SCIENTIFIC DISCUSSION

1. Introduction

Truvada, which contains 200 mg emtricitabine and 245 mg tenofovir disoproxil (equivalent to 300 mg tenofovir disoproxil fumarate or 136 mg of tenofovir) in each film-coated tablet, is intended for the treatment of Human Immunodeficiency Virus (HIV) infection in adults.

Truvada is a new fixed dose combination of previously known active substances: emtricitabine, a nucleoside reverse transcriptase inhibitor and tenofovir disoproxil (as fumarate), the oral prodrug of tenofovir, a nucleotide reverse transcriptase inhibitor.

Medicinal products containing emtricitabine (200 mg hard capsule) and tenofovir disoproxil (as fumarate) (245 mg film-coated tablet) have already been approved through the centralised procedure for the treatment of HIV infection.

Truvada has been developed using the same doses and regimens as for the individual compounds.

In the context of a chronic disease where non-adherence is a critical problem with potential consequence in terms of risk of emergence of resistance, the development of a fixed combination together with a once daily regimen could be a way of alleviating the constraints of patients.

The approved indication is:

> "Truvada is a fixed dose combination of emtricitabine and tenofovir disoproxil fumarate. It is indicated in antiretroviral combination therapy for the treatment of HIV-1 infected adults.
> The demonstration of the benefit of the combination emtricitabine and tenofovir disoproxil fumarate in antiretroviral therapy is based solely on studies performed in treatment-naïve patients."

2. Quality aspects

Composition

Truvada is presented as film-coated tablets containing a fixed combination of 200 mg of emtricitabine and 245 mg of tenofovir disoproxil (as fumarate), equivalent to 136 mg of tenofovir.

The other ingredients include croscarmellose sodium, lactose monohydrate, magnesium stearate, microcrystalline cellulose, pregelatinised starch, glycerol triacetate, hypromellose, indigo carmine aluminium lake (E132) and titanium dioxide (E171).

The tablets are packed in HDPE bottle containing silica gel desiccant with a child resistant cap containing an aluminium foil inner seal.

Active Substances

Emtricitabine

Emtricitabine is a nucleoside analogue of cytidine and is also the active substance of a centrally authorised medicinal product (EU/1/03/261/01-03). Changes to the Quality documentation for emtricitabine included in the MAA for Truvada have recently been implemented to the already authorised product via a type II variation (EMEA/H/C/533/II/12).

- Manufacture

An additional manufacturer is introduced along with an additional synthesis route. Compared to the current process involving a chiral resolution catalysed by an enzyme of porcine origin, the new

process is purely synthetic. The specifications proposed for starting materials, reagents and solvents are acceptable. In addition, satisfactory in process controls and controls on the isolated intermediates have been established in order to ensure production of an active substance of consistent quality.

The potential virus safety risk associated with the enzyme of porcine origin used in the current synthesis has been satisfactorily addressed.

Comparative batch analysis data for emtricitabine produced by the 2 processes by the different manufacturers show that the physical properties of the active remain unchanged.

The impurity profile of emtricitabine synthesised by the new process has been fully characterised. Two potential impurities appear to be specific to it, although one of them has not been observed in any of the batches produced so far.

- Specification

The active substance specification include tests for appearance, identity (IR and HPLC), assay, impurities, enantiomeric purity, residual solvents (GC), heavy metals (PhEur), sulphated ash (PhEur), water content (PhEur), particle size and clarity of solution (PhEur).

As a consequence of the introduction of the new process, changes have been made to the active substance specifications including introduction of a limit for the new impurity and for a new residual solvent. The limit for the new impurity observed at levels above the ICH identification threshold of 0.1% has been justified in relation to intake in toxicological studies. Control for residual solvents are in line with ICH recommendations. Minor changes have been made as well to the analytical procedures and are supported by satisfactory validation data.

Batch analysis data provided for 6 development and clinical batches of emtricitabine synthesised by the new process at the two synthesis sites together with 10 batches synthesised by the current synthesis comply with the specifications.

- Stability

Stability data have been provided for 4 stability batches synthesised using the new process. 1-year data under long-term conditions (25°C/60% RH – commercial packaging) is available for 2 batches. Under accelerated conditions up to 9-month data is available.

The physicochemical stability of emtricitabine synthesised by the 2 processes is comparable and support the currently authorised packaging material, retest period and storage conditions.

The applicant committed to include the first 3 commercial batches of the new process from each manufacturer into the accelerated and long-term studies.

Tenofovir

Tenofovir is a nucleotide analogue of adenosine and it is the active substance of an already centrally authorised medicinal product (EU/1/01/200/001). The Truvada MAA included only a few changes to the quality documentation compared to the one approved for the centrally authorised product referred above.

- Manufacture

A new synthesis site is introduced together with a batch size increase. Batch analysis data provided for 3 batches manufactured at the new site are within specification and support the new batch size. Confirmation has been given that there is no change to the synthesis process other than the scale of the equipment used. This will be the case as well during scale-up at the other authorised synthesis sites.

- Specification

No change has been made to the currently approved specification for tenofovir, which include tests for appearance, identity (IR, HPLC), assay (HPLC), enantiomeric purity (HPLC), impurities (HPLC), residual solvents (GC), heavy metals (PhEur), clarity of solution, water content (PhEur), particle size, and differential scanning calorimetry.
Batch analysis data provided for 3 commercial batches from each of the currently authorised manufacturer and for 3 pilot scale batches for the new manufacturer show no significant differences in quality of the active substance.

- Stability

Stability data are available for commercial batches synthesised at each already authorised synthesis site under accelerated conditions (25°C/60% RH – commercial packaging) for 6 months and under long-term conditions (5°C – commercial packaging) for 2 years. The applicant committed to enter the first 3 commercial batches synthesised at the new site into accelerated and long-term studies using the current approved stability protocol. The photostability of tenofovir has been previously demonstrated.
On this basis, the currently approved packaging material, retest period and storage conditions for tenofovir remain acceptable.

Medicinal Product

- Pharmaceutical Development

The proposed medicinal product combining the currently approved doses of emtricitabine and tenofovir into a single tablet to be administered once daily intends to support patient adherence to treatment.

Emtricitabine is a high solubility/high permeability substance, while tenofovir is a high solubility/low permeability substance. The fumarate salt of the diester prodrug of tenofovir has consequently been selected so as to increase intestinal permeability and to improve the bioavailability of this active.

Emtricitabine and tenofovir disoproxil fumarate are both susceptible to hydrolysis in aqueous solution and to a smaller extent, they degrade in high moisture/temperature conditions with a possibility of incompatibility between the 2 actives and associated degradation products. All the potential degradants are adequately controlled as part of the specification of the finished product.

A wet granulation has been chosen over a dry granulation in order to minimise the effect of the physico-chemical properties of the active substances on processing and blend uniformity. Control of the amount of unbound water during manufacture and in the finished product enhances to minimise any potential degradation. In addition, the amount of moisture in the bottle headspace is minimised by using a HPDE bottle with a low permeability in term of water vapour transmission rate, by using a cap with an aluminium foil inner seal and by including a silica gel desiccant in the packaging. The HPDE bottle and the polypropylene cap meet the general Ph. Eur. requirements for plastic primary packaging material. Confirmation has been given that the silica gel desiccant is suitable for contact with food.

The disintegration of the tablets has been satisfactorily investigated in 100 ml of water, grape juice and orange juice as an alternative mean of administration for patients who are unable to swallow the tablets. The preparation should be taken immediately.

All the excipients are commonly used for this kind of formulation and are of PhEur quality except indigo carmine blue lake, which is adequately controlled according to another standard and is an authorised colorant in the EU.

Regarding the TSE risk, the lactose monohydrate from milk of bovine origin has been considered in compliance with the current TSE requirements. The magnesium stearate and the glycerol triacetate used in the film coat are from vegetable origin.

- Bioequivalence

A bioequivalence study (GS-US-104-172) has been performed to investigate the bioequivalence of the proposed combination tablet versus the 2 separate marketed products administered concomitantly in the fasted state and to investigate the food effect. The clinical formulation used in the bioequivalence study is identical to the commercial formulation.

Bioequivalence has been demonstrated between the two formulations when administered in the fasting state. Tenofovir plasma concentration-time profiles appeared to be lower in the fasted state than in the fed state (high-fat meal or light meal). Emtricitabine pharmacokinetic parameters after ingestion of food (high-fat or light meal) were essentially the same as those for the fasting state. The CHMP considered that in order to optimise the absorption of tenofovir, it is recommended to take Truvada with food (see clinical section).

- Manufacture of the Product

The manufacturing process involves the following operations: mixing, aqueous wet granulation, fluid bed drying, milling and blending, tabletting, film coating and packaging. Adequate in-process controls have been specified.

Validation data provided for batches up to commercial scale confirm the robustness and reproducibility of the manufacturing process.

- Product Specification

The product specification includes tests controlled by validated methods for appearance, identity (UV and HPLC), assay (HPLC), impurity content (HPLC), uniformity of content (PhEur), water content (PhEur), dissolution (PhEur) and microbial limits (PhEur).

Batch analysis data provided for 7 clinical batches manufactured at the proposed commercial manufacturing site comply with the specifications and indicate consistent and reproducible manufacture.

- Stability of the Product

Stability data are provided for 4 primary stability batches. Under long-term conditions (25°C/60%RH – commercial packaging) and intermediate conditions (30°C/60% RH - commercial packaging), 1 year data is available for 1 lot, 9-month data is available for 2 lots at 9 months and 6-month data is available for 1 lot. Accelerated data (40ºC/75%RH – commercial packaging) are available up to 6 months.

Photostability studies have shown that the medicinal product is non-light sensitive.

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

3. Non-clinical aspects

Emtricitabine and tenofovir have been shown to have antiviral activity against HIV and have been investigated individually in a comprehensive programme. The applicant has provided an overview of the comprehensive information on each active substance. No additional studies were conducted with the fixed dose combination except for a 14-day repeat dose rodent toxicity study with the fixed dose combination. Previous findings with the individual components in relation to pharmacology, pharmacokinetics and toxicology are summarised in the sections below.

Pharmacology

- Primary pharmacodynamics (in vitro/in vivo)

Emtricitabine is a nucleoside analogue of cytidine. Tenofovir disoproxil fumarate (tenofovir DF) is converted *in vivo* to tenofovir, a nucleoside monophosphate (nucleotide) analogue of adenosine monophosphate. Both substances act as inhibitors of the HIV reverse transcriptase and have been shown to be active against laboratory and clinical isolates of HIV-1 (EC_{50} values for emtricitabine ranged from 0.0013 to 0.5 µM and IC_{50} for tenofovir was 2.3 µM).

Emtricitabine and tenofovir are phosphorylated by cellular enzymes through non-overlapping pathways to form emtricitabine triphosphate and tenofovir diphosphate, respectively. When both substances are incubated together *in vitro* at concentrations higher than achieved in the plasma (10 µM each), complete conversion to the fully activated forms of each substance is observed. As tenofovir diphosphate and emtricitabine triphosphate are alternative substrates for different natural substrates (dATP and dCTP, respectively), there should be no competition for incorporation by HIV reverse transcriptase and subsequent DNA chain termination. In support of this, the antiviral activity of the combination of tenofovir and emtricitabine has been found to be synergistic in multiple in-vitro assay systems. Using the LAI strain of HIV-1 at a multiplicity of infection (MOI) of 0.03 in MT-2 cells, there was demonstrable synergy between tenofovir and emtricitabine in an isobologram analysis. More recently, a study was performed using the LAI strain and a clinical HIV-1 recombinant containing a wild-type RT and protease gene (MM-317) each at MOIs of 0.03 and 0.1. Analyses by MacSynergy and by isobolograms showed that the combination was synergistic and the isobologram analysis gave p-values ≤ 0.0017 for all infections.

Tenofovir diphosphate has a prolonged intracellular half-life ranging from 12 to 50 hours inactivated and resting blood mononuclear cells (PMBCs). The intracellular half-life of emtricitabine triphosphates in PMBCs *in vivo* is approximately 39 hours. These long intracellular half-lives support a once daily dose regimen.

The antiviral activity of the combination of tenofovir and emtricitabine was demonstrated *in vivo*. In a 32 week study in the SIV macaque model, the combination of tenofovir and emtricitabine administered once daily by subcutaneous injection resulted in full suppression of plasma SIV in treated animals. The response lasted throughout the study with no detectable development of resistance.

Resistance

On serial passage of virus, resistance to emtricitabine developed rapidly (2-6 passages) as the result of a *met* to *val* or to isoleucine change at codon 184 (M184V and M184I). Emtricitabine-resistant viruses were cross-resistant to lamivudine but generally retained sensitivity to other nucleoside reverse transcriptase inhibitors (NRTIs) and non nucleoside reverse transcriptase inhibitors (NNRTIs). Moderate resistance to lamivudine (mediated by E44D or V118I) gave moderate resistance to emtricitabine. Cross-resistance of a moderate degree was also observed in association with the K65R mutation or the MDR genotype containing the T69S (SS) insertion. In a cell culture system, viruses with the K65R mutation were 8-12-fold less susceptible to emtricitabine than wild types and an enzymatic study indicated a 5-fold reduced incorporation of emtricitabine when this mutation was present.

The K65R mutation was obtained with successive passage of HIV-1 in increasing concentrations of tenofovir. The IC50 tenofovir was increased 3.4-fold for isolates with the K65R mutation and 1.5-fold for isolates with M184V. The K65R mutation confers cross-resistance to abacavir, didanosine and lamivudine. Passage of wild type virus (HXB2) with lamivudine and tenofovir or with abacavir and tenofovir did not select for M184V or M184I but did select for K65R and then later Y115F (associated with reduced susceptibility to abacavir). HIV-1 isolates with resistance to zidovudine/lamivudine, +/- abacavir with 2 to 4 thymidine analogue mutations (TAMs) were inhibited by tenofovir at concentrations within 2.2-fold the inhibitory concentration for wild-type virus.

An in-vitro evaluation of the potential for emtricitabine and tenofovir to select for resistance when used together has been performed in MT-2 cells. Concentrations began at half the EC50 values for each. Results so far have shown viral replication at 16-fold the EC50 values and development of the M184I mutation from the 4th passage. No changes at K65 have been observed. These results suggest that the use of Truvada in the clinic will first select the M184 V/I mutation and later the K65R mutation.

- Secondary pharmacodynamics

Emtricitabine, inhibited HBV production *in vitro*, in HepG2 2.2.15 cells, with EC_{50} values that ranged from 0.01 to 0.04 µM. HBV resistance to emtricitabine has been observed and is associated with mutations in the YMDD motif of the HBV polymerase.

Tenofovir is a potent and selective inhibitor of HBV. It inhibited HBV production in HepG2 2.2.15 and HB611 cells with EC_{50} values of 1.1 and 2.5 µM respectively.

The results of *in vitro* investigations suggest that emtricitabine and tenofovir have limited capacity to inhibit human DNA polymerases or to mediate cytotoxicity or mitochondrial damage.

- Safety pharmacology

No safety pharmacology studies have been conducted with emtricitabine and tenofovir DF in combination. However, neither emtricitabine nor tenofovir DF alone had significant unwanted pharmacological activity as determined in different studies carried out, investigating the effects on central nervous system, cardiovascular/respiratory, gastro-intestinal and renal systems.

- Pharmacodynamic interactions

Additive to synergistic effects were observed in combination studies with protease inhibitors and with nucleoside and non-nucleoside analogue inhibitors of HIV reverse transcriptase.

Pharmacokinetics

The pharmacokinetics profile of tenofovir DF and emtricitabine were evaluated in a variety of animal models using validated analytical methods.

- Absorption- Bioavailability

Emtricitabine
Emtricitabine was rapidly and extensively absorbed in mice, rats and cynomolgus monkeys with oral bioavailability ranging from 58% to 97% and T_{max} values of 0.5 to 2.5 hours over the dose range of 10 to 600 mg/kg. Following single dose administration of emtricitabine, half-life values ranged from 1.02 to 3.17 hours in the species. No differences were noted between single and repeat dose pharmacokinetic studies.

Tenofovir DF
Following oral absorption of the prodrug, tenofovir DF, absorption and conversion to tenofovir was rapid, with maximal concentrations of tenofovir in plasma reached between 0.25 to 1.5 hours post-dose in all species (mice, rats, woodchucks, dogs and monkeys). The oral bioavailability ranged from 20% to 46% in the species. Terminal half-life values were respectively 7 hours in rats, 9 hours in monkeys and 60 hours in dogs. No gender differences were observed. In rats and monkeys, pharmacokinetics parameters were similar after single or repeated administration. On the contrary, in dogs at the highest doses tested (10 or 30 mg/kg) systemic exposure (C_{max} and AUC) was increased 2 to 3 fold after 28 days of treatment. The values then remain constant up to end of the study (42 weeks). This could suggest an accumulation.

- Distribution

Both substances widely distributed. Both substances crossed the placenta but did not concentrate in foetal tissues. Tenofovir was excreted in milk from lactating rats (milk/plasma concentration ratio of 11-23.5 %). *In vitro,* the protein binding of both substances was very negligible and therefore interaction due to protein binding displacement would be unlikely.

- Metabolism (in vitro/in vivo) and excretion

There was limited metabolism of emtricitabine. Biotransformation included oxidation of the thiol moiety to form the 3'-sulfoxide diastereomer and conjugation with glucuronic acid to form the 2'-0-glucuronide. The 3'-sulfoxide diastereomer was the principal metabolite, which represented 2% of dose in mice, 2.7% in rats and from 6% to 11% of dose in monkeys. Other metabolites collectively represented less than 2% of dose.

In vitro, tenofovir DF was rapidly converted to monoester (tenofovir soproxil) which is the major intracellular metabolite and tenofovir. Tenofovir DF metabolism was studied *in vivo* in male rats, with ^{14}C-labeled tenofovir DF by oral and intravenous route. Tenofovir was the principal metabolite. Tenofovir soproxil formation was transitional.

In vitro studies determined that neither tenofovir disoproxil fumarate nor tenofovir were substrates for the CYP450 enzymes. Neither emtricitabine nor tenofovir inhibited *in vitro* metabolism mediated by any of the major human CYP450 isoforms involved in biotransformation of compounds. Also, emtricitabine did not inhibit uridine-5′-diphosphoglucuronyl transferase, the enzyme responsible for glucuronidation.

Tenofovir was excreted unchanged in the urine in rats and dogs and renal excretion was identified as the primary route of elimination. For emtricitabine, renal excretion of unchanged substance was the principal route of elimination in mice, rats and dogs. Data suggested that the substance was eliminated by active tubular secretion. It was considered unlikely that there would be an interaction affecting elimination.

Toxicology

- Single dose toxicity

Emtricitabine
Single doses up to 4000 mg/kg orally or 200 mg/kg i.v. in mice and rats were well tolerated without signs of target organ toxicity.

Tenofovir DF
There were no signs of toxicity in rats given oral doses up to 1500 mg/kg. In dogs, the NOEL was 30 mg/kg since treatment-related lesions in the kidneys characterised by tubular karyomegaly and/or basophilia were observed with oral doses equivalent to 90 and 270 mg/kg.

- Repeat dose toxicity

Tenofovir DF
Studies were performed in mice with doses up to 1000/600 mg/kg/day for 13 weeks, in rats for 28 days, 13 and 42 weeks with doses up to 1000 mg/kg/day, in dogs with doses up to 30 mg/kg/day for 28 days, 13 and 42 weeks and in monkeys with doses up to 600 mg/kg/day for 56 days. The target organs of toxicity were the gastrointestinal tract (primary in rodents), the renal tubular epithelium and bone. In rodents, gastro-intestinal alterations typically included inflammation of the stomach and intestines, epithelial hypertrophy or hyperplasia in the duodenum and jejunum, and villous atrophy of the ileum. These gastro-intestinal effects occurred at high doses, generally greater than 300 mg/kg/day, and are thought to be due to high local concentrations of tenofovir DF or its hydrolysis products.

With respect to renal toxicity, the dog was the most sensitive species. The karyomegaly observed in the renal tubular epithelium of all species was considered a morphologic change without pathologic consequence. Microscopic alterations in dogs treated for 42 weeks at doses ≥ 10 mg/kg/day included renal tubular epithelial karyomegaly and individual cell necrosis, tubular dilatation, degeneration/regeneration, pigment accumulation, and interstitial nephritis. The incidence, severity, and reversibility of renal histopathological changes were related to the dose and duration of treatment. Bone effects, characterised as reduced bone mineral density and content with accompanying biochemical changes, were noted in rats (≥ 300 mg/kg/day) and dogs (30 mg/kg/day). The NOEL for bone effects was 100 mg/kg/day in rats and 10 mg/kg/day in dogs. Findings in the rat and monkey studies indicated that there was a substance-related decrease in intestinal absorption of phosphate with potential secondary reduction in bone mineral density. The mechanisms of these toxicities are not completely understood.

Emtricitabine
Treatment related effects were confined to the high-dose groups and included:
- mild, reversible anaemia (mice 1 and 6 months, rats 3 months and monkeys 1 year)
- changes in various organ weight without any associated adverse histopathological effects in rodents (mice 1 and 6 months, rats 3 months)
- increased urine output (mice 6 months) and soft faeces (monkeys 1 and 3 months).

Doses produced systemic exposure much greater than those anticipated in clinic.

Emtricitabine/tenofovir DF combination
A 14-day study was conducted in male CD-rats administered by gavage emtricitabine/tenofovir DF combination product with doses up to 200/300 mg/kg/day. The ratio between the two substances was similar as the one to be used in clinics. Administration of the emtricitabine/tenofovir DF tablet did not exacerbate the known toxicities of the individual agents. The NOAEL was estimated to be 67 /100 mg/kg/day. At the highest dose tested (200/300mg/kg/day), an hyperplasia of the anterior duodenal mucosa was noted but this was considered to be due to the formaldehyde release from tenofovir DF formulation as previously observed with the individual compound.

To examine the potential for an exacerbation of renal effects in the more sensitive species, the applicant undertook to conduct a one month study of emtricitabine and tenofovir DF in combination in the dog, the results of which will be submitted as part of the follow-up measures to be fulfilled post-authorisation.

- Genotoxicity *in vitro* and *in vivo* (with toxicokinetics)

Emtricitabine was not mutagenic in a battery of tests. Tenofovir DF was positive for inducing forward mutations in the in-vitro mouse lymphoma cell assay in the presence or absence of S9 metabolic activation. Tenofovir DF was also positive in the Ames test (strain TA 1535) in 2 out of 3 studies, once in the presence of S9 (6.2 to 6.98 fold-increase) and once without S9. It was also weakly positive in an in-vivo / in-vitro UDS test in primary rat hepatocytes. There was no clastogenic effect in the mouse bone marrow micronucleus test.

A concern was raised for potential increased genotoxicity with combination antiretroviral treatment. Indeed combination of zidovudine and didanosine has been reported to potentiate genetic damage in human cells *in vitro* [Meng, 2000] and in CD-1 mice *in vivo* [Bishop, 2004]. The applicant undertook therefore to investigate *in vitro* whether co-administration of emtricitabine with tenofovir DF has the potential to enhance the genotoxic potential of tenofovir DF, the results of which will be submitted as part of the follow-up measures to be fulfilled post-authorisation.

References:
- Meng Q, Walker DM, Olivero OA, Shi X, Antiochos BB, Poirier MC, and Walker VE (2000). Zidovudine–didanosine coexposure potentiates DNA incorporation of zidovudine and mutagenesis in human cells. Proceedings of the National Academy of Sciences, 97, 12667-12671.

- Bishop JB, Witt KL, Tice RR, and Wolfe GW (2004). Genetic damage detected in CD-1 mouse pups exposed perinatally to 3'-azido-3'-deoxythymidine and dideoxyinosine via maternal dosing, nursing, and direct gavage. Environmental and Molecular Mutagenesis, 43**,** 3-9.

- Carcinogenicity (with toxicokinetics)

Emtricitabine
The studies did not show any carcinogenic potential in mice with doses up to 750 mg/kg/day nor in rats with doses up to 600 mg/kg/day. Although in these studies the highest dose of emtricitabine did not reach the maximum tolerable dose, this was considered acceptable given that animals were exposed to approximately 25 fold of human exposure.

Tenofovir DF
The rat carcinogenicity study with doses up to 300 mg/kg/day by gavage for 2 years was negative. In mice, there was a low incidence of duodenal neoplasms occurring only at the high dose of 600 mg/kg/day. At this dose, the systemic AUCss was 15 times the human one at the therapeutic dose. These findings were considered likely related to high local concentrations in the gastrointestinal tract. While the mechanism of tumour formation is uncertain, the findings are unlikely to be of relevance to humans.

- Reproductive and developmental studies

Emtricitabine
Emtricitabine had no effects on fertility at dose of 1000 mg/kg/day for the mice and 3000 mg/kg/day for the rats.

Emtricitabine was neither embryotoxic nor teratogenic in mice and rabbits (doses up to 1000 mg/kg/day).

In peri- and post natal toxicity study in mice in which emtricitabine was administered at doses up to 1000 mg/kg/day, no treatment-related effects were observed.

Overall, emtricitabine did not produce adverse effects on reproduction at dose up to 1000 mg/kg/day, a dose level at which plasma AUC exposure were about 60 fold in mice and 120 fold in rabbits higher than in humans given emtricitabine at the therapeutic dose.

Tenofovir DF
Tenofovir DF had no impact on fertility in the studies performed in rats. Tenofovir DF was neither embryotoxic in rats (doses up to 450 mg/kg/day) nor in rabbits (doses up to 300 mg/kg/day).

In peri- and post natal toxicity study in rats, administered doses up to 600 mg/kg/day, tenofovir DF significantly reduced pups survival and animal weights. The viability index was reduced in the 450 mg/kg/day group and significantly reduced in 600 mg/kg/day. A slight delay in sexual maturation was noted at $\geq$ 450 mg/kg/day and slightly longer oestrous cycles that did not affect reproductive performance. The maternal NOEL was 50 mg/kg/day and the F1 no adverse effect dose was 150 mg/kg/day.

- Local tolerance

Tenofovir DF was a severe irritant to the ocular tissue of the rabbit, a slight irritant to the skin of the rabbit and was not a contact sensitizer in guinea pigs. No studies were conducted with emtricitabine neither with the combination.

- Other toxicity studies

Emtricitabine did not produce any immunotoxic effects in CD-rats with doses up to 1000 mg/kg/day during 28 days. Given that the long-term toxicity studies with emtricitabine or tenofovir DF alone in several species, at multiples of human exposure, have not demonstrated any changes in haematology parameters or lymphoid organs that were considered to be signs of immunotoxicity, and clinical experience with emtricitabine and tenofovir DF, either alone or in combination, no immunotoxicological effects with the combination of emtricitabine/tenofovir DF would be expected. The applicant undertook however to include characterisation of the lymphocyte subsets and NK cell activity in the one month study of the combination of emtricitabine and tenofovir DF in the dog.

The potential for mitochondrial toxicity of emtricitabine was assessed as part of repeat dose toxicity studies performed in mice and cynomolgus monkeys. No treatment related mitochondrial toxicity was noted. In vitro studies in HepG2 cells are ongoing.

The potential for mitochondrial toxicity of tenofovir DF was investigated in a 28 day study in rats and in a 90 day study in woodchucks. In these studies, several parameters were assessed, including: cytochrome c oxidase, citrate synthase and mitochondrial DNA content of liver, kidney, skeletal and cardiac muscle. No evidence of mitochondrial injury was noted, based on these parameters. Moreover, in the 56 days repeat-dose toxicity study performed in monkeys, there was no sign of mitochondrial dysfunction.

The identified metabolites of either emtricitabine or tenofovir DF were assessed as part of the routine toxicology and qualification of impurities studies. The fixed dose combination tablet of emtricitabine/ tenofovir DF is not anticipated to produce any new metabolites.

The impurities and degradation products present in the fixed-dose combination tablets were qualified through the 14-day duration study in rats with crushed tablets both experimentally degraded and undegraded (high heat and humidity).

In a study assessing the efficacy against SIV infection, co-administration of tenofovir (20-30 mg/kg/day subcutaneous) and emtricitabine (50 mg/kg/day subcutaneous) to SIV-infected pig-tailed monkeys for up to 6 months caused no obvious adverse effects at dose levels many times higher than the intended clinical dosages.

Ecotoxicity/environmental risk assessment

Based on an analysis of the environmental risk posed by the use of emtricitabine, tenofovir DF, individually and in the fixed combination, no significant risk to the environment related to the use of Truvada is anticipated.

4. Clinical aspects

Introduction

Emtricitabine and tenofovir DF have already been approved as separate medicinal products for the treatment of HIV in the EU. Truvada combines the two substances with the same dose as the ones approved for the individual compounds. Consequently the clinical development programme focused on the demonstration of the absence of interaction between the two substances and of the bioequivalence between the fixed combination of tenofovir DF/emtricitabine and the individual compounds administered concurrently. At the time of the submission of the application, very preliminary data were provided on two clinical studies in antiretroviral naïve patients to support the clinical efficacy and safety of emtricitabine and tenofovir when used together within antiretroviral therapies. Further data on these studies were subsequently submitted at the time of the responses to the list of questions.

The applicant claimed that all studies were performed according to Good Clinical Practices.

An overall of the clinical studies are displayed in table 1.

Table 1: Overview of the clinical studies

Study No	Design	Population	Treatment
FTC-114 (interaction study)	Open-label single centre 3 way cross-over of tenofovir DF and emtricitabine	Healthy Volunteers Enrolled : 19 (15M, 4 F)– Completed : 17 subjects	**Treatment A**: 200 mg emtricitabine, administered once daily (QD) in the morning for 7 consecutive days (with breakfast) **Treatment B**: 300 mg tenofovir DF, administered QD in the morning for 7 consecutive days (with breakfast) **Treatment C:** 200 mg emtricitabine and 300 mg tenofovir DF co-administered QD for 7 consecutive days (with breakfast) There was no washout interval between successive treatments.
GS-US-104-172 (bioequivalence study)	Randomised, open label 4 treatment single centre, 4 way cross over.	Healthy volunteers Enrolled: 44 (26 M / 18 F) Completed: 39	***Treatment A****: Tenofovir DF 300mg (single tablet) + emtricitabine 200mg (single capsule), fasting conditions* ***Treatment B****: Tenofovir DF 300mg/ emtricitabine 200mg (combined tablet), fasting conditions* ***Treatment C****: Tenofovir DF 300mg/ emtricitabine 200mg (combined tablet), high-fat meal conditions* ***Treatment D****: Tenofovir DF 300mg/ emtricitabine 200mg (combined tablet), light meal conditions*
Main clinical studies			
GS-01-934	Phase 3, 48 weeks Randomised, open-label parallel active control 24 weeks data available	Antiretroviral naïve, HIV-1 infected adult patients Planned 500 (250 in each arm)	**Group 1**: Tenofovir DF 300mg QD + emtricitabine 200mg QD + efavirenz 600mg QD* **Group 2**: lamivudine 150 mg / zidovudine 300mg BID + efavirenz 600mg OD* *If efavirenz-associated central nervous system toxicity occurred, nevirapine 200mg BID could be substituted for EFV
M02-418 (GS-02-982)	Randomised, open-label, multicentre, active controlled study 48 weeks data available	Antiretroviral-naive, HIV-1 infected patients with plasma HIV-1 RNA levels > 1 000 copies/ml. Planned: 200 (120 in QD and 80 in BID) Randomised: 196 patients 118 in the QD group and 78 in the BID group Enrolled: 190 patients 115 in the QD group and 75 in the BID group.	**Group 1**: Lopinavir 800mg / ritonavir 200mg QD + tenofovir DF 300mg + emtricitabine 200mg **Group 2**: Lopinavir 400mg / ritonavir 100mg BID + tenofovir DF 300mg + emtricitabine 200mg

Pharmacokinetics

The pharmacokinetics profile of tenofovir DF and emtricitabine as individual compounds have already been assessed. The pharmacokinetic programme for the fixed dose combination consisted therefore of:
- one pharmacokinetic interaction study (study FTC-114) aiming at evaluating the potential interaction between tenofovir DF and emtricitabine
- one study evaluating the bioequivalence and the food influence between the fixed dose combination *versus* the commercially available individual medicinal products (study GS-US-104-172).

A brief summary of the major pharmacokinetic parameters for each individual compound is presented below for reference.

- Emtricitabine

Emtricitabine was rapidly and extensively absorbed following oral administration with peak plasma concentrations occurring at 1 to 2 hours post-dose. Under fasting conditions, the absolute bioavailability of emtricitabine from the hard capsules (200 mg) was estimated to be 93 %. Administration of emtricitabine with a high-fat meal did not affect systemic exposure (AUC_{0-inf}) of emtricitabine. Emtricitabine is therefore recommended as a once daily regimen to be administered with or without food.

Following intravenous administration the steady-state volume of distribution of emtricitabine was approximately 1.4 l/kg. After oral administration, emtricitabine widely distributed throughout the body. In vitro binding of emtricitabine to human plasma proteins was < 4% and independent of concentration over the range of 0.02 to 200 µg/ml.

There was limited metabolism of emtricitabine. The biotransformation of emtricitabine includes oxidation of the thiol moiety to form the 3'-sulphoxide diastereomers (approximately 9% of dose) and conjugation with glucuronic acid to form 2'-O-glucuronide (approximately 4% of dose).

Emtricitabine did not inhibit *in vitro* metabolism mediated by the following human CYP450 isoenzymes: 1A2, 2A6, 2B6, 2C9, 2C19, 2D6 and 3A4. Also, emtricitabine did not inhibit uridine-5′-diphosphoglucuronyl transferase, the enzyme responsible for glucuronidation.

Emtricitabine was primarily excreted by the kidneys with complete recovery of the dose achieved in urine (approximately 86%) and faeces (approximately 14%). Thirteen percent of the emtricitabine dose was recovered in urine as three metabolites. The systemic clearance of emtricitabine averaged 307 ml/min. Following oral administration, the elimination half-life of emtricitabine was approximately 10 hours.

- Tenofovir DF

After single oral administration in fasted state, tenofovir DF was rapidly absorbed, with time to peak concentration (T_{max}) of approximately 1 hour, and was converted to tenofovir. After a high fat meal, T_{max} was delayed by approximately 1 hour. C_{max} and AUC were enhanced by a high-fat meal (AUC_{0-t} and $AUC_{0-\infty}$ were 40% higher following administration with food). Tenofovir DF is recommended as a once-daily regimen to be taken with food.

Following intravenous administration, the steady-state volume of distribution of tenofovir was approximately 0.8 l/kg. After oral administration, tenofovir DF widely distributed throughout the body. *In vitro* protein binding of tenofovir to plasma or serum protein was less than 0.7 and 7.2%, respectively, over the tenofovir concentration range 0.01 to 25 µg/ml.

In vitro studies determined that neither tenofovir DF nor tenofovir were substrates for the CYP450 enzymes. Tenofovir did not inhibit in vitro metabolism mediated by any of the major human CYP450 isoforms involved in biotransformation of compounds.

Tenofovir was primarily excreted renally with approximately 70-80% of the dose excreted unchanged in urine following intravenous administration. The systemic clearance of tenofovir averaged approximately 300 ml/min. Renal clearance was estimated to be approximately 210 ml/min, which was in excess of the glomerular filtration rate. This indicated that active tubular secretion is an important part of the elimination of tenofovir. Following oral administration the elimination half-life of tenofovir was approximately 12 to 18 hours.

- Pharmacokinetic interaction between tenofovir and emtricitabine

In-vitro phosphorylation

The intracellular phosphorylation of tenofovir and emtricitabine to their active anabolites (tenofovir-DP and FTC-TP) was investigated *in vitro* for potential antagonism. Free tenofovir and the mono- and di-phosphorylated forms (PMPA, PMPAp and PMPApp) were quantified as well as FTC-TP. When tenofovir and emtricitabine were incubated together (10 μM each), similar levels of PMPApp and FTC-TP were observed at 2 and 24 hours as compared to the incubation of each alone.

Experiments were also carried out using a pro-drug of tenofovir (GS-7340) that more efficiently delivered PMPA, PMPAp and PMPApp to cells to determine if higher levels of tenofovir metabolites could affect the metabolism of emtricitabine to FTC-TP. There was no significant effect on the anabolism of emtricitabine compared with testing alone and no effects were seen on GS-7340 anabolism with and without emtricitabine.

In-vitro studies relevant to renal excretion

Both emtricitabine and tenofovir are excreted mainly unchanged in the urine with active secretion into the renal tubule. In an *in vitro* study, a *Xenopus* oocyte expression system was used to evaluate the interaction of adefovir, cidofovir, and tenofovir with another major human renal organic anion transporter hOAT3 and two cation transporters hOCT1 and hOCT2. The two latter were unable to transport any of the tested nucleoside phosphonates but hOAT3 was found to transport adefovir and tenofovir. At 100 μM, adefovir and tenofovir did not inhibit hOAT3 whereas 1,000 μM concentrations gave 23% and 38% inhibition, respectively. The maximum plasma concentrations of adefovir and tenofovir and their affinities towards hOAT1 and hOAT3 indicated that they had a low potential for interactions due to the inhibition of major renal transporters.

Co-administration in vivo

To support the combination tablet of emtricitabine and tenofovir DF, the steady state pharmacokinetics of these agents when administered alone and together were evaluated in study FTC-114 in healthy volunteers. An outline of the study is presented in table 1.

A summary of the pharmacokinetic parameters following administration alone and then combined is presented in table 2.

Table 2: Emtricitabine (FTC) and tenofovir PK parameters

Pharmacokinetic Parameter	**Geometric Mean**		**Geometric Least Square Means Ratio (90% CI)**	
	FTC	**FTC + Tenofovir DF**		
Emtricitabine PK Parameters				
AUC_{tau} (hr•ug/ml)	10.00	10.62	1.065	(0.997, 1.137)
$C_{max, ss}$ (ug/ml)	1.73	1.67	0.962	(0.872, 1.061)
$C_{min, ss}$ (ug/ml)	0.06	0.07	1.201	(1.117, 1.291)
Tenofovir PK Parameters	**Tenofovir DF**	**Tenofovir DF + FTC**		
AUC_{tau} (hr•ng/ml)	2768	2757	1.000	(0.922, 1.086)
$C_{max, ss}$ (ng/ml)	273	281	1.026	(0.951, 1.106)
$C_{min, ss}$ (ng/ml)	52	53	1.020	(0.922, 1.128)

Based on ANOVA analysis, C_{max} and AUC emtricitabine at steady state were not affected by tenofovir co-administration while $C_{min,ss}$ increased by approximately 20%. A similar analysis showed no notable effect of emtricitabine on the pharmacokinetics of tenofovir.

The data support the lack of effect on the steady state pharmacokinetics of either substance when emtricitabine and tenofovir DF are co-administered after standard breakfast that contained a substantial amount of calories and fat.

- Bioequivalence

Study GS-104-172 was an open-label, randomised, four-way crossover study in healthy volunteers. Subjects were assigned to a treatment sequence according to a computer-generated randomisation scheme. The four treatments administered to subjects on days 1, 8, 15, and 22 were:

A: emtricitabine 200 mg (single capsule) + tenofovir DF 300 mg (single tablet), fasting conditions
B: emtricitabine 200 mg/Tenofovir DF 300 mg (combined tablet), fasting conditions
C: emtricitabine 200 mg/Tenofovir DF 300 mg (combined tablet), fed (high-fat meal) conditions
D: emtricitabine 200 mg/Tenofovir DF 300 mg (combined tablet), fed (light meal) conditions

The high-fat meal contained approximately 784 kcal, comprising approximately 58% fat, 26% carbohydrates, and 16% protein. The light meal contained approximately 373 kcal, comprising approximately 20% fat, 68% carbohydrates, and 12% protein.

The results displayed in table 3 demonstrated that in a fasted state, the fixed-dose combination provides emtricitabine and tenofovir DF exposures equivalent to those provided with the individual formulation given concurrently (90 % CI for treatment B/A of AUC and C_{max} compatible with the pre-defined hypothesis of bioequivalence (80 to 125 %).

Table 3: 90% CI for GM Ratios of Emtricitabine and Tenofovir for Treatment B versus A

Pharmacokinetic Parameter	Geometric Least Squares Means		Geometric Mean Ratio (%)	90% Confidence Interval
	Treatment B (N = 39)	Treatment A (N = 39)		
Emtricitabine PK Parameters				
C_{max}	2.03	2.11	96.5	89.5–104.0
AUC_{0-t}	10.11	10.10	100.1	95.9–104.5
$AUC_{0-\infty}$	10.42	10.40	100.2	96.2–104.4
Tenofovir DF PK Parameterss				
C_{max}	240.90	256.20	94.0	85.8–103.0
AUC_{0-t}	1505.30	1505.00	100.0	94.0–106.5
$AUC_{0-\infty}$	1854.08	1848.43	100.3	94.6–106.3

a Geometric least squares means are obtained by the back-transformation of least squares means of the parameters based on the natural logarithm scale.
b Treatment B = Tenofovir DF/emtricitabine combination tablet administered to fasted subject.
c Treatment A = co-administration of Tenofovir DF and emtricitabine to fasted subject.

This study also assessed the food effect either as a high fat meal or a light fat meal on the PK parameter of tenofovir DF and emtricitabine following administration of the fixed-dose combination tablet.

As shown in table 4, plasma tenofovir concentration-time profiles differed between treatment B (fixed dose combination under fasted conditions) and treatments C (high-fat meal) and D (light meal) although profiles for treatments C and D were very similar. Concentrations were lower in the fasted state, as reported previously with tenofovir DF alone.

Table 4: Tenofovir PK after Administration of fixed dose combination tablet to Fasted and Fed Subjects

Tenofovir PK Parameter	Treatment B[a] (N = 39) Mean ± SD	Treatment C[b] (N = 39) Mean ± SD	Treatment D[c] (N = 39) Mean ± SD
C_{max} (ng/ml)	253.63 ± 83.46	293.62 ± 87.77	290.17 ± 96.34
T_{max} (hr)[d]	0.75 (0.50, 2.50)	1.50 (0.50, 4.00)	1.50 (0.50, 4.00)
AUC_{0-t} (ng•hr/ml)	1605.84 ± 534.36	2246.78 ± 603.59	2207.18 ± 575.06
$AUC_{0-\infty}$ (ng•hr/ml)	1961.07 ± 594.47	2581.82 ± 643.86	2561.12 ± 628.86

a Treatment B = Tenofovir DF/emtricitabine combination tablet administered to fasted subject.
b Treatment C = Tenofovir DF/emtricitabine combination tablet administered with a high-fat meal.
c Treatment D = Tenofovir DF/emtricitabinecombination tablet administered with a light meal.
d Median (min, max).

Administration of the combination tablet after either a high-fat or light meal was associated with a delay in the tenofovir T_{max} relative to the fasted state. Both high-fat and light meals increased C_{max} tenofovir (by approximately 16% and 13.5%, respectively) and there were corresponding increases of 35% and 34% in $AUC_{0-\infty}$. On comparing the two fed state results with fasting results, the 90% confidence intervals for the ratios of geometric means for C_{max} and $AUC_{0-\infty}$ fell outside the 80% to 125% limits except for the comparison of C_{max} between B and D (see table 5).

Table 5: Effect of food on PK of tenofovir administered as fixed dose combination tablet

	Geometric Least Squares Means			Treatment C: Treatment B		Treatment D: Treatment B	
Tenofovir PK Parameter	Treatment B[b] (N = 39)	Treatment C[c] (N = 39)	Treatment D[d] (N = 39)	Geometric Mean Ratio (%)	90% Confidence Interval	Geometric Mean Ratio (%)	90% Confidence Interval
C_{max}	240.90	279.52	273.36	116.0	105.9–127.1	113.5	103.6–124.3
AUC_{0-t}	1505.30	2162.02	2115.57	143.6	134.9–152.9	140.5	132.0–149.6
$AUC_{0-\infty}$	1854.08	2499.27	2481.29	134.8	127.2–142.9	133.8	126.2–141.9

b Treatment B = Tenofovir DF/FTC combination tablet administered to fasted subject.
c Treatment C = Tenofovir DF/FTC combination tablet administered with a high-fat meal.
d Treatment D = Tenofovir DF/FTC combination tablet administered with a light meal.

Emtricitabine pharmacokinetic parameters after ingestion of food (high-fat or light meal) were essentially the same as those for the fasting state. The 90% confidence intervals for the ratios of geometric means for C_{max} and $AUC_{0-\infty}$ after either a high-fat or light meal were contained within 80% to 120%, indicating that the rate and extent of absorption of emtricitabine after ingestion of a meal or after an overnight fast were similar. These results indicate that food intake has no notable effect on emtricitabine pharmacokinetics (see table 6).

Table 6: Food effect on PK Emtricitabine administered as the fixed dose combination tablet

	Geometric Least Squares Means			Treatment C: Treatment B		Treatment D: Treatment B	
Emtricitabine PK Parameter	Treatment B[b] (N = 39)	Treatment C[c] (N = 39)	Treatment D[d] (N = 39)	Geometric Mean Ratio (%)	90% Confidence Interval	Geometric Mean Ratio (%)	90% Confidence Interval
C_{max}	2.03	1.93	1.96	94.7	87.9–102.0	96.6	89.6–104.0
AUC_{0-t}	10.11	9.77	9.79	96.7	92.7–100.9	96.8	92.8–101.0
$AUC_{0-\infty}$	10.42	10.12	10.15	97.1	93.3–101.2	97.4	93.5–101.5

b Treatment B = Tenofovir DF/FTC combination tablet administered to fasted subject.
c Treatment C = Tenofovir DF/FTC combination tablet administered with a high-fat meal.
d Treatment D = Tenofovir DF/FTC combination tablet administered with a light meal.

A concern was raised related to the applicant's initially proposed dose recommendation to be taken with food or without food. In view of these data and the results from the clinical studies, the CHMP considered that in order to optimise the absorption of tenofovir, it was recommended to take Truvada with food. This has been included in the Summary Product Characteristics.

A concern was also raised on the fact that the bioequivalence was tested in the fasted conditions. The applicant has adequately addressed this point showing that Truvada tablets would provide similar plasma levels of emtricitabine and tenofovir to those obtained after administration of the individual formulations of emtricitabine and tenofovir (alone or concomitantly) in the fed or fasted state.

Interaction studies

No specific study has been performed with the fixed-dose combination tablet, however, no new interactions compared to the ones already known with each substance are expected.

The information on the individual compounds relevant for the fixed combination has been mentioned in the Summary of Product Characteristics.

Interaction between emtricitabine and tenofovir DF with lopinavir/ritonavir

In study M02-418 (presented in table 1), the interaction between emtricitabine and tenofovir DF with lopinavir/ritonavir (LPV/RTV) was assessed. Emtricitabine has not been evaluated previously for effects on LPV/RTV pharmacokinetics. Co-administration of tenofovir and LPV/RTV twice daily for 14 days in a previous study gave 15% decreases in C_{max} and AUC lopinavir with 30% increases in these parameters for tenofovir. Similar findings for tenofovir but not for lopinavir were obtained in another study in which co-administration was in the fed state. These results were unexpected and remain unexplained.

Overall, the comparisons between previous findings and the pharmacokinetic results from this study do not allow for further comment on whether tenofovir exerts a consistent effect on plasma levels of lopinavir/ritonavir. Also, it is not possible to make a definitive statement regarding any possible effect of emtricitabine on lopinavir/ritonavir. However, it can be assumed any additional effect of emtricitabine that might exist is unlikely to be clinically relevant.

- Special populations

Renal impairment: The pharmacokinetics of the fixed dose combination have not been studied in patients with renal impairment. Since no pharmacokinetic interaction between emtricitabine and Tenofovir DF was observed in FTC-114, it could be considered that the data obtained previously with the individual compounds could be extrapolated to emtricitabine/tenofovir DF as a fixed dose combination.

In patients with creatinine clearance [CL_{Cr}] 50-80 ml/min, the pharmacokinetics of tenofovir and emtricitabine were similar to data from healthy subjects and HIV-infected patients with normal renal function (supported by modelling data). No dose adjustment is therefore required.

In patients with CL_{Cr} 30-49 ml/min, there was a marked reduction in the renal elimination of tenofovir and higher systemic exposure. Pharmacokinetic simulations predicted that dosing with tenofovir DF 300 mg every 48 hours would limit accumulation and achieve C_{min} values similar to those observed in patients with normal renal function. Similarly, there was an increase in emtricitabine AUC and corresponding decrease in CL/F in such patients. Pharmacokinetic simulations predicted that dosing emtricitabine once every 48 h would achieve exposures that were within 20% of those in subjects with normal renal function. Since the interval in moderate renal impairment is the same for tenofovir DF and emtricitabine, i.e. 48 hours, the same recommendation for dosing interval is considered appropriate for the fixed dose combination.

In contrast, dose interval recommendations for tenofovir DF and emtricitabine in patients with severe renal impairment are different, therefore Truvada is not recommended in patients with CL_{Cr} below 30 ml/min nor in haemodialysis patients.

Because safety and efficacy of these dose interval recommendations have not been clinically evaluated in HIV-1 infected patients, a new clinical study (GS-104-0235) has been initiated to evaluate the safety, antiviral activity and pharmacokinetics of emtricitabine and tenofovir after administration as Truvada to HIV-1 infected patients with mild, moderate and severe renal impairment. The results of this study will be provided as part of the follow-up measures to be fulfilled post-authorisation.

Hepatic impairment: The pharmacokinetics of the fixed-dose combination have not been studied in patients with hepatic impairment. No dose adjustment is required for tenofovir DF in patients with hepatic impairment. Based on minimal hepatic metabolism and the renal route of elimination for emtricitabine, it is unlikely that a dose adjustment would be required for Truvada in patients with hepatic impairment.

Children: the fixed combination of emtricitabine/tenofovir DF is not recommended in children and adolescents below 18 years. The safety and efficacy of Truvada have not been established in patients under the age of 18 years.

Pharmacodynamics

- Primary pharmacology

No data pertaining to the pharmacodynamics of the fixed-dose combination of emtricitabine/tenofovir DF has been provided.

The data on the individual substances showed a correlation between the dose and the baseline susceptibility and anti-viral efficacy. Further data on resistance are presented under each clinical study.

Clinical efficacy

Main studies

The demonstration of the antiviral activity of the fixed-dose combination emtricitabine/tenofovir DF as once daily regimen is based on the results of two studies (GS-01-934 andM02-418) in which emtricitabine was co-administered with tenofovir as part of an antiretroviral regimen in antiretroviral naïve patients.

Study GS-01-934

This is an ongoing open label study aiming to compare tenofovir DF + emtricitabine to a fixed dose combination of lamivudine/zidovudine (LAM/ZDV) when each was administered in combination with efavirenz over 48 weeks.

- Study Participants

Antiretroviral naïve patients with HIV-1 RNA levels > 10,000 copies/ml were eligible. Patients were stratified on the basis of screening CD4 cell count (< or ≥ 200 cells/mm^3).

- Treatments

Patients were randomised (1:1) to receive either:
- tenofovir DF, emtricitabine and efavirenz together without regard to meals (preferably at bedtime)
- or the fixed dose tablet of LAM/ZDV in the morning with or without food and one fixed dose tablet of LAM/ZDV with efavirenz without regards to meals (preferably at bedtime).

- Outcomes/endpoints

The primary efficacy variable is the proportion of patients in each group who achieved and maintained HIV RNA < 400 copies/ml through week 48 defined by the FDA's Time to Loss of Virological Response (TLOVR) algorithm.

Missing values and treatment discontinuations are considered as failures. The secondary efficacy variable is the proportion of patients in each group who achieved and maintained HIV RNA < 50 copies/ml through week 48 defined by the FDA's Time to Loss of Virological Response (TLOVR) algorithm.

- Sample size and Statistical methods

Non-inferiority for emtricitabine + tenofovir DF compared with LAM/ZDV arm will be concluded if the lower confidence bound 95% confidence interval (CI) is greater than – 13% ("delta" of 0.13). The sample size of 500 (250 in each arm) has been determined with the hypothesis of 70% response rate for each regimen at week 48, with 85 % of power to establish non-inferiority between the two arms.

Results

- Patients disposition

In the initial application, results from the interim analysis (182 patients included) had been provided. These data have been subsequently substantiated with the results from the planned 24-week interim analysis.

At the cut-off for the planned 24-week interim analysis, all patients treated (n = 511) had completed the week 24 study visit (median: 32 weeks; maximum: 50 weeks).

Demographics were similar between treatment groups as shown in table 7.

Table 7: Patient demographics (mITT)

Characteristic	FTC + Tenofovir DF + EFV (N = 244)		LAM/ZDV + EFV (N = 243)	
Male	210	86%	210	86%
Female	34	14%	33	14%
Caucasian	138	57%	148	61%
Black	61	25%	50	21%
Hispanic	36	15%	38	16%
Asian	3	1%	3	1%
Other	6	2%	3	1%
Mean Age ± SD	37.4 ± 9.72		37.6 ± 9.07	
Plasma HIV-1 RNA (log_{10} copies/ml)				
Mean ± SD	5.03 ± 0.54		5.00 ± 0.51	
Median	5.02		4.99	
Min, Max	3.56, 6.48		3.84, 6.54	
CD4 Cell Count (cells/mm^3)				
Mean ± SD	245 ± 164		241 ± 154	
Median	233		237	
Min, Max	3, 803		2, 1191	
HIV Status, n (%)				
Asymptomatic	35	14%	26	11%
Symptomatic HIV Infection	113	46%	119	49%
AIDS	96	39%	98	40%

FTC = emtricitabine; EFV = efavirenz; LAM/ZDV = lamivudine/zidovudine as fixed dose combination tablet

Overall 85/511 patients discontinued treatment by week 24. The rate of discontinuation was 21% for LAM/ZDV (54/254) and 12% for emtricitabine + tenofovir DF (31/257) and adverse events were the commonest reason (9% and 3% respectively) for discontinuation.

There were 24/511 patients (4.7%) with detectable primary resistance mutations to NNRTIs at baseline (see below), of which 5 and 2 per group were discontinued because of sub-optimal virological responses. Because of the potential for early virological failure and the development of additional resistance mutations in these 24 patients, the applicant was advised to notify the investigators responsible and they were excluded from the primary efficacy analysis. Therefore, a mITT population was derived and efficacy analysis sets for the 24-week interim analysis were as follows:

- Primary: Modified Intent-to-Treat (mITT): All patients (a) randomised into the study (b) received at least one dose of study medication (c) had no major protocol eligibility violations and (d) no baseline NNRTI resistance.

- Secondary: Intent-to-Treat (ITT): As for mITT except for (d).

Table 8: Patient disposition

	FTC + Tenofovir DF + EFV (N)	LAM/ZDV + EFV (N)
No. of Patients Randomised and Treated	257	254
No. of Patients with Major Protocol Violations[a]	2[b]	0
No. of Intent-To-Treat Patients	255	254
No. of Patients with Baseline Primary NNRTI Resistance Mutations	13[b]	11
No. of Modified Intent-To-Treat Patients	244	243

a Prior antiretroviral therapy
b Both patients with major protocol violations also had baseline NNRTI resistance
FTC = emtricitabine; EFV = efavirenz; LAM/ZDV = lamivudine/zidovudine as fixed dose combination tablet

Efficacy results

The results at 24 weeks are presented in table 9.

Table 9: Responders (mITT) at Week 24 by FDA TLOVR Algorithm

Response Rate	FTC + Tenofovir DF + EFV		LAM/ZDV + EFV		Difference FTC/Tenofovir DF – LAM/ZDV (95% CI)[a]
HIV RNA < 400 copies/ml, n/N %					
All Patients	215/244	88%	195/243	80%	7% (+1%, 13%)
HIV RNA > 100,000 copies/ml	112/128	88%	92/119	77%	7% (–1%, 16%)
HIV RNA ≤ 100,000 copies/ml	103/116	89%	103/124	83%	6% (–3%, 15%)
CD4 < 200 cells/mm^3	86/101	85%	76/104	73%	12% (+1%, 23%)
CD4 ≥ 200 cells/mm^3	129/143	90%	119/139	86%	5%, (–3%, 12%)
HIV RNA < 50 copies/ml, n/N %					
All Patients	180/244	74%	161/243	66%	7% (–1%, 15%)
HIV RNA > 100,000 copies/ml	86/128	67%	66/119	56%	12% (+1%, 24%)
HIV RNA ≤ 100,000 copies/ml	94/116	81%	95/124	77%	5% (–6%, 15%)
CD4 < 200 cells/mm^3	65/101	64%	58/104	56%	9% (–5%, 22%)
CD4 ≥ 200 cells/mm^3	115/143	80%	103/139	74%	6% (–3%, 16%)

a The confidence interval was stratified on baseline CD4 stratum and computed under normal approximation to the binomial distribution.

Although there was a significant imbalance in the rate of premature discontinuation between the two treatment arms, mainly due to withdrawals for adverse events, the efficacy results in the various defined populations were consistent and reassuring with regard to likely comparability between the two treatment arms.

There was a concern over the choice of the 400 copies/ml cut-off, rather than 50 copies/ml which is the goal to achieve and maintain in a previously antiretroviral-naïve population. However, the applicant pointed out that defining sub-optimal responses as not achieving HIV RNA levels below 400 copies/ml at week 32 was consistent with advice on intervention from the British HIV Association and the US Department of Health and Human Services treatment guidelines.

Regarding the choice of the delta, which was also questioned, the applicant based it on consideration that the study compares two study compounds (tenofovir DF +emtricitabine) versus two active controls (LAM/ZDV) on a background of a single medicinal product (EFV). Conventionally, the delta would be justified by the contribution of the active control drugs over EFV alone. However, there are no guidelines or data on EFV monotherapy over 48 weeks. The current standard for equivalence trials based on FDA guidance is to use a delta of 10% – 12% for comparing triple antiretroviral regimens where a single agent is compared with an active control. In GS-01-934, a delta that is slightly larger than this was specified because the contribution of two substances over EFV was assumed to be larger than that of a single substance. The comparisons with LAM/ZDV at 24 weeks show a lower 95% CI that does not exceed –6 %. Therefore, it was considered that the applicant's pre-determined choice of delta at week 48 was not an issue for week 24 results.

Within the subgroups defined by viral loads and CD4 cell counts as above, proportions reaching and maintaining viral loads at the two cut-offs were consistently numerically higher for the FTC + TDF group.

Mean changes from baseline to week 24 in plasma HIV-1 RNA levels were −3.29 and −3.21 $\log_{10}$ copies/ml per group with mean changes in CD4 cell counts of 129 and 111 cells/mm^3.

Provision of the final report at 48 weeks will be provided as part of the follow-up measures to be fulfilled post-authorisation.

- Resistance

Samples for resistance testing were obtained at baseline, weeks 8, 16, 24 and early study treatment discontinuation visits. There were 24/511 with genotypic resistance to NNRTIs at baseline excluded from the mITT population. Of these, 7 met resistance analysis criteria by week 24 (3 and 4 per group) and 6/7 had the K103N mutation at baseline while one had the K101E and G190A mutations. Phenotypic resistance to efavirenz was detected in all seven patients at baseline (range: 17-fold to 188-fold reduced susceptibility). The M184V mutation developed in 6/7 patients and additional NNRTI mutations (L100I, V108I, Y188L and P225H) developed in 4/7.

In the mITT population, 32 patients met the resistance analysis criteria by week 24 (FTC+ TDF = 17; LAM/ZDV = 15). Post-baseline genotypes were obtained for 18 patients, of which 5/10 and 3/8 per group failed virologically but had virus that remained wild type or identical to the baseline sequence. M184V/I developed in 3 of 18 patients in the resistance analysis but no patient had this at baseline and M184V/I did not develop without efavirenz resistance. No patient in either arm developed the K65R mutation or had this at baseline or developed classical TAMs.

Resistance to the NNRTI class was the most common form of genotypic resistance that emerged (10/487 = 2% of the mITT population). The frequency of development of resistance to efavirenz was similar between the two treatment arms at 2% and the K103N mutation was the most common efavirenz resistance mutation that developed.

Study M02-418 (GS-02-982)

This is an open-label study primarily designed to compare once-daily and twice-daily lopinavir/ritonavir (LPV/RTV) when administered in combination with tenofovir and emtricitabine in the treatment of antiretroviral-naïve patients. Emtricitabine and tenofovir DF were to be taken once daily with food in the morning along with the lopinavir/ritonavir dose.

- Study participants

Adults HIV-infected patients with HIV RNA >1000 copies/ml and no prior antiretroviral treatment were eligible. There was no CD4 cell count restriction.

- Treatment

Patients were randomised (3:2) to receive either 800 mg lopinavir/200 mg ritonavir once daily or 400 mg lopinavir/100 mg ritonavir twice daily, each in combination with 300 mg tenofovir DF and 200 mg emtricitabine once daily.

- Outcomes/endpoints

The primary efficacy variable was the proportion of patients with HIV RNA below 50 copies/ml at week 48. This was measured using an intent-to-treat analysis in which missing values were considered failure (ITT M=F) unless the immediately preceding and following values were below 50 copies/ml.

- Sample size and statistical methods

The once daily LPV/RTV group was to be considered non-inferior to the twice daily group if the lower 95% CI around the difference at the < 50 copies/ml level (based on the normal approximation to the binomial distribution) was above –20%.

Results

- Patients disposition

Of 196 patients randomised, three per group were not treated. Of the 190 remaining, 45 (24%) discontinued from the study before week 48, mostly due to adverse events or HIV-related events. Approximately 45% of patients had baseline CD4 cell counts < 200 cells/mm^3 and 38% had baseline plasma HIV-1 RNA > 100,000 copies/ml. The mean time since HIV diagnosis was 2.3 years.

- Efficacy results

The results at 48 weeks are presented in table 10.

Table 10: Proportion with Plasma HIV-1 RNA Levels < 50 Copies/ml at Week 48 – GS-02-982

	LPV/RTV QD + Tenofovir DF + FTC		LPV/RTV BID + Tenofovir DF + FTC		Difference QD – BID (95% CI)
ITT (NC = F)[a]	81/115	70%	48/75	64%	6% (–7%, 20%)
ITT (M = F)[b]	80/115	70%	47/75	63%	7% (–7%, 21%)
Observed data[c]	80/89	90%	47/54	87%	3% (–8%, 14%)

a Intent-to-treat (ITT) non-completer equals failure (NC = F) analysis
b ITT missing equals failure (M = F) analysis: Any patient with a missing value for any reason at a given visit was considered > 50 copies/ml.
c Observed data: Missing values were excluded from the analysis.

The ITT (N=F) 48-week response rates were consistent with the 67% previously reported in Abbott study M98-863 for a twice-daily regimen of LPV/RTV and two NRTIs.

Using the TLOVR algorithm, 71% and 65% in the once and twice daily groups achieved and maintained confirmed plasma HIV-1 RNA levels < 50 copies/ml through 48 weeks (95% CI: –8%, 20%). In subgroups defined by viral load and CD4 cell count at baseline proportions of patients with HIV-1 RNA < 50 copies/ml at week 48 on once daily LPV/RTV were numerically higher in patients with ≥ 100,000 copies/ml and < 200 cells/mm^3.

The mean decrease in viral load from baseline to week 48 was –3.14 $\log_{10}$ copies/ml in the once daily and –3.00 $\log_{10}$ copies/ml in the twice daily group. Mean changes in CD4 cell counts were 185 cells/mm^3 and 196 cells/mm^3, respectively.

- Resistance

Genotypic resistance testing was conducted for all available samples in which HIV-1 RNA was > 500 copies/ml from weeks 12 to 48. Of the 15 patients with available genotypic resistance data, 3 patients demonstrated an M184V/I mutation in reverse transcriptase, indicating the development of emtricitabine resistance. The K65R mutation did not occur and no other RT mutations suggestive of NRTI resistance emerged.

With respect to a potential impact of gender in term of efficacy, very limited data from these two studies (only 14 % of patients were female) did not suggest any difference.

Regarding the possible use of emtricitabine/tenofovir DF fixed dose combination with other NRTIs

There have been reports of a high rate of virological failure and of emergence of resistance at early stage when tenofovir disoproxil fumarate was combined with lamivudine and abacavir as well as with lamivudine and didanosine as a once daily regimen. The issue of possible sub-optimal efficacy of tenofovir plus lamivudine with either abacavir or didanosine remains unresolved at present, but this is still under investigation. There is as yet no indication of a pharmacokinetic or pharmacodynamic interaction that might explain the findings. Currently, there are no data on the efficacy of emtricitabine and tenofovir DF when administered with a third NRTI. Since there is a close structural similarity between lamivudine and emtricitabine and similarities in the pharmacokinetics and

pharmacodynamics of these two agents, the same problems may be seen if Truvada is administered with a third nucleoside analogue. A warning has been included in the Summary of Product Characteristics.

Antiretroviral experienced patients

There are currently no data on the use of emtricitabine plus tenofovir DF for the treatment of antiretroviral-experienced subjects. The applicant undertook to conduct a new study (GS-US-164-0107) in these patients using emtricitabine/tenofovir DF fixed dose combination, the results of which will be submitted as part of the follow-up measures to be fulfilled post-authorisation.

HIV/hepatitis co-infection

Limited data from study GS-01-934 suggested as expected that Truvada might exert a significant effect on HBV viral loads. Only 13/511 (3%) patients were HBsAg positive at baseline. Of these, nine were randomised to tenofovir DF + emtricitabine and four to LAM/ZDV. One patient discontinued tenofovir DF + emtricitabine DF prior to week 24 because of non-compliance.

There were 4/13 patients with baseline plasma HBV DNA $\geq 1 \times 10^6$ copies/ml (three in the emtricitabine/tenofovir DF group). All patients with plasma HBV DNA $< 1 \times 10^6$ copies/ml were HBeAg negative at baseline. In the tenofovir DF + emtricitabine group, the reduction in HBV DNA ranged from 0.9 to 7.5 $\log_{10}$ copies/ml at week 24 and one seroconverted to HBeAg negative. Plasma HBV DNA for one LAM/ZDV group patient had decreased by 4.3 $\log_{10}$ copies/ml at week 24.

The mean baseline serum ALT for these patients was 67 mg/dl in the tenofovir DF + emtricitabine group and 38 mg/dl in the LAM/ZDV group. The mean changes from baseline to week 24 were –46 mg/dl and 0 mg/dl, respectively.

In addition, 26/511 patients were HCV antibody positive at baseline (10 tenofovir DF + emtricitabine). Two discontinued LAM/ZDV prior to week 24 (due to anaemia or lost to follow-up). The mean baseline serum ALT was 40 mg/dl in the FTC/TFV and 58 mg/dl in the LAM/ZDV group. Mean changes from baseline to week 24 were +25 mg/dl and +17 mg/dl, respectively.

Clinical safety

The safety of both emtricitabine and tenofovir DF has been established in adults in multiple controlled clinical trials and corroborated by several years of post-marketing experience (for tenofovir, approximately 200,000 patient-years of exposure to 31 December 2003 as calculated from sales data).

The evaluation of the safety profile of emtricitabine/tenofovir DF as fixed dose combination focused therefore on data relevant to the co-administration of emtricitabine and tenofovir.

- Patient exposure

Forty-four healthy volunteers received single doses of emtricitabine/tenofovir DF as fixed dose combination tablets in the bioequivalence study GS-US-104-172 and 19 subjects received single concomitant doses of the current formulations of emtricitabine and tenofovir DF in the interaction study FTC-114.

Interim 24-week safety data for the study GS-01-934 safety dataset (n = 511) are presented (last 24 week visit 2 July 2004). The median time on study regimen was approximately 32 weeks for both treatment groups. For deaths, serious adverse events (SAEs) and discontinuations due to adverse events, all data received and entered into the database as of 16 July 2004 are provided.

As of the cut-off for the 48 weeks analysis of studyM02-418, data on 190 patients are available. The overall median time on study regimen was 378 days (range 4-378 days).

- Adverse events

Study FTC-114
The total and treatment-related adverse events reporting rates were slightly higher (total 6/18 subjects; 33%) with the combination compared with each substance alone (4/18 and 5/18) due to reports of gastrointestinal symptoms and headache.

Study GS-104-172
There were too few adverse events reported during this bioequivalence study for any comment to be made on rates or types of events.

Study GS-01-934 24-Week Safety Data

At the cut-off for the 24-week interim analysis, the difference in discontinuation rates prior to week 24 (12% and 21%) was mainly attributed to the higher rate of withdrawal due to adverse events in the LAM/ZDV group (3% (8/257) in tenofovir DF + emtricitabine versus 9% (22/254) in LAM/ZDV). The most frequent AE implicated was anaemia in 13 patients of the LAM/ZDV group.

The most frequent adverse events (reported in ≥ 5% of each treatment group) were dizziness, nausea and diarrhoea for tenofovir DF + emtricitabine compared to nausea, dizziness and insomnia for LAM/ZDV.

Adverse events considered possibly or probably related to treatment (see table 11) were reported more frequently for the LAM/ZDV group (45%) than for tenofovir DF + emtricitabine (30%). Incidences of the possibly/probably related events of nausea (11% *vs* 25%), vomiting (2% *vs* 7%) and anaemia (0% *vs* 6%) were at least 5% higher in the LAM/ZDV group. Two patients (< 1%) in the tenofovir DF + emtricitabine group compared with 16 patients (6%) in the LAM/ZDV group had grade 3 / 4 adverse events that were considered to be possibly or probably related to study treatment. The difference was mainly due to occurrences of anaemia and fatigue.

Table 11: Adverse Events Possibly or Probably Related to Study Treatment - GS-01-934

Adverse Event (Preferred Term)	Tenofovir DF + FTC + EFV (N = 257)		LAM/ZDV + EFV (N = 254)	
	N	%	n	%
Number of Patients Experiencing any Related Adverse Event	76	30%	115	45%
Nausea	28	11%	63	25%
Diarrhoea	14	5%	14	6%
Fatigue	8	3%	13	5%
Headache	7	3%	13	5%
Rash	5	2%	0	0%
Vomiting	5	2%	19	7%
Abdominal Distension	4	2%	3	1%
Skin Hyperpigmentation	5	2%	1	< 1%
Dizziness	3	1%	9	4%
Flatulence	3	1%	4	2%
Loose Stools	3	1%	5	2%
Abdominal Pain	2	<1%	4	2%
Abdominal Pain Upper	2	<1%	4	2%
Decreased Appetite	2	<1%	5	2%
Dysgeusia	1	<1%	5	2%
Dyspepsia	2	<1%	4	2%
Anaemia	0	0%	14	6%

There was one death reported up to the cut-off for the 24-week interim analysis – a patient in the LAM/ZDV group died due to progressive multifocal leukoencephalopathy considered to be a consequence of AIDS. Twelve tenofovir DF + emtricitabine patients (5%) and 18 LAM/ZDV patients (7%) experienced serious adverse events (SAEs). While seven LAM/ZDV patients had anaemia reported as a SAE, no other event was reported by more than one patient and these seven were the only SAEs considered related to study drugs.

There was no evidence of clinically significant renal events in the tenofovir DF + emtricitabine group and similar numbers (2%) per treatment group had events classified within the Renal and Urinary Disorder Order Class. The incidence of serum creatinine and phosphorus abnormalities was low and comparable between the two treatment groups.

Up to the cut-off, three tenofovir DF + emtricitabine and one LAM/ZDV patient had fractures but all were trauma-related and none were considered by the investigator to be related to study drugs.

There was one report of metabolic acidosis in the LAM/ZDV group and no reports of pancreatitis in either group. There were also single reports of abdominal obesity, hypercholesterolaemia and hypertriglyceridaemia in the LAM/ZDV group. Peripheral neuritis/neuropathy was reported for four tenofovir DF + emtricitabine patients but the patient's medical history and concurrent medications provided an alternative explanation for the events and none was serious or led to treatment discontinuation.

Skin hyperpigmentation was reported for five tenofovir DF + emtricitabine patients and three LAM/ZDV patients, of which all and one per group was considered to be related to the NRTI components of the regimen. However, all were of grade 1 severity and no intervention was required.

The overall incidence and severity of abnormal renal and hepatic parameters were similar between the two treatment groups. Two patients per group had a grade 1 serum creatinine elevation and one LAM/ZDV patient had a grade 2 creatinine elevation. A patient in the tenofovir DF + emtricitabine group had a grade 4 serum creatinine abnormality reported and treatment was interrupted but repeat testing 6 days later demonstrated a serum creatinine 0.8 mg/dl. Therefore, it was thought that there might have been a laboratory error and treatment was resumed without problems. The mean change in calculated creatinine clearance at week 24 was – 0.9 ml/min for the tenofovir DF/emtricitabine group and 8.03 ml/min for the LAM/ZDV group.

Study M02-418

At the cut-off 48-week analysis, 45 patients (24%) had prematurely discontinued from the study, mostly (20 patients) for AEs/HIV-related events. These were mostly gastrointestinal, particularly diarrhoea and nausea (six patients for each).

The most common AEs considered possibly or probably related to treatment were diarrhoea and nausea. Two deaths occurred during the first 48 weeks of the study (lymphoma-like syndrome and AIDS) but were not considered to be treatment-related. SAEs were reported for 18 patients, of which 11 were in the twice-daily LPV/r group. In three patients, these SAEs (one case of worsening diarrhoea, one hepatitis and one nephritis) were assessed by the investigator as possibly or probably related to lopinavir/ritonavir, tenofovir DF and/or emtricitabine.

The incidence of renal laboratory abnormalities in M02-418 was similar to the 48-week findings of study GS 99-903, in which tenofovir DF was administered in combination with efavirenz which had no effect on serum tenofovir levels, and lamivudine. However, nephritis was reported for one patient and abnormal renal function was reported for another during the first 48 weeks of the study.

No bone fractures were reported during the 48-week phase of the study. Four patients reported skin discoloration, of which one was a case of hypopigmentation and was considered by the investigator to be unrelated to therapy and one was considered possibly related.

Overall the data from the studies did not show new patterns of adverse reactions compared to experience with each agent. Very limited do not indicate any difference in the safety profile depending of the gender.

- Specific adverse reactions

Renal excretion by a combination of glomerular filtration and tubular secretion is the primary route of elimination of tenofovir and emtricitabine. In preclinical studies with tenofovir, at exposure similar or higher than that achieved following the recommended daily dose in humans, some evidence of nephrotoxicity was observed in all species (dog, rat and monkey). The clinical safety data did not indicate a causal association between tenofovir DF and renal events. Post-marketing spontaneous adverse reaction data indicated that tenofovir DF may in rare circumstances lead to renal events including renal failure, Fanconi Syndrome and other proximal tubulopathies. As for tenofovir DF as individual, monitoring of renal function is recommended before taking tenofovir DF/emtricitabine as the fixed dose combination tablet and every 4 weeks during therapy.

In the toxicology studies of tenofovir, bone abnormalities were reported. The clinical safety data demonstrated bone toxicity, including a reduction in bone mineral density with prolonged use of tenofovir DF. Post-marketing safety data support the conclusions from clinical trials. The effects of long term administration on bone metabolism and their clinical relevance are currently unknown. As for tenofovir DF as individual, a warning has therefore been added in the Summary of Product Characteristics of the fixed dose combination tablet.

Clinical safety data and post-marketing surveillance demonstrate a low risk of mitochondrial toxicity and metabolic effects with tenofovir DF and emtricitabine. However, class labelling warnings regarding mitochondrial toxicity, lactic acidosis, metabolic abnormalities and lipodystrophy are included in the Summary of Product Characteristics.

5. Overall conclusions, benefit/risk assessment and recommendation

Quality

The quality of the fixed dose combination tablet of emtricitabine/tenofovir DF was considered acceptable when used in accordance with the conditions defined in the Summary of Product Characteristics. Only a few changes were made to emtricitabine and tenofovir DF active substances already authorised via the centralised procedure. The pharmaceutical form selected was adequate taken into account the properties and the stability of the active substances and it is a well-accepted pharmaceutical form for oral administration. The excipients were commonly used for this kind of formulation and the packaging material is well documented. The manufacturing process enhanced to obtain reproducible finished product batches. Stability tests under ICH conditions indicated that the product was stable for the proposed shelf life. At the time of the CHMP there were some outstanding issues which had no impact on the benefit/risk profile. The applicant committed to provide the necessary information as follow-up measures to be fulfilled post-authorisation.

Non-clinical pharmacology and toxicology

Emtricitabine is a nucleoside reverse transcriptase inhibitor and tenofovir disoproxil fumarate is the salt of the prodrug of tenofovir, a nucleotide reverse transcriptase inhibitor. Both compounds have been shown potent antiretroviral activity against HIV-1 *in vitro* and are active *in vivo* in several animal retrovirus models.

The combination of tenofovir and emtricitabine consistently showed synergistic anti-HIV-1 activity *in vitro*. The antiviral activity of the combination of tenofovir and emtricitabine was demonstrated *in vivo* in SIV-infected macaques. In-vitro resistance selection experiments with the combination demonstrated the initial development of the M184I mutation and high-level resistance to emtricitabine, but preserved susceptibility to tenofovir.

Neither tenofovir DF nor emtricitabine had significant unwanted pharmacological activity as determined in a variety of in-vitro and in-vivo safety pharmacology studies.

The pharmacokinetics of the fixed dose combination have not been evaluated in preclinical studies. This was considered acceptable in view of the clinical data which showed that absorption characteristics of each active substance were not affected by co-administration or combination into a single dosing form.

Tenofovir was not a substrate or inhibitor of any CYP450 enzymes. Only a small fraction of the dose eliminated in urine was metabolised. Emtricitabine was subject to phase I metabolism and to some direct conjugation, both to a limited extent. Therefore, it is unlikely when the drugs are co-administered that there would be any metabolism-mediated interaction. Tenofovir and emtricitabine are primarily excreted by the kidney and clearance is by glomerular filtration with active tubular secretion. It is considered unlikely that there would be an interaction affecting elimination.

The two substances exhibited different patterns of target organ toxicity. Specifically, the only significant effect of emtricitabine identified at dose levels constituting large clinical multiples was a minor anaemia. In contrast, extensive non-clinical investigations of the toxicity of tenofovir DF showed that the target organs were gastrointestinal, bone and kidney.

Administration of the emtricitabine/tenofovir DF tablet did not exacerbate the known toxicities of the individual agents, as demonstrated in the 14-Day rat toxicology study. The applicant undertook to investigate the toxicity of the combination of emtricitabine and tenofovir DF in the dog, a species considered "more sensitive" to the renal effects of tenofovir DF, in a study of longer duration. The results will be presented as part of the follow-up measures to be fulfilled post-authorisation.

Given that there are no signs of immunotoxicity with the individual agents and that there is no known pharmacokinetic interaction or overlapping toxicities, no immunotoxicological effects of the combination would be expected, however, the applicant will further look at this aspect in the future dog study.

Emtricitabine was not mutagenic, meanwhile tenofovir DF was positive in 2 out of 3 in vitro studies but was negative in an *in vivo* micronucleus test. Because the combination of zidovudine and didanosine have been reported to potentiate genetic damage in human cells in vitro, the applicant will conduct further studies to assess whether co-administration of emtricitabine with tenofovir DF has the potential to synergistically enhance the genotoxic potential of tenofovir, the results of which will be submitted as part of the follow-up measures to be fulfilled post-authorisation.

Efficacy

Pharmacokinetics

The pharmacokinetics profile of the fixed dose combination has not been assessed, however the pharmacokinetics profile of tenofovir DF and emtricitabine have already been assessed as individual compounds. In addition, there was no pharmacokinetic interaction between tenofovir DF and emtricitabine identified in in-vitro and in vivo studies.

As a pre-requisite to support this fixed dose combination, study GS-104-172 established bioequivalence between the fixed dose combination tablet of emtricitabine/tenofovir DF and the same dose of emtricitabine and tenofovir DF administered separately in a fasted state. This study also assessed the effect of food and based on the available data, in order to optimise the absorption of tenofovir, the fixed dose combination tablet of emtricitabine/tenofovir DF should be taken with food as recommended in the Summary of Product Characteristics.

With respect to patients with renal insufficiency, based on the recommendations for the individual compounds, dosing interval adjustment of fixed dose combination tablet of emtricitabine/tenofovir DF is required in patients with moderate renal impairment (creatinine clearance between 30 and 49 ml/min). It is not recommended however for patients with severe renal impairment (creatinine clearance < 30 ml/min) and in patients who require haemodialysis. A new clinical study has been

initiated to evaluate the safety, antiviral activity and pharmacokinetics of emtricitabine and tenofovir after administration as fixed dose combination tablet to HIV-1 infected patients with mild, moderate and severe renal impairment, the results of which will be submitted as part of the follow-up measures to be fulfilled post-authorisation. The pharmacokinetics information on the individual components which are relevant for the fixed dose combination has been included in the Summary of Product Characteristics.

Clinical efficacy

The efficacy of emtricitabine and tenofovir DF as individual compounds has already been assessed in antiretroviral naïve and experienced patients. There are no clinical data obtained with the emtricitabine/tenofovir DF as fixed dose combination.

To support the fixed combination, the applicant provided the results from two studies in antiretroviral naïve patients where tenofovir DF was co-administered with emtricitabine.

Preliminary 24-week data from an ongoing, open label clinical study (GS-01-934) in antiretroviral naïve patients treated with tenofovir DF plus emtricitabine and efavirenz once daily showed similar antiviral effect to the fixed dose combination of lamivudine/zidovudine twice daily with efavirenz once daily. The percentage of patients who achieved and maintained a viral load < 400 copies/ml was 88% (215/244) in the tenofovir DF plus emtricitabine group and 80% (195/243) in the zidovudine/lamivudine group (p-value = 0.019); the corresponding percentages for a viral load < 50 copies/ml were 74% (180/244) in the tenofovir DF plus emtricitabine group and 66% (161/243) in the zidovudine/lamivudine group (p-value = 0.075). The applicant undertook to submit the final results at 48 weeks as part of the follow-up measures to be fulfilled post-authorisation.

In the second open label study, patients were treated once daily with emtricitabine and tenofovir DF in combination with lopinavir/ritonavir given once or twice daily. At 48 weeks, 70% (81/115) and 64% (48/75) of patients demonstrated HIV RNA < 50 copies/ml with the once and twice daily regimens of lopinavir/ritonavir, respectively.

There are currently no data on the use of emtricitabine plus tenofovir DF in antiretroviral-experienced patients. The applicant undertook to submit the results of a planed study in this population using emtricitabine/tenofovir DF as fixed dose combination.

In term of resistance, it has been seen *in vitro* and in some HIV-1 infected patients due to the development of the M184V/I mutation with emtricitabine and the K65R mutation with tenofovir. No other pathways of resistance to emtricitabine or tenofovir have been identified. Emtricitabine-resistant viruses with the M184V/I mutation were cross-resistant to lamivudine but retained sensitivity to didanosine, stavudine, tenofovir, zalcitabine and zidovudine. The K65R mutation can also be selected by abacavir, didanosine or zalcitabine and results in reduced susceptibility to these agents plus tenofovir and emtricitabine. Patients with HIV-1 expressing three or more thymidine analogue associated mutations (TAMs) that included either the M41L or L210W reverse transcriptase mutation showed reduced susceptibility to tenofovir DF.

There have been reports of a high rate of virological failure and of emergence of resistance at early stage when tenofovir DF was combined with lamivudine and abacavir as well as with lamivudine and didanosine as a once daily regimen. At present the reason for this finding is unknown. Since emtricitabine has a close structural similarity with lamivudine the CHMP agreed to add a warning in the Summary of Product Characteristics as the same problems may be seen if Truvada is administered with a third nucleoside analogue.

Limited clinical experience in patients co-infected with HIV and HBV suggests that treatment with emtricitabine and tenofovir DF in antiretroviral combination therapy to control HIV infection also results in a reduction in HBV DNA.

Although it has been demonstrated with the published scientific literature accumulated so far that a reduction of the pill burden and/or frequency of the pill burden could be translated into improved adherence, no data have been provided in term of adherence. The applicant undertook therefore to substantiate the potential benefit in term of adherence of this fixed dose combination in ongoing/planned studies, as part of the follow-up measures to be fulfilled post-authorisation.

Clinical Safety

The safety profile of tenofovir DF and emtricitabine as individual components have already been well defined. The adverse reactions reported with Truvada were consistent with the known safety profiles of the substances given separately. Thus far, the data from concomitant administration of the two actives do not seem to point to additional problems.

Benefit/risk assessment

Given the prior approval of the two active substances and the fact that there is no evidence of a pharmacodynamic or pharmacokinetic interaction that might impact on patients safety or efficacy, the combination table of emtricitibine/tenofovir DF might represent a benefit in terms of convenience for selected patients.

Overall considering that:

- there are no major concern raised on the quality and non-clinical aspects of the dossier submitted to support the approval of this fixed combination,
- as a mandatory pre-requisite to support this fixed dose combination of emtricitabine/ tenofovir DF, the bioequivalence has been demonstrated between the fixed combination and both substances administered individually at the same dose.
- preliminary 24 weeks data of study GS-01-934 and 48 weeks data of study M02-418 has provided efficacy data in antiretroviral naïve patients for tenofovir DF associated with emtricitabine once daily as part of an antiretroviral regimen
- there is no evidence of deterioration of the safety profile of the fixed dose combination over the individual compounds.
- simplified schedule regimens are especially critical when considering that HIV infection has become a chronic disease and that non-adherence negatively impacts on the response to treatment. Emtricitabine/tenofovir DF encompassing not only the advantage of a fixed dose combination but also of a once daily regimen has the potential to simplify the daily life of patients.

the CHMP considered that the benefit/risk ratio of the fixed dose combination emtricitabine/tenofovir DF as a one daily regimen to be taken in combination with other antiretroviral agents was favourable.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the benefit/risk ratio of Truvada in the treatment of in the treatment of HIV infection was favourable and therefore recommended the granting of the marketing authorisation in the following indication:

> "Truvada is a fixed dose combination of emtricitabine and tenofovir disoproxil fumarate. It is indicated in antiretroviral combination therapy for the treatment of HIV-1 infected adults.
> The demonstration of the benefit of the combination emtricitabine and tenofovir disoproxil fumarate in antiretroviral therapy is based solely on studies performed in treatment-naïve patients."