SCIENTIFIC DISCUSSION

1. Introduction

As of the end of 2004, an estimated 39.4 million people worldwide – 37.2 million adults and 2.2 children younger than 15 years – were living with Human Immunodeficiency virus (HIV) infection/Acquired Immunodeficiency Syndrome (AIDS).

Current treatment options consist of four different mechanistic classes of compounds:

- NRTIs (nucleoside/nucleotide reverse transcriptase inhibitors) inhibiting the reverse transcriptase (RT) of HIV by structural similarity with the substrate of RT.
- NNRTIs (non-nucleoside reverse transcriptase inhibitors) inhibiting the reverse transcriptase of HIV without being nucleosides analogues.
- PIs (protease inhibitors) inhibiting the HIV protease which is an enzyme required for the assembly and release of mature HIV particles from the cell after the replication cycle.
- Entry inhibitors: there is currently only one compound authorised which is a fusion inhibitor, (inhibits fusion of the HIV with CD4+ cells, hence infection of cells).

Regimens containing one or more of these agents are required for building combination antiretroviral therapies. The choice of the combination regimens depends on several factors such as the status of the patient, particularly in terms of plasma viral load (HIV RNA), CD4 cell counts, previous treatment(s), prior relapse and intolerance to treatment. The long-term use of all these products is, however, hampered by different factors such by the emergence of resistance, by potential toxicity and in some cases by inconvenient dosing schedules or formulations. Further therapeutic agents are therefore needed, particularly in patients who have failed their therapy and who have no or few remaining treatment options.

PREZISTA, which contains darunavir, a protease inhibitor, has been developed for highly treatment-experienced patients who have failed more than one PI regimen.

A so-called full application has been submitted for registration. PREZISTA is available as 300 mg film-coated tablets.

The recommended dose is 600 mg to be co-administered with low dose of ritonavir (100 mg as pharmacokinetic enhancer) twice daily.

The approved indication is: PREZISTA, co-administered with 100 mg ritonavir in combination with other antiretroviral medicinal products for the treatment of human immunodeficiency virus (HIV-1) infection in highly pre-treated adult patients who failed more than one regimen containing a protease inhibitor (PI).
This indication is based on week-24 analyses of virological and immunological response from 2 controlled dose range finding Phase II trials and additional data from uncontrolled studies (see section 5.1).
In deciding to initiate treatment with PREZISTA co-administered with 100 mg ritonavir careful consideration should be given to the treatment history of the individual patient and the patterns of mutations associated with different agents. Genotypic or phenotypic testing (when available) and treatment history should guide the use of PREZISTA".

2. Quality aspects

Introduction

PREZISTA is presented as film-coated tablets containing 300 mg of darunavir (as ethanolate). The other ingredients include microcrystalline cellulose, colloidal anhydrous silica, crospovidone, magnesium stearate, poly(vinyl alcohol) – partially hydrolysed, macrogol 3350, titanium dioxide (E171), talc and sunset yellow FCF (E110).
The tablets are packed in high-density polyethylene (HDPE) bottles closed with polypropylene (PP) child resistant closures.

Drug Substance

Darunavir is a white to off-white hygroscopic powder. Its solubility in organic solvents varies significantly and it is very slightly soluble in aqueous solution (solubility increases with decreasing pH). Therefore, the particle size is likely to be important to the rate and possibly to the extent of absorption of darunavir.
It contains 5 chiral centres, however the manufacturing process leads, in a consistent way, to the single enantiomer 3*R*, 3a*S*, 6a*R*, 1*S*, 2*R*. The absolute configuration has been confirmed by X-ray diffraction analysis.
Under commercial synthesis conditions, darunavir is isolated as a crystalline ethanolate (1:1 solvate).
It can exist as a non-solvated amorphous form and as a hydrate form as well. Investigations of conditions under which inter-conversion between the different polymorphs occur showed that the hydrate form can be formed under conditions of high relative humidity and that both solvates can be converted into the amorphous form when subject to heat and/or extremely low relative humidity.
Detailed information on quality/control of materials used in the synthesis, as well as on the synthesis itself, has been provided by the way of an active substance master file (ASMF).

- Manufacture

Darunavir is synthesised at two different sites from two starting materials in a 3-step synthesis followed by crystallisation in ethanol.
Satisfactory specification and associated methods have been provided for the starting materials, key intermediate, reagents and solvents in the restricted part of the ASMF.
The stereo-isomeric purity of the active is ensured by using starting materials of high stereo-isomeric purity and by controlling the enantiomeric purity of critical intermediates. Moreover, the configuration of the 3*R*, 3a*S*, 6a*R*, 1*S*, 2*R* enantiomer is energically favourable, no stereogenic centre is formed during manufacture and absence of interconversion during synthesis and storage has been demonstrated.
Process impurities originating from each starting material/reagent/solvent and during the synthesis have been adequately discussed. No degradation products have been detected during stability studies.
Validation data provided for 3 consecutive full-scale batches confirm robustness and reproducibility of the process.

- Specification

The active substance specification includes tests controlled by validated methods for appearance, identity (IR and HPLC), assay (HPLC), impurity content (HPLC), ethanol (GC), residual solvents (GC), residue on ignition, water content, heavy metals and particle size.
The formation of the desired enantiomer is ensured by controlling the enantiomeric purity of the starting materials and of the intermediates (see above). Therefore, the omission of a chiral

assay for the claimed enantiomer and a chiral purity test in the specification has been supported in this particular case.
Darunavir particle size specification is within the particle size range of tablets used in clinical trials and found to be bioequivalent (see drug product).
Impurity limits in the specification are justified by toxicology studies.
Batch analysis data provided for the nine production-scale batches, including the 3 validation batches, confirm satisfactory compliance and uniformity with the proposed specification.

- Stability

Under accelerated conditions (40°C/75% RH - commercial packaging) and long-term conditions (25°C/60% RH - commercial packaging), respectively 6-month data and up 1-year data have been provided for three batches manufactured using the commercial synthesis.
The parameters tested included appearance, assay, purity, stereo-isomeric purity, ethanol, water content and microbial purity
The proposed retest period is supported by the presented data when darunavir is stored in the proposed packaging.

Drug Product

- Pharmaceutical Development

The objective of the pharmaceutical development was to obtain an immediate-release solid oral dosage form, the poor water-solubility, the polymorphism and the physical characteristics of darunavir being the critical parameters to take into account.

After a 20 mg/ml oral solution (TF019), numerous solid oral dosage forms were developed and used in clinical studies:

- several capsule formulations (TF038, TF042, TF043, and TF044 - TF051 and TF052),
- a powder for oral suspension (TF041),
- several direct compression tablet formulations (TF036, F001, F002, and F012, F014, F016).

The tablet formulations showed a lower bioavailability compared to the capsule formulations. However, the tablet was selected for further development due to the possibility of incorporating a high drug load, a favourable stability profile/storage conditions and convenience for the patient.

The excipients have been selected based on their compatibility with darunavir: sodium lauryl sulphate initially included as a surfactant (400 mg tablet (TF036, F001) and proportional 200 mg tablet (F002)) was removed from subsequent formulations (400 mg film-coated tablet (F012 and F014) and proportional 300 mg film-coated (F016 commercial formulation)) as it yielded to a new degradation product. In addition, crospovidone was introduced in the latest formulations to improve manufacturability during the compression process and the tablets were film-coated to aid taste masking, product identification and in order to reduce tablet friability.
All the excipients are of PhEur quality except the spray-dried mixture of microcrystalline cellulose and colloidal silicon dioxide and the film-coating. However, they are controlled according to acceptable standards. With regards to the TSE risk, PREZISTA does not contain any component of animal/human origin.

Given the limited solubility of darunavir and the finished product manufacturing method (direct compression with ~52 % of active), adequate control of darunavir particle size is of importance. An acceptable specification has been established based darunavir particle size of

tablets used in clinical trials and that were shown to have comparable bioavailability in bioequivalence studies (study TMC114-C154 and study TMC114-C156).
With regards to polymorph conversion, it has been shown that the manufacturing process/storage does not have a significant impact on ethanolate/hydrate conversion. Moreover, the bioavailability of tablets containing varying amounts of darunavir ethanolate/darunavir hydrate was determined to be within the range of bioequivalence (TMC114-C148).

Satisfactory specification has been provided for the HDPE bottles and the polypropylene cap lined with foil induction seal. The suitability of the primary packaging selected has been confirmed by stability studies.

Different formulations have been used in clinical studies (see above). In general relative bioavailability studies showed that the oral solution (TF019) and the clinical trial tablet formulations (e.g. TF036) meet criteria for bioequivalence under relevant clinical conditions (study TMC114-C118). However, the final tablet commercial formulation (F016) is about 20 % more bioavailable than the clinical trial tablets formulations (e.g. F001, F002) (study TMC114- C166) (see clinical sections).

- Manufacture of the Product

The manufacturing process includes the following steps: dry blend, direct compression, film-coating and packaging.
Satisfactory in-process controls have been defined. The effect of the manufacturing process on the potential for ethanolate/hydrate conversion has been established.

- Product Specification

The product specification includes tests controlled by validated methods for appearance, identity (HPLC and IR), identification of colorants, assay (HPLC), degradation products (HPLC), dissolution, uniformity of dosage units (PhEur) and microbial purity (PhEur).
The dissolution method has shown to be discriminatory towards important aspects of the formulation i.e. active substance particle size and finished product formulations differences.).
Solvate conversion during manufacture has been investigated.
Batch analysis data provided for 3 batches manufactured at the commercial manufacturing site comply with the specifications and indicate consistent and reproducible manufacture.

- Stability of the Product

Stability data have been provided for the 3 batches manufactured at the commercial manufacturing site.
Under accelerated conditions (40°C/75% RH– intended packaging) and under long-term conditions (25°C/60% RH and 30°C/70% RH – intended packaging), 6-month data and 1-year data have been provided
The parameters tested included: appearance, assay, purity, stereo isomeric purity, dissolution, microbial purity, ethanol content and water content.
The observed changes were small, and not likely to have a significant effect on efficacy and safety of the product when used according to the directions in the SPC.
The data provided support the proposed shelf life and storage conditions as defined in the SPC.

Discussion on chemical, pharmaceutical and biological aspects

The active substance is well characterised and documented. The pharmaceutical form selected is adequate taking into account the properties and the stability of the drug substance. The excipients are commonly used for this kind of formulation and the packaging material is well documented. The manufacturing process enhances to obtain reproducible finished product batches. Stability tests under ICH conditions indicate that the product is stable for the proposed shelf life. At the time of the CHMP opinion, there were minor unresolved quality issues having no impact on the benefit-risk- balance of the product. The applicant committed to resolve it as follow up measure after the opinion, within an agreed timeframe.

3. Non-clinical aspects

Introduction

Darunavir is a new human immunodeficiency virus (HIV) protease inhibitor (PI). Early non-clinical development investigated the safety of darunavir as a single agent. However, early clinical trials showed that systemic exposure to darunavir was enhanced by co-administration with the CYP3A4 inhibitor ritonavir (RTV), and therefore the non-clinical safety programme also includes bridging studies with the combination.

All pivotal non-clinical studies were claimed to have been conducted in accordance with principles of Good Laboratory Practices (GLP).

Pharmacology

- Primary pharmacodynamics (*in vitro/in vivo*)

Darunavir as a protease inhibitor inhibits the cleavage of HIV encoded gag-pol polyproteins in virus infected cells, thereby preventing the formation of mature and infectious new virions. It was selected for its potency against wild type HIV-1 and HIV strains resistant to currently approved PIs.

HIV-1 protease inhibition steady state kinetics.
The inhibitory constant (K_i) for darunavir was <0.09 nM, in comparison to <0.10 nM for lopinavir, and higher for the other PI's (ranging from 0.09 to 0.24 nM). Mutants harbouring the V82A or the I84V mutation were also tested, for the V82A, the increase in K_i for darunavir (1.3-fold) was lower than the published increases for all the other PI's (from 1.7 to 11.2-fold). For the I84V PR, the increase in K_i for darunavir (3.2-fold) was lower than the published data for ritonavir, nelfinavir, and saquinavir (11.2-fold, 3.5-fold, and 10.7-fold, respectively).

Inhibition of the activity of human proteases by darunavir
There were no significant inhibitory activities of darunavir (i.e. inhibition ≥ 50%) with any of the human cellular proteases (pepsin, renin, and human cathepsin D and E).

Thermodynamics of darunavir-HIV-1 protease interaction
Darunavir binds tightly to HIV-1 protease to form highly stable complex with both wild type and mutant proteases.

In vitro activity of darunavir (in T-cell lines).

	HIV-1*	HIV-2*	simian immunodeficiency virus SIV*	HIV-2/EHO**
EC_{50} values	2.29 - 6.26 nM	4.70 - 8.49 nM	9.28 nM	6 nM

*MT4-cells; **MT2 cells

In vitro activity in HIV-1 infected primary cells

EC_{50}	1.2 (0.7 ng/ml) to 5.0 nM (2.7 ng/ml).
EC_{90}	2.8 nM (1.5 ng/ml) to 8.0 nM (4.4 ng/ml)

In vitro antiviral activity against primary isolates (in human peripheral blood mononuclear cells, PBMC's).

	HIV-1 Group M	HIV-1 Group O
EC50	<0.10 – 4.28 nM	1.59 – 2.54 nM

In vitro cytotoxicity.
The 50% cytotoxic concentration (CC_{50}) was found to be greater than 100 μM.
With a median EC_{50} of 3.8 nM using the MTT assay in MT4 cells, a selectivity index ($SI=CC_{50}/EC_{50}$) >26000 was determined.

Resistance

In vitro selection from wild type HIV-1	- After 208 passages over 2 years replicating viruses could not be selected in the presence of darunavir at concentrations above 220 nM - Mutations at codons 37, 55, 41, 70, 71, 74, 77, and 85 - Only 3 resulted in emerging viruses with a darunavir fold change (FC) >4.
In vitro selection from PI-resistant HIV-1	- Duration of the passaging cultures ranged from 162 to 251 days with darunavir concentrations at the end of selection ranging from 1.20 to 12.00 μM (300 to 3000-fold the darunavir EC_{50} value against HIV-1/IIIB) - baseline viruses had EC_{50} values ranging from <0.15 to 110.3 nM. - 22 darunavir in vitro selection mutations of those 11 mutations not yet reported to be associated with resistance to proteases (L11I, I15V, G16E, L23I, S37N, L63P, V82I, T91A, T91S, Q92R, and L76V) - resulting emerging viruses had a darunavir FC ranging from 52.9 to 641.0 nM - 8/9 viruses resistant to darunavir retained susceptibility to tipranavir (FC <4).
Antiviral activity against a screening panel of 20 protease inhibitor-resistant recombinant viruses	18 of 20 were susceptible to darunavir
Antiviral activity of darunavir against 3309 recombinant protease inhibitor resistant HIV-1 generated from clinical samples (decreased susceptibility - FC>4 - to at least one protease inhibitor such as amprenavir, atazanavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, or tipranavir)	Phenotyping data - EC_{50} values ≤ 10 nM in 78% of isolates - EC_{50} >100 nM in 3% of the isolates. - FC ≤ 4 in 80% of the isolates - FC > 10 in 10% of isolates Genotypic data available for 1113 of the protease resistant viruses. Subgroups of viruses with up to 3 primary protease mutations and/or up to 8 protease resistance-associated mutations had a median fold change ≤4 for darunavir (which was lower than all other PIs except tipranavir to which it was equivalent).

- Secondary pharmacodynamics

No separate secondary pharmacodynamic studies were performed.

- Safety pharmacology

In vitro, hERG current was not inhibited (up to 10 μM, which is similar to 10-fold the human free maximum plasma level) and the cardiac action potential (sheep isolated cardiac Purkinje fibres) remained unaffected after 10 μM darunavir.
In conscious telemetered beagle dogs, darunavir (120 mg/kg; oral) did not affect cardio-haemodynamic and ECG parameters (at comparable C_{max} and little lower AUC values).
At oral dose up to 2000 mg/kg in rats, darunavir did not show any effect on gastrointestinal transit time, neurobehavioral and motor activity, or respiration.
Plasma levels were not measured in these studies but data obtained in toxicity studies in dogs and rats at the same doses showed that C_{max} was higher in dogs but similar in rats compared to values in human following therapeutic doses, and AUC was lower in both species. Although the safety pharmacology studies did not indicate any potential hazard with darunavir, there is no safety margin.

- Pharmacodynamic drug interactions

Combinations with current antiretrovirals were studied in an anti-HIV-1/IIIB MT4 cell-based assay. The data indicate additivity with all nucleoside/nucleotide reverse transcriptase inhibitors, all tested non-nucleoside reverse transcriptase inhibitors. A modest *synergistic* effect with amprenavir, nelfinavir and ritonavir was observed whereas additive effect was described for the combination with atazanavir, indinavir, lopinavir, saquinavir, and tipranavir and with the fusion inhibitor enfuvirtide.

Pharmacokinetics

The pharmacokinetics of darunavir has been evaluated *in vitro* and in several species (mice, rats, dogs and rabbits), that were also used in the non-clinical pharmacology and toxicology studies. The effect of ritonavir, as a pharmacokinetic enhancer for darunavir, was also investigated in several studies. The methods of analysis used to assay darunavir were adequate and validated. No interference between the analysis of darunavir and ritonavir was observed.

- Absorption- Bioavailability

Following oral administration, darunavir was rapidly absorbed in all animal species (T_{max} 0.5-6 h). Elimination half-life was also rapid with half-lives generally less than 5h. Oral bioavailability of darunavir ranged from 37 to 58% in rats and 60 to 122% in dogs.

The exposure in animal species was low compared to exposure in humans. Several attempts were made to increase darunavir exposure for instance by testing other routes of administration.

In mice, AUC values increased more than dose-proportionally up to 450 mg/kg/day and less than dose-proportionally at higher dose levels. Repeated oral dosing resulted in a decrease in AUC (by up to 3-fold at high dose levels), likely due to induction of liver enzymes involved in the metabolism of darunavir.
In rats, C_{max} and AUC values, after repeated administration, increased more than dose-proportionally up to 100 mg/kg/day and less than dose-proportionally at higher dose levels. As in mice, repeated oral administration resulted in a decrease in systemic exposure to darunavir (by up to 2.5-fold at high dose levels). No consistent differences in plasma kinetics were observed between males and females.
In Beagle dogs, systemic exposure increased dose-proportionally up to 80 mg/kg but above this dose level, no further increase was observed.

Ritonavir increased AUC of darunavir by up to 15-fold in rabbits, 4-fold in rats and 2-fold in mice as well as maintained exposure on repeating dosing. No effect of RTV was observed in dogs. Ritonavir had only limited effect on the pharmacokinetics of darunavir in animals (mice, rats, and dogs), whereas a 14-fold increase in systemic exposure was observed in human when a single dose of 600 mg darunavir was administered with 100 mg ritonavir compared to darunavir alone.

Darunavir exposure (plasma, brain, and liver concentration) was much higher in juvenile rats (aged 12 or 26 days) probably due to incomplete maturation of the blood-brain barrier and liver enzymes involved in the elimination of darunavir. It was nonetheless lower on day 26 than on day 12, presumably reflecting the development of drug metabolising enzymes and the blood-brain barrier.

- Distribution

In adult rats, the tissue distribution of ^{14}C-darunavir was extensive and rapid. The highest concentrations of radioactivity were measured in the liver and adrenal gland. No undue retention or accumulation was observed, except in melanin-rich tissues such as the pigmented parts of the eye although binding was reversible.
Co-administration of ritonavir with darunavir had little effect on tissue distribution although it reduced exposure of pigmented tissues and exposure was lower in pregnant female rats.

Darunavir crossed the placenta in pregnant rats, with levels of radioactivity found in foetuses being about 20% of that in maternal blood and is secreted in rat milk.

The plasma protein binding was moderate to high in all tested species and was concentration dependent. At 0.5 μg/ml, plasma protein binding ranged from 63 % in rabbits to 95 % in rats.
In human the plasma protein binding was relative constant (~ 94%) up to a concentration of 4.7 μg/ml, whereas about 75% was bound at 18.8 μg/ml.

- Metabolism (*in vitro/in vivo*)

Darunavir is metabolised by Phase I and Phase II biotransformation mechanisms. A large number of metabolites were detected *in vitro* using animal and human hepatocytes and microsomal preparations. The metabolic pathway was qualitatively similar in rats, dogs and humans. The most prevalent pathway was the Phase I biotransformation including carbamate hydrolysis, aliphatic hydroxylation at the isobutyl moiety and aromatic hydroxylation at the aniline moiety. Dogs were most representatives of human with carbamate hydrolysis predominating in both species.

Darunavir was mainly metabolised by CYP3A. In mice and rats darunavir treatment induced hepatic microsomal CYP3A4. UDP-GT activity was additionally induced in rats. In dogs, no induction effects were observed.

Darunavir is presented as a single enantiomer but no chiral inversion occurs *in vivo*.

- Excretion

Following a single dose in rats, most radioactivity was excreted within 24 h and was almost complete in 96h. The predominant route of excretion for 14 C-darunavir was via the faeces and amounted to 94% in rats, 86% in dogs and 82% in humans. Urinary excretion was about 4% of the administered dose in rats and dogs but was higher in humans (12.2%). Unchanged darunavir was mainly excreted in feces and amounted to up to 12.3% in rats, 26% in dogs and 6.8% in humans. In rats, darunavir was also excreted via milk.
In a biliary excretion study in Sprague-Dawley rats, an average of 54% of the radioactivity was excreted in the bile during the 24h period after darunavir administration.

- Pharmacokinetics drugs interaction

Six anti-HIV compounds were investigated for inhibitory effects in human liver microsomes. The results showed that saquinavir and amprenavir are predicted to be minor to moderate inhibitors of darunavir metabolism in vivo, whereas delavirdine, ritonavir, indinavir and nelfinavir are likely to be strong inhibitors.

Darunavir also inhibits P-gp and may therefore inhibit transepithelial permeation of P-gp substrates.

Toxicology

A comprehensive toxicological programme was conducted in mice, rats, dogs and rabbits.
Due to a more rapid clearance in animals, the maximum achieved exposure in animal studies was low compared to the human therapeutic level, and adding ritonavir could not much further enhance it. Likely, this difference was caused by the fact that ritonavir is a much stronger inhibitor of CYP3A4 in humans than in animals.

- Single dose toxicity

Single dose toxicity studies were performed in mice, rats and dogs. The maximum achieved exposure was low, with an exposure at the highest dosages being lower or only slightly higher than the human exposure.
In mice, treatment related mortality and macroscopic findings (distended or fluid-filled gastro-intestinal tracts) occurred at oral doses ≥ 280 mg/kg.
In rats, there was no treatment mortality or toxicologically relevant observations with oral doses up to 471 mg/kg. In a second study, higher doses were used but the systemic exposure did not really increase to that observed with a dose of approximately 500 mg/kg.
In dogs, vomiting was the most obvious adverse effect occurring at the doses of 301 mg/kg limiting the maximum tolerated dose to 75 mg/kg. The NOAEL in the IV dog study was 10 mg/kg.
Acute toxicity studies have not been conducted with the combination of darunavir and ritonavir which is acceptable in view of the repeated dose bridging studies which supersede the need for additional acute toxicity studies.

- Repeated dose toxicity

Repeated dose oral toxicity of darunavir was studied up to 6 months in rats and up to 12 months in dogs. In addition studies have been conducted with the administration of both darunavir and ritonavir. In dogs, exposure to darunavir did not increase and the combination was poorly tolerated therefore studies longer than 2 weeks were not conducted whereas in mice the co-administration of darunavir with ritonavir was evaluated up to 6 months.

In the rat, the main target organs/systems were the hematopoietic system, the blood coagulation system, the liver and thyroid.
In the 6 months rat study, changes in the hematopoietic system consisted of increased reticulocyte and platelet counts, decreased haemoglobin levels and haemotocrit (at ≥ 20 mg/kg/day). An increased spleen weight and extramedullary haemotopoiesis was observed at ≥ 100 mg/kg/day and increase in red blood cell counts (RBC) at 500 mg/kg/day. These changes are indicative of increased turnover of red blood cells and this may be associated with the observed increased bilirubin levels in serum (at ≥ 20 mg/kg/day) and in the deposition of lipofuscin pigment in the hepatocytes (at ≥100 mg/kg/day). A toxic effect on the red blood cell system in rodents may be a class related effect, since it has also been observed with other protease inhibitors. Increased white blood cell counts were also seen, but only in males. There were also changes in the blood coagulation system at 500 mg/kg/day consisting of increased platelet counts and a prolonged PT and APPT times. These changes were not associated with any evidence of bleedings. The mechanism behind this effect is unknown,

however the relevance of this finding is probably low since increased platelet counts have not been reported in other species and no related adverse events have been reported in clinical studies. In the kidney, lipofuscin pigment in the proximal tubules was seen in males and females at ≥100 mg/kg/day. Several clinical biochemistry parameters were altered by treatment with darunavir, mainly increases in bilirubin (at ≥20 mg/kg/day), some liver enzymes, cholesterol, protein and reduced triglyceride levels were confined to 100 and 500 mg/kg/day. Changes in serum cholesterol and lipid are known adverse reactions of protease inhibitors probably secondary to the effects on the liver.
Many of the above-mentioned effects were also seen in the 2 week and 3 month rat study. Based on the 6 month rat study, the no adverse effect level (NOAEL) for darunavir was 20 mg/kg/day, leading to a safety margin less than 1. At this dose, there were slight increases in reticulocyte count, potassium and bilirubin in the males in the absence of histopathological changes.

In dogs, there were only limited effects of treatment with darunavir up to the highest doses in the 3 and 6 months studies. Hepatocellular pigment, vacuolation, with a limited increase in alkaline phosphatase levels were seen at 12 months of treatment. The NOAEL was considered to be 30 mg/kg with exposure, leading to a safety margin less than 1 for human exposure.

Combination studies with darunavir and ritonavir
Ritonavir had a modest effect on darunavir exposure, expressed as AUC, in rats (4-fold increase), but not in dogs. In these studies, there was evidence of an additive effect of the combination of darunavir and RTV. In rats, the combination of darunavir with 50 or 75 mg/kg/day RTV led to a small increase in effects on RBC parameters, liver and thyroid, compared to exposure to darunavir alone. At higher doses of darunavir (≥100 mg/kg/day), some very mild in grade pancreatic changes were observed in males (increased of islets fibrosis in the pancreas). There was no safety margin, since, based on AUC values, this effect was seen at about 1.5 times of the recommended clinical dose of darunavir+ rtv (600/100 mg b.i.d.). The clinical relevance of this finding is not clear. PT and APPT times were decreased, after an initial increase.
In dogs, the combination of darunavir and RTV was poorly tolerated, causing a severe weight loss and markedly reduced food consumption. These effects were far less marked at 10 or 20 mg/kg/day. Frequent vomiting was an issue of concern, because it could lead to wide variation in systemic exposure. Liver enzymes were increased in a study with RTV only.
In rats and dogs, slight changes were noticed in thymus weight. These changes were not significant and not dose-related and as such they were considered not to be clinically relevant.

- Genotoxicity

Darunavir was not mutagenic in the Ames assay at concentrations up to 3330 µg/plate, with or without metabolic activation. Darunavir was not clastogenic in an in vitro chromosomal aberration test in primary human lymphocytes at concentrations up to 333 µg/ml, with or without metabolic activation nor in an oral micronucleus study in mice with doses up to 20000 mg/kg. The exposure was not measured in this study but based on other studies, it was expected to be only just similar to the human exposure, due to impossibility to increase the exposure in animals, limiting the value of the study. Nonetheless taken together all the results and the fact that darunavir belongs to a class of products which have been shown to be non-genotoxic till now, no further data was considered necessary. Genotoxicity studies with the combination darunavir with ritonavir were not performed which is acceptable as neither compound alone was genotoxic.

- Carcinogenicity

Two carcinogenicity studies in rats (doses up to 500 mg/kg/day) and mice (doses up to 1000 mg/kg/day) are ongoing. The submission of the results of these studies post-approval was considered acceptable in view of the clinical benefit for the intended patient population.

- Reproduction toxicity

A fertility and early embryonic development study was performed in rats. There was no effect on male or female fertility up to doses producing effects on body weight gain (1000 mg/kg/day). In females, the number of corpora lutea and implantations decreased at 1000 mg/kg/day and as a result also the number of live foetuses. The NOAEL was 200 mg/kg/day.

Embryo-foetal development studies were performed in rats, rabbits and mice. The mice were chosen as a third species, because darunavir exposure in the rabbit appeared to be very low and the applicant justified that mainly for practical reasons, it was not possible to apply a twice-daily dosing scheme in rodents. In mice, embryo-foetal development was also investigated in combination with ritonavir, to increase exposure to darunavir. Darunavir was not embryotoxic or teratogenic in rats (NOAEL = 1000 mg/kg/day), rabbits (NOAEL = 1000 mg/kg/day) or mice.

Pre- and postnatal development was investigated in rats, also in combination with ritonavir.
In F1 animals, body weight gain was reduced, especially during lactation but also post-weaning. A slight delay was observed in the opening of the eyes and ears. In combination with ritonavir, the percentage of pups that exhibited the startle response on lactation day 15 was reduced. Furthermore, the combination of darunavir and ritonavir caused a reduced pup survival during lactation at 1000/50 mg/kg/day. The NOAEL for the F1 generation was 40 mg/kg/day.

- Local tolerance

As darunavir is administered orally, no studies evaluating local tolerance were considered necessary
A local lymph node assay was provided showing that darunavir did not cause skin sensitisation in mice.

- Other toxicity studies

In juvenile rats that were directly dosed on days 12 – 25 of life, mortality was increased already at the lowest dose of 40 mg/kg. Effects included a distended abdomen, an unsteady gait and in some of the animals convulsions. The increased toxicity in juvenile rats was probably due to a higher exposure as already highlighted in the pharmacokinetic section.

No immunotoxicity was observed in a T-cell dependent antibody response test in rats after administration of darunavir at exposures which were similar to or just below human exposures (higher dose 500 mg/kg). Also in combination with ritonavir (100/50 mg/kg) no immunotoxicity was observed (exposure of darunavir below human exposure).

The impurities have been sufficiently toxicologically qualified.

- **Ecotoxicity/environmental risk assessment**

An assessment of the risk was performed. Phase I calculations resulted in a PEC of 6 µg/l. As the action limit was exceeded, the applicant conducted a Phase II assessment. The risk to the aquatic environment, the terrestrial compartment and the STP is acceptable. An assessment of bio-concentration factor is not needed. Darunavir is neither very persistent and very bioaccumulative (vPvB) nor (persistent bioaccumulative and toxic (PBT). The limit of quantification is 54 µg/l hence not suitable for monitoring of drinking water quality. The water-sediment study is ongoing, the results of which will be provided post-authorisation.

Summary of environmental properties of darunavir

darunavir	**CAS Number: 206361-99-1**			
PBT (persistent bioaccumulation toxicity) assessment	P: yes	B: no	T: not assessed	**not PBT not vPvB**
Physical-chemical properties of darunavir ethanolate				
	logKow	2.47	23°C	
	water solubility	0.16-0.19 mg/l	pH 4-9 20 °C	
	molar mass	548		
Analytical methods				
	Purpose	Method	LOQ	0.1 µg/l achieved?
	Verification	HPLC-UV	54 µg/l	no
Environmental fate and behaviour				
Adsorption	Soil type / sludge	Kd	Koc	unit
	activated sludge	75.3	345	l/kg
	sandy loam	42	993	l/kg
	loam	4.12	389	l/kg
	loamy sand	18.1	933	l/kg
	sandy clay loam	9.5	265	l/kg
	clay	8.18	732	l/kg
Degradation	Ready test	not readily biodegradable	no primary degradation	OECD301B
	Hydrolysis	no hydrolysis	OECD111	
Ecotoxicological information				
	Species	Duration	Criterion	Value [mg/l]
	Pseudokirchneriella subcapitata	72 hours	NOEC	43
	Daphnia magna	48 hours	EC50	>44
	Oncorhynchus mykiss	96 hours	LC50	>38
	Daphnia magna	21 days	NOEC	19
	Pimephales promelas	32 days	NOEC	≥9.4
	Activated sludge		EC50	>1000

4. Clinical aspects

Introduction

The clinical programme consisted of:

- studies aiming to characterise the pharmacokinetic profile of darunavir following single and multiple administration, with or without low dose of ritonavir;
- two phase II, randomised, open label, controlled proof-of-concept studies in treatment experienced patients (TMC114-C201 and TMC114-C207).
- two phase IIb, randomised, partially blinded, controlled, dose finding studies in 3-classes experienced patients (TMC114-C202 and TMC114-C213 also known as POWER1 and POWER2)
- two open label trials in protease inhibitors experienced patients (TMC114-C215 and C208. The analysis of data from subjects who initiated treatment at the recommended dose is known as the POWER3 analysis).

The design of the two controlled studies, which have become the main trials have been discussed in the context of a Scientific Advice.

At the recommended dose of 600 mg to be co-administered with low dose of ritonavir (100 mg as pharmacokinetic enhancer) twice daily with food, the approved indication is:

> "PREZISTA, co-administered with 100 mg ritonavir is indicated in combination with other antiretroviral medicinal products for the treatment of human immunodeficiency virus (HIV-1) infection in highly pre-treated adult patients who failed more than one regimen containing a protease inhibitor (PI).
> This indication is based on week-24 analyses of virological and immunological response from 2 controlled dose range finding Phase II trials and additional data from uncontrolled studies (see section 5.1).
> In deciding to initiate treatment with PREZISTA co-administered with 100 mg ritonavir careful consideration should be given to the treatment history of the individual patient and the patterns of mutations associated with different agents. Genotypic or phenotypic testing (when available) and treatment history should guide the use of PREZISTA."

Good Clinical Practices

The clinical studies included a statement regarding conduct in accordance with Good Clinical Practices. The applicant provided a statement to the effect that clinical trials conducted outside the Community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Pharmacokinetics

The pharmacokinetics of darunavir administered alone or with low-dose ritonavir were evaluated in thirty-five Phase I trials involving 748 healthy subjects, as well as in 1 Phase I trial in HIV-1 infected patients. In addition, data derived from patients involved in other studies were used in 3 population pharmacokinetic analyses. A large number of interactions studies have also been submitted, some reflecting the variability in the formulations used throughout the development of darunavir (oral solution, suspension, capsules and tablets).
The analytical methods used to measure darunavir and ritonavir have been adequately validated.

Absorption
Under fed conditions, the oral bioavailability of darunavir administered alone was approximately 37%. When combined to ritonavir it increased to approximately 82%, indicating that ritonavir strongly inhibits the first pass metabolism.
Following oral dose of 600 mg darunavir co-administered with 100 mg ritonavir twice daily (bid), darunavir was rapidly absorbed with maximal peak plasma concentrations observed after about 2.5 – 4 h (Cmax 5627 ± 923.5 ng/ml, AUC∞, 92340 ± 20020 ng.h/ml). Low dose ritonavir increased the dose-normalised Cmax and AUC_{last} of darunavir by 191% and 1316%, respectively, giving a near to 14-fold increase in darunavir exposure.

As for other protease inhibitors, darunavir is a P-gp substrate.

Systemic exposure increased dose proportionally following ritonavir boosted single doses ranging 400 mg-1200 mg but not after multiple dosing. This non-linearity may be due to induction of metabolising enzymes and/or saturable plasma proteins binding with increasing concentrations. There was no indication that the elimination half-life was dependent of dose or affected by repeated administration. Steady state was achieved within 4 days with or without ritonavir boosting and no accumulation was observed. With ritonavir, no indication of auto-induction or time dependency was observed in the steady state predose levels of darunavir.

Inter-individual variability in darunavir pharmacokinetics (AUC and C_{max}) was estimated between 30 – 60% after un-boosted single dose and about 35 % after multiple dose administration. When boosted, the inter-individual variability was reduced to about 25 – 35% after single and multiple doses. There is currently no data on intra-individual variability but this will be further addressed post-authorisation.

Bioequivalence and effects on food

Opposite food effects on the bioavailability of darunavir were observed for the clinical trial formulation TF019 (oral solution) and TF036 (400 mg tablet). Whereas food decreased the mean C_{max} and AUC_{last} values obtained with the oral solution by 33% and 13%, respectively, (with 90% CI around the ratios that did not span 1.0 and p values < 0.05), it increased these values seen with the tablets by 35% and 42%, respectively (with 90% CI around the ratios that did not span 1.0 and p values < 0.05). The mechanism for the observed opposite effect of food on the bioavailability of darunavir for the solution compared to tablet has not been elucidated. The solution TF019 was nonetheless an early clinical formulation not intended for commercial use.

The food effect was further looked at in study C143 where the F014 tablet (commercial/Phase III 400-mg tablet formulation) was administered with 100 mg ritonavir b.i.d., under fasted conditions and different fed conditions. Because the F014 tablet is dose proportional with the F016 commercial/Phase III 300 mg film-coated tablet, the results can be extrapolated. The results of this study are displayed in table 1:

Table 1- results from study C143

Treatment	A standard breakfast	B fasted	C high fat breakfast	D nutritional drink rich in proteins	E croissant with coffee
			Ratio (90% CI)		
		B vs. A	**C vs. A**	**D vs. A**	**E vs. A**
C_{max}	-	0.69 (0.62 – 0.77)	1.10 (0.97 – 1.24)	0.99 (0.86– 1.14)	0.99 (0.87 – 1.11)
AUC_{0-t}	-	0.70 (0.62 – 0.79)	1.04 (0.96 – 1.14)	0.99 (0.88 – 1.13)	0.98 (0.86 – 1.10)
AUC_{inf}	-	0.70 (0.62 – 0.80)	1.04 (0.95 – 1.13)	1.04 (0.93 – 1.16)	0.98 (0.87 – 1.11)

Based on the results, which showed that food increased darunavir bioavailability, darunavir should be taken with ritonavir as recommended in the Summary of Product Characteristics. The Phase IIb efficacy and safety trials required food intake.

An extensive bioavailability/bioequivalence programme was carried out to characterise the difference between formulations.

In order to bridge the early oral solution TF019 formulation to the clinical trial tablet formulation (F001 uncoated 400 mg tablet), a cross-over design trial was conducted which showed that darunavir exposure was within the limits of bioequivalence for the oral solution and clinical trial tablet, when administered with food and with low-dose ritonavir.

In another single dose trial under fasted conditions, no bioequivalence could be shown between the F002 uncoated 200 mg tablet and the F016 commercial/Phase III 300 mg film-coated tablet (AUC_{0-t}: ratio 1.33, 90% CI 1.22 – 1.47, C_{max} : ratio 1.33, 90% CI 1.21 – 1.47), and between the F001 uncoated 400 mg tablet and the F016 tablet (AUC_{0-t}: ratio 1.34, 90% CI 1.23 – 1.47, C_{max} : ratio 1.23, 90% CI 1.13 – 1.33). The effect was similar for the 200 and the 400 mg tablet, indicative for a similar bioavailability of the 200 mg tablet compared to the dose proportional 400 mg tablet. Sparse sampling data from patients treated with F001 tablets and then switched to F016 tablets also confirmed higher bioavailability (37%) for the commercial formulation.

To address the concern over non-demonstration of bioequivalence, the applicant conducted two additional trials (C166 and C168). Study C166, an open-label, randomised crossover trial, aimed to assess the rate and extent of absorption of darunavir following administration of the clinical trials formulation (F001, F002) and the F016 commercial/Phase III formulation at 3 dose levels, all in presence of low dose of ritonavir with food. The F016

commercial/Phase III tablet showed a 21% greater bioavailability compared to the clinical trial tablets F001/F002. In the absence of low-dose ritonavir and under fed conditions, the commercial/Phase III tablets exhibited comparable exposure to the clinical trial tablet (F001/F002) (trial C168). At the 600-mg dose level, the darunavir exposure was comparable for the 2 formulations (13% higher for commercial/Phase III tablet). Similarly, at the 1200-mg dose level, the darunavir exposure was comparable for the 2 formulations (8% higher for commercial/Phase III tablet).
The approach of conducting single-dose bioavailability trials was acceptable because of lack of time dependency for darunavir combined with low-dose ritonavir. The additional bioavailability trials showed that the administration of food diminishes the differences in exposure between commercial/ Phase III and the clinical trial tablets from 35% to 21%, in line with the described differences in patients in clinical trials. In addition, the difference in exposure between the commercial/ Phase III tablet and clinical trial tablet (21%) was similar at the 600-mg dose of darunavir whether the clinical trial tablets were administered as 200-mg + 400-mg tablets or as 3 x 200-mg tablets.
Additional data from study C166, also under clinical relevant conditions, bridge the F001 and the F016 tablets, and therefore the TF019 and the F016 formulations. As noticed before, the commercial F016 formulation had a 21% higher bioavailability than the F001 clinical trial formulation. This may also account for the TF019 (vs. F016).
These data bridge also bioequivalence data from the oral TF019 solution, as this solution is bioequivalent with the TF036 tablet (= F001 tablet), under clinically relevant conditions. The F016 tablets can therefore be expected to be supra-bioavailable to the oral solution to a similar extent that they are supra-bioavailable to the TF036 tablets used in the Phase IIb clinical studies.

Distribution
The volume of distribution was about 88 l after i.v. administration of darunavir alone, and about 131 litres when co-administered with ritonavir.
Animal data indicate that darunavir distributes over the whole body, but high concentrations were observed in liver, adrenal gland, and the eye. Protein binding is concentration dependent and decreased from 96 to 92% over the clinical relevant concentration range of 0.1 – 7 µg/ml. The concentration dependency of plasma protein binding is within the physiologically relevant range. The Cmax of darunavir in human is about 10 µg/ml, indicating that during a time-interval around the Tmax the free fraction can be up to 4 times higher than at other time intervals associated with lower darunavir concentrations.
Darunavir binds predominantly to α1-acid glycoprotein, and to a lesser extend to albumin. The blood to plasma concentration ratio was 0.64, indicative for some distribution to red blood cells.

Metabolism and elimination
In-vitro and in-vivo data indicate that darunavir undergoes extensive metabolism mainly by the CYP3A4 pathway. When radiolabelled darunavir was given without ritonavir about 50% of the radioactivity in plasma was due to darunavir and the remaining 50% was due to biotransformation products. After boosting, plasma exposure of unchanged compound increased to about 80%.

When un-boosted, darunavir is mainly excreted in faeces (85%) and about 12% of the administered dose could be recovered in urine, mainly as metabolites. Clearance was about 33 l/h and the elimination half-life about 12 hours.
After boosting with ritonavir, over 6 days, about 14% of the administered dose could be recovered in urine and about 80% in faeces, of which 41% was as unchanged. Relative low percentages of compound identification were obtained, but this may be due to difficulties of metabolite identification in faeces. When boosted, a significant inhibition was observed in the carbamate hydrolysis, aliphatic hydroxylation and aromatic hydroxylation. In addition excretion of glucuronide metabolites was increased.

RTV boosting decreased considerably the plasma clearance of darunavir to 6 l/h, while the elimination half-life was not greatly affected (16 h).

Special population

Preliminary pharmacokinetic data indicated that darunavir pharmacokinetics in subjects with mild hepatic impairment (n = 8, Child Pugh A) and moderate hepatic impairment (n = 3, Child Pugh B) were comparable to those in healthy subjects (n = 8) following 600 mg darunavir with 100 mg ritonavir (b.i.d). Ritonavir pharmacokinetics in subjects with mild hepatic impairment were comparable to those in healthy subjects, while ritonavir exposure in subjects with moderate hepatic impairment was approximately 60% higher than that in healthy subjects. Waiting for the final results of this study, to be provided post-authorisation, no dose adjustments are recommended for darunavir or ritonavir in mild and moderate hepatic impaired patients. In the absence of data, darunavir is contraindicated in patients with severe hepatic impairment.

Considering that the elimination of darunavir via the renal route is minimal, no impact of the impairment of the renal function is anticipated on the pharmacokinetics of darunavir. No dose adjustment is therefore recommended in patients with renal impairment.

The pharmacokinetics of darunavir have not yet been evaluated in children/adolescents but the applicant undertook to submit the results of the planned studies as part of the follow-up measures to be fulfilled post-authorisation.

Based on the population pharmacokinetic analyses, no impact of gender, weight or race is anticipated on the pharmacokinetics of darunavir, which would require special precautions. For the elderly, because only limited data is available, a warning has been included in the Summary of Product Characteristics requiring caution.

Pharmacokinetics in target population

In HIV infected patients a less than dose proportional increase was observed at steady state when darunavir was given with low dose ritonavir. With the 600 + 100 mg b.i.d. dose, trough levels were obtained well above the theoretical target for minimum concentrations to be maintained (550 ng/ml). Darunavir exposure in HIV infected patients was higher than that observed in healthy subjects. The mean steady state Cmax levels ranged from 6008 –9200 ng/ml (10.1 – 15.5 µg/ml). The population pharmacokinetic analyses suggested that this may in part be explained by the higher α1-acid glycoprotein levels in HIV infected patients compared to healthy subjects.

Pharmacokinetic interaction studies

In vitro data showed that darunavir is a strong inhibitor of CYP3A4 and to a lesser degree CYP2C9, CYP2C19, CYP2D6. CYP1A2 and CYP2B6 were only slightly inhibited at the highest darunavir concentration (100 µM) while no inhibition of CYP2A6 and CYP2E1 could be detected. Darunavir appeared also to induce CYP enzymes. In vitro studies showed that darunavir may also inhibit P-gp.

In vivo interactions involving CYP3A4 metabolism are likely to be extensive and most of the submitted interaction studies focussed on the possible interaction on this level.

An overview of the results from studies interaction is presented in table 2.

Table 2: Interaction studies (↑ = increase, ↓ = decrease, ↔ = no change)

Co-Admin	Co-Admin Compounds	DRV/RTV	N	Substance assessed	LS Mean Ratio % (90% CI) C_{max}	AUC	C_{min}
Co-Administration With Other Protease Inhibitors							
Ritonavir	Titrated: 300 to 600 mg b.i.d. over 6 days	Darunavir 800 mg single dose (oral solution)	9	darunavir	↑ 197 (140-277)	923 (662-1288)	-
Atazanavir	300 mg q.d.	400/100 mg b.i.d.	13	darunavir	↔ 102 (96-109)	103 (94-112)	101 (88-116)
				atazanavir	↔ 89	108	152
					(78-101)	(94-124)	(99-234)
	Atazanavir did not statistical significant affect serum levels of darunavir and vice versa						
Indinavir	800 mg b.i.d.	400/100 mg b.i.d.	9	darunavir	↑ 111 (98.2-126)	124 (109-142)	144 (113-182)
				indinavir	↑ 108	123	225
					(95-122)	(106-142)	(163-310)
	Indinavir increases the serum levels of darunavir as a result of additional CYP3A4 inhibition and vice versa						
Lopinavir/ Ritonavir	400/100 mg b.i.d.	300/100 mg b.i.d. (oral solution)	9	darunavir	↓ 61 (51-74)	47 (40-55)	35 (29-42)
				lopinavir	↑ 122	137	172
					(112-132)	(127-149)	(146-203)
	Lopinavir/ritonavir decreases the serum levels of darunavir as a result of CYP3A4 induction whereas darunavir/ritonavir increases the serum levels of lopinavir as a result of additional CYP3A4 inhibition.						
Saquinavir	1200 mg single dose	Darunavir 1200 mg b.i.d.	8	darunavir	↑ 121 (103-142)	144 (136-153)	-
		(oral solution)		saquinavir	↑ 498	533	-
					(316-783)	(333-853)	
Saquinavir/ Ritonavir	1000 mg b.i.d.	400/100 mg b.i.d.	14	darunavir	↓ 83 (75-92)	74 (63-86)	58 (47-72)
				saquinavir	↔ 94 (78-113)	94 (76-117)	82 (52-130)
	Saquinavir decreases the serum levels of darunavir as a result of CYP3A4 inhibition. Darunavir/ritonavir may increase the levels of saquinavir to a great extend, as a result of CYP3A4 inhibition. . When saquinavir is used boosted, no significant effect were seen.						
Co-Administration With Other Antiretrovirals							
Efavirenz	600 mg q.d.	300/100 mg b.i.d. (oral solution)	12	darunavir	↓ 85 (72-100)	87 (75-101)	69 (54-87)
				efavirenz	↑115	121	117
					(97-135)	(108-136)	(101-136)
	Efavirenz decreases the serum levels of darunavir as a result of CYP3A4 induction whereas darunavir/ritonavir increases the serum levels of efavirenz as a result of CYP3A4 inhibition.						
Etravirine	800 mg b.i.d.	600/100 mg b.i.d.	20	darunavir	↑126 (117-135)	123 (116-131)	113 (105-

							123)
				etravirine	↓ 66	67	56
					(52-84)	(52-86)	(40-78)
Nevirapine	200 mg b.i.d.	400/100 mg b.i.d.	8	darunavir	↑ 140 [a] (114-173)	124 [a] (97-157)	102 [a] (79-132)
				nevirapine	↑ 118	127	147
					(102-137)	(112-144)	(120-182)
	Darunavir/ritonavir increases the serum levels of nevirapine as a result of CYP3A4 inhibition. This difference was not considered to be clinically relevant.						
Tenofovir	300 mg q.d.	300/100 mg b.i.d. (oral solution)	12	darunavir	↑ 116 (94-142)	121 (95-154)	124 (90-169)
				tenofovir	↑ 124	122	137
					(108-142)	(110-135)	(119-157)
	Darunavir/ritonavir increases the serum levels of tenofovir. Ritonavir effect on MDR-1 transport in renal tubuli has been a proposed mechanism. A recommendation for monitoring of renal toxicity has been included in the SPC.						
Co-Administration With Other Drugs							
Clarithromycin	500 mg b.i.d.	400/100 mg b.i.d.	17	darunavir	↔83	87	101
				Clarithromycin	↑126	157	274
	Darunavir/ritonavir increases the serum levels of clarithromycin as a result of CYP3A4 inhibition and possible P-gp inhibition. Levels of the metabolite 14-OH-clarithromycin were not detectable.						
Ketoconazole	200 mg b.i.d.	400/100 mg b.i.d.	14	darunavir	↑121 (104-140)	142 (123-165)	173 (139-214)
				Ketoconazole	↑211 (181-244)	312 (265-368)	968 (644-1455)
	Ketoconazole as potent inhibitor as well as substrate of CYP3A4, increased plasma concentrations of darunavir. Simultaneously, plasma concentrations of ketoconazole was increased by darunavir.						
Paroxetine	20 mg q.d.	400/100 mg b.i.d.	16		↔97 (92-102)	102 (95-110)	107 (96-119)
				Paroxetine	↓64 (59-71)	61 (56-66)	63 (55-73)
Sertraline	50 mg q.d.	400/100 mg b.i.d.	13	darunavir	↔101 (89-114)	98 (84-114)	94 (76-116)
				Sertraline	↓56 (49-63)	51 (46-58)	51 (45-57)
	In these studies there was a clear effect on the free fraction of paroxetine confirming that displacement from protein AAG may partly explain the interaction between darunavir with ritonavir and paroxetine. For sertraline this was less evident.						
Ranitidine	150 mg b.i.d.	400/100 mg b.i.d.	16	darunavir	↔ 96	95	94

					(89-105)	(90-101)	(90-99)
Omeprazole	20 mg q.d.	400/100 mg b.i.d.	16	darunavir	↔ 102 (95-109)	104 (96-113)	108 (93-125)
	The studies did not show any interactions.						
Digoxin	0.4 mg single dose	600/100 q12h	14	Digoxin		↑ 60%	ND
	Darunavir/ritonavir increases the serum levels of digoxin, possibly due to inhibition of Pgp.						
Pravastatin	40 mg single dose	600/100 mg b.i.d. (oral solution)	14	pravastatine	↑163 (95-282)	181 (123-266)	-
	In this study, the increased exposure of pravastatin was only seen in a subset of subjects. The mechanism of this interaction is unknown as well as its clinical relevance.						
Atorvastatin	10 mg q.d. a	300/100 mg b.i.d. (oral solution)	15	atorvastatin	↑ 56 (48-67)	85 (76-97)	181 (137-240)
	Darunavir/ritonavir increases the serum levels of atorvastatin as a result of CYP3A4 inhibition.						
Sildenafil	25 mg single dose	400/100 mg b.i.d.	16		↑62 (55-70)	97 (86-109)	-

Hormonal contraceptive					
Ethinylestradiol Norethindrone	35µg/1mg q24h	600/100 q12h	ethinylestradiol	↓ 44%	↓ 62%
			Norethindrone	↓ 14%	↓ 30%

A study was conducted in healthy volunteers with rifabutin however it was prematurely discontinued due to adverse events. Although no conclusion could be drawn, since rifabutin is an inducer and substrate of CYP450 enzymes, increase in rifabutin exposure and decrease in darunavir exposure can be expected.
In addition to the drug interaction studies completed, the applicant has performed a drug screen for the HIV-1 infected patients enrolled into C215 treated with F016 at 600/100 mg b.i.d. Differences in median darunavir AUC_{24h} values ranged from -22% (gemfibrozil) to 33% (clotrimazole). No apparent effects on darunavir exposure were seen on co-administration with any of the NRTIs. There was a trend towards somewhat higher plasma concentrations of darunavir (up to approximately 20%) when azole anti-fungal agents were co-administered. Concomitant systemic use of clotrimazole and darunavir co-administered with 100 mg ritonavir may increase plasma concentrations of darunavir. Caution is warranted and careful clinical monitoring is recommended, when co-administration of systemic clotrimazole is required.

Considering the concentration dependent protein binding indicative for saturation of binding, interactions due to drug-protein displacement cannot be ruled out. A warning has therefore been included in the SPC. In addition the applicant undertook to further measure unbound and total darunavir concentrations to elucidate possible displacement problems.

Based on the different elimination pathways of the other NRTIs zidovudine, zalcitabine, emtricitabine, stavudine, lamivudine, that are primarily renally excreted, and didanosine and abacavir which metabolism is not mediated by CYP450, no interactions are expected.

Based on the results of the studies, the appropriate warnings and recommendation have

therefore been included in the SPC.

The applicant undertook to provide data of ongoing interaction studies using the recommended clinical dose and the commercial formulation either as part of the specific obligations (rifabutin and didanosine) or the follow-up measures (methadone, lopinavir/ritonavir, rifabutin, didanosine, buprenorphine/naloxone, carbamazepine, in vivo cocktail trial to determine the effects of steady state darunavir with low dose ritonavir (600/100 mg bid) on the metabolism of CYP probe substrates 2D6, 2C19 and 2C9 and further investigation on potential inhibition of CYP 2C8) to be fulfilled post-authorisation.

Pharmacodynamics

Darunavir Resistance profile

The influence of baseline genotype and phenotype on the virologic outcome and on the development of mutations to darunavir/ritonavir treatment was studied in the pooled data from the clinical trials TMC114-C202, TMC114-C213, TMC114-C215 and TMC114-C208 (see next section for details of study designs). Phenotypic and genotypic testing was performed using Antivirogram and VirtualPhenotype testing methods respectively.

Conservative analyses, using 3 response parameters (≥ 1 $\log_{10}$ decrease in viral load, viral load > 50 copies/ml, and change in $\log_{10}$ viral load at Week 24) to assess the influence of individual protease mutations at baseline on the virologic outcome at Week 24, show that the following protease mutations at baseline were associated with a lower than overall response to darunavir + rtv (600+100 mg b.i.d.):
- decrease of $\geq$1.0 $\log_{10}$: V11I, V32I, I47V, I50V, I54L or M, L76V and/or I84V
- change versus baseline in $\log_{10}$: V11I, V32I, I47V and/or I54L or M
- viral load < 50 copies/ml: V11I, V32I, L33F, I47V, I50V, I54L or M, G73S, L76V, I84V and/or L89V.

Among those, V11I, V32I, I47V and I54L or M, were identified using all 3 parameters of response. The table 3 presents the observed effects in mean change *versus* baseline in $\log_{10}$ Viral Load.

Table 3 Change vs. Baseline in log10 Viral Load (NC = F) at Week 24 (copies/ml) by Presence of Individual Protease Mutations at Baseline - TMC114-C202/C213/C215/C208

	TMC114/RTV 600/100 mg b.i.d.	**control**
Protease Mutation	**N Mean (SE)**	**N Mean (SE)**
Overall	376 - 1.74 (0.07)	123 -0.48 (0.08)
V11I	40 -1.18 (0.21)	11 -0.77 (0.34)
V32I	36 -0.82 (0.22)	23 -0.39 (0.18)
I47V	51 -1.00 (0.18)	20 -0.30 (0.15)
I54L	27 -1.19 (0.26)	16 - 0.75 (0.32)
I54M	31 -0.66 (0.21)	14 -0.24 (0.20)

In addition, a reduced virologic response (decrease $\geq$1.0 $\log_{10}$ in viral load versus baseline at Week 24 or a viral load < 50 copies/ml at Week 24) was observed in subjects with ≥ 7 PI resistance-associated mutations (any change at positions 30, 32, 36, 46, 47, 48, 50, 53, 54, 73, 82, 84, 88, or 90), or in subjects with ≥ 10 PI resistance-associated mutations from the 2004

IAS-USA PI resistance associated mutations list at baseline. Nevertheless, the response rate in all subgroups (by type and number of mutations at baseline) was generally higher in the darunavir + rtv groups compared to the response rate in the control group.

Table 4 Response to Darunavir + rtv (600 + 100 mg b.i.d). by Baseline Genotype – ITT-TLOVR Analysis of Trials TMC114-C213, TMC114-C202, and TMC114-C215/TMC114-C208

Number of Baseline Mutations[a]	≥ 1 Log_{10} Decrease in Viral Load at Week 24		Viral Load < 50 Copies/ml at Week 24	
	N	n (%)	N	n (%)
All	377	252 (67)	377	157 (42)
<7	317	226 (71)	317	142 (45)
≥7	57	24 (42)	57	13 (23)

a Any change at positions 30, 32, 36, 46, 47, 48, 50, 53, 54, 73, 82, 84, 88, or 90.

Table 5 Response to Darunavir + rtv (600+100 mg b.i.d) by Baseline Genotype –ITT-TLOVR Analysis of Trials TMC114-C213, TMC114-C202, and TMC114-C215/TMC114-C208

Number of Baseline Mutations[a]	≥ 1 Log_{10} Decrease in Viral Load at Week 24		Viral Load < 50 Copies/ml at Week 24	
	N	n (%)	N	n (%)
All	377	252 (67)	377	157 (42)
<10	299	215 (72)	299	136 (45)
≥10	75	35 (47)	75	19 (25)

a PI resistance-associated from the 2004 IAS-USA PI resistance associated mutations list.

Response to PREZISTA co-administered with ritonavir (600/100 mg b.i.d.) by baseline genotype*: As treated analysis of POWER 1, 2 and 3.

Number of mutations at baseline*	Change in log_{10} viral load at week 24	Proportion of subjects with ≥ 1 log_{10} decrease at week 24	Proportion of subjects with < 50 copies/ml at week 24
0 – 2	-2.1	78% 213/274	50% 138/274
3	-1.12	45% 26/58	22% 13/58
≥ 4	-0.46	27% 11/41	10% 4/41

* Number of mutations from the list of mutations associated with a diminished response to PREZISTA/ritonavir (V11I, V32I, L33F, I47V, I50V, I54L or M, G73S, L76V, I84V or L89V)

Mutations at V32I, L33F, I47V, I54L and L89V developed in viruses from at least 10% of the patients with virological failure (never reaching >1.0 log_{10} decrease or rebound from a >1.0 log_{10} decrease). The development of these mutations was associated with a median darunavir FC increase of 8.14 at endpoint compared to baseline.

Mutations at V32I and I54L were the most common mutations that developed in > 20% of the virological failures. Overall, in the rebounders a median increase of 8.14 at endpoint compared to baseline was found in darunavir FC.

Cross-resistance

The data from laboratory studies in clinical isolates are under the pharmacology section.

The baseline phenotypic data of trials C202, C213, and C215/C208 showed that for each individual PI (indinavir, ritonavir, nelfinavir, saquinavir, amprenavir, lopinavir, atazanavir), resistance was > 79% for the subset of patients sensitive to darunavir (FC ≤ 10, N=661). In contrast, viruses from almost all of the subjects resistant to darunavir (FC > 10, N = 287) were also resistant to the other PIs. An analysis of the cross-resistance between darunavir and amprenavir showed that the majority (63%) of samples with decreased susceptibility to amprenavir remained susceptible to darunavir, irrespectively of previous use

of (fos)amprenavir. Virological response data for patients who have failed amprenavir or who harbour viruses resistant to amprenavir indicated that darunavir+ rtv at the recommended dose was effective in most of these patients. There is currently not enough experience to conclude on potential cross-resistance with tipranavir.

Relationship plasma concentration and effect

PK/PD relationship was investigated on the basis of the dose finding studies TMC114-C202 and TMC114-C213. Logistic regression models were applied, including the covariates baseline log_{10} viral load, number of sensitive NRTIs in the OBR, darunavir log_{10} FC at baseline, and use of enfuvirtide in the OBR. The pharmacokinetics of darunavir (AUC24h and C0h) were statistically significantly associated with all response parameters at Week 24. However, these relationships were not as strongly correlated as darunavir log_{10} FC at baseline and use of enfuvirtide in the OBR.

The inhibitory quotient (IQ), reflecting the ratio between the concentration of darunavir achieved in plasma and the darunavir FC at baseline, was the strongest predictor of virologic response. The relationship between IQ and percent virologic response is driven primarily by the darunavir FC at baseline and less by exposure to darunavir. Therefore darunavir FC at baseline was considered to be the most important prognostic factor with regard to response.

Secondary pharmacology

In study C153, darunavir/ritonavir (F001 tablets) was given at 1600/100 mg once daily and at 800/100 mg twice daily for 7 days and ECG changes were compared with those seen during dosing with moxifloxacin 400 mg once daily or placebo, each for 7 days, in a 4-way crossover design. Dosing was after standard meals on testing facility days and pharmacokinetic data were obtained on days 1 and 7. Darunavir elicits small increases in QTc and PR intervals compared to placebo, which are probably not of clinical significance.

The PK/PD did not show any relationship found between exposure and safety parameters, vital signs, or ECG parameters. Therefore there is no need for therapeutic drug monitoring to guide the use of darunavir in clinical practice.

Clinical efficacy

Table 6 Overview of clinical trials

Trial (Location)	Design	Indication Studied/ Population	Doses of darunavir+ rtv (formulation)	Treatment Duration	Number of Patients (24 Sept 2005)
Proof-of-Principle Trials					
TMC114-C201 (Europe)	Phase IIa, randomised, open-label, controlled trial	HIV-1 infection/ PI-experienced patients	400/- mg b.i.d. 800/- mg b.i.d. 800/- mg t.i.d. 1200/- mg t.i.d. (oral solution)	Short term (14 days)	34
TMC114-C207 (Europe)	Phase IIa, randomised, open-label, controlled trial	HIV-1 infection/ PI-experienced patients	300/100 mg b.i.d. 600/100 mg b.i.d. 900/100 mg q.d. (oral solution)	Short term (14 days)	50
Controlled Trials					
TMC114-C202 (USA, Argentina)	Phase IIb, Randomised, controlled partially blinded, 2-part hybrid trial	HIV-1 infection/ 3-class experienced patients	400/100 mg q.d. 800/100 mg q.d. 400/100 mg b.i.d. 600/100 mg b.i.d. (tablet)	Long term (144 weeks[a])	319
TMC114-C213 (Australia, Austria, Belgium, Brazil, Canada, France, Germany, Hungary, Italy, Portugal, Spain, Switzerland, United Kingdom)	Phase IIb, randomised, controlled, partially blinded, trial	HIV-1 infection/ 3-class experienced patients	400/100 mg q.d. 800/100 mg q.d. 400/100 mg b.i.d. 600/100 mg b.i.d. (tablet)	Long term (144 weeks[a])	318
Non-Randomised Trials					
TMC114-C215 (Australia, Europe, USA, Canada , Brazil)	Phase IIb, open-label trial	HIV-1 infection/ 3-class experienced patients	400/100 mg b.i.d 600/100 mg b.i.d. (tablet)	Long term (144 weeks[a])	431
TMC114-C208 (Europe)	Phase IIb, open-label trial	HIV-1 infection/ PI-experienced patients	400/100 mg b.i.d. 600/100 mg b.i.d. (tablet)	Long term (144 weeks[a])	29

a Trials are still ongoing.

Dose ranging studies

In these early studies, patients randomised to the darunavir treatment group received the TF019 oral solution (20 mg/ml) as a substitute for all PIs in the failing therapy. Patients randomised to the control group continued their current therapy.

In study TMC-C201 darunavir was administered in the fasting state and without RTV in doses of 400 mg b.i.d, 800 mg b.i.d, 800 mg t.i.d, 1200 mg t.i.d. The NRTI regimen was to remain unchanged until the end of the treatment period**.** Due to availability of data from pharmacokinetic studies that showed a boosting effect of RTV, only 34 patients (30 males with mean age around 40 years) were enrolled. The median duration of HIV infection was 7.33 years (0.1 - 21 years) and half of the patients had CDC classification C disease. Ten patients (29.4%) were taking a single PI at screening, 18 (52.9%) a single boosted PI, 1 (2.9%) a double PI and 5 (14.7%) a double boosted PI. Of the 34, 33 had clade B virus with a

baseline median log_{10} viral load of 4.12 and median CD4 count of 320 x 10^6/l. Overall, 16 patients (47%), including 4 in the control group, had virus resistant to all currently approved PIs while 12 (35.3%) had virus susceptible to 2 or more. The median fold change in EC_{50} values (FC) for darunavir at baseline for those who started treatment was 1.60 (range 1.20 to 2.56 for darunavir groups but the median FC for the control group was 9.80).

A dose related anti-viral activity (change in log_{10} viral load from baseline on Day 14) was observed: the median changes in log_{10} viral load from baseline were -0.313, -0.818, -1.124, and -0.691 log_{10} copies/ml for the increasing dose groups, respectively versus +0.210 log_{10} copies/ml for the control group.

In study TMC114-C207 darunavir was co-administered with RTV in the fed state to HIV infected patients in doses of 300/100 mg b.i.d., 600/100 mg b.i.d., 900/100 mg q.d.). The study design was generally similar to that of C201, including the fact that patients on failing regimens that included NNRTIs were not eligible. There were 50 patients randomised and treated in the study of which 46 were male with a mean age of around 40 years. The median baseline log_{10} viral load was 4.26 copies/ml, the median baseline CD4 count was 305 x 10^6/l and 23 patients were of CDC classification C. At screening, 40 were taking a boosted PI of which 27 were taking lopinavir/ritonavir. Phenotypic data indicated that 25 patients (51%) had virus resistant to all currently approved PIs while 13 had virus that was susceptible to 2 or more PIs. The median fold resistance value for darunavir was 1.70 with a range for other PIs from 5.30 to 92.40. Patients had a broad range of protease mutations at baseline with a median of 3 primary PI mutations and a median of 6 primary and secondary mutations.

A dose related anti-viral activity was observed. The DAVG (time averaged difference) of the plasma viral load response over a 2-week treatment observed in each darunavir/ritonavir group were statistically superior to the control arm ($p<0.001$ for all comparisons). -0.56 log_{10} for the 300/100 mg b.i.d. group, -0.70 log_{10} for the 900/100 mg q.d. group, and -0.81 log_{10} for the 600/100 mg b.i.d. group, *versus* -0.03 log_{10} for the control group.
At the highest dose (600 mg/100 mg b.i.d) the highest darunavir exposure was achieved and the mean trough darunavir concentration were approximately 2000 ng/ml (compared to 350 ng/ml with 1200 mg t.i.d darunavir in the C201 study).

On the basis of these studies the applicant considered that combination of darunavir with ritonavir was justified. The applicant further provided data to bridge bioequivalence between the oral solution and the commercial/Phase III tablet. The *proof of principle* studies and their relevance to dose finding Phase IIb studies can therefore be considered acceptable.

Main studies

The demonstration of efficacy of the proposed dose regimen for boosted darunavir is based on two ongoing Phase II randomised, controlled, partially blinded trial studies:

TMC-114-**C202:** investigating the dose response of darunavir with low dose of ritonavir in 3-class-experienced HIV-1 infected patients, followed by an open-label period on the recommended dose of darunavir with low dose of ritonavir.

TMC-114-**C213**: investigating the efficacy, safety, and dose-response relationship of darunavir with low dose ritonavir in 3-class-experienced HIV-1 infected patients, followed by an open-label period on the recommended dose of darunavir with low dose of ritonavir

Methods

- **Study Participants**

In study TMC-114-C202, the main inclusion criteria were:

- Male or female HIV-1 infected patients aged 18 years and older;
- Patients receiving a PI-containing regimen at screening, initiated at least 8 weeks prior to screening, with plasma HIV-1 RNA > 1000 copies/ml (assayed by RNA PCR Standard specimen procedure);
- "Three-class-experienced" defined as follows:

o The initial protocol stated that patients had to have received at least two different PIs for at least 3 months but was changed in February 2004 to at least one PI for at least 3 months. Patients were to have at least 1 primary PI mutation at screening as defined by the IAS-USA guidelines (i.e. D30N, M46I/L, G48V, I50V/L, V82A/F/T/S, I84V, L90M). The original protocol set a 30% limit on the proportion of patients that might be enrolled with ≥ 3 primary PI mutations but this was later removed by amendment and any patients previously excluded could be re-screened.

o Prior treatment was also to be with ≥ 2 NRTIs for at least 3 months in total and ≥ 1 NNRTI in a failing regimen (based on virological response or tolerability) but patients on antiretroviral therapy that contained an NNRTI at screening were excluded. An amendment of February 2004 allowed for patients with documented NNRTI resistance to be enrolled even if there was no history of NNRTI use.

o Investigational agents counted as prior exposure but were not allowed in screening or optimised background regimens (OBR).

Main exclusion criteria were:

- Presence of any currently active AIDS defining illness (Category C conditions according to the Center for Disease Control [CDC] Classification System for HIV Infection 1993) with the following exceptions: - Stable cutaneous Kaposi's Sarcoma and - Wasting syndrome due to HIV infection
- Previously demonstrated clinically significant allergy or hypersensitivity to any of the excipients of the investigational medications, with the exception of sulphonamide allergy although darunavir is a sulphonamide;
- Patients with clinical or laboratory evidence of active liver disease, liver impairment/dysfunction or cirrhosis irrespective of liver enzyme levels.

In study TMC-114-C213, the main inclusion/exclusion criteria were the same as for trial C202, with the exception that patients co-infected with hepatitis B or C were allowed to enter the trial if their condition was clinically stable and would not require treatment during the study period. Patients diagnosed with hepatitis A at screening were not allowed in the trial.

Treatment

The trials included:
- A screening period, which lasted a maximum of 6 weeks (Part S + A) and included a 2-week period (Part A) after randomisation during which the patients remained on the regimen used at screening.

In study C202, for patients randomised to the darunavir/ritonavir treatment groups, the trial included:
- A functional monotherapy period (2 weeks) during which patients substituted the PI(s) in the regimen from screening for one of four treatment groups of darunavir + rtv (Part B1) but had to remain on their background NRTIs with or without enfuvirtide.

The four darunavir+rtv treatment groups were as follows: 400/100 mg q.d., 800/100 mg q.d., 400/100 mg b.i.d. and 600/100 mg b.i.d.

During the dose-finding parts of these studies darunavir was supplied as 200 mg (F002) or 400 mg (F001) tablets, each coming from several different batches. In the 600/100 mg b.i.d. group 1x400 mg + 1x 200 mg darunavir tablet was taken twice daily while only 400 mg tablets were used in the other groups. Patients were to take darunavir+ rtv orally within 30 minutes after completion of a meal every 12 or 24 hours as allotted. Whenever possible, the OBR and darunavir+ rtv were taken at the same time.

Investigators were provided with genotypic viral resistance data in order to construct an OBR for each patient according to these results and treatment history.

- Prior to randomisation, and based on the screening resistance data and prior antiretroviral (ART) history, the investigator selected a PI(s)-regimen and an OBR consisting of NRTIs with or without enfuvirtide.
- If the 3-class experienced patients had virus that showed resistance to all 3 classes it was advised to add a new class, if possible.
- If patients had virus resistant to all PIs it was recommended to use a new treatment regimen that included either a boosted PI or dual (boosted) PI combination. Patients randomised to the control group received the pre-selected PI(s) during the treatment period while all the others received their assigned darunavir+ rtv regimen instead. At least one PI had to be added to the OBR for control group patients.
- Patients on NNRTIs at screening were not eligible and NNRTIs were not allowed in any group during the study.
- Tipranavir was not allowed in the control group.
- Abacavir was disallowed during the trial. However, if patients receiving abacavir at screening without problems of tolerance for at least 8 weeks could continue with this as part of their OBR but could not discontinue abacavir and restart after screening.
- Enfuvirtide was allowed in all groups.

The OBR (for control and darunavir+ rtv groups) and the investigator-selected PI(s) regimen (for controls) were not to be changed until the end of the treatment period unless this was necessary due to tolerability/toxicity reasons. Medications that might be expected to have a clinically significant interaction with darunavir were banned.

Both studies are still ongoing and have a treatment duration of 144 weeks.

- **Objective**

In the original protocols, the primary objective was to evaluate the dose-response relationship of antiviral activity of the darunavir +ritonavir dose regimens at 24 weeks.
Based on planned interim analyses (week 16 and 24) which showed a higher antiviral activity of darunavir+ritonavir than expected (as presented under the results), all randomised patients switched to the recommended dose of darunavir+ritonavir (600 mg+100 mg b.i.d) and the primary objective was amended to compare all darunavir+ritonavir dose groups (TMC114-C202 + TMC114-C213) with control at Week 24 by means of confirmed virologic response.

- **Outcomes/endpoints**

Following amendment of the objective of the studies, the primary endpoint was switched from change in viral load from baseline, to virologic response defined as at least 1.0 $\log_{10}$ decrease in viral load versus baseline at week 24.

The main secondary outcomes included:

- Proportion of patients with at least 1.0 $\log_{10}$ / 0.5 $\log_{10}$ drop (time to loss of virologic response = TLOVR) in plasma viral load (compared to baseline) at all other time points, and time to achieve this.
- Proportion of patients with plasma HIV-1 RNA levels < 50 copies/ml / < 400 copies/ml (TLOVR) at each time point, and time to achieve this.

- DAVG (time averaged difference) of $\log_{10}$ plasma viral load over 24 weeks**,** which was defined as the AUC of the change in $\log_{10}$ plasma viral load from baseline divided by the time treated in the trial. Plasma viral load values below 50 copies/ml (assay detection limit) were scored as 49 in the calculation of the DAVG.

Virological failure was defined as lack or loss of response in the trial: less than 0.5 $\log_{10}$ reduction in plasma HIV-1 RNA from baseline beyond Week 12 (1 confirmatory result required with either a planned or an unscheduled visit). These patients could be eligible for the roll-over trial (TMC114-C215).

- **Sample size**

Power calculation in the original protocols were based on the original primary antiviral activity parameter - change in plasma viral load at Week 24. When the primary efficacy parameter was amended the sample size was not revisited.

The study reports stated that to detect at least a 0.5 $\log_{10}$ change in plasma viral load decrease at Week 24 of the highest compared to the lowest darunavir dose group (with a common SD of 1), with 80% power at a significance level of 5% (one-sided), 51 patients were required per treatment group. To account for the effect of discontinuations on the primary parameters, 60 per group were needed for intent to treat (ITT) analysis.

- **Randomisation**

A central randomisation (IVRS) system was used for the dose-finding part of the trial.
Three stratification factors were identified for randomisation and were subsequently used in the statistical analysis as covariates in the analysis models:
- Screening plasma viral load (< 20000, ≥ 20000 copies/ml)
- Use of enfuvirtide (part of the OBR or not)
- Number of Primary PI mutations at baseline (1, 2, 3 or more)

Given the low numbers per treatment group an adaptive minimisation technique with biased coin assignment was used to try to achieve balance across treatment groups in each stratum.

- **Blinding**

The dose-finding part of the study was partially blinded. Patients knew whether they were in the control or in the treatment group with darunavir+ ritonavir and patients in the darunavir+ritonavir groups as well as the investigators and sponsor were aware of whether a once or twice daily regimen was administered. Nonetheless none was aware of the actual dose allotted within the once or twice daily groups.

- **Statistical methods**

All statistical tests were interpreted at the 5% two-sided significance level.
The significance level alpha was adjusted accounting for the Week 24 formal testing. There was no formal adjustment for the first (Week 16) interim analysis.

The intent-to-treat population was used as primary population and comprised all patients randomised and treated with baseline or post-baseline data, regardless of their compliance with the protocol or their eligibility.

Planned interim analyses

There were 2 Data Safety Monitoring Board interim analyses at weeks 8 and 12 with the objective of protection of human subjects. There were two other formal pre-specified combined interim analyses of both trials at 16 and 24 weeks to define the recommended dose which would have to be used in confirmatory studies (at week 16 when 150 patients in each

trial had completed 16 weeks of treatment and at week 24 when 150 patients in each trial had completed 24 weeks of treatment).

The primary analysis of trial C213 was performed as planned per protocol, i.e., when 300 subjects reached Week 24 or discontinued earlier. Based on the planned visit dates, a cut-off date of 1 February 2005 was determined. All data up to this cut-off date were included in the primary analysis of this trial. Due to over-recruitment, there were 318 subjects in the trial, of which 17 did not reach Week 24 or discontinued earlier at the time of the 1 February 2005 cut-off. The applicant decided not to wait for these last 17 subjects, as they would not influence the conclusions.
Trial C202 included a more resistant population, and a clinically significant difference would be observed between the lowest dose of darunavir+rtv and the recommended dose. The applicant used therefore same data cut-off date.
From 1 February 2005 onwards, subjects randomised to darunavir+rtv 400/100 mg q.d., 800/100 mg q.d., or 400/100 mg b.i.d. were switched to the recommended dose of darunavir+ rtv 600/100 mg b.i.d.
An additional analysis was performed with a later cut-off date (September 2005).
Although both interim analyses were not intended to stop or change the design of the current trial, the significance level alpha was adjusted accounting for the Week 24 formal testing by means of the O'Brien and Fleming method, using alpha = 0.0054 for the Week 24 interim analysis, and 0.0492 for the week 24 primary analysis.
The nominal significance level (alpha) was adjusted for multiple comparisons versus control by means of Bonferroni-Holms multiple testing procedure.

RESULTS

Patients flow

The cut-off date for the primary efficacy analysis was 1 February 2005.

For study TMC114-C202, 278 patients were included in the primary analysis, of whom 201 had reached 24 weeks of treatment or discontinued earlier.
For study TMC-114-213, 318 patients were included of whom 301 patients had reached week 24 or discontinued earlier.

Patient disposition for both studies is shown in tables 7 and 8 respectively.

Table 7: Patients disposition - Study C202

Number of subjects	**400/100 q.d.**	**800/100 q.d.**	**400/100 b.i.d.**	**600/100 b.i.d.**	**controls**	**total**
Screened	-	-	-	-	-	583
Not randomised – not treated	-	-	-	-	-	289
Randomised – not treated	1	2	4	2	7	16
Randomised – treated	57	56	55	57	53	278
Discontinuations - Reason, n (%)	**17 (29.8)**	**12 (21.4)**	**10 (18.2)**	**12 (21.1)**	**34 (64.2)**	**85 (30.6)**
Adverse event/HIV related event	5 (8.8)	4 (7.1)	4 (7.3)	5 (8.8)	2 (3.8)	20 (7.2)
Lost to follow-up	0	1 (1.8)	0	2 (3.5)	1 (1.9)	4 (1.4)
Withdrew consent	1 (1.8)	1 (1.8)	2 (3.6)	0	4 (7.5)	8 (2.9)
Virological failure	8 (14.0)	5 (8.9)	3 (5.5)	4 (7.0)	25 (47.2)	45 (16.2)
Sponsor's decision	0	1 (1.8)	1 (1.8)	0	0	2 (0.7)
Ineligible to continue	1 (1.8)	0	0	0	1 (1.9)	2 (0.7)
Non-compliant	2 (3.5)	0	0	1 (1.8)	1 (1.9)	4 (1.4)

Table 8: Patients disposition - Study C213

	DRV/RTV (mg)				**Control**	**All subjects**
	400/100 q.d	**800/100 q.d**	**400/100 b.i.d**	**600/100 b.i.d**		
Subjects screened	-	-	-	-	-	697
Not randomised	-	-	-	-	-	363
Not treated	1	1	2	2	10	16
Randomised – treated	64	63	63	65	63	318
Discontinuations – Reason, n (%)	7 (10.9)	3 (4.8)	12 (19.0)	3 (4.6)	39(61.9)	64 (20.1)
AE/HIV related AE	2 (3.1)	1 (1.6)	8 (12.7)	1 (1.5)	4 (6.3)	16 (5.0)
Lost to follow-up	0	0	0	0	1 (1.6)	1 (0.3)
Withdrew consent	1 (1.6)	0	0	0	0	1 (0.3)
Virological failure	3 (4.7)	2 (3.2)	4 (6.3)	2 (3.1)	34 (54.0)	45 (14.2)
Other	1 (1.6)	0	0	0	0	1 (0.3)

Whereas about 5-20% discontinued from each darunavir + rtv regimen (lower than in C202), 62% discontinued in the control group (similar to C202). In both studies, the main reason for trial discontinuation was virological failure, which occurred most frequently in the control groups.

Conduct of the study

In study C202, major protocol deviations occurred in 3 patients in darunavir + rtv groups (1%) – 2 who took an additional PI for some of the treatment period and 1 took once daily

instead of twice daily assigned therapy. One patient in the control group did not have at least 1 primary PI mutation at screening.
In study C213, major protocol deviations occurred in 11 patients including 6 in the darunavir + rtv groups with disallowed changes to the OBR or, in 1, continuation of lopinavir/ritonavir for the first 8 days. Three patients in the control group had disallowed changes to the OBR and 1 took abacavir while it was not included in the selected OBR.

The limited number of protocol violations was not considered to have influenced the outcome of the trials.

Baseline Characteristics

Demographic data and baseline disease characteristics were comparable across the treatment groups in both studies. Nonetheless in C213 there was a higher percentage of patients co-infected with hepatitis B or C virus in the control group compared to the darunavir+rtv groups (21% versus 12%).

The population included in study C202 had more advanced disease than the one included in C213 as shown in table 9 by baseline disease characteristics, treatment experience, and baseline CD4+ cell count.

Table 9: Overview of Relevant Baseline Characteristics in Trials TMC114-C202 and TMC114-C213

Parameters	TMC114-C202 N = 278	TMC114-C213 N = 318
Baseline Disease Characteristics		
log_{10} viral load, mean (SD)	4.66 (0.76)	4.48 (0.78)
CD4+ cell count (x 10^6/l), median (range)	106 (1; 1274)	179 (3; 816)
Duration of HIV-1 infection (years), mean (SD)	13.2 (3.94)	11.6 (4.20)
Previous ARV Experience, n (%)		
PI: ≥ 2	271 (97.5)	307 (96.5)
NNRTI: ≥ 1	270 (97.1)	302 (95.0)
NRTI: ≥4	263 (94.6)	304 (95.6)
FI: 1	63 (22.7)	34 (10.7)
Baseline Genotype and Phenotype		
≥ 1 susceptible PI, n (%)	81 (29.5)	115 (37.3)
≥ 1 susceptible NRTI, n (%)	258 (94.2)	300 (97.4)
Darunavir FC, median (range)	4.9 (0.1; 470.4)	3.5 (0.0; 503.2)
Number of primary PI mutations, median (range)	3 (0; 5)	3 (0; 6)
Number of PI resistance-associated mutations, median (range)	8 (1; 13)	8 (0; 13)
Number of patients with ≥ 3 primary PI mutations at baseline, n (%)	182 (66.2)	178 (56.0)
Hepatitis B or C co-infected		
Not co-infected	272 (97.8)	274 (86.2)
Co-infected	6 (2.2)	44 (13.9)

In C202, based on the Antivirogram data at screening:
- 70.5% of all patients were infected with virus resistant to all commercially available PIs and 15.3% had virus susceptible to only 1 PI (see table 10).
- 94% had virus susceptible to at least one NRTI.
- 49% had virus susceptible to one or more NNRTIs (but NNRTIs were banned from the OBR).

In the control group 33% had virus that was susceptible to at least 1 PI at baseline. During the treatment period 21% received a PI to which their virus was susceptible at baseline compared to 6% at screening. Also, 15% of controls were treated with no ART that was predicted to be active based on susceptibility testing results and 42% received only one agent predicted to be active.

Table 10. TMC114-C202: Phenotypic Sensitivity to Commercially Available PIs at Baseline Based on Antivirogram Number of patients with sensitive drugs

	Darunavir+ rtv (mg)				Control	All patients
Parameter	**400/100 q.d**	**800/100 q.d**	**400/100 b.i.d**	**600/100 b.i.d**		
PI						
All ARV therapy	**N = 56**	**N = 55**	**N = 55**	**N = 57**	**N = 52**	
0 sensitive	43 (76.8)	40 (72.7)	38 (69.1)	38 (66.7)	35 (67.3)	194 (70.5)
1 sensitive	8 (14.3)	9 (16.4)	7 (12.7)	11 (19.3)	7 (13.5)	42 (15.3)
> 1 sensitive	5 (8.9)	6 (10.9)	10 (18.2)	8 (14.0)	10 (19.2)	39 (14.2)

Table 11 TMC114-C202: Susceptibilities of viruses to the investigator-selected regimens- During treatment

Number to which viruses susceptible	**400/100 q.d. N = 56**	**800/100 q.d. N = 55**	**400/100 b.i.d. N = 55**	**600/100 b.i.d. N = 57**	**Control N = 52**	**All patients N = 275**
Investigator-selected PI						
0	0	0	2 c	1 c	41 (78.8)	44 (80.0)
1	0	0	0	0	11 (21.2)	11 (20.0)
> 1	0	0	0	0	0	0
NRTI in OBR	**N = 56**	**N = 52**	**N = 54**	**N = 55**	**N = 52**	**N = 269**
0	18 (32.1)	18 (34.6)	19 (35.2)	21 (38.2)	16 (30.8)	92 (34.2)
1	24 (42.9)	26 (50.0)	19 (35.2)	20 (36.4)	25 (48.1)	114 (42.4)
> 1	14 (25.0)	8 (15.4)	16 (29.6)	14 (25.5)	11 (21.2)	63 (23.4)
Enfuvirtide in OBR						
resistant	12 (37.5)	6 (19.4)	6 (20.7)	8 (28.6)	8 (32.0)	40 (27.6)
susceptible	20 (62.5)	25 (80.6)	23 (79.3)	20 (71.4)	17 (68.0)	105 (72.4)
OBR* + investigator-selected PI for controls						
0	11 (19.6)	8 (15.4)	13 (24.1)	15 (27.3)	8 (15.4)	55 (20.4)
1	22 (39.3)	23 (44.2)	15 (27.8)	17 (30.9)	22 (42.3)	99 (36.8)
> 1	23 (41.1)	21 (40.4)	26 (48.1)	23 (41.8)	22 (42.3)	115 (42.8)

c protocol violators due to use of other PI(s) in combination with DRV/RTV
*in the darunavir groups the rate reflects ONLY the OBR (i.e. count does not include darunavir)

Forty nine percent of patients had 3 or more primary PI mutations at baseline (based on the March 2003 IAS-USA list). When using the updated list, this figure was 66%.
The incidence of the primary mutations L33F/I, M46I/L, V82A/F/L/S/T, I84A/C/V, L90M was at least 30% or higher. Overall, the control group was comparable to the darunavir+ rtv groups in terms of: demographic and baseline disease characteristics, genotyping and phenotyping, including PI susceptibility, previous ARV experience and optimisation of the OBR

In the control group, all PI regimens included ritonavir as a pharmacokinetic enhancer. The most frequently used PI was amprenavir or fosamprenavir (42%) followed by saquinavir (30%) and lopinavir (28%).
In study C213**,** based on the screening Antivirogram data:

- 63% had virus resistant to all available PIs and 17% had virus susceptible to only one PI. See the table 12.
- 97% had virus susceptible to at least one NRTI.
- 45% had virus susceptible to one or more NNRTIs. However, NNRTIs were banned.

In the control group 43% had virus that was susceptible to at least 1 PI at baseline. During the treatment period 33% (see table below) received a PI to which their virus was susceptible at baseline compared to 10% at screening. As in C202, 15% of controls were treated with no ART that was predicted to be active based on susceptibility testing results and 26% in C213 received only one agent to which the virus was predicted to be susceptible.

Table 12. TMC114-C202: Phenotypic Sensitivity to Commercially Available PIs at Baseline Based on Antivirogram Number of patients with sensitive drugs

	TMC114/rtv (mg)				**Control**	**All patients**
Parameter	**400/100 q.d.**	**800/100 q.d**	**400/100 b.i.d**	**600/100 b.i.d**		
PI						
All ARV therapy						
0 sensitive	38 (62.3)	39 (62.9)	42 (70.0)	39 (60.9)	35 (57.4)	193 (62.7)
1 sensitive	8 (13.1)	11 (17.7)	8 (13.3)	13 (20.3)	12 (19.7)	52 (16.9)
> 1 sensitive	15 (24.6)	12 (19.4)	10 (16.7)	12 (18.8)	14 (23.0)	63 (20.5)

Table 13. TMC114-C202: Susceptibilities of viruses to the investigator-selected regimens only-During treatment

Nb to which viruses susceptible	400/100 q.d.	800/100 q.d.	400/100 b.i.d.	800/100 b.i.d.	Control	All patients
PI						
Investigator-selected PI(s)						
0	0	1 (100.0)c	0	0	40 (66.7)	41 (67.2)
1	0	0	0	0	18 (30.0)	18 (29.5)
> 1	0	0	0	0	2 (3.3)	2 (3.3)
NNRTI						
In the OBR						
0	10 (16.4)	15 (24.2)	11 (18.6)	15 (23.4)	15 (24.6)	66 (21.5)
1	29 (47.5)	27 (43.5)	29 (49.2)	33 (51.6)	20 (32.8)	138 (45.0)
> 1	22 (36.1)	20 (32.3)	19 (32.2)	16 (25.0)	26 (42.6)	103 (33.6)
Fusion inhibitor (enfuvirtide)						
In the OBR						
resistant	6 (22.2)	2 (7.4)	6 (20.7)	7 (25.0)	6 (25.0)	27 (20.0)
susceptible	21 (77.8)	25 (92.6)	23 (79.3)	21 (75.0)	18 (75.0)	108 (80.0)
Total susceptible*						
OBR and investigator-selected PI (s)						
0	5 (8.2)	9 (14.5)	5 (8.3)	13 (20.3)	9 (14.8)	41 (13.3)
1	21 (34.4)	19 (30.6)	25 (41.7)	21 (32.8)	16 (26.2)	102 (33.1)
> 1	35 (57.4)	34 (54.8)	30 (50.0)	30 (46.9)	36 (59.0)	165 (53.6)

c protocol violators due to use of other PI(s) in combination with DRV/RTV, *in the darunavir groups the rate reflects ONLY the OBR (i.e. count does not include darunavir)

In the control group, almost all PI regimens (97%) were co-administered with ritonavir as a pharmacokinetic enhancer. The most frequently used PI was lopinavir (46%) followed by saquinavir (38%) and amprenavir or fosamprenavir (25%).

Outcome

Interim analysis

The combined interim analysis at week 16 showed an unexpectedly high decrease in viral load in increasing with the dose in the darunavir+ rtv groups. This was confirmed at the 24 week interim and primary analyses. These results supported the selection of darunavir+ rtv 600/100 mg b.i.d. as the optimal dose in treatment-experienced patients.

Table 14: Observed Mean Change Versus Baseline in log_{10} Plasma Viral Load (Copies/ml) (ITT - NC = F) at Week 24 in the Week-16 and -24 Interim Analyses and 24-Week Primary Analyses of Trials TMC114-C202 and TMC114-C213

Trial	Treatment	Week-16 Interim Analyses			Week-24 Interim Analyses			Week-24 Primary Analyses		
		N	Mean (SD)	p[a]	N	Mean (SD)	p[a]	N	Mean (SD)	p[a]
TMC114-C202	400/100 q.d.	18	-0.72 (1.27)	ND	35	-1.05 (1.53)	ND	40	-1.20 (1.43)	0.0037
	800/100 q.d.	19	-1.04 (1.30)	ND	32	-1.11 (1.48)	ND	41	-1.31 (1.44)	0.00076
	400/100 b.i.d.	16	-0.93 (1.43)	ND	31	-1.54 (1.50)	ND	39	-1.35 (1.43)	0.00038
	600/100 b.i.d.	20	-1.77 (1.40)	ND	32	-1.77 (1.42)	ND	39	-1.71 (1.44)	0.000003
	control	26	-0.12 (0.82)	-	35	-0.12 (0.76)	-	42	-0.29 (0.73)	-
TMC114-C213	400/100 q.d.	16	-1.44 (1.44)	ND	31	-1.53 (1.33)	ND	60	-1.78 (1.28)	<0.00001
	800/100 q.d.	17	-1.82 (1.43)	ND	29	-1.77 (1.29)	ND	58	-1.83 (1.31)	<0.00001
	400/100 b.i.d.	16	-1.18 (1.37)	ND	33	-1.41 (1.37)	ND	61	-1.69 (1.24)	<0.00001
	600/100 b.i.d.	14	-2.01 (1.28)	ND	32	-1.92 (1.15)	ND	60	-2.02 (1.12)	<0.00001
	control	23	-0.14 (0.65)	-	39	-0.41 (0.97)	-	60	-0.63 (0.98)	-
TMC114-C202 + TMC114-C213	400/100 q.d.	34	-1.06 (1.38)	ND	66	-1.28 (1.45)	<0.001	100	-1.55 (1.36)	ND
	800/100 q.d.	36	-1.41 (1.40)	ND	61	-1.43 (1.42)	<0.001	99	-1.61 (1.38)	ND
	400/100 b.i.d.	32	-1.05 (1.38)	ND	64	-1.47 (1.42)	<0.001	100	-1.56 (1.32)	ND
	600/100 b.i.d.	34	-1.87 (1.34)	ND	64	-1.85 (1.29)	<0.001	99	-1.90 (1.25)	<0.00001
	control	49	-0.13 (0.74)	-	74	-0.27 (0.89)	-	102	-0.49 (0.89)	-

Primary Outcome

The results of both trials for the primary efficacy parameter (i.e. at least 1.0 log_{10} decrease in viral load versus baseline at week 24) are shown in the table below.

Table 15: Comparison of the Virologic Response Rate at Week 24 (ITT-TLOVR) Defined as a Decrease of 1.0 log10 or More in Plasma Viral Load Versus Baseline for Trials C202 and C213

	TMC114-C202		TMC114-C213		TMC114-C202/ TMC114-C213	
Treatment group	N	n (%)	N	n (%)	N	n (%)
400/100 mg q.d.	40	18 (45)	60	42 (70.0)	100	60 (60.0)
800/100 mg q.d.	41	20 (48.8)	60	43 (71.7)	101	63 (62.4)
400/100 mg b.i.d.	39	21 (53.8)	61	42 (68.9)	100	63 (63.0)
600/100 mg b.i.d.	39	24 (61.5)	60	46 (76.7)	99	70 (70.7)
Control	42	6 (14.3)	60	15 (25.0)	102	21 (20.6)

N: number of patients at Week 24; n: number of responders

All darunavir+ rtv treatment groups showed significantly higher virological response rates than the control group. The higher daily dose level of darunavir+ rtv (600/100 mg b.i.d.) tended to result in higher virological response rate than the lower daily dose levels.

- The applicant provided, 95% 2-sided confidence intervals for the difference in percentage of response observed in both treatment arms as shown in table 16.

Table 16: Virologic Response at Week 24 (TLOVR - ITT): Estimated Difference in Proportion of Response (≥ 1.0 log10 Viral Load Reduction) Between the Darunavir+ rtv Treatment Groups and Control – Trials TMC114-C202 and TMC114-C213 – Primary Analyses (Cut-Off 1 February 2005)

Trial	Comparison	Estimated Difference[a]	95% CI[b] of the Difference
TMC114-C202	400/100 mg q.d. vs Control	31.6	[12.5 ; 50.7]
	800/100 mg q.d. vs Control	34.4	[15.6 ; 53.3]
	400/100 mg b.i.d. vs Control	39.8	[20.4 ; 59.2]
	600/100 mg b.i.d. vs Control	48.4	[29.5 ; 67.3]
TMC114-C213	400/100 mg q.d. vs Control	51.8	[35.7 ; 67.9]
	800/100 mg q.d. vs Control	53.3	[37.3 ; 69.3]
	400/100 mg b.i.d. vs Control	48.5	[31.9 ; 65.2]
	600/100 mg b.i.d. vs Control	58.0	[42.9 ; 73.2]

[a] Percent response estimated from a logistic regression model including treatment and all stratification variables (use of enfuvirtide and number of primary mutations as stratified, and baseline $\log_{10}$ viral load as a covariate).
[b] Standard errors to compute the confidence intervals are approximated by the delta method.

There was initially a concern over the reliability and robustness of the results mainly in relation to level of optimisation of the OBRs and choice of PI in the control arm. The applicant's analyses clearly showed that having at least one PI in the regimen to which the virus was susceptible was an important determinate of response. Also, responses to enfuvirtide were improved when patients were on at least one ART to which the virus was susceptible. The applicant showed that the measures taken to ensure objectiveness and reliability of the results and avoiding results-driven changes of endpoints during the different amendments of the protocols of these studies were satisfactory. The outlined procedures and value of resistance data in determining the ART for treatment-experienced patients were consistent with the state of the art. The selection of specific NRTIs in the OBR and the predicted susceptibility of the selected NRTIs were comparable for the control and darunavir+ rtv groups, and this was in line with current clinical practice in treating pre-treated HIV-1 infected patients. The choice of the regimen was also affected by the treatment history of the patient and toxicity of the ARV agents. Details of the anti-viral susceptibilities of all the patients in C202 and C213 who did not receive any ART in their OBR that was predicted to be active against the patient's virus (except for darunavir in the test group) were provided. There was a very small minority of patients who did not use the recommended susceptible NRTI (6/23 versus 2/20 responders in the control) but this is unlikely to have modified the conclusions of the trials. The applicant provided also reassuring information with respect to the choice of PI in the control group. The greater majority of all control patients 95/122 (78%) did not have a susceptible control PI option based upon vircoTYPE HIV-1. Of the 27 patients who had at least 1 susceptible PI, only 2 patients did not select a PI for which vircoTYPE HIV-1 predicted susceptibility for acceptable reasons.
The overall enfuvirtide use, supporting the efforts made to offer optimal OBR to the patients, was similar in control arm and darunavir groups and accounted approximately for 45% - 40% in trials C202 and C213, respectively.
The rational for not using NNRTIs in both trials was considered acceptable and in line with present guidelines. NNRTIs generally should not be used following the development of NNRTI-resistance due to nearly universal cross-class resistance among the currently available NNRTIs, pre-treatment or failure with an NNRTI rapidly leads to resurgence of archived

resistant mutants when an agent of the class is reintroduced. The proportion of patients pre-treated with NNRTI was >95% in the studied patient population.
The explorative comparative analysis of the overall antiretroviral efficacy of the regimens in the control arms of previous independent trials (RESIST, TORO) as well as C202 and C213 showed marked similarity. Issues such as compliance (very low non-compliance for the PI component) and very low discontinuation rates through weeks 12-16 did not appear to be a source of bias.

The sensitivity analysis performed, comparing virologic response in the subgroup of patients using darunavir+ rtv without any active drugs in the background versus control patients who were 'truly optimised' (i.e., those receiving ≥ 2 active drugs in the OBR), confirm the favourable efficacy of darunavir+ rtv regimen.

In addition clarifications of all formal interim analyses that were performed on both trials, their purpose, and consequent actions and informing the regulatory authorities were provided. The change in primary endpoint to response rate, which is in line with the CHMP HIV guideline is considered a more conservative endpoint than change in $\log_{10}$ viral load and the results are considered robust.
As to the correction for inflation, when multiplicity is taken even in the most conservative approach, conclusions did not change. However, this only holds when during the trial, based on knowledge of the results, selection of likely responders did not occur. Although in- and exclusion criteria were amended during the trial, given that the effect size decreases from the first interim analysis to the final analysis selection was considered unlikely.

Secondary efficacy endpoint results and other analyses.

In both trials, results obtained for the other virologic response categories (defined as a decrease of viral load by at least 0.5 $\log_{10}$ relative to baseline, or proportion of patients with a viral load < 400 or < 50 copies/ml) confirmed the findings of the primary efficacy parameter.

Table 17. Proportion of patients with viral load < 50 copies/ml at Week 24 in Trials TMC114-C202 and TMC114-C213

	TMC114-C202		**TMC114-C213**	
Treatment group	**N**	**n (%)**	N	**n (%)**
400/100 mg q.d.	40	7 (18)	60	26 (43)
800/100 mg q.d.	41	8 (20)	60	29 (48)
400/100 mg b.i.d.	39	14 (36)	61	30 (49)
600/100 mg b.i.d.	39	15 (39)	60	32 (53)
Control	42	3 (7)	60	11 (18)

With respect to immunological parameters (mean change CD4+ from baseline; % CD4+ cell count; mean change CD8+ from baseline and mean change from baseline ratio CD4+/CD8+) the results for the darunavir+ rtv treatment groups were better than for the control in both trials. A dose response relationship was more obvious in trial C213 than in trial C202.

Sensitivity analyses (including all subjects in the darunavir+ rtv groups regardless of compliance, while in the control group only subjects who were compliant were included - as determined by the compliance questionnaire and by measurement of plasma levels) were performed the results of which corroborated the outcome and conclusions for both trials. The difference in non-compliance cannot explain the large differences observed between the

darunavir+ rtv and control group, and therefore, this sensitivity analysis confirms the overall superiority of darunavir+ rtv over control.

The applicant provided an updated 24-week analysis which included all randomised subjects in both trials C202 and C213 in the group that received the recommended dose of darunavir/ritonavir at initial randomisation (cut-off of 24 September 2005). The estimated response rates remained stable and confirmed the conclusions from the primary analysis (table 19).

Table 18 Reasons for Discontinuation - TMC114-C202 + TMC114-C213 (cut-off 24 September 2005)

Parameter	**TMC114-C202 + TMC114-C213**	
	Darunavir+ rtv 600/100 mg b.i.d	**Control**
N	131	124
Discontinuations - Reason, n (%)	28 (21.4)	100 (80.6)
Adverse event/HIV related event	12 (9.2)	6 (4.8)
Subject ineligible to continue the trial	0	1 (0.8)
Subject lost to follow-up	3 (2.3)	2 (1.6)
Subject non-compliant	1 (0.8)	2 (1.6)
Subject reached virologic endpoint	11 (8.4)	83 (66.9)
Subject withdrew consent	1 (0.8)	6 (4.8)

Number of primary PI mutations (updated)		
N	131	122
0	1 (0.8%)	0
1	11 (8.4%)	13 (10.7%)
2	53 (40.5%)	33 (27.0%)
≥3	66 (50.4%)	76 (62.3%)

Table 19 Efficacy Results for Trials TMC114-C202 and TMC114-C213 Observed in the Primary Analysis (Cut-Off 1 February 2005) and in the Updated Analysis (Cut-Off 24 September 2005)

Efficacy Parameter, n (%) for Viral Load, Mean (SD) for CD4	Primary Efficacy Analysis (Cut-off 1 February 2005)						Updated Efficacy Analysis (Cut-off 24 September 2005)					
	TMC114-C202		TMC114-C213		TMC114-C202/ TMC114-C213		TMC114-C202		TMC114-C213		TMC114-C202/ TMC114-C213	
	600/100 mg b.i.d. N = 39	Control N = 42	600/100 mg b.i.d. N = 60	Control N = 60	600/100 mg b.i.d. N = 99	Control N = 102	600/100 mg b.i.d. N = 66	Control N = 61	600/100 mg b.i.d. N = 65	Control N = 63	600/100 mg b.i.d. N = 131	Control N = 124
Decrease of ≥ 1.0 $\log_{10}$ in Viral Load Versus Baseline	24 (61.5)	6 (14.3)	46 (76.7)	15 (25.0)	70 (70.7)	21 (20.6)	42 (63.6)	8 (13.1)	50 (76.9)	18 (28.6)	92 (70.2) (62%; 78%)[f]	26 (21.0) (14%; 28%)[f]
Viral Load < 400 copies/ml	19 (48.7)	4 (9.5)	40 (66.7)	15 (25.0)	59 (59.6)	19 (18.6)	37 (56.1)	7 (11.5)	45 (69.2)	16 (25.4)	82 (62.6) (54%; 71%)[f]	23 (18.5) (12%; 25%)[f]
Viral Load < 50 copies/ml	15 (38.5)	3 (7.1)	32 (53.3)	11 (18.3)	47 (47.5)	14 (13.7)	25 (37.9)	5 (8.2)	34 (52.3)	10 (15.9)	59 (45.0) (37%; 54%)[f]	15 (12.1) (7%; 18%)[f]
Mean change (SD) Versus Baseline in CD4+ Cell Count (x 10^6 cells/l)	58.9* (66.9)	11.7 (105.6)	124.1 (139.5)	20.4 (107.1)	98.4 (120.3)	16.8 (106.1)	67.3 (79.1)	9.7 (92.2)	118.0 (136.2)	24.7 (106.5)	92.4 (113.6) (73; 112)[f]	17.3 (99.6) (0; 35)[f]

N = number of subjects at Week 24; n = number of responders; [f] 95% confidence intervals.

$p < 0.001$ for all comparisons versus control (except * $p < 0.005$)

In response to CHMP request, the applicant provided updated assessment of the magnitude of effect size (with confidence intervals) and this confirms the efficacy of the recommended darunavir+ rtv regimen (table 20).

Table 20 Virologic Response at Week 24 (TLOVR - ITT): Estimated Difference in Proportion of Response (< 50 copies/ml) Between the Darunavir+ rtv Treatment Groups and Control – Trials TMC114-C202 and TMC114-C213 – Updated Analyses (Cut-Off 24 September 2005, Pre-Switch)

Trial	Comparison	Estimated Difference[a]	95% CI[b] of the Difference
TMC114-C202	400/100 mg q.d. vs Control	12.9	[1.2 ; 24.5]
	800/100 mg q.d. vs Control	14.8	[2.3 ; 27.4]
	400/100 mg b.i.d. vs Control	28.7	[13.6 ; 43.7]
	600/100 mg b.i.d. vs Control	30.0	[16.3 ; 43.8]
TMC114-C213	400/100 mg q.d. vs Control	32.4	[16.3 ; 48.6]
	800/100 mg q.d. vs Control	45.9	[29.7 ; 62.2]
	400/100 mg b.i.d. vs Control	34.3	[18.1 ; 50.5]
	600/100 mg b.i.d. vs Control	47.4	[31.9 ; 62.9]

[a] Percent response estimated from a logistic regression model including treatment and all stratification variables (use of enfuvirtide and number of primary mutations as stratified, and baseline $\log_{10}$ viral load as a covariate).
[b] Standard errors to compute the confidence intervals are approximated by the delta method.

The applicant provided in a synoptic form an update of the efficacy data up to end May 2006 (see table 21). The updated information shows the efficacy of darunavir+ rtv at Week 72 in 65 subjects in each trial. Although virologic response tended to decrease over time with the primary efficacy endpoint (≥ 1.0 $\log_{10}$ drop in viral load), response was sustained for up to at least Week 72 for the most stringent secondary efficacy endpoint (< 50 copies/ml).

Table 21 Efficacy Results in Trials TMC114-C202 and TMC114-C213 at Week 72, and Trial at Week 48 – darunavir+ rtv 600/100 mg b.i.d. in combination with an OBR. Updated Analyses (Cut-Off End of May 2006)[a]

	TMC114-C202		TMC114-C213	
Parameter	End of May 2006 cut-off Week 72 N = 65	End of May cut-off Week 48 N = 66	End of May 2006 cut-off Week 72 N = 65	End of May cut-off Week 48 N = 65
Decrease of ≥ 1.0 $\log_{10}$ in plasma viral load, n (%)	32 (49)	34 (52)	45 (69)	47 (72)
Plasma viral load < 50 copies/ml, n (%)	22 (34)	23 (35)	33 (51)	37 (57)
Change in $\log_{10}$ plasma viral load (copies/ml), Mean (SD)	1.35 (1.37)	1.44 (1.36)	1.86 (1.23)	1.93 (1.19)
Change in CD4+ cell count (x 10^6/ml), Mean (SD)	105 (135.24)	77 (100.6)	118 (134.52)	130 (146.2)

[a] Efficacy results at Week 48 in trials TMC114-C202 and TMC114-C213 and at Week 24 in trial TMC114-C215 (data up to the final MAA cut-off date) provided for reference.

In addition, subgroup analyses confirm the favourable efficacy of darunavir+ rtv 600/100 mg b.i.d: In both trials virologic response was greater in the darunavir+ rtv group compared to control taking into account the number of susceptible ARVs (excluding or including PIs) in the OBR based on descriptive analyses, although results should be taken with caution due to the very small numbers involved (but the trend is consistently favourable for the test regimen). The same holds for analysis based on a logistic regression model, with covariates baseline $\log_{10}$ viral load, number of primary PI mutations and the number of sensitive ARVs.

Influence of other factors on Darunavir Efficacy

Region. There were some differences between regions which can be explained by differences in patient disease characteristics and prior ART experience.

Gender. A total of 109 female patients were treated with darunavir. The response (change in viral load) was greater in females than in males. This was primarily due to better prognostic factors; the median baseline darunavir FC was 1.7 in females and 4.3 in males.

Age. There were no differences between the different age categories in adults. However, the number in the eldest age category (Age>50) was rather small (187) and the patients cannot really be considered elderly.

Supportive data

Studies C208 and C215 were originally designed to provide darunavir+ rtv to patients who previously participated in trials with darunavir, and to provide data on the efficacy and safety of darunavir+ rtv treatment. These trials were changed to evaluate the long-term safety and tolerability of darunavir+ rtv (600/100 mg b.i.d.) compared to control up to 48 weeks. Due to the small number of patients in trial TMC114-C208, data from the 2 trials were combined by the applicant resulting in 3 independent groups of patients for analysis

- **De novo** (N=327): patients starting treatment directly with the recommended dose
- **Control/darunavir** (N=59): patients who were randomised to control in the original trials but who were subsequently treated with any darunavir+ rtv dose other than the recommended dose.
- **Darunavir/darunavir** (N=74): patients who failed on darunavir and rolled-over from any darunavir dose in the preceding trials to the recommended dose.

At the time of submission of the application only summary efficacy data were available but the data on 327 patients who were included in the de novo group in C215/C208, and reached Week 24 and 48 or discontinued earlier (data up to May 2006) were subsequently provided.

Comparison of the *de novo* group with patients initially randomised to the darunavir+ rtv 600 mg b.i.d. group in trials C202 and C213 suggested similarity in terms of demographics and previous ARV experience, with the exception that more patients had used tipranavir previously in C215/C208 (31.5%) compared to C202 (5%) or C213 (3.1%). In the *de novo* group 98.5% of the patients had been pre-treated with ≥ 1 NNRTI. The percentage of patients with 3 or more primary PI mutations at baseline (based on the March 2005 IAS-USA list 28) was >70%. Overall, the median number of primary PI mutations was 3, with a range of 0 to 6 and the median number of PI resistance-associated mutations was 9, with a range of 0 to 13. The median FC in the de novo group was 3.2 for darunavir and 2.6 for tipranavir. The FC for other PIs was higher (all > 20), reflecting the degree of treatment experience of the subjects in these trials. Results of these studies are presented in table 22.

Table 22 Efficacy Results in Trial C215 at Week 24 and at Week 48 – darunavir+ rtv 600/100 mg b.i.d. in combination with an OBR, updated Analyses (Cut-Off End of May 2006)

	TMC114-C215	
Parameter	**End of May 2006 cut-off Week 48 N = 324**	**MAA cut-off Week 24 N = 246**
Decrease of ≥ 1.0 log_{10} in plasma viral load, n (%)	197 (60.8)	160 (65.0)
Plasma viral load < 50 copies/ml, n (%)	146 (45.1)	98 (39.8)
Change in log_{10} plasma viral load (copies/ml), Mean (SD)	-1.64 (1.39)	-1.65 (1.36)
Change in CD4+ cell count (x 10^6/ml), Mean (SD)	106.8 (128.2)	79.8 (99.3)

In the original submission, the subset of patients who had previously received tipranavir (TPV), lopinavir (LPV) and amprenavir (APV) was analysed The Antivirogram data revealed that prior experience with these PIs resulted in loss of susceptibility, defined as a FC > 3, FC>10 and FC> 2.5 for TPV, LPV and APV respectively.
- In patients from the de novo group of trials TMC114-C202, TMC114-C213 and TMC114-C215/C208 who were resistant to TPV, the median darunavir FC increased to 7.4; thus, the majority of the patients resistant to TPV had a darunavir FC < 10. In patients who were failing TPV/RTV at screening, the darunavir median FC was 3.6.
Darunavir retained antiviral activity in most of the patients who had previously used TPV/RTV, where the observed decrease in viral load at Week 24 was -1.40 log_{10} copies/ml. In the patients who switched from TPV/RTV to darunavir+ rtv (used TPV at screening), the decrease in HIV-1 RNA was observed to be -1.64 log_{10} copies/ml; thus, darunavir+ rtv demonstrates significant added antiviral activity to that of TPV/RTV. Although TPV resistance at baseline was also associated with an increase in darunavir FC, the observed change in viral load was still -1.38 log_{10} copies/ml at Week 24.

- The majority of patients (392; 86%) enrolled in TMC114-C202, TMC114-C213 and TMC114-C215/C208 that were in initially randomised to the darunavir+ rtv 600/100 mg b.i.d. groups had used LPV before. Patients who were resistant to LPV, the median darunavir FC was 4.30; thus, the majority of the patients resistant to LPV had a darunavir FC < 10.

Darunavir+ rtv retained antiviral activity in most of the patients who had previously received LPV/RTV, where the observed decrease in viral load at Week 24 was -1.7 log_{10} copies/ml. The virologic response to darunavir+ rtv was consistent in patients who switched from LPV/RTV to darunavir+ rtv (used LPV at screening) and in patients with resistance to LPV at baseline.

-Previous use of APV did not impact *in vitro* susceptibility of darunavir as the median FC of darunavir was 6.3 for patients who previously used APV in the novo group. The mean change in viral load at Week 24 (NC=F) amounted to –1.51.

Special population

Results from a subanalysis on viral load per hepatitis B or C co-infection status for patients who initiated treatment at the recommended dose darunavir+ rtv 600/100 mg b.i.d. in the trials C202, -C213, or C215/C208 are presented in table 23.

Table 23 Virologic Response at Week 24 by Hepatitis B or C Co-infection Status: Proportion of Subjects With ≥ 1 log_{10} Viral Load Reduction and Viral Load < 50 copies/ml (TLOVR), and Change in log_{10} Viral Load Versus Baseline (NC = F) in Subjects who Initiated Treatment at the Recommended Dose – Trials TMC114-C202, TMC114-C213, and TMC114-C215/TMC114-C208

Group/Parameter	≥1 log_{10} Decrease in Viral Load		Viral Load < 50 copies/ml		Change in log_{10} Viral Load vs Baseline	
	N	n (%)	N	n (%)	N	Mean (SD)
Not co-infected with hepatitis B or C	261	179 (68.6)	261	110 (42.2)	261	-1.75 (1.297)
Non-active co-infection with hepatitis B or C	3	3 (100.0)	3	1 (33.3)	3	-2.80 (0.508)
Active co-infection with hepatitis B or C	46	28 (60.9)	46	21 (45.7)	46	-1.67 (1.455)

N = Total number of subjects; n = number of subjects with virologic response.

Results do not suggest any adverse impact of the co-infection on the virological response rate to darunavir but the number of co-infected patients are small.

The efficacy of darunavir with low dose of ritonavir has not been yet studied in patients under 18 years of age but the applicant provided the protocol of a study the results of which will be submitted as part of the follow up measures to be fulfilled post-authorisation.

The efficacy of darunavir with low dose of ritonavir has not been yet established in treatment-naïve HIV-1 infected patients. A controlled phase III trials is currently ongoing in this population (darunavir + rtv regimen: 800/100 mg q.d.) against a fixed PI comparator lopinavir/RTV.

Clinical safety

The clinical safety database derived from several studies in healthy subjects and in HIV infected patients. The safety data of darunavir 600 mg with ritonavir 100 mg twice daily are limited to the analyses of data from 2 ongoing phase IIb trials (C202, C213), complemented by data of de novo patients from 2 open-label trials (C215/C208). A total of 810 patients received treatment with the recommended dose of darunavir+ rtv 600/100 mg b.i.d.:
- 458 patients started immediately on the recommended dose, this included 327 patients who were treated with the formulation to be marketed (de novo group);
- 352 patients switched from a lower dose to darunavir+ rtv 600/100 mg b.i.d. (switched group).

Darunavir being a protease inhibitor, the safety profile was assessed during the clinical studies, considering some of the abnormalities known with this class.

Table 24 Number of patients in safety database

Patients included in clinical trials with darunavir[a]	1948
Patients[a] exposed to darunavir	1783
Patients on recommended dose	810
HIV-1 infected patients on recommended dose for at least 6 months	375 (350 initiated darunavir at the recommended dose, 25 received recommended dose after dose switch)
HIV-1 infected patients on recommended dose for at least 48 weeks	92 (all initiated darunavir at the recommended dose)
Total patient years of exposure of darunavir	828
Total patient years of exposure of recommended dose of darunavir	446

[a] Healthy volunteers and HIV-1 infected patients.

In the initial submission the applicant focussed on the 458 patients who initiated therapy with the recommended dose in these trials (de novo patients i.e., who had never received darunavir+rtv at any dose before). An overall safety analysis of the principal adverse event (AE) and laboratory safety findings was subsequently provided based on the pooled analysis of safety data obtained with any dose of darunavir+rtv in trials C202, C213, C215/C208.

Adverse events

The testing of darunavir alone or in combination with low-dose ritonavir in healthy subjects did not reveal special concerns. However, in many of the trials and the interaction trials the dose used was generally lower than the recommended dose.
The most commonly reported AEs were headache and gastrointestinal (GI) disorders. In addition, skin and subcutaneous disorders (including rash-related AEs) and AEs related to bilirubin abnormalities were commonly reported after repeated dosing of darunavir alone. Rash-related AEs led to permanent treatment discontinuation in 30% (13) of subjects during treatment with darunavir alone compared to 2% (5) of subjects during treatment with darunavir + rtv.

A phase I specifically designed to evaluate the effect of darunavir+ rtv on ECG parameters, using higher doses (800/100 mg b.i.d. and 1600/100 mg q.d.), did not evidence any impact on QTc prolongation. However, there were small increases in QTc and PR intervals compared to placebo for which the clinical relevance is unknown.

In the dose-finding part of the phase II trials, the small numbers of tested patients revealed no clear relationship with darunavir+ rtv dose (or pharmacokinetics).

Pooled data from C202 and C213
Data were provided for 637 patients up to the time of switch or up to the cut-off of September 2005 (see tables 25 and 26).

Table 25: Pooled data from C202 and C213

	400/100 q.d. N = 129	**800/100 q.d. N = 127**	**400/100 b.i.d. N = 126**	**600/100 b.i.d. N = 131**	**Total DRV N = 513**	**Controls N = 124**
Mean exposure (weeks)	***43.95***	***45.48***	***44.02***	***62.29***	***49.03***	***31.54***
≥ 1 AE	122 (94.6)	119 (93.7)	118 (93.7)	131 (100)	490 (95.5)	117 (94.4)
≥ 1 SAE	19 (14.7)	24 (18.9)	24 (19.0)	26 (19.8)	93 (18.1)	17 (13.7)
≥ 1 AE leading to death	5 (3.9)	2 (1.6)	5 (4.0)	5 (3.8)	17 (3.3)	1 (0.8)
≥ 1 AE leading to permanent discontinuation	8 (6.2)	7 (5.5)	12 (9.5)	11 (8.4)	38 (7.4)	6 (4.8)
≥ 1 grade 3 or 4 AE	34 (26.4)	39 (30.7)	37 (29.4)	48 (36.6)	158 (30.8)	36 (29.0)

Table 26 AEs in > 10% in any group (C202 + C213)

Preferred Term, n (%)	**Darunavir/ritonavir (mg)**					**Control N=124**
	400/100 q.d. N=129	**800/100 q.d. N=127**	**400/100 b.i.d. N=126**	**600/100 b.i.d. N=131**	**Total Darunavir N=513**	
Mean exposure (weeks)	***43.95***	***45.48***	***44.02***	***62.29***	***49.03***	***31.54***
Injection site reaction	40 (31.0)	32 (25.2)	32 (25.4)	36 (27.5)	140 (27.3)	27 (21.8)
Diarrhoea	27 (20.9)	20 (15.7)	34 (27.0)	26 (19.8)	107 (20.9)	35 (28.2)
Headache	26 (20.2)	26 (20.5)	28 (22.2)	19 (14.5)	99 (19.3)	25 (20.2)
Nausea	25 (19.4)	18 (14.2)	18 (14.3)	24 (18.3)	85 (16.6)	16 (12.9)

Fatigue	16 (12.4)	17 (13.4)	19 (15.1)	16 (12.2)	68 (13.3)	21 (16.9)
Nasopharyngitis	14 (10.9)	22 (17.3)	14 (11.1)	18 (13.7)	68 (13.3)	13 (10.5)
Upper respiratory tract infection	14 (10.9)	13 (10.2)	20 (15.9)	16 (12.2)	63 (12.3)	8 (6.5)
Insomnia	20 (15.5)	19 (15.0)	10 (7.9)	5 (3.8)	54 (10.5)	6 (4.8)
Cough	18 (14.0)	14 (11.0)	8 (6.3)	12 (9.2)	52 (10.1)	8 (6.5)
Herpes simplex	9 (7.0)	12 (9.4)	15 (11.9)	16 (12.2)	52 (10.1)	2 (1.6)
Pyrexia	13 (10.1)	14 (11.0)	9 (7.1)	7 (5.3)	43 (8.4)	16 (12.9)

The majority of AEs (92%) were grade 1 to 2 in severity. The profile of grade 3 or 4 AEs was similar for the different darunavir + rtv groups and was similar to that in the control group. Immune reconstitution syndrome was reported in three patients and was a SAE in one of these. Injection site reactions were associated with use of enfuvirtide.

The incidence of rash-related AEs was 8% in darunavir + rtv and control groups while the incidence of rash-related AEs per 100 patient years of exposure was lower with darunavir + rtv (8.1; control: 13.3) and there was no apparent relationship with darunavir dose. The single case considered to be a SAE and leading to discontinuation of darunavir + rtv was a toxic skin eruption considered grade 3 and probably related to treatment by the investigator. Apart from this case and one case of grade 3 erythema multiforme on darunavir + rtv, which also led to discontinuation and was considered possibly drug-related, all rash-related AEs were grade 1 or 2.

Pooled data from all dosing at 600/100 mg b.i.d. in C202, C213 and C215/208

The number of patients totalled 810 but the duration of exposure to this dose was very variable due to the different potential origins of the patients.

Table 27 Pooled data from all dosing at 600/100 mg b.i.d. in C202, C213 and C215/208

	De novo					
	C202/213 **N = 131**	***C215/208*** **N = 327**	***All*** **N = 458**	***Switched*** **N = 352**	***All = switched*** **N = 810**	***controls*** **N = 124**
Mean exposure (weeks	63.5	23.9	35.2	20.2	28.7	31.5
≥ 1 AE	131 (100)	289 (88.4)	420 (91.7)	217 (61.6)	637 (78.6)	117 (94.4)
≥ 1 SAE	27 (20.6)	42 (12.8)	69 (15.1)	25 (7.1)	94 (11.6)	17 (13.7)
≥ 1 AE leading to death	5 (3.8)	6 (1.8)	11 (2.4)	5 (1.4)	16 (2.0)	1 (0.8)
≥ 1 AE discontinuation	12 (9.2)	8 (2.4)	20 (4.3)	4 (1.1)	24 (3.0)	6 (4.8)
≥ 1 grade 3/ 4 AE	48 (36.6)	83 (25.4)	131 (28.6)	41 (11.6)	172 (21.2)	36 (29.0)

Treatment discontinuation due to AEs was infrequent: 4% in the 600/100 mg b.i.d. de novo group and 5% in the control group.

Treatment-emergent AEs in > 10% during treatment with 600/100 mg b.i.d. are displayed in table 28. The incidence of AEs was lower for those who switched from a lower dose of darunavir+ rtv to the recommended dose than seen in the *de novo* patients. Incidences of nausea, diarrhoea, vomiting, nasopharyngitis, sinusitis, bronchitis, herpes simplex, headache and pyrexia tended to decrease over time.

Table 28 AEs in > 10% of de novo or control group patients during treatment
C202 + C213 + C215/C208

Preferred Term, n (%)	**C202/213 (A)**	**C215/C208 (B)**	**Switched (C)**	**Total A, B, C**	**controls**
	N = 131	**N = 327**	**N = 352**	**N = 810**	**N = 124**
Mean exposure (weeks)	***63.5***	***23.9***	***20.2***	***28.7***	***31.5***
Any AE	***131 (100)***	***289 (88.4)***	***217 (61.6)***	***637 (78.6)***	***117 (94.4)***
Injection site reaction	36 (28)	61 (19)	11 (3)	108 (13)	27 (21.8)
Diarrhoea	26 (20)	45 (14)	22 (6)	93 (11.5) 71	35 (28.2)
Nausea	24 (18)	33 (10)	14 (4)	(8.8)	16 (12.9)
Nasopharyngitis	18 (14)	37 (11)	12 (3.4)	67 (8.3)	13 (10.5)
Headache	20 (15)	31 (10)	7 (2.0)	58 (7.2)	25 (20.2)
Fatigue	17 (13)	15 (5)	6 (1.7)	38(4.7)	21 (16.9)
Pyrexia	8 (6)	17 (5)	8 (2.3)	33(4.1)	16 (12.9)

Safety data in patients who switched darunavir dose and/or formulation did not reveal a clear relationship between before and after the switch. However, headache and GI events were more commonly reported after switch in the lower dose groups, compared to the higher dose groups. There appeared to be no relationship between safety parameters and darunavir formulation. The safety profiles before and after the switch to the commercial darunavir formulation (F016)- in patients previously exposed to various doses of the clinical trial formulations- seemed to be consistent with each other.

The safety profile in the novo group was similar to that of PIs within the tested control group receiving OBR.

Serious adverse event/deaths/other significant events

The applicant gave additional overall overview of the principal AE and laboratory safety findings of the overall pooled analysis of safety data obtained with any dose of darunavir+ rtv in trials C202, C213, C215 and C208. This analysis included 924 patients in darunavir/ritonavir versus 124 in the control group and the mean duration of treatment for this group was considerably shorter than for the total darunavir+ rtv group (31.5 versus 46.6 weeks) due to a high attrition rate in the control group of trials C202 and C213.The findings of this overall safety analysis are consistent with the overall safety profile of darunavir+ rtv based on the pooled analysis of safety data from patients who initiated treatment with the recommended dose of darunavir+ rtv 600/100 mg b.i.d. in these trials initially presented.

In total, 25 deaths after treatment with any dose of darunavir/ritonavir (924) were reported. Overall mortality rate was 3.0 patients per 100 patient years exposure vs. 1.3 patients per 100 patient years exposure in the control group. However, the control group consisted only of a

small number of patients (124) and there was a high frequency of early dropouts for virologic failure. In the darunavir groups, all deaths, except from 3 cases, occurred after at least 18 weeks of treatment. None of these deaths were considered at least possibly related to darunavir + rtv treatment. Most cases (16 of 25, 64%) occurred in patients with advanced HIV disease (with baseline CD4+ cell count below 50 x 10^6 cells/l). The data are reassuring but any future case with fatal outcome will be discussed in PSURs.

Overall, 15% of the patients in the 600/100 mg b.i.d. de novo group reported SAEs. Most types of SAEs occurred at isolated cases. The most common SAE was pneumonia but this occurred in only 1 % in the de novo group. Discontinuations due to AEs were infrequent (4% in the 600/100 mg b.i.d. de novo group; 5% control).

The incidence of AEs of special interest for HIV patients using PI containing ART regimens was generally low, mostly grade 1 or 2 in severity. The most common grade 3 or 4 laboratory abnormalities were decreased white blood cells count (7% in the 600/100 mg b.i.d. de novo group), and increased triglycerides (9%), amylase (7%), and total cholesterol (5%).

No SAE of hepatotoxicity was observed in the dose-finding studies. Abnormalities in liver function tests were mainly observed for AST and ALT. Graded increases in AST and ALT were observed in 23% and 21% of the patients in the 600/100 mg b.i.d. de novo group, respectively. Most graded individual liver abnormalities were grade 1 or 2 in severity. The incidence of grade 3 or 4 increases in ALT and AST was low (2%); and the mean values for AST and ALT decreased overtime. Liver abnormalities observed with darunavir/ritonavir were generally mild or moderate, and similar or lower to that observed in patients receiving commercially available PIs (excluding tipranavir) in the control group. There was also 1 case of grade 4 hepatotoxicity 12 days after the start of treatment with darunavir+ rtv 600/100 mg b.i.d. in the short-term Phase IIa trials. A liver biopsy showed a histologic image compatible with an acute hepatitis of medicamentous origin. The subject discontinued the trial during the follow-up period and the SAE resolved without sequelae.

Hyperglycaemia and exacerbation of existing diabetes mellitus have been reported in patients receiving antiretroviral therapy, including protease inhibitors. Hyperglycaemia (18%, grades 1 to 3) in patients treated with the recommended dose (de novo group) occurred with a similar incidence compared to control.

The incidence of cardiac-related AEs was 6% in patients treated with the recommended dose de novo and 6% in control patients. The most common cardiac-related AE preferred term was abnormal ECG (2% in the 600/100 mg b.i.d. de novo group and control). All other AEs were reported by less than 1% of the patients.

Fat distribution and metabolic disorders have been reported with ART. AEs related to lipids occurred in 8% in the 600/100 mg b.i.d. de novo group and in 9% of controls and mostly concerned hypertriglyceridaemia (6% in the de novo group). The proportions of patients who met the criteria for intervention with respect to blood lipids at baseline were 18% and 19% in respective groups. In the subgroup who did not receive lipid lowering agents, the proportions qualifying for treatment at Weeks 12 and 24 were lower in the de novo group compared to controls (7% vs 16% and 5% vs 12%). There were 19 (4%) with a grade 3 or 4 lipid-related AE during treatment with the 600/100 mg b.i.d. None of the lipid-related AEs was considered serious or caused discontinuation.

With respect to bilirubin abnormalities, in the pooled multiple-dose Phase I trials in HIV-1 negative subjects, blood bilirubin increased and hyperbilirubinemia were reported in 22 (5.8%) and 21 (5.6%) subjects, respectively. Their incidence was higher during treatment with darunavir alone (each in 5 subjects) than during treatment with darunavir+ rtv (in 1 and 0 subjects, respectively).

Increased blood bilirubin and hyperbilirubinemia were also observed during treatment with darunavir+ rtv + coadministered products (17 and 15 subjects, respectively), and treatment with coadministered product alone (13 and 4 subjects, respectively), primarily during treatments that included indinavir, lopinavir/rtv, and atazanavir.

Diabetes mellitus/hyperglycaemia have been reported with ART including PI. The incidence of glucose-related AEs was lower in the 600/100 mg b.i.d. de novo group (2%) than in controls (6%) and most involved hyperglycaemia. Three patients had grade 3 or 4 AEs and two with diabetes had SAEs but no patients were discontinued.

During de novo treatment increases from reference were seen in mean haematocrit and haemoglobin as well as in platelet, lymphocyte, RBC, WBC and neutrophil counts. These also increased in the control group except for a decrease in haemoglobin by Week 24. However, graded haematology abnormalities in the de novo group included decreases in WBC count (22%), neutrophil count (22%) and lymphocyte count (13%) and increases in PTT (11%). Respective percentages for the control group were 29%, 27%, 23% and 7%, respectively. Grade 3 or 4 laboratory abnormalities were mainly related to WBC count (6% in the de novo group), neutrophil count (5%) and lymphocyte count (4%).

Immune reconstitution syndrome is a risk of effective ART. Four cases were reported in the dose-finding studies. Since there was a difference in the incidence of herpes simplex virus infections in the darunavir/ritonavir treatment group compared to control (10.8 versus 2.7 events per 100 patient years of exposure) it cannot be excluded that combination ART with darunavir may cause an inflammatory reaction to opportunistic pathogens.

In HIV-1 infected patients, the incidence of rash- and liver-related AEs darunavir tended to be higher in men than in women while the incidence of cardiac-related AEs was slightly higher in women. None of these differences were considered to be clinically relevant. Darunavir contains a sulfonamide moiety, and it cannot be excluded that its sulfonamide moiety contributes to rash. Subjects in clinical trials with darunavir have therefore been monitored for signs and symptoms of rash. In the total population of the Phase IIb trials, the incidence of rash was comparable for darunavir+ rtv patients with or without documented sulphonamide allergy (8.1% versus 7.2%). Most events did not lead to permanent discontinuation, hence no new investigations into the etiology and/or mechanism of rash were pursued.

A few cases of pancreatitis grade 3 or 4 have been observed. Increases in pancreatic amylase were reported in 28% of the 458 subjects who initiated treatment on the recommended dose 600/100 mg b.i.d. in trials C202, C213, and C215/C208, while increases in lipase were reported in 14% of these subjects. In the control group of trials TMC114-C202 and TMC114-C213, the incidence of increased amylase was 27% and the incidence of increased lipase was 9%. The incidence of grade 3 or 4 laboratory abnormalities in pancreatic amylase was comparable between these 2 groups (7% versus 5% respectively), while grade 3 or 4 abnormalities in lipase were more frequently observed in subjects treated with darunavir+ rtv (4% versus 1% respectively). Pancreatitis seems to be a potential risk because in a few cases a possible and doubtful relationship to the trial medication has been mentioned, although they seem to have been resolved after temporary interruption of the trial medication.

Safety related to drug-drug interactions and other interactions

Initially there were concerns raised regarding the safety data reported from the Phase I drug interaction studies since these did not employ the final formulation and dose. There are no data from the Phase II studies on the safety of co-administration with NNRTIs, as they were banned from the trials as well as medications that might be expected to have a clinically significant interaction with. The applicant provided nonetheless an analysis of safety in the pooled group of HIV-1 infected subjects who received darunavir+ rtv at any dose in the Phase

IIb trials C202, C213, and C215/C208 (in total 924 subjects) according to any potentially interacting concomitant medications. The results did not suggest any specific safety concerns emerged following concomitant administration of darunavir+ rtv and any of the concomitant medications in the studied population but these should be viewed with caution due to the small numbers involved.

Safety in special populations

In general, the overall safety profile did not differ relevantly or systematically for the subgroups by age, gender, race and region. However, the number patients older than 50 years was rather small (187) and the patients cannot really be considered elderly.

Thirteen percent of darunavir+ rtv-treated subjects and 16% of subjects in the control group had active hepatitis B or C virus coinfection. An additional 2% and 5% had a non-active co-infection. Hepatitis C co-infection was more common than hepatitis B co-infection.
In the group of subjects who initiated treatment with the recommended dose in trials C202, C213, and C215/C208, the incidence of liver-related AEs did not differ in subjects without or with active hepatitis B or C co-infection (6%). Among subjects treated with any darunavir+ rtv dose, there was a higher incidence of liver-related AEs in subjects with active co-infection (13%) than in subjects without co-infection (8%). This was mainly related to a higher incidence of increased AST, ALT and GGT. The incidence of these AEs was between 5% and 6% in subjects with active hepatitis B or C coinfection and between 2% and 3% in subjects without hepatitis B or C co-infection. The subgroups of subjects with non-active hepatitis B or C co-infection were too small to allow meaningful conclusions.
Similar to the control group, the co-infected patients with hepatitis B or C had a slightly higher rate of liver-related AEs (13%) vs. all other patients treated with darunavir (8%). Therefore, it is recommended to monitor liver-related AEs according to standard practice in HBV or HCV co-infected patients. This is in line with ART experience in this special group of patients.

Drug exposure to darunavir during pregnancy occurred in four patients and in all cases study medication was stopped immediately. The follow up of the cases reported: 1 induced abortion during the first trimester, 1 continued taking ART (not darunavir) foetus died due to intrauterine asphyxia, 1 gave birth a healthy term male babyand 1 no information. The applicant undertook to provide results from the Antiretroviral Pregnancy Register in the PSURs.

5. Pharmacovigilance

- **Detailed description of the Pharmacovigilance system**

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

- **Risk Management Plan**

The applicant submitted a risk management plan, including a risk minimisation plan.

Table 29 Summary of the risk management plan

Safety issue	**Proposed pharmacovigilance activities**	**Proposed risk minimisation activities**
Risk identified		
Rash	Ongoing assessment of rash-related AEs in the Phase III trials	Warning in section 4.4 and listed in 4.8 of the SPC

	Routine post-marketing surveillance and report in PSURs	
Hepatoxicity	Ongoing assessment in the Phase III trials Routine post-marketing surveillance and report in PSURs Comparative frequency of hepatotoxicity reported in external independent HIV database if signal safety detected	Warning in section 4.4 SPC and monitoring of liver functions
Lipid abnormalities	Ongoing assessment in the Phase III trials Routine post-marketing surveillance and report in PSURs Annual comparative frequency of lipid abnormalitiesreported in external HIV database if signal safety detected	Class labelling in section 4.4 and 4.8 of the SPC
Hyperglycaemia	Ongoing assessment of rash-related AEs in the Phase III trials Routine post-marketing surveillance and report in PSURs Annual comparative frequency of hyperglycaemia reported in external HIV database if signal safety detected	Class labelling in section 4.4 and 4.8 of the SPC
Viral resistance and data on cross resistance	Annual reports of virologic failures occurring in Phase III and Expanded Access Programme in PSURs	Information in 5.1 of the SPC
Potential risks		
Pancreatitis	Analysis of events in the Phase III trials Routine post-marketing surveillance and report in PSURs	-
Coronary events and cardiovascular conduction abnormalities	Report on cardiac conduction safety data based on ECG analysis from Phase III trials Routine post-marketing surveillance and report in PSURs Comparison of events in clinical trials and EAP to external HIV cohort	Listed in 4.8 of the SPC
Immune reconstitution	Analysis of events in the Phase III trials Routine post-marketing surveillance and report in PSURs	Class labelling warning in section 4.4 of the SPC
hyperbilirubinaemia	Analysis of events in the Phase III trials Routine post-marketing surveillance and report in PSURs	-
Lipodystrophy	Analysis of events in the Phase III trials Routine post-marketing surveillance and report in PSURs	Class labelling in section 4.4 and 4.8 of the SPC
Limited or missing information		
Pregnancy	Routine post-marketing surveillance and report in PSURs Results from the Antiretroviral Pregnancy Registry annually	
Hepatitis B/C con-infected patients	Report from patients enrolled in Phase III trials and expanded access programmes	Warning in section 4.4 and 4.8
Children/adolescents	Ongoing paediatric development	
Elderly	Increased number in Phase III trials and expanded access programmes	
Hepatic impaired patients	Pharmacokinetics study in hepatic impaired patients	

The safety specification covers all the issues that are considered relevant for the use of darunavir in clinical practice. Within the pharmacovigilance plan, most of the activities planned are routine pharmacovigilance practices with report in the PSUR according to the normal PSUR schedule, using the International birth date (23 June 2006).
The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information.

6. Overall conclusions, risk/benefit assessment and recommendation

Quality

The quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way.

Non-clinical pharmacology and toxicology

Darunavir is a protease inhibitor with potent activity against wild type HIV-1 and PI resistant HIV. Darunavir showed antiviral activity against laboratory strains and clinical isolate compatible with clinical use for the treatment of HIV.

The pharmacokinetics of darunavir has been adequately studied but low systemic exposures compared to the intended human exposure were achieved in all species, consequently the margin of safety derived from animal studies is absent or low.

In chronic toxicity studies, the key target organs/systems identified in rodents were the haematopoietic system, the blood coagulation system, liver and thyroid. A variable but limited decrease in red blood cell-related parameters was observed, together with increases in activated partial thromboplastin time. Changes were observed in liver (hepatocyte hypertrophy, vacuolation, increased liver enzymes) and thyroid (follicular hypertrophy). In the dog, no major toxicity findings or target organs were identified. The safety margin for darunavir, expressed on the basis of Cmax and AUC, was close to or less than one. In juvenile rats, there were deaths at the lowest dose, while the same was not observed in adult rats even at the highest dose. Exposure was much higher in juvenile rats than what was observed in adult rats. Further studies are planned in juvenile rats, the results of which will be provided post-authorisation.

Darunavir did not impact on mating or fertility in rats at exposure levels below (AUC - 0.5 fold) of that in human at the clinically recommended dose. The number of corpora lutea and implantations were decreased in the presence of maternal toxicity. There was no teratogenicity with darunavir in rats and rabbits when treated alone nor in mice when treated in combination with ritonavir. The exposure levels were lower than those with the recommended clinical dose in humans. In a pre- and postnatal development assessment in rats, darunavir with and without ritonavir, caused a transient reduction in body weight gain of the offspring pre-weaning and there was a slight delay in the opening of eyes and ears. Darunavir in combination with ritonavir caused a reduction in the number of pups that exhibited the startle response on day 15 of lactation and a reduced pup survival during lactation. These effects may be secondary to pup exposure to the drug via the milk and/or maternal toxicity. No post weaning functions were affected with darunavir alone or in combination with ritonavir.
Considering these data and the lack of data in humans, darunavir + rtv should not be used in pregnant women unless the benefits outweigh the risks. Considering that darunavir is excreted

in rats milk and in the absence of human data, darunavir + rtv should not be used during pregnancy.

Darunavir was not genotoxic in a standard battery of tests. The lack of final results from the carcinogenicity studies was addressed and in accordance with the CHMP on the need for Carcinogenicity Studies of Pharmaceuticals (CPMP/ICH/140/95), and draft Guideline on Carcinogenicity Evaluation of Medicinal Products for the Treatment of HIV Infection EMEA/194898/2006, because darunavir is intended for the treatment of patients with limited treatment options, the CHMP considered a marketing authorisation could be granted prior the availability of these results. However the applicant undertook to submit the final results as part of the follow-up measures to be fulfilled post-authorisation.

There was no evidence of immunotoxicity or antigenicity associated with darunavir.
The applicant performed an environmental risk assessment. Results from ongoing studies will be provided as part of the follow-up measures to be fulfilled post-authorisation.
The salient findings from the preclinical studies have been reflected in the SPC.

Efficacy

The pharmacokinetics profile of darunavir co-administered with ritonavir has been defined in healthy volunteers as well as in HIV-1 infected patients. Overall exposure of darunavir (600 mg bid) was increased by approximately 14-fold when co-administered with ritonavir (100 mg bid). When darunavir (tablet formulation) is administered with ritonavir, food increased bioavailability by about 30%. Therefore darunavir should be taken with food, as used in the clinical trials and recommended in the SPC.
The pharmacokinetics profile of darunavir has not yet been assessed in children and adolescents and only preliminary data are available in patients with hepatic impairment. Waiting for the final results of this study, no dose adjustments are recommended for darunavir or ritonavir in mild and moderate hepatic impaired patients. In the absence of data, darunavir is contraindicated in patients with severe hepatic impairment. Considering that the elimination of darunavir via the renal route is minimal, no impact of the impairment of the renal function is anticipated on the pharmacokinetics of darunavir. No dose adjustment is therefore recommended in patients with renal impairment. There are only limited data in elderly and therefore darunavir+rtv should be used with caution in this population.
Darunavir exposure was higher in HIV-infected patients compared to healthy volunteers. The population pharmacokinetic analyses suggested that the higher α1-acid glycoprotein levels in HIV infected subjects compared to healthy subjects could partly explain this difference.
The relevant pharmacokinetics data, and recommendations, have been reflected in the Summary of Product Characteristics.
In vitro studies showed that darunavir is mainly a substrate for CYP3A4, and also substrate for P-gp. Darunavir may also have the potential to inhibit P-gp. Darunavir inhibited CYP3A at clinical relevant plasma concentrations. Darunavir appeared also to induce CYP enzymes. In vivo interactions with medicinal products that induce or are metabolise by CYP P450 isoenzymes activity are likely to be extensive. The applicant completed a number of studies nonetheless almost all of them used doses of darunavir lower than the recommended dose (darunavir+ rtv 400/100 mg b.i.d. and some with 300/100 mg b.i.d. in the fed state instead of the recommended dose of 600/100 mg) and/or formulation less bioavailable than the final one to be marketed. The commercial tablet formulation showed about 21% increase in exposure compared to some clinical trial formulations. The observed interactions are in line with those observed for other PIs given with low-dose ritonavir but due to the limitations of the studies, the effects may be underestimated. In addition because darunavir binds predominantly to α1-glycoprotein, protein displacement of medicinal products highly bound to that protein cannot be ruled out. All the relevant information on interactions and adequate warnings have been reflected in the SPC, including recommendation for clinical monitoring of safety if necessary. The applicant undertook to provide additional data to further characterise the interaction

profile of darunavir as part of specific obligations and follow-up measures to be fulfilled post-authorisation.

The 600/100 mg dose is the recommended dose for treatment of HIV infected subjects. Dose rationale is mainly based upon the virological responses observed in the two dose-finding studies (see below) and the fact that a less than dose proportional increase in exposure is observed over the 400/100 and 600/100 b.i.d. dose range.

The resistance profile of darunavir was studied *in vitro* and *in vivo*. Based on the data from Phase IIb studies, susceptibility to darunavir was correlated with both the type and number of protease mutations. Mutations at V32I, L33F, I47V, I54L and L89V developed in viruses from at least 10% of the patients with virological failure (never reaching >1.0 $\log_{10}$ decrease or rebound from a >1.0 $\log_{10}$ decrease). Mutations at V32I and I54L were the most common mutations that developed in > 20% of the virological failures. The applicant undertook to provide additional data on the emergent of resistant strains and on cross-resistance as part of the follow-up measures to be fulfilled post-authorisation.

The demonstration of the clinical efficacy of darunavir is based on the primary week-24 analyses of virological response from 2 controlled dose range finding Phase II trials (studies C202 and C213 also known as Power 1 and 2) in which darunavir co-administered with 100 mg ritonavir plus an optimised background regimen (OBR) was compared to a control group receiving an investigator-selected PI(s) regimen plus an OBR. These randomised, controlled trials consisted of an initial dose-finding part and a second long-term part in which all patients randomised to darunavir + 100 mg ritonavir received the recommended dose of 600/100 mg b.i.d. The protocols of these studies were amended (including the primary endpoint) to become pivotal. HIV-1 infected patients who were eligible for these trials had previously failed more than 1 PI containing regimen. The OBR consisted of at least 2 NRTIs with or without enfuvirtide (ENF). Patients were treatment-experienced (average time since HIV-1 infection diagnosis was 13.2 years in C202 versus 11.6 years in C213; 71% of the patients in the C202 trial and 63% of patients in the C213 trial were infected with virus resistant to all commercially available PIs at the time of the trials; the median CD4+ cell count at baseline was 106 in the C202 trial versus 179 x 10^6 cells/ml the C213 trial).
In the pooled analysis, patients treated with darunavir+ rtv at the recommended dose 600/100 mg b.i.d. (n=131) had significantly higher virological response rates (defined as at least 1.0 $\log_{10}$ decrease in viral load versus baseline at week 24) than the control group (70 % versus 21 %). In both trials, results obtained for the other virologic response categories (defined as a decrease of viral load by at least 0.5 $\log_{10}$ relative to baseline, or proportion of patients with a viral load < 400 or < 50 copies/ml) confirmed the findings of the primary efficacy parameter. At 24 weeks, for instance, the percentage of patients with viral load < 50 HIV RNA copies/ml were 45% (59/131) in the darunavir + ritonavir group versus 12 % (15/124) in the control group. For patients reaching week-48 or discontinued earlier, the 48-week analysis indicates that proportions of patients with at least 1 log drop decrease over time (from 69% to 61%); however, the same percentage of patients were undetectable (< 50 HIV RNA copies/ml) at week-24 and week-48 respectively (45%). The limitations in the design of the proof of principles studies were not considered *per se* major in the light of the demonstrated favourable efficacy results.
In addition, results from the open-label trials C208/C215 (POWER 3) using similar inclusion and evaluation criteria as in the controlled trials enrolled patients who were even more advanced (with median CD4+: 115 x 10^6 cells/l and virologically patients had median 3 primary PI mutations, 9 resistance associated mutations) than those enrolled in trials C202 and C213 were provided. Thirty two percent of these patients had also failed the most recently approved PI tipranavir. In the group of patients who started directly treatment with the recommended dose of darunavir + ritonavir**,** virological response rate defined as at least 1.0 $\log_{10}$ decrease in viral load versus baseline at week 24 accounted for 65 % (160/246).

Additional data supported the 24 week efficacy profile of darunavir+ rtv (in 327 patients) at the recommended dose with the to be marketed formulation. The provided week 48 results suggest that the virological response is sustained within the observed follow-up period.

In a limited numbers of hepatitis B or C co-infected patients, the virological response rate to darunavir does not appear affected.

The efficacy of darunavir has not been established in children and adolescents nor in HIV-infected treatment naïve patients.

Safety

The data on clinical safety of darunavir 600 mg co-administered with ritonavir 100 mg twice daily are mainly based on the analyses of data from 2 ongoing phase IIb trials (C202, C213), complemented by data of de novo patients from 2 open-label trials (C215/C208). The safety database, especially with regard to the final recommended regimen and formulation, comprises therefore a limited number of patients.
The most commonly reported adverse reactions of any grade were diarrhoea, nausea, and headache . Protease inhibitors class related events were prospectively looked at (e.g cardiac related, lipid-related, liver-related and glucose related adverse events). So far the safety profile seems consistent with other PI. Nonetheless the lack for head to head comparative phase III studies versus comparators in similar therapeutic indications precludes a definitive conclusion related to the safety profile of darunavir compared to other PIs.
In the total population of the Phase IIb trials, the incidence of rash was comparable for darunavir/rtv patients with or without documented sulphonamide allergy (8.1% versus 7.2%). There was no apparent relationship with darunavir dose. Discontinuation due to adverse reaction were infrequent. There was one case of SAE (toxic skin eruption) leading to discontinuation of darunavir + rtv. In the patients who switched from the clinical trial formulation to the commercial formulation there did not seem to be an increased risk of AEs
Because patients were not necessarily taking potentially interacting medicinal products during the clinical studies (for instance NNRTIs were excluded), the safety data obtained may not be wholly applicable to the more general use of darunavir.
As expected for antiretrovirals, patients co-infected with hepatitis B or C can have an increased frequency of treatment-emergent liver function abnormalities but the limited control data do not allow assessment of any differences between the darunavir and control groups. The standard antiretroviral warning and monitoring labelling text have been included in the SPC.
There is a need to further characterise the safety profile. This has been addressed in the risk management plan and final results from ongoing studies will be submitted as part of the follow-up measures to be fulfilled post-authorisation. From the safety database all the adverse reactions have been included in the Summary of Product Characteristics.
Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately address these.

- User consultation

The Applicant performed a user consultation test, which was found acceptable.

Risk-benefit assessment

The available efficacy data from the controlled Phase IIb trials C202 (POWER 1) and C213 (POWER 2) on darunavir + rtv at the recommended dose 600/100 mg b.i.d. in the targeted heavily pre-treated HIV-1 infected (with multi-PI resistance) patient population (n=131) support the conclusion that it is adequately efficacious. In addition, the open trial C215/C208 (POWER 3) using similar inclusion and evaluation criteria as in the controlled trials enrolled

patients in 327 patients who were even more advanced confirms the 24 week efficacy profile of darunavir+rtv at the recommended dose with the to be marketed formulation. The provided week 48 versus week 24 results suggest that the virological response is sustained within the observed follow-up period.
The present safety data on darunavir+rtv do not suggest a safety profile which would preclude approval of darunavir. The safety database with the recommended dose and the formulation to be marketed is still limited. Therefore, the data obtained may not be wholly applicable to the more general use of darunavir. In addition there are limitations in the available interaction data and some effects may be underestimated hence additional monitoring may be warranted as recommended in the SPC.
Overall, the data presented support a clinical benefit for darunavir in combination with low dose ritonavir in the treatment of HIV-1 infected highly pre-treated adult patients who failed more than one regimen containing a protease inhibitor (PI). Taking into account the observed safety profile, the risk-benefit of darunavir in the sought indication is considered positive. However, there are insufficient comprehensive clinical data available in particular regarding safety data for the optimal use of boosted darunavir in the targeted population in clinical practice. The CHMP reviewed the request from the applicant for a conditional marketing authorisation, and considered, that the PREZISTA (darunavir) falls within the scope of Regulation (EC) No 507/2006, with particular reference to Article 2, based on the following grounds:
PREZISTA is indicated for the treatment of HIV-1 infection in highly pre-treated adult patients who failed more than one regimen containing a protease inhibitor. Thus, PREZISTA is a medicinal product, which aims at the treatment of a seriously debilitating and life-threatening disease.

The CHMP considered, that PREZISTA fulfils the requirements of Article 4 of Regulation (EC) No 507/2006 based on the following grounds:

(a) The clinical benefit of darunavir has been demonstrated. This is based on evidence from two randomised, controlled studies comparing the safety and efficacy of darunavir + low dose ritonavir with other ritonavir-boosted protease inhibitor combinations. Patients in both arms of these trials also used other anti-HIV agents (nucleoside reverse transcriptase inhibitors) with or without enfuvirtide. In these studies, patients on the recommended dose of darunavir + ritonavir experienced higher rates of reduction of their HIV viral load than patients on other ritonavir-boosted protease inhibitor combinations (70 % versus 21 % achieved virological response defined as at least 1.0 $\log_{10}$ decrease in viral load versus baseline at week 24). The used objective laboratory endpoint of plasma HIV RNA and derived efficacy parameters such as change in HIV RNA from baseline, ≥ 0.5 $\log_{10}$ decrease from baseline, ≥ 1 $\log_{10}$ decrease from baseline, and proportions of patients with viral load < 400 or < 50 copies/ml are all reliable virological outcome measures for the sought target HIV-1 infected population. Additional data from the open-label trial support the efficacy of darunavir with low dose ritonavir with evidence of sustained responses within the observed follow-up period.
The safety profile was considered acceptable. The limited database is nonetheless recognised and supplementary safety data in a controlled fashion derived from randomised controlled Phase III trials are necessary.
Therefore based on the CHMP review of data on quality, safety and efficacy, the CHMP considered that the risk-benefit balance of PREZISTA as defined in Article 1(28a) of Directive 2001/83/EC, for the treatment of HIV-1 infection in highly pre-treated adult patients who failed more than one regimen containing a protease inhibitor, was positive.

b) The CHMP considers that the following data will provide comprehensive clinical data:
- the final study reports from the studies POWER 1, 2 and 3
- the final study report from the ongoing study C214 (randomised, controlled, open-label trial to compare the efficacy, safety and tolerability of darunavir with low dose of ritonavir versus lopinavir/ritonavir in treatment-experienced HIV-1 infected subjects)

- the final study reports from the ongoing interaction studies with rifabutin and didanosine as well as an analysis assessing the effect of coadministered nevirapine and efavirenz on darunavir from study C214 ; in addition estimation of intra-subject variability.
- the data from the darunavir treatment arm that do not receive the candidate NNRTI (TMC125) for the two following studies:

-study C206: Phase III randomised, double-blinded, placebo-controlled trial to investigate the efficacy, tolerability and safety of TMC125 as part of an ART including darunavir with low dose ritonavir and an investigator-selected OBR in HIV-1 infected subjects with limited to no treatment options.

- study C216: Phase III randomised, double-blinded, placebo-controlled trial to investigate the efficacy, tolerability and safety of TMC125 as part of an ART including darunavir with low dose ritonavir and an investigator-selected OBR in HIV-1 infected subjects with limited to no treatment options. T

- study C208: open label trial of darunavir with low dose of ritonavir in HIV-1 infected subjects who were randomised in the trials C201, C207 or in sponsor selected Phase I trials
- study C209: open-label safety study of darunavir in combination with low dose RTV and other ARVs in highly experienced HIV-1 infected patients with limited or no treatment options

These ongoing clinical trials using darunavir+rtv at the presently recommended dose will provide comprehensive clinical data for the optimal use of boosted darunavir in the targeted population. Because these studies are ongoing the CHMP considers that it is likely that the applicant will be in a position to provide the comprehensive clinical data.
The design of the studies in heavily pre-treated patients using an investigational NNRTI (trials C206 and C216) is as such that they can generate only supportive data for safety of PREZISTA. They are not designed as confirmatory Phase III trials for PREZISTA.

(c) No satisfactory methods of treatment that have been authorised, or exist in the Community for patients with advanced disease and limited to no remaining treatment options. Despite other agents that have shown activity in this setting, such as tipranavir, there remains a large unmet medical need in the treatment of this patients population.
Based on the available data, the CHMP considers that darunavir with low dose of ritonavir will be a component of combination antiretroviral therapy for patients with advanced disease and limited to no remaining treatment options, thus addressing the unmet medical need.

(d) New treatments having an effect in terms of relevant endpoints are of immediate relevance to patients with advanced disease and limited to no remaining treatment options. Although comprehensive data are not available to allow to precise the optimal use of darunavir with low dose ritonavir in the targeted population, in view of the efficacy of the product and the poor prognosis, lack of comprehensive data poses no risks that outweigh the benefits. Therefore the CHMP considers that the benefit to public health of the immediate availability on the market of the medicinal product concerned outweighs the risk inherent in the fact that additional data are still required.

In addition to the specific obligations, the applicant undertook to provide additional data as part of follow-up measures to be fulfilled post-authorisation including (cross)-resistance data, interaction data, data in patients with hepatic impairment, in children and adolescents and in HIV-infected treatment-naive patients.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that pharmacovigilance activities in addition to the use of routine pharmacovigilance were needed to investigate further some of the safety concerns. No

additional risk minimisation activities were required beyond those included in the product information.

Due to the nature of the disease, darunavir + ritonavir treatment should be initiated by a physician experienced in the management of HIV infection.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of PREZISTA was favourable and therefore recommended the granting of the conditional marketing authorisation in the following indication:

> PREZISTA, co-administered with 100 mg ritonavir is indicated in combination with other antiretroviral medicinal products for the treatment of human immunodeficiency virus (HIV-1) infection in highly pre-treated adult patients who failed more than one regimen containing a protease inhibitor (PI).
> This indication is based on week-24 analyses of virological and immunological response from 2 controlled dose range finding Phase II trials and additional data from uncontrolled studies (*see section 5.1 of the Summary of Product Characterisitcs*).
> In deciding to initiate treatment with PREZISTA co-administered with 100 mg ritonavir careful consideration should be given to the treatment history of the individual patient and the patterns of mutations associated with different agents. Genotypic or phenotypic testing (when available) and treatment history should guide the use of PREZISTA.