SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Fuzeon. This scientific discussion has been updated until 1 November 2004. For information on changes after approval, please refer to module 8b.

1. Chemical, pharmaceutical and biological aspects

Composition

Fuzeon is presented as a powder for solution for injection and a powder and solvent for solution for injection containing 90 mg of enfuvirtide, as active substance. It is intended for single-dose subcutaneous administration following reconstitution with water for injections.

The other ingredients include sodium carbonate anhydrous, mannitol, sodium hydroxide, hydrochloric acid (and water for injections).

Fuzeon is supplied in type I glass vials. The closure system consists of a rubber stopper and a seal with a flip-off cap. It is available in three presentations, one including only powder vials, the second one including powder vials and solvent vials and the third one including powder vials, solvent vials, syringes with safety features of 1 ml and 3 ml and alcohol swabs.

Active substance

Enfuvirtide is a chemically synthesised 36-amino acid peptide. Its sequence is derived from the HIV-1 glycoprotein gp41.

The active substance is insoluble in pure water and its solubility is pH dependent. It is insoluble to very slightly soluble in most organic solvents.

Enfurvitide is a chiral molecule composed of naturally occurring L-amino acid residues. Diastereoisomers and epimers produced during the synthesis are considered as impurities. The enantiomer consisting of solely D-amino acids is unlikely because of the nature of the synthesis process in which the enantiomeric purity is part of the acceptance criteria for the amino acids starting materials.

Enfuvirtide can exist in four solid amorphous forms, but only the intended commercial synthesis process has produced two of them.

Concerning aggregation, this increases with the enfuvirtide, sodium chloride and buffer concentrations. It aggregates more at lower pH than at higher pH. However the nature of aggregation was reversible in all cases. The effect of mannitol on aggregation is minimal.

Enfuvirtide is synthesised using a standard Fmoc (Fluorenyl-methoxycarbonyl) solid phase synthesis of three main fragments, followed by a solution phase condensation of these fragments and purification of the deprotected crude enfuvirtide by chromatography and precipitation. Satisfactory specifications and associated methods have been provided for the starting materials.
During the development phase, the route of synthesis was modified due to demand on capacity from a conventional linear solid-phase peptide synthesis to a process involving fragments condensation. The active substance used for phase II and III clinical trials was synthesised by this latter process. In a third step the purification has been optimised.
The new route of synthesis was accompanied by a slight increase in related impurities. However, all impurity limits in the specification have been justified by toxicology studies and the impurity profile is similar in the toxicological/clinical batches and full-scale commercial batches.
The other impurities include residual solvents and reagents and have also been qualified with reference to EU guidelines.
Batch analysis data have been provided for 17 pilot scale batches (9 registration batches and 8 clinical batches) and three full-scale validation batches synthesised using the commercial synthesis process.

Results confirmed satisfactory compliance and uniformity with the proposed specification and demonstrates that the impurity profile is homogenous. Data from 5 development batches have also been provided for comparison.

Specification

The active substance specification includes test for appearance, identity (HPLC and peptide mapping), solution test, water content, heavy metals (Ph. Eur), residual solvents, residual acetate, residual trifluroacetate, assay (HPLC, min. 90.0%), peptide content, purity (HPLC, min 90%), impurities (HPLC), amino acids analysis (hydrolysis and HPLC), endotoxin content and specific optical rotation.
The analytical methods used in routine controls are suitably described and validated.
The specification limit for the purity has been adequately justified (see other paragraphs).

Stability

Stability data were provided for three pilot batches synthesised at the commercial synthesis site. 12 months data are available under long-term conditions (4ºC – packaging intended for commercialisation). Under accelerated conditions, studies have been performed during 3 months (40ºC/75% R.H. – packaging intended for commercialisation) and 6 months (25ºC/60% R.H. – packaging intended for commercialisation).
Stability studies under stressed conditions have also been performed in solution (pH, heat, light and chemical oxidation) and in the solid state (heat, humidity and light).

The results obtained support the proposed retest period of 12 months under refrigerated conditions (periods of higher temperature over 8°C and below or equal 25°C may occur for logistic reasons and should not exceed 10 days) in double polyethylene bags sealed and placed into tightly closed HDPE container.

Other ingredients

All the excipients comply with the Ph. Eur. requirements.
Regarding the TSE risk, Fuzeon includes no component of ruminant origin. The conventional viral safety risk has been satisfactorily addressed.
The syringes are CE marked and approved for the intended use.
The type I glass vials and the butyl rubber cap used as primary packaging material for the powder and the solvent vials meet the general Ph. Eur. requirements.

Product development and finished product

Because of the long-term instability of the liquid dosage forms tested, Fuzeon has been developed as a single dose lyophilised powder to be reconstituted extemporaneously with water for injections. The stability of enfuvirtide formulated using a carbonate buffer in the pH 9-9.5 range is adequate to permit enough time for solution manufacture and lyophilisation, and for use after reconstitution.
The choice of the excipients was based on compatibility test results and previous experience gained from similar products. The solid-state compatibility of the peptide with the excipients in the formulation is being assessed as part of the on-going stability studies. No incompatibility has been shown.
The overfills of 20% for the powder vials and of 0.2ml for the solvent vials are suitable to allow the withdrawal of the nominal volume of solvent and of reconstituted solution.
The reconstitution time has not been considered as a critical parameter during the development and this is reflected by an unusual reconstitution time of up to 45 minutes.

Bioequivalence has been established between a 50 mg/ml formulation used in early phase II studies, and I and the 100 mg/ml formulation used in phase III studies, which is intended for marketing.

The manufacturing process of the powder vials consists of four steps: preparation of the bulk solution, prefiltration and first sterile filtration, sterilisation of primary packaging materials, final sterile filtration and filling into vials, freeze-drying and capping of vials.

Terminal sterilisation of the product is not possible. The aseptic processing is controlled by bioburden determination on samples before sterile filtrations and the sterility test is part of the release testing.
The entire manufacturing process, including the aseptic manufacturing and lyophilisation, has been satisfactorily validated at commercial scale.
The manufacture of the solvent vials is carried out according to established manufacturing procedures commonly used for the manufacture of sterile products by terminal sterilisation.

Batch analysis data provided for 3 registration batches produced at the commercial manufacturing site comply with the specifications and indicate consistent and reproducible manufacture.

Specification

The finished product specification includes test for container, appearance and coloration, reconstituted solution (reconstitution time, appearance and degree of coloration (Ph. Eur.), pH, particulate matter (Ph. Eur.)), water content (Ph. Eur.), uniformity of mass (Ph. Eur.), identity (HPLC and UV or SDS-PAGE), assay (HPLC - 93-105% release and 90-105% shelf life), degradation products (HPLC), bacterial endotoxins (Ph. Eur.) and sterility (Ph. Eur.).

Stability

Stability of the product before reconstitution

Long term and accelerated stability studies were conducted on three batches manufactured at the intended manufacturing site. Twelve months data are available for batches stored under long-term storage conditions (5ºC and 25ºC/60% R.H., inverted and upright position). The data from accelerated storage conditions cover 12 months at 30ºC/75% R.H. and 6 months at 40ºC/75% R.H. A photostability study was also performed.

The methods used for product stability have been validated, and shown to be stability indicating.

The results presented support the proposed shelf life and storage conditions defined in the Summary of Product Characteristics.

In-use stability of the reconstituted solution

In use stability of the reconstituted solution was tested during 48 hours at 5°C and at room temperature, in upright and inverted position and also for light stability. A microbiological challenge testing by inoculation was also performed at refrigerated conditions and at room temperature.

The reconstituted solution is physically and chemically stable for up to 48 hours at 2-8°C and up to 24 hours at room temperature. Light sensitivity was detected. Microbiological challenge indicated a static behavior only under refrigerated conditions.

The methods used for drug product stability have been validated, and shown to be stability indicating.

The results presented support the proposed shelf life and storage conditions defined in the Summary of Product Characteristics. It is noted that the product has an unusually long reconstitution time.

2. Toxico-pharmacological aspects

Pharmacodynamics

- Mechanism of action

The replication of HIV is initiated by the attachment of the virus to the membrane of the target cell. The receptors on the cell surface include the CD4 –receptor and two co-receptors (CXCR4 or CCR5). The viral structure gp120 is the attachment site and another viral structure, gp41, is the anchor

between the gp120 structure and the viral trans-membrane attachment. Fusion between gp120 and the cell membrane enables the viral RNA to enter into the target cell.

Gp41 has three main regions: a fusion peptide at the N terminal and two heptad repeat regions (HR1 and HR2). At attachment between the CD4 receptor of the target cell and gp120, a conformational change in gp120 is evoked, which opens up for a high-affinity binding site to the co-receptor. The interaction between gp120 and the co-receptor results in further conformational changes that lead to the exposure of the fusion domain of gp41. A conformational change in gp41 leads to an attachment between the two HR regions, after which the virus-to-host cell membrane fusion can take place. The primary sequence of enfuvirtide derives from the sequence of amino acid residues 638-673 of the env gene, equivalent to residues 127-162 of the ectodomain of gp41 of the HIV1LAI clade. This sequence of amino acid is claimed to closely mimic the HR2 region of gp41 and to bind to the HR1 region, thereby preventing the intra-molecular protein-protein interactions between the HR1-HR2 domains of gp41 that are necessary for virus-to-host cell membrane fusion. Thus, the blocking of the HR1 region of gp41 prevents viral-target cell membrane fusion.

The antiviral activity of enfuvirtide was evaluated *in vitro* and *in vivo*.

- *In vitro* studies

The anti-HIV activity of enfuvirtide was initially noted for its potency in inhibiting HIV-1 gp41 induced cell-cell fusion. In a series of experiments in different assays for cell-cell confirmed, IC_{50} values for enfuvirtide against HIV1 isolates ranged from 0.11nM to 5.56nM and IC_{90} values from 0.31nM to 111nM. However, the activity against HIV-2 was about 1000 fold lower.

Enfuvirtide inhibited infection with cell-free virus at concentrations 10 to 100 times higher than those needed for inhibition of cell-cell fusion. A variety of assay protocols confirmed the antiviral potency of enfuvirtide against acute infection with HIV-1 of human lymphoblastoid cell lines, peripheral blood mononuclear cells and monocyte/macrophage cells, giving IC_{50} values in the range of 0.089 nM to 620 nM and IC_{90} values in the range of 17.8 nM to 1182 nM.
These antiviral potency values include data for experiments using different clades of HIV-1 (A through G) and virus with different co-receptor specificities.

There is no evidence for cytotoxic activity of enfuvirtide at concentrations considerably in excess of those at which antiviral activity is seen.

- *In vivo* studies

Antiviral activity *in vivo* was studied in the HuPBMC-SCID mouse model of HIV-1 infection. Enfuvirtide, at doses of 67 mg/kg/day or higher administered intraperitonally, completely blocked recovery of infectious virus from all tissues tested and dramatically reduced viral DNA load by as much as 6 logs10 or more. Inhibition of HIV infection was dose-dependent. At a daily intraperitonal dose of 2 mg/kg in SCID mice, antiviral activity was seen only in some of the experiments, and in those cases only in some of the studied compartments. This dose gives an exposure approximately half of that given by the recommended dose in human. This issue and the implications on the dose selected for clinical use will be addressed as follow-up measure to be fulfilled post-authorisation.

- Viral resistance

Enfuvirtide, binds to a different target than reverse transcriptase and protease inhibitors retained activity against virus isolates and recombinant virus constructs carrying resistance mutations to some or all of the three classes of current antiretroviral agents suggesting the lack of cross resistance.

Enfuvirtide resistant viruses could be selected *in vitro*. Five virus passages over 5 or 6 weeks selected enfuvirtide resistant virus. Resistance was accompanied by genotypic change(s) in the gp41 gene, mapping to amino acid region 36-38. Studies on site-directed mutant viruses demonstrated that substitutions at other parts of gp41 (within aa 36-41) may decrease the sensitivity to enfuvirtide.

Appearance of enfuvirtide resistant virus was also shown in the clinic as further discussed in the clinical part of this document.

- Secondary pharmacodynamics

It has additionally been shown that enfuvirtide can act as an agonist on a cell surface receptor on host cells formyl peptide receptor (FPR) with a constant of affinity of 1200 nM (5.4 µg/ml). FPR is involved in innate immune functions, initiating an inflammatory response at a site of bacterial infection by recruiting phagocytic cells, primarily neutrophils and monocytes. The full picture of the biology of FPR and its homologues is not yet known and other functions are believed to be associated with this family of receptors.
The interaction between enfuvirtide and FPR was shown to result in chemoattraction of monocytes and neutrophils and inhibition of IL-12 production by monocytes, but not dendritic cells.
No studies have addressed the activity of enfuvirtide on FPR *in vivo* and the consequences of such activity for the clinical use remain to be defined. The applicant undertook to further explore this finding as part of the specific obligations to be fulfilled post-authorisation.
A series of close analogues of enfuvirtide were studied for antiviral activity but none of them had significantly better activity than enfuvirtide.

- Pharmacodynamic interactions

In vitro enfuvirtide acts additively to synergistically in combination with representatives of the three classes of current antiretroviral agents. No evidence of antagonism was found.

- General and safety pharmacology programme

Enfuvirtide did not affect the spontaneous locomotor activity nor the general behaviour and physiological status in male mice receiving subcutaneous doses up to 50 mg/kg. The conclusions are hampered by the lack of pharmacokinetics data in this species. However, as discussed in the pharmacokinetics section, enfuvirtide penetrated the central nervous system to a small extent.
In beagle dogs, there was no evidence of effect on the cardiovascular and respiratory functions, after intravenous doses of 5 mg/kg enfuvirtide. Transient reductions in QRS amplitude were observed at 15 and 50 mg/kg. The S-T segment was transiently elevated in one animal at 50 mg/kg. There were no effects on the QT or QTc intervals.

Pharmacokinetics

The pharmacokinetics profile of enfuvirtide was evaluated in the rat and monkey, species used for toxicology studies. Enfuvirtide was mainly administered subcutaneously, which is the intended route for administration in humans although intravenous, continuous infusion and intramuscular administration have also been used in some studies. Most of the studies used the formulation intended for marketing.
Enfuvirtide was measured in biological samples by various immunoassay techniques between different studies. Later in the development programme an LC/MS/MS method was introduced. These methods were in most cases not validated but considered suitable by the performance of the calibration standards, weakening the confidence in the data and rendering comparisons between different studies difficult.

Absorption

After subcutaneous injection of low doses to rats, enfuvirtide was rapidly absorbed (T_{max} 1-2 hours) but the extent decreased with increasing the dose as evidenced by less than anticipated increases in C_{max}. The bioavailability was dose-dependent (ranging from 36 % at a dose of 3.2 mg/kg to as low as 2.4 % at the highest dose of approximately 400 mg/kg), limiting the possibility to achieve high plasma concentrations. This limits the value of the rat for toxicological evaluations. However, toxicokinetic data from an embryotoxicity study in rats, further discussed in the toxicology section, showed that reasonable exposure could be achieved by injections of high doses of enfuvirtide.

In the monkey, enfuvirtide was readily absorbed after a subcutaneous injection with a peak concentration occurring within 1-3 hours after dosing. C_{max} and AUC values increased approximately proportionally to the dose. The absolute bioavailability was high (58 to 133 % over doses ranging from 0.4 to 6.7 mg/kg) and relatively dose-independent.
The calculated apparent clearances for enfuvirtide ranged from 0.029 to 0.058 l/hr/kg and appeared to be independent of dose administered, and half-life values ranged from 3-4 hours.

The intravenous pharmacokinetic profiles of enfuvirtide were comparable between rats, monkeys and humans, characterised by small volumes of distribution, low systemic clearances and short terminal half-lives.

The pharmacokinetic profile in monkeys was similar after intramuscular or subcutaneous administration with a peak concentrations occurring within 1-3 hours and absolute bioavailability of approximately 80 %.

Distribution

There is no data on the protein binding in animals but the applicant undertook to provide further data post-authorisation and discuss the importance in relation to enfuvirtide activity.

Distribution of enfuvirtide was evaluated using Quantitative Whole Body Autoluminography, which could not discriminate between intact enfuvirtide and peptide fragments or free amino acids. High amounts of radioactivity were found in well-perfused tissues. Enfuvirtide showed poor penetration to the central nervous system.
Enfuvirtide showed equal distribution between lymph and plasma. The presence in lymph nodes was demonstrated with an immuno-histochemical method, but enfuvirtide could not be detected with LC/MS/MS. Lymph nodes are important reservoirs of HIV and the levels of enfuvirtide in this tissue are likely important for the clinical efficacy.
Placental transport and transport to milk was studied in rats using ^{3}H-enfuvirtide. The transport was stated to be minimal (about 3 % of the administered radioactivity was secreted into milk after 48 hours). However, the actual numbers suggest that reasonable amounts of radioactivity reached these compartments. The appearance of radioactivity was delayed in these compartments, and it is not possible to conclude about the amount of intact peptide.

Metabolism

As a synthetic peptide consisting of naturally occurring amino acids, degradation of enfuvirtide to smaller peptides and free amino acids occur through the action of peptidases/proteinases primarily in the liver and the kidney.

Ro 50-6343 was identified as a major metabolite in rat plasma. This metabolite represents a minor change to enfuvirtide. Ro 50-6343 was shown to be present at relatively low levels in monkeys and human plasma (AUC ratio 0.08 – 0.31 and 0.05-0.15 respectively) as compared in rats where AUC values for Ro 50-6343 were equal or higher than those of enfuvirtide. The antiviral potency of the metabolite is approximately 20 % of that of enfuvirtide. The applicant undertook to clarify whether the immunoassays could discriminate between the metabolites and enfuvirtide and address the risk of overestimating exposure in the toxicity studies.

Excretion

Following subcutaneous of 3H-enfuvirtide in rats (202 mg/kg), radioactivity was slowly eliminated at a constant rate. The apparent clearance for enfuvirtide ranged from 0.023 l/hr/kg to 0.058 and appeared to be independent of the dose administered.
The mean total radioactivity collected over 168 h was approximately 92 %. The recoveries in urine, faeces and expired air were approximately 6 %, 14 % and 12 % respectively. A significant portion of enfuvirtide catabolites (approximately 2/3) remained to be excreted seven days after dosing suggesting

that the radiolabel was incorporated into intermediary pathways as either peptide fragment or amino acid.

Interactions

In vivo data demonstrated a low potential for enfuvirtide to influence cytochrome P450 isozymes. Repeated cutaneous injections of enfuvirtide to rats did not induce hepatic metabolism as assessed by increases in liver weight, or the content or activities of CYP1A2, 2B1/2 or 3A1/2. The potential for metabolic interactions with enfuvirtide is therefore low.

Toxicology

The toxicological programme included single dose toxicity studies in rats, repeated dose toxicity studies in rats and monkeys, genotoxicity, reproduction toxicity studies in rats and rabbits, local tolerance and ecotoxicity.
Enfuvirtide was initially considered for intravenous injection but this was later changed to the subcutaneous route. This is reflected in the toxicity studies where both routes were used. The main repeated dose toxicity studies were a 6-month study in the rat and a 9-month study in the cynomolgus monkey, both using the clinical protocol of subcutaneous injections twice daily.
The pivotal toxicity studies were performed in compliance with Good Laboratory Practices, however with exceptions.

Single dose toxicity

Enfuvirtide was lethal after a single intravenous injection of 100 mg/kg in rats. The cause of death appeared to be lung damage. Macroscopic and microscopic changes of the lungs were seen at doses ≥ 20 mg/kg. Mild signs of possible intolerance were seen in female monkeys receiving 50 mg/kg.

Repeated dose toxicity

The repeated dose toxicity was evaluated in rats after intravenous injection (28 days) or subcutaneous injection (6-months) and monkeys after intravenous injection (28-days) or subcutaneous (28-days and 9 months).

Microscopic changes in the lung were also seen in rats after intravenous injection. These changes (microgranuloma and inflammatory loci) were not considered treatment related by the pathologist. However, the increased frequency in the high-dose group (10 mg/kg) and similar findings in the single-dose toxicity study could suggest a relation. This applicant undertook to further investigate this finding post-authorisation. There were slight decreases in leucocytes at the end of the treatment and minor changes in red cells parameters at the end of the recovery period in males treated with 10 mg/kg. Small reversible increases in absolute and relative thyroid/parathyroid weights were seen in both gender at 10 mg/kg.

The chronic 6-month repeated dose study in rats receiving twice daily subcutaneous injections (up to 34.5 mg/kg/day for the 1st month and up to 30 mg/kg/day for the following 5 months) resulted in an exposure to enfuvirtide which was only 70% of the expected therapeutic exposure. The only reported adverse effect was injection site reactions (subdermal haemorrhages, subdermal chronic inflammation/fibrosis and degeneration of the cutaneous muscle on the injection sites in males). There were very low amounts of anti-enfuvirtide antibodies. Later studies in the rat with higher doses showed that higher exposures were attainable. A study on pregnant rats showed an exposure to enfuvirtide 8.9-fold higher than the therapeutic exposure.

The chronic 9-month repeated dose study in cynomolgus monkeys (twice daily subcutaneous injections with doses up to 10 mg/kg/dose) resulted in an exposure 2.8 times higher than therapeutic exposure. The only reported adverse effect was injection site reactions (haemorrhage, oedema and inflammatory infiltrate with plasma cells and eosinophile). Other changes were an increased prominence of splenic follicular germinal centers in all animals treated with 20 mg/kg/day, and depletion of cortical lymphocytes in the thymus. The spleen effect was considered related to an ongoing immune response to enfuvirtide, whereas the thymus effect was considered related to

experimental stress. However, there was a higher frequency of thymic lymphocyte depletion in the animals treated with enfuvirtide. The applicant undertook to further address this issue post-authorisation.

The exposure in rats is below the expected clinical exposure. The exposure in the monkey study is above the expected clinical exposure but the safety margin is narrow. The applicant undertook to further address this issue as part of the special obligation to be fulfilled post-authorisation.

Genotoxicity

The genotoxic potential of enfuvirtide was evaluated in a battery of *in vitro* tests (reverse gene mutation assay in bacteria and forward gene mutation in Chinese hamster ovary cells without and with metabolic activation) and *in vivo* micronucleusassay in mice. No genotoxic activity was found. The clastogenicity study was not conducted as recommended in applicable guidelines. However, the ICH guideline on preclinical safety evaluation of biotechnology products is applicable to synthetic peptides, and for these products genotoxicity testing is not needed.

Carcinogenicity

No carcinogenicity studies have been performed, but the CPMP considered the justification provided by the applicant insufficient. A host activity of enfuvirtide could be postulated from the finding that enfuvirtide acts as an N-formyl peptide receptor (FPR) agonist *in vitro* and an interaction of enfuvirtide with formyl peptide receptors *in vivo* cannot be excluded. A host activity of enfuvirtide is also suggested by the injection site reactions and the lung injury after intravenous injection of enfuvirtide in rats. Thus, the applicant undertook to conduct carcinogenicity studies, the results of which will be provided post-authorisation.

Reproduction toxicity

Enfuvirtide administered subcutaneously in rats with doses up to 30 mg/kg/day did not affect the fertility or the embryo-foetal development but in the absence of toxicokinetic data, these results are of limited value.
In one rat study performed with higher doses (up to 500 mg/kg), enfuvirtide was not embryotoxic nor teratogenic. The toxicokinetic analysis demonstrated that plasma concentrations and plasma exposure at the highest dose was approximately 8.9 times greater than the expected therapeutic exposure.
As for the rats, the study in rabbit showed that treatment starting on Gestation day 6 study did not result in any embryotoxic or teratogenic effects with doses resulting in an exposure 3.2-4.4 times higher than the expected therapeutic exposure.
Efuvirtide administered subcutaneously in rats with doses up to 30 mg/kg/day did not affect the peri or post-natal development off the offspring but in the absence of toxicokinetic data, these results are of limited value.
Thus no firm conclusions can be made regarding the effect of enfuvirtide on fertility, early embryonic or post-natal development. Enfuvirtide should therefore not be used during pregnancy unless the potential benefit justifies the potential risk to the foetus as mentioned in the Summary of Product Characteristics and the applicant undertook to further evaluate the reproduction toxicity as part of the special obligation to be fulfilled post-authorisation.

Local tolerance

Inflammatory reactions were seen at the injection site in the subcutaneous repeated dose studies as already highlighted. Local reactions were commonly seen in control animals but tended to be more severe in enfuvirtide treated animals. The reports did not clearly describe the kinetics of the reactions and in most cases the analysis of the reaction site was performed after a long period of treatment. A 72-hr study was performed with subcutaneous infusion of enfuvirtide. An inflammatory response at the infusion site was evident within this time period.

The injection site reaction was further characterised in 2 weeks study in mini-pigs receiving subcutaneous injections of two different strengths (50 and 100 mg/ml). Subcutaneous masses were

evident from day 8 in one study and day 4 in a second study. Microscopic findings in the repeated dose toxicity studies and the mini-pig studies were similar, although more severe in the latter. They consisted of non-specific changes (haemorrhage, oedema, necrosis and fibrosis, cutaneous muscle degeneration) and a mixed inflammatory infiltrate composed of macrophages, lymphocytes and other cell types. In more severe cases, granulomas were found, characterised by a necrotic centre surrounded by multinucleated giant cells and foamy macrophages with distended cytoplasm.

The mechanism for the local inflammation and granuloma formation has not been elucidated yet but the applicant undertook to further explore this finding post-authorisation.

The guinea pig maximisation test showed a skin sensitisation response indicative of a potential for causing delayed type hypersensitivity.

Other studies

Antigenicity
Methods for antibody analysis did not allow quantitative measurements. Antibodies were readily detected in monkeys treated with enfuvirtide intravenously or subcutaneously. Only very low titres were seen in the rat. No studies allowed determination of the neutralising activity of the antibodies. Thus, the possible influence of neutralising antibodies on the outcome of the toxicity studies cannot be assessed. However, antibodies to enfuvirtide are commonly seen in patients treated with enfuvirtide and do not appear to affect efficacy or safety. There was no change in the pharmacokinetic parameters over time, suggesting that the antibodies did not alter systemic clearance or elimination of enfuvirtide.

Immunotoxicity
Immunotoxicity of enfuvirtide was not specifically addressed. Studies on immune parameters were limited to haematology and histopathology in the repeated dose studies where no major changes associated to the immune system were seen. However, there were findings in the toxicity studies, which could suggest a potential immunotoxicity of enfuvirtide. Therefore, the applicant undertook to further investigate the immunotoxicity potential of enfuvirtide as part of the specifique obligations to be fulfilled post-authorisation.

Impurities
Most of the impurities have been toxicologically qualified. For the others based on the peptide structure and the low level for individual impurities/degradants the lack of qualification data was not considered as a safety concern.

Environmental risk assessment

An assessment of the environmental risk was performed and no significant risk to the environment related to the use of enfuvirtide is anticipated.

3. Clinical aspects

The clinical programme consists of:
- 8 completed phase I and clinical pharmacology studies
- 3 completed phase II studies
- 2 ongoing confirmatory phase III studies evaluating the efficacy and safety of enfuvirtide in combination with an optimised background regimen in HIV-1 infected patients with prior experience and/or documented resistance/intolerance to each of the 3 classes of approved antiretroviral medicinal products.

In addition there are three ongoing access studies including patients who have participated in previous studies with enfuvirtide or another fusion inhibitor in development and one ongoing open-label safety study in patients unable to construct a viable antiretroviral regimen among approved agents. The clinical programme includes also two ongoing studies in paediatric patients aged from 3 to 16 years.

As of the cut-off date for the submission of the application, a total of 1,537 patients had been included. All studies were performed in HIV-1 infected patients since administration to healthy volunteers may induce anti-enfuvirtide antibodies that cross react with HIV gp41. This may result in a false positive HIV test with the anti HIV Elisa test.
All studies were claimed by the applicant to be performed according to Good Clinical Practices.

An overview of these studies is displayed in table 1.

Table 1: Overview of clinical studies

Protocol Number	Study Design	Dose and duration	Number of subjects/ patients
Absorption/Bioavailability Studies			
NP16370/T20-506	Influence of subcutaneous (SC) injection site (arm, abdomen, thigh). Cross-over design	90 mg bid 7 days	12
NV16059/T20-208	Comparison carbonate formulations Sequential cross-over design	90 mg bid 14 days	19
NP16220/T20-501	PK of subcutaneous (SC) and intravenous (IV) administration 4-way cross-over, single dose	SC : 45, 90, 180 mg IV 90 mg	12
Pharmacokinetic/ Tolerability Studies			
TRI-001	Single and multiple dose PK, IV administration Parallel group study (n=4/group).	3, 10, 30, 100 mg bid 14 days	17
TRI-003	PK of continuous subcutaneous infusion or subcutaneous injection. Single and multiple dose, parallel group study (n=13/group)	12.5-100 mg bid 4 weeks	78
Interaction Studies			
NP16221/T20-502	Influence of enfuvirtide on the metabolic activities of cytochrome P450 isozymes	90 mg bid 7 days	12
NP16324	Influence of saquinavir (soft capsules) + ritonavir on PK of enfuvirtide	90 mg bid 7 days	12
NP16325/T20-504	Influence of ritonavir on the PK of enfuvirtide.	90 mg bid 7 days	12
NP16334/T20-505	Influence of rifampicin on the PK of enfuvirtide.	90 mg bid 7 days	12
Pediatric Studies			
NV16056/T20-310	A Phase I/II PK and safety study of enfuvirtide in combination with an optimized background in HIV-infected children and adolescents.	2 mg/mg	20
NV16060/T20-204	A Phase I/II study of enfuvirtide, a fusion inhibitor, in HIV-1-infected children.	15, 30, 60 mg/kg	12+12
Patient Studies in HIV-1 Positive Adults			
T20-206	A controlled Phase II trial assessing three doses of enfuvirtide in combination with abacavir, amprenavir, ritonavir, and efavirenz in HIV-infected-adults. PK profile at week 4 and trough values up to week 48	50, 75 and 100 mg bid 48 weeks	45
T20-205	A Phase II roll-over protocol for HIV-infected adults with prior enfuvirtide treatment. PK profile at week 4 and trough values up to week 96	50 mg bid 96 weeks	70
Main studies			
T20-301	Open-label, randomized, Phase III assessing enfuvirtide in combination with optimized background treatment (OB) versus OB treatment in HIV-1 infected patients with prior experience and/or prior documented resistance or intolerance to each of the 3 classes of antiretrovirals.	90 mg bid 48 weeks (24 weeks reported)	491
T20-302	Open-label, randomized, Phase III assessing enfuvirtide in combination with OB treatment versus OB treatment in HIV-1 infected patients with prior experience and/or prior documented resistance or intolerance to each of the 3 classes of antiretrovirals.	90 mg bid 48 weeks (24 weeks reported)	504

Clinical pharmacology

Pharmacodynamics

Mechanism of action

As presented in the pre-clinical section of this document, enfuvirtide acts through selective inhibition of fusion of the virus with CD4 expressing cells, thus preventing the entry of HIV-RNA into target cells and precluding the initiation of the reverse transcription process and the replication cycle of the virus.

Dynamic studies

The relation between dose and antiviral effect was evaluated in two phase I/II short term monotherapy studies (TR-001-TR003) and in two phase II combination studies (T20-206 and T20-208). These studies are presented in the clinical efficacy section of this document, under dose response studies.

Viral resistance

The viral target for binding of enfuvirtide is the HR1 region of gp41, hence mutations in that region might lead to enfuvirtide resistance. Results from *in vitro* and phase II studies showed that:

- HIV-1 gp41 aa 36-45 are highly conserved in virus from enfuvirtide naïve patients.
- Specific mutations in the HIV gp41 aa 36-45 have been shown by *in vitro* selection and site-directed mutagenesis to cause resistance/reduced susceptibility to enfuvirtide.
- Mutations in HIV-1 aa 36-45 ectodomain giving resistance to enfuvirtide are selected *in vivo*.
- The incidence with which aa 36-45 mutations are selected is influenced by the efficacy of the total antiretroviral treatment regimen.

Only post-treatment plasma samples from patients experiencing virological failure up to 24 weeks have been analysed so far.

Analyses of pooled data from study T20-301 and T20-302, after 24 weeks of therapy, showed that among patients with paired genotype and phenotype data from baseline and with virological failure, the large majority showed greater than 4-fold increase in pEC_{50} (EC_{50} of patient viral Env to inhibition by T-20) at virological failure (187/204, 91.7%). At genotypic evaluation, almost all (185/187, 99 %) had substitutions in gp41 aa 36-45.
The most common mutations were V38A, N43D, N42T, V38V/A, G36D, G36V, V38M, Q40H, N/S42T, and L45M. Many of these occurred in mixed populations with wild-type virus. Combinations of more than one mutation were seen in viruses from 93 (42.7%) patients, but only Q40H plus L45M occurred as a relatively frequent combination (10 cases, 4.6%, including mixed populations with wild-type or with other substitutions). It is unclear whether multiple substitutions correlate to higher EC_{50} values. Specific mutations associated with high degree resistance have not been identified.

Further data are needed to attempt to correlate specific mutation patterns with high degree phenotypic resistance and *in vivo* kinetics. In addition mutation in the HR2 region should be analysed. The applicant undertook to explore these issues post-authorisation.

Pharmacokinetics

The pharmacokinetics of enfuvirtide has been determined in 186 adult HIV patients included in phase I/II studies and in 115 adult HIV patients included in phase II studies. Pharmacokinetic data are also available in 32 children. No specific studies have been performed in special populations. The influence of demographic factors on pharmacokinetics was evaluated in a population pharmacokinetic analysis, involving data from 628 patients enrolled in the confirmatory phase III studies. Enfuvirtide plasma concentrations have been determined using several validated analytical methods, of which LC/MS/MS was used in the majority of studies.

Absorption and distribution

Enfuvirtide is absorbed slowly after subcutaneous administration, with maximum plasma concentration reached after on average 5-7 hours. The mean absorption time increased with dose from a mean value of 7.26 hours for a 45 mg dose to a mean of 9.79 hours for a 180 mg dose. At steady-state the plasma-concentration time profile presents low fluctuation.

The absolute bioavailability is 84% for the 90 mg dose administered subcutaneously.
The effect of injection site location (abdomen, thigh and arm) on steady-state pharmacokinetics of enfuvirtide was evaluated in study T20-506. Compared with the abdomen, the relative bioavailability of enfuvirtide was similar from the thigh but higher (17%) from the arm. The difference in absorption is unlikely to be clinically relevant. Enfuvirtide can therefore be injected at any of these sites and no dose adjustment is required for rotation of injection sites as recommended in the Summary of Product Characteristics.

Bioequivalence has been established between the formulation used in early phase I and II studies, and the formulation used in the confirmatory phase III studies, which is intended to be marketed.

Enfuvirtide has a low volume of distribution. The mean steady state volume of distribution after administration of 90 mg of enfuvirtide intravenously was 5.5 ± 1.2 l. The protein binding of enfuvirtide is about 92 % in plasma of HIV infected patients. Enfuvirtide is bound predominantly to albumin and to a lower extent to α-1 acid glycoprotein. *In vitro* studies showed that 50-75 % of enfuvirtide was associated with the red cells.
The potential distribution of enfuvirtide to other tissue and fluid is unknown.

After subcutaneous administration, the pharmacokinetics of enfuvirtide are dose-proportional over the range of 45 to 180 mg and time-independent.
Based on the population pharmacokinetic analysis, the inter and intra-individual variability in CL/F is about 30% and in V/F about 55%.

In the population pharmacokinetics the overall mean predicted C_{trough} value in the target population is 3.0 µg/ml and the overall mean predicted AUC_{12h} value is 54 µg·h/ml, which are comparable with the actual overall values determined in the various studies.

Metabolism and elimination

Like most peptides, the metabolism of enfuvirtide is unknown and, for technical and safety reasons, it has not been examined using traditional radio-label studies. *In vitro* three metabolites were detected, one of which has also been identified *in vivo* at concentrations much lower than parent compound (AUC ranging from 2.4 to 15 % of the enfuvirtide AUC). The lack of documentation regarding *in vivo* metabolism/catabolism is acceptable, given that enfuvirtide is a peptide composed of 36 naturally occurring L-amino acid residues. Enfuvirtide is likely to be subject to catabolism by peptidases, proteinases, and other enzymes.
Enfuvirtide being a polypeptide is not expected to be excreted in urine but following filtration is expected to be catabolised to constituent amino acids which are reabsorbed. The applicant undertook, however, to provide urinary excretion data post-authorisation.

After intravenous administration, the systemic clearance of enfuvirtide was low, 1.4 ± 0.28 l/h and the elimination half-life was 3.2 hours.
After single dose of 90 mg administered subcutaneously, the mean elimination half-life was approximately 3.8 h, suggesting absorption rate limited elimination.

Special population

The influence of age, gender, weight, race, renal and hepatic function on the pharmacokinetics of enfuvirtide was evaluated in a population pharmacokinetic analysis.

Body weight and gender were identified as covariates affecting inter-patient variability in clearance. The apparent clearance of enfuvirtide was 20% lower in female than in male patients irrespective of weight and was increased with increased body weight, irrespective of gender (20 % higher in a 100 kg and 20 % lower in a 40 kg body weight relative to a 70 kg reference patient). These effects of limited clinical relevance do not warrant any dose adjustment.
Race and age (up to 67 years) do not seem to influence the pharmacokinetics of enfuvirtide nor did the presence of circulating gp41 antibodies that cross-react with enfuvirtide.
There are no data in patients with hepatic impairment, and therefore enfuvirtide should be used with caution in these patients. Few patients in the confirmatory studies were co-infected with hepatitis B/C. The addition of enfuvirtide did not increase the incidence of hepatic events. However a warning on the increased risk for severe and potentially fatal hepatic adverse events in patients with chronic hepatitis B/C treated with antiretroviral therapy has been added in the Summary of Product Characteristics.
Limited data showed that in patients with creatinine clearance > 35 ml/min, the pharmacokinetics of enfuvirtide was not altered to a clinically relevant extent and therefore no dose adjustment is warranted. There are no data for patients with creatinine clearance below 35 ml/min or those receiving dialysis and therefore enfuvirtide should be used with caution as reflected in the Summary of Product Characteristics.

Studies in children are ongoing and only limited data are available in 32 paediatric patients aged 3 through 16 years with doses ranging from 0.5 to 2.5 mg/kg. A dose of 2 mg/kg bid (maximum 90 mg bid) provided enfuvirtide plasma concentrations similar to those obtained in adult patients receiving 90 mg bid dosage. In 20 paediatric patients ranging in age from 5 to 16 years and receiving the 2 mg/kg bid dose into the upper arm, anterior thigh or abdomen, the mean steady-state AUC was 51.4 ± 22.8 μg*h/ml, C_{max} was 5.81 ± 2.35 μg/ml, and C_{trough} was 2.82 ± 1.46 μg/ml. Pharmacokinetic data is very limited for paediatric patients under the age of six years.

Pharmacokinetic data are not available in the elderly.

Interaction studies

The potential for enfuvirtide to inhibit cytochrome P450, evaluated both *in vitro* and *in vivo*, showed a low potential for enfuvirtide to interact with concomitantly administered medicinal products, requiring no dose adjustment as mentioned in the Summary of Product Characteristics.

In an open-label, sequential, crossover study conducted in Thailand on the effect of rifampicin on enfuvirtide pharmacokinetics, C_{max} and AUC_{τ} were unchanged while C_{trough} was 15% lower with compared to without rifampicin.

In an open-label, sequential, crossover study the mean steady-state enfuvirtide plasma concentration-time profile after a 4-day treatment with a booster dose of ritonavir was higher than the control profile, an effect which was most prominent during the first half of the sampling period. C_{max}, C_{trough} and AUC_{τ} were increased 24, 14 and 22%, respectively. The mechanism of interaction is unknown but these changes were not considered clinically relevant.

In an open-label, sequential, crossover study, there was a slight increase in enfuvirtide pharmacokinetic parameters during concomitant administration of saquinavir+ low dose of ritonavir (day 7). C_{max}, C_{trough} and AUC_{τ} were increased 7, 26 and 14%, respectively. These changes were not considered clinically relevant.

The enfuvirtide AUC data from studies which allowed protease inhibitors (PIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs) were summarised and compared with AUC data from studies which did not allow PIs and NNRTIs. The AUC values of enfuvirtide were unaffected by co-administration with PIs and NNRTIs.

Clinical efficacy

Dose-response studies and main clinical studies

Dose response studies

The relationship between the dose and the antiviral effect of enfuvirtide was investigated in two monotherapy studies (TRI-001 and TRI-003). The plasma HIV-1 RNA reduction was evident and similar between the two studies after two weeks treatment. The maximal decrease in viral load (1.5 $\log_{10}$ copies/ml) was achieved within 7-14 days of treatment with 100 mg bid administered intravenously or subcutaneously.
The antiviral effect of enfurvirtide in the combination therapy setting was examined in two Phase II studies (T20-206 and T20-208). In the T20-206 study, patients were NNRTIs naive and received a fixed background of antiretroviral (ARV) medicinal products consisting of efavirenz (600 mg QD), amprenavir (1200 mg bid), abacavir (300 mg bid), and ritonavir (200 mg bid) without and with 50, 75, or 100 mg bid doses of enfuvirtide. In the T20-208 study, patients were triple-class experienced and received an optimised regimen of ARVs, chosen by the investigator or patient, based on patient's prior history and genotypic resistance assessment, and 75 or 100 mg bid doses of enfuvirtide. Both studies were designed to identify dose related response trends. The increase in antiviral response was minimal in each of the doses ≥ 75 mg bid, suggesting that the 90 mg bid dose is approaching the upper portion of the enfuvirtide dose response curve. In spite of differences in the patient population and background ARV regimen, the magnitude of effect was consistent between the two studies. At week 16, viral load declined by approximately 2 to 2.5 $\log_{10}$ copies/ml, regardless of the dose.

The maximally effective dose has not been established as no more than 100 mg could be dissolved in 1 ml of buffer considered the maximal practical injection volume for chronic subcutaneous use (100 mg bid equivalent to 90 mg delivered). The applicant undertook to discuss the possibility to increase the daily dose as part of the follow-up measures to be fulfilled post-authorisation.

No plasma concentration-effect studies have been performed but the applicant undertook to further address this issue as part of the follow-up measures to be fulfilled post-authorisation.

In the analysis of antibody status at baseline and during exposure, most HIV infected patients showed a weak antibody response to gp41, but the presence of circulating gp41 antibodies that cross-react with enfuvirtide did not appear to influence the kinetics nor the activity of enfuvirtide. Enfuvirtide is also poor antigen in the dosages used in the clinical studies and until now no neutralising antibody response has been documented. As for other efficient antiretroviral regimens the anti gp41 antibody titre seems to decline over time also in patients treated with enfuvirtide. The database is still limited and the applicant undertook to provide further data on anti-enfuvirtide reactive antibody as part of the follow-up measures to be fulfilled post-authorisation.

Main studies: T20-301 and T20-302 in heavily pretreated patients

1. Description of the studies

These ongoing studies are randomised, open-label, active-controlled, parallel-group, multicentre performed in America and Europe/Australia designed to assess the efficacy and safety of enfuvirtide (90 mg bid subcutaneous) in combination with an optimised background regimen of antiretroviral agents. The study duration is 48 weeks, but after this treatment phase, an optional 48-week Treatment Extension Phase, and a 4-week Follow-up Phase for safety are planned. For this application, efficacy analyses at 24 weeks are presented substantiated by preliminary results at 48 weeks.

The studies are open-labelled as dermal reactions at the injection site were likely to be prevalent in the treatment group and it was considered unethical to give patients in the control group a substance without antiretroviral activity but giving raise to prominent injection site reactions. These considerations discussed at CPMP scientific advice were considered acceptable.

Patients were eligible to enter in these studies if they met the following inclusion criteria:

1. At least 16 years of age.
2. Prior experience and/or documented resistance to each of the three classes of approved antiretroviral agents
3. On current, stable pre-study antiretroviral regimen (unchanged medication and doses) for ≥ 4 weeks.
4. Non-decreasing viral load, defined as:

- Plasma HIV-1 RNA measured at screen 1 of ≥ 5000 copies/ml.
- Plasma HIV-1 RNA measured at screen 2 of ≥ 5000 copies/ml and not decreased since screen 1 measurement by more than 1.0 $\log_{10}$.

Both studies have the same design allowing for meta-analyses. Differences in entry criteria between the two studies include prior duration of antiretroviral exposure (at least 6 months in T20-301 and at least 3 months in T20-302) and the number of previous protease inhibitors (at least 2 in T20-301 and at least 1 in T20-302).

Qualifying patients were randomised to one of two treatment groups: OB (optimal background therapy) or enfuvirtide + OB in a 1:2 ratio (OB: 175 patients and T-20+OB: 350 patients in each of the two main studies). The randomisation was stratified by screening viral load (< 40 000 copies/ml or ≥ 40 000 copies/ml) and by whether the patient´s OB regimen included newly approved/investigational antiretrovirals. The choice of OB regimen was individualised by the investigator and the patient prior to randomisation, based on the patient's prior treatment history, viral genotypic and phenotypic resistance testing results if available.

2. Objectives/Endpoints

The primary efficacy endpoint was the mean change in viral load from baseline to week 24 as assessed by $\log_{10}$ transformed plasma-HIV RNA levels. The objective of the studies was to demonstrate that a deliverable dose of 90 mg bid of enfuvirtide given subcutaneously and added to an OB regimen provides an additional drop in plasma HIV-1 RNA of at least 0.5 $\log_{10}$ copies/ml (as measured by least squares mean, LSM) compared with 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 at week 24.

Different categorical responder analyses were included as secondary efficacy endpoints:

- percentage of patients achieving at least 1 $\log_{10}$ decrease from baseline in HIV-RNA but > 400 copies/ml
- percentage of patients achieving < 400 copies/ml
- percentage of patients achieving < 50 copies/ml

Additional secondary efficacy parameters included change from baseline in CD4 cell counts, virological failure rate, time to virological failure, change from baseline in physical function and mental health scales of MOS-HIV questionnaire, and percentage of patients who had either an AIDS defining events or died.

The criteria for virological failure were:

1) <0.5 $\log_{10}$ copies/ml decrease from baseline either on two consecutive measurements ≥ 14 days between the first and the third measurements, starting at week 6 and 8 or any time after week 8.

2) < 1.0 $\log_{10}$ copies/ml decrease from baseline on consecutive measurements starting at week 14 or 16 or any time after week 16.

This criterion consisted of two parts; patients achieved ≥ 2.0 $\log_{10}$ copies/ml decrease from baseline on consecutive measurements and had HIV-1 RNA rebound from the average of the two lowest values by > 1.0 $\log_{10}$ copies/ml on consecutive measurements starting at week 6 or 8.

Patients in the OB group who experienced virological failure were allowed to switch to enfuvirtide+OB (revised) no earlier than after 8 weeks of OB treatment. Patients in the

enfuvirtide+OB treatment with virological failure after week 8 were allowed to change their OB regimen. Patients with virological failure who chose not to switch or continue with T20 + OB could remain on study for up to 1 month.

3. Statistical analysis

Efficacy data were analysed for two populations:

- Intent-to-Treat (ITT, all randomised patients who received at least one dose of study medications and who had at least one post-treatment viral load measurement)
- Restricted Treated (RT, all patients in the ITT population who had self reported adherence ≥ 85% to OB regimen during the treatment period and who had no major protocol violations).

Missing data were handling using the Last-Observation Carried Forward (LOCF) method. For patients who met switch criteria for virological failure or rebound, their last two (failure/rebound) observations were carried forward to the planned analysis time point. All primary efficacy analyses were performed based on the original randomised treatment regimen.
Separate analyses were conducted for patients who switched from OB to enfuvirtide +OB. An analysis of covariance model was used to analyze the viral load data. The model included the following terms: stratum (four combinations of baseline viral load category and use of other experimental antiretroviral drugs), treatment, treatment by stratum interaction, and covariate (genotypic/phenotypic sensitivity score).

To confirm the robustness of the conclusions drawn from the primary analysis, sensitivity analyses were performed on changes in $\log_{10}$ HIV-1 RNA copies/ml as follows:
1. Modified last observation carried forward (LOCF) for premature withdrawals (pre-planned)
2. Treat premature withdrawals and virological failures as failure (*post hoc*)
3. Use of LOCF for premature withdrawals and OB switch patients only (*post hoc*).

The studies were powered (80 %) to demonstrate a treatment effect in terms of a standard HIV-1 efficacy assessment criterion, the percentage of patients with a HIV-1 RNA level < 400 copies/ml at week 24. The expected minimum treatment difference between OB and T20+OB in this variable was a 15% absolute difference.

RESULTS

Efficacy results at 24 weeks are presented substantiated by preliminary results at 48 weeks.

4. Study populations/accountability of patients

Patients included were heavily pretreated with antiretroviral agents and most patients harboured virus resistant to many of these. Most patients had an advanced HIV infection with low CD4+ cell counts.
The demographic and baseline characteristics are displayed in tables 2 and 3.

Table 2. Demographic baseline data

Study		T20-301		T20-302	
		T20+OB	OB	T20+OB	OB
Number of patients		326	165	335	169
Sex	Female	25 (7.7%)	13 (7.9%)	43 (12.8%)	21 (12.4%)
	Male	301 (92.3%)	152 (92.1%)	292 (87.2%)	148 (87.6%)
Race	White	274 (84.0%)	135 (81.8%)	316 (94.3%)	161 (95.3%)
	Black	39 (12.0%)	20 (12.1%)	14 (4.2%)	4 (2.4%)
	Other	13 (4.0%)	10 (6.1%)	5 (1.5%)	4 (2.4%)
Ethnicity	Hispanic	45 (13.8%)	21 (12.7%)		
	Non-Hispanic	281 (86.2%)	144 (87.3%)		
Age (years)	Mean	42.6	42.1	42.1	43.2
Weight (Kg)	Mean	75.6	76.5	68.9	68.8
Height (cm)	Mean	176.4	175.7	175.2	174.6
BMI (Kg/m^2)	Mean	24.2	24.8	22.4	22.5
Baseline viral load (log $_{10}$ copies/ml)	Mean	5.1	5.1	5.1	5.1
Baseline CD_4 count (cells/µl)	Mean	121.3	108.9	150.6	146.2
Previous AIDS defining events	Yes	273 (83.7%)	148 (89.7%)	250 (74.6%)	138 (81.7%)
Genotypic Sensitivity Score (N,%)	Mean	1.9	1.9	1.6	1.7
Phenotypic sensitivity score (N, %)	Mean	1.7	1.8	1.4	1.4

Table 3. Summary of prior Triple Class Experience-ITT population

	Study T20-301		Study T20-302	
	T-20+OB	OB	T-20+OB	OB
Number of patients	326	165	335	169
Number of prior ARVs (mean)	12.29	11.90	12.06	12.01
Median (min:max)	12.00 (6.00 : 17.00)	12.00 (6.00 : 17.00)	12.00 (6.00 : 19.00)	12.00 (6.00 : 16.00)
Duration (years)				
Mean	7.11	7.30	7.59	7.65
Median (min:max)	6.65 (1.13 : 15.74)	6.8 (1.11 : 13.94)	7.39 (1.62 : 15.26)	7.44 (1.97 : 14.09)

There were no significant differences for the baseline and demographic characteristics between treatment groups as well as for clinical, virological and immunological variables. Distribution of OB treatment was also quite similar between groups, as well as mutations associated with resistance to currently approved antiretroviral agents.

The number and disposition of patients analysed are displayed in table 4.

Table 4: Disposition of patients

Number of Patients	Protocol T20-301			Protocol T20-302		
	Total	T20+OB	OB	Total	T20+OB	OB
Planned	525	350	175	525	350	175
Randomised	501	332	169	512	341	171
Treated	495	328	167	508	338	170
In ITT population	491	326	165	504	335	169
In RT population	420	276	144	413	279	134

A significant number of patients remained on original randomised treatment. The rate and the reasons for discontinuation, mainly for safety, were similar for both groups.
When both studies are combined, a total of 537 out of 995 ITT patients met the criteria for virological failure (301 out of 661 patients in the enfuvirtide+OB treatment group and 236 out of 334 patients in the OB treatment group). Out of the 236 patients experiencing virological failure in the OB treatment group, 195 switched to enfuvirtide+OB treatment group (these patients were not included in the efficacy results).

5. Efficacy results

The main efficacy results obtained after 24 weeks of treatment are presented in tables 5 (study T20-301) and 6 (study T20-302).

Table 5. Change in log_{10} HIV-1 RNA and $CD4^+$ T cell count from baseline to week 24; ITT population (LOCF), study T20-301

			Treatment difference (T20+OB – OB)		
Treatment	**N**	**LSM***	**LSM**	**95% CI**	**p-value**
Week 24 change from baseline in log_{10} HIV-1 RNA					
T20+OB	326	-1.696	-0.933	(-1.271; -0.594)	<0.0001
OB	165	-0.764			
Week 24 change from baseline in $CD4^+$ T cell count					
T20+OB	320	76.224	44.1	(22.5; 65.8)	0.0001
OB	163	32.086			

* LSM = least squares mean

Table 6. Change in log_{10} HIV-1 RNA and CD4+ T cell count from baseline to week 24, ITT population (LOCF), study T20-302

			Treatment difference (T20+OB – OB)		
Treatment	**N**	**LSM**	**LSM**	**95% CI**	**p-value**
Week 24 change from baseline in log_{10} HIV-1 RNA					
T20+OB	335	-1.429	-0.781	(-1.072; -0.491)	<0.0001
OB	169	-0.648			
Week 24 change from baseline in $CD4^+$ T cell count					
T20+OB	332	65.489	27.5	(3.7; 51.3)	0.0236
OB	165	38.005			

Substantial suppression of HIV-1 RNA was evident in both treatment groups during the first 24 weeks of treatment, but the enfuvirtide+OB treatment group had a statistically significant greater decrease at week 24 (LSM difference of –0.93 log_{10} copies/ml and 0.78 log_{10} copies/ml in the two studies respectively, both p-values <0.0001). These findings were confirmed by the sensitivity analyses pre-specified in the protocol and also by the *post hoc* analyses previously mentioned.

Exploratory analysis performed across trials (meta-analysis of studies T20-301 and T20-302.).

The percentage of patients responding in each response category at week 24 is presented in table 7.

Table 7: Summary of Meta-Analysis Results of the Main Efficacy Endpoints (ITT population).

Efficacy endpoints	**T-20+OB**	**OB**	**Treatment Diff.**	**95% CI**	**p-value**
HIV-1 RNA (log_{10}copies/ml) LSM difference	NA	NA	-0.846	-1.066; -0.626	< 0.0001
HIV-1 RNA < 50 copies/ml Percent responders	15.9	6.3	2.99*	1.81; 4.93	< 0.0001
HIV-1 RNA < 400 copies/ml Percent responders	32.7	15.0	2.97*	2.08; 4.23	< 0.0001
HIV-1 RNA≥1.0 log_{10} decrease from Baseline Percent responders	47.2	24.9	2.80*	2.08; 3.77	< 0.0001
$CD4^+$ T cell count (cells/µl) LSM difference	NA	NA	36.6	20.7; 52.6	< 0.0001

*= od Note that 2 visits were required for confirmation of virological response

With respect to quality of life no differences were seen at 24 weeks in the MOS-HIV physical and mental health scores between groups. Adherence to each treatment was good and similar between both treatments as further explained in the safety section of this document.

Time to Virological Response/failure

The Kaplan-Meier curves of the time to reach virological response (< 50 copies/ml, < 400 copies/ml and ≥ 1.0 $\log_{10}$ below baseline) show that the response was greater in the enfuvirtide+OB treatment group than in the OB treatment for the proportion of responders over time (p<0.0001, log-rank test). The median time to at least a 1.0 $\log_{10}$ decrease from baseline in HIV-1 RNA was estimated to be 8 days in the enfuvirtide+OB treatment group and 92 days in the OB treatment group.

The percentage of patients with virological failure at week 8 (before patients in the OB regimen were allowed to switch to enfuvirtide+OB) was twice as great in the OB group (36.8%) as in the enfuvirtide+OB group (17.5%). Kaplan-Meier estimates of the median time to virological failure in the OB treatment group was approximately 76.5 days, whereas the median time to treatment failure in the enfuvirtide+OB could not be estimated.

However, it was noted that only 15.9% versus 6.3% of the patients in the enfuvirtide + OB and OB treatment groups respectively had undetectable HIV-RNA levels (< 50 copies/mL) after 24 weeks of treatment. In addition, the proportion of patients with virological failure after 24 weeks of treatment is also substantial (45.5%) but lower than in the control group (77%). Early virological failure (<0.5 $\log_{10}$ copies/ml decrease at week 8) were seen in 17.5 % *versus* 36.8 % of the patients in the enfuvirtide + OB and OB treatment groups respectively.

Additional analyses

Both strata of patients, dichotomised by baseline HIV-RNA level (cut-off=40 000 copies/ml), had a significant decrease in HIV-RNA level when enfuvirtide was added. Patients with baseline CD4 count less than 100 cells/µl also had a significant decrease in HIV-RNA level when treated with enfuvirtide and OB, compared with patients treated with OB only. The same result holds true for patients with CD4 cell count > 100 cells/µl.

Most patients with the genotypic sensitivity score (GSS) of 0, 1 or 2 have primary mutations to all existing classes of antiretroviral products. These patients have no or few drugs in their OB regimen to which the virus is sensitive and it is therefore not surprising that there is a high rate of protocol defined virological failure. However regardless of GSS subgroup, the change in HIV RNA from baseline and the proportion of patients with HIV RNA < 400 copies/ml at week 24 was greater on the enfuvirtide+OB arm than OB treatment arm (table 8).

Table 8: Percentage of responders related to number of active ARVs in background regimen at 24 weeks

	HIV-1 RNA <400 copies/ml		HIV-1 RNA <50 copies/ml	
No. of active ARVs	FUZEON + OB %	OB %	FUZEON + OB %	OB %
0	8.9	0.0*	3.6	0.0
1	29.9	7.4*	13.9	4.2*
≥2	41.6	23.0*	20.6	9.3*

*p-value for the difference between the treatment arms <0.05

Durability of response (48 week data)

Following a CPMP concern raised on the durability of the response, the applicant submitted preliminary longer-term results. After 48 weeks of treatment, 18.3% of the patients had a complete response, defined as viral load < 50 copies/ml, which is higher than after 24 weeks of treatment (15.9%). The median time to virological failure was 278 days for enfuvirtide+OB versus 77 days for

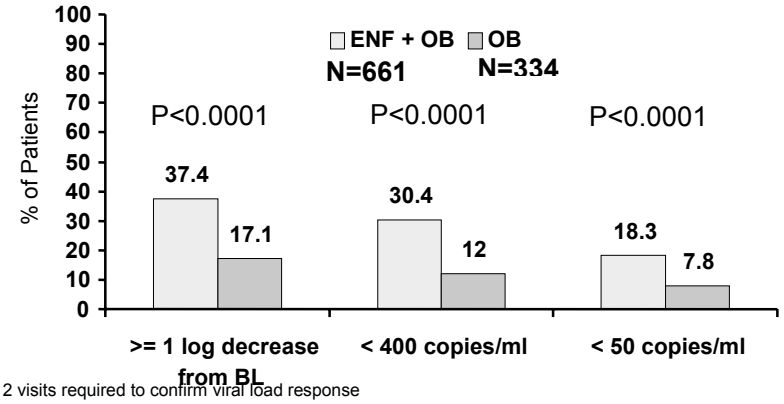
OB alone. Patients meeting the virological failure criteria were 52.3% and 77.8% respectively for enfuvirtide+OB and OB alone respectively (table 9 and figure 1).

Table 9: Number and Percent of Virological Responders by Status at Week 48 (ITT population, pooled analysis, 17 January 2003)

HIV-RNA Response Category At Week 48	ENF+OB N= 661	OB N= 334
<50 copies/ml at week 48	**121 (18.3%)**	**26 (7.8%)**
Maintained week 24 response	69 (10.4%)	15 (4.5%)
New response	52 (7.9%)	11(3.3%)
Response at week 24	105 (15.9%)	21 (6.3%)
< 400 copies/ml at week 48	**201 (30.4%)**	**40 (12.0%)**
Maintained week 24 response	173 (26.2%)	34 (10.2%)
New response	28 (4.2%)	6 (1.8%)
Response at week 24	216 (32.7%)	50 (15%)
≥ 1.0 log drop from baseline at week 48	**247 (37.4%)**	**57 (17.1%)**
Maintained week 24 response	240 (36.3%)	54 (16.2 %)
New response	7 (1.1%)	3 (0.9%)
Response at week 24	312 (47.2)	83 (24.9%)

Premature withdrawals and virological failures prior to Week 48 were considered to be non-responders (i.e., dropouts or virological failures = failure).

Figure 1Percent Responders at Week 48 (17 January 2003, ITT population, pooled analysis, dropouts and virological failures=failure)



2 visits required to confirm viral load response

AIDS-defining events

The overall incidence of AIDS defining events in ITT populations after 24 weeks of therapy was 6.8% in the enfuvirtide+OB treatment group versus 4.8% in the OB group and no beneficial effects on AIDS defining events were observed.

Preliminary data showed that the percentage of patients with a confirmed AIDS defining event by week 48 was 8.9 % (59/661) in the enfuvirtide + OB group and 5.4 % (18/334) in the OB group (table 8). Adjusting for exposure, this represents 10.6 and 11.1 events per 100 patients years respectively and there was no statistically significant difference between treatment groups. However, in the switch group (from OB to enfuvirtide+OB), AIDS defining events were more common (exposure adjusted incidence 17.6 per 100 patient years) but this group also had the lowest CD4+ count.

Table 10: Exposure-adjusted Incidence of Confirmed AIDS Defining Events by Body System and Trial Treatment, 48 weeks, 11 February 2003

	no. pts with event/100 pt-yrs		
	ENF + OB (n=661)	**Switch (n=222)**	**OB (n=334)**
exposure to study drug	**555.85 Pt.-yrs**	**119.50 Pt.-Yrs**	**162.23 Pt.-Yrs**
all body systems			
total pts with at least one AE	10.6	17.6	11.1
viral infection			
total pts with at least one AE	3.8	5.0	3.7
cytomegalovirus	2.0	2.5	0.6
herpes zoster	1.6	2.5	1.8
progressive multifocal leukoencephalopathy	0.2	-	0.6
herpes simplex virus	-	-	0.6
fungal infections			
total pts with at least one AE	3.6	5.0	3.1
candidiasis	2.3	1.7	2.5
cryptococcosis	0.4	-	-
histoplasmosis	-	-	-
coccidioidomycosis	-	-	-
neoplastic diseases			
total pts with at least one AE	1.6	4.2	1.2
non-hodgkin's lymphoma	0.5	1.7	1.2
kaposi's sarcoma	0.5	0.8	-
lymphoma of the brain	0.5	1.7	-
bacterial infections			
total pts with at least one AE	1.1	2.5	1.2
mycobacterium avium intracellulare	0.9	2.5	1.2
mycobacterium kansasii mycobacterium scrofulaceum and other atypical mycobacteria	0.2	-	-
mycobacterium tuberculosis	-	-	-
mycobacterium unidentified species	-	-	-
salmonella bacteremia	-	-	-
other conditions			
total pts with at least one AE	0.7	0.8	1.8
slim disease or HIV wasting syndrome	0.2	0.8	1.2
recurrent pneumonia	0.2	-	0.6
cervical carcinoma	-	-	-
HIV dementia	0.4	-	-
visceral leishmaniasis	-	-	-
parasitic infections			
total pts with at least one AE	0.9	3.3	1.2
pneumocystis carinii	0.5	0.8	-
toxoplasmosis	-	0.8	0.6
cryptosporidiosis	0.4	1.7	0.6
isosporiasis	-	-	-

NOTES: ENF+OB virological failures who remained on ENF+OB are included in ENF+OB exposure. OB virological failures who did not remain on OB are excluded from OB exposure (censored from time of failure. OB virological failures who switched to ENF+OB comprise the exposure to Switch. Multiple occurrences of the same adverse event in one individual counted only once

The incidences of AIDS defining infections, including recurrent pneumonia, and tumours were low in each treatment group. Cytomegalovirus (CMV) disease had a higher incidence in enfuvirtide treated patients than in OB treated patients but the absolute numbers are small and most of the patients had had signs and symptoms of CMV disease prior to study entry.

The final results of the studies were provided as part of the follow-up measures to be fulfilled post-authorisation. They confirmed the preliminary 48-week data submitted to obtain the Marketing Authorisation.

Clinical studies in special populations

The antiviral effect of enfuvirtide in children is investigated in an ongoing, open label, single-arm study (T20-310) and in study T20-204. T-20 is given at the dose of 2mg/kg subcutaneously bid in combination with optimised antiviral therapy for 48 weeks. Thirty-nine children have been exposed to enfuvirtide. Children included are 3-16 years old and HIV-1 positive. Results will be submitted as part of the follow-up measures to be fulfilled post-authorisation.

Clinical safety

Patient exposure

- 24-week data from main phase trials involving 858 patients (663 randomised to enfuvirtide+OB, 195 switches from OB) exposed to at least one dose of enfuvirtide as add-on to OB.
- Supportive data from 295 additional patients treated with enfuvirtide in other trials (167 at the recommended dose).
- Data for 39 paediatric patients

After 24 weeks of treatment in the main trials and due to the design of these trials, the total number of patient years of exposure to the randomised treatment regimen was approximately 2.6 times higher in the enfuvirtide+OB treatment group than in the OB group.

The safety data for enfuvirtide were updated in the beginning of December 2002 and altogether 1,541 patients were included, of whom 1,401 had received the proposed dose of enfuvirtide. Of these patients, 913 have received enfuvirtide for 24 weeks and 569 have received enfuvirtide for 48 weeks.

Adverse events and serious adverse events/deaths

In the overall summary, 81.1 % of patients treated with enfuvirtide+OB and 71.9% of patients treated with OB alone had adverse events that were considered related to the medications given. Besides local injection site reactions and eosinophilia, the adverse event profiles of the enfuvirtide+OB and OB treatment groups were similar in the phase III studies. The most frequently reported adverse events were diarrhoea (26.8% and 33.5% for enfuvirtide+OB and OB groups respectively), nausea (20.1% and 23.7%), fatigue (16.1% and 17.4%), and headache (11.8% and 11.1%).

A summary of events reported at higher incidences with T-20+OB vs. OB is given in Table 11.

Table 11 Adverse Events reported by more patients on T20+OB than on OB alone before and after adjustment for exposure. Randomised population.

	N (%)		No. Patients with Event/100 Patient-years	
	T20+OB (N=663)	**OB (N=334)**	**T20+OB (N=663)**	**OB (N=334)**
Adverse events				
Peripheral neuropathy	89 (13.4)	21 (6.3)	13.9	12.8
Sinusitis	57 (8.6)	8 (2.4)	**8.9**	**4.9**
Appetite decrease	50 (7.5)	8 (2.4)	7.8	4.9
Skin papilloma	38 (5.7)	4 (1.2)	5.9	2.4
Myalgia	36 (5.4)	8 (2.4)	5.6	4.9
Lymphadenopathy	32 (4.8)	2 (0.6)	**5.0**	**1.2**
Pneumonia	23 (3.5)	1 (0.3)	**3.6**	**0.6**
Conjungtivitis	22 (3.3)	4 (1.2)	3.4	2.4
Pancreatitis	22 (3.3)	4 (1.2)	3.4	2.4
Hypoaesthesia	17 (2.6)	2 (0.6)	2.7	1.2
Vertigo	19 (2.9)	1 (0.3)	3.0	0.6
Weakness	15 (2.3)	1 (0.3)	2.3	0.6
Special interest adverse events				
Peripheral neuropathies	93 (14.0)	23 (6.9)	14.5	14.1
Hepatic toxicity clinical	15 (2.3)	2 (0.6)	2.3	1.2
Diabetes mellitus and hyperglycaemia	27 (4.1)	5 (1.5)	4.2	3.1
Bronchospasm and obstruction	13 (2.0)	2 (0.6)	2.0	1.2
Bone pathology	10 (1.5)	2 (0.6)	1.6	1.2
Thrombosis or phlebitis	7 (1.1)	1 (0.3)	1.1	0.6

When adjusted for treatment duration, modestly higher incidences in the enfuvirtide treatment group were seen for peripheral neuropathies (14.5 versus 14.1 patients/100 patient-years), hepatic toxicity clinical (2.3 versus 1.2 patients/100 patient-years) and diabetes mellitus with hyperglycaemia (0.042 versus 0.031 patients/100 patient-years).

In the pooled studies T30-301 and 302, 10.0% of patients in the enfuvirtide+OB treatment group had a serious adverse event related to treatment.
The most frequently reported serious adverse events in the enfuvirtide+OB treatment group were increased creatine phosphokinase, increased GGT and pancreatitis/pancreatitis acute, all with frequency of < 3% and in the same range as in the OB treatment group.

Deaths have been reported in 28 patients (22 in the enfuvirtide+OB group and 6 in the OB group). The causes of death were varied and none of the events were uncommon for an HIV infected population. One death (suicide) was related to enfuvirtide and considered caused by the patient's inability to self inject enfuvirtide rather than caused by its pharmacological effects.

Discontinuation due to adverse events

Adverse events leading to withdrawal from treatment occurred throughout the first 24 weeks of the studies. No specific pattern with respect to the timing of withdrawals was observed.
Overall, 28.5% and 41.7% of the patients in the enfuvirtide+OB and OB treatment groups, respectively, discontinued the studies. The withdrawal rates adjusted for exposure were 9.8 and 6.7 patients/100 patient-years, in the enfuvirtide+OB and OB groups, respectively.
The most frequent adverse event causing withdrawal was gastrointestinal disorders affecting 2% and depression (1%) in each treatment group.

Infectious events
A summary of the exposure-related incidences of bacterial infection showed that the overall incidence of bacterial infections was comparable between treatment groups. However, some infections were more prevalent in enfuvirtide treated patients as shown in Table 10, in particular for pneumonia for which the difference between the enfuvirtide+ OB arm and the OB arm was statistically significant ($p = 0.02$). During the oral explanation, an analysis of time to event for pneumonia was presented which did not identify a specific pattern.

Table 12: Rates of adverse events related to bacterial infections in pooled studies T20-301 and T20-302: January 17, 2003 preliminary data.

Adverse event	ENF+OB (813.47 Pt.-Yrs)	Switch (213.82 Pt.-Yrs)	OB (163.41 Pt.-Yrs)
Bacterial infections	17.1	20.6	18.4
Pneumonia	4.9	4.7	0.6
Skin infection (not at injection site)	5.5	7.0	3.1
Sinusitis	5.0	8.9	1.2
Sepsis	1.6	1.4	1.2

Injection site reactions
The overall most common adverse event was injection site reaction, affecting almost every patient on enfuvirtide (98.3%) and seen mainly during the first treatment week (85.6%). There was no evidence of an increase in severity over time for either overall pain and discomfort or any of the signs and symptoms of a local injection site. The most frequent signs were erythema (91.7%), induration (90.6%), nodules and cysts (81.4%). Overall, 96.1% of the patients experienced some pain or discomfort and 11.5% had injection site reactions that required analgesics or limited usual activities. Most patients had grade 1-2 (90.6%) injection site reactions. The remaining patients had grade 3 reactions and none had grade 4 reactions. Few patients (4.1%) discontinued treatment due to injection site reactions.

These results were confirmed at week 48 – final data, submitted post-authorisation (Table 13).

Table 13: Summary of Individual Signs/Symptoms Characterising Local Injection Site Reactions in studies TORO 1 and TORO 2 combined (% of patients)

	n=663		
Withdrawal Rate due to ISRs	4%		
Event Category	**FUZEON +Optimised background[a]**	**% of Event comprising Grade 3 reactions**	**% of Event comprising Grade 4 reactions**
Pain / discomfort	96.1%	11.0%[b]	0%[b]
Erythema	90.8%	23.8%[c]	10.5%[c]
Induration	90.2%	43.5%[d]	19.4%[d]
Nodules and cysts	80.4%	29.1%[e]	0.2%[e]
Pruritus	65.2%	3.9%[f]	NA
Ecchymosis	51.9%	8.7%[g]	4.7%[g]

[a]Any severity grade
[b]Grade 3= severe pain requiring analgesics (or narcotic analgesics for ≤ 72 hours) and/or limiting usual activities; Grade 4= severe pain requiring hospitalisation or prolongation of hospitalisation, resulting in death, or persistent or significant disability/incapacity, or life-threatening, or medically significant.
[c]Grade 3= ≥ 50 mm but < 85 mm average diameter; Grade 4= ≥ 85 mm average diameter.
[d]Grade 3= ≥ 25 mm but < 50 mm average diameter; Grade 4= ≥ 50 mm average diameter.
[e]Grade 3= ≥ 3 cm; Grade 4= If draining.
[f]Grade 3= refractory to topical treatment or requiring oral or parenteral treatment; Grade 4= not defined.
[g]Grade 3= > 3 cm but ≤ 5 cm; Grade 4= > 5 cm.

The pathology of injection site reactions was investigated in biopsies from seven patients. All biopsies showed pathological changes, also in one patient lacking clinical reaction. All biopsies showed inflammatory infiltrate consistent with hypersensitivity reaction, with eosinophils, histocytes and rare lymphocytes. Vasculitis was not seen. All cases were positive for enfuvirtide immuneperoxidase staining, with increased positivity where the inflammation was greatest, but also along collagen fibres. No correlation between clinical picture and inflammatory response could be seen.

Systemic hypersensitivity reactions
Five serious adverse events suggestive of systemic hypersensitivity reactions (rash, fever, vomiting, nausea, chills, rigors, low blood pressure and elevated serum liver transaminases in various combinations, and possibly immune complex reaction, respiratory distress and glomerulonephritis) to enfuvirtide have been reported across the clinical trials. The reactions included allergic reactions and glomerulonephretis. The reactions started 7 to 56 days after initiation of enfuvirtide. Rash and fever were the most prevalent symptoms. Three of these cases had a positive re-challenge reaction to enfuvirtide. Risk factors that may predict the occurrence or severity of hypersensitivity to enfuvirtide have not been identified. A warning to discontinue in patients developing signs/symptoms of a systemic hypersensitivity reaction treatment has been added in the Summary of Product Characteristics.

Laboratory findings

In general, at week 24, there were no major differences in laboratory findings between the two treatment groups. However treatment-emergent grade 3 or 4 increase in amylase (7.1 % in enfuvirtide+OB and 4.2 % in OB) and lipase (8.2 % in the enfuvirtide+OB versus 5.4 % in OB) were reported at higher rates in the enfuvirtide+OB group.

Treatment-emergent eosiphilia was more prevalent in the enfuvirtide+ OB group (10.1%) compared to the OB group (2.4 %). Through week 48, treatment-emergent eosinophilia [greater than the Upper Limit of Normal (ULN) of > 0.7 x 10^9/l] occurred at a higher rate amongst patients in the Fuzeon containing group (12.4 per 100 patient years) compared with OB alone regimen (5.6 per 100 patient years). When using a higher threshold for eosinophilia (>1.4 x 10^9/L), the patient exposure adjusted rate of eosinophilia is equal in both groups (1.8 patients with event per 100 patient-years).

Table 14 shows the treatment emergent laboratory abnormalities that occurred at a rate of at least 2 patients per 100 patient-years of exposure and that occurred more frequently (either as a grade 3 or 4

laboratory abnormality) among patients receiving Fuzeon+OB regimen than among patients on the OB alone regimen to week 48 of the pooled studies TORO 1 and TORO 2 (final data).

- **Table 14: Exposure adjusted Grade 3 & 4 laboratory abnormalities among patients on Fuzeon+OB and OB alone regimens, reported at more than 2 patients with event per 100 patient years**

Laboratory Parameters Grading	Fuzeon+OB regimen per 100 patient years	OB alone regimen per 100 patient years
Total Exposure (patient years)	**557.0**	**162.1**
ALAT		
Gr. 3 (>5-10 x ULN)	4.8	4.3
Gr. 4 (>10 x ULN)	1.4	1.2
Hemoglobin		
Gr. 3 (6.5-7.9 g/dL)	2.0	1.9
Gr. 4 (<6.5 g/dL)	0.7	1.2
Creatinine phosphokinase		
Gr. 3 (>5-10 x ULN)	8.3	8.0
Gr. 4 (>10 x ULN)	3.1	8.6

Safety related to drug-drug interactions and other interactions

More than 20 % of patients with neuropathy in the enfuvirtide+OB treatment arm did not receive any NRTIs associated with neuropathy.

Safety in special populations

Few patients included in the phase III studies were co-infected with hepatitis B (8%, 55/661), C (11%, 70/661) or both (0.6%, 4/661). In these patients the addition of enfuvirtide did not increase the incidence of hepatic events, serious adverse events or grade 3 or 4 liver function abnormalities.

Preliminary data in less than 50 children treated with enfuvirtide suggest that the adverse event profile is similar to that in adults.

Quality of life

The quality of life was assessed in patients treated with enfuvirtide according to a Subcutanous Injection Survey (SIS) protocol. SIS comprises a questionnaire of 18 items measuring the patient's assessment of self-injection in HIV clinical trails. Patients were assessed according to the protocol on two occasions, at 8 and 24 weeks of treatment.

The majority of patients, highly motivated, had no difficulties in handling and coping with the subcutaneous injections.
Adherence to treatment has been registered continuously during the first 24 weeks of studies. Patients in both arms had a high degree of adherence, 93.3 % (enfuvirtide only), 88.5 % (enfuvirtide+OB) and 88 % (OB only).

4. Overall conclusions, benefit/risk assessment and recommendation

Quality

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. The active substance is well characterised and documented. The pharmaceutical form selected is adequate taking into account the properties and stability of the active substance. The excipients are commonly used in this kind of formulation and the packaging material is well documented. The validation of the manufacturing process also ensures reproducibility of the product.

Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Stability tests under ICH conditions indicate that the product is stable for the proposed shelf life. At the time of the CPMP opinion there were some outstanding quality issues which had no impact on the benefit/risk profile. The applicant undertook to provide the necessary information as follow-up measures within an agreed timeframe, and to submit variations if required following the evaluation of this additional information.

Preclinical pharmacology and toxicology

Enfuvirtide presents an antiviral activity both *in vitro* and *in vivo* compatible with a potential clinical use for the treatment of HIV infection against a large number of different HIV-1 strains, with no major influence of clade or co-receptor specificity.

The primary mode of antiviral action of enfuvirtide by binding to gp41 HR1 preventing the subsequent binding of gp41 HR2 and gp41 refolding. This in turn inhibits membrane fusion. HIV-1 with reduced sensitivity to enfuvirtide has been selected *in vitro* and *in vivo*. Genotypic changes related to enfuvirtide resistance appear to map to the aa 36 to 41 region of the HR1 domain of gp41. No cross-resistance with the three currently approved classes of antiretroviral medicinal products was evidenced.

The pharmacokinetic, absorption, disposition and metabolism of enfuvirtide has been characterised in rats and monkeys, but in some aspects the characterisation is limited or suboptimal since classical approaches are not readily or generally applicable to a synthetic polypeptide.
The pharmacokinetic parameters of enfuvirtide following intravenous injection are generally similar in rat, monkey and human, characterised by small volumes of distribution, low systemic clearances, and short terminal half-lives. Enfuvirtide bioavailability after subcutaneous injection is similarly high in primates and humans, but dose-dependent in the rat, as a decreasing fraction is absorbed with increases in dose. As a synthetic peptide consisting of naturally occurring amino acids, degradation of enfuvirtide to smaller peptides and free amino acids occur through the action of peptidases/proteinases primarily in the liver and the kidney. Enfuvirtide does not inhibit the activity of cytochrome P450 isozymes in human hepatic microsomes, and therefore the potential for interactions between medicinal products and enfuvirtide is low.
At the time of the CPMP opinion there were some outstanding issues which had no impact on the benefit/risk profile and for which the applicant undertook to provide the necessary information as follow-up measures to be fulfilled post-authorisation.

It was predicted that enfuvirtide would show a low order of toxicity, based on its virus-specific mechanism of action, and its molecular structure of a synthetic peptide of naturally-occurring L-amino acids. This view is reflected in the toxicology program. Although no major safety concerns could be identified in the toxicity studies, the performance of the studies was in most cases suboptimal with respect to exposure. Data do suggest however that enfuvirtide may have host activity on cells in the immune system through the formyl peptide receptor complex, which could have consequences for safety and efficacy. The applicant therefore undertook to further address the effect on immune functions, the potential to activate the formyl peptide receptor *in vivo* as well as carcinogenicity as part of the special obligations to be fulfilled post-authorisation. Enfuvirtide was not embryotoxic nor teratogenic in rats or rabbits. However, no firm conclusions can be made regarding the effect of enfuvirtide on fertility, early embryonic development or on post-natal development due to low exposure compared to expected therapeutic exposure. Enfuvirtide was not genotoxic. At the time of the CPMP opinion there were some outstanding issues which had no impact on the benefit/risk profile and for which the applicant undertook to provide the necessary information to further characterise the toxicity profile of enfuvirtide as specific obligations and follow-up measures to be fulfilled post-authorisation

Efficacy

The relationship between dose and antiviral effect of enfuvirtide was investigated in two monotherapy studies. The plasma HIV-1 RNA reduction was evident and similar between the two studies after 2 weeks of treatment (1.5 $\log_{10}$ copies/ml in the 100 mg treatment group). Further investigations in the

two phase II studies where enfuvirtide was combined with antiretroviral treatment confirmed the efficacy of enfuvirtide 75 mg – 100 mg in further reducing HIV-1 RNA levels by 1 - 1.5 $\log_{10}$ copies/ml after 16 weeks of combination therapy.

The dose of enfuvirtide selected for confirmatory trials, 90 mg bid by subcutaneous injection has been reasonably justified. It has not been shown to be the maximally effective dose however it is rather limited by product solubility and tolerable injection volume and preclinical and clinical data suggest that the dose may be suboptimal. Therefore the applicant undertook to discuss the possibility to increase the daily doses of enfuvirtide as part of the follow-up measures to be fulfilled post-authorisation.

Analyses of pooled data from the main studies T20-301 and 302 showed that after 24 weeks of treatment more than 90% of patients with virological failure harboured viruses with reduced enfuvirtide susceptibility and mutations in the area aa 36 – 45 of gp41 were identified in over 90% of the viruses with phenotypic resistance. Further discussion on resistance development to enfuvirtide will be provided with the 48-week results of these studies.

The pharmacokinetics profile of enfuvirtide was characterised in HIV infected patients. After subcutaneous administration enfuvirtide is slowly absorbed and the absolute bioavailability is 84% after 90 mg dose. The absorption is comparable after injection at different sites (abdomen, thigh, arm). Enfuvirtide has a low volume of distribution, is bound to protein (92%). There are no *in vivo* data on the metabolism but considering that enfuvirtide is a peptide composed of 36 naturally occurring L-aminoacid residues, it is likely to be catabolised by peptidases, proteinases and other enzymes. Data on urinary excretion will be provided as part of the follow-up measures to be fulfilled post-authorisation. No dose adjustment is required for patients with creatinine clearance above 35 ml/min. There is no data in patients with impaired hepatic function, severe renal impairment and only limited in patients with moderate renal impairment. Limited data in children suggest that administration of 2 mg/kg bid will give similar exposure as in adults. The pharmacokinetic documentation was considered satisfactory and information in the Summary of Product Characteristics adequately reflects the data.

The clinical efficacy of enfuvirtide is based on the results of two main ongoing, randomised, open label studies (T20-301 and T20-302) on heavily pre-treated patients comparing enfuvirtide in combination with optimised background regimen (OB) versus OB alone. Data at 24 weeks showed the benefits of enfuvirtide+OB treatment over OB treatment alone. A significant higher reduction in $\log_{10}$ HIV RNA was observed in patients who received enfuvirtide+OB versus OB alone (LSM difference of –0.93 $\log_{10}$ copies/ml and –0.78 $\log_{10}$ copies/ml in the 2 studies, both p values < 0.0001). Several sensitivity analyses performed confirmed the robustness of the results. Results in most subgroups tended to favour enfuvirtide+OB and in many cases the differences between treatment groups were statistically significant.
The percentage of patients with undetectable viral load (< 50 copies/ml), although low, was significantly better in the enfuvirtide+OB group than OB group alone (15.9% versus 6.3%).
In all subgroups of patients, the results favoured enfuvirtide+OB versus OB.
Data at 48 weeks were provided which confirmed the durability of the response. After 48 weeks of treatment 18.3% of the patients had a complete response (below 50 copies/ml). The treatment response is related to the degree of viral resistance in the backbone treatment but was superior to OB alone in all genotypic sensitivity score groups. AIDS related events were most common in patients switching therapy to enfuvirtide treatment, a result not unexpected as this patient group was most immunocompromised.

At the time of the CPMP opinion there were some outstanding issues for which the applicant undertook to provide the necessary information to further characterise the efficacy profile of enfuvirtide as follow-up measures to be fulfilled post-authorisation, including the final results of ongoing main studies.

Safety

The safety profile has been defined in over 1,000 patients, included over 500 patients who received enfuvirtide for 48 weeks. Overall injection site reactions of usually moderate severity and eosinophilia were events that most clearly differentiated enfuvirtide+OB from comparator. Hypersensitivity reactions were observed with low incidence. A few of these were serious, although no anaphylactic reactions have been reported. There were indications that bacterial infections like pneumonia and sinusitis may be more prevalent with enfuvirtide.

Preliminary data in children suggest a similar safety profile as the one observed in adults. Most common adverse events were mild to moderate injection site reactions and only two serious adverse events have been observed cellulitis and injection site reaction.

At the time of the CPMP opinion there were some outstanding issues for which the applicant undertook to provide the additional information to further characterise the safety profile of enfuvirtide as follow-up measures to be fulfilled post-authorisation.

Benefit/risk assessment

The ongoing confirmatory trials were designed according to CPMP advice and show incontrovertible, clinically relevant efficacy of enfuvirtide as add-on to an individually optimised backbone of antiretroviral medicinal product on HIV RNA reduction and CD4+ levels at 24 weeks. For the population studied, heavily treatment-experienced patients with few remaining treatment options for which there is an unmet medical need, this is acceptable for licensing under exceptional circumstances according to CPMP Note for Guidance. Preliminary data at 48 weeks confirmed the durability of the response.

The safety issues specific to enfuvirtide are ubiquitous injection site reactions of usually moderate severity, and generalised hypersensitivity reactions, a few of which have been serious, although no anaphylactic reactions have been reported. A concern was raised with increased incidence with enfuvirtide of bacterial infections, in particular pneumonia.

The final results of the main studies to be submitted as part of the follow-up measures to be fulfilled post-authorisation will provide further information on the long-term efficacy and safety of enfuvirtide.

During an oral explanation in front of the CPMP, the applicant addressed the issue of time to event for infection with increased incidence in the enfuvirtide arm, in particular pneumonia and CMV disease. The reason for the increased rate observed is unknown and the analysis of time to event did not identify a specific pattern. Therefore the CPMP agreed to include a warning in the Summary of Product Characteristics and the applicant undertook to further address the potential impact of enfuvirtide on the risk for bacterial infections, especially in view of the preclinical indication of host activity on the immune system and the apparent increase in the incidence of pneumonia, and to conduct a prospective observational cohort study to further assess the risk of pneumonia.
The applicant presented the ongoing paediatric programme. The experience in children is limited particularly in children under the age of 6 years of age. The data in a limited number of patients showed that the 2 mg/kg twice daily provides exposures comparable to those seen in adults and that the safety profile was comparable to adults. The CPMP considered that there was insufficient experience in children to recommend an indication in children but agreed, based on the limited pharmacokinetics data to include in the Summary of Product Characteristics the dosage recommendations currently used in the clinical studies in children. In addition the applicant undertook to provide the results on the ongoing study and to explore the potential for alternative dosage forms which may also allow flexible dosing in children as part of the follow-up measures to be fulfilled post-authorisation.

Finally, the applicant presented the strategies on the supply of the product considering the current production capacity. The applicant undertook to keep the CPMP informed on the availability of the product on the market on regular basis.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the benefit/risk profile of Fuzeon was favourable in the treatment of HIV-1 infected patients for the following indication:

> "Fuzeon is indicated in combination with other antiretroviral medicinal products for the treatment of HIV-1 infected patients who have received treatment with and failed on regimens containing at least one medicinal product from each of the following antiretroviral classes**,** protease inhibitors, non-nucleoside reverse transcriptase inhibitors and nucleoside reverse transcriptase inhibitors, or who have intolerance to previous antiretroviral regimens. In deciding on a new regimen for patients who have failed an antiretroviral regimen, careful consideration should be given to the treatment history of the individual patient and the patterns of mutations associated with different medicinal products. Where available, resistance testing may be appropriate.*"*