Session 1

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Plenary Abstracts

ABSTRACT P1

Antiviral Therapy 2004; **9:**S1.

Intracellular resistance to HIV replication

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The past 3 years has seen important advances in our understanding of the mechanisms of intracellular resistance to retroviral replication. The first resistance gene described was FV-1, a Gag-like cellular protein, that blocks the replication of murine leukaemia virus post-entry. More recent studies on the Vif accessory protein of HIV led to the identification of the cellular cytidine deaminase APOBEC3G as a potent innate inhibitor of HIV replication. In HIV that is deleted for Vif (Δvif), APOBEC3G is encapsidated into the virions during virus assembly. When the virus infects new target cells and initiates reverse transcription, the encapsidated enzyme attacks the minus-strand of the reverse transcripts, modifying cytosines to uracil and resulting in G→A hypermutation on the subsequently synthesized plus-strands. APOBEC3G-induced G→A mutation has molded the HIV-1 genome over evolution resulting in an enrichment for A nucleotides. In cells infected with wild-type virus Vif induces the degradation of APOBEC3G, preserving the integrity of the viral genome. Most recently, studies aimed at understanding the resistance of primate cells to HIV-1 led to the identification of Trim5-alpha. Primate, but not human Trim5-alpha blocks HIV infection at reverse transcription by a mechanism that is not yet known. Current understanding of these host factors will be discussed with an emphasis on APOBEC3G.

ABSTRACT P2

Antiviral Therapy 2004; **9:**S2.

Monkey business: insights into AIDS virus pathogenesis from studies in non-human primates

JD Lifson

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The error-prone replication mechanism employed by the primate lentiviruses is capable of generating extensive diversity in a virus population. In the face of environmental selection pressures, this process can produce viruses that are adapted, with varying degrees of fitness, to a wide variety of immunological and pharmacological environments. Drug-resistant mutant viruses and immune escape mutant viruses selected by both humoral and cellular arms of the immune system are well documented examples of this process. Non-human primate models offer multiple advantages in efforts to understand the processes that drive and control such selection *in vivo*. These include the ability to inoculate animals at defined times, via defined routes, with defined doses of defined viruses of known sequence and pathogenicity. Compared to studies of HIV-infected patients, non-human primate models may also provide opportunities for more extensive sampling of blood or tissues, especially at defined early time points relative to inoculation, as well as greater flexibility for more intensive experimental interventions such as initiation, modification or cessation of antiretroviral therapy (ART), or immunological perturbation (as in depletion of CD8+ lymphocytes through administration of mAbs). The presentation will review illustrative examples of selection processes *in vivo* in SIV infected macaques, including selection of mutants following transmission of wild-type viruses and development of reversions or compensatory mutations in macaques inoculated with mutant viruses. Model systems presented will include observational natural history studies, as well as interventional studies, such as a model of transient ART during primary infection and therapeutic vaccination regimens in chronically infected macaques. Additional systems showing promise for the study of resistance to antiretroviral drugs *in vivo* in non-human primates may also be presented.

Session 1
Resistance to New Antiretroviral Agents

ABSTRACT 1

Antiviral Therapy 2004; **9:**S5.

A molecular model of HIV-1 integrase inhibitor resistance

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This study was designed to understand the potential for cross resistance between structurally diverse inhibitors of HIV-1 integrase. We have previously shown that inhibitors of integrase strand transfer such as the 1,3 diketones (diketo acids or DKAs) and napthyridines, share a common mechanism of action in which the critical pharmacophore is involved in sequestering divalent metal ions in the active site. This is consistent with the observation that mutations associated with diketo acid resistance mapped to residues 66, 151, 153, 154 and 155, which are proximal to the active site residues that coordinate divalent metal. We have now generated viruses that are resistant to a potent inhibitor from the napthyridine class (L-870810). These viruses contain an entirely new constellation of mutations at positions 74, 121 and 125. In studies using recombinant viruses, these mutations engender a significant loss of susceptibility to the napthyridine but do not affect activity of the DKAs. Conversely, with the exception of 155, mutations associated with resistance to the DKAs do not engender napthyridine resistance. Like the DKA mutations, the napthyridine mutations also localize to the integrase active site, however, residues associated with DKA and napthyridine resistance map to discrete regions of the active site and define remarkably distinct ligand binding surfaces, which extend in opposing directions distal to the metal binding residues. This observation together with molecular modelling studies of these inhibitors suggest a molecular basis for their discordant resistance profiles, and the role of N155 in mediating cross class resistance and maintaining the architecture of the integrase active site. The proposed model also provides a rationale for developing integrase inhibitors with complementary resistance profiles.

ABSTRACT 2

Antiviral Therapy 2004; **9**:S6.

Novel small-molecule compounds which inhibit strand transfer activity of HIV-1 integrase

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OBJECTIVE: Integration of the proviral DNA into the host genome is essential event in the human immunodeficiency virus type 1 (HIV-1) replication life cycle. Therefore, integrase (IN), which plays crucial role in this integration event, has been the attractive target of anti-HIV drugs. Up to now, a number of inhibitory compounds have been reported, yet none has been successful in clinical treatment usage. In this study we attempted to find new IN inhibitory compounds, and screened a small molecule-compound library.

METHODS: In-house strand-transfer assay was constructed to screen IN inhibitory compounds. In brief, biotinylated 31 bp donor DNA was mixed with recombinant IN, followed by incubation with digoxigenin (DIG), labelled 29 bp target DNA and the test compound. After 1 h incubation at 37°C, integrated product was captured by streptavidin-coated 96 well plate, and quantified by alkaline phosphatase-conjugated anti-DIG antibody and CSPD chemiluminescence detection system. Lineweaver-Burk plot analyses and intercalation assays were performed to clarify the mechanism of inhibitions. To evaluate *in vitro* virus replication suppressions, single replication assays using HeLa/CD4/LTR-EGFP cell line were performed.

RESULTS: We tested 12 000 small-molecule compounds and discovered one compound, carbazole derivative, with potent strand-transfer inhibitory activity. To analyse structural determinants of the strand transfer inhibitory activity, we chemically synthesized 15 derivatives with different side chains on the carbazole structural backbone. Among these 15 compounds, eight derivatives have shown potent strand-transfer inhibitions. IC_{50}s of these eight compounds ranged from 0.78 to 5.3 μM. The result of Lineweaver-Burk plot analyses indicated the carbazole derivatives as competitive inhibitor of strand transfer. No intercalation activities were observed. In HeLa/CD4/LTR-EGFP cell culture assay, IC_{50}s of the eight compounds ranged from 0.49 to 1.92 μM. However, these eight derivatives demonstrated cytotoxicity (CC_{50}=1.97 to 5.04 μM) in this HeLa cell culture.

CONCLUSION: We have successfully found novel small-molecule IN inhibitory compounds carbazole derivatives. Though their strong cytotoxicity may limit carbazole derivatives to be used in clinical at this moment, it can be the lead compound for developing novel IN inhibitors. In addition, analysing IN inhibitory mechanisms of carbazole may give more detailed information of HIV-1 IN structure and function.

ABSTRACT 3

Antiviral Therapy 2004; **9:**S7.

In vitro development of resistance against styrylquinolines of HIV-1 by emergence of integrase mutations

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BACKGROUND: Styrylquinolines (SQLs) have been reported to be HIV-1 integrase inhibitors *in vitro*. Although integrase is unique to the virus and does not have a host cell counterpart, numerous integrase inhibitors have been shown to inhibit multiple viral and non viral proteins. To get further insights into the SQL target, our aim was to determine the *in vitro* development of HIV-1 resistance to FZ41 lead compound of the SQLs family, and evaluate fitness.

METHODS: CEM 4fx cells were infected with NL43 wild-type virus in the presence of increasing concentrations of FZ41. Resistant viruses obtained were sequenced. Identified mutations were introduced into the NL43 strain by site-directed mutagenesis and phenotypically evaluated by determining FZ41 IC_{50} on P4 cells. Selected HIV-1 strains in the presence of FZ41 were examined for their ability to replicate in HeLa P4 cells in comparison with NL43 in the absence of drug.

RESULTS: Two mutants emerged after incubation in step by step increased FZ41 concentrations and a final selection with 20 µM: one contained a single mutation C280Y, the other contained two mutations V165I-V249I. IC_{50}s confirmed that the selected mutations conferred resistance to SQLs. Double mutant IC_{50} was nearly ninefold higher than that of wild-type virus. IC_{50} of the other one, C280Y mutant was fivefold higher than that of the wild-type. Of note, IC_{50} of the diketo analogue L731-988 was not modified for either of these two mutants. Replication kinetics of the mutants, as evaluated by p24 measurements, was lower relative to the replication of HIV-1 NL43 strain. Seven days post-infection, for, respectively, C280Y and V165I-V249I mutants, only 68% and 5% of the p24 level of the control was detected.

CONCLUSION: Selection of resistance FZ41 is associated with the emergence of mutations at three residues within HIV-1 integrase, that have been previously involved for two of them with integrase non-catalytic function or drug interaction. With an *in vitro* resistance pattern different from those of diketo analogues, SQLs represent a new family of integrase inhibitors.

ABSTRACT 4

Antiviral Therapy 2004; **9**:S8.

Selection for and characterization of HIV-1 isolates resistant to the maturation inhibitor PA-457

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BACKGROUND: PA-457 is the first in a new class of antiretrovirals that inhibit HIV replication by disrupting virus maturation. PA-457 blocks a late step in Gag processing that results in defective core condensation and the release of non-infectious virus particles. Specifically, PA-457 disrupts the conversion of the capsid precursor, p25 (CA-SP1), to mature CA protein, p24. PA-457's mechanism of action (MOA) is distinct from that of protease inhibitors in that it appears to directly target the Gag precursor protein rather than the protease enzyme that is responsible for Gag processing.

METHODS: PA-457-resistant virus isolates were selected by continuous culture in the presence of increasing concentrations of compound. Genotyping of resistant virus and preparation of molecular clones with resistance-conferring mutations were carried out using standard methods. PA-457 resistance was characterized using cell-based activity assays and *in vitro* analysis of Gag processing.

RESULTS: *In vitro* selection generated PA-457-resistant virus. Genotypic analysis of these isolates revealed two independent patterns of resistance-conferring mutations. Consistent with our MOA studies these mutations mapped to residues flanking the Gag CA-SP1 cleavage site. An A to V change at either the first or third residues at the N-terminus of SP1 (A1V or A3V) resulted in a resistant phenotype. Both the A1V and A3V mutants exhibited reduced replicative fitness compared to WT, however, for the A3V virus a second point change in the C-terminus of capsid restored near-WT levels of replication. While these mutations resulted in a decrease in PA-457 activity, these viruses remained sensitive to all classes of approved HIV drugs.

CONCLUSION: These results support and extend previous observations that PA-457 is a specific inhibitor of CA-SP1 cleavage, with no activity against other Gag processing events. Characterizing the determinants of PA-457 activity is the first step in defining the molecular target for this novel HIV maturation inhibitor.

ABSTRACT 5

Antiviral Therapy 2004; **9:**S9.

In vitro resistance profile of small molecule HIV attachment inhibitors

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BACKGROUND: A small molecule inhibitor, BMS-488043, of HIV-1 attachment has demonstrated clinical antiviral efficacy. A comprehensive knowledge of the *in vitro* resistance profiles for this class will greatly aid our understanding of potential clinical resistance development and mechanism of action.

METHODS: Phenotypic and genotypic analysis was performed on viral variants selected against several compound analogues using multiple viral strains.

RESULTS: Selected resistance substitutions span the entire envelope, with >9 residues identified at CD4 contact sites. Remarkably, resistant envelopes carrying double contact site substitutions situated distantly in the CD4 binding pocket were also observed, suggesting that compounds interact with critical CD4 binding residues in a pre-CD4 form of gp120. Also, many of the selected substitutions overlap with the epitopes of CD4 binding site antibodies and the residues affecting viral susceptibility to sCD4. Furthermore, three other frequently occurring envelope changes were either at the V1V2 stem or line the CD4 binding pocket. To further elucidate the inhibitor binding location, gp120 variants carrying CD4 binding pocket mutations were generated. Six were shown to be severely defective in compound binding and resistant to compound inhibition of sCD4 binding. Of the emerging substitutions external to the CD4 binding pocket, two common envelope substitutions at M434 and F423 overlapped with the CD4i epitopes. In addition, many selected substitutions are situated at the CCR5 binding sites (in C4 and V3). In fact, interaction of the HIV-1 V3 loopwith a component of the CD4 binding site at the C4 region has been suggested. The V68A change near the N terminus of gp120, and many changes at gp41 may also indirectly influence the shape of compound interaction sites. Together, the data support initial findings that the attachment inhibitors bind to gp120 and function through interference of the gp120/CD4 interactions in the envelopes studied. Results of labelled compound binding and sCD4 competition further support this hypothesis.

CONCLUSION: The cumulative data strongly suggest that compound binding affects the residues in the CD4 pocket of gp120. Also, viral susceptibility to HIV-1 attachment inhibitors can be attributed to multiple interactions between various regions of gp120 and gp41, parallel to that observed for viral resistance to neutralization antibodies.

ABSTRACT 6

Antiviral Therapy 2004; **9**:S10.

In vitro escape of R5 primary isolates from the CCR5 antagonist, UK-427,857, is difficult and involves continued use of the CCR5 receptor

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BACKGROUND: The CCR5 antagonist, UK-427,857, is currently in clinical development as a member of a new class of antiretrovirals targeting HIV co-receptor binding. We have performed *in vitro* serial passage experiments of R5 isolates in the presence of increasing concentrations of the compound in an attempt to understand the pathways that may lead to UK-427,857 resistance.

METHODS: Six R5 HIV-1 primary isolates were serially passaged through mitogen-stimulated PBL in the presence of increasing concentrations of UK-427,857 for up to 20 weeks. Virus cultures that replicated in the presence of high concentrations of UK-427,857 (>1000-fold the parental virus) were generated and stocks were characterized for co-receptor tropism. Env-recombinant pseudotyped viruses were also generated from these stocks and their susceptibility to UK-427,857 was assessed using the PhenoSense HIV Entry Assay. Individual *env* clones of resistant variants were sequenced and compared to sequences of parental viruses and drug-free passaged controls.

RESULTS: High-level resistance to UK-427,857 was achieved in 3/6 virus cultures. Two resistant viruses (CC1/85res and RU570^{res}) continued to use the CCR5 co-receptor. The third virus (SF162^{res}) acquired reduced susceptibility to UK-427,857 in both the drug-treated and drug-free passaged control cultures: in each case the resistant variants selected during passaging were able to use CXCR4 as its entry co-receptor. The CC1/85res and RU570^{res} viruses exhibited increased sensitivity to a CCR5-specific mAb (2D7), suggesting altered envelope recognition of the external face of the co-receptor. The infectivity of the RU570^{res}, but not the CC1/85res, virus was impaired relative to the parental virus. Strain-specific mutations were identified in the gp160 V3 loop regions of CC1/85res and RU570^{res}. Resistance to UK-427,857 could not be generated in three R5 virus cultures (92BR017, 92BR018 and 92BR023) during the course of this study.

CONCLUSIONS: Resistance to UK-427,857 was either slow to emerge or did not develop during this study, suggesting there is considerable selective advantage *in vitro* for continued use of the CCR5 co-receptor in a UK-427,857-sensitive manner. Furthermore, our results indicate that gp160 mutations associated with UK-427,857 resistance may be strain-specific, suggesting that the context of the V3 loop is crucial for CCR5 recognition. These results offer promise for the efficacy and durability of UK-427,857-containing HAART.

ABSTRACT 7

Antiviral Therapy 2004; **9:**S11.

Suppression of X4- and dual-tropic HIV-1 variants during a short course of monotherapy with the CXCR4 antagonist AMD3100

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BACKGROUND: HIV-1 co-receptor antagonists represent a new opportunity for antiretroviral intervention. In a prior retrospective analysis of a Phase I/II study of the CXCR4 antagonist AMD3100, several subjects with X4R5-tropic virus at baseline developed R5-tropic virus on treatment. The present study was conducted to determine whether baseline X4R5 viruses represent mixed mono-tropic or pure dual-tropic variants and to describe changes in virus populations associated with the addition and removal of drug pressure.

METHODS: Virus populations at baseline (day 0), on treatment (day 11) and off treatment (days 18, 39) were characterized by determining the co-receptor tropism of 25–40 envelope clones per time point using an envelope pseudo-virus infectivity assay. Gp160 sequences were determined for at least 10 clones per time point.

RESULTS: Clonal analysis was performed on the virus populations of three study subjects exhibiting X4R5 tropism at baseline and R5 tropism on treatment. In patient one, co-receptor utilization was 66%-R5 and 34%-X4 at baseline, 100%-R5 at day 11 (on treatment) and day 18 (off treatment), returning to 65%-R5 and 35%-X4 on day 39 (off treatment). In patient two, co-receptor utilization was 55%-R5, 10%-X4 and 35%-dual at baseline, predominantly R5 at day 11 (97%) and day 18 (92%), returning to 32%-R5, 3%-X4 and 65%-dual by day 39. In patient three, the co-receptor utilization was 67%-R5, 27%-X4 and 6%-dual at baseline, 100%-R5 at day 11, predominantly R5 at day 18 (97%), remaining R5 (100%) at day 39. Sequence analysis of individual clones distinguished R5 viruses from X4 and dual-tropic viruses, largely based on differences in the V3 region. Envelope sequences selected under drug pressure were closely related to variants pre-existing in the baseline population.

CONCLUSION: During a short course of monotherapy with AMD3100, X4- and dual-tropic variants were suppressed, accompanied by a concomitant increase in the proportion of R5-tropic variants in the viral population. The suppression of dual-tropic variants merits additional investigation as this observation could have significant implications for the use of CXCR4 inhibitors in the clinic. Shifts in co-receptor tropism, both on and off treatment most likely result from selection of pre-existing variants from within the baseline quasispecies.

ABSTRACT 8

Antiviral Therapy 2004; **9:**S12.

Withdrawal of fusion inhibitors from a failing antiretroviral regimen results in reversion to enfuvirtide susceptibility

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BACKGROUND: *In vitro* growth competition assays and observations of decay of enfuvirtide-resistant viral populations after short-term monotherapy have suggested substantial decreases in fitness of enfuvirtide-resistant variants. However, the dynamics of these populations after interrupting chronic enfuvirtide treatment have not been examined.

METHODS: Patients failing an enfuvirtide-containing regimen participated in a 10-day T-1249 replacement study (T1249-102). Subsequently, patients opted to resume or discontinue ENF while continuing their failing background regimen pending enrolment in a T-1249 chronic dosing study. We compared genotype (GT) in gp41 aa 36–45, and enfuvirtide phenotype (PT) from patients while on a failing enfuvirtide regimen (baseline, prior to T-1249) and after resumption or discontinuation of ENF following short-term T-1249 therapy.

RESULTS: Patients studied (n=18) had documented resistance to enfuvirtide and had been failing an enfuvirtide-containing regimen for a median of 61 weeks. Eight resumed enfuvirtide (Resumed) for a median of 82 days while 10 interrupted fusion inhibitors (FI, Interrupted) for a median of 114 days (range 46–230). At baseline, median HIV RNA and geometric mean (GM) change in enfuvirtide $FCIC_{50}$ (normalized IC_{50}) were 4.86 log_{10} copies/ml and 133.9-fold for Resumed patients and 4.98 log_{10} copies/ml and 120.5-fold for Interrupted patients. Relative to T-1249 baseline, at the end of the observation period HIV RNA did not change in Resumed patients (–0.05 log_{10} copies/ml) while it increased a median of +0.21 log_{10} copies/ml in Interrupted patients. Similarly, GM enfuvirtide $FCIC_{50}$ increased 1.1-fold for Resumed while for Interrupted it decreased by 10.0-fold (P=0.006). Decreases in enfuvirtide $FCIC_{50}$ >10-fold were seen in 0/8 Resumed and 6/10 Interrupted patients; two Interrupted patients had changes between two- and 10-fold. The two Interrupted patients who showed no changes in enfuvirtide susceptibility were among those with the shortest interruption of FI (46–57 days). In Interrupted patients, reversions to enfuvirtide susceptibility were generally associated with GT reversions.

CONCLUSION: Following chronic enfuvirtide treatment, interruption of FI resulted in replacement of FI-resistant populations by more susceptible ones. These findings extend earlier observations that fitness disadvantages observed in ENF-resistant viruses *in vitro* result in parallel fitness disadvantages *in vivo*.

ABSTRACT 9

Antiviral Therapy 2004; **9:**S13.

A human monoclonal antibody blocks HIV entry by a T20-like mechanism

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As a validated target for anti-HIV drugs, the highly conserved Heptad Repeat 1 (HR1) of gp41 is also a potential target for neutralizing antibodies in either a therapeutic or prophylactic setting. Though no HR1-directed neutralizing monoclonal antibodies have been described to date, we have now identified such an antibody using phage display. Bacteriophage bearing human single-chain antibodies (scFvs) were selected for binding to the gp41 HR1 region, which is the target of T20. scFvs encoded by bound phage were expressed, purified and screened using a high-throughput HIV entry assay. One scFv, D5, inhibited entry in this assay and also blocked HIV replication in single- and multiple-cycle assays. Upon conversion into a human IgG1, D5 retained antiviral potency equivalent to that of the scFv on a molar basis.

Biochemical studies demonstrated that the D5 epitope maps to the C-peptide binding groove on HR1. Further, D5 prevented binding of an epitope-tagged C peptide to HR1 *in vitro*, suggesting that D5 blocks HIV entry via a T20-like mechanism.

D5 IgG1 neutralized a variety of HIV isolates, but potency varied considerably and some isolates were almost completely resistant. Resistant isolates contained amino acid polymorphisms in the HR1 region. However, these polymorphisms did not confer resistance when transferred to a sensitive isolate, and in biochemical studies D5 bound equally well to wild-type and polymorphic gp41 mimetics. Thus, D5 resistance was caused by regions of the envelope glycoprotein outside HR1.

This study marks the first time that a synthetic antigen has been used to select a broadly-neutralizing monoclonal antibody. Discovery of D5 provides proof-of-concept that the gp41 HR1 region is accessible to human IgG, that an IgG directed at HR1 can block HIV entry, and that it is possible to design synthetic antigens bearing an HR1-derived neutralizing epitope. Collectively, these observations lay the foundation for identification of therapeutic mAbs directed at HR1 and for design of immunogens capable of eliciting antibodies in vaccinees.

Acknowledgements: The HIV Antibody Discovery Team includes Z An[1], E Bianchi[2], D Bramhill[1], G Ciliberto[2], J Cook[1], R Cortese[2], D Eckert[1], E Emini[1], R Hampton[1], D Hazuda[1], R Hrin[1], S Jarantow[1], J Joyce[1], P Kim[1], S Lane[3], S Lennard[3], C Lloyd[3], D Lowe[3], M Lu[1], P Lu[1], M McElhaugh[1], J Osbourn[3], M Patel[1], A Pessi[2], W Schleif[1], J Shiver[1], W Strohl[1], T Vaughan[3] and H Zhang[1]. 1 Merck Research Laboratories, West Point, Pa. ; 2 IRBM P. Angeletti, Pomezia, RM, Italy; 3 Cambridge Antibody Technology, Cambridge, UK.

ABSTRACT 10

Antiviral Therapy 2004; **9:**S14.

Cross-resistance profile of the novel lysine-containing HIV-1 protease inhibitor PL-100

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BACKGROUND: The rapid emergence of drug-resistant strains of HIV is a major issue in HIV/AIDS treatment. New viral inhibitors with distinct structural properties are urgently needed to address this problem. Our approach has been to synthesize a series of protease inhibitors (PIs) based on an L-lysine scaffold. The cross-resistance profile of one selected compound, PL-100, was determined using a selected series of 14 HIV isolates with known reduced susceptibility to other PIs.

METHODS: A series of L-lysine derivatives was first evaluated using recombinant HIV protease enzymatic assays and then in cell culture antiviral assays using wild-type HIV-1 (NL4-3) grown in MT4 cells. One of the most active compounds of the series, PL-100, was further characterized. Activity against a panel of 14 multi-PI-resistant strains was evaluated using the PhenoSense assay (ViroLogic). For comparison, saquinavir, indinavir, nelfinavir, amprenavir and lopinavir were tested in parallel.

RESULTS: PL-100 inhibited HIV protease with a Ki of less than 100 pM, and was highly specific for this particular aspartic protease. The compound inhibited wild-type HIV with an EC_{50} of 8 nM, while cytotoxicity (CC_{50}) in MT4 cells was 34 µM. In the panel of resistant viruses, there was broad cross-resistance among the approved PIs tested, with a range in median fold-change (FC) of 8.2-fold to 54-fold for the different PIs. In comparison, PL-100 had a median FC of 4.5-fold. Between 11 and 13 isolates had >2.5-fold reduced susceptibility to one of the five approved PIs, and four to 11 isolates had FC >10-fold. In comparison, nine isolates had FC >2.5 and two isolates had >10-fold reduced susceptibility to PL-100. In six of the 14 isolates, the FC for PL-100 was the lowest of the PIs tested, compared to two for APV, three for IDV, two for SQV, and one for LPV. Linear regression between PL-100 and the approved PIs revealed a weak correlation with amprenavir (r^2=0.51) and lopinavir (r^2=0.49).

CONCLUSION: PL-100 has potent anti-protease and antiviral activity against wild-type HIV-1, and has a favourable cross-resistance profile as compared to the PIs tested in this study.

ABSTRACT 11

Antiviral Therapy 2004; **9:**S15.

Phenotypic and genotypic resistance to a new protease inhibitor, 640385, in HIV-1 virus samples from subjects failing amprenavir

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BACKGROUND: 640385 is a new protease inhibitor (PI) in clinical development with potency against multiple PI-resistant clinical isolates. Because 640385 shares structural features with amprenavir, early assessment of any potential cross-resistance was desirable. HIV-1 isolates from subjects experiencing virological failure on amprenavir-containing regimens were examined for resistance to 640385 and amprenavir.

METHODS: Thirty clinical isolates obtained from subjects experiencing virological failure to an amprenavir-containing regimen and having amprenavir-associated resistance mutations (I54L/M *n*=10, I50V *n*=10, V32I+I47V *n*=5, I84V *n*=2, V32I *n*=1, I54L+I84V *n*=1, I54M+I84V *n*=1) were sent to ViroLogic for phenotypic evaluation (PhenoSense™). Isolates had additional protease substitutions (mean of 3.7 IAS mutations, mean of 7.9 changes from HXB2). Isolates containing mixtures at amprenavir-associated amino acid positions were excluded.

RESULTS: The mean fold increase of IC_{50} for the 30 viruses was 10.8-fold change (FC) to amprenavir and 2.0-FC to 640385. Of the 13 viruses (13/30, 43%) with >10-FC to amprenavir (mean 17.7-FC), none (0/13, 0%) had >10-FC to 640385 (mean 3.1-FC). Of the 17 (17/30, 57%) viruses with ≤10-FC to amprenavir (mean 5.5-FC), none (0/17, 0%) had >2 FC to 640385 (mean 1.2-FC). Of the viruses harbouring I54L/M or V32I+I47V mutations, none had >2-FC to 640385 (I54L/M: amprenavir mean 4.7-FC vs 640385 1.1-FC; V32I+I47V amprenavir mean 8.8-FC vs 640385 1.4-FC). Of the viruses harbouring the I50V mutation, 5/10 had <2-FC, 4/10 had 2 to ≤3-FC and 1/10 had >3-FC (7.9-FC) to 640385 (I50V: amprenavir mean 13.4-FC vs 640385 2.7-FC). Viruses harbouring the I84V mutation in the absence of other amprenavir-associated mutations had limited reduction in susceptibility to 640385 (1.4- and 2.1-FC). Both viruses having the I54L/M+I84V mutations had >3-FC to 640385 (3.9- and 6.0-FC, respectively).

CONCLUSIONS: In a panel of HIV-1 isolates specifically selected for presence of amprenavir resistance, there was minimal evidence for cross-resistance between 640385 and amprenavir despite their chemical similarity. Although I54L/M+I84V mutations may contribute to decreased 640385 susceptibility, I54L/M mutations in the absence of I84V, and conversely I84V mutations in the absence of I54L/M and V32I+I47V do not appear to be associated with 640385 resistance. Similarly, the I50V mutation did not appear associated with 640385 resistance.

ABSTRACT 12

Antiviral Therapy 2004; **9**:S16.

In vitro selection and characterization of resistance to the new HIV protease inhibitor GW640385

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BACKGROUND: GW640385 is a new HIV-1 aspartyl protease inhibitor (PI) with potency against multiple PI resistant clinical isolates. *In vitro* passage of wild-type (HXB2, IC_{50}=0.5 nM) virus with GW640385, was carried out in MT-4 cells at both high and low selection pressure. The aim of this study was to elucidate the impact of individual mutations, selected during *in vitro* passage, upon GW640385 anti-HIV activity.

METHODS: Viral RNA was extracted from each passage and the protease (PRO)/cleavage sites (CS) (p7/p1–p1/p6) were subjected to population and clonal sequence analysis to confirm linkage between resistance mutations. Site-directed mutant viruses were constructed containing individual or combinations of the selected substitutions to determine the contribution of each mutation to GW640385 resistance.

RESULTS: Low pressure *in vitro* passage (13 passages up to 5 nM, ~10×IC_{50}) led to the selection of PRO substitutions: Q58E, A71V, V82I and CS R452K. Phenotypic evaluation of individual mutations, or combinations thereof, demonstrated no more than a 2.55-fold reduction in susceptibility to GW640385. High pressure *in vitro* passage (15 passages up to 120 nM, ~240×IC_{50}) led to the selection of PRO substitutions: L10F, G16E, E21K, A28S, M46I, F53L, A71V and CS L449F, P453T. All the site-directed mutants grew successfully in cell culture except for the A28S variants. Phenotypic evaluation of these individual variants showed that each had minimal impact on GW640385 activity (up to twofold decrease in susceptibility). Viruses containing the A28S mutation, either alone or in combination with other PRO/CS changes, failed to replicate or replicated poorly and could not be phenotypically evaluated. Recombinant virus generated from cloned virus from the original passage experiment also either failed to grow or grew very poorly. One virus that grew poorly was phenotypically evaluated (L10F, G16E, K20T, A28S, M46I, A71V, L449F, P453T) and had high-level resistance (>20-fold decreased susceptibility) to GW640385.

CONCLUSION: The *in vitro* passage of HXB2 in the presence of GW640385 has identified amino acids associated with decreased susceptibility to GW640385. High pressure passage has led to the selection of the A28S protease mutation which was associated with a dramatic reduction in replicative capacity of the virus.

ABSTRACT 13

Antiviral Therapy 2004; **9:**S17.

Antiviral activity and resistance profile of AG-001859, a novel HIV-1 protease inhibitor with potent activity against protease inhibitor-resistant strains of HIV

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BACKGROUND: The availability of highly active antiretroviral therapy for patients who have previously failed protease inhibitor (PI)-containing regimens is limited by the substantial amount of cross-resistance that exists among the currently approved PIs. A series of novel allophenylnorstatin-containing HIV-1 PIs, represented by AG-001859, has been identified, which demonstrate potent antiviral activity against strains of HIV resistant to the currently approved PIs.

METHODS: The *in vitro* antiviral activity of AG-001859 was characterized in biochemical and cell-based assays against wild-type and PI-resistant strains of HIV.

RESULTS: AG-001859 is a potent, tight binding inhibitor of both wild-type HIV protease and an I84V/L90M mutant HIV protease, exhibiting a Ki of <0.1 nM against both enzymes. In cell-based assays, AG-001859 demonstrated potent antiviral activity against several strains of wild-type HIV, including laboratory adapted and primary isolates, with EC_{50} values ranging from 14 to 60 nM. The antiviral activity of AG-001859 was only moderately attenuated in the presence of 50% human serum (3.7-fold change in EC_{50}, P=0.013). The antiviral activity of AG-001859 was also evaluated against a panel of 44 PI-resistant HIV-1 variants (PhenoSense™) containing a variety of primary and secondary amino acid substitutions that confer broad cross-resistance to the approved PIs (mean number of PI-resistance substitutions = 5; range 3–11). The median EC_{50} value against these PI-resistant variants was 34 nM (range 5.3–420 nM), and the median fold-change relative to wild-type HIV was 1.6 (range 0.3–19.8). In contrast, viruses in the panel demonstrated median fold-change values of 9.3, 9.6 and 17.0 to indinavir, lopinavir and nelfinavir, respectively. AG-001859 demonstrated potent activity against all primary and secondary PI-resistance substitutions evaluated, and there was no correlation between the antiviral activity of AG-001859 and the number of PI-resistance substitutions present.

CONCLUSIONS: AG-001859 is one compound in a series of novel allophenylnorstatin-containing HIV-1 PIs which demonstrates potent antiviral activity against strains of HIV resistant to the currently approved PIs. The pharmacokinetics and safety of several compounds from this series are currently being evaluated in Phase I studies.

ABSTRACT 14

Antiviral Therapy 2004; **9:**S18.

Highly potent HIV protease inhibitors with broad activity against MDR strains

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BACKGROUND: A majority of drug-experienced patients in North America harbour HIV that is resistant to one or more FDA-approved antiretroviral agents. Recent studies suggest that the effectiveness of salvage therapies that include lopinavir/ritonavir and amprenavir/ritonavir regimens may be diminished for patients with more than 6–7 PI mutations at baseline. The epidemiological increase of MDR HIV strains poses a challenge to the development of new antiretroviral agents targeted to mutant protease and reverse transcriptase enzymes. There is an urgent need for potent and broad-spectrum PIs that be used to treat MDR HIV strains and to prevent the development of MDR HIV in primary treatment settings.

METHODS: We have implemented a structure-based approach for the design of novel HIV protease inhibitors (PIs) with high potency against WT and MDR viruses and evaluated their activity against WT and MDR mutant forms of HIV PR in enzyme-based assay and cell-based antiviral assays.

RESULTS: We have designed and synthesized a series of HIV-1 PR inhibitors (SPI inhibitors) that interact with a conserved substructure of the enzyme's active site that is relatively unaffected by mutations known to cause HIV PI drug resistance. In a cell-based antiviral assay, five SPI inhibitors exhibited an average IC_{50} value for WT HXB2 HIV of 6.6 nM (range: 4.5–10 nM). More importantly, SPI inhibitors retained potency against a panel of seven recombinant HIV-1 viruses derived from MDR patient clinical isolates containing 10–17 mutations in PR region. The average IC_{50} value against these mutants was 15.7 nM (range: 7.6–24 nM). For comparison, seven FDA-approved HIV PIs, APV, IDV, LPV, RTV, NFV, SQV and ATV have an average IC_{50} value for WT of 31 nM (3.5–70 nM) and an average IC_{50} value against mutants of more than 2100 nM (range: 159 to >5440 nM). All SPI inhibitors have CC_{50} values in MT4 cells more than 10 000 nM, which corresponds to a selectivity index of >1000. Addition of 40% of human serum increased the IC_{50} by 2.4- to 7.8-fold. A 5.7- to 9.0-fold increase in IC_{50} was observed in the presence of 45% human serum supplemented with 1 mg/ml of human alpha-acid glycoprotein which is similar to the increase seen for most other PIs.

CONCLUSIONS: SPI inhibitors are highly active against WT and MDR HIV isolates, and have the potential for further development.

ABSTRACT 15

Antiviral Therapy 2004; **9**:S19.

Analysis of time of failure genotype and phenotype from NNRTI-experienced patients treated with capravirine

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BACKGROUND: The safety and efficacy of capravirine, a novel NNRTI, were evaluated in combination with nelfinavir and two NRTIs in two Phase 2 studies in HIV-infected, NNRTI-experienced patients. Although both studies were discontinued early, patients with a viral load <400 copies/ml were given the option of continuing on open-label capravirine, nelfinavir and NRTIs. Of the 36 patients who elected to continue therapy, 16 (44%) remain virologically suppressed (VL <50 copies/ml) following 39–49 months of therapy. There were 20 cases in which the patient discontinued from the study early: six were due to virological failure, five were due to adverse events, and nine were for non-study related reasons.

METHODS: HIV phenotype and genotype were determined for each patient at study entry and at the time of virological failure (TOF).

RESULTS: Six of the 36 patients (17%) have discontinued from the study due to virological failure. The median time to virological failure was 19 months (range 4–27 months). Two of the six patients did not develop any new genotypic changes or further reduction in susceptibility to either capravirine or nelfinavir, despite receiving therapy for 11 and 15 months. Three of the six patients developed new or increased levels of resistance to both capravirine and nelfinavir at TOF. Virus isolated from these patients contained one, two or three new NNRTI-resistance associated substitutions located at different positions in reverse transcriptase (101, 108, 190 and/or 188) as well as substitutions at positions 20, 30, 36 and/or 88 of protease. TOF genotype data is not available for the one remaining patient; however, phenotype data indicate the development of nelfinavir resistance (8.2-fold), and no measurable change in NRTI or capravirine susceptibility.

CONCLUSIONS: NNRTI-experienced patients treated with capravirine as part of a HAART regimen can achieve long-term suppression of viraemia. Results from the present study support previous *in vitro* and *in vivo* findings demonstrating that a single mutation conferring high-level resistance does not rapidly emerge during capravirine therapy. Rather, varied patterns of substitutions slowly emerge, indicating a high genetic barrier to resistance. Phase 2b studies evaluating capravirine in NNRTI-experienced patients are currently underway.

ABSTRACT 16

Antiviral Therapy 2004; **9**:S20.

In vitro resistance development for a second-generation NNRTI: TMC125

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BACKGROUND: TMC125 is a NNRTI under clinical development with potent *in vitro* antiviral activity against many NNRTI resistance mutations. We describe the development of resistance mutations under selective pressure of TMC125.

METHODS: Wild-type (HXB2) and K103N viruses were grown in the presence of increasing drug pressure. Emerging viral supernatants were amplified in MT4 cells, proviral DNA was extracted, amplified and sequenced. Viral RNA was isolated for clonal analysis. Site-directed mutants were generated with the QuickChange kit (Stratagene) and tested in a pseudotyped single-cycle assay.

RESULTS: As a control, HXB2 was passaged with increasing efavirenz (EFV) concentrations. EFV selected for L100I, K103N, L214F and P225H. A combination of L100I, K103N and L214F conferred reduced susceptibility with a >1000-fold increase in IC_{50}. Other amino acid changes included T39A, I94L, H96L, S156P, D192N, G196R, E203K, Q207R, T216A and S268G. TMC 125 selected for T39A, E138K, V179F, Y181C, L214F, F227L, M230I/L; with polymorphisms at: E40K, K70R, Q91L, L109M, R125G, A158T, Q174P, G196R, N265T, D256G and E291K. Clonal analysis from virus selected at 360 nM passage concentration revealed 9/24 clones containing the triple mutant E138K+V179F+Y181C. The virus pool from this passage step showed an IC_{50} of 514 nM (>700-fold change). At 10 μM, TMC125 selected for mutations at E138K, V179F, Y181C, L214F and M230L. The quadruple mutant V179F+Y181C+L214F+ M230L showed decreased susceptibility to EFV, CPV, GSW678248 and TMC125 by 133-fold, >600-, >600- and >850-fold, respectively. TMC125 selected mutants from K103N included: K102Q, E122K, Y146S, Y181C, while the K103N reverted to wild-type. Site-directed mutagenesis studies to introduce identified positions for single-cycle assay testing are in progress.

CONCLUSIONS: TMC125 lost susceptibility with mutants: V179F, Y181C, L214F and M230L. In combination, these mutations conferred a >800 shift in susceptibility. The mutation patterns selected conferred cross-resistance to EFV, CPV and GSW678248.

ABSTRACT 17

Antiviral Therapy 2004; **9:**S21.

Dioxolane-thymine nucleoside (DOT) against drug-resistant HIV-1 mutants and its molecular mechanism

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BACKGROUND: The majority of AIDS patients are currently taking nucleosides as part of a combination therapy. Therefore, the development of nucleoside-resistant mutants of HIV-1 is a serious problem for the management of HIV infection.

METHODS: Since the discovery of βD-dioxolane-2,6-diaminopurine (DAPD or Amdoxovir) is active against AZT- and 3TC-resistant mutants, several other nucleosides with a dioxolane moiety have been synthesized in our laboratories, and their anti-HIV activity against drug-sensitive and drug-resistant mutants was determined, and their molecular mechanisms have been studied by molecular modelling.

RESULTS: Among the series of dioxolane nucleosides, the thymidine (DOT) showed significant and interesting anti-HIV activity against nucleoside-resistant mutants, as shown below. It was found from the molecular modelling studies that the dioxolane moiety plays a significant role in stabilizing the binding between the mutant HIV RT and the nucleoside TP.

Resistance strains	EC_{50} (μg)	IC_{50} (μg/ml)	TI	Activity
D67N, K70R, K103N, I135L, M184V, K219E, R284K, C355F, R356K	2.44	>100	>41	Active
M41L, E122K, M184V, T215Y	1.13	>100	>42	Active
M41L, D67N, K70R, T215Y, K219Q Other mutations not associated with AZT/R=V60I, K83R, H208Y, L239V, R356K G359S	0.29	>100	>345	Highly active
LAI M184V	0.29	>40	>137	Highly active

CONCLUSION: DOT is significantly active against nucleoside-resistant HIV-1 mutants. Thus, additional biological studies are warranted to determine the full potential of DOT as a potential anti-HIV agent (Supported by NIH AI32351, AI25899 and Veterans Affairs).

ABSTRACT 18

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Impact of residue 50 substitutions on phenotypic susceptibility to protease inhibitors

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BACKGROUND: Resistance to the HIV-1 protease inhibitor (PI) atazanavir (ATV) is associated with a signature protease (PR) substitution, I50L. PR substitutions at four primary positions (32, 50, 54 and 84), including I50V, are associated with resistance to amprenavir (APV). The impact of these substitutions on phenotypic susceptibility to other PIs may impact future treatment options.

METHODS: The Virologic database containing 24 125 clinical samples with matching genotypes and phenotypes was analysed to identify those with the following PR substitutions: I50L, I50V, V32I+I47V or I54L/M, alone or in combination with one or two other primary PR substitutions (D30N, G48V, V82A/T/S/F, I84V or L90M). The mean, median, and range of phenotypic susceptibility (FC, fold change in IC_{50} compared to NL4-3), as well as the percentage of samples with phenotypic susceptibility above the assay cutoff (clinical cutoff when available), were determined. Assay cutoffs for APV, ATV, indinavir (IDV), nelfinavir (NFV), ritonavir (RTV), and saquinavir (SQV) were 2.0–2.3, 2.1–2.5, 2.5 and 1.7-fold, respectively. Clinical cutoffs for RTV-boosted IDV and RTV-boosted lopinavir (LPV/r) were both 10-fold.

RESULTS: As expected, 100% of samples with I50L only (*n*=20) were above the assay cutoff for ATV (median FC=9.0), while none demonstrated resistance to any of the other PIs (100% below cutoff, median FC <1). Of five samples with I50L and one other primary PI mutation, three had D30N, one V32I and one L90M. In contrast, the I50V substitution alone (*n*=29), which conferred APV and RTV resistance in all cases, was also sometimes associated with resistance to ATV (6% over cutoff), LPV/r (41%), IDV (21% assay, 0% clinical), NFV (41%) and SQV (34%). Samples with other characteristic APV substitutions, V32I+I47V or I54L/M, demonstrated similar or lower levels of resistance compared to I50V alone, with the exception of ATV and NFV, for which resistance levels were similar or slightly higher.

CONCLUSION: These data indicate that the I50L substitution confers ATV-specific reductions in phenotypic susceptibility without cross-resistance to other marketed PIs. In contrast, APV-selected PI substitutions reduce phenotypic susceptibility to other PIs, in some cases, including LPV/r. Early use of ATV may preserve future treatment options.