



**Key Presentations on  
HIV Drug Resistance & Resistance Testing**



**Determination of Phenotypic Clinical Cutoffs for Atazanavir and Atazanavir/Ritonavir from AI424-043 and AI424-045**

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**Background:** Atazanavir (ATV) (400mg daily) and ATV/r (ATV 300mg daily with ritonavir 100 mg daily) are widely used in combination treatment of HIV-1. The PhenoSense assay currently utilizes a biologic cutoff for ATV at 2.3 fold change (FC). The current study seeks to define a phenotypic clinical cutoff for ATV and ATV/r, above which virologic response to therapy begins to decrease.

**Methods:** Data from two clinical trials in subjects with prior PI failure, BMS AI424-043 (ATV+2NRTIs) and AI424-045 (ATV/r+TDF+1NRTI), were included for all subjects treated through Week 24 having baseline phenotype, baseline viral load  $\geq 400$  copies/mL, and viral load at Weeks 12 and/or 24 (ATV n=131 and ATV/r n=111). ATV phenotyping (PhenoSense) was performed at baseline and correlated with virologic outcome within each study. Efficacy outcomes focused on change in viral load from baseline and the proportion of subjects with HIV-1 RNA levels  $< 400$  copies/mL at Week 24. Logistic regression and Fischer's exact tests were used to identify the ATV and ATV/r FC cutoffs.

**Results:** For the 043 and 045 studies, the median baseline HIV-1 RNA value was 15,477 and 29,898 copies/mL, respectively, and the median (range) baseline FC to ATV was 1.2 (0.4-90) and 1.2 (0.2-57), respectively. In 043, the proportion of subjects with HIV RNA  $< 400$  copies/mL at 24 weeks was relatively constant below 2.2-fold but was reduced at higher FCs ( $p=0.001$ ). The proportion with HIV RNA  $< 400$  copies/mL at 24 weeks with baseline ATV FC  $< 2.2$  or  $\geq 2.2$  was 76% and 45%, respectively. In 045, the proportion of subjects with HIV RNA  $< 400$  copies/mL at 24 weeks was relatively constant below 5.2-fold but was reduced at higher FCs ( $p<0.0001$ ). The proportions with HIV RNA  $< 400$  copies/mL at 24 weeks among those with baseline ATV FC  $< 5.2$  or  $\geq 5.2$  was 77% and 12%, respectively.

**Conclusions:** In these PI experienced study populations the overall treatment responses to ATV and ATV/r were high. Optimum responses to ATV were observed at FCs  $< 2.2$  in the 043 cohort and to ATV/r at FCs  $< 5.2$  in the 045 cohort.

Oral Presentation

### Reduced Susceptibility to Protease Inhibitors (PI) in the Absence of Primary PI Resistance-Associated Mutations

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**Background:** The majority of clinical samples submitted for phenotypic and genotypic resistance testing that demonstrate reduced protease inhibitor (PI) susceptibility also have one or more “primary” PI-selected resistance-associated mutations (RAMs). However, occasionally samples are observed with unexplained PI resistance.

**Methods:** Primary PI RAMs were defined as any change vs. wild-type at positions 23, 24, 30, 32, 46, 47, 48, 50, 54, 82, 84, 88, 90, with the following exceptions: I54V and N88D (not reported to occur without other primary mutations) and V82I (known polymorphism in PI-naïve patients). PI susceptibility (fold-change [FC] in IC<sub>50</sub>), gag (positions 418-500) and PR genotype were determined with the PhenoSense and GeneSeq assay, respectively (ViroLogic, Inc.). Fisher's exact test was used to determine genotypic changes in gag or PR associated with reduced PI susceptibility; mixtures were counted as mutant, and variables with  $p < 0.005$  were considered significant.

**Results:** A small group of samples with no primary PI RAMs but at least one PI with  $FC > 5$  was identified ( $n=125$ ; 0.5% of samples lacking PI RAMs); 28 had at least one PI with  $FC > 10$ . The median/maximum FC to amprenavir, indinavir, nelfinavir, ritonavir, saquinavir, lopinavir, and atazanavir were 2.4/25, 3.1/15, 9.6/65, 5.2/75, 2.7/52, 2.4/28, and 3.6/41, respectively. The number of samples with  $FC > 5$  to 1, 2, 3, or  $\geq 4$  PIs was 89 (mostly nelfinavir), 17 (mostly nelfinavir and ritonavir), 11, and 8, respectively. Compared to 3956 samples with no PI RAMs and all PIs  $FC < 5$ , PR mutations over-represented in samples with at least one PI  $FC > 5$  included: L10IV, I13V, L19V, K20IMT, A22V, M36IV, N37D, I54V, H69R, A71ITV, G73S, T74KS, V82I, N83D, N88D and I93L. In gag, several changes including K418ER, A431V, I437V, L449IP, P453L, and E482G were associated with PI  $FC > 5$ .

**Conclusions.** Although rare, reduced susceptibility to PIs in excess of 5-fold in the absence of primary mutations in PR can be observed in clinical samples. Accumulation of secondary mutations and/or polymorphisms in PR, as well as changes in the C-terminal region of gag, are potential contributors to reduced PI susceptibility and may modulate response to PI treatment.

Poster Presentation

**Differences in Enhanced Susceptibilities to NNRTIs and to Zidovudine (ZDV) in Site Directed Mutants (SDMs) Bearing K65R, L74V or M184V**

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**Background:** We have previously demonstrated that NNRTI HS is associated with thymidine analog mutations (TAMs). We recently described NNRTI HS in clinical isolates the NRTI mutations K65R, M184I/V, and T69D/N/S. Here we use SDMs to define the NNRTI susceptibility patterns of specific nonTA NAMS and compare these to ZDV susceptibility patterns

**Methods:** NL4-3 SDMs were created with the single mutations K65R, L74V and M184V. Multiple replicates of each SDM, K65R (n=18), L74V (n=20) and M184V (n=17), were tested in a single cycle phenotyping assay (PhenoSense). The mean fold change (FC) values were compared to 20 replicates of the NL4-3 reference using the t-Test.

**Results:** For the three NNRTIs the mean FCs for the NL4-3 reference ranged 0.95-1.01. Relative to reference all SDMs had lower mean FCs for each NNRTI ( $p < 0.0001$ ). The mean FC (SD) for EFV with K65R, L74V and M184V was 0.56 (0.03), 0.67 (0.02) and 0.65 (0.03), respectively. The mean FC (SD) for NVP with K65R, L74V and M184V was 0.53 (0.05), 0.59 (0.06) and 0.67 (0.05), respectively. The mean FC (SD) for DLV with K65R, L74V and M184V was 0.54 (0.03), 0.68 (0.06) and 0.65 (0.05), respectively. For EFV, K65R showed lower mean FC than both L74V ( $p < 0.0001$ ) and M184V ( $p < 0.0001$ ). L74V showed lower mean FC to NVP than to EFV or DLV ( $p < 0.0001$  for both). By contrast the mean FC (SD) to ZDV with M184V, L74V and K65R was 0.33 (0.04), 0.49 (0.07) and 0.60 (0.05), respectively. The mean ZDV FC was greater for M184V than L74V ( $p < 0.0001$ ) and was greater for L74V than K65R ( $p < 0.0001$ ).

**Conclusions:** The non-TA NAMS K65R, L74V and M184V enhance susceptibilities to NNRTIs and to ZDV. Discrete differences between these mutations exist such that EFV susceptibility is enhanced by  $K65R > L74V$  or M184V. ZDV susceptibility is enhanced by  $M184V > L74V > K65R$ . These observations may have relevance to models exploring the mechanisms of RTI HS.

*Poster Presentation*

**Selection of the HIV-1 Reverse Transcriptase Mutation K70E in Antiretroviral-Naïve Subjects Treated with Tenofovir/Abacavir/Lamivudine Therapy**

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**Background:** The high incidence of virologic non-response observed with the treatment-naïve subjects of the ESS30009 tenofovir/abacavir/lamivudine study arm has been associated with selection for K65R and M184V reverse transcriptase (RT) coding changes (Gallant *et al*, 43rd ICAAC, 2003, Abstract #H-840). Here, the identification of additional treatment-emergent coding changes possibly representing alternative resistance pathways associated with the observed non-response to this regimen is described.

**Methods:** Plasma HIV drug susceptibility and population genotyping were performed by ViroLogic. HIV clonal analysis was performed at GlaxoSmithKline.

**Results:** There were 81 subjects with both baseline and post-baseline (pre-therapy switch) genotypes on the TDF arm. Available on-therapy time points with genotype ranged from Weeks 4 through 30. The most commonly selected IAS USA-defined NRTI mutations detected at any time point, by population genotype, were M184I or V (78/81, 96%), K65R/K (43/81, 53%), and/or Y115F/Y (9/81, 11%). However, the K70E mutation was also selected in plasma-derived virus from 8/81 subjects (10%) at time points between week 8 to week 20 ( $p < 0.001$ ). At the time of mutation selection, all subjects were either experiencing viral rebound or had never responded to therapy. By population genotype, the K70E mutation (all as K/E mixtures) was selected along with M184I/V (8/8), K65R (7/8, all mixtures), and/or Y115Y/F (2/8). No subject on either arm ( $n=318$ ) had detectable K70E at baseline. Clonal analysis was done for one sample with a population genotype of K65K/R+K70K/E+Y115F+V118I+M184V (V118I present at baseline). K70E (15/22, 68%) was never detected on the same clones with K65R (6/22, 27%), while Y115F (21/22, 95%) was selected with either mutation.

**Conclusions:** The K70E RT mutation was selected in 10% of the antiretroviral-naïve subjects receiving ABC/3TC/TDF regimen with both baseline and post baseline (pre-therapy switch) genotype. This mutation was also previously reported to be selected in adefovir passage experiments and to be associated with adefovir resistance, and/or with selection in heavily antiretroviral-experienced subjects, it was also selected in one treatment-naïve patient treated with TDF/3TC/EFV, and in one antiretroviral-experienced subject treated with ABC/3TC/TDF/ddI. These data suggest an alternate pathway for tenofovir resistance, while the clonal data suggests antagonism and exclusivity between K65R and K70E at the genomic level.

Poster Presentation

**Gag Mutations Identified in Wild-type HIV-1 that Impact Viral Replication Capacity (RC) May Represent Cytotoxic T-Lymphocyte (CTL) Escape Mutations**

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**Objectives:** Previous analyses of the relationship between RC and sequence changes in the C-terminus of gag in 168 wild-type (WT; lacking resistance mutations in protease and RT) viruses identified several mutations associated with high or low RC. The objectives of this study were to 1) confirm these observations in a larger dataset, 2) compare gag mutations in WT viruses with those in viruses from patients known to be naïve to antiretroviral therapy (ART), and 3) to determine whether gag mutations affecting RC lie within known CTL epitopes.

**Methods:** The C-terminal gag sequence (positions 418-500) from 2650 WT viruses from the ViroLogic database, 108 viruses from ART-naïve patients in the AIEDRP cohort, and 247 baseline samples from ART-naïve patients in ESS30009, was determined. Mutations (vs. NL4-3) were correlated with RC as determined by the ViroLogic PhenoSense assay. RC values corresponding to the 15<sup>th</sup> and 85<sup>th</sup> percentiles were used to define “low” and “high” RC respectively. Gag mutations were compared to CTL epitopes listed in the LANL database.

**Results:** All of the previously described relationships were confirmed in the larger dataset except for mutations near position 458 (PTAP insertions) and at codon 466. Mutations at positions 454 and 479 were observed to be statistically significantly related to RC (low or high, respectively) only in viruses from patients known to be ART-naïve, while mutations at 9 of 17 previously described positions were significant in both datasets. High (>99.5%) sequence conservation was observed at the p7/p1 and p1/p6 cleavage sites, and the *vpr* packaging signal, except at positions 449 and 490. Only mutations between positions 465 and 479 occurred in a region not previously described as an epitope for CTL recognition.

**Conclusions:** The observation that gag mutations impact RC in WT viruses was confirmed in a larger dataset, and the many of the same mutations were significant in viruses from patients known to be ART-naïve. Most mutations occurred at known CTL epitopes, suggesting that evasion of host immunity may provide the selective pressure to maintain gag mutations that impair RC in WT viruses; other mutations may represent unrecognized epitopes or sites with as yet unknown functional importance.

Poster Presentation

**Susceptibility Measurements Using Resistance Test Vectors with or without Patient-Derived C-terminus of RT, RNaseH and Integrase are Largely Concordant**

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**Background:** Currently available drug susceptibility assays are limited to protease (PR) and reverse transcriptase (RT), and do not capture C-terminal regions of RT, RNaseH or integrase (IN). Here we describe a modification of a single cycle replication assay (PhenoSense HIV) that enables evaluation of IN inhibitors using a patient derived amplicon containing the entire polymerase (*pol*) gene.

**Methods:** Resistance test vectors (RTVs) containing the entire *pol* gene from 27 patient viruses were constructed. Susceptibility results (fold change in  $IC_{50}$  or FC) were compared to RTVs containing the standard PR/RT amplicon (codons 1-305 of RT) from the same samples. Susceptibility to PR inhibitors (PIs), nucleoside RT inhibitors (NRTIs) and non-nucleoside RT inhibitors (NNRTIs) was measured using the PR/RT and *pol* RTVs. Effects of IN, RNaseH, or C-terminal regions of RT were investigated by exchanging different domains of *pol*.

**Results:** Overall there was no difference in susceptibility to PIs, NRTIs or NNRTIs between the 2 types of RTV (t test,  $p > 0.3$ ). In most cases there was no change in classification i.e. hyper-susceptible ( $FC < 0.4$ ), sensitive, or reduced susceptibility ( $FC > 2.5$ ). Only one sample showed reduced susceptibility (NVP FC 2.8) with the *pol* RTV not detected with PRRT (FC 0.3). One patient virus with NNRTI resistance (K103N, P225H; wild-type at other NNRTI positions including 318) exhibited 6- to 10-fold higher FC to NNRTIs when the *pol*-based RTV was used (e.g. EFV FC 202-fold vs. 20-fold). Exchange of different domains of *pol* indicated that the C-terminus of RT (after codon 305) and/or RNaseH are essential for this increased resistance. Unique mutations in the C-terminus of RT of this sample are being investigated.

**Conclusions:** With rare exceptions, concordant measurements were obtained with recombinant viruses that express either the entire patient derived *pol* or only PR and incomplete RT fragments. In one sample, where enhanced NNRTI resistance was observed, molecular analysis revealed that domains in *pol* after codon 305 were essential for this resistance. Capturing the entire patient *pol* gene has the potential to retain protein-protein interactions from the native virus. Novel mutations within this region could induce enhanced NNRTI resistance by causing structural changes in the NNRTI-binding pocket of RT.

*Oral Presentation*

### Emerging Populations of Enfuvirtide Resistant HIV-1 Contain Multiple Variants that Compete for Dominance

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**Background:** Recently, we described the rapid emergence of an HIV-1 variant containing a rare single amino acid substitution (V38E) that confers high level resistance to enfuvirtide (ENF). In the current study, we conducted a detailed analysis of multiple env clones to characterize this rapidly emerging resistance virus population.

**Methods:** Heptad repeat 1 and 2 (HR1, HR2) envelope sequences were determined for ~40 envelope clones derived from virus populations at days 10 and 40 of ENF treatment. Based on HR1 sequences, approximately 10-12 clones from each timepoint were selected for ENF susceptibility testing using a single cycle HIV-1 env pseudovirion assay.

**Results:** ENF susceptibilities (IC<sub>50</sub> fold-change) at d0, d10 and d40 were 0.4, 7.6 and 400, respectively. Population genotyping identified the wildtype G-I-V motif at amino acid positions 36-38 of HR-1 at d0, a mixed G/D-I-V/E motif at d10, and a resistant G-I-E motif at d40. HR1 sequences were used to classify molecular clones derived from the d10, and d40 viruses. The relatedness of all clones was confirmed by phylogenetic analysis of gp160 sequences. D10 clones consisted of susceptible and resistant variants: G-I-V(14%), D-V-V(2%), D-I-V(18%), G-I-A(18%), G-I-E(46%). No other mutations in HR1 were observed. By d40, all clones were G-I-E. No double mutants were observed at either time point and all clones were R5-tropic. ENF susceptibility varied by genotype; G-I-V(FC=0.6), D-I-V(FC=27), G-I-A(FC=21), D-V-V(FC=49), G-I-E(FC=583), and consistent for multiple clones of like genotype. Infrequent mutations in HR2 did not contribute to significant reductions in ENF susceptibility. The infectivity of env clones varied, but consistent reductions in clones that contained ENF resistance mutations were not apparent in this dataset. Additional analyses are ongoing to attempt to identify compensatory mutations outside of HR1.

**Conclusions:** ENF resistance was associated with the early emergence of multiple variants containing distinct mutations in HR1. G-I-E variants exhibiting the largest reductions in susceptibility were prevalent at d10 and predominated by d40. The apparent lack of differences in env infectivity suggest that selection for G-I-E variants was driven by ENF pressure, however the contribution of infectivity will require a more thorough analysis of potential compensatory mutations.

Poster Presentation

**Properties of *In Vitro* Generated HIV-1 Variants Resistant to the CCR5 Antagonists SCH 351125 and SCH 417690**

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**Introduction:** As small molecule CCR5 antagonists progress in clinical development, an increasing need to understand the mechanism of HIV-1 resistance to these molecules exists. We have investigated the *in vitro* development of resistance to two compounds, SCH 351125 and SCH 417690, using different HIV isolates and cultures systems and phenotypically and genotypically characterized the resistant variants.

**Methods:** HIV-1 isolates were serially passaged in PM-1 cells or primary PBMCs in the presence of increasing concentrations of SCH 351125 or SCH 417690 and replication was monitored weekly by measurement of p24 antigen. Susceptibility was measured periodically in the cultures using primary cell infections or by generation of pseudotyped reporter viruses harboring the gp160 sequences from treated or control cultures (PhenoSense assay). Viruses were further characterized for coreceptor tropism and cross-resistance to other antiviral compounds and CCR5 inhibitors. Sequence analysis was performed on envelope clones from resistant cultures and in some cases recombinant viruses were tested for susceptibility to the compounds.

**Results:** Resistant viruses emerged in some but not all cultures after 12-16 weeks of continuous passaging. These viruses remained CCR5-tropic in primary cells and were unable to infect PBMCs from donors lacking functional CCR5. However, one isolate (D1/85.16) replicated in U-87-CXCR4 cells suggesting that the presence of X4-tropic virus in the culture. Clonal analysis of gp120 regions revealed that in most cases, an accumulation of mutations in the V3-loop correlated with a sequential decrease in sensitivity to CCR5 antagonists. Different viruses displayed unique patterns of V3 mutations although most were clustered in the V3 cap region. One resistant clone isolated displayed no changes in V3 suggesting that other regions of gp120 may contribute to resistance. Infectivity studies demonstrated that highly resistant viruses replicate less efficiently than control viruses.

**Summary:** 1) Resistance to CCR5 inhibitors emerges slowly in culture and is associated with the sequential accumulation of multiple mutations in gp120. 2) Resistant viruses remain CCR5-tropic in primary cells although CXCR4-tropic variants were detected in one culture. 3) Resistant viruses showed cross-resistance to other CCR5 inhibitors but were sensitive to other drug classes. 4) Mutational patterns differed among individual resistant viruses but typically clustered in the V3-loop.

Poster Presentation

**Susceptibility to Inhibitors of CD4-Env Binding Correlates with Membrane Fusion: Implications for HIV-1 Entry**

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**Background:** Both cell-surface CD4 and the CD4 binding site (CD4-bs) on HIV-1 envelope represent targets for entry inhibitor development. X4-tropic laboratory-adapted viruses are highly sensitive to inhibition by soluble CD4 and are strong inducers of membrane fusion (syncytia formation). In this study, we evaluated primary virus envelope proteins for susceptibility to inhibitors of CD4-gp120 binding and ability to induce membrane fusion.

**Methods:** HIV-1 Envelopes were amplified from patient plasma and transferred to an expression vector. Susceptibility to CD4-bs inhibitors (sCD4 and IgG-b12; Scripps) and anti-CD4 monoclonal antibody (B4) was assessed using a single-cycle, pseudovirion assay. Membrane fusion was measured by co-culturing env-positive effector cells and CD4/CCR5/CXCR4-positive CEM Luc-GFP and luciferase reporter cells (5.25). gp160 env sequences were generated by the dideoxynucleotide chain termination method.

**Results:** In a survey of 64 patient viruses, increased susceptibility to CD4-bs inhibitor (sCD4) correlated with increased membrane fusion and decreased susceptibility to CD4 inhibitors (mAb B4). To begin to define genetic and structural determinants that confer differential sensitivity to CD4-gp120 inhibition and membrane fusion, 16 envelope molecular clones derived from a single virus population were sequenced and tested for susceptibility. Phylogenetic analysis revealed two related virus populations that also segregated based on sensitivity to CD4-gp120 inhibitors. Clonal and chimeric envelopes containing shorter gp120 variable loops and fewer glycosylation sites exhibited increased susceptibility to CD4-bs inhibitors (sCD4 and IgG-b12), decreased susceptibility to anti-CD4 antibody and induced higher levels of membrane fusion. Envelope clones were susceptible to R5 inhibitor and fusion inhibitor.

**Conclusions:** Susceptibility of HIV-1 to CD4-bs inhibitors correlates inversely with susceptibility to CD4 inhibitor and the efficiency of membrane fusion. Envelope molecular clones exhibiting differential susceptibility to CD4 inhibition were distinguished by alterations in gp120 glycosylation and length of variable regions. Based on these observations, we hypothesize that envelope conformations influence the degree of CD4-dependency that is required to mediate membrane fusion and virus entry.

*Oral Presentation*

**PL-100 and Its Derivatives, a Novel Class of Potent Human Immunodeficiency Virus Type 1 Protease Inhibitors: Resistance Profile and Pharmacokinetics**

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**Background:** We previously reported PL-100 as a potent, specific and non-cytotoxic HIV-1 protease inhibitor (PI). A small study was initially conducted to probe the cross-resistance profile of PL-100 against 14 multi-PI-resistant HIV isolates. Here, we have further characterized cross-resistance profiles of PL-100 and its derivative, PL-337 against 49 additional HIV isolates with reduced susceptibility to approved PIs. We also studied emergence of mutations conferring resistance to PL-100 in vitro. Furthermore, we developed a phosphorylated pro-drug, PPL-100 from PL-100 to improve its pharmacokinetic profile.

**Methods:** Cross-resistance profiles of PL-100 and PL-337 were established using PhenoSense™ assay (ViroLogic Inc). For comparison, atazanavir, saquinavir, indinavir, nelfinavir, amprenavir, and lopinavir were tested in parallel. For selection of resistance mutations to PL-100, we used blood mononuclear cells with a standard procedure of progressive increments of PI concentrations. Viral kinetics and genotyping were analyzed by established methods. Water solubility of PPL-100 was determined by HPLC/UV and LC/MS methods and pharmacokinetic profile of PPL-100 was evaluated in rats.

**Results:** In cross-resistance profiling against 63 diverse PI-resistant viruses, PL-100 and PL-337 had a median EC<sub>50</sub> fold-change (FC) of 3.6 and 4.6, respectively. The median EC<sub>50</sub> FC of the 6 approved PIs ranged from 8.1 (indinavir) to 23 (saquinavir). The percentage of resistant strains with EC<sub>50</sub> FC <10 to PL-100 and PL-337 were 76 and 72, respectively. In comparison, the percentage of strains with FC < 10 to approved drugs ranged from 27 (nelfinavir) to 54 (indinavir). PL-100 and PL-337 shared a very similar cross-resistance pattern, distinct from approved PIs. After 25 weeks of passaging under PL-100 selective pressure, preliminary data revealed a novel pattern of mutations (K45R, M46I, T80I, and P81S), some of them potentially polymorphic, in the PR gene of laboratory-adapted strain IIIB. The impact of these mutations on PL-100 antiviral activity is currently under investigation. PPL-100 was >1000-fold more water-soluble than PL-100 and showed 2 to 3-fold improvement over PL-100 in oral bioavailability and other parameters.

**Conclusions:** The combination of a favorable cross-resistance profile, and improved solubility and pharmacokinetics confirms the potential of PPL-100 as a novel PI for treatment of patients infected with PI-resistant HIV-1 strains.

Poster Presentation

**Antiviral Profile of BILR 355 BS against a Large Panel of Clinical Isolates with Mutations Conferring Resistance to Currently Available Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)**

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**Background:** BILR 355 BS is a novel NNRTI with potent antiviral activity against wild-type HIV-1 and recombinant viruses with  $\geq 1$  mutations conferring phenotypic resistance to the currently available NNRTIs (nevirapine, efavirenz, delavirdine). To further characterize its antiviral profile, BILR 355 BS was tested against several clinical isolates associated to treatment failures with first-generation NNRTI regimens.

**Methods:** Two sets of clinical isolates were tested with BILR 355 BS and first-generation NNRTIs at ViroLogic using the PhenoSense™ methodology which constructs recombinant viruses by incorporating the amplified PR and RT regions of isolates into the luciferase-containing retroviral vector. Set A consisted of 225 randomly selected NNRTI-resistant viruses with a fold-change (FC)  $\geq 10$  against at least one of the currently available NNRTIs (inclusion criteria). Set B consisted of 139 isolates selected from the 2NN clinical study (most being baseline and time of first-failure matched isolates).

**Results:** BILR 355 BS had an EC<sub>50</sub> value of 6nM in the PhenoSense™ assay against the reference NL4-3 HIV-1 wild-type strain. Within Set A, 141/225 (63%) of the isolates had FC  $\leq 10$  toward BILR 355 BS (22% for efavirenz, 1.7% for nevirapine). Of those isolates with only one NNRTI-associated mutation, 67/76 (88%) had FC  $\leq 10$  with BILR 355 BS compared with 52/74 (70%) and 22/75 (31%) with 2 or  $\geq 3$  mutations respectively. Within the 2NN isolates (Set B), 47/50 (94%) of those with only one NNRTI mutation had FC  $\leq 10$  toward BILR 355 BS (58% for efavirenz, 16% for nevirapine) compared with 12/21 (57%) and 2/3 for those with 2 or  $\geq 3$  NNRTI mutations respectively. 89% (108/121) of the single and double mutant isolates containing the K103N mutation had FC  $\leq 10$  (both sets combined). Consistent with earlier observations, the majority of isolates with FC  $>10$  had  $\geq 3$  NNRTI-associated mutations or contained either the low frequency V106A or Y188L mutation.

**Conclusion:** The susceptibility of clinical isolates to BILR 355 BS was tested. Results show that it maintains potent antiviral activity against a majority of isolates associated with NNRTI treatment failures, supporting its continued development for treatment of NNRTI-resistant HIV.

Poster Presentation

## **A Systematic Analysis of the Fitness Effects of Mutations Associated with Resistance to Protease Inhibitors**

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Protease inhibitor (PI) resistance generally develops in multiple steps, with viruses bearing multiple mutations often having improved replicative capacity and decreased PI susceptibility when compared to viruses bearing single mutations. While the fitness contributions of a subset of resistance mutations have been analyzed (e.g. the association of some primary resistance mutations with fitness losses), a systematic analysis of the fitness and PI resistance effects of single mutations in protease (PR) has not been done.

Therefore, we have constructed viral clones bearing most of the major PI resistance associated mutations (thirty-one mutants with single mutations, forty-two with two mutations, and five bearing multiple (3-6) resistance mutations). The specific infectivity (SpIn) of the mutant viruses (a measure of virus fitness) was determined using a luciferase reporter cell line to measure infectivity, which was normalized to the mass of p24 in the inoculum. The replication capacity (RC), a related measure of virus fitness, and the susceptibility to a panel of seven PIs, have also been measured for these same mutants. Both the SpIn and RC measurements demonstrate that fitness changes are broadly categorizable by mutation class, with primary mutations causing larger fitness losses than secondary mutations. With the exception of PI specific resistance interactions (e.g. D30N and Nelfinavir), the PI susceptibility of these viruses are closely related to their fitness, with primary mutations generally demonstrating increased PI susceptibility.

The degree to which the Gag intermediate NC-p1 is processed to NC by these mutant viruses is also related to their measured fitness, suggesting that the fitness effects of mutations in PR are related to reduced processing. We have also examined the ability of each of the three most common secondary mutations (L10I, L63P, and A71V) to compensate for fitness losses associated with primary mutations. The addition of any of these mutations to a primary mutation generally leads to improved fitness relative to viruses bearing single primary mutations, suggesting a largely non-specific mechanism of compensation, although there are some exceptions. The addition of a secondary mutation also leads to decreases in PI susceptibility that correlate with the fitness changes they engender.

*Poster Presentation*