SCIENTIFIC DISCUSSION

This module reflects the initial scientific discussion for the approval of Viread. This scientific discussion has been updated until 1 February 2004. For information on changes after this date please refer to module 8B

1. Introduction

VIREAD contains the active substance tenofovir disoproxil fumarate (tenofovir DF or TDF), which has an antiretroviral activity for the treatment of Human Immunodeficiency virus (HIV) infection.

Substantial improvements in the antiretroviral treatments over the past few years led to significant decrease in morbidity and mortality due to HIV infection. Current antiretroviral agents target two specific viral enzymes: reverse transcriptase (nucleoside reverse transcriptase inhibitor (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs)) and protease (protease inhibitors (PIs) Combination therapy, especially triple regimens, is considered to be the standard of care of HIV-1 infected patients. The antiretroviral agents already authorised within the European Union include:

NRTIs: zidovudine (ZDV), didanosine (ddI), zalcitabine (ddC), stavudine, lamivudine and abacavir
NNRTIs: nevirapine and efavirenz
PIs: ritonavir, indinavir, saquinavir, nelfinavir, amprenavir and lopinavir

For the treatment of HIV infected children, the available registered options are lamivudine, stavudine, nelfinavir, ritonavir, indinavir, amprenavir, nevirapine, efavirenz and lopinavir.

The long-term use of all these products is, however, limited by emergence of resistance, by toxicity and by inconvenient dosing schedules or formulations. An increasing number of patients are failing their current antiretroviral regimen. Antiretroviral agents susceptible to multi-resistant HIV are not yet available and choosing the best "salvage" therapy for patients who have failed one or more treatment regimens has become a difficult issue. Further therapeutic agents are therefore clearly needed that will re-establish virological suppression in these patients.

Tenofovir disoproxil fumarate is a salt of an oral prodrug of tenofovir. Tenofovir is a nucleoside monophosphate (nucleotide) analogue, a new class of agents, which has an *in vitro* antiviral activity against retroviruses and hepadnaviruses by inhibiting the reverse transcriptase enzyme hence, by DNA chain termination. Because tenofovir was not well absorbed from the intestine, the prodrug, tenofovir disoproxil, was developed to increase the bioavailability. Viread is available as a film-coated tablet, containing 245 mg of tenofovir disoproxil (as fumarate), equivalent to 300 mg tenofovir disoproxil fumarate or 136 mg tenofovir.

The approved indication has been extended further to the assessment of an additional clinical study conducted in treatment-naïve patients and provided post-authorisation, and is currently as follows:

"Viread is indicated in combination with other antiretroviral medicinal products for the treatment of HIV-1 infected adults over 18 years of age.

The demonstration of benefit of Viread is based on results of one study in treatment-naïve patients, including patients with a high viral load (> 100,000 copies/ml) and studies in which Viread was added to stable background therapy (mainly tritherapy) in antiretroviral pre-treated patients experiencing early virological failure (< 10,000 copies/ml, with the majority of patients having < 5,000 copies/ml).

In deciding on a new regimen for patients who have failed an antiretroviral regimen, careful consideration should be given to the patterns of mutations associated with different medicinal products and the treatment history of the individual patient. Where available, resistance testing may be appropriate".

The recommended dose is one 245 mg tablet daily taken orally with a meal. In exceptional circumstances in patients having particular difficulty in swallowing, Viread can be administered following disintegration of the tablet in at least 100 ml of water, orange juice or grape juice.

2. Part II: Chemical, pharmaceutical and biological aspects

As the quality variations submitted since the marketing authorisation was granted had no major impact on the safety/efficacy of Viread, the quality scientific discussion below reflects the data submitted in support of the initial marketing authorisation. See "Steps taken after granting the Marketing Authorisation" for information on quality variations.

Composition

Viread is formulated as immediate release film-coated tablets containing 245 mg of tenofovir disoproxil (as fumarate), equivalent to 136 mg of tenofovir. The excipients are those commonly used in this type of product: pregelatinised starch (binder); croscarmellose sodium (disintegrant); lactose monohydrate (filler); microcrystalline cellulose (filler); magnesium stearate (lubricant); and a proprietary hypromellose-based film-coating (lactose monohydrate, glycerol triacetate, hypromellose, titanium dioxide [E171], indigo carmine lake [E132]).

The tablets are presented in high density polyethylene (HDPE) bottles with aluminium foil induction seals and polypropylene child-resistant caps. Each bottle contains 30 tablets and includes a canister of silica gel as a desiccant to reduce the headspace moisture, and polyester fibre to prevent tablet chipping in transit.

Active substance

Tenofovir disoproxil fumarate (tenofovir DF) is a diester prodrug of the purine based nucleotide analogue, tenofovir. Tenofovir DF is obtained by introduction of labile esters on the phosphonate group of tenofovir. This (isopropoxycarbonyloxy)methoxy ester is utilised as a promoiety in order to increase lipophilicity and enhance the oral bioavailability of the parent compound. The physicochemical characteristics of tenofovir DF with respect to salt selection, hygroscopicity, dissociation constant, partition coefficient, solubility, solution and solid state have been studied.

Tenofovir disoproxil fumarate is manufactured as an anhydrous crystalline form using a linear synthesis which has been well described, including a flow diagram. The starting material adenine is subjected to a two-step modification to form the skeleton of the final molecule, which is modified with protecting groups. A deprotection step is performed prior to the final step, which consists of esterification, salt formation and final purification. In the final purification step, crude tenofovir disoproxil solution is washed with water, then concentrated, treated with silica gel, and filtered. A solution of fumaric acid in 2-propanol is added to the resulting oil, to produce the fumarate salt which is then crystallised, isolated by filtration, and rinsed with isopropyl ether. Following isolation, the product is dried at not more than 45°C to a solvent content (LOD or GC) of not more than 0.5 %. The dry product is milled to break up any aggregates.

The proposed specification for the starting material adenine is generally adequate, but some amendments are required.

Tenofovir DF contains a single chiral centre at the C-11 position (C-2 of the propyl side-chain) and the defined method of synthesis routinely produces the R-enantiomer, arising from the use of (R)-1,2-propylene carbonate in the first reaction step. Proof of the structure has been provided by means of elemental analysis, UV, IR, NMR, MS, and single crystal X-ray diffraction.

Two polymorphic forms have been identified by X-ray powder diffraction and DSC, a ‘high’ melting polymorph (115 - 118°C) and a ‘low’ melting polymorph (112 - 114°C). The melting enthalpies, intrinsic dissolution rates and solubility of these crystal forms are indistinguishable, and therefore these solid-state differences are unlikely to result in clinical consequences.

The specification for the active substance includes relevant tests for: appearance: identity (IR & HPLC); assay by HPLC (97-101 % tenofovir DF, non-chiral); enantiomeric purity by HPLC (not less than 98 % of the R-isomer); 14 potential related impurities are described of which 8 are

controlled in the specification by HPLC; organic volatile impurities; and heavy metals. Physical tests include: clarity of solution; water content; DSC (main endotherm characterisation); and particle size. 9-propenyladenine (9-PA) is a process-related impurity which is mutagenic. Although the amounts found in batches of the drug substance have been monitored and limited throughout development, a routine test and limits for this impurity should be included in the active substance specification. Three manufacturers of the active substance have been nominated. Although the same synthetic methods are used at each of the sites, slightly different solvents may be used, therefore some further justification of the proposed residual solvent specifications is required.

Analytical validation data for all analytical methods are provided and take into account current guidelines. Details of the reference standards are provided.

Batch analyses data are presented for a total of 39 batches of tenofovir DF used in toxicological, clinical and stability studies, with precise impurity profile, however some further clarification is required.

Stability of the active substance

Tenofovir DF shows excellent physicochemical stability when stored at 5ºC for up to 36 months (three lots, packaged in polyethylene bags, sealed, and then placed into tightly capped HDPE bottles), the primary route of chemical degradation being hydrolysis. There was no significant loss in purity or increase in total impurity and degradation product content after storage under accelerated storage conditions (same packaging, at 25ºC/60 %RH & 30ºC/60 %RH) for up to 6 months.

Tenofovir DF active substance is specified to be stored under refrigeration at 2 - 8ºC. Tenofovir DF is to be stored in polyethylene bags, which are placed into tightly closed HDPE containers and the proposed retest period of 24 months is supported.

Other ingredients

All the excipients in the product comply with the appropriate specifications and monographs of the current PhEur and are widely used for the manufacture of solid oral dosage forms.

Information has been provided to demonstrate that the CPMP is satisfied that the materials, lactose monohydrate, magnesium stearate (vegetable source) and the proprietary film-coating (Opadry II Y-30-10671-A) are in compliance with the latest EU guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products.

Satisfactory control specifications and certificates are provided for the packaging materials. The bottles and closures are controlled according to the general PhEur requirements for plastic containers and closures.

Product development and finished product

The fumarate salt of the diester prodrug of tenofovir is chosen to increase the intestinal permeability and to improve the bioavailability of the active substance. The choice for a tablet presentation, and the rationale for both the proposed qualitative and quantitative composition of the formulation has been presented.

The processing parameters, including those for the film-coating, have been investigated and optimised. The free moisture in the tablets is minimised both during the manufacturing process and in the packaging. The HDPE resin used for the primary packaging (bottles) is thick and was selected based upon moisture vapour transmission data, as the product must be protected from extended periods of exposure to high moisture conditions. The use of 1 gram of silica gel (in a canister) per bottle was established based upon stability data. Induction sealing of the bottle (with aluminium foil) also reduces the available moisture.

Film coated tablets of different strengths have been used in clinical trials and the formulations for these have been presented. Bioequivalence of the 34 % w/w active clinical formulation and the intended commercial formulation was examined in an open-label pharmacokinetic study in healthy volunteers. The results demonstrated that the intended commercial formulation is bioequivalent to the early clinical formulation prototype.

The manufacturing processes have all been well described. Manufacture commences with a conventional wet granulation process, followed by a drying step to dry the granules (to LOD ≤ 3 %) to reduce the intragranular moisture content. After compression, the bulk uncoated tablets are tested for hardness and friability. Finally the film-coating (aqueous based) is applied.

The industrial batch size has been stated to be up to 1000 kg. The frequency of in-process control testing remains to be fully clarified.

Nine lots of up to 230 kg in size have been manufactured and used for validation studies, and although the process has been shown to be robust and to result in consistent product some points for clarification remain and some further validation data are also required.

Product specification

The product specification contains the relevant tests and limits for a product of this type. Tests include appearance, identification of the active substance (HPLC & UV), assay (96 – 105 % at release, 90 – 105 % during shelf life, by HPLC), and limits for 10 named related impurities/degradates. Unspecified impurities are limited to not more than 0.2% each. In addition there are also tests for content uniformity (PhEur), dissolution, water content and microbial limits (PhEur).

The proposed specification limits for total impurities and degradation products, in both the release and shelf-life specifications are very high and remain to be tightened or further justified by reference to the original toxicological studies.

The analytical methods are described and suitably validated, in accordance with current guidelines.

Batch analyses results on 10 batches are provided.

Stability of the product

Long-term and accelerated stability studies were conducted on nine batches of tenofovir DF tablets, 245 mg. The stability batches were produced at a scale that is greater than one-tenth of the intended commercial scale, were identical in the composition, used the same manufacturing process, and were packaged into the same container-closure system as the intended commercial product.

Long-term stability studies were conducted at 25ºC/60 %RH and 12 months data are available for two batches and 9 months data for three batches.

The results indicate an acceptable long-term stability. The tablets remained within the product specifications when stored for up to 12 months at 25ºC/60 %RH. A statistical analysis was performed to estimate the total impurity and degradation product content at the proposed expiration dating period of 24 months. The stability data provided however do not yet support the claimed limit of 8.0 % for impurities/degradation products in the shelf-life specification.

No significant change in physicochemical stability was observed for tenofovir DF tablets stored for 6 months at 40ºC/75 %RH. The drug product remained within the product specifications over the 6 month study duration. No significant change in physicochemical stability was observed for tenofovir DF tablets exposed to artificial daylight fluorescent lamps.

On the basis of the long-term and accelerated stability data and the statistical analyses, the proposed shelf life, that is, 24 months with no specific storage condition, is acceptable. However clarification of some of the stability data, and some additional data are required.

3. Part III: Toxico-pharmacological aspects

Pharmacodynamics

Mechanism of action

Tenofovir is a nucleotide analogue (i.e., a nucleoside monophosphate analogue). Compared to nucleoside analogues, it does not require an initial phosphorylation reaction, which is often rate limiting, to be converted to the active metabolite: tenofovir diphosphate (PMPApp). PMPApp efficiently inhibits both RNA- and DNA-directed HIV-1 reverse transcriptase (RT) activities by direct binding competition with the natural deoxyribonucleotide substance and, after incorporation into DNA, by premature termination of DNA synthesis.

Intracellular constitutively expressed enzymes convert tenofovir to PMPApp through two phosphorylation reactions. This conversion occurs both in activated cells as well as in non proliferating lymphocytes and macrophages.

In resting human peripheral blood mononuclear cells (PBMCs), the half-life of PMPApp was approximately 50 hours, whereas the half-life in activated PBMCs was approximately 10 hours. Tenofovir diphosphate is a weak inhibitor of cellular polymerases α, β, and γ, with kinetic inhibition constants (K_i) that are more than 200-fold higher against human DNA polymerase α (5.2 µmol) and more than 3,000-fold higher against human DNA polymerase β and γ (81.7 and 59.5 µmol, respectively) than its K_i against HIV-1 RT (0.02 µmol).

In vitro antiviral activity

The *in vitro* antiviral activity and cytotoxicity of tenofovir and tenofovir DF were evaluated in different cell types.

The concentration of tenofovir required for 50 % inhibition (IC_{50}) of wild-type HIV_{IIIB} is 1-6 µmol in MT-2 or MT-4 cells (based on inhibition of viral cytopathic effect) and 0.2-0.4 µmol in PBMCs (based on inhibition of virus production). Tenofovir had an IC_{50} of 0.04 µmol against $HIV\text{-}1_{BaL}$ in primary monocyte/macrophage cells. The mean tenofovir IC_{50} was 0.9 µmol against a panel of 10 wild type clinical isolates. Due to its increased cellular permeability, the anti-HIV activity of tenofovir DF was increased by 17 to 90-fold over that of tenofovir.
The mean IC_{50} of tenofovir against HIV-1 subtypes A, C, D, E, F, G, and O in PBMCs were within two-fold of subtype B (0.55 to 2.2 µmol).
In different cell types, tenofovir exhibited no or low cytotoxicity. For instance, in PBMCs and MT-2 cells, CC_{50} values of tenofovir were above 1 µmol. The selectivity index for tenofovir was therefore superior to 2,000 ($SI = CC_{50}/IC_{50}$).
Tenofovir was active against HIV-2 *in vitro*, with a potency similar to the one against HIV-1. *In vitro*, it has also an antiviral activity against a broad spectrum of retroviruses and hepadnaviruses.

Tenofovir showed *in vitro* minor to moderate synergy with didanosine and nelfinavir, but a strong synergy with zidovudine, amprenavir and all non-nucleoside reverse transcriptase inhibitors tested. The other combinations were additive, and no significant antiviral antagonism was observed.

As nucleoside analogues are associated with mitochondrial toxicity and production of lactic acidosis, the potential effects of tenofovir were evaluated *in vitro*. At concentration up to 300 µmol, tenofovir did not have any effect on the synthesis of mitochondrial DNA nor on the production of lactic acidosis

but additional *in vivo* studies are ongoing to confirm these findings, the results of which will be provided as specific obligations to be fulfilled post-authorisation.

In vivo antiviral activity

The antiviral activity of tenofovir was confirmed in *in vivo* animal models, including SIV infected macaques.

Resistance

The K65R mutation in RT was obtained *in vitro* with successive passage of HIV-1 in increasing concentrations of tenofovir. The K65R mutation, which is also selected by zalcitabine, didanosine and abacavir, was associated with a limited phenotypic resistance to tenofovir (3 to 4-fold reduced susceptibility). Tenofovir remained active (IC_{50} within 2 fold of the wild type) against recombinant mutant molecular clones of HIV-1 expressing didanosine (L74V), zalcitabine (T69D), zidovudine (D67N + K70R or T215Y), multinucleoside (Q151M) and abacavir/lamivudine (M184V) resistance.

The susceptibility results with molecular clones of HIV-1 were confirmed and extended with phenotypic analyses of a panel of recombinant HIV-1 clinical isolates from antiretroviral experienced patients. Clinical HIV isolates expressing MI84V mutation alone showed mild reduced susceptibility to tenofovir (0.7-fold). Viral clones carrying both zidovudine-associated mutations and M184V mutation were more susceptible to tenofovir as compared to the ones carrying zidovudine-associated mutations (fold of resistance ranging from 0.9-3.7 fold versus 0.9-8.4 fold respectively). The susceptibility to tenofovir of recombinant HIV-1 isolates containing the 69 insert was 5.6 to 34.9-fold increased (>10-fold for five isolates), which demonstrates the high resistance of these viruses to tenofovir. On the other hand, the susceptibility to tenofovir of isolates resistant to lamivudine and expressing the mutation Q151M complex ranged from 0.6 to 3.3-fold increases, which suggests that the susceptibility for multi-compounds resistant viruses was maintained. Cross-resistance is unlikely with non-nucleoside reverse transcriptase inhibitors, as they bind to a structurally distinct portion of RT; the same for protease inhibitors, due to the different enzyme target.

General and safety pharmacology programme

Studies did not reveal any adverse effects on the central nervous system of rats dosed at 500 mg/kg or on the cardiovascular system of dogs dosed at 30 mg/kg. In rats receiving doses of 500 mg/kg, there was evidence of renal effects, suggesting that kidney may be the target organ for toxicity of tenofovir DF. Overall, there was no evidence that tenofovir had any significant effect on any of the major organ systems at doses exceeding the proposed therapeutic dose, which is equivalent to 6 mg/kg/day based on a 50 kg person.

Pharmacokinetics

The pharmacokinetic profile of tenofovir and tenofovir DF was determined, using validated testing methods, in several species (mice, rats, dogs and monkeys) following oral administration. Studies were conducted with tenofovir free base and tenofovir disoproxil in both the citrate and fumarate salt forms. All experiments with the citrate salt used solutions, thereby assuring comparability to experiments in which the fumarate salt was employed (the citrate and fumarate salts are completely dissociated in solution).

Absorption and distribution

Following oral administration, the absorption of tenofovir DF was rapid with a maximum tenofovir plasma concentration obtained within 0.25 to 1.5 hours post dose in all species and declined in a biphasic manner. The observed terminal half-life values were approximately 7, 9 and 60 hours in rats, monkeys and dogs respectively.

The bioavailability of tenofovir DF was moderate and dependent of the species, ranging between 10 and 40 % (10-20 % in rodents and 30-40 % in dogs and monkeys). It depended also upon the

co-administration of food, as food increased the absorption. The bioavailability seemed partially dose-dependent as it decreased with increasing doses.

Doses less than 100 mg/kg exhibited linear pharmacokinetics. In monkeys receiving 250 mg/kg, Cmax and AUC were less than dose proportional, suggesting decreased absorption, whereas in dogs, values appeared to be linear with dose. Following repeated oral administration, no accumulation was observed except in dogs for which a 2-3 fold increase in both Cmax and AUC occurred after 28 days of treatment.

The volume of distribution was high in all species (more than 1.0 l/kg), suggesting that tenofovir distributes widely. The highest concentrations were present in bile, kidney and liver of dogs. Tenofovir did not appear to cross the brain barrier but crossed the placenta in monkeys.

The protein binding was not evaluated in animals, but was found to be low in human plasma.

Metabolism and elimination

Tenofovir DF was rapidly converted into tenofovir in plasma and liver, much more slowly in intestine. Tenofovir was metabolised intracellularly to tenofovir diphosphate with a $T_{1/2}$ over 50 hours in monkeys PBMCs. *In vitro*, tenofovir disoproxil, tenofovir soproxil and tenofovir were detected, with tenofovir soproxil being the major metabolite observed intracellular.
Following oral administration of tenofovir DF in rats and dogs, tenofovir disoproxil was metabolised by non specific esterases to tenofovir. No other metabolites than tenofovir and tenofovir soproxil were detected. *In vitro*, tenofovir DF did not have any inhibiting or inducing effect on CYP3A4, 2D6, 2C9, 2E1 and 1A2. However it induced CYP 1A1 and 2B.

In all species, the primary route of elimination was renal, mainly as unchanged substance. Following intravenous injection of ^{14}C-tenofovir in dogs and rats, more than 90 % of the administered dose was recovered in urine by 72 hours post dose, primarily as the unchanged substance. Renal clearance values in rats were 2-4 times the rate of the creatinine clearance (1580 ml/hr/kg versus 314 ml/hr/kg), suggesting the involvement of both glomerular filtration and tubular secretion in the excretion of tenofovir. The tubular secretion was particularly important in monkeys as renal clearance was 4-fold higher than creatinine clearance.
Following oral administration of ^{14}C-tenofovir in dogs, the amount of the dose recovered in urine was approximately 25 % to 28 % at 24 hours post dose; 15 % to 20 % of the dose remained in tissues. These values were close to the total amount of dose absorbed.
Excretion into milk was moderate. Indeed in rats receiving 600 mg/kg/day, tenofovir concentration in milk represented less than 23.5 % of the corresponding tenofovir plasma concentration.

Toxicology

A conventional toxicological programme using tenofovir disoproxil fumarate was carried out in rats, mice, rabbits and rhesus monkeys. Based on the pharmacokinetic data, these species were considered relevant models for toxicology studies, since all the animals achieved adequate exposure to the active compound. Tenofovir and tenofovir DF were administered orally, which is the intended clinical route of administration. Most of the studies have been conducted according to Good Laboratory Practices. Toxicokinetic studies showed that exposure achieved in animals resulted in safety factors between 2 and 10 for a specific target organ according to species evaluated.

Single dose toxicity
The acute toxicity of tenofovir DF was evaluated at doses up to 1500 mg/kg in rats and up to 270 mg/kg in dogs. There were no deaths or clinical abnormalities recorded in rats. In dogs, treatment-related lesions in the kidneys were observed with doses equivalent to 90 and 270 mg/kg. These were characterised by tubular karyomegaly and/or basophilia.

Repeated dose toxicity

Repeated dose toxicity studies were conducted in mice (13 weeks), rats (up to 42 weeks) and dogs (up to 42 weeks). In long-term studies, the highest doses of tenofovir DF administered were 1000 mg/kg in rats and 30 mg/kg in dogs.

In rats, the target organs were the gastro-intestinal tract (intestinal epithelial hypertrophy/slight to moderate intestinal hyperplasia) and kidney (renal tubular epithelial karyomegaly and pigment accumulation at dose of 30 mg/kg/day). It was suggested that gastro-intestinal lesions could be rodent specific occurring at the highest doses as no signs were reported in either monkeys or in dogs. It was agreed however that this finding should be further investigated. Changes in serum chemistry parameters, including dose-related slight to moderate changes in cholesterol, triglycerides, ALT, AST and creatinine were also reported in the 300 mg dose group. All these effects were reversible after treatment discontinuation. The no-observed effect-level (NOEL) determined was less than 30 mg/kg, and therefore no overall safety margins could be established.

In dogs, the main target organ was the kidney. Dose-related histopathological changes included tubular dilatation and interstitial nephritis reported in the 10 and/or 30 mg/kg/day dose groups and slight to moderate renal tubular karyomegaly in all treated groups. Proteinuria, glycosuria, creatinine augmentation and increased urine volume were observed in the 30 mg/kg/day dose group throughout the study. NOAEL was less than 3 mg/kg and no overall safety margin could be established.

A concern was raised as tenofovir DF was found to be nephrotoxic at high doses in all species. The signs reported were specific to a decreased glomerular filtration rate as well as proximal tubular impairment. It was shown that tenofovir is transported into tubular cells by the human organic anion transporter 1 (hOAT1), as cidofovir. Cytotoxicity studies were performed to assess the effect of tenofovir in several *in vitro* models for renal proximal tubular toxicity compared to other nucleotide analogues (cidofovir and adefovir). Results suggested a less marked nephrotoxicity profile of tenofovir, probably due to a lower interference of tenofovir with essential intracellular functions. Another study revealed that tenofovir showed negligible direct cell toxicity in normal human renal proximal tubule epithelial cells.

Decreases in bone mineral content and bone mineral density, consistent with increased bone resorption were also reported in dogs (30 mg/kg/day) and rats (1000 mg/kg/day). This was further supported by findings of hypercalciuria and hyperphosphaturia, loss of bone mineral content and bone density. Bone changes did not seem to completely reverse during the recovery period. Bone mineral loss resulting in pathologic osteomalacia was also observed in juvenile monkeys treated with 30 mg/kg s.c., after more than 4 months of treatment. To further explore the bone toxicity mechanism and its relation with renal toxicity, specific *in vitro* and *in vivo* studies were conducted. *In vitro*, tenofovir DF was not cytotoxic to osteoblast-like cells. *In vivo*, hypophosphataemia appeared to be caused by deteriorated intestinal phosphate absorption or an impaired renal phosphate reabsorption. The effects of tenofovir DF on phosphate homeostasis in rhesus macaques were investigated in a 56-day study, the results of which were provided as a specific obligation post-authorisation. Renal toxicity did not seem therefore to be directly associated with the effect on bone. Phosphate supplementation given in rats and monkeys appeared to overcome the impairment, resulting in increased urinary phosphorus excretion, but the benefit of phosphate supplementation has not been evaluated in clinical studies.

Reproduction toxicity

Tenofovir DF did not affect fertility and general reproductive performance of the male and female rats at doses up to 600 mg/kg. Tenofovir DF was neither embryotoxic nor teratogenic in rats (doses up to 450 mg/kg/day) and in rabbits (doses up to 300 mg/kg/day). The NOEL for maternal effects was 150 mg/kg/day in rats and 100 mg in rabbits respectively. In peri- and post natal toxicity study in rats, tenofovir DF significantly reduced pups survival and animal weights. The viability index in rats was reduced in the 450 mg/kg/dose group and significantly reduced in 600 mg/kg/day. Pup weights were also decreased in these groups and there was a slight delay of sexual maturation that did not affect reproductive performance. The non effect dose was 150 mg/kg. In a non GLP study in rhesus macaques, there was evidence of reduced birth weight and several bone lengths at a dose of

30 mg/kg/day, that correspond to 25-times the human exposure. Based on these results, tenofovir DF should be used in pregnant women only if the potential benefit outweighs the potential risks to the foetus as recommended in the Summary of Product Characteristics.

Mutagenicity

The mutagenic potential of tenofovir DF was evaluated in a standard battery of *in vitro* and *in vivo* tests. Tenofovir DF was positive in the *in vitro* mouse lymphoma assay, equivocal in the Ames tests (positive in one assay using the strain of *Salmonella typhimurium* TA 1535 with or without activation). Tenofovir DF was negative in the *in vivo* micronucleus assay in mouse (doses up to 2000 mg/kg orally). To clarify these findings and complete the information on the genotoxicity of tenofovir DF, additional *in vitro* and *in vivo* UDS test on rats hepatic cells have been performed in order to assess the ability of tenofovir DF (or metabolites) to cause DNA damage by measuring UDS induced in vivo in primary hepatocytes cultured in vitro. The tenofovir DF was considered as weakly positive in this assay, showing that tenofovir can induce DNA damage and supporting the previous mutagenic potential results. These results will have to be considered in the light of the final assessment of the rodent carcinogenicity studies.

Carcinogenicity

The carcinogenic potential of tenofovir DF has been evaluated in rats and in mice. Long-term carcinogenicity studies were ongoing at the time of the original CPMP Opinion. Preliminary results (12 months and 22 months data in mice and rats) did not raise any particular concerns since no external gross evidence of carcinogenicity was reported. The final results were submitted as part of specific obligations and were fulfilled post-authorisation. In these studies, animals were administered tenofovir DF once daily by oral gavage for 2 years. The high doses in these studies were selected based on the endpoint of gastrointestinal/kidney/bone toxicity in the rat (0, 30, 100, 300 mg/kg) and on the maximum concentration that could be reliably delivered in mice (0, 100, 300, 600 mg/kg).

Final report on the 104-week oral carcinogenicity study in rats:

Lifetime daily administration of tenofovir DF at dosage levels of up to 300 mg/kg/day to Sprague-Dawley rats did not reveal major concerns in carcinogenicity. Nevertheless, an increased incidence of the sub-cutaneous tissue/lipoma in males and uterus polyp/endometrial stroma in females have been observed. In the light of historical control data, it was concluded that the 5% incidence of sub-cutaneous tissue/lipoma in male could be considered incidental in origin. Furthermore, based on the fact that the increase in uterus polyp/endometrial stroma in females was minimal compared to reported values and that there was no evidence of any treatment-related effect (non-neoplastic and neoplastic) on the reproductive organs (uterus, ovaries and vagina), the increase in incidence of the benign tumour could be considered to be of no toxicological significance.

Final report on the 104-week oral carcinogenicity study in mice:

CD-1 mice were treated for 104 weeks with tenofovir DF and were exposed to the maximal possible dose, since over 600 mg/kg, gastro-intestinal tract toxicity lead to the death of the animals. However at this dosage, body weight gain was not significantly reduced and survival was comparable among the different control and treated groups.

- Duodenal neoplasms have been observed in few animals receiving very high dose of tenofovir DF (600 mg/kg in mice *versus* 4-5 mg/kg in humans). These are probably the consequence of hyperplasia, resulting from irritation, in response to the formaldehyde released from tenofovir DF. The high dose and the small area exposed in mice are conditions that will not occur in humans. Furthermore, it should be pointed out that mouse adenocarcinomas have not been observed in rats, despite the observation of hyperplasia in this species. Altogether, the duodenal hyperplasia and low incidence of duodenal tumours observed at the highest dose in the mouse carcinogenicity study are not considered to present a significant carcinogenic risk for humans.
- A statistically significant increase of hepatocellular adenoma has been observed in female animals in the high dose group relative to controls. This is probably in relation to the treatment in light of the non-neoplastic hepatocellular lesions observed. However, this observation was present in only one sex and one species and no carcinomas were observed, suggesting that this finding does not seem to represent a risk for humans.

In conclusion, given the results from the rat carcinogenicity assay, which reveal no major concerns, the fact that tenofovir DF is positive only at the highest dose tested (600 mg/kg/day) in the mouse carcinogenicity study, the margin of safety and the likelihood that the observed tumours could result from local high concentrations of tenofovir DF, it can be considered that there are no significant concerns, regarding the carcinogenic potential of tenofovir DF in patients.

Local tolerance
Tenofovir DF was a severe irritant to the ocular tissue and a slight irritant for the skin. By contrast tenofovir was not a contact sensitizer in guinea pigs.

Impurities
All the impurities of synthesis or degradation origin were present in material tested in toxicology studies and therefore were toxicologically qualified.

Environmental risk assessment
The predicted environment concentration value was below the defined threshold of 0.01 µg/l, suggesting that it is unlikely to pose a risk to the environment at the proposed dose recommendation.

Other
As tenofovir DF is an ester prodrug, a concern was raised with respect to the potential release of two molecules of formaldehyde, a known toxic compound, for each molecule of tenofovir generated upon hydrolysis. The distribution of formaldehyde in the body is currently unknown. The applicant, however, estimated a high safety margin between the maximum dose that could be absorbed in patients (0.6 mg/kg for 60 weighted patient) and the NOAEL for chronic toxicity and carcinogenicity of formaldehyde based on published literature.

4. Part IV: Clinical aspects

The clinical programme aimed to investigate the antiviral activity of tenofovir DF either as monotherapy or in combination with other antiretroviral agents in antiretroviral naïve and experienced HIV-1 infected adult patients.
The initial application for the marketing authorisation focused however only on clinical data to support the use of tenofovir DF, in combination with other antiretroviral medicinal products, in multi-experienced HIV-1 infected adult patients who expressed multi-resistance at baseline.
Post authorisation, as part of the clinical programme, the Marketing Authorisation Holder has submitted the results of a double blind pivotal study in antiretroviral naïve patients.
All the clinical trials were performed according to the Good Clinical Practices and agreed international ethical principles. An overview of the clinical programme is presented in table 1.

Table 1: Overview of the clinical programme

Study	Type of Study	Doses	Duration of Treatment	No. of Patients Randomised	Study Design
Clinical Pharmacology					
701	Pharmacokinetic, efficacy and safety study in treatment experienced and naïve patients	1 mg/kg, 3 mg/kg (IV)	8 days	Tenofovir: 1mg/kg =8 3mg/kg =8 Placebo =4	Randomised, double-blind placebo-controlled
909	Drug interaction study in healthy volunteers	300 mg	Multiple dose	TDF 300 mg=103	Randomised, open-label, multiple dose
914	Bioequivalence and food effect study in healthy volunteers	300 mg	Single dose	TDF 300 mg=40	Randomised, open-label, 3-period
Dose Evaluation					
901	Pharmacokinetic, efficacy and safety study in treatment experienced and naïve patients	75, 150, 300, 600 mg	28 days	TDF 75mg =20 TDF 150 mg =8 TDF 300 mg =8 TDF 600 mg=10 Placebo =13	Randomised, double-blind, multicentre, placebo-controlled with extension phase
Clinical Efficacy and Safety					
902	Intensification study in treatment experienced HIV-infected patients	75, 150 300 mg	48 weeks, + extended dosing	TDF 75mg =54 TDF 150 mg =51 TDF 300 mg =56 Placebo =28	Randomised, double-blind, multicentre, placebo-controlled with extension phase
907	Intensification study in treatment experienced HIV-infected patients	300 mg	48 weeks	TDF 300mg =368 Placebo =184	Randomised, double-blind, multicentre, placebo-controlled
908	Open-label safety in HIV-infected patients with advanced disease	300 mg	Up to 96 weeks	TDF300 mg =296	Open-label, multicentre, extended dosing study
Dose Adjustment in specific populations - *Post-Marketing Authorisation programme*					
919	Pharmacokinetic in subjects with normal renal function, or with varying degrees of renal impairment, including subjects undergoing haemodialysis.	300 mg	Single dose	TDF 300 mg =41	Open-label, parallel-group study
Clinical Efficacy and Safety - *Post-Marketing Authorisation programme*					
903	Comparative study: TDF + 3TC + EFV *versus* d4T + 3TC + EFV in treatment naive HIV-infected patients	300 mg	144 weeks	602 (ratio 1:1)	Randomised, double-blind, parallel, multicentre, placebo-controlled

To support the initial authorisation, approximately 1,050 HIV-infected patients received tenofovir disoproxil fumarate alone (study 901) or in combination with other antiretroviral agents (studies 902, 907 and 908).

The current approved indication is in combination with other antiretroviral agents in HIV infected patients over 18 years of age.

Clinical Pharmacology

Antiviral activity

Tenofovir DF is a nucleotide analogue, which had an antiretroviral activity demonstrated *in vitro* and *in vivo* in animal models as presented in the Pharmaco-toxicological part of this document.

Results from early clinical studies support the potential clinical benefit of tenofovir DF monotherapy in suppressing plasma HIV RNA levels.
In study 701, short term daily intravenous administration of tenofovir DF at doses of 1 mg/kg and 3 mg/kg in HIV-1 infected patients, who were either treatment naïve or experienced, resulted in a reduction of HIV RNA levels by 0.6 and 1.1 $\log_{10}$ copies/ml at day 14, respectively. The viral suppression was maintained for up to one week following treatment discontinuation (-0.20 and -1.07 $\log_{10}$ copies/ml respectively). There were no statistically significant differences between tenofovir DF and placebo groups with respect to CD4 cell counts.
A significant decrease compared to placebo was also demonstrated in the early dose ranging study 901, which will be further detailed under the section dose ranging studies.

Resistance profile

As described in the Pharmaco-toxicological part of this document, *in vitro* resistance data suggested that tenofovir DF presents a favourable resistance profile. To further characterise the genotypic and phenotypic profile of tenofovir, resistance data were collected from the main clinical studies: 902 (virology ITT population of 184 patients), 907 (virology ITT population of 253 patients) and 903 (virology AT population of 37 patients).

- Genotypic analysis

Study 902 and 907

In both studies, the distribution of resistance mutations at baseline was similar across treatment groups and was consistent with the extensive treatment experience of the patients (see table 2).

Table 2: Baseline genotypic analysis

	Study 902	**Study 907**
1 or more primary nucleoside-associated resistance mutations in RT	94 %	94 %
1 or more PI-associated resistance mutation in RT	57 %	58 %
1 or more primary NNRTI-associated resistance mutations in RT	32 %	48 %
Lamivudine/abacavir associated M184V/I mutations	66 %	68 %
Lamivudine/abacavir associated M184V/I mutations + typical ZDV associated resistance mutations (mean of 2.8 mutations)	47 %	44 %

In addition, 1 patient in study 902 and 5 in study 907, all of them from the tenofovir arms expressed the K65R RT mutation (mutation associated with zalcitabine, didanosine and abacavir in vivo and also selected by tenofovir DF in vitro) at baseline.

HIV-RNA response by baseline resistance mutation

In study 902, for patients receiving tenofovir DF in addition to their existing regimen, a comparable decline in HIV-RNA was observed by week 48 whatever the genotype measured at baseline (- 0.62 $\log_{10}$ copies/ml $DAVG_{48}$). In particular, a comparable virologic response was observed between patients resistant to or susceptible to zidovudine (-0.57 $\log_{10}$ copies/ml versus -0.61 $\log_{10}$ copies/ml, $DAVG_{48,}$ respectively).

In study 907, patients in the virological substudy receiving tenofovir DF in addition to their existing regimen demonstrated a statistically significant mean decrease in HIV-RNA level by week 24 (-0.59 $\log_{10}$ $DAVG_{24}$ *versus* - 0.03 $\log_{10}$ copies/ml, $p < 0.0001$) and by week 48 (-0.56 $\log_{10}$ $DAVG_{24}$

versus - 0.7 log_{10} copies/ml, p <0.0001) compared to placebo, despite the presence of extensive RT resistance mutation at baseline. The highest reduction in viral load was observed in patients without zidovudine resistance but with the M184V mutation among all genotypic groups at week 24 (-0.97 log_{10} $DAVG_{24}$, p <0.0001) and at week 48 (-0.90 log_{10} $DAVG_{48}$, p <0.0001). The difference with other groups was not statistically relevant. Patients with K65R mutation at baseline did not respond to tenofovir DF (+ 0.12 log_{10} mean $DAVG_{24}$). Treatment with tenofovir DF resulted in infrequent development of resistance to tenofovir, as only 8/274 patients (3%) developed the K65R mutation by week 48.

Study 903

Patients with virologic rebound or suboptimal therapy were analyzed for the development of resistance to tenofovir and all other RT inhibitors (As treated population). Distribution of patients with HIV expressing NRTI- and NNRTI-Associated Resistance Mutations at Virologic Failure (n = 37, As Treated Population) is shown in table 3:

Table 3: NRTI- and NNRTI-Associated Resistance Mutations at Virologic Failure (n = 37, AT)

	TDF+3TC+EFV (N = 299)		d4T+3TC+EFV (N = 301)		
	n	**%**	**n**	**%**	p-Value[a]
Patient Samples Analyzed	22	7.4%	15	5.0%	0.23
Any NNRTI Resistance Mutation[b]	14	4.7%	10	3.3%	0.41
K103N	9	3.0%	6	2.0%	0.44
M184V/I	8	2.7%	8	2.7%	1.00
Other NRTI Resistance[c] (non M184V/I)	9	3.0%	3	1.0%	0.08
K65R	7	2.3%	2	0.7%	0.10
No Resistance Mutations Detected	5	1.7%	4	1.3%	0.75

a - Fisher exact test.
b - NNRTI resistance mutations = L100I, K103N, V106A/M, V108I, Y181C/I, Y188C/L/H, or G190A/S/E/Q in RT.
c - NRTI resistance mutations = M41L, A62V, K65R, D67N, T69D/N, K70R, L74V/I, V75T, F77L, Y115F, F116Y, Q151M, M184V, L210W, T215Y/F, or K219Q/E/N in RT.

Four percent of patients analysed had HIV expressing resistance mutations to the NNRTI class without significant difference between treatment groups (4.7% versus 3.3 % in tenofovir DF group versus d4T group respectively p=0.41). Among NRTI-associated resistance mutations, M184V/I occurred in 2.7% of patients with no difference between treatment groups. The most common other NRTI-associated mutation was the K65R mutation, occurring more frequently in the tenofovir DF group (2.3%) compared with the active control group (0.7%).

The potential development of reverse transcriptase mutations (non K65R) selected by tenofovir in naïve patients was of particular interest. Of note, 5 patients over the 22 who failed with the tenofovir DF containing regimen displayed NRTI-associated resistance mutations (non M184V/I and non K65R). One patient had a K219Q mutation that was detected at baseline. Some substitutions occurred in positions already identified but with amino-acids changes different from those known to induce genotypic resistance (D67G+K70E+V75L, K219R). Two patients had additionally developed an A62V mutation and one of these patients also developed a Y115F mutation. The impact on HIV resistance for the additional development of these RT mutations is unknown and needs to be further investigated by the Marketing Authorisation Holder.

- Phenotypic analysis

Study 902 and 907

The influence of the phenotype at baseline on the virological response at week 24 was also assessed in both studies using Virco Antivirogram TM assay (n = 54 in study 902 and n = 85 in study 907). In particular, the impact of zidovudine-resistant HIV at baseline in the virological response was assessed. Results are displayed in table 4.

Table 4: Virological Response measured by mean $DAVG_{24}$ log_{10} copies/ml to tenofovir DF by baseline tenofovir and zidovudine susceptibility

	Baseline tenofovir susceptibility fold change from wild type		**Baseline zidovudine susceptibility fold change from wild type**		
	< 4-fold	> 4-fold	< 4-fold	> 4-fold	> 10-fold
Study 902	-0.55	-0.17	- 0.73	-0.43	
Study 907	-0.27	-0.08	-0.72	-0.39	- 0.17

A correlation between the tenofovir susceptibility at baseline and the virological response to 300 mg tenofovir DF was demonstrated in both studies. The small number of patients with baseline tenofovir susceptibility of 3 and 4 fold precludes defining a potential clinical breakpoint for tenofovir. In study 907, tenofovir DF patients with baseline tenofovir susceptibility within 3-fold of wild-type responded with -0.42 to -0.72 log10 decreases in HIV RNA through week 24 and maintained a similar response through week 48.
The more zidovudine-associated mutations (also know as thymidine-analogue-associated mutations or TAM) were present at baseline, the less was the virological response to tenofovir DF. However, even with more than 4-fold zidovudine-associated mutations, a mean 3-fold reduced susceptibility to tenofovir was reported in both studies.

An integrated analysis of the effects of pre-existing baseline ZDV resistance mutations has been performed for both studies (n = 80 in study 902 and n = 253 in study 907). The ZDV mutations included in this analysis were M41L, D67N, K70R, L210W, T215Y/F and K219Q/E/N. Patients with HIV expressing the D67N, K70R or K219Q/E/N mutations responded to tenofovir DF 300 mg similarly to those patients without these mutations. In contrast, patients with HIV expressing the M41L, L210W or T215Y/F mutations showed diminished responses, although all of these responses were statistically significant when compared to placebo patients with the same mutations (Table 5). Upon further analysis, only the M41L and L210W mutations were uniquely associated with reduced responses as patients expressing T215Y/F in the absence of M41L or L210W had a mean HIV RNA response of -0.70 log10 copies/ml (n=25).

Table 5: HIV RNA responses by baseline TAM [1] in Studies 902 and 907 (ITT)

Baseline Mutations	**Change in HIV RNA [2] (n)**		**Net Treatment**	**Effect [3] P-Value [4]**
	TDF 300 mg	**Placebo**		
D67N	-0.53 (79)	-0.02 (32)	-0.51	<0.0001
No D67N	-0.62 (143)	-0.04 (52)	-0.58	<0.0001
K70R	-0.71 (67)	-0.0 (32)	-0.71	<0.0001
No K70R	-0.54 (155)	-0.05 (52)	-0.49	<0.0001
K219Q	-0.60 (57)	0.0 (19)	-0.60	<0.0001
No K219Q	-0.58 (165)	-0.04 (65)	-0.55	<0.0001
M41L	-0.26 (81)	+0.01 (32)	-0.27	<0.0001
No M41L	-0.78 (141)	-0.05 (52)	-0.73	<0.0001
L210W	-0.17 (46)	+0.04 (15)	-0.21	0.0254
No L210W	-0.70 (176)	-0.04 (69)	-0.66	<0.0001
T215Y/F	-0.35 (106)	+0.05 (39)	-0.40	<0.0001
No T215Y/F	-0.80 (116)	-0.09 (45)	-0.71	<0.0001
T215Y/F No M41L or L210W	-0.70 (25)	+0.22 (8)	-0.92	ND

1 TAMs are M41L, D67N, K70R, L210W, T215Y/F or K219Q/E/N in RT.
2 Average HIV RNA change from baseline through week 24 (DAVG24) in log10 copies/ml.
3 Difference between DAVG24 values of TDF- versus placebo-treated patients.
4 Wilcoxon rank sum test comparing TDF- and placebo-treated patients.

The effect of the number of baseline ZDV mutations was also analysed. Patients with HIV expressing no ZDV mutations or just 1 or 2 mutations had mean HIV RNA responses of greater than -0.66 log10 copies/ml (Table 6). It was confirmed that HIV RNA responses to tenofovir DF were diminished in the presence of >3 of the defined ZDV mutations. However, a specific effect of the M41L or L210W mutations was also observed among these patients.

In the absence of either of these mutations, patients with HIV expressing >3 ZDV mutations had an HIV RNA response of -0.67 log10 copies/ml (Table 6). In the presence of M41L or L210W, responses in patients with >3 ZDV mutations were diminished (-0.21 log10 copies/ml) but were still statistically superior to placebo patients with the same mutations (p=0.0126). Thus, it appears that the magnitude of the response to tenofovir DF therapy depends on both the number and specific type of ZDV mutations present at baseline.

Table 6: HIV RNA responses by Type and Number of Baseline TAMs [1] in Studies 902 and 907 (ITT)

Baseline Mutations	**Change in HIV RNA [2] (n)**		**Net Treatment Effect [3]**	**P-Value [4]**
	TDF 300 mg	**Placebo**		
No TAMs	-0.80 (68)	-0.11 (29)	-0.69	< 0.0001
Any TAM	-0.50 (154)	(81)	-0.50	< 0.0001
1-2 TAMs	-0.66 (55)	-0.04 (33)	-0.62	< 0.0001
≥3 TAMs including M41L or L210W	-0.21 (57)	+0.01 (29)	-0.22	0.0126
≥3 TAMs without M41L or L210W	-0.67 (42)	+0.07 (19)	-0.74	< 0.0001

1 TAMs are M41L, D67N, K70R, L210W, T215Y/F or K219Q/E/N in RT.
2 Average HIV RNA change from baseline through week 24 (DAV_{G24}) in log_{10} copies/ml.
3 Difference between DAV_{G24} values of VIREAD- versus placebo-treated patients.
4 Wilcoxon rank sum test comparing TDF- and placebo-treated patients.

In study 907, patients with K65R RT mutation at baseline, did not respond to tenofovir DF therapy (+0.12 log10 mean $DAVG_{24}$).

In studies 902 and 907, patients with HIV expressing the M184V mutation showed stronger responses to tenofovir DF than patients without M184V with $DAVG_{24}$ values of -0.65 versus -0.48 log_{10} copies/ml and -0.64 versus -0.40 log_{10} copies/ml respectively. However, when the net effect from the placebo group was subtracted and other RT mutations considered, results were not statistically significantly different. Phenotypic analysis showed an approximate 2-fold increase in tenofovir susceptibility associated with the M184V mutation among all analysed patients or among those patients with baseline zidovudine resistance mutations. Although suggestive, these data do not firmly demonstrate hypersusceptibility to tenofovir of viral strains harbouring the 184 mutation.

Development of nucleoside associated RT mutations

The analysis of development of resistance to NRTIs in tenofovir-treated patients led to different results between studies 902 and 907.
In the post-baseline genotypic analysis of the virology substudy 902, a trend to a higher rate of mutations in the RT gene was observed in patients treated with tenofovir. Overall, 26 % and 43 % of patients in the tenofovir group developed RT mutations at weeks 24 and 48 respectively as compared to 14 % of patients developing RT mutations in the placebo group at week 24. This proportion was particularly high in the 150 mg treatment arm (37 % and 57 % of patients at 24 and 48 weeks respectively). The majority of the patients (63 of 79) developed typical zidovudine-associated mutations while taking either zidovudine, stavudine, or abacavir concomitantly. By contrast the post-baseline genotypic data of study 907, revealed that, at week 24, fewer patients in the tenofovir DF treatment arm developed nucleoside-associated RT mutations compared to the placebo arm though this difference was not statistically significant (15 % versus 22 %, respectively). Overall data showed a statistically significant mean HIV RNA decrease of - 0.41 log_{10} copies/ml at week 24, suggesting that tenofovir DF still had an anti-HIV activity despite the development of these mutations. Phenotypic analysis showed that in those patients that developed new mutations to NRTI at 24 week of treatment, mean fold change in tenofovir susceptibility (as compared to basal susceptibility) was 2.5 fold in the tenofovir arm and 1.2 fold in the placebo group. This change is just at the limit of what is considered a threshold of low resistance level (> 2.5-3 fold) that represents the inter-assay variation of the phenotypic test.

Selection of HIV-1 virus with reduced susceptibility to tenofovir

The development of primary resistance mutations to tenofovir was infrequent and only 4 patients in study 902 and 5 in study 907 developed K65R mutation. This mutation was associated with low phenotypic resistance (2.8 to 3.9-fold increased IC_{50} of the wild type HIV-1). Among the 5 patients with K65R mutation in study 907, there was a high variation in response to tenofovir DF therapy (- 1.10 to + 0.72 $\log_{10}$ copies/ml).
Owing to the limited number of patients as well as the co-administered regimen therapies, the impact of the K65R mutation is currently unknown.
Limited data showed that reduction in tenofovir susceptibility during tenofovir treatment was generally not associated with viral rebound (4 in 12 patients who had > 2.5 fold change in tenofovir susceptibility) and if any, the rebound was not associated with genetic changes.

Study 903

Table 7: Phenotypic Susceptibility to NRTIs at Virologic Failure (n=37, A T Population)

	Mean Fold Change from Wild-Type Control						
	TFV	d4T	ZDV	3TC	ddI	ddC	ABC
Tenofovir DF Group (n=22)	1.7	0.9	0.8	>22	2.5	1.9	1.9
With K65R (n=7)	**3.7**	1.4	0.9	>44	5.4	4.2	4.1
Without K65R (n=15)	0.7	0.7	0.7	>11	1.1	0.8	0.9
With M184V (n=4)	3.6	1.4	0.8	>56	7.5	4.6	4.4
Without M184V (n=3)	3.8	1.4	1.0	29	2.6	3.7	3.7
Active Control Group (n=15)	0.9	1.5	1.0	>21	2.2	1.2	1.6
With K65R (n=2)	1.8	3.5	0.9	>19	4.4	1.8	2.8
Without K65R (n=13)	0.8	1.1	1.0	>21	1.8	1.2	1.5

The K65R mutation was associated with a mean 3.7-fold reduction in susceptibility to tenofovir. This was the only mutation identified that was associated with decreased susceptibility to tenofovir. The virologic failure in patients treated with TDV/3TC/EFV regimen was mainly due to NNRTI-associated or 3TC resistance mutations.

At this stage, considering the limited number of patients who develop the K65R mutation (n=7) and that phenotypic resistance does not correlate with virological response, the CPMP could not draw any conclusion.
Clarifications on the relation of genotypic and phenotypic resistance specifically in terms of the K65R mutation on the predictability of clinical efficacy of second line treatment in case of virological failure is to be provided and analysed. The 144 weeks data of the ongoing 903 study are awaited to further assess the response to subsequent treatments in patients receiving tenofovir and developing the K65R mutation.

A recommendation has been added to the Summary of Product Characteristics to not introduce tenofovir in antiretroviral experienced patients with strains harbouring the K65R mutation.

Pharmacodynamic/Pharmacokinetic relation

The correlation between the decrease in viral load and tenofovir intracellular concentrations is currently unknown but will be further evaluated as part of the follow-up measures to be fulfilled post-authorisation.

Pharmacokinetics

The pharmacokinetic profile of tenofovir DF was determined after single and multiple doses of tenofovir and tenofovir DF administered intravenously and orally, in HIV-1 infected patients (n = 93) and healthy volunteers (n = 143), respectively. The pharmacokinetics development comprises the following Phase I/II clinical studies:

- Study 914: bioequivalence and food study in healthy volunteers
- Study 909: interaction study in healthy volunteers
- Study 701: pharmacokinetic and efficacy evaluation in naïve and experienced patients following intravenous infusion over 1 hour
- Study 919: pharmacokinetic in non HIV-infected subjects with normal renal function, or with varying degrees of renal impairment, including subjects undergoing haemodialysis (submitted and assessed post-authorisation)

Pharmacokinetic data in patients were also obtained from clinical studies 901 and 907.

Two validated HPLC assays, including a more sensitive assay using a mass spectrometric detection, were used for measurements of tenofovir in human serum and urine. The pharmacokinetic profile was determined with doses ranging from 1 and 3 mg/kg, when administered intravenously, and 75 to 600 mg, when administered orally.

Absorption and distribution

Because tenofovir had a low bioavailability due to the presence of a phosphonate group that makes it negatively charged at neutral pH, tenofovir DF was developed.
After single oral administration in fasted state, tenofovir DF was rapidly absorbed, with time to peak concentration (T_{max}) of approximately 1 hour, and converted to tenofovir. Serum tenofovir levels decreased in a biphasic manner with a terminal half-life between 12 and 15 hours, allowing for a once daily dose regimen.

The bioavailability from urinary excretion was estimated to be equivalent to 15-25 %, following a single dose of tenofovir DF 300 mg in fasted patients. High fat food had a significant impact on the bioavailability, increasing it by approximately 30 to 40 %. After a high fat meal, T_{max} was delayed by approximately 1 hour and C_{max} was enhanced by 14 % (335 ng/ml in fed state versus 296 ng/ml in fasted state, respectively). Therefore it is recommended to take tenofovir DF with food as mentioned in the Summary of Product Characteristics. However, administration of tenofovir DF with a light meal did not have a significant effect on the pharmacokinetics of tenofovir.

The pharmacokinetic profile was dose-proportional after intravenous and oral administration within the range 75 to 600 mg. Most of the pharmacokinetic parameters after tenofovir administration seemed to be independent of the dose with the exception of the terminal half-life, which consistently increased over time. In addition repeated intravenous administration of 3 mg/kg tenofovir resulted in an apparent reduction of the serum and renal clearances. However no clinically relevant dose accumulation was reported.

In vitro study demonstrated that tenofovir is practically unbound to plasma proteins (0.7 % in human plasma). Following single intravenous administration of 1 mg/kg or 3 mg/kg, volume of distribution averaged 0.8 to 1 l/kg at steady state.

Metabolism and elimination
Following absorption, tenofovir DF is rapidly converted to tenofovir, which is then metabolised intracellularly to the active metabolite, tenofovir diphosphate. No other metabolite than tenofovir and tenofovir soproxil were identified.

As already mentioned in the Pharmaco-toxicological part of this document *in vitro* studies showed that, at clinically relevant concentrations, tenofovir and tenofovir DF did not inhibit or induce the major CYP-450 isoforms (CYP 3A4; 2D6; 2C9; 2E1 and 1A2). However, *in vitro*, tenofovir DF induced CYP 1A1 and 2B.

The terminal elimination half-life of tenofovir in serum averaged approximately 17-18 hours, which together with the long intracellular half-life in PBMC (> 24 hours) supports once daily dosing.

Tenofovir DF was mainly eliminated renally as unchanged tenofovir, and represented more than 80 % of the administered dose after repeated intravenous administration of 3 mg/kg. The estimated mean

renal clearance (160 ml/hr/kg) exceeds creatinine clearance (~ 75 ml/hr/kg) indicating that tenofovir is eliminated by glomerular secretion and active tubular secretion, which confirmed the pre-clinical observations.
After oral administration, 17 % to 23 % of the administrated dose is recovered unchanged in urine in the fasted and fed state, respectively.

A subgroup analysis of study 903 in antiretroviral naïve patients, showed no relevant gender or ethnic influence on the virological response to tenofovir. This analysis supports previous conclusions derived from subgroup analysis performed in study 907, in antiretroviral experienced patients (combining 903 and 907 studies, where efficacy data are derived from 170 non Caucasians and 100 females).

Special population

- Patients with hepatic impaired function

Tenofovir and tenofovir DF are not metabolised by liver enzymes. Although minimal impact of hepatic impairment is expected, a phase I, open-label, parallel-group study evaluating the pharmacokinetics of tenofovir following administration of a single dose of tenofovir DF 300 mg in 24 fasted non-HIV infected adults subjects with normal and impaired hepatic function (3 groups with 8 subjects in each group: normal hepatic function, moderate or severe hepatic impairment) has been undertaken, to determine whether Viread specific dosing recommendations are needed in patients with hepatic impairment.

Subjects with hepatic impairment were stratified using the Child-Pugh-Turcotte (CPT) classification system. The groups studied were subjects with moderate (CPT class B – CPT score: 7-9) and severe (CPT class C -CPT score > 9) hepatic impairment caused by viral-induced (non-hepatitis B) liver cirrhosis. The control group consisted of healthy volunteers with normal hepatic function.

Systemic exposures of tenofovir were quite similar in subjects with moderate hepatic impairment (AUC_{0-t} and $AUC_{0-\infty}$ were +15% to +16%) and slightly increased in subjects with severe hepatic impairment (AUC_{0-t} and $AUC_{0-\infty}$ +31% to +37%), relative to subjects with normal hepatic function.
Overall, tenofovir exposures ($AUC_{0-\infty}$) were not substantially altered in the subjects with hepatic impairment. The slight increase in tenofovir exposure in patients with marked hepatic impairment does not justify any dosing adjustment with regard to hepatic function.

Appropriate recommendations have been included in the Summary of Product Characteristics.

- Paediatric population

No pharmacokinetic data in children are currently available but the applicant undertook to evaluate the pharmacokinetic profile in this population.

- Patients with renal impaired function

Considering that tenofovir is mainly secreted at the proximal tubule of the kidney, study GS-01-919, a phase I, open-label, parallel-group study, has been undertaken to evaluate the pharmacokinetics of tenofovir DF following administration of tenofovir DF 300 mg in subjects with normal renal function and varying degrees of renal function impairment, including subjects undergoing haemodialysis.

Subjects were divided in 5 groups (Table 8), according to their kidney function assessed with creatinine clearance (Cl_{cr}):

Table 8

Renal function	Cl_{cr}	N
Normal	>80ml/min	3
Mild impairment	50-79 ml/min	10
Moderate	30-49 ml/min	8
Severe	10-29 ml/min	11
End Stage	Haemodialysis	9

The pharmacokinetic analyses were performed in the 40 subjects who completed the study.
Tenofovir Pharmacokinetic parameters following a single administration of tenofovir DF 300 mg for each study group are summarized in the table 9.

Table 9: Single-dose tenofovir PK parameters in subjects with varying degrees of renal function (Median; range)

Renal function	**Cl_{cr}-ml/min**	**AUC0-inf -ng.h/ml-**	**C_{max} (ng/mL)**	**Cl/F -ml/min-**	**Cl_{renal} - ml/min-**
Normal **(N = 3)**	86.5 (82.5-101.0)	2057 (2015-2481)	346 (300–360)	1098 (911-1121)	246 (209-275)
Mild impairment **(N = 10)**	64.2 (51.7-79.8)	2907 (1531-4577)	324 (261–425)	777 (494-1477)	167 (128-213)
Moderate Impairment **(N = 8)**	33.8 (31.8-42.6)	5400 (2532-10320)	402 (153–566)	424 (219-893)	92 (67-153)
Severe Impairment **(N = 11)**	18.6 (12.2-27.8)	17461 (5823-30203)	528 (381–1025)	129 (75-388)	32 (10-109)
End Stage **(N = 9)**	haemodialysis				**Cl_{hd} 134 ml/min**

Haemodialysis subjects
The median extraction coefficient of the dialyzer, was approximately 54% and the median serum haemodialysis clearance was 134 ml/min. By measuring the amount of tenofovir recovered in dialysate during haemodialysis, 10% (range 2% to 16%) of the tenofovir DF dose (as determined using 135.6 mg of tenofovir contained in a 300 mg dose of tenofovir DF) was removed during haemodialysis. In comparison, 20% of an administered dose of tenofovir was recovered in nonhaemodialysis subjects over a 96-hour urine collection period.
Assuming a similar relative oral bioavailability in haemodialysis subjects, this corresponds to removal of approximately 50% of the estimated orally bioavailable dose of tenofovir in a 300 mg dose of tenofovir DF, indicating the efficiency with which haemodialysis removes tenofovir from the serum.

Pharmacokinetic modeling and dosing recommendations
Using a two-compartment model, tenofovir concentrations profiles at steady state were simulated to predict exposures, following both once daily dosing and with longer dosing intervals, in subjects with moderate or severe renal impairment.
The PK modelling estimations showed that, in subjects with moderate or severe renal impairment, a once daily dosing of tenofovir DF 300 mg resulted in a significant accumulation of tenofovir (e.g. median simulated tenofovir C_{trough} values of 123 and 482 ng/ml respectively, representing approximately
221 and 865% (relative ratio) higher trough concentrations compared with subjects with CL_{cr} ≥50ml/min).

Considering the results of the modeling, the following dosing interval adjustments are proposed in patients with Cl_{cr}<50ml/min:

	Creatinine Clearance (ml/min)*		Haemodialysis Patients
	30-49	10-29	
Recommended 245 mg Dosing Interval	Every 48 hours	Every 72 to 96 hours	Every 7 days following completion of a haemodialysis session**

* Calculated using ideal (lean) body weight
**Generally, once weekly dosing assuming three haemodialysis sessions per week, each of approximately 4 hours duration or after 12 hours cumulative haemodialysis.

Since Viread is only available as a 245 mg tablet, recommendations for subjects with renal impairment are only based on adjustment to the dosing interval. Revised guidance, which would refer to dose reduction rather than increase in dosing interval, is pending further investigations of alternative dosage formulations.

Recommendations have been included in the Summary of Product Characteristics as follows: "The proposed dose interval modifications are based on limited data and may not be optimal. The safety and efficacy of these dosing interval adjustment guidelines have not been clinically evaluated. Therefore, clinical response to treatment and renal function should be closely monitored in these patients".
No dosing recommendations could be drawn for non-haemodialysis patients with creatinine clearance < 10 ml/min.

Interaction studies
As above mentioned, *in vitro studies* demonstrated that tenofovir disoproxil fumarate is not a substrate for major isoforms of CYP450 system, apart from its effect on CYP2B and CYP1A1. Metabolic interactions with substrates, inhibitors or any substances affected by this enzymatic system, including most protease inhibitors or non nucleoside reverse transcriptase inhibitors, are therefore not expected. Interaction study agents representative of the three antiretroviral classes (NRTIs, PIs and NNRTIs) was carried out.

- Antiretroviral agents

In study 909, potential interactions between tenofovir DF administered at 300 mg once daily during 7 days with lamivudine, didanosine administered as chewable buffered tablet, indinavir, lopinavir/ritonavir and efavirenz were evaluated. This was a multiple dose, crossover, open label and randomised study conducted in male and female healthy volunteers.

Moreover, study 932, was conducted to evaluate whether the pharmacokinetics of didanosine, administered as 400 mg enteric coated gastro resistant capsules and the pharmacokinetics of tenofovir DF with a light meal were affected by their co-administration. Neither lamivudine nor didanosine administration had any significant impact on tenofovir DF pharmacokinetic parameters. Tenofovir DF decreased Cmax and delayed Tmax of lamivudine although no difference in lamivudine exposure was reported. On the other hand, when didanosine gastro-resistant capsules were administered 2 hours prior to or concurrently with tenofovir disoproxil fumarate the AUC for didanosine was on average increased by 48% and 60% respectively. The mean increase in the AUC of didanosine was 44% when the buffered tablets were administered 1 hour prior to tenofovir. In both cases the pharmacokinetic parameters for tenofovir administered with a light meal were unchanged.

Since didanosine is also secreted at the proximal tubule of the kidney, there is a competition for elimination. During the clinical studies where 30% of patients received tenofovir DF in combination with antiretroviral regimen containing didanosine, there was no evidence of a higher incidence of pancreatitis, peripheral neuropathy or associated laboratory abnormalities. No recommendation could be drawn at this stage with regard to a specific dosage adjustment when these medicinal products are co-administered but close monitoring for didanosine-related adverse events, including, but not limited to, pancreatitis and peripheral neuropathy was recommended, as indicated in the Summary of Product Characteristics.

Indinavir had a minor but not clinically relevant effect on Cmax and Tmax of tenofovir DF. Tenofovir DF delayed the Tmax (1.42 versus 0.99 hours respectively) and reduced the Cmax of indinavir with no significant reduction in indinavir exposure. Although no pharmacokinetic interactions were observed with indinavir, the risk of decrease in tenofovir renal clearance when co-administered with indinavir should be considered in patients susceptible to develop nephrotoxicity. A warning has therefore been included in the Summary of Product Characteristics.

Co-administration of tenofovir DF with lopinavir/ritonavir resulted in a statistically significant increase in tenofovir DF mean serum concentrations (Cmax and AUC0-t increased by approximately

30 %). By contrast, tenofovir induced a slight reduction in the exposure to lopinavir/ritonavir, although Cmin of lopinavir remained unchanged (Cmax and AUC0-t of lopinavir reduced by 15 %). The co-administration resulted also in a statistically significant lower mean peak of ritonavir concentrations (both Cmax and AUC0-t decreased by 30 %) and Tmax was unchanged. The mechanism for this unexpected interaction and the clinical relevance of these results are currently unknown but will be further investigated post-authorisation.

- Other medicinal products

Since *in vitro* interaction study showed that tenofovir DF induced CYP2B and that co-administration with methadone (metabolized by CYP2B6) may be required in practice, the applicant undertook to further investigate this potential interaction.

As tenofovir DF is primarily excreted renally, there may be potential increased concentrations of tenofovir and/or medicinal products that decrease or compete for renal clearance (including those with the same renal transporter hOAT1) when co-administrated. These should therefore not be co-administered unless necessary (with monitoring of the renal function) as recommended in the Summary of Product Characteristics.

Bioequivalence

The intended commercial formulation of tenofovir disoproxil fumarate (1 x 300 mg tablet) was shown to be bioequivalent to the formulation used during the clinical development (4 x 75 mg).

Clinical Efficacy

The antiviral clinical efficacy was evaluated in the following clinical studies:

- Study 901: Phase I/II dose ranging study in naïve and pre-treated patients
- Study 902: Phase II, 48 weeks duration as double blind, in antiretroviral experienced patients
- Study 907: Phase III, 48 weeks duration including 24 weeks as double blind and 24 weeks open, in antiretroviral experienced patients.
- Study 903: Phase III, 144 weeks duration as double blind, in antiretroviral naïve patients.

In both studies 902 and 907, after 48 weeks treatment period, patients remaining on tenofovir could continue into open-label protocols.
Results from studies 902 and 907 were provided to support the efficacy of tenofovir DF administered in combination with other antiretroviral agents for the treatment experienced HIV infected patients with detectable viral load. They were designed as intensification, placebo controlled studies where tenofovir DF was added to stable background antiretroviral treatment.
At the time of the submission of the application, efficacy results for 48 weeks and 24 weeks of treatment were available for study 902 and 907 respectively. In addition interim data up to 96 weeks for patients continuing the long-term open label protocol of study 902, were presented. The efficacy results of study 907 for 48 weeks of treatment have been assessed post authorisation.
In addition, the 48 weeks efficacy data of study 903 have been also submitted post-marketing authorisation to support the efficacy of tenofovir DF administered in combination with lamivudine and efavirenz compared with the combination of stavudine, lamivudine and efavirenz, in treatment naïve HIV infected patients, including 43% of patients with high viral load (> 100,000 copies/ml).

Dose-response studies and main clinical studies

Dose response study

Pre-clinical toxicology studies supported the evaluation of 75 mg daily as the initial dose. Study 901 was the first dose ranging study of tenofovir DF. The design of the study is described in table 10.

Table 10: Design of study 901

Title of the study	randomised, double-blind, placebo-controlled, multiple dose levels study of tenofovir DF
Time of analysis	Part A and B: 35 days → Part C: 12 months → Extended dosing : 12 months
Study design	Part A : Day 1-7 – double-blind / single dose / observation Part B : Day 8-35 – double-blind / daily study drug treatment Cohort 0 ➢ TDF 75 mg QD or placebo once daily Cohort 1 ➢ TDF 150 mg (2 x 75 mg) or placebo once daily Cohort 2 ➢ TDF 300 mg (4 x 75 mg) or placebo once daily Cohort 3 ➢ TDF 600 mg (8 x 75 mg) or placebo once daily Cohort 6 ➢ TDF 75 mg or placebo + HU 500 mg BID HU = hydroxyurea which exhibits *in vitro* synergy in the inhibition of HIV-1 virus replication Part C: 12 months / open label Cohort 6 (part B completed), Single arm ➢ TDF 75 mg or placebo + HU 500 mg + *HAART regimen* Extended dosing: additional 12 months / open label*: TDF 300 mg QD + potent HAART regimen* Cohort 3 + part B completed, no dose-limiting toxicity Cohort 6 + part C completed, no dose-limiting toxicity
Food consumption	Tenofovir DF taken with breakfast and on days 8, 15 and 53, with high-fat breakfast
Number of patients analysed	N = active (pre-treated + naive) Total: 59 (39+20) (Part A 59; Part B 56) Cohort 0: 12 Cohort 3: 10 Placebo : 11 Cohort 2: 8
Population	HIV-1 infected patients (naive and pre-treated); HIV-1 RNA level ≥ 10,000 copies/ml CD4 cell count ≥ 200 cells/mm ^{3}
Primary Endpoints	Pharmacokinetic parameters and anti-HIV-1 RNA and CD4 cells counts between baseline/day 35

The antiviral effect of tenofovir was dose proportional up to 300 mg. At day 35, a significantly greater decrease in HIV-1 RNA levels was observed in all active treatment groups compared to placebo. The greatest median decrease in HIV-1 RNA occurred in the group who received tenofovir DF 300 mg compared to placebo (-0.85 $\log_{10}$ copies/ml versus – 0.01 $\log_{10}$ copies/ml respectively, p = 0.036, ITT population n = 59). No additional antiviral effect was evidenced with the 600 mg dose (- 0.80 $\log_{10}$ copies/ml, p = 0.0002 versus placebo, ITT population). At day 35, naïve patients seemed to have greater decreases in HIV-1 RNA levels compared to experienced patients (- 1.57 versus – 0.61 $\log_{10}$ copies/ml in the 300 mg tenofovir DF arm and –1.40 versus – 0.97 $\log_{10}$ copies/ml in 600 mg tenofovir DF group). The addition of hydroxyurea to tenofovir DF did not provide any additional antiviral effect. No real improvement in CD4 cell counts was observed in any of the treatment groups.

Using a four parameters logistic model, doses of 300 mg and 600 mg were estimated to provide 90 % and 99 % of the maximal antiviral effect, defined as -1.0 $\log_{10}$ copies/ml with a dose of 115 mg representing 50 % of the maximal effect.

HIV RNA levels obtained in Part C and the extended dosing phase seemed to be maintained however the results should be interpreted with caution due to the small sample sized in each cohort (between 3 and 7 patients), the absence of controlled group and the potential impact of highly active antiretroviral treatment that patients started on.

Although the exposition was limited, tenofovir DF 300 mg seemed to be well tolerated in the extended phase (> 1 year). Gastro-intestinal disorders such as diarrhoea were the most common adverse events reported.

Overall, the pharmacokinetic parameters defined in this study were consistent with the pharmacokinetic profile described previously. A dose-proportional pharmacokinetics of tenofovir following oral administration was observed and the long terminal half-life supported a once daily dosage regimen. Although the sample size is limited, particularly in the 600 mg dose group, the dose of 300 mg daily was supported by a significant viral load decrease compared to placebo with an acceptable safety profile. Results from study 902, presented below further substantiated the dose of 300 mg daily.

Main studies

- The overview of clinical studies 902 and 907 in adults is displayed in the table 11.
- The overview of clinical study 903 is displayed in the table 15.

Study 902 and 907

Table 11: Overview of the clinical studies 902 and 907

	Study 902	Study 907
Design	randomised, double blind, multicentre, placebo-controlled	randomised, double blind, multicentre, placebo-controlled
Population	Stable antiretroviral regimen (no more than 4 antiretroviral agents) for 8 weeks prior to enrolment Antiretroviral experienced patients (> 4 years) HIV RNA ≥ 400 and ≤ 100,000 copies/ml	Stable antiretroviral regimen (no more than 4 antiretroviral agents) for 8 weeks prior to enrolment Antiretroviral experienced patients (> 4 years) HIV RNA ≥ 400 and ≤ 10,000 copies/ml
Dosage regimen	Tenofovir DF 75 mg, 150 mg, 300 mg QD Or Placebo + stable antiretroviral therapy After 24 weeks post-randomisation, patients receiving placebo were crossed over to tenofovir 300 mg for the remainder of the 48 weeks treatment duration.	Tenofovir DF 300 mg QD Or Placebo + stable antiretroviral therapy After 24 weeks post-randomisation, patients receiving placebo were given open-label tenofovir 300 mg for the remainder of the 48 weeks treatment duration.
Study duration	48 weeks (at the end of 24 weeks, patients on placebo and TDF 75 mg crossed over in a blinded fashion to TDF 300 mg) + extended open label extended phase	24 weeks double blinded Follow-up 24 weeks open label + extended open label extended phase
N randomised	189 randomised (2:2:2:1) 54 in the TDF 75 mg group 51 in the TDF 150 mg group 56 in the TDF 300 mg group 28 in the placebo group	552 randomised (2: 1) 368 in the TDF group 184 in the placebo group
Age	18 to 65 years	18 to 65 years

The sample size of study 902 was calculated on the basis of safety rather than efficacy criteria (difference in the proportion of patients in each of the treatment groups with Grade 3 or higher adverse events).
In both studies, patients were stratified according to HIV-RNA levels (< 20,000 copies/ml and ≥ 20,000 copies/ml for study 902 and < or ≥ 5,000 copies/ml for study 907), CD4 cell counts (< or ≥ 200 cells/ml) and number of antiretroviral products prior to study entry (< 4 or ≥ 4).

In study 902, patients were encouraged to continue their baseline antiretroviral treatment, in addition to the assigned study compound for at least 4 weeks post-randomisation. Thereafter changes in the background antiretroviral therapy were allowed (addition of a new antiretroviral agent, discontinuation of background antiretroviral agent or clinically relevant interruption for 30 days or more). The criterion used in deciding to change the background therapy was the viral load level remaining above the limit of quantification at week 12 following randomisation. By contrast, the protocol of study 907 encouraged no changes in the background antiretroviral treatment for at least 24 weeks post-randomisation.

Although this medicinal product was developed before the revision of the Points to Consider for the assessment of an anti-HIV medicinal product (CPMP/602/95 rev.3), the development is in line with the spirit of the recommendations. Indeed, the design corresponds to an intensification therapy, the population enrolled is antiretroviral multi-experienced with extensive resistance at baseline.

Even if this population does not correspond to a deep salvage, it is in accordance with the definition of the early failure described in the Points to Consider document.

Endpoints/assays

The primary efficacy endpoint was the treatment effect on viral load as measured by the time-weighted average change from baseline of $\log_{10}$ plasma HIV-1 RNA levels up to week 24 ($DAVG_{24}$). In study 902, the DAVG up to week 4 ($DAVG_4$) was a co-primary endpoint.

The secondary endpoints were:

- DAVG up to week 48 post-randomisation ($DAVG_{48}$)
- the proportion of patients with plasma HIV-1 RNA levels at or below quantification limits (≤ 400 copies/ml and ≤ 50 copies/ml) during the study period
- CD4 cell counts measured by mean change from baseline and DAVG

In both studies, genotypic and phenotypic analyses were performed in a subset of patients as already discussed in the Clinical Pharmacovigilance section of this document.

Statistical analysis

For the DAVG analysis of viral load and CD4 cell counts, DAVG(t) is defined as the patient time-weighted average value of $\log_{10}$ copies/ml or CD4 cell counts between the first visit post start of the study medication and week t, minus the baseline average.

For both studies, primary efficacy analyses were performed using the intent-to-treat (ITT) population, which included all patients who were randomised and received at least one dose of study medication, with no data exclusion. Analysis of several secondary endpoints was also performed on an "as-treated" population (AT), which included all patients who received at least one dose of study medication but excluded all data after discontinuation of assigned medication and/or addition of other antiretroviral therapy.

Results

Population

The demographic and baseline disease characteristics of patients from studies 902 and 907 (ITT Population) are displayed in table 12. Three and two patients did not receive study medication in 902 and 907 respectively.

Table 12: Demographic and baseline disease characteristics (ITT population)

Characteristics	Study 902 (N = 186)	Study 907 (N = 550)
Age (years)		
Mean	41.9	41.6
Median	41.1	40.0
Range	27.3 to 62.3	22 to 70
Gender		
Male, n (%)	171 (92%)	469 (85%)
Female, n (%)	15 (8%)	81 (15%)
Race		
Caucasian, n (%)	138 (74%)	379 (69%)
Black, n (%)	24 (13%)	92 (17%)
Hispanic, n (%)	21 (11%)	68 (12%)
Other, n (%)	3 (2%)	11 (2%)
CD4 count (cells/mm^3)		
Mean (SD)	374 (235)	427 (214)
Median	331	386
Range	9 to 1240	23 to 1385
HIV-1 RNA (log_{10} copies/ml)		
Mean (SD)	3.66 (0.68)	3.36 (0.51)
Range	1.72 to 5.76	1.70 to 4.88
Prior ART experience		
Mean duration (months)	~ 55	~ 65
Background regimen	79 % exposed to ≥ 4 antiretroviral	69 % of tritherapy 22 % exposed to ≥ 4 antiretroviral

NRTI = nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor.

For both studies, the baseline characteristics were well balanced between the tenofovir and the placebo treatment groups.

The majority of patients in study 902 had AIDS or symptomatic HIV-1 infection (for instance, 59 % of patients in the tenofovir DF 300 mg group compared to 41 % of patients asymptomatic). Of note, 52 % of all tenofovir patients had less than 5,000 copies/ml and 94 % of patients had mutations associated with nucleoside reverse transcriptase inhibitors at baseline.

The population in 907 consisted mainly in asymptomatic patients (50 %) with limited viral load, including 78 % with a viral load less than 5,000 copies/ml, but a significant antiretroviral experience (overall mean duration of prior antiretroviral therapy was 5.4 ± 2.9 years). In addition 87 % of patients had a mean CD4 cell count at baseline above 200 cells/mm^3. Overall, 17 % of patients were naïve to PI and 41 % had not been previously exposed to NNRTIs. The current antiretroviral regimen duration was 3.8 ± 2.19 years in the tenofovir DF group and 3.6 ± 2.03 years in the placebo group and consisted mainly in PI + NRTI in both groups (43 % in tenofovir DF and 48 % in placebo groups respectively). As for study 902, 94 % of patients had mutations associated with nucleoside reverse transcriptase inhibitors at baseline.
In both studies, the baseline characteristics of the patients suggested that patients were treatment experienced and in early virological failure rather than as in true therapeutic failure to their baseline therapy.

Discontinuation

The percentage of treatment discontinuation was limited in both studies and accounted for 14 % in study 902 (25 % versus 11 % in the placebo and tenofovir DF 300 mg groups, respectively) and 6 % in study 907 (6 % in both placebo and tenofovir DF groups). Even during the open label phase of study 907, study drug discontinuation occurred rarely.

The main reasons for discontinuation pertained to occurrence of adverse events/intercurrent illness (5 % in study 902 and 3 % in study 907) and lost of follow-up (3 % in study 902 and less than 1 % in study 907).

Efficacy results

Study 902

Primary endpoint

The time-weighted changes from baseline $DAVG_{xx}$ in log_{10} HIV-1 RNA levels at weeks 4, 24, and 48 (ITT population n = 183) are presented in table 13:

Table 13

$DAVG_{XX}$ Date Group		**Median**	**Quartile 1 (25%) Quartile 3 (75%)**	**p-value[a]**
$DAVG_4$				
Placebo	+0.02	-0.04	-0.17, +0.20	-
75 mg	-0.22	-0.14	-0.46, -0.03	0.008
150 mg	-0.44	-0.36	-0.72, -0.19	<0.001
300 mg	-0.62	-0.56	-1.02, -0.25	<0.001
$DAVG_{24}$				
Placebo	+0.02	+0.04	-0.20, +0.42	-
75 mg	-0.26	-0.16	-0.43, +0.06	0.013
150 mg	-0.34	-0.23	-0.74, -0.06	0.002
300 mg	-0.58	-0.54	-0.96, -0.12	<0.001
$DAVG_{48}$				
Placebo crossover[b] to 300 mg (24-48 weeks)	-0.52	-0.38	-1.04, -0.17	
75 mg	-0.33	-0.29	-0.59, +0.06	-
150 mg	-0.34	-0.29	-0.77, 0.00	-
300 mg	-0.62	-0.61	-1.04, -0.25	-

[a] p-value versus placebo, Wilcoxon rank sum test, not stratified
[b] 21 of the 28 patients originally randomised to placebo crossed over to 300 mg, per protocol; this group is a non- randomised group

Results demonstrated statistically significant changes from baseline in HIV-1 RNA for each of the treatment groups compared to placebo, but at all time points, the 300 mg group achieved the maximum mean antiviral effect. The antiviral effect of tenofovir DF 300 mg added to a background therapy provided therefore a limited but clinically relevant effect on viral load as compared to placebo (- 0.58 log_{10} copies/ml at 24 weeks and - 0.61 log_{10} copies/ml at 48 weeks).
In the AT population, results were consistent except for the median value of $DAVG_{48}$ (- 0.39 log_{10} copies/ml versus 0.15 log_{10} copies/ml in the placebo arm, $p < 0.001$).

In a subanalysis, the virological impact of tenofovir was more pronounced in patients with baseline viral load ≤ 5,000 copies/ml compared to those with viral load > 5,000 copies/ml. Indeed for the 300 mg arm, $DAVG_{24}$ accounted for – 0.68 log_{10} copies/ml in patients with baseline ≤ 5,000 copies/ml compared to – 0.47 log_{10} copies/ml in patients with baseline > 5,000 copies/ml. The analysis was not performed according to the planned stratification (< or ≥ 20,000 copies/ml) and the size of the subgroup study was too limited to draw firm conclusions.

Antiviral activity was sustained through 48 weeks. The $DAVG_{48}$ value in patients with less than 4 medications in baseline background therapy for the tenofovir DF 300 mg group was – 0.61 log_{10} copies/ml compared to – 0.63 log_{10} copies/ml in patients with 4 medications.

Secondary endpoints

A greater, but not significant, viral load reduction from baseline was observed in the highest tenofovir DF group (- 0.63 log_{10} copies/ml at week 4 and – 0.68 log10 copies/ml at week 24, ITT population). This study was however not powered to detect any significant differences between the groups in terms of proportion of patients with undetectable viral load. In the ITT population, where missing data equal to treatment failure, the proportion of patients with plasma HIV RNA ≤ 400 copies/ml was 26 % in the tenofovir DF 300 mg group versus 21 % in the placebo group.
Similarly, tenofovir DF treatment did not have any impact on CD4 cell counts compared to placebo.

Changes to background therapy

Changes were consistent across all the groups. The majority of changes consisted of the addition of another antiretroviral agent to the background therapy. Differences between all the groups in time to first change to background therapy were not statistically significant using Kaplan-Meier estimates (21.3 weeks in the placebo group versus 19.3 weeks in the tenofovir DF 300 mg group). The ITT and AT population efficacy results were similar, reinforcing the limited impact of the changes in the demonstration of the efficacy of tenofovir DF ($DAVG_{24}$ for the 300 mg group equivalent to –0.58 log_{10} copies/ml versus – 0.52 log_{10} copies/ml respectively).

Long- term data

During the open label phase, where all patients were switched to tenofovir DF 300 mg (n = 135), the antiviral response seemed to be sustained up to 96 weeks. At 96 weeks, the mean change from baseline in plasma HIV RNA was – 0.98 log_{10} copies/ml in the group who received 300 mg tenofovir DF throughout the study duration (ITT population).

Study 907

Primary endpoint

The mean time-weighted average change from baseline in viral load was significantly greater for tenofovir DF compared to placebo at all time points assessed. During the open-label phase (up to week 48), decreases from baseline plasma HIV-1 RNA levels continued at all time points. After 24 weeks of treatment, using the ITT population, the mean time-weighted average change from baseline in viral load of the tenofovir DF 300 mg group was significantly greater than that of the placebo group, with a sustained virological response after 48 weeks of treatment, as shown in table 14.

Table 142:

DAVG ITT Population	**TDF**	**Placebo**	**Placebo Crossover to TDF (24-48 weeks)**	**p-value**
Week 24	**(N=367)[1]**	**(N=182)**		
Mean (∀ SD)	-0.61 (0.61)	-0.03 (0.36)		p < 0.0001
Median	-0.56	-0.02		
Interquartile Range: Quartile 1 (25%) to Quartile 3 (75%)	-1.07 to -0.15	-0.22 to 0.19		
Range	-2.17 to 1.37	-1.35 to 1.01		
Week 48	**(N=367)**		**(N=170)**	
Mean (∀ SD)	-0.57 (0.59)		-0.60 (0.75)	
Median	-0.57		-0.48	
Interquartile Range: Quartile 1 (25%) to Quartile 3 (75%)	-0.99 to -0.15		-1.05 to –0.07	
Range	-2.26 to 1.33		-2.82 to 1.17	

[1]- 367 patients in the TDF group included in the analysis (i.e., one patient with no post-baseline HIV-1 RNA data was excluded)

In term of changes in viral load from baseline, tenofovir DF induced a limited but relevant decrease compared to placebo (- 0.61 log_{10} copies/ml *versus* – 0.03 log_{10} copies/ml at 24 weeks). Efficacy data obtained after 48 weeks of treatment show a sustained virological response in the Tenofovir DF group ($DAVG_{24}$ = -0.61 log_{10} copies/mL and $DAVG_{48}$ =-0.57 log_{10} copies/mL).

Subgroup analysis

The benefit of tenofovir DF treatment on plasma HIV RNA levels was observed in patients with both viral basal load above and below 5,000 copies/ml. The proportion of patients achieving undetectable viral load was lower in patients who had a viral load ≥ 5,000 copies/ml at baseline as compared to patients with < 5,000 copies/ml (15 % versus 55 % with HIV RNA ≤ 400 copies/ml and 5 % versus 28 % with HIV RNA ≤ 50 copies/ml at week 24, respectively). However it is difficult to draw firm conclusion since 78 % of patients had a viral load < 5,000 copies/ml at baseline. Similarly, the benefit of tenofovir was less favourable in patients with a basal immunological status less than 200 cells/mm^3.

Secondary endpoints

The proportion of patients with HIV-1 RNA below the limit of quantification (≤ 400 and ≤ 50 copies/ml) was significantly greater in the tenofovir DF groups compared to placebo by the time of the first on-treatment assessment at week 2, and at all subsequent time points. At 24 weeks, 45 % of patients in the tenofovir DF group had a viral load below 400 copies/ml compared to 13 % in the placebo group ($p < 0.0001$). Using the limit of quantification 50 copies/ml, there were still 22 % of patients versus 1 % in the placebo group with undetectable viral load ($p < 0.0001$). At week 48, the proportion of patients with HIV-1 RNA levels ≤400 copies/ml and HIV-1 RNA levels ≤50 copies/ml (41% and 18% respectively in the tenofovir DF group) confirmed that the antiviral response to Viread was durable through 48 weeks: Since patients who met the inclusion criteria of HIV-1 RNA ≥ 400 copies/ml were randomised within 21 days after screening, some were found to have less than 400 copies/ml at baseline (8 % (31/368) in tenofovir DF group and 5 % (9/182) in the placebo group). At week 48, 22 of the 31 (71%) patients of the tenofovir DF group who were found to have baseline HIV RNA ≤400 copies/ml, had a sustained undetectable viral load which reflects that the lower the viral load is at baseline, the more sustained the virological response to tenofovir.

No clear immunological impact of tenofovir on CD4 cell counts could be demonstrated. Only the time-weighted average change from baseline analysis, at week 24, indicated a statistically significant difference between the tenofovir DF and the placebo groups (+ 12 .6 versus -10.6 respectively, $p = 0.0008$). A similar trend was shown at 48 weeks with a limited change from baseline (+12.5 cells/mm^3).

Change in background antiretroviral medications while on study prior to week 24

The proportions of patients having a change in background antiretroviral therapy during the double-blind phase were similar in the tenofovir DF group (11 %) and the placebo group (11 %) ($p = 0.89$). For those patients who had a change, there was no difference between the two groups in terms of the proportion receiving a new antiretroviral medication ($p = 0.38$) and the proportion discontinuing a baseline medication and not receiving a new antiretroviral medication ($p = 0.35$). Up to week 48, changes in background therapy were reported for 93/368 patients (25%) in the tenofovir DF group. In 67 patients (18%), the change was the addition of a new antiretroviral medication.

Because changes to background antiretroviral therapies were discouraged, the weekly proportion of patients discontinuing treatment was low in each arm during the first 24 weeks, ranging from 0 % to 4%. When focusing on the time to first new antiretroviral medication added during the double blind phase up to week 24, the proportion of patients was lower in the tenofovir arm than in the placebo arm although not statistically significant (21 % versus 7 %, Kaplan Meier estimate $p = 0.87$). In addition, the ITT and AT population 24 weeks efficacy results were similar, reinforcing the limited impact of the changes in the demonstration of the efficacy of tenofovir DF ($DAVG_{24}$ for the 300 mg group equivalent to –0.61 log_{10} copies/ml versus – 0.62 log_{10} copies/ml respectively).

Study 903

Table 15 Overview of clinical study 903

	Study 903
Design	Randomised, double blind, parallel, multicentre, placebo-controlled
Population	Antiretroviral-naive, HIV-1-infected patients Plasma HIV-1 RNA levels > 5 000 copies/ml at screening (less than 50% of patients with HIV-1 RNA levels > 100 000 copies/ml) Stratification according to baseline: HIV-1 RNA (> or ≤ 100,000 copies/mL) CD4 cell count (< or ≥ 200 cells/mm^3)
Dosage regimen	TDF 300 mg QD + 3TC 150 mg BID + EFV 600 mg QD +d4T placebo BID *versus* d4T 40 or 30 mg BID + 3TC 150 mg BID + EFV 600 mg QD + TDF placebo QD Nevirapine 200 mg PO BID could replace EFV in the event of EFV-associated CNS toxicity or rash
Study duration	144 weeks, double-blinded (48 and 96 weeks interim analysis to compare the safety, efficacy, and tolerability of the two treatment regimens)
N randomised	602 randomized (ratio 1:1), 600 analyzed (intent-to-treat): - 299 in tenofovir DF + lamivudine + efavirenz group and - 301 in stavudine + lamivudine + efavirenz group
Age	18 to 65 years

Endpoints/assays

The primary endpoint was the equivalence of both regimens in the treatment of HIV-1 infected antiretroviral-naive patients as determined by the proportion of patients with plasma HIV-1 RNA levels < 400 copies/ml at week 48.

The secondary endpoints were:

- To assess the equivalence of the two treatment regimens with respect to the ability to achieve HIV-1 RNA levels < 50 copies/ml at week 48,
- To compare the safety, efficacy, and tolerability of the two treatment regimens through 144 weeks of exposure (analyses conducted at weeks 48, 96, and 144).

Statistical analysis

A two-sided 95% confidence interval on the difference in treatment group response rates (tenofovir-containing arm minus stavudine-containing arm) weighted by baseline HIV-RNA and CD4 strata was constructed. A "delta" of 0.10 was chosen for the definition of equivalence.
The tenofovir-containing arm would be declared equivalent to the stavudine-containing arm if the lower confidence bound for the difference between the arms in the proportion with HIV-1 RNA below 400 copies/ml was greater than -0.10.
Patients missing plasma HIV-1 RNA values at week 48 and patients who added or switched antiretroviral medications before week 48 were analyzed as having plasma HIV-1 RNA concentrations ≥ 400 copies/ml at week 48.
Nevirapine, when substituted for efavirenz, was not considered as an addition of antiretroviral medication.

Results

Population

Distinction was made, in the study analysis, between patients who prematurely discontinued the study drugs (permanent discontinuation) (55 (18%) and 48 (15%) respectively in the tenofovir DF and d4T groups 18%) and those who prematurely discontinued the study as detailed in Table 16 [27(9%) and 28(10%)]. The difference being likely that patients who discontinued the study drug but who have the efficacy endpoint collected were part of the efficacy analyses.

Table 16: ITT population of study 903

No. of Patients	**TDF+3TC+EFV**		**d4T+3TC+EFV**		
	N	**%**	**N**	**%**	**p-Value**
Randomized	299		***303***		
Received Study Drugs	299		***301***		
Completed Study Week 48	**272**	91%	**272***	90%	
Discontinued Study Before Week 48	27	9%	28	9%	1.0*

** 273 expected with no explanation*

Table 17: Stratification (ITT population)

	TDF+3TC+EFV (N = 299)		**d4T+3TC+EFV (N = 301)**	
Number (%) of Patients	299	100%	301	100%
Randomization Stratification				
RNA ≤ 100,000 copies/ml and CD4 < 200 cells/mm^3	38	13%	38	13%
RNA ≤ 100,000 copies/ml and CD4 ≥ 200 cells/mm^3	133	44%	136	45%
RNA > 100,000 copies/ml and CD4 < 200 cells/mm^3	78	26%	77	26%
RNA > 100,000 copies/ml and CD4 ≥ 200 cells/mm^3	50	17%	50	17%

Table 18: Demographics and Baseline Characteristics (ITT Population)

Characteristic	TDF+3TC+EFV (N = 299)		d4T+3TC+EFV (N = 301)		Overall Total (N = 600)	
	n	%	N	%	n	%
	299		301		600	
Gender						
Male	220	74%	225	75%	445	74%
Female	79	26%	76	25%	155	26%
Age (Years)						
Mean ± SD	35.5 ± 8.63		35.9 ± 9.07		35.7 ± 8.85	
Race						
White	191	64%	193	64%	384	64%
Black	64	21%	53	18%	117	20%
Asian	3	1%	6	2%	9	2%
Hispanic	21	7%	23	8%	44	7%
Other	20	7%	26	9%	46	8%
Weight (kg)						
Mean (± SD)	71.77 ± 13.81		72.13 ± 14.40		71.95 ± 14.10	
Range	42.3 to 126		41.7 to 122		41.7 to 126	
Plasma HIV-1 RNA (log_{10} copies/ml)						
Mean (± SD)	4.91 ± 0.64		4.91 ± 0.61		4.91 ± 0.62	
Range	2.62 to 6.71		3.61 to 6.64		2.62 to 6.71	
CD4 Count (cell/mm^3)						
Mean (± SD)	276.0 ± 201.3		282.7 ± 200.2		279.4 ± 200.6	
Range	4.5 to 838.0		3.0 to 956.0		3.0 to 956.0	
HIV-1 Status						
Asymptomatic	196	66%	180	60%	376	63%
Symptomatic HIV-1 Infection	51	17%	64	21%	115	19%
AIDS	52	17%	57	19%	109	18%

The population enrolled with a mean age of 35 years old consisted in mainly male (74%) patients. The mean viral load at baseline is of 4.91(+/- 0.62) log copies/ml, including **43 % of patients with a viral load > 100 000 copies/ml** and 39% with a CD4 cell count at baseline < 200 cell/mm3 (mean CD4 cell count: 279+/-201). Although this patient population was treatment naïve, 19% (115/600) of the patients in the study had symptomatic HIV-1 infection and 18% (109/600) had AIDS (Table 16). The stratification on the basis of the 100,000 copies/ml viral load (Table 15) was considered as of particular interest with regard to the concern raised during the initial Marketing Application in relation to the limited number of patients with high viral load (78% of patients having a viral load <5000 copies/ml).

Efficacy

Primary endpoint

Patients who add or switch antiretroviral medications prior to week 48 were analysed as if the week 48 RNA were greater than 400 copies/ml.

Table 19: Patients with Plasma HIV-1 RNA Levels Below 400 copies/ml at week 48

ITT **Missing data = Failure** **Changes in ART= Failure**	TDF+3TC+EFV (N = 299)		d4T+3TC+EFV (N = 301)		Difference
Number (%) of Patients with Plasma HIV-1 RNA < 400 copies/ml*	239/**299**	(79.9%)	253/**301**	(84.1%)	-4.1%
Number (%) of Patients with Plasma HIV-1 RNA < 400 copies/ml by Baseline Stratum					
RNA ≤ 100,000 copies/ml and CD4 < 200 cells/mm^3	23/32	(71.9%)	25/33	(75.8%)	-3.9%
RNA ≤ 100,000 copies/ml and CD4 ≥ 200 cells/mm^3	108/129	(83.7%)	125/139	(89.9%)	-6.2%
RNA > 100,000 copies/ml and CD4 < 200 cells/mm^3	65/86	(75.6%)	60/80	(75.0%)	0.6%
RNA > 100,000 copies/ml and CD4 ≥ 200 cells/mm^3	43/52	(82.7%)	43/49	(87.8%)	-5.1%
Difference in percent **(stratum weighted)** [TDF+3TC+EFV - d4T+3TC+EFV] 95% CI for difference					-4.4% **[-10.4**%;1.5%]

With regard to stratification, antiviral response is similar in naïve patients regardless of baseline viral load, conversely to the trend observed in antiretroviral pre-treated patients.
In ITT population, it was not possible to strictly conclude to the demonstration of the non inferiority since 10.4 is definitively superior to 10 (10% predefined non inferiority margin).
However, considering that this margin was very close to the stringent predefined non inferiority margin, the efficacy demonstration has been considered as acceptable.

When the ITT analysis only considers the population with HIV RNA values at week 48 (exclusion of patients who prematurely discontinued the study: 27/299 and 28/301 in the tenofovir DF and d4T groups respectively), results are in accordance with the predefined hypothesis of non inferiority.

Secondary endpoints

Ultrasensitive test results (plasma HIV-1 RNA levels < 50 copies/ml at week 48) were in accordance with the 10% predefined non-inferiority margin, for both ITT and AT population. Moreover, in line with previous efficacy analyses, comparable results were observed between both treatment groups in term of viral load change from baseline (- 3 log copies/ml).

Clinical studies in special populations

The efficacy of tenofovir DF has not yet been evaluated in children, but the provision of clinical data in this population is part of the follow-up measures to be fulfilled post-authorisation.

Clinical Safety

At the time of the Marketing Authorisation evaluation, the safety database comprised mainly data from the three controlled clinical studies of tenofovir DF (studies 907, 902 and the extensions of these studies, and study 901) as well as data from the compassionate use study in patients with advanced disease (study 908). These studies included approximately 1,050 HIV infected adult patients who received tenofovir DF either alone (study 901) or in combination with other antiretroviral medicinal

products (studies 902, 907 and 908). The safety profile of tenofovir DF was also analysed by pooling safety data from studies 902 and 907. This approach was considered acceptable based on the similarity of study design and treatment experience of the populations.

Experience from clinical trials
Safety data were collected from study 910, an open-label multicenter study of the safety of tenofovir DF
300 mg administered orally over an extended period to HIV-1 infected patients previously treated in studies 901, 902, and 907. Patients who completed either of the two studies without dose-limiting toxicity were eligible to enroll in study 910. As of 1st May 2001, 467 patients from study 907, 102 patients from study 902 and 6 patients from study 901 have rolled over to study 910.

Experience from Post Marketing surveillance
Based on the long term data of studies 902 and 907, data of ongoing study 910 and 903 as well as data assessed in the first (31/10/01 - 30/04/02), the second (01/05/02 – 30/10/02) and the third (01/11/02 – 30/04/03) Periodic Safety Update Reports, the Summary of Product Characteristics have been updated with regard to safety.

Patient exposure

At the time of the marketing authorisation, safety results obtained with tenofovir DF 300 mg were provided for approximately 443 patients at week 24 (including 21 patients in study 902 who received tenofovir DF 300 mg after an initial 24 weeks on placebo) and for 75 patients at week 48 for the double blind phases of studies 902 and 907. A limited number of patients (n = 60) received 96 weeks of treatment. Data from 291 HIV infected patients with advanced disease who were enrolled in study 908 and received treatment with tenofovir 300 mg with a mean duration of follow-up of 29 weeks were also provided.

To answer concerns raised on the safety of tenofovir DF, especially related to the potential bone and renal toxicity, the applicant provided an Updated Safety Dataset (cut-off June 2001) comprising all patients (n = 687) who received at least one dose of tenofovir DF 300 mg in studies 902 and 907, including placebo crossover patients from both studies and 74 patients who initially received 75 mg or 150 mg tenofovir DF in study 902. The mean exposure to tenofovir DF for this updated clinical trial population was 58 weeks, with a range of 0.4 to 143 weeks (Table 20).

Table 20: Treatment Duration (Pooled Studies)

	Integrated Summary of Safety			Safety Update
	Placebo (0-24 Weeks) (N = 210)	**TDF 300 mg (0-24 Weeks) (N = 443)**	**All TDF (N = 687)**	**All TDF (N = 687)**
Weeks on Study Drug				
Mean _ SD	23.0 _ 4.0	23.0 _ 4.1	35.8 _ 30.4	92.6 _ 41.3
Median	24.0	24.0	28.1	96.0
Range	2.1 to 25.9	0.4 to 29.3	0.1 to 115.9	0.4 to 191.0

Experience from Pivotal clinical trials
The assessment of adverse reactions from pivotal clinical trials is based on:
- studies 902 and 907, in 653 treatment-experienced patients receiving treatment with tenofovir DF (n = 443) or placebo (n = 210) in combination with other antiretroviral medicinal products for 24 weeks
- and also study 903, in which 600 treatment naïve patients received treatment with tenofovir DF (n = 299) or stavudine (n = 301) in combination with lamivudine and efavirenz for 48 weeks.

Experience form Post Marking surveillance
As of 31st October 2002, the cumulative patient exposure to tenofovir is estimated to be 100,302 patients.

Adverse events and serious adverse event/death

Overall, the frequency and type of adverse events reported in the tenofovir DF and the placebo groups appeared similar during the 24-week placebo-controlled treatment phases of both studies 902 and 907.

The most frequently reported adverse events associated with tenofovir DF related to gastrointestinal disorders (nausea, diarrhoea, vomiting and flatulence). In the pooled dataset, approximately 1 % of patients discontinued tenofovir due to occurrence of gastrointestinal adverse events. In the tenofovir DF 300 mg group, 10 % and 12 % of patients reported diarrhoea and nausea respectively during the first 24 weeks of treatment. In addition asthenia, headache, vomiting and flatulence considered related to treatment were each reported by more than 5 % of patients in the tenofovir DF group. The frequency of gastrointestinal disorders reported in the placebo group was similar and safety data from study 902 did not reveal any dose effect.

The nature, frequency and severity of adverse events during the first 48 weeks of treatment in Study 903 were similar between the tenofovir DF group and the active control group. Frequently occurring adverse events (> 20% of either treatment group) were headache, viral infection, diarrhoea, nausea, dizziness, pharyngitis and rash.

The additional reactions identified from post-marketing experience are increased creatinine, renal insufficiency, kidney failure, Fanconi syndrome, proximal tubulopathy, proteinuria, acute renal failure, asthenia, dyspnoea, rash, pancreatitis, lactic acidosis, abdominal pain, allergic reaction and amylase increased.

Serious adverse events and deaths

In all the clinical studies, serious adverse events (SAEs) occurred infrequently and there was no difference between the tenofovir DF and placebo groups. SAEs that were considered related to tenofovir DF were uncommon. During the double-blind period of the phase II/III studies, only four SAEs were considered to be at least possibly related to tenofovir DF; hepatic failure (tenofovir DF 75 mg), osteopenia (tenofovir DF 75 mg), acute pancreatitis (tenofovir DF 300 mg) and lactic acidosis associated with nausea (tenofovir DF 300 mg).

In treatment-experienced patients (studies 902 and 907), the frequency of serious adverse events was higher in placebo patients (8%) compared to patients who received tenofovir DF (5%) during 24-weeks of treatment. While the incidence of serious adverse events has increased with extended treatment duration in these studies (mean duration of 93 weeks), individual events have occurred infrequently, with only pneumonia (3%) occurring in > 1% of patients.

In Study 903, 11% of patients in the tenofovir group and 10% of patients in the active control group experienced serious adverse events. In the tenofovir DF group, possibly related serious adverse events (as judged by the investigator) comprised single reports of anaemia, peripheral neuritis, bronchitis and gynecomastia. Two patients in the tenofovir DF group experienced acute kidney failure, although neither of these events was considered related to treatment.

A total of 3 deaths were reported in studies 901, 902 and 907, and one subsequent death occurred during the extension protocol 910, although none of them was considered related to tenofovir. In study 903, 5 patients (1 in the tenofovir DF group and 4 in the active control group) died during the first 48-week phase of the study. All 5 deaths were considered as not related to either stavudine or tenofovir DF.

Post-approval, during the reporting periods for the first and second PSURs (26 October 2001 to 31 October 2002), a total of 28 reports with a fatal outcome were received in the PSURs. Twenty-four of these were considered possibly related to treatment. In these 24 reports, other factors were present, such as concurrent illnesses or concomitant medication, which could have contributed to the reported events.

Laboratory findings

Laboratory abnormalities of all grades generally occurred with similar frequencies in the tenofovir 300 mg and placebo groups in study 907 (96 % versus 97 % respectively). Although preclinical studies identified small transient changes in liver enzymes in rats, a combined analysis of studies 902 and 907 (n = 443) did not reveal any significant difference between tenofovir DF 300 mg and placebo in the incidence of graded ALT elevation during 24 weeks of double blind treatment. An analysis of risk factors for elevated ALT ($\geq$ 2 grade) showed that patients with underlying hepatitis had a 2.7 fold greater risk of ALT elevations ($p < 0.0001$). The applicant will further address whether the introduction of tenofovir in co-infected patients has any influence in the evolution of HBV/HCV infections.
During the preclinical studies, administration of tenofovir DF resulted in bone abnormalities and kidney alterations. The potential bone toxicity and nephrotoxicity of tenofovir DF were therefore assessed during the clinical studies and a more specific assessment of some laboratory parameters was performed.
No significant laboratory toxicity related to treatment with tenofovir DF in both treatment-experienced and treatment-naïve patients were observed. Studies 902, 907 and 903 did not show a significant renal toxicity. In long-term data, an increased incidence of hypophosphatemia grade 1 and 2 of 18% was observed after a median duration of exposure of 93 weeks. The increased incidence of hypophosphatemia in study 902 and 907 was not confirmed in study 903. Hypophosphatemia was transient, resolving while patients continued to receive tenofovir DF or after discontinuation of the medicinal product.

Events of Special Interest

Bone abnormalities

An analysis of bone-related laboratory parameters (urinary and serum calcium, parathyroid hormone and alkaline phosphatase) did not suggest pathology associated with tenofovir administration. In study 907, there was no clinically significant difference in the fractional excretion of phosphate for tenofovir compared to placebo (mean +0.37 % versus -1.12 % respectively; median: +0.40 % versus -1.00 %).

A combined incidence of grade 1 and 2 hypophosphataemia of 12 % for tenofovir DF and 7 % for placebo was reported during the double blind treatment period of studies 902 and 907. The treatment difference was not statistically significant. The Safety Updated Dataset showed that the incidence of grade 1 and 2 abnormalities increased slightly to 15 %. The hypophosphataemia was transient. Among the pooled population of tenofovir DF treated patients, 17 received phosphate supplements at some timepoints. All of them had their abnormality resolved. The mechanism of hypophosphataemia has not yet been elucidated and its impact on the bone is unknown. It was, however, suggested that it was more likely to be related to an effect on intestinal phosphate absorption than renal toxicity. Indeed the absence of link between hypophosphataemia and proximal tubule impairment was comforted by the absence of other abnormalities usually observed in tubulopathy (hypokalemia, decreased plasma bicarbonate glycosuria). It was agreed to recommend in the Summary of Product Characteristics monitoring of serum phosphate and the applicant agreed to further evaluate the potential benefit of administering phosphate supplements in patients who develop hypophosphataemia.
Bone mineral density monitoring was performed in a subgroup of patients from study 902 (n = 62, including 11 in the placebo group). The median percentage of changes from baseline at week 24 were -2.00 % for the placebo group versus - 0.16 %, - 0.15 % and –1.19 % for tenofovir DF 75 mg, 150 mg and 300 mg respectively. These changes were mild and not statistically significant. The effects did not worsen with continued exposure to tenofovir DF up to week 48 and no dose effect relationship could be evidenced.
In the Safety Updated Dataset, a total of 27 fractures were reported in patients treated with tenofovir. In the controlled studies there have been no reports of vertical compression fractures or spontaneous fractures of weight bearing bones, and all the fractures have been associated with significant trauma.

Experience from Post Marketing surveillance

The available data are too limited to draw conclusions concerning bone toxicity. However, reduction in bone mineral density was seen in patients after 48 weeks of treatment with tenofovir DF. The Marketing Authorisation Holder has been requested to continue the monitoring of potential adverse effects / toxicity on bone metabolism as part of post-marketing specific obligations (including the collection of bone densitometric measurements, bone markers, cases of fracture and osteoporosis) from PSURs and from the ongoing clinical study in antiretroviral naïve patients.

Renal toxicity

In the short term studies 901 and 701, no acute renal toxicity associated with tenofovir was reported. The protocols of studies 902 and 907 excluded patients with renal impairment (plasma creatinine > 1.5 mg/ml and/or creatinine clearance < 60 ml/min) and those who received concomitant nephrotoxic substances.
A combined incidence of increased serum creatinine of grade 1 of 1 % was reported for tenofovir DF compared to placebo during the double-blind period of studies 902 and 907. Nearly all of these Grade 1 abnormalities remained in the normal range (< 1.5 mg/dl). No patient discontinued treatment due to an increase in serum creatinine. The Safety Updated Dataset confirmed that the incidence of increased creatinine was low (5 %) and no patient had a serum creatinine elevation above grade 1. In study 903, the incidence of increased serum creatinine was similar between the tenofovir DF group (1.7%) and the active control group (2.3%) and the incidence of hypophosphataemia was also similar between the two groups (tenofovir DF group (5.0%) and the active control group (4.0%).
The incidence of decrease in bicarbonate hypokalemia, glycosuria and proteinuria was similar between tenofovir DF and placebo groups. These data suggest that tenofovir DF is not nephrotoxic although the risk cannot be currently completely excluded. Therefore monitoring of the renal function, is recommended in the Summary of Product Characteristics. The use of tenofovir DF should be avoided with concurrent or recent use of a nephrotoxic medicinal product; if concomitant use of tenofovir DF and nephrotoxic agents is unavoidable, weekly monitoring of renal function is recommended. Finally in patients with an increase of creatinine to values higher than 2 mg/dl or decrease in serum phosphate to values below 1 mg/dl, interrupting tenofovir DF treatment should be considered.

Experience from Post marketing surveillance

As described in the PSURs, cases of renal events have been identified in the Marketing Authorisation Holder Global Safety Database (which includes serious adverse events from clinical trials and serious and non-serious adverse events reported from spontaneous sources or literature publications). Reported cases with renal impairment were difficult to assess, however tenofovir DF can be responsible for tubulopathy (including Fanconi), (acute) renal failure, tubular necrosis. Moreover, glycosuria, hypophosphatemia and proteinuria are sensitive indexes of proximal tubulopathies and are often observed in proximal tubulopathies cases. Most abnormalities were reversible after discontinuation of tenofovir DF.
Even though background noise concerning renal events in HIV patients is high, longterm data on efficacy and tolerability have been requested in patients whose renal function necessitates a reduced tenofovir DF dosage as well as a safety review of all renal disorders.
Based on post-marketing experience and ongoing review of the total Safety database for Viread, the following renal events have been added to the Summary of Product Characteristics as possible adverse reactions on the basis of seriousness, frequency and potential causal relationship with tenofovir DF: increased creatinine, renal insufficiency, renal failure, proximal tubulopathy (including Fanconi syndrome) and hypophosphataemia.

Mitochondrial toxicity

Although *in vitro* tenofovir did not have any effect on the synthesis of mitochondrial DNA nor on the production of lactic acidosis, adverse events compatible with mitochondrial toxicity were reported with tenofovir DF. For instance, 3 cases of pancreatitis were reported, 2 in study 907 and one in study 908. In these 3 cases, tenofovir was added to nucleoside analogues regimen. As already mentioned, during the double-blind period of the phase II/III studies, there were 3 SAEs related to tenofovir DF which could be considered secondary to mitochondrial toxicity; hepatic failure (tenofovir DF 75 mg), acute pancreatitis (tenofovir DF 300 mg) and lactic acidosis associated with nausea (tenofovir DF 300 mg). The safety data from clinical trials suggested that tenofovir DF has a low potential for mitochondrial toxicity, and data from the treatment-naïve population (study 903) were similarly reassuring with little evidence of tenofovir DF-related mitochondrial toxicities.
The assessment of clinical safety data from ongoing clinical studies and during post-marketing experience continues to demonstrate a low risk of mitochondrial toxicity with tenofovir DF. Lactic acidosis and pancreatitis were identified as possible adverse reactions and were included in the Summary of Product Characteristics.

Lactic acidosis

Evidence from adequate and well-controlled clinical studies in both treatment-experienced and treatment-naïve HIV-infected patients have demonstrated a low risk of lactic acidosis with tenofovir DF. Following the review of the first (26.10.01-30.04.02) and second (01.05.02-31.10.02) PSURs, lactic acidosis was identified as a possible adverse reaction on the basis of seriousness, frequency of reporting or potential causal relationship with tenofovir DF of cases in the Clinical Safety Database. Since patients involved in these cases had been diagnosed with HIV for many years and in most cases antiretroviral combination regimens included medications implicated in the development of lactic acidosis (such as didanosine, stavudine), these events could have been complications of long-term antiretroviral NRTI therapy and therefore, the potential contribution of tenofovir DF is difficult to assess. The same conclusions were given further to the review of the third PSUR (01/11/02 – 30/04/03). Overall, the assessment of clinical safety data from ongoing MAH sponsored clinical studies and during post-marketing experience, continues to demonstrate a low risk of lactic acidosis with tenofovir DF. However, considered against the background of NRTI-associated lactic acidosis and the revised class labelling, the CPMP has decided to strengthen the warning section of the product information of Viread with regard to lactic acidosis. Furthermore, the SPC has been updated to highlight that the risk of occurrence of lactic acidosis, a class effect of nucleoside analogues, is low for tenofovir DF as indicated by preclinical and clinical data.

Long term safety profile

In the extended phase of study 902 (median time on study medication of approximately 90 weeks), tenofovir DF continues to be well tolerated: 19% patients have discontinued study. The two most common ≥ grade 3 adverse events were asthenia and depression; the three most common ≥ grade 3 laboratory abnormalities were elevations of AST, triglycerides and creatine kinase. No patient developed ≥ grade 2 elevation in serum creatinine. One death occurred due to liver failure, which was not considered related to tenofovir DF. The results should however be considered with caution as the number of patients exposed were limited. The Updated Safety Dataset (including 687 patients who received tenofovir DF at the dose of 300 mg daily for a mean of 58 weeks) substantiated the absence of potential nephrotoxicity and bone toxicity related to the use of tenofovir.

Continuous assessment of Viread long-term safety profile is performed throughout PSURs and the product information updated accordingly.

Safety profile of Viread in combination therapy

Treatment with a combination of at least three antiretroviral drugs can induce a characteristic syndrome termed lipodystrophy or fat redistribution syndrome containing peripheral fat wasting (including accentuation of facial folds) and central adiposity. Metabolic disturbances such as hyperlipidaemia and insulin resistance also often appear. PIs were originally believed to be the causal agents. NRTIs have also been implicated. In addition, lipodystrophy has also been observed with protease-inhibitor-sparing regimens. The emerging picture is that of a connection between visceral lipomatosis and protease inhibitors and lipoatrophy and NRTIs correlating with different possible mechanisms e.g. effects on lipoprotein production and adipocyte differentiation. Non-drug factors are also of importance e.g. increasing age, duration and severity of HIV infection.
Following evaluation of data submitted by all MAHs of antiretroviral medicinal products, a class labelling, which harmonises the information on lipodystrophy for all three classes of antiretroviral products, has been agreed and implemented in the product information for all antiretroviral medicinal products. The wording presents as much as possible of the presently available knowledge; it gives a description of the condition (although there is at present no clear definition of lipodystrophy), information about causality and surveillance measures. The higher risk of developing lipodystrophy with long-term therapy as well as importance of factors such as age and disease related factors is mentioned.

Liver impairment in HIV positive patients

Further to the discussions held by the *Ad-hoc Group of Experts on Anti-HIV medicinal products* in November 2001, the CPMP agreed that liver impairment was of increasing concern in HIV positive patients both in the form of adverse hepatic effects in patients with normal liver function prior to antiretroviral treatment (ART) and as regards patients with chronic liver disease treated with ART.
In January 2002 the CPMP requested the MAH for all authorised anti-retroviral medicinal products to conduct a retrospective review of clinical trials and post marketing data relating to the use of their product(s) in patients with hepatic impairment and/or HBV/HCV co-infection. Following review of the submitted responses and discussions held during the CPMP meeting and the Pharmacovigilance Working Party meeting in October 2002, the CPMP adopted a list of questions (including general, product specific and SPC wording recommendations).

The review of the MAHs' responses has essentially confirmed that co-infected patients and patients with underlying liver disorders are at increased risk for adverse events, essentially confined to liver events. Overall, there is a disturbing lack of general and product specific knowledge (e.g. relevant pharmacokinetic data in patients with liver impairment), but there are ongoing activities.
For some of the products still undergoing drug development, the MAHs have confirmed that co-infected patients will not be excluded from participation in the studies. The CPMP stressed that whenever feasible a minimum number of co-infected patients should be included in forthcoming studies in order to provide a reasonable basis for a relevant safety (and efficacy) analysis.

Following the review of responses submitted by all MAHs of antiretroviral medicinal products, a class labelling on "liver disease" has been agreed and implemented in the product information for all antiretroviral medicinal products.

The SPC of Viread has been reworded in accordance with the CPMP recommendations to include, in section 5.2, data on AUC, Cmax and Cmin (including CV) derived from patients. These PK results in patients give a more reliable description of the concentrations achieved in clinical practice at the recommended dose.

Future studies with regard to liver disease

The MAH plans to evaluate safety of Viread in patients with liver disease or hepatitis B/C co-infection with two ongoing studies (subset safety analysis of data from the "French ATU de cohorte", which ended in December 2001 and study ACTG 5127). The MAH has committed to continue to not exclude co- infected patients from participation in clinical trials.
In addition, with respect to the request for follow-up as regards liver disorders and ART through cohort studies, the CPMP noted that the HAART oversight committee is active and will provide proposals for agreement.

Compassionate use

In this population with advanced disease, 89 cases of grade 3 or 4 adverse events were reported. There were 13 cases (4%) of pneumonia (community acquired Pneumonia, *Pneumocystis carinii*) all grade 3-4, among those 5 were fatal. None of these cases were considered related to tenofovir DF. A total of 15 deaths were reported that were considered probably related to disease progression.

Safety in specific populations

The safety profile of tenofovir DF in children is currently unknown as well as in patients with hepatic impairment.

5. Overall Conclusion and benefit risk assessment

Quality

In general the quality dossier has been well presented and indicates that the active substance and product are manufactured and controlled in a relevant way in compliance with current EU guidelines. Satisfactory information has been provided to show that these manufacture and control processes are under control and routinely and consistently generate a product of uniform quality likely to have a reproducible performance in the clinic. However, at the time of the opinion, the CPMP found that a number quality issues were still unresolved. These issues were considered to have no impact on the benefit/risk balance of the product when used according to the Summary of Product Characteristics. It was agreed that they should be resolved as follow up measures to be submitted post-authorisation.

Preclinical pharmacology and toxicology

Tenofovir DF presented an antiviral activity both *in vitro and in vivo*, compatible with a potential clinical use for the treatment of HIV-1 infection.

Based on *in vitro* data, the mitochondrial toxicity appears not to be a major concern compared to nucleoside analogues. These preliminary findings will however be further substantiated through additional data provided as specific obligations to be fulfilled post-authorisation.

The pharmacokinetic profile of tenofovir has been adequately characterised in the different species studied. Following oral administration, tenofovir DF is rapidly absorbed and converted to tenofovir. Tenofovir is mainly excreted renally. Tenofovir is not metabolised through CYP450 system and did

not have any potential effect on major CYP isoforms except for inducing CYP1A1 and 2B.

The toxicological profile tenofovir DF was assessed using a complete battery of toxicological studies. Identified target organs were gastro-intestinal tract, bone and kidney. The toxic effects occurred at exposure levels comparable or slightly higher than that measured in humans with 300 mg dose of tenofovir DF. Safety margins ranged therefore between 2 and 10 according to species. Although toxicity in some target organs has a very low safety margin, this has to be put in balance with clinical experience derived from the clinical studies. A concern was raised however with respect to the potential nephrotoxicity and bone toxicity of tenofovir DF. Although the currently available data, including safety data, do not evidence that tenofovir DF is nephrotoxic and bone toxic, appropriate recommendations have been included in the product information and the Marketing Authorisation Holder has been requested to closely monitor cases of renal disorders, bone disorders and osteoporosis.
Reproduction toxicity studies indicated that tenofovir DF did not affect fertility parameters and is not embryotoxic nor teratogenic although it reduced the viability index and weight of pups in peri and post natal studies.
Tenofovir was genotoxic in some tests therefore potentially carcinogenic. However, given the results from the long-term oral carcinogenicity study in mice and rats, it was considered that there are no significant concerns regarding the carcinogenic potential of tenofovir DF in patients.
Other outstanding preclinical issues were identified, but the CPMP considered that they did not have any impact on the benefit/risk balance of the product when used according to the Summary of Product Characteristics. It was agreed that they should be resolved as follow up measures to be submitted post-authorisation.

Efficacy

The pharmacokinetic profile of tenofovir DF was adequately defined, mainly in male HIV infected patients. Tenofovir DF was rapidly absorbed, converted into tenofovir and mainly eliminated renally as unchanged. Food enhanced the low bioavailability of tenofovir DF, and therefore it is recommended to take tenofovir DF with food. Of interest, the metabolism of the tenofovir DF did not interfere with the CYP 3A4, which limits the potential for pharmacokinetic interactions. An unexpected interaction with lopinavir/ritonavir has however been observed. Some remaining issues with respect to potential interactions with other medicinal products were identified that the applicant agreed to clarify as part of follow-up measures to be fulfilled post-authorisation. In addition, the applicant has provided additional data to characterise the pharmacokinetic profile of tenofovir DF, particularly with respect to patients with renal impairment and the potential impact of gender and/or race. No pharmacokinetic data in children are currently available but the Marketing Authorisation Holder has undertaken to evaluate the pharmacokinetic profile in this population.

Tenofovir exhibits a long half-life, over 12 hours, with an intracellular T1/2 >24 hours in PBMC, allowing once daily dosing, which presents an advantage for adherence. The choice of the dose, one tablet of 245 mg tenofovir disoproxil once daily, has been appropriately substantiated during clinical trials. The one daily dosage regiment was considered of interest in terms of compliance.

The clinical benefit of tenofovir containing regimen in term of virological suppression was mainly based, at the time of the Marketing Authorisation, on two placebo-controlled studies performed in antiretroviral multi-experienced patients with extensive resistance mutations at baseline and designed as intensification trials (902 and 907). This intensification design corresponds to a pragmatic approach in the management of early treatment failure. Although, this compound was developed before the revision of the Points to Consider Document in the assessment of anti-HIV medicinal product, the development is in line with the spirit of the guideline.
The majority of patients experienced an early virological failure (< 10, 000 copies/ml with the majority of patients having < 5,000 copies/ml) and received tenofovir DF in addition to a triple regimen. At week 24, in the ITT population, a limited but clinically relevant decrease in viral load was observed in comparison with placebo (-0.6 log10 copies/ml), and was consistent in both 902 and 907 studies. This effect appeared to be sustained at 48 weeks, based on the limited data from study 902 and the durability of the virological response in this population was confirmed by the 48-week final data

from study 907, submitted as part as post-marketing specific obligation. Clarifications provided by the applicant gave reassurance on minimal impact of the change in antiretroviral therapy during the studies on the overall efficacy results. Subanalyses (immunological and virological parameters at baseline) were performed but the results were not sufficiently robust to draw any firm conclusions.

A concern was raised however on the limited clinical data in patients with high viral load at baseline, but the applicant undertook to further evaluate the clinical benefit of tenofovir DF in this subpopulation as part of specific obligations to be fulfilled post-authorisation.

In addition, a study conducted in antiretroviral naïve patients was submitted post-authorisation. This trial investigated the antiviral effect of Viread used in combination with lamivudine and efavirenz *versus* stavudine, lamivudine and efavirenz, in treatment-naïve patients with a significant proportion of patients with a plasma viral load > 100 000 copies/ml (stratification) at baseline (43%). In these treatment-naïve patients, comparable results were observed between both treatment groups in term of viral load change from baseline. Based on the 48-week data of this study, the indication has been extended to antiretroviral naïve patients. The 144-weeks data of this ongoing study are awaited to further assess the durability of the virological response.

The favourable resistance profile has been further substantiated through a significant amount of genotypic and phenotypic data derived from clinical studies. Overall a low level of resistance to tenofovir DF has been demonstrated *in vitro* and the NRTI genotypic resistance at baseline did not influence the virological response to tenofovir. The resistance profile was therefore considered of interest in antiretroviral experienced patients.
Of particular interest, the emergence of resistance is limited (3% at 48 weeks in pretreated patients). Based on the data derived from clinical studies, the introduction of tenofovir should be discouraged in patients with strains harbouring, at baseline, the K65R mutation, which is the primary mutation associated with tenofovir, as observed with abacavir. However, the clinical breakpoints of tenofovir have not been determined. The Marketing Authorisation Holder is investigating this issue as well as the appropriate guidance that should be provided when K65R is emerging during the time course of treatment with tenofovir.
The resistance profile of the drug is described in the Summary of Product Characteristics and additional data are expected post-authorisation to further characterise the resistance profile of tenofovir DF and the clinical relevance.

The clinical benefit in children and adolescents under 18 years of age is currently unknown but the applicant presented a paediatric programme, for which the results of the studies will be submitted post-authorisation.

Other clinical outstanding issues were identified and will be resolved as follow up measures to be submitted post-authorisation.

Safety

Overall the tolerance of tenofovir DF was good and the post-marketing safety data did not raise any new concern. The undesirable effects reported with tenofovir DF were mainly gastro-intestinal disorders, dizziness, and hypophosphatemia. During post-approval use of tenofovir DF, possible adverse reactions have also been identified, such as: asthenia, pancreatitis, lactic acidosis, dyspnoea, rash and renal disorders.
The safety profile of the tenofovir DF raised some concerns with respect to nephrotoxicity and bone toxicity. Hypophosphataemia was observed in preclinical and in clinical studies 902 and 907. The mechanism is currently unknown but it was suggested that hypophosphataemia is most likely to be related to an effect on intestinal phosphate absorption than tubular toxicity. The benefit of a phosphorus supplementation will be further investigated in patients experiencing hypophosphataemia in clinical studies. Additional Safety Updated Data as well as post-marketing safety data gave re-assurance of the absence of nephrotoxicity and bone toxicity of tenofovir DF. However the need for monitoring renal toxicity and serum phosphate has been included in the Summary of Product Characteristics.

The applicant undertook to perform a close post-marketing surveillance targeting these particular issues (safety review of all renal disorders and collection of bone densitometric measurements and bone markers).

Benefit/Risk Assessment and recommendation

There is an unmet medical need for new therapeutic options for the treatment of antiretroviral experienced patients in particular with the increasing problem of resistance.

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered that the benefit/risk profile of tenofovir DF in combination with other antiretroviral medicinal products was favourable in the treatment of HIV-infected adult patients experiencing early virological failure. Results from studies which were ongoing at the time of the evaluation of the marketing authorisation have been provided, including study in naïve patients, to complete the assessment in terms of efficacy (durability of the response and impact on CD4) and safety profile of tenofovir DF. Considering that other additional preclinical and clinical efficacy and safety data should be submitted to further define the efficacy and safety profile of tenofovir DF, the marketing authorisation for Tenofovir 245 mg film-coated tablets remains under exceptional circumstances, as initially recommended by the CPMP.

Further to the assessment of study in naïve patients, the indications are currently:

> "*Viread is indicated in combination with other antiretroviral medicinal products for the treatment of HIV-1 infected adults over 18 years of age.*
>
> *The demonstration of benefit of Viread is based on results of one study in treatment-naïve patients, including patients with a high viral load (> 100,000 copies/ml) and studies in which Viread was added to stable background therapy (mainly tritherapy) in antiretroviral pre-treated patients experiencing early virological failure (< 10,000 copies/ml, with the majority of patients having < 5,000 copies/ml).*
>
> *In deciding on a new regimen for patients who have failed an antiretroviral regimen, careful consideration should be given to the patterns of mutations associated with different medicinal products and the treatment history of the individual patient. Where available, resistance testing may be appropriate*".

Based on the CPMP review of data on quality, safety and efficacy, the CPMP considered by majority decision that the benefit/risk profile of Viread was favourable, in combination with other antiretroviral agents, in the treatment of HIV infected patients over 18 years of age.