of calculating the PSS.

Thus, PSS is the sum of the sensitivity scores for all the drugs in a patient's OB regimen, and the higher the PSS, the more likely the regimen was to be effective. [redacted], the PT score was set to be equal to the GT score for Tenofovir.

For non-genotypic or phenotypic samples, GSS and PSS were considered as missing unless they were used as the covariate in the analysis of covariance model. In those cases, the missing PSS or GSS score was replaced by the total number of ARVs, in a patient's OB regimen divided by 2.

For all of the patients in the T-20+OB arm, gp41 genotyping and enfuvirtide susceptibility was attempted prior to the start of enfuvirtide dosing. In this report genotypic data were presented on patients who met virologic failure through study week 24. The applicant stated that phenotypic susceptibility to enfuvirtide and the correlations between genotypic and phenotypic changes on treatment would be presented in a separate

report as part 2 at a later date. The applicant also stated that analysis of the 48-week data would be presented in a separate submission.

The primary objectives of the resistance analysis are:

1. To characterize the susceptibility of baseline patient plasma virus Env to inhibition by enfuvirtide
2. To characterize changes in susceptibility of patient plasma virus Env to inhibition by enfuvirtide between baseline and protocol-defined virologic failure

The secondary objectives of the resistance analysis are:

1. To examine the association between substitutions in plasma virus gp41 at amino acid positions 36-45 and changes in Env susceptibility to enfuvirtide between baseline and virologic failure.
2. To examine the prevalence of virus R5, X4 or dual receptor tropism and the prevalence of non-B clade virus for baseline Envs
3. To estimate the incidence of changes in coreceptor tropism between baseline and virologic failure
4. To assess association between the viral load decreases during treatment and the following characteristics for baseline Envs: susceptibility to enfuvirtide, baseline Env coreceptor tropism, Env clade and substitutions in gp41 amino acids 36-45 different from HXB2.
5. To examine the relationship between virologic factors including coreceptor tropism (R5 and X4) and Env clade on susceptibility to enfuvirtide at baseline.

Methods used for genotypic and phenotypic analysis of HIV Envelope

Genotypic and phenotypic analysis of HIV Envelope gene gp160: The applicant employed a commercial testing laboratory, [redacted], for genotyping and testing of phenotypic susceptibility to enfuvirtide. The assay, an experimental proprietary procedure called [redacted] Assay, is not approved. This assay developed for the evaluation of phenotypic and genotypic resistance to enfuvirtide is similar to the methodologies previously used to determine the genotypic and phenotypic resistance to HIV RT and PR inhibitors.

[redacted]

For the combined 301 and 302 clinical studies paired phenotypic data at baseline and virological failure were available for 207/301(68.8%) patients through week 24. However, the fold change in susceptibility on treatment could only be assessed for 206 patients as one patient switched tropism between X4 and R5.

Table 14. Emergence of resistance and virological failure in patients on Fuzeon treatment

	Clinical Study		
	301	**302**	**301+302**
Patients in ITT	326	335	661
Patients with baseline enfuvirtide PT[1]	300	312	612
Patients meeting VF[1] criteria	136	165	301
VF patients with PT at time of VF[#]	97	116	213
VF patients with BL and VF PT	94*	113	207*
VF patients with BL[1] and VF, GT[1] and PT	94*	111	205*

1= abbreviations: PT= phenotype; GT= genotype; VF= virologic failure; BL= base line

Plasma sampling for resistance testing generally occurred after virologic failure.

*Samples from ninety-three patients in study 301 and 206 for the combined studies were evaluable for fold-change due to coreceptor switch from X4 to R5 for Env from one patient. Similarly, 93 for study 301 and 204 for the combined studies had paired GT and PT and were evaluable for fold-change in susceptibility.

Baseline viral Env susceptibility to enfuvirtide: The applicant provided a histographic representation of the baseline pEC_{50} values (pEC_{50} = Patient viral Env EC_{50}, defined as the concentration of enfuvirtide required for 50% inhibition of recombinant virus carrying patients plasma-derived Env) with percentage of Envs in each bar on the Y-axis and the actual pEC_{50} values on the X-axis (Interim report, Module 1 page 39-41). The data indicate that the log of pEC_{50} values is approximately normally distributed around a geometric mean of 0.259 μg/ml. Sixteen of the 612 (2.6%) Envs from the pooled isolates had pEC_{50} values greater than 2 standard deviations above the geometric mean (>1,956 μg/ml), while 8 (8/612, 1.3%) pEC_{50} values more than 2 standard deviations below the geometric mean (0.034μg/ml). In the combined studies, the geometric mean plus 2 standard deviations was approximately 7.5 fold higher than the geometric mean. [The

geometric mean pEC_{50} values in study 301 and 302 were 0.259 µg/ml (n=300) and 0.260 µg/ml (n=312), respectively]

HIV Env susceptibility to enfuvirtide at virologic failure: The data presented in Table 15 show the pEC_{50} values and the fold changes from baseline at virologic failure. The overall geometric mean pEC_{50} at the time of virologic failure for the combined studies was 5.645 µg/ml (range ————µg/ml). Therefore, the geometric mean fold increase in pEC_{50} from baseline was 21.8 fold (range ————.

Table 15. pEC_{50} and fold-change from baseline at virologic failure

Study	301		302		301+302	
	EC_{50} at VF	**Fold change at VF**	**EC_{50} at VF**	**Fold change at VF**	**EC_{50} at VF**	**Fold change at VF**
N	97	93	116	113	213	206
Geo. Mean (µg/ml)	7.189	26.05	4.611	17.15	5.645	20.71
Min-Max	[ ]	[ ]	[ ]	[ ]	[ ]	[ ]

The summary of pEC_{50} fold increase from baseline at virological failure presented in Table 16 for the combined 301 and 302 studies, at the time of virologic failure shows that most patient Env recombinant viruses (159/206, 77.2%) had greater than a 10-fold increase in pEC_{50} from baseline. A similar percentage of Envs, 13.6% (28/206) and 9.2% (19/206), had 4- to 10-fold changes and less than 4-fold changes, respectively.

Table 16. Summary of pEC_{50} fold increase from baseline at virological failure

	301	**302**	**Overall**
Less than 4-fold	5 (5.4%)	14 (12.4%)	19 (9.2%)
4-10 fold	11 (11.8%)	17 (15%)	28 (13.2%)
Greater than 10-fold	77 (82.8%)	82 (72.6%)	159 (77.2%)

The results indicate that among patients with paired Env genotype and phenotype from baseline and virologic failure a large majority (90.8%) showed greater than 4-fold increase in pEC_{50} at virologic failure through 24 week of treatment. The applicant stated

that of the 17 patients with evaluable Env showing less than 4-fold increase in pEC_{50} on treatment, Envs from 7/17 (41.2%) had substitutions in gp41 aa 36-45 at the time of virologic failure and Envs from 10/17 (58.8%) did not have these mutations.

In the combined studies, viral Env susceptibility to enfuvirtide was available for nearly all (612/661, 92.6%) patients at baseline, and a majority (207/301, 68.8%) of patients at virological failure.

Genotypic resistance: The applicant evaluated the correlation between the decrease in susceptibility to enfuvirtide and specific substitutions in gp41 at amino acids 36-45 at virologic failure. The data presented in Table 17 show that the geometric mean fold changes observed ranged from 7.2-fold for the viruses harboring the G36G/D mixture to 42-fold for the most common substitution V38A (n=27). The second most common substitution N43D (n=19) showed a geometric mean fold-change of 26.4 fold. A single substitution classified as a mixture generally had lower fold-changes than their corresponding full substitutions. The genotypic changes in gp41 amino acids 36 to 45 were observed with decreasing frequency at amino acid positions 38, 43, 36,40, 42 and 45.

Table 17. Genotypic changes in phenotypically resistant HIV isolates.

	pEC_{50} fold change from baseline		
	N	**Geo. mean**	**Min-Max**
V38A	27	41.60	
N42T	3	38.10	
V38V/A	8	36.48	
G36D	6	32.39	
Q40H, L45L/M	3	28.88	
Q40Q/H, N43N/D	3	28.44	
N43D	19	26.38	
V38V/A, Q40Q/H	3	23.12	
Q40H	4	18.94	
V38M	6	15.20	
V38V/A, N43N/D	3	13.01	
G36G/D, N43N/D	4	11.16	
N43N/D	5	8.63	
G36G/D	3	7.17	
None	12	1.55	

The magnitude of resistance observed with clinical samples are consistent with previous site-directed mutagenesis studies which indicated that multiple substitutions within amino acid residues 36-45 of gp41 generally confer greater decrease in susceptibility to enfuvirtide than those observed for viruses with single substitution.

Baseline coreceptor tropism and clade designation: The applicant evaluated the coreceptor usage of the baseline isolates using MAGI [redacted] assays described earlier. Table 18 shows the coreceptor use of the baseline recombinant virus from study 301 and 302 and from the combined studies.

Table 18. Coreceptor tropism of recombinant viruses at baseline

Clinical study	301 n=300	302 N=312	301+302 N=612
Baseline virus coreceptor tropism			
Patients with only R5 tropic virus	186 (62.0%)	19 (61.5%)	378 (61.8%)
Patients with only R4 tropic virus	1 (3.7%)	12 (3.8%)	23 (3.8%)
Patients with only R5X4 tropic virus	103 (34.3%)	108 (34.6%)	211 (34.5%)
Baseline viral clade			
No evaluable baseline genotype	3 (1.0%)	0	3 (0.5%)
Clade B virus	294 (98%)	297 (95.2%)	591 (96.6%)
Non-B clade virus	1 (0.3%)	11(3.5%)	12 (2.0%)
Indeterminate clade*	2 (0.7%)	4 (1.3%)	6 (1.0%)

*No clade designation could be assigned for a total of 6 isolates due to the indeterminate sequence in the Env region used to determine the clade

The data in the Table 18 indicate that in the combined 301 and 302 studies baseline viruses from 378/612 (61.8%) patients were R5 tropic, while viruses from 23/612 (3.8%) patients were X4 tropic and viruses from 211/612 (34.5%) were dual tropic at baseline.

With regard to clades, 591/612 (96.6%) of the baseline viruses were classified as belonging to clade B. Viruses from 12 patients (2.0%) were classified as belonging to non-B clades. No clade could be assigned for the viruses from 6 patients and 3 patients had no evaluable baseline genotype.

Changes in viral coreceptor tropism during treatment: Table 19 shows the summary of the number of patients with changes in viral Env coreceptor tropism on treatment. Viral Env from a total of 42/207 (20%) patients had a change in coreceptor tropism from

baseline during treatment. Recombinant virus from 13/207 (6.3%) of the patient's virus Env changed tropism from R5 to R5X4 and 24/207 (11.6%) of patient's virus Env changed from R5X4 to R5. Viral Env from 1 patient changed tropism from X4 to R5 and none changed in the reverse direction.

Table 19: Summary of changes in viral tropism in the combined studies 301 and 302

Coreceptor tropism at baseline	Coreceptor at virological failure			
	X4	R5	R5X4	Total
X4	4	1	1	6 (3%)
R5	0	113	13	126 (61%)
R5X4	3	24	48	75 (36%)
Total	7 (3%)	138 (67%)	62 (30%)	207

The applicant analyzed the relationship between the HIV-1 RNA $\log_{10}$ change from baseline and viral tropism (Table 20). The data indicate that patients harboring these viruses experienced similar reductions in viral load. It is unclear if the changes in coreceptor use reflect clinically significant changes or are a result of the assay, i.e. the use of recombinant clones.

Table 20. Mean decline in $\log_{10}$ HIV-1 RNA at week 24 by baseline coreceptor tropism.

	X4	**R5**	**R5 X4**	**P-value**
N	23	378	211	0.4056
Least square mean	-1.34	-1.55	-1.64	
Standard error	0.243	0.059	0.086	

The summary virology data from phase 3 clinical studies indicate Fuzeon is an effective antiviral agent. However, 45.5% of the patients were virologic failures by week 24 of treatment. Of these, 90.8% harbored virus with a ≥4-fold decrease in susceptibility to enfuvirtide. The emergence of resistance is not surprising because the viral target, HIV gp160, is the most highly variable gene of HIV and single amino acid substitutions can confer clinically meaningful shifts in susceptibility.

CONCLUSIONS:

A range of anti-HIV activity studies of enfuvirtide using a variety of virus-cell infection test systems provided a broad perspective into the antiviral activity of enfuvirtide. The summary results from these studies show that enfuvirtide inhibits HIV-1 replication at a concentration of approximately 1.0 μg/ml. Pharmacokinetic studies showed that at the prescribed dose of 90 mg, BID, the steady state plasma concentration of enfuvirtide was 4 μg/ml which appears adequate for exerting antiviral activity in vivo. Accordingly in the clinical studies, Fuzeon was effective as an anti-HIV drug. Consistent with the pharmacokinetic data is the observation that ninety percent of HIV isolates from virologic failure patients exhibited a >4 fold shift in susceptibility. Thus, the in vitro antiviral activity is predictive of in vivo activity and is consistent with the antiviral efficacy observed in clinical studies.

The inherent genetic variation with predominant single base mutations and the emergence of resistance to antiretroviral agents in HIV is a well recognized phenomenon. The target of enfuvirtide in HIV, the viral envelope, is even more mutation prone than other HIV genes because of selective immune pressure on the envelope in addition to the inherent genetic variation. Therefore, the expectation of treatment of HIV infection with a drug that targets HIV envelope proteins is for a shorter duration with relatively rapid emergence of escape mutants. Accordingly, enfuvirtide resistant virus in vitro emerged within 5 cycles of virus passage in cells, and in the clinical studies 45.5% (301/661) of treated patients experienced virologic failure within 24 weeks of treatment. Among the virologic failures 90.8% of the failures were attributed to the emergence of resistance to enfuvirtide. The genetic basis for the resistance was due to mutations that occurred in the gp41 binding site of enfuvirtide, at amino acid positions 36-45.

The mechanism of HIV entry and its blockade by enfuvirtide involve the participation of the viral determinants as well as the cellular determinants. Predictably, the virus for its part displayed its facile escape from the drug's pressure by altering its genome. What is remarkable and unresolved is the effects of adaptation of the virus to the host by acquisition of the ability to switch and/or broaden its tropism. How these gains of function influence the viral transmissibility, cell/tissue tropism and replication efficiency has not been addressed. Prior evidence in the literature suggested that change in viral tropism from the use of the CCR5 to the CXCR4 coreceptor leads to changes in HIV pathogenesis. In short, is there a change in the viral virulence with changes in the viral tropism? There were no switches from CCR5 to CXCR4 but others were observed. The applicant should be asked to address these aspects in future studies.

Analysis of HIV isolates from virologically failed patients indicate that the failure was due to the emergence of resistance mutations at the drug binding site in gp41 at aa 36-45 in a majority (90.8%) of these patients. However, in 10 patient isolates, the drug failure could not be attributed to mutations at the drug-binding site. Evidence in the published literature indicates that the escape mechanism against enfuvirtide was not only exclusive to the gp41 region as the applicant implied but also due to alterations beyond gp41, in certain domains in the gp120 of the HIV envelope (4 and additional references therein). In addition it has been reported that resistance could be due to alterations in coreceptor expression levels (4). Thus, the applicant should be requested to investigate the potential causes of virologic failure due to genotypic changes at sites beyond the drug-binding site, which may cause/contribute to resistance. In addition, resistance due to alterations in expression levels should also be explored.

Microbiology Label: FDA version of the microbiology label

MICROBIOLOGY

Mechanism of Action

Enfuvirtide interferes with the entry of HIV-1 into cells by inhibiting fusion of viral and cellular membranes. Enfuvirtide binds to the first heptad-repeat (HR1) in the gp41subunit of the viral envelope glycoprotein and prevents the conformational changes required for the fusion of viral and cellular membranes.

Antiviral Activity In Vitro

The in vitro antiviral activity of enfuvirtide was assessed by infecting different CD4+ cell types with laboratory and clinical isolates of HIV-1. The IC_{50} (50% inhibitory concentration) for enfuvirtide in laboratory and primary isolates representing HIV-1 clades A to G ranged from 4 to 280 nM (18 to1260 ng/ml). The IC50 for baseline clinical isolates ranged from 0.089 to 107 nM (0.4 to 480 ng/ml) by the cMAGI assay (n=130) and from 1.56 to 1680 nM (7 to 7530 ng/ml) by a recombinant phenotypic entry assay (n=612). Enfuvirtide was similarly active in vitro against R5, X4, and dual tropic viruses. Enfuvirtide has no activity against HIV-2.

Enfuvirtide exhibited additive to synergistic effects in cell culture assays when combined with individual members of various antiretroviral classes, including zidovudine, lamivudine, nelfinavir, indinavir, and efavirenz.

Drug Resistance

HIV-1 isolates with reduced susceptibility to enfuvirtide have been selected in vitro. Genotypic analysis of the in vitro-selected resistant isolates showed mutations that resulted in amino acid substitutions at the enfuvirtide binding HR1 domain positions 36 to 38 of the HIV-1 envelope glycoprotein gp41. Phenotypic analysis of site-directed mutants in positions 36 to 38 in an HIV-1 molecular clone showed a 5-fold to 684-fold decrease in susceptibility to enfuvirtide.

In clinical trials, HIV-1 isolates with reduced susceptibility to enfuvirtide have been recovered from subjects treated with FUZEON in combination with other antiretroviral agents. Post treatment HIV-1virus from 185 subjects exhibited decreases in susceptibility to enfuvirtide ranging from 4-fold to 422-fold relative to their respective baseline virus and exhibited genotypic changes in gp41 amino acids 36 to 45. Substitutions in this region were observed with decreasing frequency at amino acid positions 38,43, 36, 40, 42, and 45.

Cross-resistance

HIV-1 clinical isolates resistant to nucleoside analogue reverse transcriptase inhibitors (NRTI), non-nucleoside analogue reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI) were susceptible to enfuvirtide in cell culture.

Roche version of the microbiology label
Microbiology
Mechanism of Action

[

]

Antiviral Activity In Vitro

[

]

[redacted]

Cross-resistance

[redacted]

Phase 4 considerations:

1. Please evaluate the effect of possible alterations in viral tropism on virulence. Submit the completed evaluation within 18 months of the date of this letter.

2. In patients who failed virologically in Studies T20-301 and T20-302, please sequence the entire gp160 sequence of paired isolates to identify the genetic determinants that contribute to the phenotypic resistance. Submit your results within 15 months of the date of this letter

RECOMMENDATIONS: The applicant's studies on the in vitro antiviral activity of enfuvirtide showed that the drug is an inhibitor of HIV replication. Consistent with the in vitro observation, at the prescribed dose of 90-mg BID, the drug product Fuzeon inhibited HIV replication in vivo as quantified by reduction of the viral RNA in the plasma of treated patients. With respect to microbiology the drug is recommended for approval.

Narayana Battula, Ph.D.
Microbiologist

Concurrence:
HFD530/Assoc. Dir. Farrelly, J. _____

HFD530/TL Micro. O'Rear, J. _____

REFERENCES:

1. Burkhard P. et al., (2001) Coiled coils: a highly versatile protein folding motif. Trends Cell Biol. 11:82-8

2. Wild CT. et al., (1994) peptides corresponding to a predictive α-helical domain of human immunodeficiency virus type 1 gp41 are potent inhibitors virus infection. Proc. Natl. Acad. Sci.USA. 91: 1970-74

3. Chou TC, Talalay P. (1984) Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul. 22:27-55

4. Reeves JD et al., (2002) Sensitivity of HIV-1 to entry inhibitors correlates with envelope/coreceptor affinity, receptor density, and fusion kinetics. Proc. Natl. Acad. Sci.USA. 99: 16249-54

Appendix-1
GLOSSARY OF ABBREVIATIONS

aa	Amino Acids
AIDS	Acquired Immunodeficiency Syndrome
ARV	Antiretroviral (drugs)
cMAGI	CCR5 expressing derivative of Multinuclear Activation of Galactoside Indicator cells
EFV	Efavirenz
GST	Genotypic Sensitivity Score
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HR1, HR2	HIV gp41 heptad repeat regions 1 and 2
IDV	Indinavir
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
OB	Optimized background Therapy
PBMC	Peripheral Blood Mononuclear Cells
PI	HIV Protease Inhibitor
PR	HIV Protease
PST	Phenotypic Sensitivity Score
RT	HIV Reverse Transcriptase
XTT	2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl]-2H-tetrazolium hydroxide

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)

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/s/

Narayana Battula
4/3/03 04:15:04 PM
MICROBIOLOGIST

Fuzeon review. Jules and Jim signed off on the hard copy

Julian O Rear
4/5/03 12:40:55 PM
MICROBIOLOGIST

James Farrelly
4/8/03 08:56:59 AM
PHARMACOLOGIST

Product Quality Microbiology Review

Review for HFD- 530

February 27, 2003

NDA: 21-481

Drug Product Name
Proprietary: Fuzon™ for Injection
Non-proprietary: enfuvirtide
Drug Product Classification: viral fusion inhibitor (antiviral)

Review Number: 1

Subject of this Review
Submission Date: September 13, 2002
Receipt Date: September 18, 2002
Consult Date: November 4, 2002
Date Assigned for Review: November 7, 2002

Submission History (for amendments only): N.A.
Date(s) of Previous Submission(s): N.A.
Date(s) of Previous Micro Review(s): N.A.

Applicant/Sponsor
Name: Hoffman – La Roche Inc.
Address: 340 Kingsland Street
Nutley, NJ 07110

Representative: Robin L. Conrad
Telephone: 973-582-3676

Name of Reviewer: James L. McVey

Conclusion: The application is incomplete in that most of the sterilization process validation data is not provided.

APPEARS THIS WAY
ON ORIGINAL

Product Quality Microbiology Data Sheet

A. 1. **TYPE OF SUPPLEMENT:** N.A.
2. **SUPPLEMENT PROVIDES FOR:** N.A.
3. **MANUFACTURING SITE:**

Table 4 Commercial Manufacturers

Manufacturing Address	Process	Establishment #	Date for PAI Readiness
F. Hoffmann-La Roche Ltd. Grenzacherstrasse 124 4070 Basel Switzerland	• Manufacture of Vials • Release and Stability Testing of Vials	FEI # 3002807200 CFN# 9692013	11/1/02
[redacted]	[redacted]	[redacted]	11/1/02
Hoffmann-La Roche Inc. 340 Kingsland Street Nutley, New Jersey 07110-1199	• Stability Testing • Labeling • Secondary Packaging **Sterile Water for Injection**	CFN# 2210844	9/1/02
[redacted]	[redacted]	[redacted]	9/1/02

4. **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** 90 mg/vial for reconstitution with 1.1 mL of sterile WFI to yield approximately 1.2 mL.
5. **METHOD(S) OF STERILIZATION:** [redacted]
6. **PHARMACOLOGICAL CATEGORY:** Antiviral.

B. **SUPPORTING/RELATED DOCUMENTS:** T-20 Fusion Inhibitor IND [redacted] pre-NDA sponsor prepared minutes submitted on July 7, 2002. [redacted] DMF for A Letter of Reference for this DMF is stated to be provided in section A.2.6 (Container/Closure) . The letter dated June 12, 2002 is found in the DMF.

C. **REMARKS:** [redacted]; production at [redacted] plants for manufacture of the drug product and a [redacted] for [redacted] production are the subjects of this review.

filename: 21481r1

Executive Summary

I. Recommendations

A. Recommendation on Approvability – This application is recommended for approval pending resolution of the product quality microbiology issues.

B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable – N.A.

II. Summary of Microbiology Assessments

A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology – Formulated product is ——— and ——— at —— separate plants. A —— plant manufactures the ———. The package may contain sterile syringes and alcohol wipes as well.

B. Brief Description of Microbiology Deficiencies - The lack of validation data for the sterilization of the product, the equipment and the container/closures is the major issue. The applicant provides protocols and acceptance criteria without actual verification data in most cases. This is consistent for both aseptic filling plants.

C. Assessment of Risk Due to Microbiology Deficiencies – Risk (to human health) is high that a non sterile product could be shipped if validation data is not completed and acceptable.

III. Administrative

A. Reviewer's Signature _____

B. Endorsement Block

Review Microbiologist. J.L. McVey
Microbiology Supervisor. P.H. Cooney

C. CC Block

cc:
DFS
HFD- 805/McVey/21481

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Peter Cooney
2/27/03 11:14:10 AM
MICROBIOLOGIST

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Product Quality Microbiology Review

Review for HFD- 530

March 11, 2003

NDA: 21-481 Amendment

Drug Product Name

Proprietary: Fuzon™ for Injection
Non-proprietary: enfuvirtide
Drug Product Classification: viral fusion inhibitor (antiviral)

Review Number: 2

Subject of this Review

Submission Date: September 13, 2002
Receipt Date: September 18, 2002
Consult Date: November 4, 2002
Date Assigned for Review: November 7, 2002

Submission History (for amendments only): Amendment submission dated March 7, 2003 and received March 10, 2003.
Date(s) of Previous Submission(s): See above
Date(s) of Previous Micro Review(s): Review number 1, dated February 27, 2003. FAX with Microbiology concerns sent February 27, 2003.

Applicant/Sponsor

Name: Hoffman – La Roche Inc.
Address: 340 Kingsland Street
Nutley, NJ 07110
Representative: Robin L. Conrad
Telephone: 973-582-3676

Name of Reviewer: James L. McVey

Conclusion: This application is recommended for approval from a product quality microbiology perspective.

A. 1. **TYPE OF SUPPLEMENT:** Original Application.

2. **SUPPLEMENT PROVIDES FOR:** n.a.

3. **MANUFACTURING SITE:** Hoffman La Roche in Basel, Switzerland and ——— for the ———product. ——— for the ———.

4. **DOSAGE FORM, ROUTE OF ADMINISTRATION AND STRENGTH/POTENCY:** 90 mg/vial for reconstitution with 1.1 mL diluent.

5. **METHOD(S) OF STERILIZATION:** ———

6. **PHARMACOLOGICAL CATEGORY:** Antiviral.

B. **SUPPORTING/RELATED DOCUMENTS:** Original submission dated September 13, 2003.

C. **REMARKS:** Additional concerns would be raised about ——— at —— plants and ———, testing at the ——— plant if this drug product were not considered very important to human health. These concerns are judged to be sufficiently small with respect to risk to human health that they are not considered approvability issues for this application. The Hoffman – La Roche processes for sterilization of components and equipment were previously approved in NDA 19-661/S-028 on September 23, 2002 under similar circumstances (priority drug).

filename: 21481r2.doc

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Executive Summary

I. Recommendations

A. Recommendation on Approvability – Recommended for approval.

B. Recommendations on Phase 4 Commitments and/or Agreements, if Approvable – n.a.

II. Summary of Microbiology Assessments

A. Brief Description of the Manufacturing Processes that relate to Product Quality Microbiology – see first review.

B. Brief Description of Microbiology Deficiencies – none.

C. Assessment of Risk Due to Microbiology Deficiencies – Minimal risk to human health is perceived from the approval of this drug product.

III. Administrative

A. Reviewer's Signature _____

B. Endorsement Block
Review Microbiologist. J.L. McVey
Microbiology Supervisor. P.H. Cooney

C. CC Block
DFS
HFD- 805/McVey/21481r2

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