Reviews in Antiviral Therapy & INFECTIOUS DISEASES 3 2013

JOURNAL OF ABSTRACTS AND CONFERENCE REPORTS FROM INTERNATIONAL WORKSHOPS ON INFECTIOUS DISEASES & ANTIVIRAL THERAPY

Abstract Book

14th International Workshop on Clinical Pharmacology of HIV Therapy

22 - 24 April 2013, Amsterdam, The Netherlands

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Abstracts

Oral presentations

Abstract: O_01

Novel Drugs and Formulations

Effects of gender, race, age and BMI on the pharmacokinetics of long-acting rilpivirine (RPV-LA) after a single IM injection in HIV-negative subjects

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Introduction: Investigation into the determinants for penetration of long-acting agents in the genital tract has important implications for effective PrEP. RPV-LA is a parenteral formulation containing 300mg per mL, allowing for prolonged plasma exposure and potentially monthly or less frequent dosing. In this post-hoc analysis we determined the effect of demographics on the pharmacokinetics (PK) of RPV-LA in the plasma and female genital tract (cervicovaginal fluid & tissue; CVF & VT) after a single IM injection in HIV-negative subjects.

Materials and Methods: SSAT040 is a prospective, open-label stratified-dose exploratory study which investigated RPV PK in healthy volunteers with low behavioural risk for HIV. Volunteers were aged 18-50 and >50% of females were of self-identified African or African-Caribbean ethnicity. Participants received a single IM dose (gluteus maximus) at 300, 600 or 1200mg (20 subjects per arm), and a small sub-study of males (n=6) received an IM dose of 600mg. RPV concentrations [RPV] in plasma were collected on days 0 (0, 4 and 8 hr), and between days 1 to 84. CVF, self-collected by direct aspiration, was determined at similar times from 8 hr onwards. Biopsies of vaginal tissue (VT) were taken at two timepoints, on either day 7, 14, 28 or 56. [RPV] were determined by HPLC-MS/MS and PK parameters (AUC_{84d}, C_{max}) were calculated using WinNonLin. PK parameters were log transformed and univariate and multivariate linear regression analyses performed to determine the effects of gender, weight, BMI, age and ethnicity on RPV systemic and compartmentalised PK (dose normalised to 300mg).

Results: Sixty-six participants were included: 29 were black African/Caribbean, 30 Caucasian and 7 other. Median (range) weight and BMI of the subjects were 76.3 kg (61.0-95.0) and 24.6 kg/m2 (20.4-30.0) in males, and 66.2 kg (47.8-100.4) and 23.6 kg/m2 (17.1-34.7) in females. BMI did not differ significantly between the sexes (P=0.750). Male and female subjects were aged 39.5 years (27.2-41.0) and 29.3 years (18.3-48.9), respectively (P=0.717). Female sex (-32.2%; P=0.013) and higher BMI (kg/m2; -2.3%; P=0.028) were independently associated with lower RPV plasma exposures and C_{max}. However, no clear relationship was observed between AUC_{84d} and BMI. There was no evidence of an effect of age or ethnicity on RPV systemic PK. In the female genital tract, age <40 years (-32%; P<0.031) and a BMI >25 kg/m2 (-40%; P<0.004) were associated with lower RPV AUC_{84d} and C_{max} in CVF, independent of systemic [RPV]. BMI was a predictor of [RPV] in VT; with higher BMI associated with lower [RPV] in VT.

Conclusions: These preliminary data, from a relatively small cohort, show that absorption of RPV-LA into the systemic circulation is influenced by BMI. In addition, the effect of gender is potentially due to differences in adipose tissue distribution between males and females. Younger women with higher BMI showed decreased RPV concentrations within vaginal fluid; however, changes in pH due to the stage of the menstrual cycle, level of mucus production and permeability of the vaginal epithelium, throughout the study duration, are also likely to impact on drug distribution in the female genital tract.

Conflict of interest
Received travel grant from Janssen Pharmaceutica

Abstract: O_02

Novel Drugs and Formulations

Plasma and tissue GSK1265744 pharmacokinetics following long-acting parenteral administration in healthy male and female subjects

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Background: GSK1265744 is a potent integrase inhibitor (INI) in development as a long-acting parenteral (LAP) nanosuspension formulation for HIV treatment and prevention. Intramuscular and subcutaneous injection of GSK1265744 100 to 800mg in healthy subjects has produced plasma concentrations associated with robust short-term antiviral activity following oral monotherapy. Infrequent LAP antiretroviral administration may offer individuals at high risk for HIV exposure an alternative to daily pre-exposure prophylaxis (PrEP) regimens. Evaluating distribution into tissues may give insight to the dosing strategy of GSK1265744 for PrEP. Plasma GSK1265744 PK parameters and concentrations in various tissues are presented.

Materials & Methods: Sixteen healthy male and female subjects were randomized to one of two cohorts (4 males and 4 females/cohort) and received GSK1265744 400mg IM either as a single injection or split into two 200mg IM gluteal injections. Serial plasma sampling was performed for 12 weeks with monthly follow-up sampling until individual plasma concentrations fell below 0.01µg/mL. Rectal (males only) and cervicovaginal tissue biopsies were performed at Weeks 2 and 8 (unsplit injection group) or Weeks 4 and 12 (split injection group). Concentrations were determined by LC/MS/MS. Plasma GSK1265744 parameters were estimated by noncompartmental analysis and summarized by descriptive statistics.

Results: GSK1264744 400mg IM was well-tolerated, and no SAEs were reported throughout the study. All subjects provided plasma samples and tissue biopsies through Week 12. Mean (SD) plasma GSK1265744 AUC(0-W4), AUC(0-W12), and AUC(0-∞) were 336 (188)µg·h/mL, 1058 (549)µg·h/mL, and 2447 (677)µg·h/mLfollowing unsplit administration, and 737 (392)µg·h/mL, 1965 (808)µg·h/mL, and 2775 (1043)µg·h/mL following split administration. Mean (SD) plasma Cmax was 0.752 (0.431) µg/mL and 1.57 (0.736) µg/mL following unsplit and split administration, respectively. Median tmax occurred at Week 9 following unsplit administration and Week 2 following split administration. Median (range) GSK1265744 tissues concentrations across visits were 108 (<50 – 505)ng/g in cervical tissue, 160 (<50 – 247)ng/g in distal vaginal tissue, 164 (<50 – 904)ng/g in proximal vaginal tissue, and <50 (<50 – 197)ng/g in rectal tissue. Median plasma GSK1265744 at biopsy visits were 620ng/mL in females and 702ng/mL in males. Assuming a tissue density of 1g/mL, median individual tissue:plasma ratios ranged from 16-25% in the female genital tract and was 7.5% in rectal tissues in males. Median concentrations in vaginal tissue approached the plasma protein-adjusted IC90 for GSK1265744 of 166ng/mL.

Conclusions: Observed GSK1265744 plasma concentrations were, as predicted, lower than the target therapeutic range yet permitted evaluation of tissue partitioning and the impact of split dosing. GSK1265744 AUC(0-W4), AUC(0-W12), and Cmax were approximately two-fold higher and tmax occurred earlier following split administration of GSK1265744 400mg IM as compared to the single injection, indicating that splitting the LAP IM dose increases the rate of absorption. Similar AUC(0-∞) values between groups indicate extent of absorption is not affected by dose splitting. GSK1265744 was detected in all tissues evaluated, with an apparent higher distribution into cervicovaginal tissue compared to rectal tissue. These data suggest therapeutic range GSK1265744 plasma concentrations will produce tissue drug concentrations that exceed protein-adjusted IC90 upon repeat LAP administration and support its further investigation for PrEP.

Conflict of interest
Employee of GlaxoSmithKline

Abstract: O_03

Pharmacokinetics for Pediatrics, Pregnancy and other Special Populations

Prediction of antiretroviral drug penetration into the female genital tract using a novel QSAR model

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Introduction: The exposure of oral antiretroviral (ARV) drugs in the female genital tract (FGT) is not predicted by drug class or basic physicochemical properties. An efficient method of predicting drug penetration into these tissues would be useful in selecting ARV candidates for such applications as HIV pre-exposure prophylaxis or HIV eradication. Here we describe a novel quantitative structure-activity relationship (QSAR) model and cluster analysis with this capability.

Materials & Methods: A literature search from 1950-2012 was conducted in PubMed, EMBASE, and Web of Science to identify studies reporting PK data for any drug in cervical or vaginal tissue (CT or VT), or cervicovaginal fluid (CVF). When reported, we used the ratio of CT, VT, or CVF to blood plasma as a measure of penetration (PR). We calculated the PR if not explicitly reported. A database was created with PRs, chemical structures, SMILES string, volume of distribution, and protein binding. Predicted interactions with the MRP2, MRP4, and MDR1 efflux transporters were estimated using previously validated QSAR transporter models. PR was modeled using both computed chemical descriptors and pharmacokinetic parameters and different machine learning techniques (k-nearest neighbor, random forest, and multiple linear regression). Hierarchical chemical clusters were identified and analyzed *in silico* without regard to PR values. Rilpivirine (RPV) and dolutegravir (DTG) were used for prospective investigation.

Results: Penetration data were collected for 58 compounds (20 ARVs) representing 17 drug classes. Prediction performance of derived QSAR models was found to be unstable. The model with the highest predictive accuracy explained ~50% of the variability (R^2_{test}=0.47) with a mean absolute error (MAE) of 0.39 log-units, which is equivalent to a 2.5-fold difference in PR. Low plasma protein binding and low MRP4 affinity were associated with higher FGT penetration (PRs >1.5). Cluster analysis revealed concordant PR trends for certain chemotypes, such as fluoroquinolones and macrolides. The model overestimated RPV PR by only 0.5 fold, but overestimated DTG PR by 6 fold. This suggests the model may be useful for compound screening for only certain drug classes.

Conclusions: Using 58 compounds and 7 parameters, we have developed modestly predictive QSAR models of FGT penetration, with the k-nearest neighbor model being the most successful. Incompatibility of data obtained from multiple sources may be a contributing factor in the quality of the models. But, in consensus with the identification of chemical clusters, this represents an improvement upon the current approach of clinical testing. This model also identified MRP4 as a new mechanism of FGT disposition: a finding we have recently confirmed in vaginal and cervical tissues using protein and mRNA.

No conflict of interest

Abstract: O_04

Pharmacogenetics

CYP2B6 genotype but not rifampicin co-administration explains variability in long term efavirenz clearance and plasma exposure among HIV patients

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Introduction: Use of rifamycins in patients co-treated with antiretroviral therapy (ART) is marred with drug-drug interactions caused by induction of the activity of the CYP enzymes and the transporter protein, P-gp. Efavirenz, a drug constituted in first-line highly active antiretroviral therapy (HAART) is mainly metabolised through CYP2B6 pathway with minor involvement of CYP3A4/5, CYP2A6, and UGT2B7. Efavirenz exhibits significant auto-induction resulting in its increased clearance over time and this is predicted by *CYP2B6*6* gene polymorphism. Simillary, Rifampicin, a rifamycins commonly used in tuberculosis (TB) treatment induces CYP2B6 enzymes with considerable variation between subjects. With an estimated 60% TB-HIV co-infection in some parts of Africa, a deeper understanding of the efavirenz-rifampicin interactions is considered important in managing patients of these populations. We aimed at studying the effect of CYP2B6, CYP3A5 and ABCB1 genotypes and rifampicin treatment on long-term enzyme induction in Ugandan TB co-treated HIV positive patients.

Materials and methods: We performed a comparative study to investigate effect of *CYP2B6*, *CYP3A5, ABCB1* genotypes and rifampicin cotreatment on short versus long-term efavirenz autoinduction and apparent oral clearance in Ugandan HIV patients. ART naïve HIV patients (n=263) with (n=157) or without (n=106) TB-coinfection participated. Rifampicin and efavirenz based therapy were used for TB and HIV respectively. *CYP2B6*, *CYP3A5* and *ABCB1* genotypes were determined. Efavirenz apparent oral clearance was determined on days 14, 56, 84 112, 140, 168, 196 and 224.

Results: Effect of *CYP2B6*6* genotype on enzyme induction and efavirenz clearance was persistent at all time points while effect of *CYP2B6*11* was observed on days 14 and 56 ($p<0.01$). Rifampicin increased efavirenz clearance on day 14 ($p<0.01$). Regardless of rifampicin use, enzyme induction is pronounced in *CYP2B6*1/*1* genotypes. Effect of rifampicin on efavirenz apparent oral clearance was significant only during early therapy while efavirenz autoinduction was long-term lasting about 6 months, and its extent was determined by CYP2B6 genotype

Conclusion: Inter-subject variability in Efavirenz clearance due to enzyme induction during efavirenz – rifampicin co-treatment is mainly explained by CYP2B6*6 and *11 genotypes. Efavirenz auto-induction is paradoxically less marked with rifampicin than during efavirenz only treatment, and this should allay anxieties related to reduction of efavirenz concentrations during HIV-TB co-treatment.

No conflict of interest

Abstract: O_05

Pharmacogenetics

CYP2B6 and NAT2 genetic polymorphisms enlighten the pharmacokinetics interaction of efavirenz with rifampicin and isoniazid - ANRS 12154 trial

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Background: The impact of anti-tuberculosis (anti-TB) treatment on efavirenz (EFV) pharmacokinetics (PK) is poorly understood and there are currently conflicting reports in the litterature with some studies reporting increased metabolism of EFV in the presence of rifampicin and others reporting just the opposite. This study investigate the interaction between EFV and anti-TB treatment adjusting on genetic polymorphisms involved in both drugs PK.

Materials & Methods: 307 Cambodian patients coinfected with HIV and tuberculosis were included in the CAMELIA ANRS1295–CIPRAKH001 clinical trial. EFV 600mg daily and stavudine + lamivudine were administered in addition to standard anti-TB treatment (rifampicin + isoniazid for 6 months with ethambutol + pyrazinamide for the first 2-months). Blood samples were drawn on average 14h post EFV intake at weeks 2 and 6 after the onset of antiretroviral therapy and at weeks 22 (EFV+anti-TB drugs) and 50 (EFV alone) after the onset of the anti-TB treatment. 10 patients also participated to an intensive pharmacokinetic study during a dosing interval after week 50.

EFV concentrations were measured in plasma by HPLC with UV detection and analyzed with a one compartment model with zero-order delayed absorption and linear elimination using the SAEM algorithm implemented in the software NONMEM 7.1,2.
Due to their relation to EFV PK, the following SNPs were genotyped: CYP3A5 A6986G (rs776746), ABCB1 C3435T (rs1045642), CYP3A4*1B (rs4646437), CYP2B6 G516T (rs3745274), CYP2B6 C1459T (rs3211371), CYP2B6 (rs4803419), CYP2A6 (rs8192726). In relation to rifampicin, the polymorphisms OATP1B1 T521C (rs4149056), PXR (rs6785049) and PPARa (rs4253728) were genotyped and for the drug isoniazid we genotyped the smallest set of SNPs within NAT2 that enables the most efficient classification of individuals into rapid and slow acetylors: NAT2 (rs1801280, rs1801279, rs1799930, rs1799931).

Results: CYP2B6 G516T and rs4803419 polymorphisms were the most significant covariates. EFV CL/F for patients carrying the CYP2B6 516 GG, GT and TT genotypes were 12.5, 8.8 and 2.5 L/h on EFV alone. On EFV+anti-TB drugs, a decrease in EFV CL/F was observed in NAT2 'slow' metabolizers (40% of the patients in the study) whereas an increase was observed in NAT2 'rapid' metabolizers (P-value<10^{-3}). The extent of the decrease in patients NAT2 'slow' also differed according to the 3 different CYP2B6G516T genotypes. Predicted EFV mid-dose concentrations in patients carrying CYP2B6 516 TT and classified NAT2 'slow' were 11.5 mg/L on EFV+anti-TB drugs and 9.3 mg/L on EFV alone.

Conclusions: These data suggest that the inducing effect of rifampicin on EFV metabolism is counter balanced by a concentration dependent inhibitory effect of isoniazid on minor non CYP2B6 biotransformation pathways. Pharmacogenetics of EFV and anti-TB drugs explain controversial data reported on this drug-drug interaction.

Some of the data were presented at the 13th International Workshop on Clinical Pharmacology of HIV Therapy in April 2012 in Barcelona.

No conflict of interest

Abstract: O_06

Pharmacogenetics

Impact of drug transporter genetic polymorphisms on tenofovir exposure and creatinine clearance in HIV-infected Adults in Thailand

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Background: Tenofovir disoproxil fumarate (TDF) use has been associated with a decline in creatinine clearance (CrCL) and kidney tubular dysfunction (KTD). Genetic variants in ATP-binding cassette transporters (ABCC2, ABCC4 and ABCC10) have been shown to be associated with CrCL decline and/or KTD; however, the relationship between tenofovir (TFV) plasma exposure, ABCC genetic variants and kidney function remains unclear. We developed a population pharmacokinetic model to describe tenofovir concentrations in HIV-infected adults and investigated the association of drug transporter genetic polymorphisms with tenofovir exposure, and decline in kidney function.

Methods: Retrospective analysis in antiretroviral naive HIV-infected adults in Thailand who initiated a tenofovir-based HAART regimen. Subjects were participating in the PHPT Observational cohort study [ClinicalTrials.gov: NCT00433030]. Stored plasma and DNA samples from consenting patients were analyzed. Plasma tenofovir concentrations measured at 1, 6, 12, 24 and 36 months after treatment initiation were used to develop the PK model. Pharmacokinetic parameters were estimated using non-linear mixed effects regression models (NONMEM Version VI). DNA samples were analyzed for single nucleotide polymorphisms (SNPs) in ABCC2 (rs717620, rs2273697, rs8187694, rs3740066, rs8187710), ABCC4 (rs1751034), and ABCC10 (rs9349256, rs2125739). Glomerular filtration rate (eGFR) was calculated at baseline, and then every 3 months for up to 5 years using the Cockcroft–Gault equation.

Results: 238 HIV-infected adults (58% female) were included: at treatment initiation median (interquartile range) age was 36 years (31-42), weight 52 kg (48-59), serum creatinine (SCr) 0.8 mg/dL (0.7-0.9), creatinine clearance (CrCL) 89 mL/min (74-105), viral load 4.8 $\log_{10}$ copies/mL (4.3-5.2) and CD4 count 144 cell/mm^3 (91-208). A total of 1,089 tenofovir concentrations were available. A two-compartment model with first-order absorption/linear elimination best described TFV concentrations. Ka was fixed to 0.56 hr-1 for model stability. Tenofovir oral clearance (CL/F) was influenced by CrCl. Final population estimates (interindividual variability in percentage) of tenofovir CL/F, Vc/F, Q and Vp/F were 51.4 L/hr (20%), 251 L (161%), 130 L/hr, and 1410 L, respectively.
The Minor Allele Frequency for ABCC2 -24C>T, 1249 G>A, 3563 T>A, 3972 C>T, and 4544 G>A were 0.200, 0.042, 0.004, 0.227, 0.004, respectively; ABCC4 3463 A>G was 0.187; and ABCC10, rs9349256 and rs2125739 were 0.076 and 0.498, respectively. No significant association between the ABCC2, ABCC4 or ABCC10 polymorphisms and tenofovir AUC were observed. Combining hetero- and homozygous variants, a significantly higher AUC was observed at 6 months, 1 and 2 years for ABCC4 3463 A>G compared to wild-type (6 months 2.56 vs. 2.74 mcg.h/mL, p=0.03). In a multivariate analysis, the ABCC4 3463 AG/GG genotypes remained significantly associated with higher TFV AUC after adjusting for baseline viral load, CD4 count and ABCC SNPs. ABCC genetic polymorphisms were not associated with decline in creatinine clearance (defined as 25% from baseline) in univariate or multivariate analysis adjusted for baseline characteristics.

Conclusion: ABCC4 3463 AG/GG variants were associated with higher tenofovir exposure. No SNPs were associated with significant decreases in CrCL over time; however, markers of KTD were not assessed. It is possible that ABCC10 and ABCC2 are better indicators of tenofovir susceptibility in kidney tubular cells than plasma.

No conflict of interest

Abstract: O_07

Drug Drug Interactions

Evaluation of the drug interaction potential between the pharmacokinetic enhancer cobicistat and tenofovir disoproxil fumarate in healthy subjects

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Background: Cobicistat (COBI) is a pharmacokinetic (PK) enhancer approved for use as a booster of the HIV-1 integrase inhibitor elvitegravir (EVG) when given as the EVG/COBI/emtricitabine (FTC)/tenofovir disoproxil fumarate (TDF) single tablet regimen Stribild™ (STB). COBI is also in regulatory review as a PK enhancer of the HIV-1 protease inhibitors atazanavir and darunavir. In vitro studies have indicated that prodrug TDF is a substrate of the intestinal secretory transporter P-glycoprotein (P-gp) and COBI is a P-gp inhibitor. The present study evaluated the PK and potential for drug interaction between COBI and TDF when administered alone or in combination.

Materials & Methods: This was a randomized, two-cohort, 2 x 2 crossover (per cohort) study in healthy subjects with seven-day treatment periods separated by a washout. In Cohort 1, subjects (n=32) received COBI 150 mg QD alone or in combination with TDF 300 mg QD. In Cohort 2, subjects (n=14) received TDF 300 QD alone or in combination with COBI 150 mg. Safety assessments were performed throughout dosing and during follow-up. PK assessments were performed on first and final day of each treatment period. Statistical comparisons of COBI and tenofovir (TFV) exposures were made using geometric mean ratios (GMR) and associated 90% confidence interval (CI) bounds of 70-143% (>95% power to conclude no PK alteration), with COBI plus TDF serving as the test treatment and COBI or TDF administered alone serving as the reference treatment.

Results: Study treatments were generally well tolerated. Forty-five of the 46 enrolled subjects completed study drug and one prematurely discontinued study drug due to adverse events (AEs) (skin rash (Grade 2) while receiving TDF). No Grade 3 or 4 AEs were observed and renal function parameters were within normal range. In treatments containing COBI, a slight decrease (~10 mL/min) in estimated glomerular filtration rate (eGFR) was observed, consistent with the known inhibitory effect of COBI on creatinine secretion via inhibition of the renal MATE-1 transporter. In Cohort 1, following multiple-dose administration of COBI plus TDF vs COBI alone, COBI PK was unaffected (GMR (90% CI) C_{max}: 100 (95.4, 105), AUC_{tau}: 100 (94.7, 106), and C_{tau}: 107 (94.1, 121). In Cohort 2, following the first dose of COBI plus TDF vs TDF alone, TFV C_{max} and AUC were modestly higher, consistent with the inhibition of intestinal P-gp-mediated efflux of TDF by COBI (GMR (90% CI) C_{max}: 142 (123, 165), AUC_{inf}: 111 (104, 118), and AUC_{last}: 107 (96.6, 118). No further increase in TFV exposure was observed upon repeat dosing, indicating the higher exposure was driven by increased oral bioavailability consistent with P-gp inhibition. TFV half-life was similar between treatments of COBI plus TDF vs TDF alone, indicating that TFV elimination was unchanged.

Conclusions: COBI PK is unaffected upon coadministration with TDF. Consistent with the known inhibitory effect of COBI on P-gp-mediated intestinal efflux of TDF, TFV exposures were modestly higher following COBI plus TDF vs TDF alone, and comparable to historical data following STB or TDF dosed with other P-gp inhibitors (rilpivirine) or ritonavir-boosted protease inhibitor regimens.

Conflict of interest
Employee and stockholder in Gilead Sciences

Abstract: O_08

Pharmacokinetics for Pediatrics, Pregnancy and other Special Populations

Pharmacokinetics, metabolism and excretion of tenofovir alafenamide

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Background: Tenofovir alafenamide (TAF) is a cell targeted prodrug of tenofovir (TFV), a nucleotide analog that inhibits human immunodeficiency virus type 1 (HIV-1) reverse transcription. In preclinical studies in mice, rats and dogs, ~ 25% of the administered dose was recovered in the urine, predominantly as TFV (90%), with unchanged TAF present at minimal levels (≤ 3.4%) following oral administration of TAF. The objective of the present study was to determine the mass balance/recovery, metabolite profile, and pharmacokinetics of TAF and its metabolite TFV in humans following administration of a single, oral dose of radiolabeled TAF.

Methods: Healthy male subjects (N = 8) were administered a single dose of TAF (25 mg) containing 100 µCi of [^{14}C]TAF . Blood, urine, and feces samples were collected up to a maximum of 21 days or until one of the following criteria were met: >90% of administered dose recovered in feces and urine and <1% of dose was present in consecutive sampling intervals; plasma radioactivity in consecutive samples were ≤ 2-fold background or urine/feces sampling was discontinued. Levels of TAF and TFV were measured using LC/MS/MS and total radioactivity assessed by LSC. Metabolite profiling was performed in select urine, feces, and plasma samples. Safety assessments were performed throughout the study.

Results: All 8 subjects were evaluated in the study. No drug-related adverse events were observed and all observed laboratory abnormalities were mild in severity. The total mean (SD) recovery of [^{14}C]-radioactivity was 84% (2.4%) (47% (4.6%) in feces and 36% (5.6%) in urine). TFV was the predominant species in feces (99% of total quantified radioactivity) and urine (86%). Peaks in plasma radioactivity were observed at ~ 2 hours post-dose, where TAF was the primary species (~73%) again and at ~24-48 hours, where uric acid was the predominant species (~98%). The whole blood-to-plasma concentration ratio of [^{14}C]-radioactivity increased from 0.6 at 0.25 hours post dose to 2.4 at 216 hours post dose, driven by low plasma radioactivity at later time points. Over the 96-hour period following TAF administration, the predominant species circulating in plasma was uric acid. Additionally, low levels of other metabolites in the purine catabolism pathway, including xanthine, hypoxanthine, and adenine, were observed in plasma.

Conclusion: TAF was extensively metabolized and only minimal amounts were excreted unchanged in urine. In contrast, the metabolite TFV was predominant species observed in urine and feces. Uric acid was the predominant species observed in plasma over time but did not change total circulating plasma uric acid level.

Conflict of interest
Employee of Gilead Sciences.

Abstract: O_09A

Drug Drug Interactions

Pharmacokinetics of cenicriviroc when administered with and without ritonavir, darunavir/ritonavir or atazanavir/ritonavir

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Introduction: Cenicriviroc (CVC) is a novel, once-daily, potent dual C-C chemokine receptor type 5 (CCR5) and type 2 (CCR2) antagonist. CVC is metabolised via CYP3A4 and CYP2C8, and is currently being evaluated for the treatment of HIV infection (Study 652-2-202; NCT01338883). The primary objectives of 3 Phase 1, multiple-dose, open-label, fixed-sequence, crossover studies were to determine the plasma pharmacokinetics (PK) of CVC when administered with and without ritonavir (RTV), darunavir/ritonavir (DRV/r) or atazanavir/ritonavir (ATV/r) (Studies 652-1-104, 652-1-107 and 652-1-108, respectively). The secondary objectives of these studies were to determine the safety and tolerability of CVC when administered with and without the protease inhibitors.

Materials & Methods: A total of 20 healthy subjects were enrolled in each study and received study medication. Subjects received CVC 50 mg once daily (qd) on Study Days 1–10, followed (without any washout) by CVC 50 mg qd with RTV 100 mg qd (Study 104), DRV/r 800/100 mg qd (Study 107) or ATV/r 300/100 mg qd (Study 108) on Study Days 11–20. Blood samples were collected over the 24-hour dosing interval on Days 10 and 20, and also before each dose, for determination of plasma CVC concentrations. PK analyses of plasma CVC were conducted using noncompartmental methods. Paired analysis of variance (ANOVA) was performed on log-normal (ln)-transformed steady-state PK parameters (C_{max}, C_{min} and $AUC_{0–24}$) to evaluate the significance, if any, of a drug interaction. The ratios of least-squares (LS) geometric means of the concomitant administration relative to the administration of CVC alone were calculated for these parameters.

Results: All subjects completed Study 107; 18 of the 20 subjects completed Studies 104 and 108: 2 subjects withdrew consent from Study 104 and 2 subjects discontinued Study 108 due to moderate rash. Co-administration of CVC with RTV, DRV/r or ATV/r resulted in a significant increase in plasma CVC exposure. The ratios of LS geometric means of the concomitant administration relative to the administration of CVC alone for C_{max}, C_{min} and $AUC_{0–24}$, respectively, were as follows: 2.39, 5.24 and 3.55 in Study 104 (CVC and RTV); 2.17, 4.17 and 3.13 in Study 107 (CVC and DRV/r); 2.55, 5.75 and 3.89 in Study 108 (CVC and ATV/r). In all 3 studies, steady-state plasma concentrations of CVC were achieved after 6 days of administration of CVC alone and after 9, 7 and 7 days during co-administration of CVC with RTV, DRV/r or ATV/r, respectively. CVC alone and in combination with RTV, DRV/r or ATV/r was generally well tolerated and no serious or unexpected adverse events were reported. No clinically relevant laboratory abnormalities were observed with CVC alone or in combination with RTV or DRV/r. Hyperbilirubinemia, an expected laboratory abnormality with ATV, was observed following co-administration of ATV/r with CVC, and resolved after completion of dosing.

Conclusions: When co-administered with CVC, RTV 100 mg, DRV/r 800/100 mg and ATV/r 300/100 mg significantly increased plasma CVC exposure. Phase 3 studies to evaluate the clinical efficacy of CVC in combination with guideline-recommended HIV agents are being planned.

Conflict of interest

Employee of Tobira with investment options

Abstract: O_09B

Drug Drug Interactions

Pharmacokinetic interactions between cenicriviroc and efavirenz

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Introduction: Cenicriviroc (CVC), a novel, once-daily, potent dual C-C chemokine receptor type 5 (CCR5) and type 2 (CCR2) antagonist, is metabolised via CYP3A4 and CYP2C8, and is under evaluation for the treatment of HIV infection (Study 652-2-202; NCT01338883). A Phase 1, multiple-dose, open-label, fixed-sequence, crossover study was undertaken to assess 2-way pharmacokinetic (PK) interactions between CVC and efavirenz (EFV) (Study 652-1-109).

Materials & Methods: 40 healthy subjects were enrolled into 2 parallel groups (20 subjects/group). Study design Group 1: a 3-period (10 days/period), 3-treatment crossover evaluating the effect of EFV on CVC PK. Treatments: Period 1, CVC 200 mg once daily (qd); Period 2 (following Period 1 without washout), CVC 200 mg qd co-administered with EFV 600 mg qd; Period 3 (after washout of 14 days), CVC 200 mg qd on Day 1 and CVC 400 mg qd on Days 2–10, co-administered with EFV 600 mg qd. Study design Group 2: a 2-period (10 days/period), 2-treatment crossover evaluating the effect of CVC on EFV PK. Treatments: Period 1, EFV 600 mg qd; Period 2 (following Period 1 without washout), EFV 600 mg qd co-administered with CVC 400 mg qd. CVC was administered immediately after dinner and EFV at bedtime. Serial plasma CVC or EFV samples were collected (24-hour profile after the last dose of each period) for calculation of C_{max}, C_{min} and $AUC_{0–24}$. Ratios of least-squares (LS) geometric means of concomitant administration relative to administration of CVC or EFV alone were determined for these parameters. Safety and tolerability were assessed.

Results: 19 subjects were evaluable for CVC PK in Group 1 (3 periods) and 20 subjects for EFV PK in Group 2 (2 periods). Co-administration of EFV with CVC 200 mg resulted in a significant decrease in plasma CVC exposure compared with CVC alone: the ratios of LS geometric means for C_{max}, C_{min} and $AUC_{0–24}$ were 0.77, 0.52 and 0.57, respectively. Doubling the CVC dose to 400 mg offset the interaction when CVC and EFV were co-administered; the ratios of LS geometric means for C_{max}, C_{min} and AUC_{0-24} for EFV and CVC 400 mg co-administration compared with CVC 200 mg alone were 1.23, 0.85 and 0.98, respectively. Co-administration of EFV and CVC 400 mg did not result in a significant change in EFV exposure compared with EFV alone: the ratios of LS geometric means for C_{max}, C_{min} and $AUC_{0–24}$ were 1.07, 0.96 and 1.01, respectively. CVC 200 mg alone and CVC 200/400 mg co-administered with EFV were generally well tolerated in both groups; no subject discontinued due to an AE, there were no serious or unexpected AEs and no clinically relevant laboratory abnormalities were observed.

Conclusions: Co-administration of EFV with CVC 200 mg resulted in a statistically significant drug interaction, which was offset by doubling CVC dosage to 400 mg. CVC 400 mg had no effect on EFV exposure. Phase 3 studies to evaluate the clinical efficacy of CVC in combination with guideline recommended HIV agents are being planned.

Conflict of interest
Employee of Tobira with investment options

Abstract: O_10

PK-PD of Drug Efficacy and Toxicity

A comprehensive model linking plasma and intracellular phosphate pharmacology for ZDV and 3TC

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Introduction: The cellular pharmacology of NRTI *in vivo* is complex and not well understood. This is exemplified by the poor characterization of relationships between systemic/intracellular NRTI-PK and PK-PD to date. This work builds a model linking plasma and intracellular phosphate concentrations of both ZDV and 3TC, and associates modeled concentrations to PD outcomes.

Methods: Participants received standard ZDV/3TC doses in an observational PK study. Paired plasma and PBMC samples were collected at 2, 5, and 8 hours post-dose on days 1, 3, 7, and 12. Fat biopsies were collected before first dose and at day 12 for mitochondrial DNA (mtDNA) counts. Plasma and intracellular mono-, di-, and tri-phosphates were determined by LC/MS/MS. Candidate genes relevant to ZDV/3TC pharmacology were evaluated for single nucleotide polymorphisms (SNPs). ADAPT 5 software was used for population-PK, while pharmacodynamic analyses utilized Phoenix WinNonLin. The effect of covariates such as HIV-serostatus, sex, race, weight, creatinine clearance, and SNPs were assessed by AIC and visual predictive checks.

Results: Forty-three subjects participated: 23 HIV+, 14 women, and 10 African-Americans. An indirect stimulatory Emax/EC50 model best linked plasma concentrations to intracellular ZDV/3TC-MP concentrations. Phosphorylation/ dephosphorylation rates were first order. Emax estimates were within observed ZDV (8670 fmol/M) and 3TC (3.03 pmol/M) MP concentrations. EC50 were similar to estimated steady-state plasma concentrations (150 (ZDV) and 1141 (3TC) ng/mL). ZDV plasma CL and V_p were associated with weight. 3TC plasma CL was associated with creatinine clearance, race, and HIV-serostatus, while Q was associated with sex. ZDV-MP was significantly elevated in HIV-infected individuals, as both the MP to DP phosphorylation rate (47%, $p<0.0001$) and the MP elimination rate (46%, $p=0.001$) were reduced. DP to TP phosphorylation rates for ZDV (19%, $p=0.01$) and 3TC (28%, $p<0.0001$) were lower in HIV-infected subjects. ZDV-TP dephosphorylation rate estimates decreased in African-Americans (19%, $p=0.03$). BCRP and other transporter/kinases were univariate, but not multivariate, predictors of intracellular ZDV/3TC. PD outcomes were different according to HIV status. Neutrophil counts decreased in HIV- individuals (range 0.9 to -4.2 $x10^9$/L) but did not change in HIV+ subjects (range -2.0 to 2.0 $x10^9$/L). Fitted ZDV-TP Cmax levels were associated with decreases in adipose mtDNA through an inhibitory Emax/EC50 curve. The modeled decline in HIV-negative was -125 to -430 copies, and that for HIV-positive was 657 to -789, over the ZDV-TP Cmax concentration range of the study.

Discussion: A model linking ZDV/3TC plasma to intracellular phosphates via indirect stimulation and first order intracellular kinetics was developed. The model confirms and extends previous analyses by enabling covariate testing on specific steps in the biological system. The effect of HIV-infection significantly decreased the DP to TP phosphorylation rates for ZDV and 3TC resulting in lower TP concentrations, despite increased 3TC plasma and ZDV-MP concentrations. African-American race was associated with higher ZDV-TP, via a decreased TP dephosphorylation rate. HIV-status was predictive of distinct PD. Higher ZDV-TP was associated with greater loss in mtDNA, suggesting a concentration-effect relationship.

No conflict of interest

Abstract: O_11

PK-PD of Drug Efficacy and Toxicity

A viral dynamics model describes monotherapy and combination data with raltegravir, including differences between BID and QD dosing in QDMRK study

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Background: Raltegravir is an integrase strand transfer inhibitor (InSTI) used in combination with other antiretroviral agents for treatment of HIV. Understanding of the relationship between raltegravir PK and treatment outcome was advanced by analyses of data from the QDMRK study, in which 800 mg once-daily (QD) raltegravir was shown to be not non-inferior to 400 mg twice-daily (BID) raltegravir following 48 weeks of treatment in combination with tenofovir and emtricitabine. These data were further used to develop a viral dynamics model that describes the results of both short-term monotherapy and long-term combination therapy with raltegravir in treatment-naïve HIV-infected patients.

Materials & Methods: A PK/PD viral dynamics model for raltegravir + nucleoside or nucleotide analog reverse-transcriptase inhibitors (NRTIs) was developed to relate short- and long-term efficacy for raltegravir to its concentration-time profile. First, nonlinear mixed effects modeling was conducted based on short-term HIV RNA data from monotherapy studies with raltegravir as well as published monotherapy data for dolutegravir (GlaxoSmithKline). Next, model parameters were refined using simulations to match long-term response rates observed in QDMRK and STARTMRK, Phase III studies of raltegravir co-administered with NRTIs to treatment-naïve patients. In the compartmental PK/PD viral dynamics model, uninfected CD4+ cells are infected by the virus, leading to the production of actively infected cells, latently infected cells, or long-lived infected cells. Actively and long-lived infected cells generate virus, and latently infected cells are activated to become actively infected cells. The effect of InSTIs and NRTIs in the model is to inhibit the infection of the target cells by the virus. The model was improved by inclusion of an additional effect for InSTIs on the inhibition of the conversion rate from latently infected to actively infected CD4+ cells. A simple Emax model was used to describe the dependence of inhibition on drug exposure. Resistant virus strains were also included in the model, with resistance to each drug class characterized by an IC50 multiplier (resistance step size).

Results: An IC50 value of 3.5 ng/mL (158% interindividual variability) for raltegravir inhibition of viral infectivity was identified as being most consistent with the available short and long term efficacy data. The final model was able to describe the inter-arm differences observed in the QDMRK study reasonably well, including the percentages of patients in each arm who failed with mutations associated with resistance to raltegravir.

Conclusions: A PK/PD viral dynamics model was developed for raltegravir that relates the concentration-time profile to treatment outcome. This model could be used to assess the likely impact on efficacy of changes in the raltegravir concentration-time profile, e.g., for a pediatric or other formulation of raltegravir with a concentration-time profile that differs from that of the currently marketed formulation.

Conflict of interest
Employee of Merck and stockholder

Abstract: O_12

PK-PD of Drug Efficacy and Toxicity

Clinical trial simulations of effectiveness of reduced-dose efavirenz therapy: impact of adherence, pharmacogenetics and rifampin co-administration

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Background: Effectiveness of low dose efavirenz is currently being evaluated in clinical trials as a viable strategy for cost-effective HIV treatment in resource limited settings. Here, we evaluate this strategy by performing extensive clinical trial simulations and incorporating an exhaustive efavirenz knowledge-base including true adherence patterns, pharmacokinetics, pharmaco-dynamics, pharmacogenetics, risk of resistance development, and expected drug-drug interaction with the commonly co-administered TB drug rifampin.

Methods: Clinical trial simulations (CTS) were performed using a comprehensive adherence-Pharmacokinetic/Pharmacodynamic-clinical outcome model. Dosing histories were generated by sampling from the Medication Event Monitoring System (MEMS) Database that contains actual, detailed dosing histories from patients, most of whom were participating in clinical trials. Four dose levels were used: 800mg, 600mg, 400mg and 300mg. Efavirenz pharmacokinetics were simulated from parameters estimated from meta-analysis of all published efavirenz population pharmacokinetic models. The pharmacogenetic impact of decreased clearance with the CYP2B6 polymorphic variant 516 G>T was built into the model, as well as effect of increased clearance with concomitant rifampin administration. The implemented HIV-dynamic model represented by three interrelated compartments: non-infected T cells, infected T cells, and free virions, was based on the approach by *Wu et al (2005)* and adapted from *Perelson & Nelson (1999)*. Increasing risk of viral resistance over time was modeled after method proposed by *Huang & Rosencranz (2003),* which suggests increasing EC50 over time. All models and simulations scenarios were implemented in MATLAB R2012b and sensitivity analysis was performed. Each CTS included 10,000 patients, repeated 100 times for each scenario, and run in hourly time integration steps over a six month treatment period.

Results: CTS results elaborate the complex interplay between dose, adherence, pharmacogenetics and rifampin concomitant administration to determine whether low dose efavirenzis effective or not in a given simulated subject. In the patient population receiving a 400 mg reduced dose at the expected population median adherence level of 80%, 17-35 % of the patients will be at high risk of viral failure. Concomitant rifampin administration further increases this risk up to 22-42%. With the standard 600 mg dose (at the same level of adherence, 80%), the estimated risk of viral failure is 10-20%, increasing up to 15-28% percent when administered with rifampin. Consistent with clinical observations, simulated subjects with the CYP2B6 polymorphic genotype exhibited more favorable results, in general showing lower risk of treatment failure after receiving target 400mg dose in absence of rifampin.

Conclusions: CTS results indicate that a reduced-dose (400 mg) efavirenz treatment alternative requires very high adherence (>90%) levels to be effective, if it is to be recommended. Patients on concurrent rifampin therapy are less well-suited to receive a reduced-dose regimen, regardless of adherence level. Patients with CYP2B6 polymorphic genotype maintain low viral load after receiving 400mg dose; however a high adherence level (>80%) is necessary. Overall, our work, incorporating all important aspects of efavirenz based treatment, suggests that low dose efavirenz may not be a desirable alternative for optimizing the cost-effectiveness of HIV treatment, and is likely to result in increased proportion of patients with viral failures and resistance development.

No conflict of interest

Abstract: O_13

Pharmacokinetics for Pediatrics, Pregnancy and other Special Populations

Establishing darunavir dosing recommendations in treatment-naïve and treatment-experienced pediatric patients

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Background: DELPHI established the twice-daily darunavir/ritonavir dose in treatment-experienced children 6 to <18 years old (AIDS 2009;23:2005–13). To further establish pediatric dosing recommendations, two phase II, open-label, single-arm, 48-week trials with darunavir/ritonavir were conducted to assess darunavir pharmacokinetics in treatment-experienced children (ARIEL) and treatment-naïve HIV-1-infected adolescents (DIONE).

Methods: In ARIEL (TMC114-TiDP29-C228; NCT00919854), children (3 to <6 years; 10 to <20kg) initially received twice-daily darunavir/ritonavir 20/3mg/kg with ≥2 active antiretrovirals. After 24 weeks, children could optionally participate in a substudy to assess once-daily darunavir/ritonavir (40/7mg/kg, <15kg; 600/100mg, ≥15kg) pharmacokinetics for 2 weeks, then resume twice-daily dosing. In DIONE (TMC114-TiDP29-C230; NCT00915655), adolescents (12 to <18 years; ≥40kg) received once-daily darunavir/ritonavir 800/100mg and 2 NRTIs. Intensive pharmacokinetics was conducted after 2 weeks. Sparse sampling was also conducted in both trials. Darunavir plasma concentrations were measured by a validated LC-MS/MS method. Darunavir population pharmacokinetic parameters were calculated by Bayesian feedback using an adult model adapted for pediatrics. In both trials, the aim was to achieve a darunavir AUC range within 80% to 130% of that in adults (i.e. 62.3µg•h/mL or 89.7µg•h/mL for twice- or once-daily dosing, respectively). Modeling and simulation was used to determine dosing recommendations for once-daily dosing in children 3 to <12 years old. Pharmacokinetic/pharmacodynamic relationships were graphically analyzed.

Results: In ARIEL (main study), before dose adjustment, twice-daily darunavir (20/3mg/kg) resulted in darunavir geometric means (SD) AUC_{12h} of 63.8 (26.7) (overall; N=19), 65.5 (25.7) (10 to <15kg; N=10) and 62.1 (29.4) (15 to <20kg; N=9) µg•h/mL, equivalent to 102%, 105%, and 100% of adult AUC_{12h}, respectively. A dose adjustment was made to twice-daily 25/3 mg/kg (<15 kg) and 375/50 mg (15 to <20kg) post Week 2. With these adjusted doses, darunavir geometric means (SD) AUC_{12h} in ARIEL at Week 48 were 78.5 (25.1) (overall; N=19), 89.1 (20.7) (10 to <15kg; N=5) and 75.1 (26.4) (15 to <20kg; N=14) µg•h/mL, equivalent to 126%, 143% and 121%, respectively, of adult exposure. With once-daily darunavir/ritonavir in ARIEL (substudy, N=10), darunavir geometric mean (SD) AUC_{24h} was 115 (40.6) µg•h/mL; 128% of adult AUC_{24h}. In DIONE, the darunavir AUC_{24h} geometric mean (SD) was 80.7 (23.6) µg•h/mL (N=12) at Week 48, equivalent to 90% of the adult AUC_{24h}. In ARIEL and DIONE, no apparent relationships between Week-48 darunavir AUC or C_{0h} and achieving viral load <50 copies/mL or change in $\log_{10}$ viral load from baseline were seen. For adverse events of interest, no relationships were observed with either Week-48 darunavir AUC or C_{0h} in both trials.

Conclusions: ARIEL demonstrated that twice-daily darunavir/ritonavir regimens of 20/3mg/kg (<15kg) and 375/50mg (15 to <20kg) in treatment-experienced children aged 3 to <6 years achieved darunavir exposure comparable with that in treatment-experienced adults receiving twice-daily darunavir/ritonavir 600/100mg. Simulation of once-daily darunavir/ritonavir regimens suggested that the following doses of darunavir/ritonavir: 35/7mg/kg (10 to <15kg); 600/100mg (15 to <30kg); and 675/100mg (30 to <40kg) were comparable with once-daily dosing at 800/100mg in adults. For treatment-naïve adolescents (12 to <18 years; ≥40kg; DIONE), 800/100mg once-daily darunavir/ritonavir resulted in comparable exposure to darunavir in adults.

Conflict of interest
Employee of Janssen.

Abstract: O_14

PK-PD of Drug Efficacy and Toxicity

Antiretrovirals cerebrospinal fluid concentrations above drugs IC95s are associated with CSF viral loads below 50 copies/ml

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Introduction: Antiretroviral drugs are generally effective in suppressing HIV replication in the central nervous system (CNS); nevertheless low-level replication in the cerebrospinal fluid (CSF) is not an uncommon finding whose clinical impact could be significant. IC50 has been generally used to define appropriateness of CSF exposure of anti-HIV drugs, however, the relationship between CSF pharmacokinetics and pharmacodynamics has not been fully assessed for antiretrovirals. Our aim was to evaluate the use of IC95 as a pharmacodynamic cut off in the clinical setting.

Materials & Methods: Patients undergoing spinal tabs for clinical reasons were enrolled between 2010 and 2012 after signing a written informed consent. To be included patients should not present genotypic resistance to NNRTIs, protease inhibitors, raltegravir and R5-tropism (maraviroc intakers). HIV RNA was measured through Real Time PCR; CSF concentrations were analyzed through a validated ULPC/MS-MS method. IC50s and IC95s were derived from the paper by Acosta and coll. [in ng/ml respectively IC50 and IC95 (1.7-6.5) for ATV, (0.4-1.9) for DRV, (3.1-17) for LPV, (3.6-44) for RAL, (32-253) for NVP, (1.3-4.7) for EFV] or the EC90 (0.06-10.7) for MVC. Data are expressed as medians (interquartile ranges) and non-parametric tests were adopted for all the analysis.

Results: 150 samples from 113 patients were collected; subjects were mainly male (80, 70.8%), aged 44 years old (38-51) and of 20.7 Kg/m2 (18.7-23.5) of BMI. 85 (60.3%) and 64 (44.4%) samples showed respectively a plasma and CSF viral load below 50 copies/ml. 90 (60%) samples presented CSF levels above IC95, 52 (34.7%) between IC95 and IC50 and 8 (5.3%) below IC50. CSF concentrations were 1.37 times (0.54-9) the IC95 values with the highest ratio for darunavir twice daily [18.4 (15.8-24.7)], darunavir once daily [11.3 (5.8-16.8)] and efavirenz [6.4 (3-11.2)]. Cerebrospinal fluid concentrations above IC95s were significantly associated with a higher probability of a CSF viral load below 50 copies/ml [p=0.02, OR 2.22 (1.11-4.42)]; the IC50 cut off was not associated with viral suppression (p=0.67).

Conclusions: In the clinical setting, the CSF concentrations to IC95 ratio showed to be a very promising PK/PD parameter. Drugs concentrations above the IC95s, in fact, were significantly associated with a better viral control, as opposite to the use of IC50 the most frequently reported threshold in previous papers. This ratio showed a remarkable variability according the different drugs, being the highest values reported for darunavir (both twice and once daily) and efavirenz. Therefore, CSF drug concentrations to IC95 ratio could be suggested to compare drug exposure in the cerebrospinal fluid.

Conflict of interest
Travel grants and speaker's honoraria from Janssen-Cilag, Abbott, BMS, MSD, Viiv

Abstract: O_15

Drug Drug Interactions

Pharmacokinetic (PK) interactions between Boceprevir (BOC) and Atazanavir/r (ATV/r) or Raltegravir (RAL) in HIV/HCV coinfected patients (pts).

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Background: There are PK interactions between BOC and HIV PIs in healthy individuals. The aim of this ANRS HC27 substudy was to investigate the impact of BOC on ATV/r and RAL PK in HIV/HCV co-infected pts.

Methods: In this multi-center open-label phase II trial, treatment-experienced HIV/HCV genotype 1 pts well suppressed on ART (HIV VL <50 cp/mL) received peg-IFNα2b (1.5 µg/kg/wk) + RBV (800 to1400 mg/d) + BOC (800 mg tid) after a 4-week lead-in phase (peg-IFN+RBV). Allowed ARVs were: TDF, FTC/3TC, and ATV/r (300/100 mg qd) or RAL (400 mg bid).PK parameters Ctrough(Cτ), Cmax and AUC0-8h were determined at baseline and after 4 weeks of BOC (W8). Drugs levels were measured by LC-MS/MS. PK calculations were done by a non-compartmental method.

Results: 12 pts completed the PK study, 7 with ATV/r and 5 with RAL. *See table for results.*

Conclusions: With the limitations of this small study, there was a trend towards lower ATV PK parameters when ATV/r was combined with BOC that was significant only for AUC. There was substantial variability in RAL PK parameters with a trend towards higher RAL $AUC_{0\text{-}8h}$ and Cmax and lower RAL Cτ which were not statistically significant. BOC PK was unaffected. Pending more data, carefully monitoring of HIV replication in pts under ATV/r or RAL-based regimens receiving BOC would be appropriate.

No conflict of interest

Drug	$AUC_{0\text{-}8h}$ *(µg/L.h)* (mean ± sd)	Median	Cτ *(µg/L)* (mean ± sd)	Median	Cmax *(µg/L)* (mean ± sd)	Median
ATV (D0)	13211.7 ± 6620.4	13420	763.8 ± 754.4	553	2412.8 ± 1513.7	2380
ATV (W8)	6533.5 ± 3177.8	5862	507.7 ± 485.9	419	1418.5 ± 792.9	1300
R = ATV W8/D0(1)	*0.49*	*0.43*	*0.66*	*0.76*	*0.59*	*0.55*
RAL (D0)	5900.8 ±6167.5	3982.6	337.7 ± 348.5	201	1387 ± 1478.5	816
RAL (W8)	9248.6 ±3032.2	10510	153.9 ± 115.5	122	3556 ± 1407.8	4110
R = RALW8/D0	*1.57*	*2.63*	*0.45*	*0.61*	*2.56*	*5.03*
BOC (+ ATV/r)	4169.7 ± 1048.3	4416.3	154.3 ± 89.2	157	1314.6 ± 458.2	1370
BOC (+ RAL)	5310 ± 2643.3	4116.3	118.2 ± 79.5	88.1	1039.7 ± 574.2	1050

(1) p<0.01; all other comparisons show no statistical differences

Abstract: O_16

Drug Drug Interactions

The effect of boceprevir and telaprevir on dolutegravir pharmacokinetics, in healthy adult subjects.

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Background: Dolutegravir (DTG) is an investigational HIV integrase inhibitor that that delivers predictable plasma exposures without the need for a booster. DTG is primarily metabolized via UDP-glucuronosyltransferase (UGT)1A1 with a minor component of cytochrome P450 (CYP)3A4. DTG is a substrate for P-glycoprotein (Pgp) but because of its high permeability, no alteration in absorption would be expected by co-administration of Pgp inhibitors. Boceprevir (BCV) and Telaprevir (TVR) have been demonstrated to be strong CYP3A4/5 inhibitors and as such may inhibit DTG metabolism through this minor pathway.

Materials & Methods: This was a single-center, randomized, open-label, two-cohort, two-period, one way interaction study in healthy adult subjects. In the first treatment period, all subjects received DTG 50 mg q24h for 5 days (Treatment A). In Period 2, subjects received DTG 50 mg q24h with either BCV 800 mg q8h (Treatment B) for 10 days or TVR 750 mg q8h (Treatment C) for 10 days. There was no washout between treatment periods. Serial PK samples were collected after each period and safety assessments were performed throughout the study. Plasma DTG concentrations were determined by HPLC-MS/MS. Plasma PK parameters were determined by non-compartmental methods (WinNonlin 5.2). The ratios of geometric least squares (GLS) means (90% CI) comparing selected PK parameters with and without TVR or BCV were estimated by analysis of variance (ANOVA) using the SAS Mixed Linear Models procedure.

Results: A total of 32 subjects were enrolled in the study. Twenty-eight subjects completed the study as planned, 4 subjects were withdrawn due to AEs (2 non-drug related, 2-drug-related (increased ALT and increased Grade 1 serum creatinine) which all resolved within 3 weeks). The most commonly-reported drug-related AEs were headache, dysgeusia, and maculopapular rash. No consistent, treatment related or clinically significant changes in mean or median hematology and clinical chemistry were observed in the study. Co-administration of BCV had no effect on plasma DTG AUC(0-τ) or Cmax, and increased DTG Cτ by 8%. Co-administration of TVR increased DTG plasma exposures compared to administration of DTG alone: AUC(0-τ), Cmax, Cτ, increased by 25%, 19%, and 37%, respectively.

Conclusions: The combination of DTG with BCV or TVR was generally well tolerated. Neither BCV nor TVR had a clinically significant effect on plasma DTG exposure. DTG can be co-administered without dose-adjustment with these commonly prescribed drugs for the treatment of HCV/HIV co-infection.

Conflict of interest
Employee of GSK and stockholder

Abstract: O_17

Drug Drug Interactions

The effect of boceprevir and telaprevir on the pharmacokinetics of maraviroc: an open-label, fixed-sequence study in healthy volunteers

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Introduction: Maraviroc (MVC) is a chemokine co-receptor type 5 (CCR5) antagonist indicated for the treatment of CCR5-tropic HIV-1 infection. MVC is a substrate for CYP3A4/P-gp, and when co-administered with potent CYP3A4/P-gp inhibitors, MVC exposure is increased. The primary objective of this study was to evaluate the effect of boceprevir (BOC) and telaprevir (TVR) on the pharmacokinetics (PK) of MVC.

Materials & Methods: In this open-label, single-center, fixed-sequence, Phase I study, healthy adults aged 18-55 years received MVC 150 mg twice daily (BID) for 5 days (Period 1), followed by BOC 800 mg three times daily (TID) plus MVC 150 mg BID for 10 days (Period 2), then TVR 750 mg TID plus MVC 150 mg BID for 10 days (Period 3), with a 10-day washout between Periods 2 and 3. Blood samples were collected for PK analysis over 12 hours (MVC) or 8 hours (BOC and TVR) on Day 5 of Period 1 and Day 10 of Periods 2 and 3. Parameters included AUC_{12} (MVC) or AUC_8 (BOC and TVR), C_{max}, and plasma concentration at 12 hours (C_{12}; MVC) or 8 hours (C_8; BOC and TVR) postdose. Safety and tolerability were assessed.

Results: Fourteen volunteers (all male) were enrolled and thirteen completed all three treatment periods. The majority (86%) were white with a mean age of 33.3 years and mean weight of 79.3 kg. The ratios of the geometric means (90% confidence intervals) for MVC AUC_{12}, C_{max}, and C_{12} were 3.02 (2.53-3.59), 3.33 (2.54-4.36), and 2.78 (2.40-3.23), respectively, for MVC+BOC versus MVC alone, and 9.49 (7.94-11.34), 7.81 (5.92-10.32), and 10.17 (8.73-11.85), respectively, for MVC+TVR versus MVC alone. The geometric mean MVC average exposure (C_{avg}) with MVC 150 mg BID in combination with BOC was 151 ng/mL, and 465 ng/mL with TVR co-administration. In the presence of MVC, BOC AUC_8, C_{max}, and C_8 were 5404 ng·h/mL, 192 ng/mL, and 80.7 ng/mL, respectively, while TVR AUC_8, C_{max}, and C_8 were 21980 ng·h/mL, 3533 ng/mL, and 1943 ng/mL, respectively. The overall incidence of adverse events (AEs) was 43%, 100%, and 92% for Periods 1, 2 and 3, respectively. All were mild-to-moderate in severity with the exception of a severe event of asthmatic crisis (pre-existing condition) following the completion of MVC+BOC treatment (unrelated), which led to the discontinuation of one volunteer. Dysgeusia (50%) and pruritus (29%) were the most common AEs during treatment with BOC+MVC, and fatigue (46%) and headache (31%) were most common during treatment with TVR+MVC. No serious AEs or deaths occurred.

Conclusions: MVC exposure was increased in the presence of BOC or TVR. These findings are consistent with evidence that both BOC and TVR are inhibitors of CYP3A. BOC and TVR exposures when co-administered with MVC were consistent with historical data. MVC co-administered with BOC or TVR was generally well-tolerated with no unexpected safety findings. MVC should be dosed at 150 mg BID when co-administered with either BOC or TVR, consistent with current dose recommendations with other potent CYP3A inhibitors. No dose adjustment for BOC or TVR is warranted with MVC.

Conflict of interest
Employees of Pfizer Inc, and hold stock/stock options in Pfizer Inc.

Abstract: O_18

Pharmacology of other viral diseases

Early ribavirin concentration is a critical response factor in the sub-population of patients infected by HCV-1 and unfavourable IL28B genotype

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Introduction: The current standard of care (SOC) therapy for chronic hepatitis C (CHC) is the combination of pegylated interferon-α (Peg-IFNα) and ribavirin (RBV). HCV genotype 1 and 4 infected patients have always had the worst sustained virological response (SVR) rate, nearly 45%, compared with genotypes 2 or 3, near to 75%. Today, new protease inhibitors for the treatment of HCV genotype 1 are available. However, there are several issues concerning efficacy and safety of the standard therapy which, with the new concomitant drugs, could become more important. There is not a clear knowledge about the factors which cause influence the clinical response to therapy in HCV genotype 1 infected patients. Important factors to achieve SVR already identified are the IL28B gene polymorphisms, but also RBV pharmacokinetics seem to play some role in the clinical response determination. Our aim was to investigate the relationship between RBV early (1 month) plasma concentrations and the achievement of early virological response (EVR; HCV RNA not-detectable at 3 months of therapy, strongly related to SVR). Moreover, we tried to define a early RBV concentration cut-off value, specific for HCV genotype 1 infected patients with unfavourable IL28B profile, over which there is a better probability to achieve EVR.

Materials and Methods: Our sample consisted in 67 naive CHC HCV genotype 1 infected patients, undergoing Peg-IFNα plus RBV treatment. Main inclusion criteria were no concomitant interacting drugs, no hepatic or renal function impairment, and self-reported adherence > 95%. Blood sampling at the end of dosing interval (Ctrough) was performed at 4 weeks of therapy after written informed consent was given. Plasma RBV concentrations were measured using a validated HPLC-UV method, while patients genotyping for IL28B polimorphisms (rs8099917 T>G and rs1297860 T>C) was performed using TaqMan Genotyping Array. Statistical Analysis was made with SPSS 20.0: statistical significance of the correlation between plasma RBV concentration and EVR achievement was checked using Spearman correlation test. Early Plasma RBV cut-off value was determined using Receiver Operating Characteristic (ROC) curve.

Results: At 3 months of therapy, 43/67 (64.2%) patients achieved EVR. Considering the whole sample, RBV concentrations were not significantly related to clinical response. The genotype TT of single nucleotide polymorphism (SNP) rs8099917 T>G resulted significantly associated with the obtainment of EVR.
Stratifying patients according to this SNP genotype, a concentration over 1800 ng/mL was associated with good chances to achieve EVR (sensibility 69%; specificity 82%), in the subset of patients which have an unfavourable IL28B genetic profile (genotypes GG and TG).
RBV concentrations were not significantly correlated to EVR in patients with TT genotype.

Conclusions: For the first time, an early 'genotype-specific' cut-off of plasma RBV concentration were determined for the prediction of virological response at 3 months of therapy. These results show how genetics are the most important factors for the determination of response to therapy, but early RBV exposure is critical in patients which are genetically predisposed to fail treatment.
In conclusion, these results underline the importance of the identification of IL28B genetic profile and the crucial role that Therapeutic Drug Monitoring of RBV might have in a genetic-specific subset of patients, in order to grant them an optimal pharmacokinetic exposure, information also useful for a weighted approach to therapy with protease inhibitors.

No conflict of interest

14th International Workshop on Clinical Pharmacology of HIV Therapy

22 – 24 April 2013, Amsterdam, The Netherlands

Abstracts

Poster presentations

Abstract: PP_01

Drug Drug Interactions

Antiretroviral prescribing errors and potential adverse drug-drug interactions are common with hospitalization of hiv-infected patients

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Background: Reports suggest that antiretroviral therapy (ART) prescribing errors often occur with hospitalization of HIV-infected patients. Identification and prevention of this problem has the potential to reduce patient harm and healthcare-associated costs.

Methods: A retrospective medical record review of adult HIV-infected patients discharged from a midwestern US academic health center between 1/1/2009 and 12/31/2011. Patients were screened for admission greater than 24 hours and receipt of outpatient ART. The initial inpatient ART regimen was compared to outpatient ART. Errors were documented as omission; underdose; overdose; duplicate therapy; incorrect scheduling and/or incorrect therapy. Potentially adverse drug-drug interactions were also recorded. Time to error correction was recorded as <24 hours, <48 hours, >48 hours or never corrected. Relative risks (RR) were computed to compare error rates, with statistical significance defined as $p < 0.05$. A multivariate regression analysis was performed to compare error correction within 24 hours for admissions with a drug omission (the most common error type) to those admissions without a drug omission. Relative risks were also computed for the patient characteristics gender and race, with calculations based on error incidence upon admission.

Results: A total of 416 admissions and 177 patients met study population criteria. Overall, 289 medication errors were identified within 146 admissions (35% of admissions). ART was prescribed with contraindicated medications on 51 occasions. Only nonnucleoside reverse transcriptase inhibitors (NNRTI) and protease inhibitors (PI) were involved with drug-drug interactions. The most common prescribing error was drug omission (69%). At an error rate of 31% nucleoside reverse transcriptase inhibitors (NRTI) were associated with increased risk of error when compared to PIs (RR 1.32; 95% CI 1.04, 1.69) and coformulated drugs (RR 1.59; 95% CI 1.19, 2.09). While 31% of errors were detected and corrected within the first 24 hours, over half (55%) were never remedied during hospitalization. Of those errors that were never corrected, the most common error type was drug omission (accounting for 58%), and the drug class most likely to never be corrected was PIs (43%). Multivariate regression analysis demonstrated that admissions containing a drug omission were 7.4 times more likely to have all errors corrected within 24 hours than admissions without a drug omission. Lastly, increased risk of admission error was observed for Blacks (43%) when compared to Whites (27%) (RR 1.53; 95% CI 1.06, 2.23) but no significant differences between Whites and other minorities or between men and women were observed.

Conclusion: Errors in inpatient ART were common and the majority was never detected. The most common errors involved medication omission, and NRTIs had the highest rate of prescribing error. Medication omission and PIs were associated with the most prolonged time to correction, with drug omission associated with increased probability for error correction within the first 24 hours of hospitalization. Interventions for error prevention and correction such as those provided by HIV pharmacists could preclude errant or suboptimal ART during hospitalization.

No conflict of interest

Abstract: PP_02

Drug Drug Interactions

Increased plasma and intracellular ribavirin concentrations associated with telaprevir use

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Background: Ribavirin's (RBV) major dose-limiting toxicity is hemolytic anemia. Patients receiving telaprevir (TVR) with peginterferon alfa and RBV (PR) have twice the rate of anemia as those receiving PR alone. The mechanism for this increased incidence of anemia is unknown. We compared RBV plasma and intracellular (peripheral blood mononuclear (PBMC) and red blood cell (RBC)) concentrations at steady state (SS) in those receiving TVR+PR vs. PR alone to determine if increased RBV exposures in the presence of TVR may explain the increased incidence of anemia.

Materials & Methods: RBV pharmacokinetics (PK) were determined in an ongoing study of HCV-infected, genotype 1, treatment naive subjects receiving TVR+PR or PR alone (NCT01097395). RBV dosing was weight based (1 vs. 1.2g daily). Subjects underwent 12-hr intensive PK sampling for RBV at SS (wks 9-14) following observed dosing in the fasted state. RBV in plasma was measured using a validated HPLC-UV assay and RBV mono- (RMP), di (RDP), and triphosphate (RTP) in PBMC and RBC were measured using a validated LC/MS/MS assay. Log-transformed dose (mg/kg) adjusted RBV plasma area under the concentration time curve (AUC0-12) and RMP, RDP, and RTP in PBMC and RBC were compared (unpaired t-tests) in those receiving TVR+PR vs. PR alone.

Results: Twenty-one (14 male) HCV-infected subjects (mean±SD age 50.5±8.8yrs and weight 79±15kg), 16 on PR alone and 5 on TVR+PR underwent intensive PK sampling. Dose (mg/kg) adjusted plasma RBV AUC0-12 at SS was 1.54-fold higher in those receiving TVR+PR vs. PR alone (p=0.002). RMP, RDP, and RTP in RBC were 3.3- (p=0.003), 2.3- (p=0.0005), and 2.4-fold (p=0.001) higher, respectively in those receiving TVR+PR vs. PR alone. RMP, RDP, and RTP in PBMC were 2.5- (p=0.003), 3- (p=0.006), and 2-fold (p=0.04) higher, respectively in those receiving TVR+PR vs. PR alone. In patients on TVR+PR, RMP, RDP, and RTP concentrations declined after stopping TVR. Baseline hemoglobin was 15.5 g/dL in both groups, but decreased -5.5g/dL in those on TVR+PR vs. -4.3g/dL on PR alone (p=0.17) during treatment. Besides TVR use, no other variables (CrCl, age, gender, race) were associated with plasma or intracellular RBV PK.

Conclusions: RBV plasma AUC0-12 and RMP, RDP, and RTP in PBMC and RBC were significantly higher in those receiving TVR+PR vs. PR alone. We speculate that increased RBV exposures due to TVR might be a factor in the anemia observed during TVR-based antiviral therapy of HCV. Further research is needed to confirm these findings and elucidate the mechanism and clinical implications for this unexpected interaction.

Abstract will be presented/was presented as oral presentation at the CROI 2013 meeting

No conflict of interest

Abstract: PP_03

Pharmacokinetics for Pediatrics, Pregnancy and other Special Populations

Changes in tenofovir population pharmacokinetics during pregnancy

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Introduction: Tenofovir (TFV), a once daily nucleotide reverse transcriptase inhibitor, is a common component of Combination Antiretroviral Therapy (CART) used in HIV-infected pregnant women. The pharmacokinetics (PK) of tenofovir may be affected by the physiologic changes occurring during pregnancy, such as altered gastrointestinal function and increased glomerular filtration and body water. Previous studies found a 25-33% reduction in TFV exposure during pregnancy. The objective of this study was to perform a population PK analysis of TFV during pregnancy and postpartum to determine the mechanism(s) underlying reduced TFV exposure during pregnancy.

Materials and Methods: IMPAACT P1026s is an ongoing, prospective, non-blinded study of antiretroviral (ARV) PK in HIV-1-infected pregnant women receiving ARVs for routine clinical care [ClinicalTrials.gov Identifier: NCT00042289]. This analysis included data from P1026s cohorts receiving 300 mg TFV disoproxil fumarate (135.6 mg TFV) daily, either as Viread® or co-formulated with emtricitabine (Truvada®) or emtricitabine/efavirenz (Atripla®). Steady-state TFV PK profiles were collected at 30-36 weeks gestation (3rd Trim) and 2-12 weeks postpartum (PP), and optionally during the second trimester between 20-26 weeks gestation (2nd Trim). PK samples were taken pre-dose and 1, 2, 4, 6, 8, 12, and 24 hours post-dose. Plasma TFV concentrations were determined by a LC-MS-MS method. Population PK analyses were performed using NONMEM version 6.2 (FOCEI subroutine). The potential impact of pregnancy stage, serum creatinine, concomitant ARVs (ritonavir boosted PIs), albumin, and age were assessed as potential covariates in the model during a univariate screen which was followed by a multivariate assessment. Individual subject's PK parameters were estimated using an empiric Bayesian approach.

Results: Eighty-six steady-state PK profiles were collected encompassing 650 plasma TFV concentrations from 46 women during the 2nd trimester (n=7), the 3rd trimester (n=41), and 2-12 weeks postpartum (n=38). 54 women (63%) were receiving ritonavir boosted PI. The TFV concentration data were best described using a two-compartment model with first-order absorption and elimination. Allometric scaling of PK parameters resulted in a better fit than unscaled PK parameters and was included in the analysis prior to other covariates. Absorption was rapid and essentially complete prior to the first PK sample at 1 hour so KA was fixed at 7 hr^{-1} ($t_{1/2}$ absorption = 10 min) to promote model stability. Inter-occasion variability was included for F for each subject visit. Age, pregnancy state, serum creatinine, and albumin were significantly associated with CL/F during the univariate screen but only serum creatinine (SCr) remained a significant covariate when added to subject size in the final model. The final model parameters and intersubject variabilities (%CV) were:

- CL/F ($L/h/kg^{0.75}$) = 2.03 L hr^{-1} * (SCr / 0.6 mg dL^{-1})$^{-0.517}$ (24%)
- V2/F (L/kg) = 6.84 (35%)
- V3/F (L/kg) = 8.22 (43%)
- Q/F ($L/h/kg^{0.75}$) = 4.84

The post-hoc AUC estimates were significantly lower during the 3rd Trim versus PP (geo. mean 2.38 vs 2.90 mcg*h/mL, p=0.009) with a 3rd Trim:PP ratio of 0.83 (0.75-0.91 90% CI).

Conclusions: Changes in TFV pharmacokinetics during pregnancy are related to weight gain and to reduction in SCr associated with enhanced glomerular filtration.

No conflict of interest

Abstract: PP_04

PK-PD of Drug Efficacy and Toxicity

Rilpivirine concentrations in plasma and cerebrospinal fluid after switching from nevirapine-containing cART

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Background: Pharmacokinetic (PK) parameters such as cerebrospinal fluid (CSF) exposure and plasma exposure following a switch in combination antiretroviral (cART) therapy are often unknown for recently licensed antiretroviral agents. We assessed the plasma and CSF exposure and the safety and tolerability of rilpivirine (RPV) after switching from nevirapine (NVP)

Materials and Methods: Neuro-asymptomatic HIV-infected male subjects receiving stable cART comprising of tenofovir, emtricitabine, NVP (245/200/400 mg all once daily) with undetectable plasma HIV RNA and absence of significant co-morbidities were recruited. NVP was switched to rilpivirine 25 mg daily for 60 days. On days 0, 3, 7, 14, 28, and 60 safety and trough plasma concentrations (Cmin) were assessed. Plasma HIV RNA was also assessed regularly during the study. On day 60, lumbar puncture (LP) examination was undertaken 4 to 8 hours post dose. Both plasma and CSF samples were analysed by HPLC-MS/MS. Geometrical mean (GM) Cmin were calculated as were the number of subjects with both NVP and RPV Cmin below thresholds proposed to be associated with maximal virological efficacy (3000 and 20.3 ng/mL for NVP and RPV, respectively). GM CSF:plasma ratio of RPV was calculated and RPV CSF concentrations were compared to the EC_{50} and EC_{90} RPV concentrations for wild-type virus (0.27 and 1.35 ng/mL, respectively).

Results: Thirteen patients were enrolled and twelve underwent the LP procedure. Study medication was well tolerated, no clinically significant adverse events were reported and plasma HIV RNA remained undetectable during the study period.
GMs for NVP Cmin were 4510 ng/mL (95% CI: 4050-5023), 580 ng/mL (95% CI: 372-904), 93 ng/mL (95% CI: 50-173) and 40 ng/mL (95% CI: 18-85) on days 0, 3, 7 and 14 after switch, respectively. GMs for RPV Cmin were 30.8 ng/mL (95% CI: 24.7- 38.3), 36.7 ng/mL (95%CI: 30.4-44.4), 46.7 ng/mL (95% CI: 36.1-60.6), 58.6 ng/mL (95% CI: 50.2- 68.3) and 59.6 ng/mL (95% CI: 50.5-70.3) on days 3, 7, 14, 28 and 60, respectively. In 3 subjects, on 4 occasions, both NVP and RPV Cmin were under the proposed thresholds associated with maximal virological efficacy (on day 3 RPV Cmin was 14 and 19 ng/mL, on day 7 RPV Cmin was 17 ng/mL and on day 14 RPV Cmin was 18 ng/mL; on all of these time points NVP Cmin was below 724 ng/mL).
The overall GM RPV CSF concentration was 0.81 ng/mL (95% CI: 0.68-0.96) representing a mean CSF:plasma ratio of 1.2% (95% CI: 1.0 to 1.5%). No CSF RPV concentrations were below the EC_{50} of 0.27 ng/mL. Mean CSF:plasma ratio varied from 1.0% (95% CI: 0.87 to 1.2%) at 4 hours post dose to 1.5% (95% CI: 0.45 to 4.7%) 8 hours post dose.

Conclusion: Switching therapy from NVP-containing cART to RPV-containing cART was safe and well tolerated. Effective plasma concentrations of RPV or NVP were observed in the majority of subjects throughout the study sampling times. RPV concentrations in CSF were between the EC_{50} and EC_{90} for wild-type virus.

Conflict of interest
The study has received funding from Janssen Pharmaceutical Companies.

Abstract: PP_05

PK-PD of Drug Efficacy and Toxicity

Utilising In Vitro-In Vivo extrapolation to investigate efavirenz penetration into the central nervous system

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Background: Adequate concentrations of efavirenz (EFV) in the CNS are necessary to maintain the suppression of viral replication but high concentrations may increase the likelihood of developing central nervous system (CNS) toxicity. Despite the widespread use of EFV, its penetration into the CNS has not been fully characterised. The aim of this investigation was to evaluate EFV penetration into the cerebrospinal fluid (CSF) and brain using an *in vitro in vivo* extrapolation (IVIVE) simulation and to compare the prediction with available data from rodents and humans.

Materials & Methods: The physicochemical properties of EFV (including log P, molecular weight, pKa) were gathered from the literature while permeation across Caco-2 cells and protein binding were determined experimentally. A novel open source 6 compartment physiologically based pharmacokinetic model was generated describing the exchange between plasma, CSF and brain tissue. Pharmacokinetic (PK) simulations were performed using the differential equation solver Berkeley Madonna (v8.3.18). A once daily 600mg dose of EFV was simulated over 21 days for predictions of PK characteristics at steady state. Simulations were then compared with human data from the literature and rodent data. Wistar rats were administered with EFV by oral gavage (10mg/kg, 4µCi/mg, 0.5% methylcellulose vehicle). Plasma samples were collected over 300 minutes post dosing and analysed using a 3100 TR liquid scintillation counter (IsoTech, Uk). In order to test for a correlation between predicted tissue to plasma ratios and those obtained from rodent data, simple linear regression was performed using SPSS (v20). Statistical significance was defined as $P<0.05$.

Results: A simulation of 50 patients predicted PK parameters in agreement with literature data. Median plasma and CSF EFV were 2420 ng/ml (IQR 1573 – 3975) and 18.2 ng/ml (IQR 13.4 – 23.7). These predictions were in the previously reported range described by Best *et al.* 2010; Median plasma and CSF concentrations of 2145 ng/ml (IQR 1384 – 4423) and 13.9 ng/ml (IQR 4.1 – 21.2) respectively. The model also predicted EFV to accumulate in brain tissue (median brain tissue concentration 6256 ng/ml IQR 5893 – 6531) with a brain to plasma ratio of 2.6. Rodent brain tissue to plasma ratio was 8.5, showing accumulation of EFV in brain tissue (plasma 119 ng/ml, brain tissue 1016 ng/ml). The predicted tissue distribution (brain, muscle, gut, liver and spleen) correlated well with experimental data from rodents ($r^2 = 0.83$; $P = 0.02$).

Conclusions: The IVIVE model and rodent data presented here indicate that EFV accumulates within brain tissue. Although useful, measurement of CSF concentrations may be an underestimation of the penetration of antiretrovirals into the brain. Limitations associated with obtaining tissue biopsies and paired plasma and CSF samples from patients makes IVIVE an attractive tool for probing drug distribution.

No conflict of interest

Abstract: PP_06

PK-PD of Drug Efficacy and Toxicity

Pharmacokinetics and pharmacodynamics of once-daily etravirine and darunavir/ritonavir in early treatment-experienced subjects

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Background: The long elimination half-life of etravirine (~41 hours) supports its use for once-daily dosing. In the SENSE trial, virologic response was similar in antiretroviral-naïve patients treated with etravirine 400mg once daily or efavirenz, both with 2 nucleoside reverse transcriptase inhibitors. The Intelence aNd pRezista Once A Day Study (INROADS, NCT01199939) was designed to assess the safety, antiviral activity and pharmacology of etravirine 400mg with darunavir/ritonavir 800/100mg as a once-daily, nucleoside-sparing regimen in treatment-experienced or treatment–naïve subjects with transmitted resistance. The results of a pharmacokinetic substudy at Week 4, population pharmacokinetics over 48 weeks and a pharmacodynamic evaluation are presented.

Materials & Methods: INROADS was a Phase 2, single-arm, open-label, multicenter, 48-week trial evaluating etravirine 400mg (two 200mg tablets) and darunavir/ritonavir 800/100mg, both once daily, in HIV-1–infected adults who were treatment experienced or treatment naïve with transmitted resistance. Sparse sampling for etravirine and darunavir plasma concentrations were collected over 48 weeks: two blood samples were taken at Weeks 4 and 24 each and one sample was taken at Weeks 12, 36 and 48 from all subjects. Bayesian feedback for individual etravirine and darunavir AUC_{24h} and C_{0h} were performed using previously developed population pharmacokinetic models (NONMEM). In addition, subjects had the option to participate in a substudy that evaluated etravirine, darunavir and ritonavir pharmacokinetics over 24 hours at Week 4. For the substudy, blood samples were taken at predose and at 1, 2, 3, 4, 6, 9, 12 and 24 hours post dose. Pharmacokinetic parameters were assessed for etravirine, darunavir and ritonavir using non-compartmental analysis (WinNonlin).

Results: Forty-nine subjects (80% male, 49% Black, median age 45 years) were included in the population pharmacokinetic analysis. Mean etravirine and darunavir AUC_{24h} were 14,900 and 104,000 ng·h/mL, respectively, and C_{0h} were 436 and 2790 ng/mL, respectively. Of the nine subjects enrolled in the substudy, seven had full pharmacokinetic data available. Median t_{max} were 4.0, 3.0 and 4.0 hours post dose for etravirine, darunavir and ritonavir, respectively. Mean C_{max}, AUC_{24h}, and C_{min} were respectively, 766 ng/mL, 8786 ng·h/mL and 148 ng/mL for etravirine; 12,190 ng/mL, 132,536 ng·h/mL and 1529 ng/mL for darunavir; and 729 ng/mL, 6521 ng·h/mL and 39.1 ng/mL for ritonavir. There was no apparent relationship between darunavir or etravirine AUC_{24h} or C_{0h} and virologic response (% <50 copies/mL at week 48 or change in viral load from baseline to week 48). The number of subjects with viral load >50 copies/mL at week 48 was higher in subjects with etravirine AUC_{24h} values below the median (7/25 versus 2/24). There was no apparent relationship between darunavir or etravirine AUC_{24h} or C_{0h} and safety laboratory values, with the exception of fewer lipid disturbances overall in the lowest quartile of darunavir exposure; and a higher incidence of gastrointestinal adverse events among patients with higher darunavir AUC_{24h}.

Conclusions:
Pharmacokinetic/pharmacodynamic analyses of the etravirine/darunavir/ritonavir-containing dual-therapy regimen administered once daily for treatment-experienced or treatment-naïve subjects with transmitted resistance show that adequate darunavir and etravirine exposures are obtained and these are effective in virologic control.

Conflict of interest
Employees of Johnson & Johnson

14th International Workshop on Clinical Pharmacology of HIV Therapy

22 – 24 April 2013, Amsterdam, The Netherlands

Abstracts

Posters

Abstract: P_02

Drug Drug Interactions

Co-administration of efavirenz increases intracellular concentrations of once daily ritonavir-boosted darunavir in healthy volunteers

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Introduction: Novel 2-drug nucleoside sparing regimens are under investigation, including combinations of non-nucleoside reverse transcriptase inhibitors (NNRTI's) and protease inhibitors (PI's), because of toxicities resulting from use of nucleosides (NRTI's) and development of viral resistance. Pharmacokinetic (PK) interaction studies are important to determine optimal combination regimens, and plasma concentration measurements are commonly performed to guide dosing decisions. However, PI's and NNRTI's exert their effect within cells. We therefore investigated the intracellular (IC) PK of once daily ritonavir (RTV)-boosted darunavir (DRV/r) and efavirenz (EFV) alone and in combination.

Material & Methods: This was an open-label single-sequence three-period PK study in 12 healthy HIV-seronegative adults, ages 24-49 years, five females. Participants received DRV/r 900/100 mg once daily for 10 days (period 1) and PK sampling for IC DRV and RTV was performed on day 10. Efavirenz (600 mg once daily) was added for 14 days (period 2) and PK sampling for IC DRV, RTV, and EFV was performed on day 24. DRV/r was stopped and EFV continued from day 25 to 38 (period 3). Pharmacokinetic sampling for IC EFV was performed on day 38. Samples were drawn pre-dose (0h) and 4, 8, 12 and 24 hours post-dose.PBMCs were isolated by using ficoll paque and washed twice with cold PBS.IC concentration of 3 drugs were determined by HPLC-MS/MS. PK parameters were calculated using Phoenix® and WinNonlin®, and geometric mean ratios (GMR) and 90% CI generated.

Results: All three drugs exhibited marked interindividual variability in IC PK parameters (CV%: 42-240%). IC DRV $AUC_{0\text{-}24h}$, C_{max} and C_{24h} at the end of periods 1 and 2 were 166 vs 372 µM.h, 16 vs 43 µM, 4.5 vs 5.2 µM, respectively. IC DRV $AUC_{0\text{-}24h}$ and C_{max} were significantly increased following addition of EFV (period 2 vs period 1) with GMR (90% CI) of 2.24 (1.2-4.0) and 2.63 (1.2-5.77), respectively; IC DRV C_{24h} was not significantly altered [GMR 1.16 (0.5-2.5)]. RTV IC PK parameters were not significantly influenced by co-administration of EFV with GMR (90% CI) of 1.38 (0.9-2.1), 1.6 (0.88-2.93), and 1.23 (0.83-1.83) for $AUC_{0\text{-}24h}$ C_{max} and C_{24h}, respectively. EFV IC $AUC_{0\text{-}24h}$ and C_{max} were not significantly altered in the presence of DRV/r [period 2 *vs.* period 3 GMR's (90% CI) 1.31 (0.99-1.73) and 1.2 (0.71-2.0) respectively]. However, IC EFV C_{24h} was significantly increased by DRV/r with GMR (90% CI) 2.39 (1.41-4.1).

Conclusions: Once daily RTV-boosted DRV IC $AUC_{0\text{-}24h}$ and C_{max} GMR's were significantly increased in the presence of EFV in healthy volunteers, and the EFV IC C_{24h} was significantly increased by DRV/r. The mechanisms and clinical significance of these IC interactions are unknown. These interactions suggest that once daily DRV/r and EFV may provide a promising alternative for patients unable to take NRTI's.

Conflict of interest
Funding for this study was provided by the National Healthcare Group CEO Special Research Fund.
C.F. has served on scientific advisory boards for Bristol-Myers Squibb, Gilead Sciences Merck, Roche, Vertex, and ViiV Healthcare. P.P. has served on the scientific advisory board for Tibotec.

Abstract: P_03

Drug Drug Interactions

A multiplex LC-MS/MS assay for the simultaneous therapeutic drug monitoring of Ribavirin, Boceprevir and Telaprevir

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Background Up to 210 million individuals worldwide are chronically infected by HCV, and due to the common route of transmission, prevalence of HCV is more frequent in HIV-infected individuals than in the general population. New direct acting antivirals (DAAs) that inhibit hepatitis C virus (HCV) replication are now used for the treatment of chronic hepatitis C infection. A marked pharmacokinetic variability and a high potential of drug-drug interactions between DAAs and numerous drug classes has been identified, notably with antiretroviral drugs. In addition, ribavirin (RBV) is commonly associated with haemolytic anaemia and often requires RBV dose adjustment, advocating for therapeutic drug monitoring (TDM) in patients under combined antiviral therapy. However, an assay for the simultaneous analysis of RBV and DAAs constitutes an analytical challenge because of the large differences in polarity of these drugs, ranging from hydrophilic (RBV) to highly lipophilic (telaprevir, TVR). Moreover, TVR is characterized by an erratic chromatographic behavior on standard octadecyl-based reversed-phase columns and must be separated from VRT-127394, its inactive 21-epimer metabolite. We have developed a convenient assay implying simple plasma protein precipitation followed by high performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for the simultaneous determination in plasma of RBV, boceprevir (BOC) and TVR as well as its metabolite VRT-127394.

Materials & Methods Plasma samples (100 µL) extraction is performed by protein precipitation with MeOH prior to quantification of drug levels by LC-MS/MS, with electro-spray ionisation in the positive mode, using the *m/z* transitions for RBV, BOC and TVR, at *m/z* 245→113, 520.4→308, *and* 680.5→322, respectively. The stable isotopic compounds RBV-$^{13}C_5$, BOC-D_9 and TVR-D_{11} are used as internal standards. Separation of RBV, BOC and TVR (eluted at 2.5, 5.5 and 8 min respectively) is obtained onto a graphite UPLC column maintained at +80°C, using a stepwise gradient elution with three mobile phases constituted of 0.1% formic acid (FA), MeOH/H_2O 1:1 (+0.1 % FA) and isopropanol +0.1 % FA, delivered at 0.3 ml/min.

Results The method is sensitive (lower limit of quantification: 25 ng/mL, 25 ng/mL and 100ng/mL for TVR, BOC and RBV, respectively), precise (inter-day CV%: 2 - 5%) and accurate (bias -2.2 to 4.3%). While BOC is stable in whole blood up to 24h at RT, RBV and TVR plasma levels are altered if whole blood (citrate) is let 1h at RT. This indicates that blood need to be centrifuged immediately after collection from patients, followed by plasma storage at -20°C prior to analysis. Conversely, the antiviral agents are stable in acidified (with formic acid 10% 5:95) plasma samples let at RT and +4ºC, up to 8h and 24h, respectively.

Conclusions This new, sensitive and simple multiplex LC-MS/MS assay has been fully validated and is used for real time TDM in the routine clinical Service and for research pharmacokinetics studies in HCV- and HCV/HIV-co-infected patients.

No conflict of interest

Abstract: P_04

Drug Drug Interactions

Pharmacokinetic interaction of the direct acting antiviral agent boceprevir with maraviroc in healthy volunteers

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Background: Boceprevir is a direct acting antiviral agent used in the treatment of hepatitis C (HCV). Its clinical efficacy is being investigated in patients co-infected with HIV and HCV. Boceprevir displays many drug-drug interactions with various medications including antiretrovirals, where it has been shown to increase or decrease CYP3A4/5 substrates. It is also a weak inhibitor of P-glycoprotein. No data are yet available on the use of this agent in combination with maraviroc, a CCR5 antagonist which is a substrate of CYP3A4 and of P-glycoprotein.

Materials and Methods: This pharmacokinetic (PK) phase 1, single center, open-label, crossover single-sequence drug-drug interaction study was conducted in healthy Caucasian males. Subjects were selected according to a strict protocol based on physical examination and laboratory tests. Subjects received maraviroc 150 mg every 12 hours for 5 days followed by co-administration of maraviroc 150 mg every 12 hours with boceprevir 800 mg every 8 hours with food for 14 days. On days 5 and 19, maraviroc plasma concentrations were determined by high performance liquid chromatography coupled to a tandem mass spectrometer before and at 0.5, 1, 1.5, 2, 4, 6, 8 and 12 hours after the morning maraviroc dose. Geometric mean ratios (GMR) of $AUC_{0\text{-}12}$ (area under the concentration-time curve for the 12 hours dosing interval), C_{max} (maximum concentration) and C_{tau} (concentration at the end of the dosing interval) were calculated. From these data, lack of interaction was concluded if the 90% confidence interval of the GMR (test/reference) fell completely within 80-125%. Individual maraviroc pharmacokinetic parameters were calculated using non-compartmental analysis (WinNonlin 6.3, Pharsight). Information regarding adverse events (AEs) was collected throughout the study and up to 7 days after the end of the study.

Results: As of January 2013, a total of 15 male participants consented to the study and 5 subjects were enrolled and completed the study (median age: 25 years; median weight: 79.6 kg; median body mass index: 24.6 kg/m^2). Boceprevir significantly increased the exposure of maraviroc with $AUC_{0\text{-}12}$ GMR - [90% confidence interval] of 2.28 [1.24-3.32] and C_{tau} GMR of 3.62 [2.64-4.60]. Boceprevir did not significantly change maraviroc C_{max} (GMR of 1.25 [0.16-2.34]). Maraviroc exposures with boceprevir were lower than historical data of maraviroc 300 mg BID without CYP3A4 inhibitors and interindividual variability was high in both treatment arms (mean [%CV]; maraviroc: $AUC_{0\text{-}12}$ 0.367 mg*h/L [58%], C_{max} 0.170 mg/L [70%] and C_{tau} 0.007 mg/L [52%]; maraviroc+boceprevir: $AUC_{0\text{-}12}$ 0.923 mg*h/L [69%], C_{max} 0.212 mg/L [62%] and C_{tau} 0.030 mg/L [69%]). Overall, the study drugs were very well tolerated and AEs reported were mild to moderate (overall incidence 80%; n=4/5). The most common AE was dysgeusia (80%), a known side effect of boceprevir. No grade 3 or 4 AEs or laboratory abnormalities were observed.

Conclusions: Co-administration of boceprevir and maraviroc resulted in significantly enhanced exposure of maraviroc. Our results suggest that boceprevir is inhibiting maraviroc's CYP3A4-mediated metabolism and/or P-glycoprotein. Given the magnitude of the interaction, maraviroc 150 mg every 12 hours is recommended when used with boceprevir.

No conflict of interest

Abstract: P_05

Drug Drug Interactions

The effect of rifampin on the pharmacokinetics of the HIV-1 attachment inhibitor prodrug BMS-663068 in healthy subjects

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Background: BMS-663068 is a phosphonooxymethyl prodrug of BMS-626529, an HIV-1 attachment inhibitor. BMS-626529 is a substrate of the P-glycoprotein (P-gp) transporter and primarily metabolized by an esterase-mediated hydrolysis pathway and to minor extent by Cytochrome P450 3A4 (CYP3A4). The study objective was to assess the effects of rifampin on the pharmacokinetics (PK) of BMS-626529 after single dose administration of BMS-663068.

Materials & Methods: This study was an open-label, one sequence, one-way interaction study in 15 healthy male subjects. On Day 1, all subjects received a single dose of BMS-663068 1200 mg in the morning with standard meal (Treatment A). On Days 6-12, subjects were administered rifampin 600 mg QD in the evening; on Day 11, subjects received a single dose of BMS-663068 1200 mg in the morning with standard meal (Treatment B). Subjects were confined to the clinical facility for the duration of the study. Serial blood samples for BMS-626529 were collected predose and up to 48 hours post-dose on Days 1 and 11. All subjects were monitored for adverse events (AEs) throughout the study. Single-dose PK parameters, including (maximum concentration [Cmax], time of Cmax [Tmax], area under the curve (AUC) from time 0 to infinity [AUC(INF)], elimination half-life [THALF], apparent oral clearance [CLT/F]) were estimated from plasma concentration versus time data for BMS-626529. PK parameters were summarized and point estimates and 90% confidence intervals (CIs) for the ratios of the geometric means for BMS-626529 Cmax and AUC(INF) with and without rifampin were constructed using linear mixed models on log-transformed data. AEs were listed and tabulated by system organ class, preferred term and treatment.

Results: Following administration of BMS-663068, the ratio of geometric means (CIs) for BMS-626529 AUC(INF) and Cmax with and without rifampin were 0.181(0.163,0.200), and 0.241(0.208,0.279), respectively. Median Tmax, mean THALF, and mean CLT/F values in the absence and presence of rifampin were 5 hr and 4 hr, 6.29 hr and 6.84 hr, and 686 mL/hr and 3841 L/hr, respectively. The similar magnitude of reduction in both AUC and Cmax in conjunction with a similar elimination rate suggests that the interaction is likely primarily mediated via presystemic P-gp induction; however, a contribution of CYP3A-mediated presystemic metabolism cannot be ruled out. Following administration of BMS-663068 alone and with rifampin, BMS-626529 was safe and well-tolerated.

Conclusions: The results of the study demonstrate the effects of the P-gp and CYP3A inducer rifampin on BMS-626529 AUC and Cmax, with a clinically significant reduction by 82 and 76% respectively.

Conflict of interest
Employee of Bristol-Myers Squibb

Abstract: P_06

Drug Drug Interactions

Pharmacokinetic interactions between raltegravir and non nucleoside reverse transcriptase inhibitors or protease inhibitors: a retrospective study

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Background: Regarding its metabolism avoiding CYP450 pathways, raltegravir (RAL) presents a low propensity to alter the pharmacokinetics of other associated drugs and mutually. Then, RAL is a cornerstone drug to construct long-term suppressive therapy using 'no boost no Nucs' strategies in patients with co-morbidities. The objectives were to describe RAL plasma concentrations according to their antiretroviral (ARV) backbone: Non Nucleoside Reverse Transcriptase Inhibitors (NNRTI) or Protease Inhibitors (PI +/- RTV) or Nucleoside Reverse Transcriptase Inhibitors (NRTI) and to compare their metabolic impact on RAL plasma concentrations.

Materials and Methods: A request from our pharmacological database was performed on 2011-2012 to look for RAL plasma concentrations in association with NNRTI, PI +/- RTV or NRTI. All HIV-infected patients with available NNRTI and PI plasma concentrations were enrolled in this retrospective study. RAL C12h (12 +/- 2 hours after the last drug intake) were determined using UPLC coupled with tandem Mass Spectrometry (LOQ < 1 ng/mL). Results are presented as median (IQR25%-75%). Statistical analysis was performed using t-test.

Results: In this study, 1,208 RAL C12h were collected: 327 without NNRTI or PI (Gp1), 442 with PI +/- RTV (Gp2: 61% darunavir/r, 10% lopinavir/r, 11% atazanavir (ATV) alone, 10% ATV/r and 8% other PI/r), 213 with NNRTI (Gp3: 77.5% etravirine (ETR), 10.5% efavirenz and 12% nevirapine) and 226 with NNRTI + PI/r (Gp4: 85% ETR and darunavir/r, 15% other associations). In the overall population, RAL C12h was 141 ng/mL (58-317). In the sub-groups, RAL C12h was 162 ng/mL (80-360) in Gp1, 125 ng/mL (51-275) in Gp2, 157 ng/mL (69-390) in Gp3 and 117 ng/mL (54-289) in Gp4. With unboosted ATV and ATV/r, RAL C12h was 239 ng/mL (154-679, n=47) and 179 ng/mL (118-316, n=45), respectively and with ETR alone, RAL C12h was 167 ng/mL (67-450, n=165). In RAL+DRV/r containing group, with and without ETR, RAL C12h was 112 ng/mL (48-292, n=174) and 100 ng/mL (44-251, n=268), respectively. Compared with the reference group (Gp1), RAL C12h was significantly lower with DRV/r ($p<0.01$) or with DRV/r+ETR ($p=0.03$) and higher with unboosted ATV ($p=0.03$). No statistical difference was found between the reference group and ETR alone ($p=0.67$), between reference group and ATV/r ($p=0.55$) and between DRV/r+ETR and DRV/r alone ($p=0.76$).

Conclusions: Therapeutic drug monitoring of ARV is yet mostly indicated to study patient variability, toxicity, drug-drug interactions (DDI) rather than virological failures as demonstrated with more than 85% of suppressed viremia in the national French database. In the overall population and despite its important variability, RAL C12h IQR25% remained above the supposed antiviral threshold of 50 ng/mL. Moreover, no pharmacokinetic interaction between ETR and RAL was found. This study also confirmed the booster effect of ATV alone on RAL exposure through its inhibitory effect on UGT1A1. Surprisingly, a lower RAL C12h (but still adequate) was observed with DRV/r compared to the reference group. This study suggests that RAL containing regimen would be safe on a pharmacokinetic concern.

No conflict of interest

Abstract: P_07

Drug Drug Interactions

Pharmacokinetic evaluation of antiretroviral and concomitant antiepileptic and psychotropic drugs therapy in HIV-infected patients

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Introduction: Central nervous system (CNS) disorders are extremely common among HIV infected patients, due to opportunistic infections, psychiatric diseases, comorbid conditions, including infection and the effects of accelerated cardiovascular disease and ageing. The presence of neurocognitive disorders is very high (more than 50% of patients). HIV may cause itself cognitive, behavioral, and motor difficulties. Moreover, seizures represent a manifestation of nervous system involvement that is common among HIV patients, with a reported frequency rate ranging from 3-11%. There is a high possibility of drug combinations available for the treatment of these concomitant pathologies with antiepileptic and psychotropic drugs (APDs). Given the paucity of data on the possible interaction between the different combinations of treatments (ARVs and APDs), at our Clinical Center we are evaluating outpatients who undergo the routine monitoring (TDM) of these therapies.

Material & Methods: This is an observational, single site prospective study, approved by local IRB. Steady-state trough plasma concentrations of levetiracetam were measured by using specific and validated HLPC assay with UV detection, citalopram, mirtazapine, quetiapine were quantified by HPLC with Mass Spectrometer Triple Quadrupole. For each APD drug we used the following suggested therapeutic range: levetiracetam 6-20 mcg/mL, citalopram 30-130 ng/mL, mirtazapine 40-80 ng/mL, quetiapine 70-170 ng/mL. Antiretroviral drugs (PIs and NRTIs) were quantified by HPLC-UV methods, RLT was measured by HPLC with fluorimetric detection.

Results*:* To date, twenty-nine adult HIV infected patients were included in the study (mean age: 39); 8 were female and 21 were males. Among them, 21 were receiving a PI based therapy, 4 a NNRTI based therapy and 4 an integrase inhibitor based therapy. Fourteen out of 29 patients (48%) had trough plasma concentration of the APDs out of the therapeutic range, in particular 1/10 receiving levetiracetam, 4/8 receiving citalopram, 4/5 receiving mirtazapine and 5/6 receiving quetiapine. Patients on lopinavir therapy (12) had all trough plasma concentration higher than the suggested range (1-6 mcg/mL), the median trough lopinavir level was 12.7 mcg/mL. The same trend was observed for atazanavir (10 pts) (median trough level 1.3 mcg/mL). Raltegravir, darunavir, amprenavir and nevirapine showed a high interpatient variability.

Conclusions: At the time of selection of antiretroviral therapy and drug therapy for neurological or psychiatric disease, it is necessary to evaluate the possible interactions, adherence and, if the case, dose adjustment. Our preliminary data, although limited, suggest the usefulness of therapeutic drug monitoring not only for ARVs, but also for psychotropic and antiepileptic medications, when given in combination. It is important to address all issues related to mental and physical health of patients in order to avoid over or underdosing of drugs, without compromising the quality of life but also guaranteeing adherence to therapy.

No conflict of interest

Abstract: P_08

Drug Drug Interactions

Effect of PH and acid-reducing agents on atazanavir intestinal permeability

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Background: Oral bioavailability of the HIV protease inhibitor, atazanavir, is highly sensitive to food intake and co-administration of acid-reducing agents such as proton-pump inhibitors (e.g., omeprazole) and H_2-receptor antagonists (e.g., cimetidine). These interactions may be mediated by changes in the gastrointestinal pH; however, to date little is known about the pH-dependence of atazanavir intestinal absorption or contribution of other mechanisms, such as intestinal drug transport or metabolism, to these interactions.

Methods: *In vitro* intestinal permeability (P_{app}) of ^{3}H-labeled atazanavir across Caco-2 cell monolayers grown on Transwell membrane inserts was examined at different luminal pH (apical pH varied from pH4.5 to pH8.5), in the presence or absence of acid-reducing agents, or in the buffered solution simulating intestinal fluid in the fed state (pH5.0, 15 mM sodium taurocholate, 3.75 mM lecithin) or fasted state (pH6.5, 3 mM sodium taurocholate, 0.75 mM egg lecithin). *In situ* permeability (P_{eff}) of atazanavir was assessed by open-loop single-pass perfusion of rat jejunum and ileum segments to further examine the effect of luminal pH, food intake, and acid-reducing agents on atazanavir intestinal absorption.

Results: In Caco-2 cells, intracellular accumulation of atazanavir after 30 min incubation was two-fold higher at acidic pH5.5 compared to neutral pH7.4 (p<0.01). Similarly, the apical-to-basolateral permeability of atazanavir across Caco-2 cell monolayers was also significantly higher at acidic apical pH compared to neutral pH (p<0.05), indicative of an enhancement in atazanavir intestinal absorption under acidic conditions. Atazanavir permeability across Caco-2 cell monolayers was also significantly higher in fed-state compared to fasted-state buffer, in agreement with the enhanced absorption of atazanavir when taken with food. *In situ* permeability of atazanavir in the jejunum and ileum was also higher at acidic luminal pH compared to neutral or basic pH, observing on average 2.7 and 2.3-fold increase in the steady-state permeability of atazanavir in the jejunum and ileum, respectively, when the pH of the luminal fluid was gradually increased from pH4.5 to pH8.5 in each animal. Similarly, *in situ* permeability of atazanavir measured in fed-state buffer was higher compared to fasted-state buffer. Omeprazole and several other proton-pump inhibitors were found to interfere with the efflux transport of atazanavir in Caco-2 cells. However, this effect was not observed *in situ*, suggesting that clinical drug-drug interactions between omeprazole and atazanavir are pH-mediated.

Conclusions: Our *in vitro* and *in situ* data demonstrate that atazanavir intestinal permeability is strongly dependent on the pH of the intestinal lumen. Changes in the gastrointestinal pH which occur after food intake (i.e., acidification) may explain the increase in atazanavir absorption when taken with food. Similarly, the alkalinization of the gastric and intestinal fluid induced by acid-reducing agents (i.e., omeprazole, cimetidine) may explain the drastic reduction in atazanavir plasma concentrations when it is co-administered with acid-reducing agents. Even though some proton-pump inhibitors such as omeprazole can also inhibit atazanavir efflux *in vitro*, their effect on pH of the gastrointestinal fluid appears to be responsible for the clinical interactions between atazanavir and acid-reducing agents.

This study is supported by the Canadian Foundation for AIDS Research.

No conflict of interest

Abstract: P_09

Novel Drugs and Formulations

Continuous intravenous infusion of enfuvirtide in an out-clinic patient with a multiresistant HIV strain and severe injection-site reactions

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Introduction: A 70 year-old out-clinic patient with highly resistant HIV treated with cART containing enfuvirtide (Fuzeon®), was not able to continue this treatment due to severe painful subcutaneous (s.c.) swellings at the injection sites, which is known to be a frequent side effect of enfuvirtide limiting its clinical use. As there were no options to switch enfuvirtide to another antiretroviral agent due to extensive viral resistance, we explored alternative routes of administration of enfuvirtide.

Methods: A review of literature showed that continuous administration of the total daily dose of 180 mg intravenously (i.v.) through a tunneled central venous catheter was the best treatment option based on pharmacokinetic/pharmacodynamic considerations. Enfuvirtide was aseptically prepared in an elastomeric pump (Accufuser® 5 ml/h, 275 ml, 180 mg enfuvirtide/24 hours). Stability of enfuvirtide in the elastomeric pump was tested. Before and after switching from s.c. to i.v. administration, multiple plasma samples were drawn to study the pharmacokinetics of enfuvirtide. All samples were quantified by an LC-fluorescence method. Subsequently, viral load and clinical condition of patient were monitored frequently.

Results: After 7 days (2-8°C) and subsequent 24 hours after starting the pump at room temperature, no significant decline was seen in enfuvirtide concentration in the elastomeric pump. Continuous i.v. administration of 180 mg enfuvirtide daily resulted in therapeutic plasma concentrations (3519 ng/ml) that were around 4 times higher than $C_{through}$ levels in this patient during s.c. use (755 ng/ml). The reached plasma concentration exceeded the recently proposed minimally effective concentration of 2100 ng/ml. During the first two months of follow up, patient remained clinically stable, with undetectable viral load and raising CD4+lymphocyte count.

Conclusions: Continuous i.v. therapy with enfuvirtide proved efficacious and safe in an HIV patient with extensive resistance mutations, who did not tolerate subcutaneous injections of enfuvirtide. Therefore, it may serve as an alternative route of administration of enfuvirtide in selected HIV patients.

No conflict of interest

Abstract: P_10

Novel Drugs and Formulations

Bioequivalence of a darunavir/cobicistat fixed-dose combination tablet (FDC) versus single agents in healthy volunteers

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Background: Low-dose ritonavir is used to boost darunavir, and cobicistat is a potential alternative booster. Compared with darunavir/ritonavir (800/100mg), previous studies showed comparable darunavir pharmacokinetics for darunavir/cobicistat (800/150mg) as either single agents (Mathias et al. IWCPHIV 2010; abstract 28), or as an FDC (Kakuda et al. IWCPHIV 2012; abstract O20). In this study, the bioequivalence of a darunavir/cobicistat FDC versus single agents was assessed.

Methods: This was a phase I, open-label, randomized, 3-panel, crossover study in healthy volunteers (TMC114IFD1003; NCT01619527). Volunteers received a single dose of darunavir/cobicistat (800/150mg) as an FDC versus darunavir (2x400mg tablets) and cobicistat (150mg tablet) under fasted (panel 1; N=74) or fed (panel 2; N=40) conditions, or as an FDC under fasted versus fed (high-fat breakfast) conditions (panel 3; N=19), with a ≥7 day washout between treatments. Darunavir and cobicistat plasma concentrations were measured over 72 hours using validated high performance liquid chromatography with tandem mass spectrometry methods. Pharmacokinetic parameters were analyzed using non-compartmental analysis (WinNonlin) and tested using the least square means (LSM) ratio and 90% confidence intervals (CI). Safety assessments were made until 7–10 days after the last drug intake.

Results: The majority of volunteers were white (95–100%) and male (54–58%) in each panel. Median age (43–46 years) and body mass index (25.1–25.2kg/m^2) were similar across panels. Darunavir C_{max} and AUC_{∞} were bioequivalent between the FDC versus single agents: LSM ratios (90% CIs) were 98.59% (93.72–103.73) and 96.00% (90.30–102.07), respectively, for fasted conditions (panel 1), and 96.76% (93.06–100.60) and 97.81% (92.81–103.05), respectively, for fed conditions (panel 2). For cobicistat, C_{max} and AUC_{∞} were also bioequivalent between the FDC versus single agents, under fasted and fed conditions, as the 90% CIs were all within 80.00–125.00%. Median t_{max} for darunavir was comparable between the FDC (3.00h [fasted]; 4.03h [fed]) and single agents (3.00h [fasted]; 4.00h [fed]), as was the t_{max} for cobicistat. Food significantly increased darunavir C_{max} (LSM ratio [90% CI]: 227.09% [205.75–250.63]) and AUC_{∞} (170.20% [148.50–195.06]) compared with fasted conditions (panel 3), and prolonged t_{max} (4.50h [fed]; 3.00h [fasted]). Cobicistat C_{max} and AUC_{∞} were not affected by food intake as the 90% CIs were all within 80.00–125.00%, although t_{max} was longer following food intake (4.98h [fed]; 2.00h [fasted]). No volunteers discontinued the study due to adverse events (AEs). All AEs were grade 1 or 2 in severity. Overall, 64 (48%) volunteers had ≥1 AE, and 27 (20%) and 26 (20%) volunteers had ≥1AE at least possibly related to darunavir and cobicistat, respectively. The most frequent AEs occurring in >5% of volunteers (n [%]) were headache (40 [30]), muscle spasms (12 [9]), and diarrhea (11 [8]).

Conclusions: Bioequivalence of the darunavir/cobicistat (800/150mg) FDC was demonstrated versus darunavir/cobicistat administered as single agents under fasted and fed conditions. Darunavir, but not cobicistat, exposure was increased by food compared with fasted conditions. The FDC of darunavir/cobicistat and the single agents were generally safe and well tolerated.

Conflict of interest
Employee of Janssen.

Abstract: P_11

Novel Drugs and Formulations

Solid drug nanoparticles of efavirenz, prediction of bioavailability from physiochemical characteristics and excipient choice

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Introduction: Formation of solid drug nanoparticles (SDNs) has potential to increase bioavailability and improve pharmacokinetics (PK) of antiretroviral drugs. Efavirenz (EFV) is characterised by low aqueous solubility and bioavailability and EFV SDN options with increased intestinal permeation may have application for dose reduction or treatment of special populations. The aim of this work was to assess how nanoparticle characteristics relate to intestinal permeability of EFV SDNs.

Methods: 146 10% drug-loaded EFV SDNs were prepared by emulsion-templated freeze-drying. Excipients included various combinations of polyvinyl alcohol, α-tocopherol polyethylene glycol succinate, hydrolysed gelatine, hydroxypropyl methylcellulose, sodium alginate, sisterna 16, sodium dodecyl sulfate, polyvinyl pyrrolidone 30k. Particle size, polydispersity and zeta potential were determined by dynamic light scattering. Apparent permeability (P_{app}) of SDNs was determined across Caco-2 monolayer relative to an aqueous solution (<0.5% DMSO). Constant of absorption (ka) was predicted using a semi-mechanistic *in vitro in vivo* extrapolation model. The effect of physiochemical characteristics and excipients on P_{app} and predicted ka was assessed with multivariate linear regression. All data are given as mean ± SD.

Results: 93% of SDNs had higher P_{app} than an EFV solution**.** Surfactant content was related to P_{app} and predicted ka. SDNs with higher surfactant content had lower P_{app} ($1.7 \times 10^{-5} \pm 0.4 \times 10^{-6}$ cm/s) and predicted ka (0.75 ± 0.13 hr^{-1}) compared to SDNs with lower content ($2.3 \times 10^{-5} \pm 0.6 \times 10^{-6}$ cm/s and 0.9 ± 0.16 hr^{-1}; $p<0.05$). In regression for SDNs with low surfactant, particle diameter (β = .141, p = 0.12), hydrolysed gelatine (β = .002, p = 0.002) and polyvinyl pyrrolidone 30k (β = .002, p = 0.24) were correlated with predicted ka. For SDNs with high surfactant, particle diameter (β = .136, p = 0.002), zeta potential (β = -0.001, p = 0.001), hydroxypropyl methylcellulose (β = -0.003, p = 0.0001), sodium alginate (β = -0.007, p = 0.006) and sisterna 16 (β = -0.006, p = 0.027) were correlated with predicted ka.

Discussion: EFV SDNs with improved intestinal P_{app} compared to EFV aqueous solution have been generated. Surfactant content, nanoparticle size and excipient choice all influenced EFV predicted Ka. Understanding how excipients and nanoparticle characteristics influence biological phenotypes will enable future rational design of SDNs with desirable pharmacological properties.

No conflict of interest

Abstract: P_12

Pharmacogenetics

Effect of drug metabolizing enzyme polymorphisms on dolutegravir pharmacokinetics: Meta-analysis across phase 1 studies

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Background: Dolutegravir (DTG) is an investigational HIV integrase inhibitor in late-stage development. DTG is primarily metabolized via UDP-glucuronosyltransferase (UGT)1A1 with a minor component of cytochrome P450 (CYP)3A. This pharmacogenetic (PGx) study was undertaken to evaluate the effect of UGT1A1 and CYP3A polymorphisms (including functional variants in the pregnane X receptor gene (PXR/NR1I2) which may influence CYP3A4 expression) on exposure in subjects receiving DTG.

Materials & Methods: Data was derived from eight phase I and one phase IIa clinical studies, where subjects received repeat DTG 50mg once daily dosing, had intensive PK assessments, and used the tablet formulation. Samples were collected from subjects who consented to participation in PGx research during each study and were analyzed for genetic markers of UGT1A1, CYP3A4, CYP3A5, and NR1I2. Pharmacokinetic (PK) parameters (CL/F, AUC(0-τ) and Cmax) of DTG were determined using non-compartmental methods. The effect of genetic variants on PK parameters was assessed

using analysis of covariance. Genetic variants were modeled on predicted functional activity (UGT1A1, CYP3A4, and CYP3A5) as well as on the number of reduced activity or minor alleles (for all genes).

Results: A total of 89 unique individuals with both genetic and PK data were included. The overall mean age was 36.9 years (SD=12.1), and the majority of subjects were male (n=78, 88%). Similar numbers of African American (n=37, 42%) and White (n=46, 52%) subjects were included. For UGT1A1, 46%, 45%, and 8% of subjects had genotypes conferring normal metabolic status (*1/*1, *1/*36), reduced metabolic status (*1/*6, *1/*28, *1/*37, *28/*36, *36/*37), and low metabolic status (*28/*28, *28/*37), respectively. One subject had an unknown status. CL/F decreased 23%, AUC(0-τ) and Cmax increased 31% and 22%, respectively, in subjects with low and reduced UGT1A1 activity compared to subjects with normal UGT1A1 activity. DTG CL/F, AUC and Cmax were similar between CYP3A4 and CYP3A5 functional groups (low, reduced, and normal metabolizers).

Conclusions: Subjects with genotypes conferring poor and reduced metabolizer status of UGT1A1 had modestly increased exposures of DTG. The increases in DTG exposure due to UGT1A1 polymorphisms are not considered clinically significant and no dose adjustment is necessary. Polymorphisms in CYP3A4, CYP3A5 and NR1I2 were not associated with differences in the PK of DTG.

Conflict of interest
Employees of GlaxoSmithKline

Abstract: P_13

Pharmacogenetics

Genotype frequencies of pregnane X receptor (PXR 63396C>T)in the Serbian population

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Introduction: The pregnane X receptor (PXR; *NR1I2*) regulates the expression of CYP3A4 and ABCB1 in response to endobiotics and xenobiotics. Three polymorphisms (44477T>C *[rs1523130]*, 63396C>T *[rs2472677]*, and 69789A>G *[rs763645]*) within putative transcription factor binding sites of *PXR* regulatory regions have been shown to influence CYP3A4 and/or ABCB1 expression and activity in primary hepatocytes and liver. Furthermore, the T allele at position 63396 was associated with plasma concentrations of atazanavir. Little is known about the frequency of allelic variants of *PXR* in the Serbian population. Thus, the aim of this study was to identify genotype frequencies of *PXR* 63396C>T in a cohort of patients recruited in Belgrade, Serbia.

Materials & Methods: Whole blood samples was taken from HIV-infected patients at the The HIV/AIDS Center, Institute of Infectious and Tropical Disease 'Dr Kosta Todorovic', School of Medicine, University of Belgrade. All subjects were unrelated Serbian Caucasians from Belgrade and Central Serbia. Ethical approval was granted and written consent was obtained from all patients. Genomic DNA was extracted from whole blood and quantified using standard methods. Genotyping for

63396C>T was carried out by PCR-based allelic discrimination at the Department of Molecular and Clinical Pharmacology, University of Liverpool, UK. Difference in genotype and allele frequencies in Serbian patients compared with other Caucasian populations was then assessed by chi square test.

Results: A total of 79 Caucasians were included in the analysis. Of these, 79 patients (75.95%) were male, the median age was 40 years (IQR, 34 – 48 years), and the median body mass index was 23.1 (IQR, 21.0 – 24.5). 16 (20.3%) patients were homozygous for the C allele, 34 (43.0%) patients were heterozygous and 29 (36.7%) patients were homozygous for the T allele. There was no statistically significant difference between allele frequency (p = 0.53; OR = 1.1) for *PXR* 63396C>T frequency between the Serbian and previously reported Caucasian German patient populations (Wyen *et al*. *J Antimicrob Chemother*. 2011. 66(9):2092-8).

Conclusions: Understanding differences in frequency between populations can contribute to the better understanding of molecular basis of the Serbian patient population in drug response.

No conflict of interest

Abstract: P_14

Pharmacokinetics for Pediatrics, Pregnancy and other Special Populations

Bilirubin as a surrogate marker for atazanavir plasma concentrations in a paediatric population

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Background: Atazanavir dose recommendations have not been extensively studied in paediatrics and present dosing may result in inadequate plasma concentrations. In adults, a positive association between bilirubin and atazanavir plasma levels has been shown. We described paediatric atazanavir dosing and investigated plasma bilirubin concentration as a possible surrogate for atazanavir therapeutic drug monitoring (TDM).

Materials & Methods: TDM data of patients ≤18 years were retrospectively analysed between Jan/2004-Jul/2011. Atazanavir minimum concentrations (Cmin) were estimated using a half-life of 10.10 and 5.58 hours with and without ritonavir respectively, for levels drawn more than 4 hours post-dose. Cmin below 0.15 mg/L was considered subtherapeutic. Associations between dose-Cmin and bilirubin-Cmin were evaluated using linear regression and receiver-operator curve (ROC), respectively.

Results: Fifteen patients, 67% female, 93% black, underwent 35 TDMs. At the time of TDM, median age was 15 years (range 8-18), weight 49 kg (range 29-74), body surface area 1.47 m2 (range 1.00-1.88). Median atazanavir dose was 4.42 mg/kg (range 3.40-10.49) and 150 mg/m2 (range 125-299); 26 (74%) boosted with ritonavir. For TDMs of patients without ritonavir (n = 5), median Cmin was 0.05 mg/L (range 0-0.25); 60% of which were subtherapeutic. For those with ritonavir (n = 19), median Cmin was 0.79 mg/L (range 0-1.85); 11% of which were subtherapeutic. Cmin could not be evaluated in a total of 11 TDMs (9 TDMs done < 4 hours post-dose, 2 TDMs with unknown time post-dose). No association between Cmin and dose was found (mg/kg– p=0.81; mg/m2– p=0.75); similar results were obtained when adjusted for ritonavir use. Bilirubin concentrations of 18 µg/L were predictive of Cmin equal to or above 0.15 mg/L with 95% sensitivity and 80% specificity (ROC area under the curve 0.91; 95%CI 0.76; 1.00).

Conclusions: Though atazanavir dose is not predictive of Cmin, bilirubin may be a surrogate marker for atazanavir Cmin in paediatric patients. These results should be confirmed in prospective studies.

Modified abstract will also be presented at CAHR (Canadian Association of HIV Research), Vancouver, April 11-14, 2013.

Conflict of interest
Alison Wong- Received honoraria for consultation (Janssen) and speaking engagement(Merck).
Jason C Brophy- Investigator-initiated research grant from Abbott .Charles J.L. la Porte- Employee at Janssen Pharmaceuticals, The Netherlands.

Abstract: P_15

Pharmacokinetics for Pediatrics, Pregnancy and other Special Populations

Population pharmacokinetics of twice daily zidovudine (ZDV) in HIV-infected children and an assessment of ZDV exposure following WHO dosing guidelines

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Background: Zidovudine (ZDV) is a common component of antiretroviral treatment for HIV in children but limited pharmacokinetic (PK) data are available on the widely applied twice daily regimen. WHO guidelines aim to provide a simplified dosing approach for children based on weight bands. In 2010, the WHO increased ZDV doses in several weight bands compared to the 2006 guidelines. Our aim was to develop a population PK model to describe ZDV plasma concentrations in HIV-infected children and to estimate the ZDV exposures obtained following WHO guidelines.

Materials & Methods: Rich and sparse ZDV plasma concentrations were combined from six paediatric clinical trials. A total of 773 concentrations were available from 100 children (n=63 girls; n=58 African, n=38 Thai; n=4 other) stable on ZDV tablets (n=54 PK profiles) or syrup (n=73 PK profiles), dosed according to WHO or National guidelines. Median (range) ZDV dose was 150mg (60-300), 9mg/kg (5.1-24.0), 214mg/m^2 (144-559). Median age, weight, serum creatinine (SCr), and haemoglobin (Hb) were 5yr (1-18), 18kg (6-59), 0.34mg/dL (0.2-0.8) and 11.6g/dL (6.3-14.5), respectively. Nonlinear mixed effects modelling (NONMEM v.7.2) was applied to estimate ZDV population PK parameters and parameter variability. The following patient characteristics that could potentially influence ZDV PK parameters were evaluated for inclusion in the model: age, weight, sex, ethnicity, SCr and Hb. Validity of the final model was assessed using visual predictive check. Comparison of ZDV exposures based on WHO 2006 and 2010 dosing guidelines were performed using Monte Carlo simulations.

Results: ZDV PK was described by a 2-compartment model with first-order (tablet; k_a 3.0h^{-1}) or zero-order (syrup; D2 0.7h) absorption. Including bodyweight on oral clearance (CL/F, Q/F) and volume of distribution parameters (Vc/F, Vp/F) using allometric scaling significantly improved the model fit. A positive linear relationship was observed between ZDV CL/F and age. ZDV CL/F, Q/F, Vc/F and Vp/F were 62, 7L/h/18.3 kg and 66, 53L/18.3 kg, respectively. Overall variability in ZDV CL/F was 49%. Using the final model, 91% of observed ZDV concentrations were within the 90% prediction interval for simulated concentrations.
Mean ZDV AUC_{0-12} was 25% higher compared to published adult data (2.81 *vs*. 2.24mg.h/L). Simulated mean AUC_{0-12} for WHO 2010 guidelines were higher than those for 2006 guidelines in the majority of weight bands due to increased doses (2006: 2.10-3.13mg.h/L *vs.* 2010: 2.52-3.55mg.h/L). The percentage of simulated ZDV AUC_{0-12} below the average adult exposure was 19-50% for 2010 guidelines compared to 25-68% for 2006 guidelines. Stratifying simulated ZDV AUC_{0-12} in children according to arbitrary low and high thresholds of half and double the average adult exposure (1.12 and 4.48mg.h/L, respectively) resulted in 0-7% and 0-3% of ZDV AUC_{0-12} below 1.12mg.h/L and 3-16% and 6-25%

above 4.48mg.h/L for 2006 and 2010 guidelines, respectively.

Conclusions: Mean ZDV exposure was higher in children than adults. Given the absence of a toxicity threshold for ZDV, information on safety and tolerability are needed with increased ZDV doses following WHO 2010 guidelines. Furthermore, the impact on intracellular ZDV triphosphate requires investigation; however, predicted plasma ZDV exposures are reassuring.

No conflict of interest

Abstract: P_16

Pharmacokinetics for Pediatrics, Pregnancy and other Special Populations

Transplacental passage of atazanavir and neonatal hyperbilirubinaemia

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Introduction: The transplacental passage of antiretrovirals (ARVs) including Atazanavir (ATV) is well documented however the extent of this transfer and its effects on the neonate are poorly understood. ATV is known to cause elevated bilirubin levels in adults and so could contribute to hyperbilirubinaemia in neonates exposed to the drug *in utero*. The aim of this study is to evaluate the transplacental diffusion of ATV and determine the incidence of hyperbilirubinaemia in the neonate.

Material & Methods: Fetal cord blood of 16 infants (15 pregnancies - 14 singleton and a twin pair) exposed to ATV *in utero* were assessed for both ATV and bilirubin concentrations. These were compared to maternal levels at time of delivery. In addition neonatal bilirubin levels in the first 24hrs of life were collected. Demographic and birth outcome data were gathered.

Results: 6 women were on ATV prior to conception while 9 commenced treatment between 19 – 33 wks gestation (median 15 wks, range 13-18.4). 13/15 women achieved HIV RNA <50 cpm at time of delivery. Mean birth weight was 3.3kg [range 2.0-4.7]; 2 babies were pre-term (twins at 35 wks); the remainder were > 37 wks gestation.
Maternal and cord ATV concentrations were available for 15 mother-baby pairs. Considerable variations in maternal ATV concentrations at delivery were noted and were most likely attributable to the differences in time since last dose. The median ATV concentration was 1250 ng/ml [range <48-3441], with 13/15 greater than the MEC of 150ng/ml. Detectable ATV levels were present in 12/15 cord samples (median 223 ng/ml [range <48-531]), 8/12 were greater than the MEC 150ng/ml, 3 were borderline. Linear regression analysis showed a significant correlation between maternal blood and cord blood ATV concentrations (R^2 = 0.632, P<0.001). The mean ratio of maternal blood concentration to cord blood concentration was 0.14 (95% CI 0.08 -0.20).
The median maternal serum total bilirubin concentration at delivery was 23.5mmol/ml [range 6-102]; median cord blood total bilirubin concentration was 34mmol/ml [range 15-89] and median neonatal total bilirubin concentration was 60mmol/ml [range 19-146]. Excluding 1 infant with an indirect bilirubin level of 146mmol/ml at 27 hours age, a significant correlation was noted between neonatal unconjugated bilirubin concentration and both maternal serum unconjugated bilirubin (R^2 = 0.693, P=0.02) and cord unconjugated bilirubin concentrations (R^2 = 0.759, P=<0.001). There was no correlation between ATV level and bilirubin concentration. No cases of hyperbilirubinaemia were noted.

Conclusions: Transplacental transfer of ATV may offer additional protection to the neonate against mother-to-child transmission of HIV, with therapeutic levels observed in the majority of cord blood samples here. Although no cases of hyperbilirubinaemia were observed in this small cohort, further study into the effects of ATV on the fetus and neonate are needed.

No conflict of interest

Abstract: P_17

Pharmacokinetics for Pediatrics, Pregnancy and other Special Populations

Rilpivirine concentrations in seminal plasma in HIV-infected patients

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Background: HIV may reside outside the systemic circulation in anatomical sanctuary sites, including seminal plasma (SP). Current evidence suggests that SP penetration of antiretrovirals (ARVs) may suppress viral replication in specific compartments, perhaps reducing both sexual transmission of HIV and the local reservoir of resistant virus. A greater understanding of ARV pharmacokinetics in SP may influence ARV selection particularly in serodiscordant couples wishing to conceive. In order to gain better understanding of ARV penetration into anatomical sanctuary sites we need sensitive and specific analytical methods. Here we describe a UPLC-MS/MS method for the quantification of rilpivirine in SP and show its application to determine rilpivirine SP concentrations from a small sub study cohort from *The Rilpivirine Cerebrospinal-fluid Study* [NCT01562886].

Materials and Methods: Eight HIV-infected adult males with a plasma HIV RNA <50 copies/mL and receiving a stable ARV regimen comprising of tenofovir, emtricitabine plus nevirapine were recruited. Nevirapine was switched to rilpivirine (25 mg daily) for 60 days. On day 59, paired plasma and semen samples were collected 24hr post dose. Plasma samples were extracted via protein-precipitation (100 µL sample, 100 µL 0.1% formic acid, and 500 µL acetonitrile). SP samples underwent liquid-liquid extraction (3 mL of hexane/ethyl acetate [80:20], evaporated to dryness and reconstituted in 100 µL of 5:1 acetonitrile:water + 0.1% formic acid). The internal standard [RPV-d4] was included in the sample extraction procedures to correct for any variation in extraction efficiency and ion suppression effects. All samples were analysed by UPLC-MS/MS. Gradient chromatographic separation on a reverse-phase Fortis™ C18 column, with detection by selective reaction monitoring in positive mode was applied. Accuracy of the methodology was monitored using quality controls at low, medium and high points on the calibration curves.

Results: Rilpivirine eluted within a 10 minute run time. Calibration curves were validated over the following concentration ranges; plasma = 5-5000 ng/mL, SP = 0.5-200 ng/mL (r^2 values >0.99; quadratic 1/x). Inter-assay variation ranged between 4.2-7.1% (plasma), 3.6-6.3% (SP) for precision and 1.7-12% (plasma), -8.03-12.5% (SP) for accuracy. Carryover was 0.03% of the lower limit of quantification. Recovery for rilpivirine was 76-81% (plasma) and 103-106% (SP).
Both plasma and SP samples were analysed in duplicate. On day 59, the rilpivirine concentration geometric means (range) were; SP = 4.9 ng/mL (2.4-9.0), plasma = 51.7 ng/mL (31.6-91.1). The geometric mean ratio for SP:plasma was 0.10 (0.05-0.21). SP and plasma concentrations were above the published EC_{50} and EC_{90} values for rilpivirine against wild-type virus (0.27 and 1.35 ng/mL) in all 8 patients.

Conclusions: Here we describe a bioanalytical method to assess rilpivirine concentrations in SP and plasma in HIV-infected subjects. We found rilpivirine SP:Plasma ratio to be similar to those reported for other NNRTIs which are highly protein bound (etravirine = 0.16, efavirenz = 0.09). The rilpivirine geometric mean ratio in SP (0.1) was greater than the plasma free fraction (rilpivirine is >99% bound). Although we do not have data on the free rilpivirine concentration in SP, the total concentration is more than 4 times above the EC_{90} value against laboratory strains of wild-type HIV-1.

No conflict of interest

Abstract: P_18

Pharmacokinetics for Pediatrics, Pregnancy and other Special Populations

Population pharmacokinetics of efavirenz in pediatric patients to inform dosing in children ≥ 3 months of age

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Background: In children and adolescents (aged ≥3 years), the current recommended efavirenz (EFV) dose is based on body weight, and ranges from 200 mg once-daily (QD) for children weighing 10 – <15 kg, to 600 mg QD for children weighing ≥40 kg, in the US. Due to limited safety and efficacy data, EFV is not currently recommended for children aged <3 years or <10 kg. The aim of this analysis was to use a population pharmacokinetic (PPK) model to assess appropriate dosing regimens for this pediatric group.

Materials and Methods: The PPK model was developed with 3,289 concentration values from 168 pediatric patients (aged 3 months–21 years [57 patients under 3 years of age]; weight at dosing: 3.3–117 kg; studies PACTG382, PACTG1021, AI266922). To enhance model stability, 1,232 concentration values from 24 healthy adults (AI266059) were included. Pediatric trials contributed 88% of subjects and 73% of observations in the analysis set. Model parameters were estimated using the NONMEM software program. A two-compartment model with first-order absorption and first-order elimination was used as the base model. The full model (including pre-specified covariates, e.g. age, weight, gender, race) underwent backward elimination to identify a parsimonious final model containing statistically significant covariates. The final model was used to simulate steady-state EFV exposures (N=1000 PK parameter sets, 100 pediatric subjects per weight category) for various dose regimens of EFV capsule-sprinkle and capsule formulations to identify dose regimens that produced comparable exposure between pediatric patients weighing <10 kg and ≥10 kg. The criteria used were target steady-state AUC levels of 190–380 μM•h and steady-state C_{max} and C_{min} of 5.2–8.2 and 1.9–2.9 μg/mL, respectively (80–125% of the reference C_{max} and C_{min} values from children weighing 10 – <15 kg).

Results: The steady-state PK of EFV in pediatric HIV patients were well described by a linear two-compartment model with first-order absorption. Weight was identified as a clinically meaningful covariate on EFV clearance, central volume and rate of absorption; age was not significant in the presence of weight. PPK simulation results suggested that the following EFV dosages (according to weight bands) produce comparable exposure to that of children ≥10 kg who received the current approved dosage: 200 mg QD (≥7.5 – <10 kg); 150 mg QD (≥5 – <7.5 kg); and 100 mg QD (≥2.5 – <5 kg). These doses produced a median AUC within the target range. Simulation results for C_{max} and C_{min} supported the weight-band determined doses above.

Conclusions: Steady state EFV concentration-time profiles in pediatric HIV-infected populations were adequately described by a linear two-compartment PK model with first-order absorption. Body weight was found to affect EFV clearance, central volume and rate of absorption. Model-based simulation was employed to determine weight-tiered EFV QD doses (capsules or capsule sprinkles) that are projected to provide AUC, C_{max}, and C_{min} for pediatric HIV-infected patients weighing ≥2.5 – <10 kg that were within target ranges. Additionally, the simulation results confirm the current QD dosing recommendations for EFV (capsule or capsule-sprinkle formulations) for pediatric patients weighing ≥10 kg (≥3 years).

Conflict of interest
Employee of BMS

Abstract: P_19

Pharmacokinetics for Pediatrics, Pregnancy and other Special Populations

Darunavir/ritonavir once daily total and unbound plasmatic concentrations in HIV-infected pregnant women

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Background: Pregnant women undergo physiological changes that can affect antiretroviral drugs pharmacokinetics (PK), especially drug distribution and union to plasmatic proteins, leading to lower protease inhibitor exposure. Ritonavir-boosted Darunavir(DRV/r) starts to be used in pregnant women thanks to its efficacy and safety, but PK data are scarce. Unbound (active) rather than total concentrations may be more reliable in these patients, but there is no data on DRV unbound concentrations when given once-daily in pregnant women.

Methods: HIV-infected women receiving treatment with DRV/r 800/100 mg once-daily. A complete steady-state12-hour PK study was performed during the third trimester and post-partum, with pre-dose and 1,2,3,4,6,8 and 12 hours after dose samples. Drug concentrations were determined by mass spectrometry. AUC and CL/F were assessed by a non-compartmental model and linear/log trapezoidal rule with WinNonlin 3.3. Descriptive values are expressed as median (interquartile range) and geometric means (GM). Geometric mean ratios (GMR) have been used to compare concentrations between periods.

Results: Eight patients were included, with 5 patients having data in both periods. Baseline (at third trimester) characteristics were: age 32 (28-35) years, race 4 black and 4 caucasian, BMI 27.3 (25.1-36.9) kg/m^2, CD4 511 (230-576) cel/mm^3, all patients had undetectable viral load that was maintained throughout pregnancy. One patient had HBV co-infection and none had positive HCV. All patients were receiving tenofovir, emtricitabine and DRV/r. Median pregnancy duration at PK study was of 31 (26-32) weeks. Post-partum determination was performed after 12 weeks.

Total Darunavir PK parameters (GM) during third trimester and post-partum were: Ctrough 832 and 1549 ng/mL (GMR 0.53 [90%CI 0.19-1.52]), Cmax 5888 and 8128 ng/mL (GMR 0.73 [90%CI 0.41-1.32]) and AUC0-24 48978 and 72444 ng·h/mL (GMR 0.69 [90%CI 0.35-1.35]). Median (IQR) Tmax were 3 (2-4) and 1 (1-3) hours and oral clearance 15 (11-25) and 9 (6-25) L/h, during third trimester and post-partum.

Unbound Darunavir PK parameters (GM) during third trimester and post-partum were: Ctrough 66 and 87 ng/mL (GMR 0.76 [90%CI 0.26-2.25]), Cmax 813 and 1148 ng/mL (GMR 0.71 [90%CI 0.44-1.16]) and AUC0-24 5623 and 7413 ng·h/mL (GMR 0.76 [90%CI 0.44-1.32]). Median (IQR) Tmax were 3 (2-4) and 1 (1-2) hours and oral clearance 140 (116-214) and 88 (73-182) L/h, during third trimester and post-partum.

There were no side effects during pregnancy and all neonates were on-term and healthy.

Conclusions: although there was a trend towards lower total and unbound DRV concentrations during the third trimester, total DRV was well above the IC_{50} for wild-type HIV when given 800/100 mg once-daily. Thus, dose adjustments or twice-daily dosing probably would not be necessary.

No conflict of interest

Abstract: P_20

Pharmacokinetics for Pediatrics, Pregnancy and other Special Populations

Atazanavir total and unbound plasmatic concentrations in HIV-HCV coinfected patients with hepatic cirrhosis

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Background: Ritonavir-boosted Atazanavir(ATV/r) is mainly metabolized in the liver. Hepatic cirrhosis can impair liver function and decrease plasmatic proteins, potentially modifying ATV/r pharmacokinetics (PK). Unbound rather than total concentrations may be more reliable in these patients, but there is scarce data on ATV unbound concentrations.

Methods: HIV patients with compensated cirrhosis (cases) and HIV controls without hepatic disease were included. A complete steady-state12-hour PK study was performed, with pre-dose and 1,2,3,4,6,8 and 12 hours after dose samples. Drug concentrations were determined by mass spectrometry. Descriptive values are expressed as median (range or IQR).

Results: 11 patients were included, 8 cases (3 ATV 300 mg, 3 ATV/r 300/100 mg and 2 ATV 400 mg) and 3 controls (ATV/r 300/100 mg). Baseline characteristics were: 82% men, age 45 (43-51) years, BMI 24.4 (21.7-28.2) kg/m^2, CD4 450 (344-609) cell/mm^3. No patients had had prior clinical decompensation, median MELD was 11 (10-12) and Child score was B in 1 case and A in 7. Median elastography values were 29.1 (19-66) kPa.

ATV PK parameters (median, range) are depicted in table 1.

ATV exposure was lower in patients with the non-boosted 300 mg dose and similar in the other 3 groups. There were no differences on ATV concentrations depending on tenofovir use. All patients had HIV-RNA<25 c/mL, except the ATV 300 mg patient with the lowest Cmin (total 60, unbound 5 ng/mL).

Conclusions: In patients with compensated cirrhosis, ATV/r total and unbound concentrations are similar to those observed in controls and in prior studies, and probably dose adjustments would not be necessary.

No conflict of interest

PK parameter		Cirrhosis ATV 300	Cirrhosis ATV 400	Cirrhosis ATV/r 300/100	Controls ATV/r 300/100
Cmin (ng/mL)	total	685 (60-706)	1260 (400-2121)	1405 (786-1602)	1061 (782-1422)
	unbound	23 (5-71)	84 (26-142)	79 (38-120)	92 (57-157)
Cmax (ng/mL)	total	4479 (2737-4649)	6153 (3429-8878)	2981 (1930-4670)	3256 (2696-4612)
	unbound	235 (220-366)	649 (649-940)	362 (283-362)	628 (530-813)
AUC 0-24h (ng•h/mL)	total	16696 (16696-51231)	69893 (37337-102448)	52336 (28197-55151)	48730 (36014-54999)
	unbound	2735 (181-2759)	5947 (4462-7432)	4354 (2249-7121)	6498 (3501-7037)
CL/F (L/h)	total	6.78 (5.85-17.97)	7.30 (3.9-10.71)	5.73 (5.44-10.64)	6.15 (5.45-8.33)
	unbound	109.67 (108.7-1652.7)	71.72 (53.81-89.63)	68.9 (42.12-133.39)	46.17 (42.63-85.68)
Unbound drug (%)		6.9 (3.8-6.9)	11.05 (7.4-14.7)	10.0 (9.6-14.8)	17.0 (15-212.6)

Abstract: P_21

Pharmacology of other viral diseases

Early ribavirin plasma concentrations as predictor of anemia onset after one month of anti-HCV therapy in ITPA stratified population

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Introduction: So far, Standard of care therapy for chronic hepatitis C (CHC) has been the combination of pegylated interferon-α (Peg-IFNα) and ribavirin (RBV). Today, new protease inhibitors for the treatment of HCV genotype 1 are available. However, there are several issues concerning efficacy and safety of the standard therapy which, with the new concomitant drugs, could become more important. One of main response-limiting factor is the discontinuation due to haemolytic anemia onset (it affects more than 30% of patients). This problem is addressed with the use of erythropoietin, increasing costs, or with RBV dose reduction, decreasing response rate.
It has been demonstrated that haemoglobin loss mainly depends on genetic factors, inosine triphosphatase (ITPA) single nucleotide polimorphisms (SNPs), and on RBV exposure. Nowadays there are not any early RBV concentration cut-off values defined, generically acknowledged, which are able to predict anemia.
In this study we investigated on this topic and determined early RBV concentration cut-off values, stratifying our sample according to ITPA genotype, over which there is a high risk of toxicity.

Materials and Methods: Our sample consisted in 168 naive CHC hepatitis patients, undergoing Peg-IFNα plus RBV treatment. Main inclusion criteria were no concomitant interacting drugs, no hepatic or renal function impairment, and self-reported adherence > 95%.
Blood sampling at the end of dosing interval (Ctrough) was performed at week 2 of therapy after written informed consent was given. Plasma RBV concentrations were measured using a validated HPLC-UV method, while patients genotyping for ITPA polimorphisms (rs6051702, rs7270101 and rs1127354) was performed using TaqMan Genotyping Array. Statistical Analysis was made with SPSS 18.0: statistical significance of the correlation between plasma RBV concentration and anemia occurrence (Hgb < 10 g/dL and/or Hgb decline > 3 g/dL after one month of therapy) were made using Spearman correlation test. Early Plasma RBV cut-off values were determined using Receiver Operating Characteristic (ROC) curve. 'P values' of at least 0.05 were assumed as significant.

Results: At 1 month of therapy, 63/168 (37.5%) patients developed anemia. Median RBV plasma concentration at 2 weeks observed was 1329 ng/mL IQR [923-1834]. Plasma RBV concentrations at 2 weeks resulted to be correlated with the onset of anemia ($p<0.001$). Considering the whole sample, a concentration over 1700 ng/mL (sensibility 53%; specificity 77%) was associated with a stronger risk of anemia ($p<0.001$).
Stratifying patients according to ITPA genotype, two cut-off values were determined: 1600 ng/mL for the group of patients not carrier of mutations (sensibility 50%; specificity 74%), and of 1900 ng/mL for patients with at least one mutated allele over the 3 SNPs (sensibility 45%; specificity 88%).

Conclusions: For the first time, early 'ITPA genotype-specific' cut-off values of plasma RBV concentration were determined to predict anemia after 1 month of treatment. These data show how the presence of at least one 'protective' mutation on ITPA gene confers resistance to anemia, and higher plasma RBV exposure is needed to develop toxicity.
With these cut-off values clinicians may provide and modulate therapy on the basis of the ITPA-genotype in order to obtain the greatest RBV exposure, reducing the onset of toxicity.

No conflict of interest

Abstract: P_22

PK-PD of Drug Efficacy and Toxicity

Darunavir TDM in treatment-experienced HIV infected patients receiving highly potent antiretroviral regimens: results from the PRIDE study

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Introduction: Darunavir (DRV) genotypic inhibitory quotient (GIQ) ≥ 2.15 mg/L/mutation, weighted mutation score GIQ (GIQ-WS) ≥ 0.6 mg/L/mutation point and virtual IQ (vIQ) ≥ 1.5 have been associated with virologic response in treatment-experienced patients. It is unclear which parameter should be used. The aim was to describe the pharmacokinetic/pharmacodynamic (PK/PD) parameters of DRV and virologic response in advanced HIV-1-infected patients receiving a potent optimized background regimen (OBR).

Material and Methods: Open-label, prospective study including HIV-1 infected consenting patients ≥ 18 years old with past virologic failure or intolerance to ≥ 3 antiretroviral (ARV) classes. All subjects had virologic failure at baseline. Subjects were started on DRV/ritonavir (600/100 mg) twice daily, plus an OBR that could include etravirine (ETV), raltegravir (RAL) and/or NRTIs. Virologic response was defined as a viral load of < 50 copies/mL at week 48. CD4+ T-cell count, adverse events and DRV, ETV and RAL trough concentrations (C_{12}) were collected at weeks 4, 12, 24 and 48. DRV GIQ_{all} and GIQ-WS_{all} were calculated as the ratio between the median of week 4, 12, 24 and 48 DRV C_{12} (C_{12-all}) and the number of DRV resistance associated mutations according to two different resistance algorithms, POWER and De Meyer weighted score, respectively. The DRV vIQ_{all} was calculated as C_{12-all} divided by the DRV fold-change in IC_{50} as per virtual phenotype multiplied by the protein-adjusted IC_{50} for resistant virus (0.55 mg/L). To estimate the potency of the regimens, the instantaneous inhibitory potential (IPP) for the combination of ARVs measured (IIP_{C12} DRV+ETV+RAL) was calculated assuming Bliss independence. DRV, ETV and RAL plasma concentrations were measured with a validated LC/MS/MS assay.

Results: Fourteen subjects were included: median age 51 years, 93% male, median time on ARVs 13 years, median number of previous PIs 4. All subjects had ≥ 2 active ARVs, with 78.6% of subjects having ≥ 3. The median DRV fold change was 1.45. In 64.3% of subjects both ETV and RAL were combined with DRV. An additional 28,6% received either ETV or RAL. NRTIs were taken by 50% of subjects. Virologic response was observed in 12 (85.7%) subjects. The median C_{12-all} of DRV, ETV, and RAL were 3.5, 0.58 and 0.071 mg/L, respectively. The DRV median VIQ_{all}, GIQ_{all} and GIQ-WS_{all} were 4.22 (IQR: 1.62-6.28), 2.55 mg/L/mutation (IQR: 1.87-3.26) and 0.91 mg/L/mutation point (IQR: 0.70-1.16). Literature-derived therapeutic targets for DRV vIQ_{all}, GIQ_{all}, and GIQ-WS_{all} were achieved in 78.6%, 66.6% and 83.3% of subjects, respectively. Subtherapeutic values were not associated with virologic failure. The two patients with detectable virus at week 48 (59 and 145 copies/mL) had therapeutic DRV parameters. The median IIP_{C12} (DRV+ETV+RAL) was 13.4 (range 7.67-18.0).

Conclusions: Among patients receiving DRV-based regimens containing ≥ 2 active drugs, subtherapeutic DRV PK/PD parameters were not associated with virologic failure. Combining DRV, ETV and RAL provides an IIP for the combination above 7, point at which viral replication is completely halted in vitro. Therapeutic drug monitoring of DRV might not be required in patients also receiving ETV and/or RAL.

No conflict of interest

Abstract: P_23

PK-PD of Drug Efficacy and Toxicity

Feasibility of nevirapine monitoring in saliva samples from HIV-1 infected Ugandan adults

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Background: In resource-poor settings where access to HIV viral load testing is limited, therapeutic drug monitoring (TDM) could serve as a tool to support adherence to antiretroviral therapy (ART). Nevirapine (NVP) is widely used in resource-poor settings and measurement of NVP concentrations in saliva has been proposed as an alternative non-invasive method for monitoring NVP systemic exposure. However, data are scarce evaluating therapeutic cut-offs for NVP in saliva. In Uganda, we evaluated the feasibility of monitoring NVP in saliva to identify patients with sub-therapeutic NVP plasma concentrations in plasma.

Materials and Methods: This was a cross-sectional pharmacokinetic sub-study of the PharmAccess African Studies to Evaluate Resistance (PASER) program. Paired plasma and stimulated saliva samples were obtained from 297 Ugandan, HIV-infected adults receiving NVP-based antiretroviral therapy. NVP concentrations were measured using a validated high performance liquid chromatography (HPLC) method at the Infectious Diseases Institute, Kampala (lower limit of quantification 0.05 mg/L). Plasma NVP concentrations <3.0 mg/L were considered subtherapeutic (US Department of Health and Human Services suggested threshold for TDM of NVP). We determined the negative and positive predicted values of different thresholds for subtherapeutic saliva concentrations of NVP for predicting values above or below the defined threshold for NVP in plasma.

Results: Median (IQR) age of participants was 39.1 (32.8-45.2) years. Three-hundred saliva and 287 plasma results were available for analysis. Median (IQR) NVP concentrations in saliva and plasma was 3.40 (2.59-4.47) mg/L and 6.12 (4.75-7.95) mg/L, respectively. A low proportion (5%, 15/287) of patients had a subtherapeutic NVP plasma concentration. The mean (coefficient of variation, %) NVP saliva-to-plasma ratio was 0.55 (62%) which was similar to results from previous studies. A cut-off value of NVP in saliva of 1.60 mg/L was associated with a negative and positive predicted value of 0.99 and 0.72, respectively and with sensitivity and specificity of 87% and 98%, respectively for predicting a subtherapeutic NVP plasma concentration.

Conclusions: Monitoring NVP concentrations in saliva using HPLC-method is feasible in a resource-limited setting. A cut-off value of NVP concentration in saliva of 1.60 mg/L has an acceptable negative and positive value plus sensitivity and specificity for predicting subtherapeutic NVP exposure.

No conflict of interest

Abstract: P_24

PK-PD of Drug Efficacy and Toxicity

Could ritonavir intracellular concentrations have a role as active drugs when used as a booster PIs?

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Introduction: Ritonavir (RTV) is a synthetic protease inhibitor that has been shown to have activities against HIV type 1 (HIV-1) and HIV-2.

It was the first protease inhibitor (PI) used in HIV+ patients. Currently, RTV is administered at low dose as a booster, in order to improve the pharmacokinetic profile of the coadministered PI. Inhibition constant (Ki) for RTV is about 10-60pM, near those of other PIs: atazanavir (ATV, 20-50pM), darunavir (DRV, 5-10pM), tipranavir (TPV, 10-90pM) or lopinavir (LPV, 5-20pM). Despite the low dosage of RTV administered to patients, we have recently reported high RTV intracellular concentrations as compared to other coadministered PIs. To date, there is not any evidence that RTV has any inhibitory effect on viral protease when administered as booster. Our aim was to investigate the intracellular molar concentrations of RTV, ATV, DRV, TPV and LPV, in order to evaluate, the binding competition between the different PIs and the protease enzyme of HIV in the clinical setting.

Materials and Methods: Patients administered with RTV booster standard dose plus one PI since at least 3 months were considered in the study. Sampling was performed after written informed consent was obtained in accordance with local ethics committee indications. Main inclusion criteria were: no concomitant interacting drugs, no hepatic or renal function impairment, self-reported adherence >95%. PBMC and plasma Ctrough of RTV and other PIs were measured in samples collected just before assumption, by validated HPLC-MS and HPLC-PDA methods, respectively. Cell count and mean cell volume were performed by a Coulter Counter instrument and data used for calculate total PBMC volume. Median value of individual measurements was considered. Values were expressed as molarity. Ki considered were from Altman et al.(2008). Biochemical considered formula to evaluate speed of enzyme-substrate reaction (V) was: $V=[V^{MAX}xS]/[K_M$ x (1+(I/Ki))+S] form Kakkar et al.(1998).

Results: One hundred patients were enrolled. Frequencies of ritonavir-boosted PIs were atazanavir, 37%; darunavir-600, 23%; lopinavir, 19%; tipranavir, 13%; and darunavir-800, 8%. Median intracellular RTV concentrations for each combination regimen were: 0.99 µM with atazanavir, 2.36 µM with lopinavir, 1.60 µM with tipranavir, 2.74 µM with darunavir 600mg/bid and 1.45 µM with darunavir 800mg/qd. Median intracellular PI concentrations were: 2.62 µM with atazanavir, 3.72 µM with lopinavir, 9.52 µM with tipranavir, 0.70 µM with darunavir 600mg/bid and 0.72 µM with darunavi 800mg/qd. The reaction rate calculated on the basis of median intracellular concentrations for each coadministration were: V_{RTV}=5.538e-5 and V_{ATV}=1.758e-5 for ATV/r; V_{RTV}=2.009e-5 and $V_{DRV-600}$=1.114e-5 for DRV 600mg/bid/r; V_{RTV}=3.798e-5 and $V_{DRV-800}$=1.111e-5 for DRV 800mg/qd/r; V_{RTV}=2.335e-5 and V_{LPV}=1.344e-6 for LPV/r ; V_{RTV}=3.442e-5 and V_{TPV}=9.240e-6 for TPV/r.

Conclusions: Our results suggest an high intracellular accumulation of RTV, and despite the low boosting dosing, intracellular RTV molar concentrations in some patients (30%) were higher then coadministered PIs. Moreover, the reaction rate calculated for RTV and PIs in each therapeutic combination showed to be only slightly faster (and potentially favourable) for boosted PIs as compared to RTV. These data may reflect a real competition to the binding site of HIV-protease between RTV and the concomitant PI. Moreover, this effect could be additive as well as competitive, therefore the existence and the possible nature of these possible intracellular interactions should be investigated in devoted in-vitro studies.

No conflict of interest

Abstract: P_25

PK-PD of Drug Efficacy and Toxicity

Pharmacokinetics of darunavir/ritonavir 600/100 mg QD within a dose-optimization program

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Background: Dose optimisation programs aim to lower antiretroviral drug costs and improve tolerability and convenience while maintaining virological efficacy. The objective of this substudy was to explore pharmacokinetic parameters of darunavir/ritonavir dosed at 600/100mg QD in the context of a dose optimization clinical trial.

Methods: Multicenter, randomized clinical trial comparing the efficacy and safety of darunavir dose reduction in HIV-infected patients with a suppressed viral load while on treatment including darunavir/ritonavir 800/100mg QD. Patients were randomized to continue on darunavir 800mg QD (800 arm) or to reduce the darunavir dose to 600mg QD (600 arm). A pharmacokinetic substudy including 15 patients allocated to each study arm was performed. A full pharmacokinetic profile was obtained from each participant at least four weeks after randomization. Darunavir concentrations in plasma were determined by using HPLC immediately before and 1, 2, 4, 6, 8, 10, 12 and 24 hours after a morning dose of darunavir/ritonavir. Individual darunavir pharmacokinetic parameters (maximum concentration [C_{max}], area under the concentration-time curve from 0 to 24 hours [AUC_{0-24}] and concentration at the end of the dosing interval [C_{trough}]) were calculated using non-compartmental analysis, and the two study arms were compared with the geometric mean ratio (GMR) and its 90% confidence interval (CI).

Results: Both arms were comparable regarding to gender, age, and body weight. Geometric mean (90% CI) darunavir C_{max}, AUC_{0-24} and C_{trough} were 6517 (5822-7294) ng/mL, 76661 (66562-88293) ng.h/mL, and 1597 (1264-2017) ng/mL for the 600 arm; and 6626 (5920-7416) ng/mL, 83988 (72924-96732) ng.h/mL, and 1835 (1453-2318) ng/mL for the 800 arm, respectively. The GMR (90% CI) for the 600 arm relative to the 800 arm was 0.98 (0.84-1.15) for C_{max}, 0.91 (0.75-1.11) for AUC_{0-24}, and 0.87 (0.63-1.21) for C_{trough}.

Conclusion: A darunavir dose reduction from 800mg QD to 600mg QD, in combination with ritonavir 100mg QD, provided comparable darunavir pharmacokinetic parameters. Despite a decrease in darunavir Ctrough in some patients, all participants maintained darunavir levels far above the concentration needed to inhibit wild-type strains of HIV. Clinical efficacy and safety of this dose optimisation strategy is under investigation.

No conflict of interest

Abstract: P_26

PK-PD of Drug Efficacy and Toxicity

The pharmacokinetic profile of rilpivirine

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Background: Relatively little is known about the pharmacokinetic (PK) profile of rilpivirine when used in daily clinical practice. Known determinants of rilpivirine PK are food, co-medication and inter-individual variation in CYP3A activity. The use of PK models in PK modelling software such as MWPharm allows us to analyse the effect of these different parameters on plasma rilpivirine concentrations. The software supports the creation of population models based on individual patient data and serum concentrations. In order to support the TDM of rilpivirine we developed such a model by means of available clinical data.

Material and methods: Clinicial data were used from a single centre HIV-treatment clinic in The Hague with 750 patients in care. Step 1: Published PK data from literature (1) on plasma concentration versus time curves at day 1 en 14 were used to construct a baseline model in MWPharm (Mediware, NL) by means of Bayesian fitting. Step 2: Data of plasma concentrations from our patients were used to parameterize the final model by means of the KINPOP module in MWPharm using iterative two-stage Bayesian population modelling (ITS).

Results: Data on 54 patients were available. 4 patients were excluded (lack of weight (n=1), a double dose (n=2), or a gastric bypass (n=1). Of the final population of 50 patients, (40 males) the age ranged from 19 to 68 years.
All samples were taken 2 or more weeks after the start of rilpivirine. 96% of samples were taken > 10h after rilpivirine dosing. Therefore based on literature the bioavailability F, absorption rate constant Ka, distribution volume V and absorption lag time Tlag were fixed on 1, 0.37 h^{-1}, 2L/kg and 0.9h respectively (1). A two compartment model best described rilpivirine PK. Mean CL/F was 7.66 L/h (SD 2.59). K12 and K21 were 7.52 10^{-2} h^{-1} (SD 0.02) and 1.78 10^{-2} h^{-1} (SD 0.005). Correlation with oral clearance was found for height (p=0.0051), lean body mass (p=0.006) and corrected lean body mass (cLBM) (p=0.006). A trend towards a lower clearance at older age and higher body surface area (BSA) was seen. With fixed F, Ka, V and Tlag the model predicted that at least 95% of patients would have plasma concentrations within the therapeutic range of 40 - 600ug/L with the standard dose of 25 mg once daily..

Conclusion and discussion: We have developed a 2-compartment PK model for rilpivirine partly based on published PK data and adapted with available patient data, to assist the TDM of this NNRTI. Predominant hepatic metabolism can explain the (trend in) correlation of CL/F with height/BSA/(c)LBM and age. The large inter individual variation in CYP3A activity might explain the large variation in CL/F. Without taking into account variation in absorption and distribution 95% of patients would have plasma concentrations within the therapeutic range with 1 dd 25 mg. Food however has shown to affect absorption. Further research, especially on the absorption and distribution phase of rilpivirine is needed to optimize our model and identify specific parameters to further explain inter individual variation.

1. Crauwels H, Vingerhoets J, Ryan R, Witek J, Anderson D.Pharmacokinetic parameters of once- daily rilpivirine following administration of efavirenz in healthy subjects. Antivir Ther. 2012;17(3):439-46.

No conflict of interest

Abstract: P_27

PK-PD of Drug Efficacy and Toxicity

The effect of rilpivirine on Modification of Diet in Renal Disease (MDRD) estimation of eGFR

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Background: The initiation of rilpivirine in naïve patients, combined with a backbone of NRTIs has been reported to cause, unlike efavirenz, a decrease in estimated glomerular filtration rate (eGRF) within weeks. The increase in plasma creatinine concentration is believed to be the result of inhibition of tubular secretion by rilpivirine. The effect is more pronounced with tenofovir than with zidovudine. To evaluate these findings in clinical practice, we retrospectively investigated the eGFR estimated by MDRD in naive patients starting on rilpivirine combined with emtricitabine and tenofovir and patients that switched from a tenofovir containing regimen. We hypothesize that there is a decrease in eGFR in patients after switch, which is less pronounced than in naïve patients.

Materials and methods: Clinicial data were used from a single centre HIV-treatment clinic in The Hague with 750 patients in care. The eGFR values of all patients that started rilpivirine combined with tenofovir and emtricitabine from May 2012 on were analysed. Data were collected from 5 months before switch up to 5 months after switch or the start of rilpivirine. Patients with less than 3 eGFR measurements before or after the start were excluded. The average difference in eGFR, compared to the last eGFR value before start was calculated. The average difference in eGFR before and after start was compared with a paired t test in switch patients. The average difference in eGFR after

start in naïve and switch patients was compared with an unpaired t test.

Results: 54 patients started with rilpivirine in the stated period, 8 naïve and 46 switch patients of which 43 switched from a tenofovir containing regimen. In naïve patients 6 (1 female, 5 male) had sufficient eGFR measurements. In switch patient 16 met these criteria. One patient was excluded due to extreme values, resulting in 15 switch patients (1 female, 14 male)
In switch patients the average eGFR before start was: 91.47 ml/min/1,73 m2 (SD 14.53). The average difference in eGFR before and after start was - 7.72 ml/min/1,73 m2 (SD 5.93) showing a significant decrease (p=0,018) in eGFR after the switch to rilpivirine. The change in eGFR was not correlated with the use of either NNRTIs (n=11) or PIs (n=4) before switch (p=0.65).
In naïve patients the average eGFR before start was 98.83 ml/min/1,73 m2 (SD 17.52). There was a significant decrease (p=0.002) of eGFR after start of -13.40 ml/min/1,73 m2 (SD 6.78). This decrease was significantly larger than in switch patients (p=0,017).

Discussion and conclusion: This relatively small study shows that the effect of rilpivirine on the change in eGFR calculated with the MDRD formula is dependent on whether or not patients were already on tenofovir containing therapy. The decrease in eGFR was largest in treatment naïve patients.

No conflict of interest

Abstract: P_28

PK-PD of Drug Efficacy and Toxicity

Poor performance of laboratories assaying newly developed antiretroviral agents: data for darunavir, etravirine and raltegravir from the KKGT Program

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Background: The International Interlaboratory Quality Control Program for Measurement of Antiretroviral Drugs in Plasma was initiated in 1999. We have recently published our experience during the first 10 years of the Program (Burger et al. TDM 2011). Since 1999, 3 newly developed antiretroviral agents have been added to the Program: darunavir (DRV), etravirine (ETR) and raltegravir (RAL). The objective of this analysis is to describe the performance of participating laboratories measuring these newer agents in 2011-2012.

Materials and Methods: Each year laboratories received 2 blind samples of human plasma spiked with either a low (<1.0 mg/L), medium (1.0-5.0 mg/L), or high (>5.0 mg/L) concentration of these drugs. Low concentrations were never below 50% of the minimum effective concentration or higher than 5 times the average peak plasma concentration. Participants were asked to report their results within 6 weeks of dispatch. Results are stored anonymously in a database. Laboratory results were standardized to percentages with reference to the nominal (true) concentration. Any result that deviated more than 20% of the nominal values was defined as inaccurate. Statistical analyses was done using IBM SPSS version 20.0.

Results: The number of laboratories that participated in the Program by the end of 2012 was 44 for DRV, 28 for ETR, and 30 for RAL. A total of 357 results was evaluable for analysis. Of these, 64 (17.9%) results were reported with >20% deviation, so 'inadequate' (7.6% too low; 10.4% too high). For comparison with other agents, the overall proportion of inadequate results when analyzing older antiretroviral agents was 11.6 ± 5.1%. The proportion of inadequate results in 2011 was 21.3% for DRV, 31.0% for ETR, and 26.3% for RAL; in 2012 there figures improved to 8.1%, 23.2% and 8.3% for DRV, ETR, and RAL, resp. (p=0.002 for 2012 vs. 2011). Taken DRV as the reference, performance for ETR was significantly lower: OR 0.462 (95% CI:

0.246 – 0.866; p=0.016); performance for RAL was not significantly different. Low concentrations were significantly more frequently reported as inadequate than medium or high concentrations: 28.6 vs. 10.6 vs. 8.8%, resp. (p<0.001). Laboratories that used LC-MS(MS) did not perform better than those using HPLC/UPLC: 41 inadequate results in 200 samples (20.5%) vs. 23 in 157 samples (14.6%)(p=0.154). Multiple logistic regression revealed that the lower range of concentrations performed worse than medium or high concentration (p<0.001).

Conclusion: Laboratories continue to have problems with adequately measuring low plasma concentrations of antiretroviral agents. This is particularly a problem for some of the newer agents with plasma concentrations in the < 1.0 mg/L range, such as ETR and RAL. This does not appear to be related to the type of equipment used. Continuous participation in external proficiency testing programs is warranted to alert laboratories to suboptimal performance of their assays.

No conflict of interest

Abstract: P_29

PK-PD of Drug Efficacy and Toxicity

Effects of HIV protease inhibitor regimen on platelet function in HIV-infected adult outpatients after 24 weeks of therapy

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Introduction: In the past, an increased rate of thromboembolic complications and cardiovascular events under antiretroviral therapy (ART) has been described. In particular, the influence of protease inhibitors (PIs) and the reverse transcriptase inhibitor abacavir on platelet activation and coagulation are discussed.

Methods: HIV-1-infected, therapy-naive adults (n=31) were investigated before and 4, 12 and 24 weeks after the start of ART, consisting either of a boosted PI or NNRTI, each plus tenofovir/emtricitabine co-medication. Administered ARVs were lopinavir (n=5), darunavir (n=6), atazanavir (n=5) and efavirenz (n=15). Platelet CD62P, CD40L (%1 cells) and PAC-1 binding [mean fluorescence intensity (MFI)] as well as monocyte CD11b (MFI) expression was assessed by flow cytometry. To investigate the influence of platelets on coagulation, the endogenous thrombin potential (ETP) [render fluorescence units (RFI)] was determined.

Results: CD11b and CD40L expression as well as the ETP AUC and time to peak remained unchanged in both study groups through 24 weeks on therapy and compared between both groups. In contrast, the mean (95%CI) MFI of PAC1 [from 0.225 (0.164-0.282) at baseline to 0.408 (0.201-0.616) at week 12, p=0.002 and 0.536 (0.305-0.768) at week 24, p=0.002] and CD62P [from 0.106 (0.092-0.120) at baseline to 0.168 (0.089-0.225) at week 12 and 0.210 (0.112-0.307) at week 24, p=0.010] as markers for a conformal change in fibrinogen receptor GIIb/IIIa and platelet degranulation increased significantly in patients taking PIs.

Conclusions: Of the evaluated therapy regimens, PI-based ART revealed significant effects on platelet function assessed under field conditions. The *ex vivo-in vitro* tests show conformal changes in the GIIb/IIIa receptor and the granular release of platelet derived groth factor, represented by significant increase in PAC-1 binding and CD62P expression. These effects could be reproduced over 24 weeks on therapy and are most likely contributing to atherosclerosis and an increased risk in CVE under PI-based ART.

No conflict of interest

Abstract: P_31

PK-PD of Drug Efficacy and Toxicity

Nevirapine dose escalation or immediate full dose when switching from efavirenz to nevirapine in HIV-infected patients in the ATHENA cohort study

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Background: When switching from efavirenz (EFV) to nevirapine (NVP) it is unclear whether NVP should be dose escalated or not because of EFV-related enzyme induction. Dose escalation of NVP after EFV treatment may be associated with temporary subtherapeutic NVP plasma levels. Immediate full dose of NVP may lead to an increased risk of toxicity (e.g. skin rash or hepatotoxicity). A retrospective analysis was conducted using data from the observational ATHENA cohort to evaluate the safety and efficacy of dose escalation vs. full dose of NVP in HIV-infected patients switching from EFV to NVP.

Materials & Methods: HIV-infected patients (≥18 years) from five Dutch hospitals with a treatment switch from EFV to NVP immediate release between 2001 and 2011 were selected from the ATHENA cohort study. Patients were required to have used at least 2 weeks of EFV treatment prior to switching, with a maximum of 1 week between the last dose of EFV and the first dose of NVP. Dose escalation (200 mg lead-in daily dose for 1-2 weeks followed by 400 mg/day in two divided doses or once daily) was compared to immediate full dose (400 mg/day in two divided doses or once daily). Safety and efficacy outcomes were toxicity-related discontinuation of NVP ≤ 12 weeks after start of NVP treatment and an undetectable viral load at week 24.

Results: In total 201 HIV-infected patients switching from EFV to NVP were included. The majority of patients (n=159, 79%) switched directly to full dose NVP. There was an increase in switching to full dose over time: in the period 2001-2005, 46.2% of the patients switched directly to full dose NVP compared to 90.6% in the period 2006-2011 (p<0.0001). At time of treatment switch, there were no differences between the dose escalation and full dose groups in the median (IQR) CD4 cell count and the proportion of patients with an undetectable viral load: 505 (315-748) cells/mm^3 vs. 500 (345-690) cells/mm^3 (p=0.96) and 66.7% vs. 63.5% (p=0.74) respectively. In the first 12 weeks after initiating NVP, 13 patients (8.2%) with full dose NVP stopped NVP due to toxicity compared to 1 patient (2.4%) in the dose escalation group (p=0.31). In a multivariable logistic regression analysis adjusting for CD4 cell count, the odds ratio for toxicity-related discontinuation within 12 weeks after starting NVP was 4.26 for patients starting with full dose (95%CI 0.52-35.25 p=0.18). No significant association was found between the starting dose of NVP and virological outcome (adjusted OR 0.97, 95%CI 0.34-2.73 p=0.95).

Conclusions: In our Dutch cohort, immediate full dose NVP after switching from EFV is more frequently used than dose escalation, especially in recent years. No significant difference was found in toxicity-related discontinuations or virological failures between the two dosing strategies.

Conflict of interest
This research was sponsored by a grant from Boehringer-Ingelheim.

Abstract: P_32

PK-PD of Drug Efficacy and Toxicity

Plasma concentrations of dual maraviroc/raltegravir combination in patients with suppressed viremia: Results from ROC'nRAL ANRS157 sub-study

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Background: Long term suppressive therapy with NRTI and PI sparing strategies are needed in patients with comorbidities. The ROC'nRAL ANRS157 study, a pilot, multicenter single-arm trial has enrolled from December 2011, patients with suppressed plasma HIV viremia, clinical lipodystrophy, nadir CD4 > 100 cells/mm^3, subtype B or CRF02 R5 tropic virus who switched from a suppressive ART to maraviroc (MVC 300mg bid) plus raltegravir (RAL 400 mg bid). In August 2012 the study was prematurely stopped given to an excessive rate of treatment failures: 7/44 patients with 5 virological failures and 2 severe adverse events (HBV rebound in a patient HBcAb+ and HBsAg-, one hypersensitivity syndrome). In a previous study in HIV-seronegative subjects, the MVC/RAL drug interaction was not considered to be clinically relevant. This PK sub-study aimed to evaluate MVC/RAL drug interactions in HIV-infected patients and whether plasma drug concentrations could be predictive of failures.

Methods: Blood samples were collected 12+/-2 hours after the last drug intake at W4, W24 and W48. Plasma protein binding was performed using an ultrafiltration assay with Centrifree® devices in duplicate (acceptability of CV < 15%). Plasma Cmin of MVC and RAL and its glucuronide metabolite (G-RAL) were determined using UPLC-MS/MS method (Acquity UPLC-Acquity TQD) after sample pretreatment (LOQ < 1 ng/mL). Results are presented as median (IQR25%-75%).

Results: Forty-four patients were enrolled with the following median baseline characteristics: 86% male, age: 55 years (50-60), nadir CD4 of 210 cells/mm3 (150-276), duration of ARV exposure 15 years (15-19), duration of viral suppression 5.2 years (4.4-7.9). Among them, 43 had plasma Cmin available. Total and free plasma Cmin were 107 ng/mL (54-165, n=75) and 46 ng/mL (23-77, n=69) for MVC and 152 ng/mL (95-411, n=78) and 33 ng/mL (20-67, n=72) for RAL, respectively. Free fractions represent 42% (37-46%, n=81) for MVC and 20% (16-26%, n=81) for RAL and the metabolic ratio of G-RAL/RAL was approximately 4.1 (2.3-6.4, n=80). Between and within variability of total Cmin were 105% and 35% for MVC and 117% and 61% for RAL, respectively. Of the 5 patients with virological failure, 2 had MVC and/or RAL Cmin < 50 ng/mL and 3 demonstrated emergences of viral resistance mutations.

Conclusion: Despite important variability of MVC and RAL plasma Cmin, the respective IQR25% values remain above the supposed antiviral threshold of 50 ng/mL 12 hours after the last drug intake. In all patients, free MVC plasma remained above 10 ng/mL, a concentration supposed to saturate all the membrane CCR5 molecules. Finally, the present PK sub-study confirms the lack of clinical relevance of the MVC/RAL drug interaction and do not explain the unexpected rate of failures. Among the several hypotheses under investigations, this pilot study suggests that, in this population of long term treated aging patients, MVC/RAL dual therapy lacks virological robustness resulting in a high rate of resistance mutations.

No conflict of interest

Abstract: P_33

PK-PD of Drug Efficacy and Toxicity

Reliability and precision of tenofovir-diphosphate (TFV-DP) in dried blood spots (DBS): a biomarker for tenofovir adherence

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Introduction: Detection of tenofovir (TFV)/emtricitabine (FTC) in plasma and PBMC was a surrogate measure of adherence and a powerful predictor of TFV-FTC efficacy in HIV prevention trials. However, the short plasma half-life of TFV/FTC and multiple drawbacks of PBMC collection underscore the need for an alternative quantitative adherence biomarker. We previously showed that TFV-DP has a 17-day half-life in red blood cells (RBC) and accumulates approximately 25-fold with consistent dosing, which is a promising characteristic for monitoring cumulative adherence. The objective of this study was to compare the precision of TFV-DP measurements in purified RBC versus DBS, and to evaluate the influence of hematocrit (HCT) and RBC concentration in blood (RBC/uL) on DBS measurements.

Materials & Methods: Adults were enrolled in a 30-day study of daily TFV-FTC. Paired DBS and RBC samples were collected from the same blood draw at 2 hours post-dose on days 3, 7 and 20, and at five time-points on day 30. RBC were harvested from CPT tubes and counted using an automated cell counter. For DBS, 25uL of EDTA blood was spotted on DBS paper, and a 3mm punch was extracted for analysis. TFV-DP was measured in RBC and DBS by a validated LC-MS/MS method. The precision of TFV-DP from RBC versus DBS was reported as %CV of concentrations from 5 time points at day 30 (concentrations do not fluctuate given the 17 day half-life). The number of RBC per DBS punch was determined from averaged TFV-DP in the paired samples (fmol/punch ÷ fmol/million RBC) and evaluated using regression analysis with averaged RBC/uL and HCT. Data are median (range), unless noted otherwise.

Results: Data were available from 17 participants (5 HIV+, 6 female, 9 African-American). HCT and RBC/uL were: 43% (36% to 50%) and 4.9 (4.0 to 6.3) million, respectively. Sixty paired RBC/DBS samples were available from the 17 participants, including 35 from 7 participants at all 5 day 30 time points. The TFV-DP %CV from the day 30 time points was lower and more consistent in DBS versus RBC, 8% (3% to 13%) versus 10% (3% to 29%). The determined number of RBC per DBS punch was 12 million (9 to 16); n=17. Fourteen of 17 (82%) values were within 20% of 12 million. RBC/uL was associated with RBC per punch, ($y=1.7x + 3.3$; $P=0.033$). The RBC/uL range from the 17 participants gave a predicted RBC per punch that was within 20% of 12 million. No relationship was observed between HCT and RBC per punch ($P=0.22$).

Conclusions: DBS is a reliable and precise measure for TFV-DP in RBC. The increased precision in DBS compared with purified RBC may reflect the absence of cell counting, and/or the overall simplicity of DBS collection and extraction. A relationship was identified between RBC/uL and RBC per punch, but the differences were within 20% of expected. No relationship between HCT and RBC per punch was identified. These results support the implementation of DBS as a reliable, precise, and convenient method for monitoring cumulative adherence to TFV-based therapy.

No conflict of interest

Abstract: P_34

PK-PD of Drug Efficacy and Toxicity

Comparisons between plasma and intracellular tenofovir/emtricitabine (TFV/FTC) with sex and race in HIV-negative volunteers

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Introduction: The combination of tenofovir disoproxil fumarate (TDF) -emtricitabine was recently approved for prevention of HIV acquisition in sero-discordant men and women, and men who have sex with men. African-Americans are disproportionately burdened by HIV acquisition. The effect of sex and race on intracellular TFV-DP and FTC-TP concentrations is not well studied. The objective of this study is to compare plasma and intracellular TFV-DP/FTC-TP pharmacology by sex and race in HIV- subjects.

Methods: Adults were enrolled in an intensive pharmacokinetic (PK) study of daily TDF/FTC for 30 days. Paired plasma and PBMC samples were collected at 1, 2, 4, 8, 24 hours after the first dose. Concentrations were determined by validated LC-MS/MS methods. Plasma concentrations are in ng/mL, while intracellular TFV-DP levels are fmol/million cells (M) and FTC-TP are pmol/M. Unpaired *t*-tests were used to evaluate sex and race differences on natural-log transformed first-dose steady-state concentration (Css=AUC_{24}/24h). Data are reported as untransformed mean±SD for women vs. men or non-African-American vs. African-American.

Results: 20 HIV- individuals participated, evenly balanced by both sex and race (5 non-African-American women, 5 African-American women, 5 non-African-American men, and 5 African-American men). No differences were observed between women and men in first dose intracellular TFV-DP Css (15.0±7.91 vs. 11.7±3.60; p=0.29); FTC-TP (3.15±0.502 vs. 3.11±0.767; p=0.75); plasma TFV (60.6±21.9 vs. 63.4±20.6; p=0.74); or plasma FTC (403±110 vs. 337±94.2; p=0.22). No differences were observed according to non-African-Americans versus African-American race for TFV-DP (12.7±3.09 vs. 14.0±8.41; p=0.99) and FTC-TP (3.18±0.571 vs. 3.08±0.713; p=0.65), although a non-significant trend was observed for plasma TFV (70.8±19.7 vs. 53.2±18.6; p=0.08) and FTC (412±89.9 vs. 328±107; p=0.09), respectively.

Discussion: This study focused on sex and race associations with TFV/FTC after the first dose, as these measures were not impacted by potential variability in adherence over the 30 day study. Plasma TFV/FTC and intracellular TFV-DP/FTC-TP were not significantly associated with sex. There was a non-significant trend for higher plasma tenofovir/emtricitabine in non-African-Americans versus African Americans, but these trends did not extend to intracellular TFV-DP or FTC-TP, the pharmacologically active drug forms. Our next step is to develop a population PK model that links plasma and intracellular moieties over all study days to evaluate the effect of co-variates such as sex and race on the plasma-intracellular relationship.

No conflict of interest

Abstract: P_35

PK-PD of Drug Efficacy and Toxicity

Distribution into cerebrospinal fluid of atripla in HIV-infected adults at two different time points

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Introduction: Atripla® is an FDA-approved single fixed dose combination tablet of efavirenz 600 mg (EFV), emtricitabine 200 mg (FTC) and tenofovir disoproxil fumarate 300 mg (TDF). The three drugs distribute into the central nervous system (CNS) to varying degrees, but whether the cerebrospinal fluid (CSF) concentrations will change over time in the same patient has never been studied. Drug passage into CNS is influenced by parameters including protein binding, molecular weight, lipophilicity, ionization, and the presence of membrane transporters. The study objective

was to investigate whether the CSF distribution of Atripla will change over time in the same patient over 12 months.

Material and methods: The CNS HIV Antiretroviral Therapy Effects Research (CHARTER) study is a multicenter, observational cohort study in the US that aims to determine the effects of potent antiretroviral therapy on HIV-associated neurological disease. Plasma and CSF samples from 14 subjects taking Atripla® were drawn at biannual study visits between June 2005 and November 2009. All drugs except efavirenz in plasma were assayed by mass spectrometry with a detection limit of 0.3 ng/mL. Plasma efavirenz was assayed by high-pressure-liquid chromatography with a detection limit of 9.77 ng/mL. Spearman's rho measured the correlations between CSF and CSF/plasma ratio changes over time within and between drugs. Wilcoxon signed-rank test compared the CSF distribution and CSF/plasma ratio at baseline to 12 months later.

Results: 14 participants (age 46 ± 9.7 years; 11 males) had plasma and CSF samples drawn 11.6 ± 5.4 and 11.8 ± 5.3 hours post-dose respectively. Median (95% CI) CSF concentration as the ratio of the follow-up assessment/baseline assessment are 1.60 (0.73 – 5.01), 0.99 (0.17 – 2.87) and 0.92 (0.33 – 3.79) for TDF, FTC and EFV respectively. Median (95% CI) CSF/plasma ratio as the ratio of follow-up/baseline assessment for TDF, FTC and EFV are 1.38 (0.71 – 10.47), 1.58 (0.03 – 6.55) and 1.24 (0.43 – 4.54) respectively. Concentrations of all three drugs in CSF at baseline were not significantly different than at 12 months, TDF concentrations in CSF did tend to increase over time (S=13.5, p=0.097). Within subject CSF drug concentrations at baseline were not correlated with 12 month concentrations of the same drug. However, EFV in CSF follow-up assessment/baseline assessment was correlated positively with TDF (r=0.73, p=0.016). CSF/plasma ratios for all three drugs were not correlated, nor were they significantly different from baseline to 12 months. EFV and FTC CSF/plasma follow-up assessment/baseline assessment were inversely correlated (r=-0.77, p=0.016).

Conclusion: The inter-subject and intra-subject variability for CSF drug concentration and CSF-to-plasma ratios of tenofovir, emtricitabine and efavirenz are large. Although the CSF concentrations of EFV and FTC did not change over time, TDF concentrations in CSF did tend to increase over time. The correlations of EFV with FTC and TDF differ in CSF drug concentration and CSF/plasma ratio which means that the factors that lead to CSF distribution of three antiretroviral drugs are different.

No conflict of interest

Abstract: P_36

PK-PD of Drug Efficacy and Toxicity

Population pharmacokinetics of cobicistat in adult healthy subjects and HIV-infected patients

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Background: Cobicistat (COBI) is a pharmacokinetic (PK) enhancer approved for use as a booster of the HIV-1 integrase inhibitor elvitegravir (EVG) when given as the EVG/COBI/emtricitabine (FTC)/tenofovir disoproxil fumarate (TDF) single tablet regimen Stribild™ (STB). COBI is also in regulatory review as a PK enhancer of the HIV-1 protease inhibitors atazanavir (ATV) and darunavir (DRV). The population pharmacokinetics (PK) of COBI and the effects of covariates such as demographics, background treatment, and formulation on COBI PK were determined.

Materials & Methods: Intensive and sparse data from clinical studies evaluating COBI (administered as single agent or coadministered with an agent as a PK enhancer) from a total of 16 studies were used (11 studies in healthy subjects (n = 302) and 5 studies in HIV-1 infected patients receiving STB or ATV/COBI plus FTC/TDF (n = 341)). A mixed effect modeling approach using NONMEM version 7.2 was applied using First order with conditional estimates (FOCE). The effect of covariates was assessed age, gender, race, HIV status (positive or negative),

formulation, body weight, body mass index, body surface area (BSA), creatinine clearance (estimated GFR), and HBV and/or HCV coinfection. Graphical assessments, accuracy and meaningfulness of parameter estimates, significant decrease in the objective function value provided by NONMEM, and predictive checks were used as model selection criteria.

Results: A one-compartment PK model with a combination of zero- and first-order absorption mechanisms, and absorption lag time provided a good description of the PK of COBI in both healthy subjects and HIV infected patients following multiple-dose administration of COBI. The typical apparent systemic clearance of COBI (CL/F) was estimated to be 15.0 L/h (relative standard error (RSE) =2.5%) with an interindividual variability (IIV) of 53% (9.5%). The apparent volume of the central compartment (Vc/F) was estimated to be 77.0 L (1.6%) with an IIV of 25% (8.5%). COBI absorption included a zero-order absorption with a duration of 1.16 hours (3.6%) with as IIV of 69% (10.5%), a first-order absorption rate constant of 0.88 h^{-1} (4.0%) with an IIV of 50% (9.5%), and a lag-time of 0.18 hours (24.9%) with an IIV of 130% (17.4%). The final model included body weight as a covariate effect on COBI volume. However, the effect was modest and not deemed to be clinically relevant, as the range of COBI exposures (AUC) within the bottom 5th percentile and upper 95th percentile of weight were associated with robust pharmacodynamic effect (boosting) and a well characterized exposure-safety profile. No other relevant effects of other covariates were observed on COBI PK.

Conclusions: A one-compartment PK model with zero- and first-order absorption mechanisms and absorption lag-time and including the effect of body weight on COBI Vc/F adequately described the PK of COBI in both healthy volunteers and HIV infected patients. No clinically relevant effects of covariates were observed on COBI PK.

Conflict of interest
Employee by and hold stock in Gilead Sciences

Abstract: P_37

PK-PD of Drug Efficacy and Toxicity

Population pharmacokinetics of tenofovir in adult healthy subjects and HIV-infected patients

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Background: Tenofovir (TFV) is a HIV nucleotide analog reverse transcriptase inhibitor approved for the treatment of HIV-1 infection as the orally bioavailable prodrug tenofovir disoproxil fumarate (TDF). TDF is used in combination with other antiretrovirals, frequently as a component of single tablet regimens (STRs), most recently, as Stribild™ (STB; also containing elvitegravir (EVG), cobicistat (COBI), and emtricitabine (FTC)). The population pharmacokinetics (PK) of TFV following administration of STB and the effects of covariates such as demographics and COBI exposure on TFV PK were investigated.

Materials & Methods: Pooled intensive and sparse data from studies of TDF or STB from a total of nine studies were used (4 studies in healthy subjects (n = 128) and 3 studies in HIV-1 infected patients receiving STB (n = 419)). A mixed effect modeling approach using NONMEM version 7.2 was applied using First Order Conditional Estimation with centering (FOCE INTER). The effect of covariates was assessed including age, gender, race, HIV status (positive or negative), body weight, body mass index, body surface area (BSA), creatinine clearance (estimated glomerular filtration rate (eGFR)), and COBI exposure (AUC_{tau}, C_{max}, and C_{trough}). Graphical assessments, accuracy and meaningfulness of parameter estimates, significant decrease in the objective function value provided by NONMEM, and predictive checks were used as model selection criteria.

Results: A two-compartment PK model with a combination of zero- and first-order absorption mechanisms and an absorption lag time provided a good description of the PK of TFV

in both healthy subjects and HIV infected patients. The typical apparent systemic clearance of TFV (CL/F) was estimated to be 42.3 L/h (relative standard error (RSE) =1.4%) with an interindividual variability (IIV) of 21% (10.9%). The apparent volume of the central compartment (Vc/F) was estimated to be 354 L (7.9%) with an IIV of 46% (34.9%). TFV absorption rate was 2.48 h^{-1} (10.8%) (IIV=103% (27.5%)) with a lag-time of 0.46 (12.7%) hours (IIV=122% (14.4%)). The final model included creatinine clearance (eGFR calculated by Cockcroft-Gault method) as a covariate on TFV clearance, consistent with TFV renal elimination. Following administration of STB, systemic COBI exposure was not a covariate on TFV PK, in line with the inhibitory effect of COBI on intestinal secretion of TDF via inhibition of the secretory transporter P-glycoprotein (P-gp). No other relevant effects of other covariates were observed on TFV PK.

Conclusions: A two-compartment PK model with zero- and first-order absorption mechanisms and absorption lag-time and including the effect of creatinine clearance (eGFR) on TFV CL/F adequately described the PK of TFV in both healthy volunteers and HIV infected patients receiving TDF or STB. No clinically relevant effects of any other covariate were observed on TFV PK.

Conflict of interest

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14th International Workshop on Clinical Pharmacology of HIV Therapy

22 – 24 April 2013, Amsterdam, The Netherlands

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