Abstract Book
8th International Workshop on Clinical Pharmacology of Hepatitis Therapy
26 - 27 June 2013, Cambridge, MA, USA

8th International Workshop on Hepatitis C Resistance & New Compounds
27 - 28 June 2013, Cambridge, MA, USA
Abstracts

8th International Workshop on Clinical Pharmacology of Hepatitis Therapy
26 – 27 June 2013, Cambridge, MA, USA

Abstracts
Abstract: O_01_PK

PK/PD of Drug Efficacy and Toxicity

Age and Gender Effects on the Pharmacokinetics of Multiple Oral Doses of MK-5172

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Background: MK-5172 is a reversible, noncovalent, competitive inhibitor of the HCV NS3/4A protease being developed for the treatment of chronic hepatitis C infection (HCV). This study was the first comparison of MK-5172 pharmacokinetics between males and females, as well as the first comparison between healthy young males and healthy elderly subjects.

Methods: Panel A (8 healthy males aged 65 to 78 years) and Panel B (8 healthy females aged 65 to 79 years) were enrolled in this double-blind, randomized, placebo-controlled, parallel-group, multiple-dose study. Both panels (6 active, 2 placebo per panel) received oral doses of 400 mg MK-5172 or matching placebo once daily for 7 days.

Results: The pharmacokinetics (PK) of MK-5172 was evaluated in 6 elderly male and 6 elderly female subjects, with historical MK-5172 PK data from healthy young males (n=6) used as comparison. Following 7 day dosing of 400 mg MK-5172, the steady-state AUC₀⁻₂₄ GMR (90% CI) for elderly females/elderly males was 1.76 (0.82, 3.81). Cmax and C₂₄ values were 90% and 29% greater in elderly females than elderly males, respectively.

Conflict of interest: Employee of Merck Sharp & Dohme

Conclusion: Multiple oral dosing of 400 mg MK-5172 for seven days was generally well tolerated in healthy elderly male and female subjects. A PK age effect was observed with an AUC₀⁻₂₄ GMR for elderly males/young males of 2.18. This may suggest an increase in bioavailability or reduced metabolic clearance in elderly. A PK gender effect in elderly subjects was observed with an AUC₀⁻₂₄ GMR for elderly females/elderly males of 1.76. The gender effect may be confounded by body weight, as females were 31% lighter than males in this study.
Abstract: O_02_PK

Novel drug formulations

Telaprevir & Adjusted dose of Ribavirin in naive CHC-G1: Efficacy And Treatment in CHC in Hemodialysis population. TARGET C Trial

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Objective: The prevalence of Chronic hepatitis C (CHC) in Hemodialysis population is 3%. Standard of care (SOC) offers reduced dose of Peg IFN Alfa (p-IFNα) and reduced Ribavirin (RBV) doses eliciting sub optimal SVR of 27%. Morbidity and mortality of CHC has impact on liver kidney transplant and graft failure. Triple therapy is SOC in CHC patients. Telaprevir is not cleared renally and hence is safe in the hemodialysis population. This study evaluated the efficacy of triple therapy with Telaprevir, adjusted dose of RBV and p-IFNα in naïve CHC-G1(CHC Genotype 1) individuals on hemodialysis as a Respond Guided Therapy (RGT)

Methods: Total of thirty five (n=35) naïve CHC-G1 were recruited and subdivided into two subgroups. Group A - (n=18): Received p-IFNα 135mcg once weekly, Telaprevir 750mg two tablets – TID for four days and three tablets BID post dialysis for three days along with RBV 400mg daily for 12 weeks followed by p-IFNα 135mcg plus RBV 400mg till 24weeks of duration. Group B- (n=17): Received p-IFNα 135 mcg once weekly with Telaprevir 750mg two tablets – TID for four days and three tablets BID post dialysis for three days (same as Group A) with RBV 200mg for 12 weeks followed by p-IFNα 135mcg with RBV 400mg till 48 weeks. The IL28B was evaluated for all individuals. Hematological, Liver and renal parameters were followed regularly during the trial. Viral load was followed to evaluate for response guided therapy (RGT) in all individuals.

Results: See table (VRVR- Very Rapid Virological Response, ETVR- End to treatment Virological Response)

Conclusion: This study demonstrates higher SVR comparing traditional SOC on hemodialysis CHC-G1 patients. The extended 48 weeks of therapy showed no added benefits. Multi-center trials to follow.

No conflict of interest

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Abstract: O_03_PK

Clinical Pharmacology in Special patient groups

Pharmacokinetics of Simeprevir (TMC435) in volunteers with severe renal impairment

A. Simion¹, S. Mortier², M. Peeters³, M. Beumont-Mauviel⁴, S. Ouwerkerk-Mahadevan⁵

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Background: Renal clearance of simeprevir (TMC435), an oral, once-daily (QD), HCV NS3/4A protease inhibitor in Phase III development for treatment of chronic HCV genotypes 1 and 4 infection, is an insignificant elimination pathway. On average, only 0.038% (range 0.009-0.138%) of the administered oral dose is excreted in urine. However, renal insufficiency can affect hepatic and intestinal metabolism as well as transport of some compounds, potentially impacting their plasma concentration and safety. This Phase I, open-label trial (TMC435-C126; NCT01381835) in HCV-negative volunteers investigated the effect of severe renal impairment on simeprevir pharmacokinetics (PK) and short-term safety.

Methods: Renal function was determined by estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease equation (MDRD). Volunteers with severe renal impairment (eGFR ≤29 mL/min/1.73m²) who were neither receiving dialysis nor expected to begin within the subsequent three months, were matched with healthy (eGFR ≥80 mL/min/1.73m²) volunteers. Simeprevir 150 mg was administered QD under fed conditions for 7 days, and simeprevir PK profiles determined up to 72 hours after end of dosing. Unbound simeprevir was measured pre-dose and 4 hours post-dose on Day 7. Clinical laboratory parameters (including renal function), cardiovascular safety, and adverse events (AEs) were monitored continuously. PK data from healthy and renal impaired volunteers were compared using LS means ratios.

Results: All volunteers completed the study (severe renal impairment, n=8; normal function, n=8). Simeprevir Cₘᵢₙ, Cₘₐₓ, and AUC₂₄ₕ were approximately 71%, 34%, and 62% higher, respectively, in volunteers with severe impairment compared with matched controls. The mean fraction of simeprevir unbound to protein in plasma pre- and post-dose was ~0.0001 for all volunteers in both groups. All AEs were grade 1/2 except for one serious AE of rhabdomyolysis in a renal impaired volunteer receiving concomitant fenofibrate.

Conclusion: Simeprevir exposure was higher in patients with severe renal impairment compared with matched healthy volunteers. Severe renal impairment had no effect on simeprevir plasma protein binding. Simeprevir was generally well tolerated in this study. Dosing recommendations based upon results of this and other studies will be presented.

Conflict of interest: Janssen employee
Abstract: O_04_PK

Late Breaker

Pharmacokinetics of Simeprevir (TMC435) in volunteers with moderate or severe hepatic impairment

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Background/aims: Simeprevir (TMC435) is an oral, once-daily (QD), HCV NS3/4A protease inhibitor in Phase III development for treatment of chronic infection with HCV genotypes 1 and 4. In Phase IIb trials, simeprevir with peginterferon/ribavirin improved SVR rates compared with placebo/peginterferon/ribavirin, including in HCV-infected patients with compensated cirrhosis. In HCV-infected patients with compensated liver disease (mild hepatic impairment), simeprevir exposure was ~2-fold greater versus healthy volunteers (historical data). This Phase I, open-label study (TMC435-C113; NCT01046058) investigated simeprevir pharmacokinetics (PK) and safety in HCV-negative volunteers with moderate or severe hepatic impairment.

Methods: Panel A included volunteers with moderate (Child-Pugh B) hepatic impairment and healthy matched controls (age, sex, race, ethnicity, body mass index, smoking status). Panel B included volunteers with severe (Child-Pugh C) hepatic impairment. Simeprevir 150 mg QD was administered for 7 days under fed conditions to Panels A and B (Panel B dose and enrollment were determined following evaluation of Panel A PK/safety data). Simeprevir PK profile was determined on Day 7 up to 48 hours post-dose. Clinical laboratory parameters, cardiovascular safety and adverse events (AEs) were monitored. PK data from heptatically-impaired volunteers in both panels were compared with Panel A controls, and with historical data from HCV-infected OPERA-1 patients with compensated liver disease (simeprevir 150 mg QD cohort).

Results: All patients completed the study. Table 1 shows LS means ratios (90% CI) for comparisons. One serious AE (pneumonia, unrelated to study drug) occurred in Panel A. There were no permanent discontinuations due to AEs.

Conclusion: Simeprevir exposure was ~2-fold higher in volunteers with moderate hepatic impairment versus matched healthy controls. Exposure in volunteers with severe impairment was higher than in both those with moderate impairment and HCV-infected patients with compensated liver disease (~2-fold and ~3-fold, respectively). Simeprevir was generally well tolerated in this study. Dosing recommendations based upon results of this and other studies will be presented.

Conflict of interest: Janssen employee
Abstract: O_05_PK

PK/PD of Drug Efficacy and Toxicity

Simeprevir (TMC435) does not prolong the QT/QTC interval in healthy volunteers

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Background: Simeprevir (TMC435) is an investigational, once daily, potent, oral HCV NS3/4A protease inhibitor currently in Phase III clinical development for the treatment of chronic HCV infection. Drug-induced prolongation of cardiac repolarization may increase the risk of life-threatening arrhythmias. Current ICH E14 guidance requires the conduct of a human thorough QT/QTc trial (TQT), to assess QTc interval prolongation and pro-arrhythmic potential of investigational drugs. This Phase I TQT study (TMC435-C117; NCT01269294) evaluated the effect of steady-state simeprevir on QT/QTc interval at therapeutic and supratherapeutic doses in healthy volunteers.

Methods: This double-blind, double-dummy, randomized, 4-period crossover, placebo- and positive-controlled trial involved 60 healthy volunteers. Trial sensitivity was established with moxifloxacin. Each subject received study medication in 4 sessions (Treatment A: simeprevir 150 mg QD for 7 days [therapeutic dose]; Treatment B: simeprevir 350 mg QD for 7 days [supratherapeutic dose]; Treatment C: a single dose of moxifloxacin 400 mg on Day 7; Treatment D: placebo for 7 days) with a washout period of ≥10 days between treatments. 24-hour Holter ECGs were performed on Days 1, 7, and 8 of each session. A non-inferiority criterion of <10 ms for the upper 90% confidence intervals (CIs) for QTcF changes from baseline at all time points was set (ICH E14 guidelines). Trial sensitivity for moxifloxacin vs placebo was achieved if the lower limit of the 97.5% CIs for QTcF changes from baseline to at least one pre-specified timepoint was >5 ms. Blood samples were collected for pharmacokinetic analysis, and safety and tolerability monitored throughout the trial.

Results: The largest differences between simeprevir and placebo in relation to changes in QTcF from baseline were observed 1 hour post-dose for simeprevir 350 mg (mean difference: 1.2 ms, 90% CI: [-0.95, 3.32]) and 3 hours post-dose for simeprevir 150 mg (mean difference: 0.8 ms, 90% CI: [-1.26, 2.79]); both were below the pre-defined non-inferiority criterion (10 ms). The criterion for trial sensitivity (moxifloxacin) was met (mean difference: 11.3 ms, 97.5% CI: [8.09, 14.49]). No consistent or clinically relevant changes over time were observed in heart rate, PR interval, QRS width, or ECG morphology with simeprevir. After administration of simeprevir 350 mg QD, mean Day 7 simeprevir exposure was approximately 10 times higher compared with simeprevir 150 mg QD. Administration of both simeprevir doses was generally safe and well tolerated during this trial.

Conclusions: Steady-state simeprevir did not prolong the QT/QTc interval at either therapeutic or supratherapeutic doses in healthy volunteers.

Conflict of interest: Janssen employee
Abstract: O_06_PK

PK/PD of Drug Efficacy and Toxicity

The Effect of Single 60 mg and 180 mg Doses of Daclatasvir on the QTc Interval in Healthy Subjects

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Background and aims: Daclatasvir (DCV, BMS-790052) is a highly selective, first-in-class, HCV NS5A replication complex inhibitor. Since some non-antiarrhythmic drugs have the ability to delay cardiac repolarization, an effect that results in prolongation of the QT interval, a thorough cardiac assessment (per E14 guidelines) is frequently undertaken during clinical development. DCV displays linear, non-time-dependent pharmacokinetics and no major metabolites have been identified; thus, single therapeutic (60mg) and supra-therapeutic doses (180mg) were considered sufficient to evaluate the effect of DCV on the QTc interval. Selection of the supra-therapeutic dose was based on the results of an interaction study with ketoconazole, a potent CYP3A4/P-gp inhibitor, which resulted in 1.6- and 3.0-fold increases in DCV Cmax and AUC, respectively.

Methods: This was a randomized, partially-blinded, placebo-controlled, positive-controlled, 4-way crossover study in healthy subjects (N=56). Fasted subjects were randomized to blinded DCV 60mg, DCV 180mg, matched placebo, or open-label moxifloxacin 400mg (positive control) in one of four sequences, with a 3-day washout between each period. Triplicate electrocardiogram measurements were obtained pre- and post-dosing. Electrocardiogram parameters including heart rate, QTc, QRS, PR intervals, and changes in waveform morphology were assessed. QTc was corrected using Fridericia's method (QTcF) and least-square mean differences in ΔQTcF from baseline between placebo and active treatment (ΔΔQTcF) calculated.

Results: No clinically relevant ΔQTcF prolongations were observed following administration of single 60mg or 180mg DCV doses; all upper-bound confidence intervals (CIs) were <5 msec. Moxifloxacin lower-bound CIs were >5 msec at 2–4-hour time points, confirming the assay sensitivity. Neither DCV dose had a clinically relevant effect on heart rate, QRS or PR intervals, or waveform morphology. No concentration-response trend was noted in a random coefficient regression model examining ΔΔQTcF versus DCV concentration. DCV pharmacokinetics were linear \([\text{C}_{\text{max}}, \text{AUC}_{(0-T)} \text{ and AUC}_{(\text{INF})}]\) were approximately 2.5-, 3.0- and 2.9-fold higher, respectively, following a single dose of 180mg vs 60mg). Both DCV doses were generally well tolerated in this population.

Conclusions: Single DCV 60mg or 180mg doses did not have any clinically relevant effect on QTc interval, electrocardiogram waveform morphology, or other electrocardiogram parameters.

Conflict of interest: Employee of Bristol-Myers Squibb.
Abstract: O_07_PK

Novel drug formulations

Romiplostim’s Effect to Optimize SVR with Telaprevir, Ribavirin, And Peg Interferon-alfa 2a in Thrombocytopenic Cirrhotics with CHC. RESTART C Trial

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Objectives: Treating CHC (Chronic hepatitis C) cirrhotic patients with thrombocytopenia is often challenging; requiring dose reduction or even discontinuation of treatment to avoid complications. Significant dose reduction affects the response guided therapy (RGT); adversely affecting outcomes. Thrombopoietin (TPO) agonists are used to avoid disruption or therapeutic failure to optimize SVR (Sustained Virological response). This study evaluated the use of TPO agonist in thrombocytopenia in cirrhotics with treatment experienced CHC-GT1 (CHC-Genotype 1) on treatment with Telaprevir, Ribavirin (RBV) and Peg Interferon-alfa 2a (p-IFNα-2a).

Methods: Total of Forty five (n=45) cirrhotic treatment experienced CHC-GT1 patients with a mean MELD of 16 and mean platelet count 95 thousand were recruited and subdivided into three groups. Group A- (n=15) Received placebo plus reduced dose of p-IFNα-2a with RBV and Telaprevir. Group B (n=15) Received Romiplostim 500 mcg lead in 1 month prior to initiation of therapy and SOC with Telaprevir. Group C (n=15) Received Elthrombopag 50mg orally daily lead in prior 15 days and SOC with Telaprevir for 12 weeks. RGT was analyzed with serial platelet counts, hemoglobin/hematocrit, absolute neutrophils count and platelet antibodies. HCV RNA quantitative count was measured at 1ST, 2ND, 4TH, 12TH 24TH, 36TH and 60TH weeks for SVR.

Results: See table. ( VRVR- Very Rapid Virological Response, ETVR- End to treatment Virological Response, R-Relapser, PR- Partial Responder, BT- Break through )

Conclusion: This study demonstrates the efficacy of Romiplostim in thrombocytopenic cirrhotics with treatment experienced CHC-GT1 in optimizing SVR (Group A- 53%, Group B- 67% and Group C- 60%). A larger trial is needed to validate.

No conflict of interest

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Abstract: O_08_PK

Drug Interactions

ABT-450/r-containing direct-acting antiviral regimens are not associated with adverse changes in serum lipids or glucose in HCV genotype 1-infected patients in the AVIATOR study

D. Cohen1, G. Liossis1, M. Knauss-Townsend1, C. Marincic1, L. Larsen1, R. Trinh1, T. Podsadecki1, B. Bernstein1

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Background: ABT-450 is a potent NS3 HCV protease inhibitor identified by Abbott and Enanta and dosed with ritonavir 100 mg (ABT-450/r); ABT-267 is an NS5A inhibitor and ABT-333 is a non-nucleoside polymerase inhibitor. A 12-week regimen of the 3 direct-acting antiviral agents (DAAs) and ribavirin (RBV) without interferon yielded high sustained virologic response (SVR) rates following 12 weeks of dosing in HCV genotype 1 (GT1)-infected non-cirrhotic patients. We examined the impact of 12 or 24 weeks of 3 DAA + RBV (ABT-450/r 150/100 mg once daily combined with ABT-267, 25mg once daily, ABT-333, 400mg twice daily, and weight-based RBV twice daily), on fasting serum glucose (SG), total cholesterol (TC), and triglycerides (TG) in patients in the M11-652 (AVIATOR) trial.

Methods: 159 treatment-naïve patients and 88 prior null responders received 3 DAA+RBV for 12 or 24 weeks. Fasting SG, TC and TG were measured at baseline and during 12 or 24 weeks of treatment in AVIATOR. The mean changes from baseline to week 12 or 24 in each parameter were calculated by previous treatment status and duration of treatment. The number of patients with Grade 3-4 elevations in any parameter was tabulated.

Results: Demographics, virologic results, and lipid results are shown in the table. Mean SG, TC and TG remained stable or decreased from baseline to end of treatment in each subgroup. Two patients had elevated SG; one at a single visit, and one at 2 non-consecutive visits. Three patients had TG elevations; two at a single visit and one at 2 non-consecutive visits. Both SG elevations and three of the four TG elevations were measured in non-fasting samples.

Conclusions: Interferon-free regimens containing ABT-450/r were not associated with clinically meaningful changes in serum glucose or lipids when administered for up to 24 weeks to HCV GT1-infected non-cirrhotic patients.

Conflict of interest: Employees of abbvie Inc.
Abstract: O_09_PK

Late Breaker

MK-5172 Pharmacokinetic/Pharmacodynamic relationship between Transaminase Levels and Plasma Pharmacokinetics following administration of MK-5172 with Pegylated Interferon alfa-2B and Ribavirin(PR) to HCV genotype (G)1 treatment naïve patients

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Background: MK-5172 is an HCV NS3/4A protease inhibitor with a high barrier to resistance. 332 treatment-naïve G1-infected patients were randomized to receive boceprevir+PR or MK-5172 100-, 200-, 400-, or 800-mg QD + PR for 12 weeks followed by 12 (RVR achieved) or 36 weeks (no RVR) of PR. At all four doses of MK-5172, therapy was highly effective with ≥86% achieving SVR24, and with >92% of patients with the HCV RNA target not detected (TND) at the last visit on record. While ALT/AST levels normalized by week 4, they increased to >2X and >5X upper limit of normal (ULN) in a minority of patients in a dose-dependent fashion. The percentage of patients with late ALT/AST levels >2X ULN was 2%, 9%, 19%, and 23% at doses of 100, 200, 400, and 800 mg, respectively. The percentage of patients with late ALT/AST levels >5X ULN was 0%, 1%, 6%, and 9% at doses of 100, 200, 400, and 800 mg, respectively. Analyses were conducted to explore the relationship between plasma pharmacokinetics (PK) and late ALT/AST elevations.

Methods: Patients in a PK sub-study provided MK-5172 plasma samples at select visits through Week 12. PK/safety correlations were analyzed, including PK endpoints for steady-state Ctrough and C2hr and two safety endpoints (late ALT/AST >2xULN or >5xULN). Logistic PK/safety regression analyses were conducted to assess the probability of the safety endpoint occurring at a given PK parameter value; the PK predictability for each endpoint was determined by performing Receiver Operating Characteristic and Negative Predictive Value analyses.

Results: Geometric mean (GM) Ctrough and C2hr values were well-correlated with both safety endpoints. A GM Ctrough and GM C2hr of 56 nM and 1470 nM, respectively, were predicted to have a ≤10% mean probability of ALT/AST >2xULN occurring, and are ~2- and ~4-fold above the upper 90% Confidence Interval (CI) for the 100-mg dose. A GM Ctrough and GM C2hr of 128 nM and 4675 nM, respectively, are predicted to have a mean probability of ≤5% of >5xULN occurring, and are ~4- and ~13-fold above the upper 90% CI for the 100-mg dose.

Conclusions: Plasma MK-5172 PK is well-correlated with key ALT/AST safety endpoints. Combined with high SVR rates for the 100-mg dose, the PK/Safety analysis supports the low risk for a hepatic safety signal and continued development of MK-5172 at a dose of 100-mg QD.

Conflict of interest: Employees of Merck.
Abstract: O_10_PK

PK/PD modeling

Combination therapy with simeprevir and TMC647055/low dose ritonavir: dose anticipation using PBPK modeling and dose optimization in healthy subjects

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Background: Simeprevir is a once daily potent hepatitis C virus (HCV) NS3/4A protease inhibitor in Phase III development, and TMC647055 is a novel HCV non-nucleoside NS5B polymerase inhibitor. The combination simeprevir 150 mg qd + TMC647055 1000 mg bid, given to HCV genotype 1 infected patients for 10 days, showed better antiviral activity than each compound alone. As the plasma exposure to both compounds decreased with repeated dosing due to CYP3A4 induction by TMC647055, addition of the CYP3A4 inhibitor ritonavir (r) at a low dose was considered in order to counteract TMC647055 CYP3A4 induction.

Methods: Physiologically based pharmacokinetic (PBPK) models were built for simeprevir and TMC647055. Simulations of the interactions upon coadministration of simeprevir, TMC647055 and ritonavir were conducted considering dose dependent effects on CYP3A4 in the gut and in the liver. Subsequently, doses needed for coadministration were predicted in order to achieve the exposure previously determined following administration of simeprevir or TMC647055 alone at doses proven to be generally safe and active. An open-label, sequential trial, consisting of three 14-day treatment periods separated by a washout period was then conducted. Ten healthy volunteers received simeprevir 150 mg qd, the combination simeprevir + TMC647055/r at the doses predicted using PBPK, and the combination at doses defined after interim analysis of the pharmacokinetic data from the first two periods. Blood samples for pharmacokinetic evaluation were collected.

Results: The doses derived from PBPK modeling were 50 mg qd simeprevir, 300 mg qd TMC647055, and 20 mg qd ritonavir. In healthy volunteers, TMC647055 exposure remained stable upon repeated administration of this dosage regimen, and TMC647055 pharmacokinetic parameters (Cmax, Cmin, AUC(0-24h)) were similar to those obtained at 1000 mg bid alone (historical data). Simeprevir exposure increased with repeated dosing but was lower than at 150 mg alone. Simeprevir and TMC647055 doses 2-fold higher were tested in the last treatment period with a ritonavir dose of 30 mg. The treatment was safe and well tolerated. TMC647055 exposure increased slightly during the treatment, and simeprevir exposure was 2-fold higher than at 150 mg alone.

Conclusions: As predicted from PBPK modeling, the CYP3A4 inducing effect of TMC647055 was counteracted by a low dose of ritonavir. This allowed qd dosing of TMC647055. A trial in HCV-infected patients is ongoing with co-administration of simeprevir and TMC647055/r at doses derived from the exposures obtained in healthy volunteers.

Conflict of interest: Employees of Janssen Pharm.
Abstract: O_11_PK

PK/PD modeling

A population model linking plasma and intracellular ribavirin pharmacokinetics in persons with Chronic Hepatitis C Virus

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Background: Ribavirin (RBV) is a vital component of many treatment regimens for chronic Hepatitis C virus (HCV). Following oral absorption, RBV is transported into all cell types where it is phosphorylated to the monophosphate (RMP), diphosphate (RDP), and triphosphate (RTP). Despite decades of use, the pharmacology of RBV, especially the intracellular pharmacology, has not been well characterized. We developed a model to determine the distribution pharmacokinetics of RBV from plasma to cells and to characterize the phosphorylation profile in peripheral blood mononuclear (PBMCs) and red blood cells (RBCs).

Methods: Twenty sevensubjects participating in a RBV PK study (NCT01097395) who received RBV and peginterferon alfa 2a, with (N=9) or without telaprevir (N=18) were included in the analysis. Intensive samples were collected after first dose and at steady-state (week 9-13), and sparse samples were collected at Weeks 1, 2, 4, 16, 24 and 48. Plasma RBV concentrations (N=503) and RMP, RDP and RTP concentrations in PBMCs and RBCs (N=220 each) were measured using validated HPLC-UV and LC-MS/MS assays, respectively. Plasma data were fit to a two-compartment model. Phosphorylation model consists of one compartment representing RMP, with uptake and phosphorylation rate constant $k_{mp}$, disappearance rate constant $k_{mpout}$, and rapid equilibrium partition coefficients for RDP/RMP ($R_{dpmp}$) and RTP/RDP ($R_{tpdp}$), respectively. The following covariates were tested: age, weight, creatinine clearance (CrCl), gender, race, and degree of fibrosis. Population analyses were performed using ADAPT 5 (MLEM).

Results: Plasma PK of RBV was adequately described by a two-compartment model. The population parameter estimates for apparent total clearance (CLt), central volume of distribution (Vc), distributional clearance (CLd) and peripheral volume of distribution (Vp) were 14.7 L/h, 722 L, 103 L/h, and 3300 L, respectively. RBV plasma terminal half-life was ~208 hours. Inter-individual variability (IIV) was estimated to be 28.5, 41.7, 57.9, and 33.0 %CV, respectively. The covariates identified were body weight on CLt, Vc and Vp, and CrCl on CLt. Intracellular RMP, RDP and RTP levels were adequately described by a one-compartment model. $k_{mp}$, $k_{mpout}$, $R_{dpmp}$ and $R_{tpdp}$ estimates were 0.913 (pmol/10^6 cells)/(μg/mL) h⁻¹, 0.504 h⁻¹, 0.432, and 7.77, respectively, for PBMC, and 0.0221 (pmol/10^6 cells)/(μg/mL) h⁻¹, 0.0185 h⁻¹, 4.23 and 8.31, respectively, for RBC. IIV for $R_{dpmp}$ and $R_{tpdp}$ ranged from 27-46 %CV, whereas IIV for $k_{mp}$ and $k_{mpout}$ ranged from 57-89 %CV.

Conclusions: We developed a linked population PK model which describes plasma RBV and intracellular RMP, RDP and RTP in PBMCs and RBCs using both non-steady state and steady state concentrations from patients undergoing RBV-based HCV treatment. Body weight and CrCl significantly predicted RBV PK. Unlike prior reports, we found no difference in RBV PK at first dose vs. steady state. There is rapid equilibrium between RMP, RDP, and RTP in PBMCs and RBCs, suggesting conversion from RBV to RMP is the rate limiting step. Conversion to RMP and RMP loss occurred faster in PBMCs vs. RBCs. This model describes the distribution PK and cell-specific phosphorylation profiles of RBV and can be extended to inform RBV dosing in the era of HCV direct acting antivirals.

No conflict of interest
Abstract: **O_12_PK**

**Novel drug formulations**

**Pharmacokinetic Modeling of the Relationship Between SVR and Plasma Concentrations of Faldaprevir or BI207127 in HCV GT1-infected Patients in SOUND-C2**

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**Introduction:** The SOUND-C2 study assessed faldaprevir (FDV) 120 mg QD plus BI207127 600 mg BID or TID with and without ribavirin for 16, 28, or 40 weeks in over 360 HCV GT1 treatment-naive patients. Outcomes varied depending on viral subtype (GT1a and GT1b) and patient IL28B genotype (CC and non-CC). All GT1b-infected patients, and GT1a-infected patients carrying a CC IL28B genotype (GT1a-CC), had high SVR rates in ribavirin-containing arms (up to 85% and 75%, respectively). Relationships between SVR12 and plasma concentrations of FDV and BI207127 were investigated in a post-hoc analysis.

**Methods:** Geometric mean trough concentrations of FDV and BI207127 were investigated as predictors of SVR12 in regression models. Data were analyzed for GT1a-CC patients and for GT1b patients (CC or non-CC) for the 16-, 28- and 40-week ribavirin-containing regimens. GT1a non-CC patients and all patients in the ribavirin-sparing arm were excluded because of their high virological failure rates. Separate logistic regression models were obtained for FDV and BI207127 for plasma concentrations obtained during different time windows in the first month of therapy.

**Results:** Patients with GT1b (any IL28B genotype) had flat or shallow relationships between trough concentrations of FDV and BI207127 and predicted SVR12. The predicted SVR12 was high throughout the concentration ranges examined. For both drugs there was a significantly greater effect of trough concentration on SVR12 in GT1a-CC patients compared with GT1b patients; GT1a-CC patients had predicted SVR12 >70% only at higher concentrations.

**Conclusions:** GT1b-infected patients carrying any IL28B genotype are predicted to achieve high SVR rates across broad plasma concentration ranges of FDV and BI207127 given in combination. The results suggest that the daily doses tested in SOUND-C2 give adequate plasma concentrations in GT1b-infected patients. GT1a-CC patients are predicted to require higher levels of FDV and BI207127. Further investigations of plasma concentration-response relationships in various patient subgroups, factors contributing to low plasma levels, and potential confounders, should be performed. Phase III trials of FDV 120mg QD plus BI207127 600mg BID plus RBV for 16 or 24 weeks are ongoing in GT1b-infected patients.

*Conflict of interest: Employee of Boehringer Ingelheim*
Abstract: O_13_PK

Drug Interactions

Effect of Multiple-Dose Ketoconazole and the Effect of Multiple-Dose Rifampin on Pharmacokinetics (PK) of the HCV NS3 Protease Inhibitor Asunaprevir

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Background: Asunaprevir (ASV) is a selective inhibitor of HCV NS3 protease currently in phase 3 development in combination with daclatasvir (DCV), a first-in-class NS5A replication complex inhibitor, and in phase 2b with DCV and the NS5B polymerase inhibitor BMS-791325. ASV shows in vitro activity against genotypes 1, 4, 5 and 6 and displays high liver:plasma concentration ratios in animal models (>40-fold). Previously presented clinical study results from coadministration of ASV with single-dose rifampin revealed marked increases in ASV C\(_{\text{max}}\) and AUC\(_{\text{tau}}\) (21-fold and 15-fold, respectively), suggesting that active uptake may account for preferential liver distribution. Subsequently, ASV was shown to be a substrate for human liver transporters OATP 1B1 and OATP 2B1 in vitro with K\(_{\text{m}}\) <1 \(\mu\)M. ASV is a substrate for CYP3A4 and P-glycoprotein. The effect of multiple dose rifampin and the effect of multiple dose ketoconazole as a model strong inducer and inhibitor, respectively, of CYP3A4 and P-gp were investigated in vivo.

Methods: Two open-label, nonrandomized one-way interaction studies of ASV with multiple-dose ketoconazole or multiple-dose rifampin were conducted in healthy male volunteers. Study AI447014 (N=19) investigated steady-state PK for ASV 200 mg twice-daily given alone (Days 1–7) or with ketoconazole (200 mg twice-daily; Days 8–14), with serial PK sampling on Days 7 and 14. Study AI447018 (N=20) investigated the effect of single-dose (results previously reported) and multiple-dose rifampin on ASV PK. For the multiple-dose assessments reported here, subjects received ASV 600 mg twice-daily alone (Days 10–16) or with rifampin (600 mg every evening; Days 17–23) with serial PK sampling on Days 16 and 23. ASV plasma concentrations were determined by LC/MS/MS methods. Non-compartmental PK were derived. Subjects were monitored for adverse events throughout the study. Ratios of geometric means (GMR) and associated 90% confidence intervals (90%CI) for ASV PK were estimated using linear mixed-effects models.

Results: Study AI447014: All subjects completed the study. Ketoconazole markedly increased geometric mean AUC\(_{\text{tau}}\) and C\(_{\text{max}}\) for ASV (GMR [90%CI]: 9.6 [8.6–10.8] and 6.9 [5.9–8.1], respectively). Study AI447018: 19/20 subjects (95%) completed the study, with one discontinuation for moderate diarrhea considered related to study drug by the investigator. Although reduced ASV exposure was anticipated, steady-state geometric mean plasma PK for ASV were variably affected when administered with multiple-dose rifampin (GMR [90%CI]: 0.79 [0.56–1.09] for AUC\(_{\text{tau}}\), and 0.95 [0.60–1.50] for C\(_{\text{max}}\)). Interestingly, subjects with no effect on, or increases in, ASV PK with multiple-dose rifampin had previously shown large increases in ASV PK with single-dose rifampin, with very high variability. Thus the effect of rifampin on liver uptake transporters confounded the assessment of metabolic induction. ASV administered alone and with rifampin or ketoconazole was generally well tolerated.

Conclusions: The effect of CYP3A4/P-gp induction by multiple-dose rifampin on ASV appeared to be confounded by inhibition of liver uptake via OATP transporters. However, ketoconazole markedly increased steady-state plasma ASV exposure, clearly demonstrating a significant interaction potential with agents that strongly influence activity of CYP3A4 and P-gp.

Conflict of interest: Employee of Bristol-Myers Squibb.
Abstract: O_14_PK

Drug Interactions

Evaluation of Drug Interaction Potential Between Daclatasvir and Sofosbuvir

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Background: Daclatasvir (DCV) is a first-in-class HCV NS5A replication complex inhibitor. Sofosbuvir (SOF) is a uridine nucleotide analogue HCV NS5B polymerase inhibitor. Study AI444-040 evaluated the all-oral, once-daily combination of DCV plus SOF, with or without ribavirin, in patients infected with chronic HCV genotype 1, 2, or 3. Data from AI444-040 demonstrated that sustained virologic response (SVR) was achieved in > 95% of patients. Both DCV and SOF have demonstrated very favorable clinical pharmacology profiles including few clinically relevant drug interactions. A sub-study of AI444-040 evaluated the effects of DCV co-administration on the pharmacokinetics (PK) of SOF and vice versa; SOF analyses included SOF major circulating metabolite and nucleoside analogue GS-331007. Concentrations of GS-331007 best correlate with SOF antiviral activity.

Methods: AI444-040 was an open-label, randomized study with 10 parallel treatment groups. Groups A and B (n=15 and n=16, respectively) were designed with a 24 week treatment period consisting of a 7 day lead-in of SOF 400 mg once-daily before initiation of DCV 60 mg once-daily coadministration (both without ribavirin). Patients were followed-up for a period of 24 weeks. Serial PK sampling occurred on Days 7 and 14. Plasma concentrations of DCV, SOF, and SOF predominant circulating metabolite GS-331007 were determined by validated LC/MS/MS methods. Non-compartmental PK parameters were derived. Pooled data of DCV exposures across all arms (n=87) were compared to historical data from study AI447-011: DCV 60 mg once-daily in combination with the NS3 protease inhibitor asunaprevir (with which there is no interaction) with or without peginterferon and ribavirin, also pooled across all study arms (n=70). Patients were monitored for adverse events throughout the study. Geometric means (GM) and associated coefficients of variation (CV%) were determined.

Results: All 31 patients in Groups A and B had evaluable PK and no early discontinuations were observed. Exposures of GS-331007 were similar with and without DCV; GM (CV%) for AUCtau was 11380 (29) and 12078 (35) ng*h/mL on Days 7 and 14, respectively. Exposures of SOF were approximately 35% higher in the presence of DCV, with GM (CV%) for AUCtau of 932 (51) and 1273 (49) ng*h/mL on Days 7 and 14, respectively. DCV exposures on Day 14 were comparable to historical data, with GMs (CV%) for AUCtau of 11702 (45) and 13439 (42) ng*h/mL, respectively. DCV administered with SOF was well-tolerated in treatment durations of 12 and 24 weeks.

Conclusions: The PK of GS-331007 was unchanged by the presence of DCV. No apparent effect of SOF on DCV PK was observed. These PK results demonstrate the absence of a clinically relevant interaction between SOF and DCV.

Conflict of interest: Employee of Bristol-Myers Squibb.
Abstract: O_15_PK

Drug Interactions

Daclatasvir, an HCV NS5A Replication Complex Inhibitor, has Minimal Effect on Pharmacokinetics of Midazolam, a Sensitive Probe for Cytochrome P450 3A4

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Background: Many drugs are metabolic substrates of cytochrome P450 3A4 (CYP3A4). The clinical development of new pharmaceuticals therefore requires assessment of their potential to alter the pharmacokinetics (PK) of other agents via the CYP3A4 system. Daclatasvir (DCV; BMS-790052) is a highly selective, first-in-class HCV NS5A replication complex inhibitor with picomolar potency, broad genotypic coverage (genotypes 1-6) in vitro, and a PK profile supportive of once-daily oral dosing. DCV 60 mg once daily is in phase 3 development as a part of combination therapy, and is under clinical investigation in multi-drug regimens – such as combinations of direct-acting HCV antivirals and in HCV-HIV coinfected patients receiving antiretrovirals – that offer a broad scope for CYP3A4-mediated PK interactions. DCV is a substrate and inhibitor of P-glycoprotein and a substrate of CYP3A4. In vitro, DCV displays modest, time-dependent CYP3A4 inhibition of uncertain in vivo relevance.

Methods: This was an open-label, non-randomized, single-sequence study (N=18) in healthy volunteers, to assess the effect of steady-state DCV on the single-dose PK of midazolam (MDZ), a sensitive CYP3A4 metabolic probe. Fasted subjects received a single oral dose of MDZ alone (5 mg) on Day 1, then repeated doses of DCV alone (60 mg once-daily) on Days 2–5, followed by a single concomitant dose of DCV with MDZ on Day 6. Serial PK samples for noncompartmental MDZ assessments were obtained through 24 hours on Days 1 and 6. MDZ concentrations were determined by a validated LC-MS/MS methodology. Ratios of geometric means (GMR) and their associated 90% confidence intervals (90%CI) for MDZ given with versus without DCV were estimated using linear mixed effects models on log-transformed data.

Results: Subjects were 94% male (17/18) and had a mean age of 29.6 (SD 7.9) years. Sixteen subjects (89%) completed the study, with two discontinuations for mild abnormal (vivid) dreams considered related to study drug by the investigator. Steady-state administration of DCV had minimal effect on the PK of a single dose of MDZ, with a GMR [90% CI] of 0.87 [0.83–0.92] for AUC[Inf] (49.0 versus 56.2 ng·h/mL) and of 0.95 [0.88–1.04] for Cmax (19.7 versus 20.6 ng/mL). The 90%CI for both parameters were entirely contained within the bioequivalence range for no effect [0.80–1.25]. DCV and MDZ administered alone or together were generally well tolerated.

Conclusions: Co-administration of steady-state DCV and single-dose MDZ had minimal effect on the plasma exposure of MDZ. Although there was a 13% reduction in geometric mean MDZ AUC[Inf], the GMR was within the standard range for bioequivalence. These data indicate that DCV will not affect the exposure of other CYP3A4 substrates, including other antiviral agents used in combination, to a clinically significant extent.

Conflict of interest: Employee of Bristol-Myers Squibb.
Abstract: O_16_PK

**Drug Interactions**

**Lack of PK interaction between the HCV protease inhibitor MK-5172 and methadone and buprenorphine/naloxone in subjects on opiate maintenance therapy**

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**Introduction:** MK-5172 is a reversible, noncovalent, competitive inhibitor of the hepatitis C virus (HCV) NS3/4A protease that is being developed for the treatment of chronic HCV infection. Intravenous drug use is one of the most common routes of HCV infection, and treatment for substance dependence in intravenous drug users frequently requires oral maintenance therapy with methadone or buprenorphine/naloxone. The aim of the present study was to evaluate the pharmacokinetic interaction of MK-5172 and methadone and buprenorphine in subjects on stable opioid maintenance therapy who are not HCV-infected.

**Methods:** This was a single-center, open-label, fixed-sequence, multiple-dose study in 24 adult male and female volunteers, ages 18-55 years (two parallel panels of 12 subjects each). Stable oral methadone (20 mg to 150 mg QD) or sublingual buprenorphine/naloxone (8/2 mg to 24/6 mg QD) was administered alone, and in combination with MK-5172 (200 mg QD) on Days 2-11. Pharmacokinetic samples for methadone and buprenorphine/naloxone were taken pre-dose and up to 24 hours post-dose on Days 1 and 10. Samples for MK-5172 pharmacokinetics were taken predose and up to 72 hours post-dose on Days 2 and 11. Since the subjects were maintained on individualized doses of methadone or buprenorphine/naloxone, the pharmacokinetic parameters for the opiates were dose-normalized for comparison purposes. Safety assessments included electrocardiograms, vital signs, clinical laboratory tests, physical examination, and adverse event monitoring.

**Results:** Coadministration of MK-5172 with methadone and buprenorphine/naloxone was safe and well tolerated, with no clinical manifestations of opiate toxicity or withdrawal observed. All 24 subjects enrolled completed the trial. Addition of MK-5172 to stable methadone maintenance therapy did not meaningfully change the AUC₀-2₄h or Cₘₐₓ of R-methadone (active enantiomer) with geometric mean ratios (GMRs) [90% confidence intervals (CIs)] of 1.09 [1.02, 1.17] and 1.03 [0.96, 1.11], respectively, nor of S-methadone with GMR [90% CI] 1.23 [1.12, 1.36] and 1.15 [1.07, 1.25], respectively. Addition of MK-5172 to buprenorphine/naloxone stable maintenance therapy did not meaningfully change the AUC₀-2₄h or Cₘₐₓ of buprenorphine with GMRs [90% CIs] of 0.98 [0.81, 1.20] and 0.90 [0.76, 1.07], respectively, nor of norbuprenorphine with GMR [90% CI] 1.13 [0.97, 1.32] and 1.10 [0.97, 1.25], respectively. The AUC₀-2₄h and Cₘₐₓ of naloxone were not meaningfully changed by addition of MK-5172 with naloxone GMR [90% CI] 1.10 [0.82, 1.46] and 1.00 [0.80, 1.27], respectively. When coadministered with methadone, or with buprenorphine/naloxone, mean steady-state MK-5172 AUC₀-2₄h and Cₘₐₓ were similar compared to historical control subjects.

**Conclusions:** There was no clinically meaningful impact of 200 mg QD MK-5172 on the pharmacokinetics of methadone or buprenorphine/naloxone maintenance therapy. When compared to historical controls, maintenance therapy with methadone or buprenorphine/naloxone did not significantly impact the pharmacokinetics of co-administered MK-5172. These results suggest that no dose adjustments of MK-5172, methadone or buprenorphine/naloxone are needed for co-administration of methadone or buprenorphine/naloxone with MK-5172.

Conflict of interest: Employee of Merck & Co.
Abstract: O_17_PK

Drug Interactions

Identification and Management of Potential Drug-Drug Interactions with Boceprevir and Telaprevir

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Introduction: When added to peginterferon alfa and ribavirin, boceprevir and telaprevir have increased the rates of sustained virologic response (SVR) to Hepatitis C virus (HCV) treatment. However, these drugs are associated with complicated dosing schedules, dietary restrictions, adverse effects, and clinically relevant drug-drug interactions. The complexity of current HCV therapy necessitates a multidisciplinary approach to patient care. Clinical pharmacists can play a vital role in the treatment of HCV by obtaining accurate medication histories from patients, identifying drug-drug interactions, and recommending appropriate management. The Hepatology and Infectious Disease clinics at the University of Colorado Hospital utilize a clinical pharmacist to identify and manage potential drug-drug interactions in persons being considered for HCV treatment. We sought to determine the frequency and type of drug-drug interactions identified in HCV mono-infected patients and HIV/HCV co-infected patients initiating boceprevir or telaprevir based HCV treatment.

Methods: A chart review was completed on patients initiating HCV-treatment in the Hepatology and Infectious Disease clinics between August 31, 2012 and April 11, 2013. The clinical pharmacist used all available resources for identifying drug interactions, including the primary literature, Lexicomp™, Micromedex™, the University of Liverpool’s hepatology drug-drug interactions database (www.hep-druginteractions.org), and when data did not exist, recommendations were made based on the clinical pharmacology of the agents involved. Drug interactions and clinical recommendations were discussed with providers and recorded in the patient’s medical record.

Results: Drug interaction screening was performed on thirty-six patients initiating HCV-based treatment. Twenty-two patients were hepatitis C mono-infected and fourteen patients were HIV and HCV co-infected. Thirty of the 36 patients had at least one drug interaction identified (83.3 percent). Seventy-one drug interactions were identified from a total of 278 medications screened (25.5 percent). The number of baseline medications per patient ranged from two to eighteen, with a mean of 7.7. Of these 71 drug interactions, 24 (33.8 percent) were with psychotropic medications, 14 (19.7 percent) were with analgesic medications, 6 (8.5 percent) were with anti-hypertensive medications, 6 (8.5 percent) were with corticosteroids (inhaled or nasal), 4 (5.6 percent) were with herbal products, and 17 (23.9 percent) were with other medication classes. As a result of the drug interactions, 16 medications were discontinued, 18 doses of medications were decreased, 14 medications were substituted for a medication without any drug interactions, and 23 medications were not dose adjusted but monitoring was increased.

Conclusions: In the first few months of our program, a clinical pharmacist identified and managed potential drug interactions in 83% of patients being considered for boceprevir or telaprevir-based HCV treatment. Successful identification and management of drug interactions is essential for optimizing therapeutic outcomes to HCV treatment.

No conflict of interest
Abstract: O_18_PK

Late Breaker

Evaluation of pharmacokinetic Drug Drug Interaction (DDI) between BMS-791325, an NS5B Non – Nucleotide Polymerase Inhibitor, Daclatasvir and Asunaprevir in triple combination in HCV Genotype 1 infected patients

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Background: Interferon and ribavirin free treatments for HCV may be achieved by combinations of direct-acting antivirals. Daclatasvir (DCV), an NS5A replication complex inhibitor, and asunaprevir (ASV), an NS3 protease inhibitor were combined with BMS-791325, a potent, selective non-nucleoside inhibitor of the NS5B polymerase, to treat HCV genotype (GT) 1-infected patients. This IFN- and RBV-free regimen was well-tolerated for 24 or 12 weeks and achieved SVR4 and SVR12 ≥89% in treatment-naive, hepatitis C virus (HCV) genotype (GT) 1 patients. All three drugs are P-glycoprotein substrates, CYP3A4 substrates, OATP1B1 inhibitors and P- glycoprotein inhibitors in vitro and/or in vivo. ASV induces CYP3A4, and is an OATP1B1 substrate. BMS-791325 appears to induce CYP3A4. BMS-794712 is the active major circulating metabolite of BMS-791325. No clinically meaningful DDI occurred previously between DCV and ASV. In this study, potential DDIs of the triple combination were assessed in a subset of patients.

Methods: Study AI443014 is a phase 2a open-label, multiple-dose study combining DCV (60mg QD), ASV (200mg BID, tablet), and BMS-791325 at two doses (75mg BID or 150mg BID) initially in 66 treatment-naïve, HCV GT 1-infected, non-cirrhotic patients for 12 or 24 weeks. Blood samples for pharmacokinetic analysis were obtained for up to 12 hours post-dose for ASV and BMS-791325 and up to 24 hours post-dose for DCV on Day 14 of combination therapy. Concentrations of all analytes were simultaneously quantified using a validated LC/MS/MS method. Non-compartmental pharmacokinetic parameters were derived using a validated program. The pharmacokinetics of all analytes in each regimen (N=12 for 75mg BID and N=18 for 150mg BID), including BMS-794712, were explored versus historical data in HCV-infected subjects graphically and using descriptive statistics.

Results: At the higher dose of BMS-791325 (150mg BID), geometric mean (CV%) AUCTAU for DCV and ASV on Day 14 was 11248 (36) and 1065 (78), respectively, versus 10700 (31) and 1528 (106), respectively, from historical data of ASV 200mg BID (N= 12) and DCV 60mg QD (N= 11) without PegIFNa/RBV in AI447011. In the 75mg BID cohort, BMS-791325 and BMS-794712 geometric mean (CV%) AUCTAU were 9554 (65) and 2364 (48), respectively, versus 9170 (34) and 2150 (35) respectively, from historical data of 75mg BID BMS-791325 with PegIFNa/RBV (N=12). Exposure of BMS-791325 at 150 mg was less than dose proportional. Metabolic ratio for BMS-794712 was ~25%. DCV exposure appears unaffected in this combination. ASV exposures appeared to be reduced by ~30% but variability was high. The range of observed AUCTAU values for all analytes overlaps considerably with historical data.

Conclusions: No clinically meaningful interaction was observed by addition of BMS-791325 to DCV and ASV and therefore no dosing adjustments are required for any of these agents when dosed together. Based on these results and clinical data to date, additional study of this triple combination, employing both doses of BMS-791325, is warranted.

Conflict of interest: Employee of Bristol-Myers Squibb.
Abstract: O_19_PK

PK/PD of Drug Efficacy and Toxicity

Development and validation of a novel dried blood spot assay for the quantification of ribavirin

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Background: Ribavirin is essential for a cure in hepatitis C (HCV) infection and pharmacokinetics/dynamics (PK-PD) information on this drug is important for appropriate dosing. However, current assays to gain PK-PD information require venous blood draw, separation of plasma and a lengthy extraction process. Dried blood spots (DBS) are a quick, low blood requirement, non-invasive and relatively inexpensive sample collection technique for PK-PD studies. Measurement of ribavirin using DBS would be a valuable method over the current methodology for quantitation because of these benefits. Use of DBS followed by liquid chromatography coupled to mass spectrometry (LC-MS/MS) was developed and validated for the measurement of ribavirin in patients with HCV.

Methods: DBS calibration standards (n=8; 0.05 µg/mL to 10.0 µg/mL) and quality controls (QCs) (n=4; 0.05 µg/mL, 0.15 µg/mL, 3.0 µg/mL and 8.0 µg/mL) were prepared in whole blood and spotted (30 µL) onto Whatman 903 protein saver cards. Spots were edge-punched at a 3 mm diameter and sonicated for 10 minutes in 200 µL of 100% methanol plus 20 µL of 0.25 pmol/µL isotopic ribavirin internal standard. Samples were then dried, reconstituted and subjected to LC-MS/MS. Each QC level was assessed with n=6 replicates in three separate runs to validate the assay for intra/inter-accuracy and precision. Stability was also assessed on QCs and patient samples. Unique characteristics of DBS including punch location, spot volume and hematocrit effects were also tested using this extraction-analysis procedure. Finally, correlation to plasma concentrations derived from a validated plasma methodology in the laboratory was performed using 15 patient samples.

Results: The calibration curve was linear (1/conc² weighted) over the concentration range with an average r² value of 0.997. The QC inter-and intra-assay accuracy was within ±9% and ±11%, respectively, with precision ≤ 8%. Overall recovery was ~70%, with no significant matrix effects observed. DBS specific tests showed that punch location, spot volume, and %hematocrit had no effects on RBV DBS results outcome. Center punch location was within ±9.1% of edge punches, spot volume from 10-60 µL resulted within ±8.6% of the control (30 µL spot) and a hematocrit range of 20%-63% tested within ±11% of the 50% hematocrit control. Standards, QCs and patient samples were shown to be stable for up to 10 months stored at -20°C. A Patient sample exposed to room temperature for up to one month tested within ±4.0% of immediate analysis of the sample. Storage condition (room temperature, 4°C, -20°C, and -80°C) was comparable (±11.5% ) for patient samples, standards and QCs. DBS concentrations were well correlated to plasma concentrations with resulting linear regression analysis of y=1.2x-0.15 and r²=0.981.

Conclusions: Ribavirin quantification in DBS was shown to be accurate, precise and stability indicating with good correlation to the validated plasma assay. Implementation of this assay will allow improved transport, storage and analysis of ribavirin patient samples and is promising for use in PK-PD studies of ribavirin in patients with HCV.

No conflict of interest
Abstract: O_20_PK

Advanced Investigations in Pharmacology

Metabolism and Excretion of Ledipasvir (GS-5885) in Humans

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Background: Ledipasvir (LDV, formerly GS-5885) is a novel NS5A inhibitor that has demonstrated potent genotype 1 anti-HCV activity and is currently being evaluated in Phase 3 studies as part of a fixed dose combination (FDC) with sofosbuvir. Preclinical data suggest that LDV is primarily excreted as unchanged drug in the feces. The present study was conducted to understand the metabolic and excretory pathways of LDV in humans.

Methods: Healthy male volunteers (n=8) were given a single oral dose of an ethanolic solution of 90 mg LDV containing 100µCi [14C]-labeled LDV in a capsule after a standardized meal. Blood, urine and feces samples were collected until prespecified standard criteria for collection of administered radiolabeled material were met. All samples were analyzed for total radioactivity, pooled plasma and excreta samples were subject to HPLC radioprofiling, and plasma samples were subject to HPLC-MS/MS analyses. Quantification of LDV was performed using a synthetic standard, and previously identified metabolites were quantified based on HPLC profiles of radioactivity. Safety was assessed by routine clinical and laboratory monitoring throughout the study.

Results: Eight subjects enrolled and received study drug. Seven subjects completed the study [1 discontinued early (Day 9) due to an adverse event (AE) unrelated to study drug (abdominal discomfort due to constipation)]. At the time of discontinuation, plasma and urine total radioactivity were below the limit of quantitation and ~85% of the total radioactive dose had been recovered; as such this subject was included in the analysis. Total recovery of radioactivity in excreta was 87 ± 7.8% (mean ± SD of dose), primarily in feces (86 ± 7.8%) with minimal excretion (1.2 ± 0.08%) in the urine. The predominant circulating species in plasma was LDV (> 98% of total radioactivity AUC). The whole blood to plasma ratio of total radioactivity ranged from 0.51-0.66 indicating exclusion of total radioactivity from erythrocytes. In all subjects, plasma and blood radioactivity were undetectable by 48 and 36 hours, respectively. LDV was the major species detected in the feces (70% of dose) with Oxy-LDV-3 (M19) accounting for 2.2% of the dose, the remaining unidentified components in the feces each accounted for a mean of less than 1.7% of the dose with 78% of the dose being quantified in the feces overall. Twelve adverse events (AEs) were reported, none were serious. All drug related AEs (n=3) were mild in severity, including change of bowel habit, headache, and photophobia. No Grade 2 or higher laboratory abnormalities occurred.

Conclusions: LDV is minimally metabolized with > 98% of systemic exposure as LDV that is primarily eliminated in the feces, with little role of renal excretion. These data indicate the potential for a favorable drug-drug interaction profile and ability for use in the setting of renal dysfunction.

Conflict of interest: Employee of Gilead Sciences
Abstract: O_21_PK

Late Breaker

Development, validation, and application of a LC-MS/MS assay for the quantification of telaprevir and its R-isomer in plasma

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Background: Telaprevir (TPV, VX 950) is a protease inhibitor (PI) used in the treatment of Hepatitis C Virus (HCV) infection. TPV epimerizes to an R-diastereomer (TPV-R, VRT-127394), which is 30-fold less potent compared with TPV. Our objectives were to develop a novel LC-MS/MS method that utilizes stable labeled internal standards for both TPV and TPV-R; to isocratically separate the TPV and TPV-R isomers; to evaluate the stability of TPV and TPV-R in acidified versus non-acidified plasma; and to apply the method to quantify TPV and TPV-R in HCV-infected patient samples.

Methods: TPV-R internal standard (TPV-R-IS) was prepared from TPV internal standard (d11-telaprevir, TPV-IS) by incubating with 1% ammonia solution at 37°C for ≥ 3 hours. Plasma calibration standards (n=10; 5 ng/mL to 5,000 ng/mL), LLOQ (5 ng/mL), and quality controls (QCs) (15 ng/mL, 300 ng/mL, and 4000 ng/mL) were prepared in acidified K2-EDTA plasma (100µL) with stable labeled internal standards. Samples were extracted with a liquid-liquid extraction procedure and subjected to LC-MS/MS. Each QC level was assessed with n=6 (LLOQ n=5) replicates in three separate runs to validate the assay for intra/inter-assay accuracy and precision. 150 plasma samples collected at variable times post-dose were obtained from HCV-infected patients participating in a clinical study. Plasma from the same blood draw was split; one aliquot was treated with 0.5% formic acid and the other aliquot was not. All 150 acidified samples were assayed for TPV/TPV-R.

Results: TPV-IS/TPV-R-IS ratio was approximately 1:1 following ammonia treatment. TPV and TPV-R was separated with isocratic chromatography using 45:5:5:0.1 (v:v:v:v) acetonitrile: isopropanol: water: ammonia as the mobile phase and Waters XBridge™ BEH Shield C18 column. The TPV/TPV-R calibration curves were fit to a quadratic (1/conc² weighted) curve over the concentration range with average r² values of 0.9983/0.9986. Accuracy values were ≤ 11.3%/10.9% for TPV/TPV-R QCs. Precision values were ≤8.6%/6.1%, respectively. Accuracy and precision for LLOQ was ≤15.4%/17.2% and ≤16.2%/15.2% for TPV/TPV-R, respectively. QCs in acidified plasma were shown to be stable for up to 8 hours at room temperature; extracted samples were shown to be stable for 8 days in the autosampler (15°C). The median (interquartile range) of TPV and TPV-R was 1841(1031, 2848) and 1121(462, 1842) ng/mL, respectively. The mean ratio of TPV-R/Total was 33.9%. Reproducibility of re-assayed (incurred) acidified samples TPV/TPV-R was ~4.7%/8.5%. There was no significant effect of acidification on TPV (-3.2% difference on average), whereas the average difference for TPV-R was 38%.

Conclusions: This sensitive and simple analytical method for TPV/TPV-R differs from prior methods because it achieves baseline separation in isocratic conditions and includes a TPV-R internal standard which enhances accuracy and precision. We have also demonstrated using patient samples that quantification of TPV does not require acidified plasma. Observed TPV and TPV-R concentrations in these 150 samples were within the range of published values.

No conflict of interest
Drug Interactions

The effect of food and different meal types on the bioavailability of simeprevir (TMC435), an HCV protease inhibitor in clinical development

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Background: Simeprevir (TMC435) is a potent, once-daily (QD), oral, investigational HCV NS3/4A protease inhibitor currently in Phase III clinical development. Simeprevir is readily absorbed when formulated as an oral solution or a capsule. In healthy volunteers, $t_{\text{max}}$ was 4 to 6 hours and $t_{\frac{1}{2}}$ ranged from 10 to 13 hours after repeated dosing. In HCV-infected patients, $t_{\frac{1}{2}}$ was approximately 41 hours, which supports a QD dosing regimen. EC$_{50}$ was 8 nM in a genotype 1b replicon cell line. This healthy volunteer study assessed the effect of food and different meal types on the pharmacokinetics (PK) of the simeprevir capsule formulation used in Phase III trials.

Methods: In a Phase I, open-label, randomized, crossover study, blood PK profiles were determined for 72 hours after administration of a single oral dose of simeprevir 150 mg. During three sessions, 24 volunteers received simeprevir formulated as a gelatin capsule under fasted or fed (standard breakfast [21 g fat, 533 kcal] or high-fat breakfast [56 g fat, 928 kcal]) conditions. Volunteers received the three treatments randomized according to a classical 6-sequence, 3-period Williams design; a washout period of at least 7 days was included between sessions. Safety and tolerability were monitored throughout.

Results: All volunteers completed the study. Under fasted, standard breakfast and high-fat breakfast conditions, mean $C_{\text{max}}$ was 817.9, 1286, and 1162 ng/mL, respectively, mean AUC$_{\infty}$ was 11460, 19450, and 17840 ng.h/mL, respectively, and median $t_{\text{max}}$ was 4.0, 6.0, and 6.0 h, respectively. Least square (LS) mean ratios for $C_{\text{max}}$ and AUC$_{\infty}$ (90% confidence intervals [CI]) were 1.60 (1.30–1.96) and 1.69 (1.36–2.08) for the standard breakfast versus fasted condition and 1.49 (1.22–1.82) and 1.61 (1.33–1.93) for the high-fat breakfast versus fasted condition. Treatment difference medians for $t_{\text{max}}$ (90% CI) were 1.5 h (1.0–2.0 h) and 1.0 h (0.0–2.0 h) for the fasted versus standard breakfast and fasted versus high-fat breakfast conditions, respectively. There were no adverse events of Grade 3 or greater severity and no serious adverse events.

Conclusions: In healthy volunteers, there was no impact of food type (high-fat versus standard) on simeprevir exposure following administration of the Phase III capsule. However, administration with food increased the relative bioavailability (61-69% increase in AUC after high-fat and normal caloric breakfasts) and delayed absorption by up to 1.5 hours, relative to values observed in the fasted state. It is recommended that simeprevir 150 mg is administered with food.

Conflict of interest: Janssen employee
Abstracts

8th International Workshop on Hepatitis C
Resistance & New Compounds
27 – 28 June 2013, Cambridge, MA, USA

Abstracts
Abstract: O_01A_R

**Novel HCV Polymerase inhibitors**

**Cross-Resistance of Sofosbuvir to Clinically-Relevant Ribavirin, NS3 Protease, NS5A, and NS5B Nucleoside and Non-nucleoside Inhibitor Mutations**

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**Introduction:** Sofosbuvir (SOF), a nucleotide prodrug of 2'-deoxy-2'-fluoro-2'-C-methyluridine monophosphate, is ultimately converted in hepatocytes to a pharmacologically-active nucleoside analog triphosphate that directly inhibits the NS5B polymerase. SOF has demonstrated pan-genotypic activity in genotype 1-6 in vitro and in vivo. SOF was previously tested with other classes of HCV antiviral inhibitors in vitro and demonstrated additive to synergistic activity in combination. As SOF will be used together with other HCV inhibitors for treatment of chronic HCV infection, cross-resistance of SOF to previously identified NS3 protease inhibitor, NS5A inhibitor, NS5B nucleoside inhibitor, NS5B non-nucleoside inhibitor, or ribavirin (RBV) resistance-associated variants (RAVs) was explored. In addition, as 30% of HIV-infected patients are also co-infected with HCV, the effect of HIV nucleoside inhibitors on SOF activity was examined.

**Methods:** Subgenomic replicons encoding previously published RBV, NS5A inhibitor, NS3 protease inhibitor, or NS5B nucleoside or non-nucleoside inhibitor RAVs were analyzed by luciferase-based EC₅₀ assay for susceptibility to SOF. In addition, the effects of increasing concentrations of the HIV nucleoside analogs tenofovir, FTC, 3TC, zidovudine, abacavir, stavudine, or didanosine on SOF activity was examined in genotype 1a replicon cells.

**Results:** SOF remained fully active against the previously-reported NS5B nucleoside inhibitor RAVs L159F and L320F both individually (1.2- to 1.8-fold) and in combination (2.3- and 2.2-fold change in EC₅₀ for genotypes 1a and 1b, respectively). SOF also remained active against the RBV-associated variants T390I and F415Y (0.9- and 1.0-fold change in EC₅₀, respectively). When tested against a panel of 15 different genotype 1a and 22 different genotype 1b clinically-observed NS3 protease inhibitor RAVs, no significant changes in SOF activity were observed (0.7- to 2.6-fold changes in SOF EC₅₀). Genotype 1 NS5B non-nucleoside inhibitor RAVs at sites I-IV similarly conferred no reduction in susceptibility to SOF. SOF also remained fully active against NS5A inhibitor RAVs in genotype 1-3 replicons encoding RAVs at amino acid positions 28, 30, 31, or 93. Analysis of the effects of HIV nucleoside analogs on SOF activity demonstrated that none of the 6 nucleoside inhibitors tested altered SOF replicon activity.

**Conclusions:** Cross-resistance analyses of SOF against clinically-relevant RBV, NS3 protease inhibitor, NS5A inhibitor, NS5B nucleoside, or NS5B non-nucleoside inhibitor RAVs demonstrates that SOF remains active against all RAVs examined including the nucleoside inhibitor RAVs L159F and L320F. Furthermore, NS5A inhibitor RAVs in multiple genotypes remain susceptible to SOF supporting the use of NS5A inhibitors in combination with SOF for broad genotypic treatment. Finally, HIV nucleoside analogs do not alter SOF replicon activity supporting the use of SOF in HIV-HCV co-infected patients.

Conflict of interest: Employees of Gilead Sciences Inc.
Abstract: O_01B_R

**Mechanism of resistance selection and evolution**

**In Vitro Selection of Resistance to Sofosbuvir in GT2a, GT2b, GT3a, GT4a, GT5a and GT6a Replicons**

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**Introduction:** Sofosbuvir (SOF; GS-7977) is the prodrug of a uridine nucleotide analog inhibitor of the hepatitis C virus (HCV) NS5B polymerase with demonstrated pan-genotypic activity against HCV in vivo and in vitro. In vitro resistance analyses of SOF were previously described for genotypes (GTs) 1a, 1b, and 2a replicons. However, as SOF will be used in combination for treatment of all HCV GTs, a thorough characterization of resistance profiles of SOF in other genotypes is warranted. In the present study, we selected and characterized resistance mutations to SOF in GT 2a, 2b, 3a, 4a, 5a and 6a replicon systems.

**Materials & Methods:** HCV GT2a, 3a, and 4a subgenomic replicons and chimeric GT2b, 5a and 6a replicons encoding the respective NS5B in a GT 1b Con-1 backbone, were passaged in the presence of increasing concentrations of SOF and selected with G418. A luciferase assay was used to monitor drug susceptibility changes to SOF. The emergence of NS5B variants during resistance selection was assessed by deep sequencing. Site-directed mutagenesis (SDM) was used to evaluate the contribution of observed NS5B variants to reductions in SOF susceptibility. SOF-selected replicon cells were also analyzed for cross resistance to other HCV inhibitors.

**Results:** S282T was the primary NS5B mutation selected in all 6 genotypes. The increased prevalence of the S282T mutation in the passaged replicon cell population correlated with reductions in susceptibility to SOF. SDM analysis of the S282T single mutant demonstrated a mild to modest reduction in SOF susceptibility (1.54- to 11.14-fold change). S282T mutants exhibited greatly reduced replication capacity ranging from 0.6% to 34% compared to wild-type replicons. Several additional variants were observed in selected replicon cell lines in conjunction with the S282T mutation, though by SDM analyses none of these variants individually conferred reduced susceptibility to SOF nor did these variants in combination with S282T enhance SOF resistance. Selected replicons remained sensitive to the NS5A inhibitor ledipasvir (GS-5885) while hypersensitivity to ribavirin was observed.

**Conclusions:** The NS5B S282T mutation is the primary SOF resistance mutation selected in vitro in GT 2a, 2b, 3a, 4a, 5a and 6a replicon cells. Levels of S282T detected by deep sequencing correlate with reductions in SOF susceptibility for all genotypes. Additional variants observed during selection did not contribute to reduced susceptibility to SOF. SOF-resistant replicons remain sensitive to SOF. SOF-resistant replicons remain sensitive to SOF. SOF-resistant replicons remain sensitive to SOF. SOF-resistant replicons remain sensitive to SOF. SDM analyses demonstrate that S282T mutants exhibit significantly reduced replication capacity as compared to wild-type replicons.

**Conflict of interest:** Employee and stock holder of Gilead Sciences, Inc.
Abstract: O_02_R

Novel HCV Polymerase inhibitors

Preclinical and in vitro resistance profile of potent nucleotide polymerase inhibitors of HCV: ALS-2200 and its single diastereomer, VX-135

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Introduction: Hepatitis C virus (HCV) NS5B polymerase inhibitor nucleotide analogs are emerging as important components of combination therapy for chronic hepatitis C (CHC). ALS-2200, a uridine analog, demonstrated potent antiviral activity with a 4.5 log₁₀ drop in HCV RNA over 7-days in HCV-infected patients. VX-135, a single diastereomer of ALS-2200, is advancing in Phase 2 clinical trials. Here, the preclinical and in vitro resistance profile of ALS-2200 and VX-135 are described.

Methods: Antiviral activities of ALS-2200 and VX-135 were evaluated in vitro using the HCV genotype (GT) 1b replicon. Studies sought to investigate the formation of the ALS-2200/VX-135 NTP in primary human hepatocytes and liver of non-rodent species. The inhibitory activity and replication capacity of ALS-2200 were determined using a transient Firefly luciferase genotype 1b (GT-1b) HCV replicon engineered to encode NS5B proteins from clinical isolates or containing nucleoside inhibitor-associated NS5B mutations. Resistance was evaluated using a stable GT-1b replicon after sequential passaging in increasing concentrations (up to 16 μM) of ALS-2200 by population sequencing.

Results: In the replicon assay, ALS-2200 and VX-135 exhibited potent antiviral activity with EC₅₀ of 150 nM and 117 nM, respectively. The EC₅₀ of ALS-2200 versus a panel of 14 replications containing NS5B regions from GT-1a or GT-1b clinical isolates ranged from 0.12 to 0.20 μM. Both compounds were rapidly activated to the NTP in vitro and in vivo. Incubation of primary human hepatocytes with ALS-2200 and VX-135 (50 μM) led to formation of 609 and 1174 pmol/million cells NTP, respectively. Following identical oral doses in dogs, comparable, high and sustained levels of NTP were formed in liver for both ALS-2200 (4.89 mM) and VX-135 (5.38 mM) at 24 hours. The NTP exhibited no significant inhibition of human DNA or RNA polymerases, was not incorporated into RNA by the human mitochondrial RNA polymerase, and ALS-2200/VX-135 did not inhibit mitochondrial protein synthesis.

Conclusions: ALS-2200 inhibited a GT-1b NS5B S96T replicon with similar potency (EC₅₀ = 0.21 μM) as the WT replicon. ALS-2200 also inhibited GT-2b WT and GT-2b NS5B C223H/V321I replicons with similar EC₅₀ values (0.09 vs. 0.07 μM). The GT-1b NS5B S282T mutation conferred a >38-fold reduction in the activity of ALS-2200 but showed reduced replication capacity to ~8% of WT levels. Passaging of a GT-1b replicon in ALS-2200 for >5 months resulted in the selection of a replicon with the NS5B amino acid substitutions S282T/T344N/E440G. Comparison of the S282T/T344N/E440G replicon to the S282T replicon revealed no differences in replication capacity or resistance to ALS-2200. In addition, ALS-2200 retained potency against >20 replicon variants resistant to NS3/4A, NS5A and non-nucleoside NS5B inhibitors.

Conflict of interest: Employee of Vertex Pharmaceuticals
Abstract: O_03_R

Mechanism of resistance selection and evolution

In vitro resistance studies of miravirsen (MIR), a novel anti-HCV therapeutic targeting the host cell factor miR-122

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Background: MIR is a β-D-oxy-Locked Nucleic Acid modified phosphorothioate anti-sense oligonucleotide targeting the liver-specific microRNA-122 (miR-122). miR-122 promotes HCV replication by direct interaction with two well conserved binding sites (S1, S2) within the 5' untranslated region (5'UTR) (Joplin, Science 2005). A third binding site (S3) in the 3'UTR has also been described (Henke, EMBO 2008). In these studies, we evaluated the development of resistance following in vitro serial passage of HCV replicon cells in the presence of fixed and escalating concentrations of MIR.

Materials and Methods: The HCV reporter cell line Huh-luc/neo-ET was serially passaged in the presence of G418 alone or G418 with fixed and escalating concentrations of MIR, SPC4729 (scrambled oligonucleotide negative control) or telaprevir for time periods up to 155 days. Concentrations ranged from 1 to 80 μM (2X to 160X the EC₅₀ concentration), 1.00 to 80 μM, and 0.60 to 6.00 μM (2X to 20X the EC₅₀ concentration) of MIR, SPC4729 and telaprevir, respectively. Colony formation and susceptibility assays were performed on passaged cells throughout the study. Sequence analysis was performed on stored samples from G418 control, MIR or SPC4729 passaged cells at days 39, 72, 117, 128 and 148 days of the serial passage study. Amplification and population-based sequence analysis of miR-122 binding sites (S1, S2 and S3) in the 5' and 3' HCV UTRs was accomplished by site-specific primed end-point RT-PCR followed by cycle sequencing.

Results: Passage of replicon cells in the presence of MIR was associated with a significant reduction of HCV RNA. Furthermore, a 25-day treatment with MIR up to 20X the EC₅₀ (10 μM) failed to give rise to distinct, resistant colonies. In contrast, replicon cells treated with telaprevir at 10X the EC₅₀ (6 μM) rapidly gave rise to resistant colonies at day 25. In dose-response assays using luciferase reporter activity as an endpoint, no reduction in susceptibility to MIR was observed for cells passaged in 10 μM MIR for 35 days. In genotype analyses, no nucleotide changes of significance (e.g., changes that were observed after passage with MIR that had not been seen following passage with SPC4729 or G418 alone) were observed in S1, S2 or S3 binding sites from cells passaged in the presence of MIR for periods up to 148 days.

Conclusions: In vitro passage of HCV replicon cells in the presence of up to 80 μM (160X the EC₅₀ concentration) of MIR, demonstrated no evidence of genotypic resistance up to 148 days of study. These results are consistent with sequence analyses of HCV RNA from Phase 2a clinical trials with MIR and provide further evidence of MIR’s high genetic barrier to resistance.

Conflict of interest: Consultant for Santaris Pharma A/S
Abstract: O_04_R

Late Breaker

Phase 2A Trial of BIT225, HCV-P7 Inhibitor, in Combination with Pegylated Interferon-alfa-2b and Ribavirin in HCV Genotype-1 Treatment Naive Subjects.

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Background: BIT225 is a novel HCV p7 ion channel inhibitor in clinical development for treatment of HCV. A phase 2a trial was designed to (i) evaluate the safety, tolerability and antiviral activity of BIT225 in combination with interferon alfa-2b and ribavirin, and (ii) characterize the pharmacokinetics of BIT225. The effects of dosing on viral E2, P7, and NS2 gene sequences were also examined.

Materials and Methods: HCV genotype 1 treatment naive subjects were randomized to receive BIT225 (200 or 400 mg BID) or placebo in combination with pegylated interferon alfa-2b and ribavirin (PEG/RBV) BID for 28 days followed by an additional 44 weeks of PEG/RBV alone. Pharmacokinetic studies were performed after single dose administrations of BIT225 on day 1 and day 28. The E2, P7 and NS2 genes were sequenced from virus isolated from plasma samples collected prior to the commencement of treatment and after 28 days of triple combination therapy.

Results: BIT225 400 and 200 mg cohorts achieved 48 week end of treatment virologic response(ETVR) of 100% and 88%, respectively compared to 75% for the cohort receiving PEG/RBV alone. BIT225 was effective against HCV genotypes 1a and 1b. BIT225 was generally well tolerated. Steady state pharmacokinetics for BIT225 was achieved by day 3 of therapy. Preliminary analysis indicates that Cmax and AUC pharmacokinetic parameters of BIT225 were approximately dose proportional with an apparent terminal half-life >16 hours on day 28, suggesting that BIT225 may be suitable for once daily dosing. Population sequencing of the HCV viral genes E2, P7 and NS2 was performed and several nucleotide changes were identified in the sequenced regions in all cohorts: There was no direct correlation of changes with BIT225 treatment.

Conclusions: The pharmacokinetic profiles and higher virologic response rates for BIT225 treatment cohorts compared to placebo demonstrate that a therapeutic level of BIT225 in the blood was achieved, sufficient to support an antiviral effect. No viral breakthrough was observed during the BIT225 treatment period and no detectable antiviral resistant viruses identified.
Abstract: O_06_R

Mechanism of resistance selection and evolution

Safety profile of Daclatasvir in combination with PegInterferon Alfa and Ribavirin in 1100 patient with chronic HCV infection treated in Phase 2 studies

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Background: Daclatasvir, a selective HCV NS5A replication complex inhibitor in phase 3 development for treatment of chronic HCV infection, has demonstrated pangenotypic activity and high sustained virologic response rates in combination with peginterferon/ribavirin (alfa/RBV) or other direct-acting antivirals. This analysis includes data from six phase 2 studies of daclatasvir + alfa/RBV in treatment-naïve and experienced patients of varying ethnicities.

Material and Methods: All available safety data through February 2012 from patients receiving daclatasvir in combination with alfa/RBV were aggregated into 3 groups by regimen and compared with placebo/alfa/RBV.

Results: Analysis included 1100 patients grouped by regimen: 1) daclatasvir 60 mg once-daily + alfa/RBV for 24 weeks (n=285); 2) daclatasvir 60 mg once-daily + alfa/RBV for durations other than 24 weeks (12, 16, or 48 weeks, n=220); 3) daclatasvir at doses other than 60 mg (3, 10, or 20 mg once-daily) + alfa/RBV for any duration (n=421); and 4) alfa/RBV for any duration (n=174). Patients were 64% male, mean age 50.5 years, 78.7% Caucasian, 8.5% black, 10.4% Asian, 52.5% HCV genotype (GT) 1a, 30.5% GT1b, 6.5% GT2, 7.3% GT3, and 2.8% GT4. The most frequent grade 1-4 adverse events were fatigue, headache, pruritis, insomnia, and nausea; each of these events occurred with similar frequency across the groups. Rates of treatment-related serious adverse events were 2.8%, 2.3%, 3.3%, and 2.9% in groups 1, 2, 3, and 4, respectively; rates of discontinuations due to adverse events were 6.3%, 5.9%, 4.0%, and 8.6%, respectively. Among grade 3-4 hematologic abnormalities, anemia was reported in 7.1% of patients treated with alfa/RBV (group 4) compared to 5.6%, 4.1%, and 5.0% of patients who received daclatasvir + alfa/RBV (groups 1, 2, and 3, respectively). Grade 3-4 neutropenia was reported in 31.0% of patients who received alfa/RBV compared to 23.2%, 23.7%, and 27.9% of patients in groups 1, 2, and 3, respectively. Rates of grade 3-4 thrombocytopenia (3.5%-5.3%) and grade 3-4 lymphopenia (12.8%-16.9%) were similar across the groups. There was no clear evidence of daclatasvir-specific safety signals and no discernible effect of daclatasvir dose or treatment duration.

Conclusions: Once-daily daclatasvir plus alfa/RBV was generally well tolerated, with a safety profile comparable to the known profile of alfa/RBV. Addition of daclatasvir to alfa/RBV did not increase the frequency of serious adverse events, discontinuations due to adverse events, or grade 3-4 lab abnormalities. These results support ongoing phase 3 clinical development.

No conflict of interest
Abstract: O_07_R

Clinical relevance and management of HCV drug resistance

Baseline Clonal Analysis of Protease Inhibitor Resistance Sites in Paired Liver and Serum Samples from Patients with Chronic Hepatitis C Infection

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Introduction: Recently approved protease inhibitors have revolutionized the treatment of chronic hepatitis C infection in genotype 1 infected patients. Efficacy of protease inhibitors is limited by the emergence of resistant viral variants on treatment. Studies of the NS3 protease region and identification of drug resistant variants have mainly focused on patient serum samples. Little is known about the role of the liver in harboring resistant viral variants. We therefore aimed to study differences in the HCV quasispecies population at known protease inhibitor resistance sites in liver versus serum samples of patients with chronic HCV genotype 1a infection with unfavorable predictors of treatment outcome.

Methods: With IRB approval and informed consent, serum samples and liver biopsy remnants were collected at the time of routine, clinically indicated percutaneous parenchymal liver biopsy. Viral RNA was extracted from serum using the QiAamp Ultrasense Virus Kit (Qiagen). RNA was extracted from homogenized liver biopsy remnants using Trizol and Chloroform. Extracted HCV RNA was reverse transcribed with genotype 1 specific primers. The protease region was amplified and the PCR product was cloned into a modified pUC vector (In-fusion, Clontech). Nine to 15 clones from liver and serum per patient were sequenced. Sequences were aligned using BioEdit Sequence Alignment Editor version 7.0.9. The following positions in the NS3 HCV protease were examined for substitutions: 36, 41, 43, 54, 55, 80, 122, 132, 138, 155, 156, 168, 170 and 175.

Results: Liver and serum protease regions were successfully amplified and sequenced from ten patients with chronic HCV genotype 1a infection. All patients were protease inhibitor treatment naïve. All patients had unfavorable predictors of treatment response such as prior non-response to interferon and ribavirin therapy, cirrhosis, HIV co-infection, status post liver transplantation, need for hemodialysis, or less favorable genetic background (IL28B CT or TT genotype). Clonal sequence analysis of 218 sequences (103 sequences from liver, 115 sequences from serum) revealed non-synonymous substitutions at sites of protease inhibitor resistance in six patients (T54S, Q80K, Q80R, S122G/N/C, I132V, V/I170T). These substitutions were present in liver and/or serum samples. Normalized amount of non-synonymous substitutions in all liver samples versus serum samples was similar (two-tailed student’s T-test: p=0.8). Synonymous substitutions at sites of protease inhibitor resistance were present in 9 patients. 172 synonymous substitutions were detected in 103 liver sequences and 190 synonymous substitutions were detected in 115 serum samples (two-tailed student’s T-test: p=0.8).

Conclusions: Clonal sequence analysis of paired baseline patient liver and serum samples revealed non-synonymous and synonymous substitutions at protease inhibitor resistance sites, some of which differed between serum and liver samples. It will need to be determined if the presence of substitutions in liver samples at baseline influences treatment outcome in this difficult to treat patient population.

No conflict of interest
Abstract: O_08_R

Technologies for assessment of resistance

Pretreatment genetic diversity in the protease-helicase region of hepatitis C virus affects resistance-barrier in patients naïve to direct antivirals

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Introduction: Genotype-specific responses to the recently approved ketoamide NS3 protease inhibitors are observed in hepatitis C virus treatment. Furthermore the rapid occurrence of resistant viral strains upon treatment with ketoamide NS3 protease inhibitors constitutes a major obstacle in the treatment of chronic hepatitis C virus infection. Whether the genetic diversity in the molecular target site of a ketoamide compound prior to treatment plays a role in treatment response and occurrence of resistance among distinct hepatitis C genotypes is poorly understood.

Methods: We retrieved worldwide NS3-sequence information of hepatitis C variants from public databases (660 dominant strains from patients naïve to direct antivirals). We applied simple measures from phylogeny, such as the number of transition and transversion nucleotide changes, the overall transition-to-transversion ratio, the estimated shape parameter, the number of segregating sites, nucleotide diversity, number of synonymous (silent) substitutions per site and non-synonymous (consecutive amino acid replacement) substitutions per site, and Shannon entropy to study the pretreatment genetic diversity and complexity in NS3-full-length as well as in the protease-helicase interface among 223, 358, 49 and 30 patients of genotype 1a, 1b, 2 and 3 respectively.

Results: To assess the genetic barrier to resistance we first analyzed the genetic diversity in full-length sequences of the NS3 protease-helicase complex and then focused on the protease-helicase interface in NS3 that is the molecular target site for peptidomimetic PIs. To do so, we selected a specific set of NS3 residues that was denoted as protease 'substrate-neighboring' residues. We found polymorphic sites more frequently in pretreatment variants of genotype 1b than 1a. A significant higher number of synonymous and non-synonymous substitutions were found in genotype 1b with transitions more frequently observed than transversions (P<0.001). Furthermore, higher average numbers of nucleotide differences per site were found in genotype 1b. Genotypes 2 and 3 showed overall significantly more nucleotide substitutions than 1a or 1b (P<0.001), as well as higher genetic diversity and Shannon entropy. Solely synonymous substitutions showed significant differences when compared between NS3 full-length and domain interface residues (P<0.001).

Conclusion: Our data suggest that high genetic diversity is not automatically linked with the rapid development of resistance mutations. Thereby the nature of a mismatch nucleotide exchange seems as the most important factor to the barrier to drug resistance. Especially the pretreatment frequency of transitions correlates to the genotype- or subtype-specific pace of resistance development in the clinical setting.

No conflict of interest
Abstract: O_09_R

Mechanism of resistance selection and evolution

Identification and Characterization of HCV Genotype 1L by Whole Genome Sequencing

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Introduction: The HCV genome is highly variable, with isolates classified into six confirmed genotypes, each of which has multiple phylogenetically distinct subtypes. During viral sequence analysis of baseline European samples from late phase telaprevir studies enrolling primarily genotypes 1a and 1b, we identified two samples reported as genotype 1 which we could not subtype based on NS3-4A sequence homology. To identify the genotype subtype of these two samples, we performed analysis of the nearly full length genome using massively parallel sequence analysis.

Methods: For the 2 untyped samples, a ~9kb amplicon was generated from nested RT-PCR of plasma samples collected before the initiation of treatment. A library for next-generation sequencing was prepared from the amplicon pool using the Nextera DNA Sample Preparation Kit. The amplicon library was sequenced using an Illumina HiSeq 2000 instrument. Fastq files were trimmed based on quality scores and filtered using the FASTX-Toolkit. Of the 4,541,684 and 3,736,418 reads generated from each plasma sample, 29% and 50% respectively were discarded because of low quality. The filtered fastq data were assembled de novo using VICUANA and then aligned to H77 using BLAST. A consensus genomic contig was generated from these alignments and used for a reference-guided assembly (BWA). The resulting assembled genomes spanned the entire amplified region of 9,079 bp, comprising ~94% of the H77 reference genome. 10,000x coverage was available across the entire genome, allowing for minority variant quantification. Maximum-likelihood phylogenetic estimates were obtained for the assembled genomes assuming a TN93 substitution model (+G+I; PhyML), with confidence of nodes evaluated using 100 replicates of the bootstrap. The potential of recombination to confound the phylogenetic signal was discounted using RDP3.

Results: Whole genome sequence (WGS) from these two isolates showed 94% nucleotide similarity to each other, yet only had weak similarity to confirmed subtypes 1a (Average ± StDev, 84.8 ± 0.75 % ), 1b ( 81.8 ± 1.47%), 1c ( 85.5 ± 0.58%), or 1g (81.5% ± 0.71) within the NS5B region commonly used for genotyping. Phylogenetic inference from WGS confirmed the monophyly of these two viral isolates and indicated their placement as a derived genotype 1 subtype, with both conclusions supported in 100% of bootstrap replicates. Phylogenetic inference from the NS5B region confirmed the placement of these viral isolates within the provisional subtype 1L clade with 94% bootstrap support.

Conclusions: We developed a WGS analytical pipeline to characterize isolates with undefined subtypes. For two isolates we confirmed the subtype as genotype 1L, which together represent the first complete genomes of this provisional subtype.

Conflict of interest: Employees of Vertex Pharm.
Abstract: O_10_R

Clinical relevance and management of HCV drug resistance

Analysis of HCV Genotype 5 and 6 NS5B Sequences from Clinical Samples

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Background: To increase efficacy and tolerability of HCV treatment across all genotypes, new direct-acting antivirals (DAA) are being developed with pan-genotypic potency and evaluated in clinical studies. However, sequencing assays supporting resistance testing have not been reported and limited sequence information is available for genotype 5 and 6. In this study, we developed a NS5B amplification/sequencing assay and analyzed the NS5B sequences from a panel of HCV genotype 5 and 6 DAA-treatment-naïve patient samples.

Methods: Viral RNA was extracted from HCV-infected genotype 5 and 6 patient plasma samples. Population and Illumina MiSeq deep sequencing were performed on the NS5B amplicons to generate nucleotide consensus sequences. For each patient sample, genotype and subtype were initially determined by the VERSANT® HCV Genotype INNO-LiPA 2.0 Assay. A BLAST alignment with the NS5B nucleotide consensus sequences determined the genotype and subtype of the highest similarity. For some samples, phylogenetic analysis was performed to confirm or correct the subtype discrepancy between INNO-LiPA and BLAST. Sequencing results were compared to the appropriate reference sequence (Genbank AF064490 for genotype 5, Y12083 for genotype 6) to identify the prevalence of amino acid variants. Deep sequencing results and phylogenetic analyses were used to examine intra-patient and inter-patient diversity.

Results: From the European HCV Database (euHCVDB), available nucleotide sequences with NS5B coverage (20 genotype 5, 19 genotype 6) were downloaded and aligned for primer design. Both genotype- and subtype-specific NS5B primers were designed and developed for cDNA synthesis and PCR amplification. NS5B amplification was attempted and resulted in the PCR amplification of 6/6 genotype 5 and 15/15 genotype 6 HCV-infected patient plasma samples. For genotype 5, NS5B sequencing confirmed available INNO-LiPA subtype result in 1/6 samples and allowed for subtype assignment in 5/6 samples for which INNO-LiPA subtype results were not available. For genotype 6, NS5B sequencing confirmed available INNO-LiPA subtype results in 3/15 samples and allowed for subtype refinement in 9/15 samples and subtype correction in 3/15 samples. NS5B sequencing results indicated that no nucleoside (NI), non-nucleoside (NNI) or ribavirin (RBV) resistance-associated mutations (RAM) were found in any genotype 5 samples. In contrast, for genotype 6, there were 10/15 samples with the NI RAM M289L, 13/15 samples with the NNI RAM V494A and 11/15 samples with the potentially RBV-associated mutation F415Y. In addition, an analysis of intra-patient variation between genotype 1 (n=3) and genotype 6 (n=6) samples showed that there was no difference in intra-patient diversity between these samples.

Conclusions: NS5B from 21/21 HCV genotype 5 and 6 DAA-treatment-naïve patient samples were successfully amplified and sequenced. NS5B sequencing information was essential for the refinement/correction of the INNO-LiPA subtype assignment in 17/21 samples. NI (M289L), NNI (V494A) and/or RBV (F415Y) RAMs were detected in 14/15 genotype 6 samples. No RAMs were detected in any genotype 5 samples. No evidence of difference in intra-patient diversity was observed between genotype 1 and genotype 6 samples.

Conflict of interest: Employees and/or stockholders of Gilead Sciences, Inc.
Abstract: O_11_R

Late Breaker

Resistance Analyses Using Deep and Population Sequencing after 3 Day Monotherapy with GS-9669, a Novel Non-Nucleoside NS5B Inhibitor in Genotype 1 HCV Patients

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Background: GS-9669, a novel NS5B non-nucleoside inhibitor (NNI, site II), displayed potent antiviral activity in HCV genotype (GT) 1 subjects during a multiple ascending dose clinical trial at 50mg, 500mg QD and 50mg, 100mg BID for 3 days. This study characterizes the virologic resistance observed in this phase I trial.

Material& Methods: The full-length NS5B gene was amplified and population sequenced for all patients (GT1a, n=50; GT1b, n=20) at baseline (BL), Day 4 (or earlier timepoint if viral load was < 1000 IU/mL) and Day 17. Illumina deep sequencing analysis was performed for GT1a (n=6) and GT1b (n=7) patients dosed with 500 mg QD at multiple timepoints through Day 17. NS5B from 39 patients was cloned into an NS5B shuttle vector and phenotypic analyses were performed.

Results: Patient isolates with M423I or M423V at BL had < 10-fold reductions in susceptibility to GS-9669 and partial treatment responses to GS-9669 with >2-log10 reduction of HCV RNA in 100-mg BID and 500-mg QD groups. M426L and V494I resistance-associated variants (RAVs) observed at BL did not affect GS-9669 susceptibility in vitro, and did not appear to affect antiviral response individually. From the on-treatment (Day 1 or 2) or end of treatment (Day 4 or 5) samples, RAVs at NS5B positions A486, R422 and L419 were detected in the majority of patients who received 500 mg QD and ≥ 50 mg BID, but only in 1/8 patients receiving 50 mg QD. Substitutions at position M423 were observed only in GT1a patients receiving low doses (50 and 100 mg BID). Deep sequencing analyses showed reduced frequency of resistant mutants through Day 17, however, most patients still had detectable RAVs at this point. New mutations in GT1b isolates (M423T, V494I I482V/N) were observed by deep sequencing but not by population sequencing. Linkage analyses for double mutants at positions 419 & 422, 422 & 423, and 419 & 423, and triple mutants at positions 419 & 422 & 423 and 482 & 486 & 494, with a cutoff of 1% demonstrated that majority of mutants were observed as single mutants, double mutants were observed in 3/13 patients at frequencies <2% and triple mutants were not observed in any patients. Phenotypic analysis demonstrated that viral isolates with multiple RAVs had reduced susceptibility to GS-9669 and VX-222, but wild-type susceptibility to other classes of HCV inhibitors including sofosbuvir (NI), GS-9451 (PI), GS-5885 (NS5A) and ribavirin.

Conclusions: Similar to other NNIs, RAVs were detected shortly after suppression of the wild-type virus. The lack of cross-resistance between GS-9669 resistant-mutants and sofosbuvir, GS-5885, GS-9451 and ribavirin, makes GS-9669 a candidate for use in combination with these inhibitors.

Conflict of interest: Employees and/or stockholders of Gilead Sciences, Inc.
Abstract: O_12_R

Late Breaker

Comprehensive Resistance Analyses in Patients Who Relapsed after Treatment with Sofosbuvir-Containing Regimens in five Phase 2 Studies

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Introduction: Sofosbuvir (SOF, formerly GS-7977), a novel HCV NS5B nucleotide inhibitor, demonstrated potent antiviral activity with broad HCV genotype coverage and high sustained virologic response (SVR) rates in patients. In vitro selection studies showed that NS5B S282T is the primary mutation selected by SOF in GT1a, 1b, and 2a replicon cells. In this study, we performed resistance analyses on patients that experienced HCV viral load relapse after treatment with SOF-containing regimens in five Phase 2 studies (P7977-0221, PROTON, ELECTRON, ATOMIC and QUANTUM arm C and G).

Methods: The HCV NS5B gene was amplified from patient serum samples at baseline and viral relapse time points. PCR products were analyzed using standard population sequencing and deep sequencing with an assay cut-off of 1%. PCR products from patient samples were also cloned into a replicon vector and tested for drug susceptibilities.

Results: Across different arms of the 5 Phase 2 studies, 671 patients received treatment with SOF monotherapy (n=10), or in combination with RBV, with or without interferon (n=661). No cases of on-SOF treatment viral breakthrough were observed. Among all SOF-treated patients, 76/671 patients qualified for resistance analysis. NS5B population sequences were obtained post-baseline for 75/76 patients and deep sequencing results were obtained for 73/76 patients. Notably, the S282T mutation was detected in only 1 patient, but not in any of the remaining 74 patients by either population or deep sequencing with a mutant detection sensitivity of 1%. The 1 patient with S282T detected during relapse had GT2b HCV infection and had received SOF monotherapy. Deep sequencing revealed >99% S282T in this patient at week 4 post-treatment, but <1% after week 12 post-treatment and subsequent timepoints. No other NS5B mutation could be identified by population or deep sequencing as being associated with SOF resistance. Population and clonal phenotypic analysis demonstrated that the GT2b S282T-containing virus was 15-20 fold less susceptible to SOF as compared to corresponding baseline virus. Phenotypic results were also obtained for another 64 patients at baseline and/or relapse and showed no change in susceptibility to SOF or RBV.

Conclusions: SOF demonstrates an exceptionally high resistance barrier as the S282T resistance mutation or other resistance mutations were not detected in any patient following SOF/RBV or SOF/PEG/RBV treatment across 5 Phase 2 studies and S282T was detected in only 1 patient after SOF monotherapy. These results support the potential use of SOF-containing regimens as a retreatment option in patients not achieving SVR after treatment.

Conflict of interest: Employees and/or stockholders of Gilead Sciences, Inc.
Abstract: O_13_R

Technologies for assessment of resistance

Persistence of HCV during and after otherwise clinically successful standard treatment for chronic hepatitis C

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Background and Aim: Resolution of chronic hepatitis C (CHC) following standard therapy with PEG-IFN/ribavirin (RBV) is considered when serum HCV RNA becomes repeatedly undetectable and liver enzymes normalize. However, long-term persistence of low levels of HCV RNA in plasma, lymphoid cells and liver has been reported when highly sensitive assays and testing of serial plasma and PBMC samples were applied. The aim was to re-analyze sequential plasma and PBMC samples from patients who resolved CHC and became HCV RNA negative by clinical laboratory testing due to PEG-IFN/RBV treatment.

Methods: Plasma samples (n=56) from 9 randomly selected patients who resolved CHC after a standard course of PEG-IFN/RBV therapy were collected before (n=11), during (range 48-68 wks; n=25) and up to 33.1 (range 12-88) wks post-treatment (n=20). PBMCs (n=23) from 3 patients before (n=5), during (n=10) and post-treatment (n=8) were also analyzed. Total RNA was extracted from 250 or 750 μl plasma and intact or PHA-stimulated PBMCs. HCV RNA was detected by RT-PCR/nucleic acid hybridization (RT-PCR/NAH; sensitivity <5 copies/μg RNA or <2 IU/ml). Clone sequence analysis of the HCV 5’-UTR from sequential plasma and PBMCs was done in 2 patients.

Results: HCV RNA was detected in 17/25 (68%) plasma and 8 of 10 (80%) PBMC samples collected from 8 of 9 patients during therapy, although only 5.4% plasma samples were positive by clinical assays. Among post-treatment HCV RNA-negative plasma samples by clinical assays, 9 of 20 (45.3%) were HCV reactive for up to 59 weeks post-treatment. Testing of RNA from 750 μl plasma increased HCV detection from 32.1% to 66.1% (37/56) compared to 250-μl samples. Testing naïve versus PHA-stimulated PBMCs enhanced HCV detection from 29.3% to 73.9% (17/23). Molecularly evident replication was found in 6/12 (50%) among PBMC reactive for virus RNA positive strand collected during or after treatment. Pre-treatment point mutations persisted in plasma and/or PBMCs during and after therapy. The frequency of HCV detection tended to decline in both plasma and PBMCs with longer follow-up.

Conclusions: HCV can persist at levels not detectable by standard clinical testing in both plasma and PBMC during and after PEG-IFN/RBV therapy. The findings imply the need for continued evaluation even after patients achieve undetectable HCV RNA post-treatment.

No conflict of interest
Abstract: O_14_R

HCV replication dynamics and pathogenesis

Reduction of mir-122 expression in IL28B CT/TT chronic hepatitis C patients who failed to pegylated-interferon plus ribavirin treatment


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Introduction: Mir-122 is highly expressed, in the liver, where it represents 70% of the total miRNAs. Mir-122 binding within hepatitis C virus (HCV) genome stimulates its replication, in vitro. A reduction of hepatic mir-122 expression has been associated with non-response (NR) in patients with chronic hepatitis C (CHC). Genome wide association studies identified a strong association between IL28B polymorphisms (rs12979860) and sustained virological response (SVR), in patients with CHC. The aim of the study was to investigate, in vivo, the association of mir-122 expression in the serum and in the liver with IL28B polymorphism and SVR.

Materials and Methods: Pre-treatment liver biopsies and serums from 133 patients with CHC were included. Sixty six patients achieved a SVR, and 64 failed to respond to the treatment, 43 were NRs and 21 were responder-relapsers (RR). 63 were complete early virological responders (cEVR) and 47 were primary non-responders (pNRs). Mir-122 expression was assessed in a total of 127 percutaneous liver biopsies and 83 serums. Mir-122 expressions in the liver and in the serum were both assessed by RT-q-PCR. IL28B rs12979860 polymorphism was analyzed by direct sequencing.

Results: A significant decrease in the mean level of hepatic mir-122 expression was observed for patients with a pNR as compared to cEVR (p=0.003) and for patients with failure to respond to the treatment (NRs+RRs) as compared to SVRs (p=0.016). In our series, patients with IL28B CC genotype had a higher hepatic expression of mir-122 than patients with CT and TT genotypes (p=0.01). Moreover, hepatic mir-122 expression was higher in CC patients when compared to CT and TT, in total patients (p=0.025) and in NRs (p=0.013). Mir-122 expression in the liver and in the serum were not associated (p=0.21). However both hepatic and serum mir-122 expression were associated with viral load at baseline (p=0.02 and 0.001 respectively) and transaminases levels (ASAT; p=0.03 and 0.009, respectively and ALAT p=0.03 and 0.004, respectively). Whereas a trend was found between hepatic mir-122 expression in Fibrosis 1 vs 2-3-4 within all HCV genotypes (p=0.06) a significant difference was observed in HCV genotype 1 patients (p=0.01).

Conclusions: We provide an analysis of mir-122 expression in patients with CHC. Patients with IL28B CT and TT genotypes who failed to PEG-IFN plus ribavirin therapy presented a reduction of hepatic mir-122 expression, at baseline, when compared to patients with CC genotype. Interestingly, viral load at baseline was associated with both hepatic and serum mir-122 expression. In patients with CHC hepatic mir-122 expression shows a 50% reduction compared to uninfected patients. Hepatic and serum mir-122 expression are not associated however both are significantly associated with viral load and transaminases values.

No conflict of interest
Author Index
<table>
<thead>
<tr>
<th>Author</th>
<th>Abstract Title</th>
<th>Abst #</th>
<th>Page #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basu, P.</td>
<td>Telaprevir &amp; Adjusted dose of Ribavirin in naive CHC-G1: Efficacy And Treatment in CHC in Hemodialysis population. TARGET C Trial</td>
<td>O_02_PK</td>
<td>4</td>
</tr>
<tr>
<td>Basu, P.</td>
<td>Romiplostim’s Effect to Optimize SVR with Telaprevir, Ribavirin, And Peg Interferon-alfa 2a in Thrombocytopenic Cirrhotics with CHC. RESTRAINT C Trial</td>
<td>O_07_PK</td>
<td>9</td>
</tr>
<tr>
<td>Bifano, M.</td>
<td>The Effect of Single 60 mg and 180 mg Doses of Daclatasvir on the QTc Interval in Healthy Subjects</td>
<td>O_06_PK</td>
<td>8</td>
</tr>
<tr>
<td>Bifano, M.</td>
<td>Daclatasvir, an HCV NSSA Replication Complex Inhibitor, has Minimal Effect on Pharmacokinetics of Midazolam, a Sensitive Probe for Cytochrome P450 3A4</td>
<td>O_15_PK</td>
<td>17</td>
</tr>
<tr>
<td>Caro, L.</td>
<td>MK-5172 Pharmacokinetic/Pharmacodynamic Relationship Between Transaminase Levels And Plasma Pharmacokinetics Following Administration Of Mk-5172 With Pegylated Interferon Alfa-2B And Ribavirin (Pr) To HCV Genotype (G) 1 Treatment-Naive Patients</td>
<td>O_09_PK</td>
<td>11</td>
</tr>
<tr>
<td>Chen, X.</td>
<td>Development, validation, and application of a LC-MS/MS assay for the quantification of telaprevir and its R-isomer in plasma</td>
<td>O_21_PK</td>
<td>23</td>
</tr>
<tr>
<td>Cohen, D.</td>
<td>ABT-450/r-containing direct-acting antiviral regimens are not associated with adverse changes in serum lipids or glucose in HCV genotype 1-infected patients in the AVIATOR study</td>
<td>O_08_PK</td>
<td>10</td>
</tr>
<tr>
<td>Dvory-Sobol, H.</td>
<td>Resistance Analyses Using Deep and Population Sequencing after 3 Day Monotherapy with GS-9669, a Novel Non-Nucleoside NS5B Inhibitor in Genotype 1 HCV Patients</td>
<td>O_11_R</td>
<td>38</td>
</tr>
<tr>
<td>Eley, T.</td>
<td>Effect of Multiple-Dose Ketoconazole and the Effect of Multiple-Dose Rifampin on Pharmacokinetics (PK) of the HCV NS3 Protease Inhibitor Asunaprevir</td>
<td>O_13_PK</td>
<td>15</td>
</tr>
<tr>
<td>Eley, T.</td>
<td>Evaluation of Drug Interaction Potential Between Daclatasvir and Sofosbuvir</td>
<td>O_14_PK</td>
<td>16</td>
</tr>
<tr>
<td>Eley, T.</td>
<td>Evaluation Of Pharmacokinetic Drug-Drug Interaction (Ddi) Between Bms-791325, An Ns5B Non-Nucleotide Polymerase Inhibitor, Daclatasvir And Asunaprevir In Triple Combination In Hcv Genotype 1-Infected Patients</td>
<td>O_18_PK</td>
<td>20</td>
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<tr>
<td>Estrabaud, E.</td>
<td>Reduction of mir-122 expression in IL28B CT/TT chronic hepatitis C patients who failed to pegylated-interferon plus ribavirin treatment</td>
<td>O_14_R</td>
<td>41</td>
</tr>
<tr>
<td>Ewart, G.</td>
<td>Phase 2A Trial of BIT225, HCV-P7 Inhibitor, in Combination with Pegylated Interferon-alfa-2b and Ribavirin in HCV Genotype-1 Treatment Naive Subjects</td>
<td>O_04_R</td>
<td>31</td>
</tr>
<tr>
<td>Fraser, I.</td>
<td>Lack of PK interaction between the HCV protease inhibitor MK-5172 and methadone and buprenorphine/naloxone in subjects on opiate maintenance therapy</td>
<td>O_16_PK</td>
<td>18</td>
</tr>
<tr>
<td>Grammatikos, G.</td>
<td>Pretreatment genetic diversity in the protease-helicase region of hepatitis C virus affects resistance-barrier in patients naive to direct antivirals</td>
<td>O_08_R</td>
<td>35</td>
</tr>
<tr>
<td>Author</td>
<td>Abstract Title</td>
<td>Abst #</td>
<td>Page #</td>
</tr>
<tr>
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<tr>
<td>Hebner, C.</td>
<td>Cross-Resistance of Sofosbuvir to Clinically-Relevant Ribavirin, NS3 Protease, NS5A, and NS5B Nucleoside and Non-nucleoside Inhibitor Mutations</td>
<td>O_01A_R</td>
<td>27</td>
</tr>
<tr>
<td>Jacobson, I.</td>
<td>Safety Profile of Daclatasvir in combination with Peginterferon Alfa and Ribavirin in 1100 patients with chronic HCV infection treated in Phase 2 studies</td>
<td>O_06_R</td>
<td>33</td>
</tr>
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<td>Jimmerson, L.</td>
<td>Development and validation of a novel dried blood spot assay for the quantification of ribavirin</td>
<td>O_19_PK</td>
<td>21</td>
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<tr>
<td>Kieffer, T.</td>
<td>Preclinical and in vitro resistance profile of potent nucleotide polymerase inhibitors of HCV: ALS-2200 and its single diastereomer, VX-135</td>
<td>O_02_R</td>
<td>29</td>
</tr>
<tr>
<td>Kieffer, T.</td>
<td>Identification and Characterization of HCV Genotype 1L by Whole Genome Sequencing</td>
<td>O_09_R</td>
<td>36</td>
</tr>
<tr>
<td>Kirby, B.</td>
<td>Metabolism and Excretion of Ledipasvir (GS-5885) in Humans</td>
<td>O_22_PK</td>
<td>24</td>
</tr>
<tr>
<td>Kocinsky, H.</td>
<td>ACH-3102, A 2nd Generation NS5A Inhibitor, Demonstrates Potent Antiviral Activity Against Genotype 1 HCV Despite Resistant Variants at Baseline</td>
<td>O_05_R</td>
<td>32</td>
</tr>
<tr>
<td>Kopf, W.</td>
<td>Pharmacokinetic Modeling of the Relationship Between SVR and Plasma Concentrations of Faldaprevir or BI207127 in HCV GT1-infected Patients in SOUND-C2</td>
<td>O_12_PK</td>
<td>14</td>
</tr>
<tr>
<td>Ku, K.</td>
<td>Analysis of HCV Genotype 5 and 6 NS5B Sequences from Clinical Samples</td>
<td>O_10_R</td>
<td>37</td>
</tr>
<tr>
<td>Langness, J.</td>
<td>Identification and Management of Potential Drug-Drug Interactions with Boceprevir and Telaprevir</td>
<td>O_17_PK</td>
<td>19</td>
</tr>
<tr>
<td>Lassmann, B.</td>
<td>Baseline Clonal Analysis of Protease Inhibitor Resistance Sites in Paired Liver and Serum Samples from Patients with Chronic Hepatitis C Infection</td>
<td>O_07_R</td>
<td>34</td>
</tr>
<tr>
<td>Michalak, T.</td>
<td>Persistence of HCV during and after otherwise clinically sucessful standard treatment for chronic hepatitis C</td>
<td>O_13_R</td>
<td>40</td>
</tr>
<tr>
<td>Mo, H.</td>
<td>Comprehensive Resistance Analyses in Patients Who Relapsed after Treatment with Sofosbuvir-Containing Regimens in five Phase 2 Studies</td>
<td>O_12_R</td>
<td>39</td>
</tr>
<tr>
<td>Ouwerkerk-Mahadevan, S.</td>
<td>Pharmacokinetics of Simeprevir (TMC435) in volunteers with moderate or severe hepatic impairment</td>
<td>O_04_PK</td>
<td>6</td>
</tr>
<tr>
<td>Ouwerkerk-Mahadevan, S.</td>
<td>The effect of food and different meal types on the bioavailability of simeprevir (TMC435), an HCV protease inhibitor in clinical development</td>
<td>O_20_PK</td>
<td>22</td>
</tr>
<tr>
<td>Patick, A.</td>
<td>In vitro resistance studies of miravirsen (MIR), a novel anti-HCV therapeutic targeting the host cell factor miR-122</td>
<td>O_03_R</td>
<td>20</td>
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<tr>
<td>Reitmann, C.</td>
<td>Age and Gender Effects on the Pharmacokinetics of Multiple Oral Doses of MK-5172</td>
<td>O_01_PK</td>
<td>3</td>
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<tr>
<td>Rouan, MC.</td>
<td>Combination therapy with simeprevir and TMC647055/low dose ritonavir: dose anticipation using PBPK modeling and dose optimization in healthy subjects</td>
<td>O_10_PK</td>
<td>12</td>
</tr>
<tr>
<td>Author</td>
<td>Abstract Title</td>
<td>Abst #</td>
<td>Page #</td>
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<tr>
<td>Simion, A.</td>
<td>Pharmacokinetics of Simeprevir (TMC435) in volunteers with severe renal impairment</td>
<td>O_03_PK</td>
<td>15</td>
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<tr>
<td>Simion, A.</td>
<td>Simeprevir (TMC435) does not prolong the QT/QTC interval in healthy volunteers</td>
<td>O_05_PK</td>
<td>17</td>
</tr>
<tr>
<td>Wu, L.</td>
<td>A population model linking plasma and intracellular ribavirin pharmacokinetics in persons with Chronic Hepatitis C Virus</td>
<td>O_11_PK</td>
<td>13</td>
</tr>
<tr>
<td>Xu, S.</td>
<td>In Vitro Selection of Resistance to Sofosbuvir in GT2a, GT2b, GT3a, GT4a, GT5a and GT6a Replicons</td>
<td>O_01B_R</td>
<td>29</td>
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</tbody>
</table>
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