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Key Presentations on HIV Drug Resistance, Resistance Testing and Replication Capacity



A Case of Recent Infection by a Multi-Drug-Resistant, Dual-Tropic HIV-1 in Association with Rapid Progression to AIDS

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A gay man in his late 40's from NYC presented on 12/16/04 with pharyngitis and fatigue. He was found to be HIV-1-positive, although five previous tests performed between 9/00 and 5/03 were negative. In retrospect, he recalled having fever, pharyngitis, fatigue, and myalgia of one-week duration in early 11/04, approximately 2 weeks following unprotected anal intercourse with multiple partners. Evaluations on 1/13/05 confirmed the positive HIV-1 serology, and revealed marked lymphopenia, a CD4 T-cell count of 65 cells/mm³, and a plasma viral load of 99,200 copies/ml. One week later, his CD4 T-cell count was 28 cells/mm³, and plasma viral load was 232,000 copies/ml. The patient had lost 4 kilogram of body weight over the past 3 weeks. Although a CD4 T-cell count was not available when he was HIV-1 negative, records showed that he had multiple normal lymphocyte counts cells before and on 5/03. Viral genotyping and phenotyping studies demonstrated that the patient harbored an HIV-1 that was resistant to NRTI, NNRTI, and PI, but sensitive to efaviridine (T-20). Furthermore, the viral quasispecies in his plasma and PBMC could use both CCR5 and CXCR4 as the coreceptor. This virus grows well without loss of fitness, and causes the formation of syncytia in vitro.

It is clear from the available evidence that this patient is recently infected by an HIV-1 that is not easily treated using standard antiretroviral therapy. Moreover, his rapid clinical and immunological deterioration is of concern. While it is possible that this patient is genetically predisposed to rapid disease progression, the thought of a virulent virus is inescapable. The progression to symptomatic AIDS in this case has occurred over a period of, at most, 20 months. In fact, his acute illness in 11/04 suggests that this marked immunological depletion may have taken place over only 4 months. That this patient has had hundreds of sexual partners has triggered the NYC Department of Health to issue an alert to physicians and to begin tracing of sexual contacts. The public health ramifications of this case should be obvious.

Poster Presentation

Thursday, February 24, 6:00pm – 7:00pm



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Clinician Selection of ARV Regimens Is Influenced by the Type of Resistance Test Information Provided

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Introduction: The clinical utility of combined phenotype (PT) and genotype (GT) resistance test results (PTGT) compared to PT or GT alone for the selection of antiretroviral (ARV) regimens in treatment-experienced patients has not been demonstrated. We sought to determine if the provision of PTGT test results changed ARV choices by a group of experienced HIV clinicians compared to having the same information given by GT or PT separately.

Methods: HIV clinicians (n=109) attending regional advisory meetings recommended ARV regimens for 10 patients based only on actual GT, PT, or PTGT results. No clinical information was provided, and resistance test results were given in random order. All samples had at least 1 drug with PT resistance (mean 11.3 drugs, range 6 to 18). Discordance between tests was defined as having occurred when one test led to the choice of a particular ARV while the comparator test did not. Optimal regimens and acceptable ARV choices were determined by a consensus of the authors and was compared to the regimens chosen by the participants to determine how many “acceptable” ARVs were chosen based on GT, PT or PTGT.

Results: There was substantial discordance seen in the three-way comparisons of ARV choices based on GT, PT or PTGT for all ARVs and all 10 samples. The average discordance for all samples between GT and PT was 19% (range 0-87), 11% (range 0-37) for PT vs. PTGT, and 18% (range 0-83) for GT vs. PTGT. When analyzed by ARV, median GT vs. PT discordance ranged from 41% for TDF to 2% for NFV, PT v. PTGT ranged from 27% for ddI to 1% for ATV and NFV, and GT vs. PTGT ranged from 36% for TDF to 2% for NFV. Choice of ARV regimen based on PTGT yielded the highest number of acceptable ARVs. The % of regimens with 3 or more acceptable drugs ranged from 36.7 to 96.33 (median 65.1) for GT, 42.3 to 99 (median 85.8) for PT and 47.7 to 97.3 (median 86.2) for PTGT (p < 0.001 for main effect of test in log linear models).

Conclusions: Experienced clinicians make different regimen choices based on the information provided by GT, PT, or PTGT. It appears from this study that clinicians are more likely to select optimal regimens based on PT or PTGT combined results compared to GT but further study is needed to determine if the differences in ARV choice by type of resistance test results in improved clinical outcomes.

Poster Presentation

Friday, February 25, 1:30pm – 3:30pm



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Prediction of Early HIV-1 RNA Reduction in the Jaguar Study Using Phenotypic Susceptibility to Didanosine

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Background: Previous studies have shown that the range of phenotypic fold change (FC) in didanosine (ddl) susceptibility is remarkably narrow, challenging its use as a predictor of virologic response. We investigated whether and how the FC in ddl as measured by the ViroLogic PhenoSense assay predicts virologic response to ddl in the Jaguar study.

Methods: Patients experiencing a virologic failure on combination therapy were randomised in the Jaguar study to receive ddl (n=110) or ddl-placebo (n=58). We considered two virologic outcomes: the magnitude of reduction in HIV-1 RNA from day 0 to week 4 (W4) and a protocol-defined virologic response - a reduction of $\geq 0.5 \log_{10}$ or W4 viral load (VL) < 50 copies/ml. Phenotypic susceptibility score (PSS) to ddl was defined as partially continuous or continuous and association with HIV-1 RNA reduction was evaluated using linear regression. Fischer's exact and non-parametric tests for ordered alternatives were used to quantify the strength of association between discrete categories of FC in ddl and virologic outcomes.

Results: Ninety-eight patients randomised in the ddl arm had both FC in ddl and week-4 virologic response available. The median decrease in HIV-1 RNA was 0.57 (Inter Quartile Range, 0.15 to 1.02) and the median FC in ddl was 1.65 (IQR 1.46 to 1.97). Partially continuous PSS was poorly predictive of week 4 virologic response ($p=0.13$) while continuous PSS was predictive of HIV-1 RNA reduction at week 4 ($P<0.001$) with a moderate proportion of variation explained, $R^2=0.12$. At very low FC (≤ 1.3), 15 of 18 (83%) patients responded compared with 33 of 66 (50%) patients with $1.3<FC<2.2$, and 4 of 14 (29%) patients with $FC\geq 2.2$ ($p=0.006$). Median decrease in VL was 1.01, 0.50 and 0.10 in patients with $FC \leq 1.3$, between 1.3 and 2.2, and 2.2 or higher, respectively ($p<0.0001$).

Conclusions: The relationship between phenotypic fold change to ddl and virologic response describes a continuum of susceptibility. At low fold change values (≤ 1.3) in these highly experienced patients, the majority of patients responded. These patients also experienced the largest drops in viral load from baseline. Patients with intermediate FC values ($1.3<FC<2.2$) had approximately a 50% probability of responding while patients with fold changes ≥ 2.2 had a lower probability (29%) of responding to ddl. As such, these data suggest the existence of a FC range of intermediate probability of response not appreciated previously.

Oral Presentation

Thursday, February 24, 12:15pm



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Atazanavir Resistance in a Protease Inhibitor (PI) Naïve Patient Treated with Atazanavir/Ritonavir Associated with Development of the N88S Mutation in Protease

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Introduction: I50L is the signature protease mutation in PI naïve individuals failing unboosted ATV. Although *in vitro* data identified N88S as an ATV resistance mutation it has not been observed without I50L in clinical studies of ATV in PI naïve individuals. We describe a case in which N88S emerged independently on ATV/r therapy and which was associated with high level ATV resistance.

Methods: The patient had a viral load (VL) of 6,547 copies (c)/mL and a CD4 count of 445 cells/mm³ on non-PI HAART. Barring a brief prior exposure to SQV associated with VL suppression he was PI naïve. Prior to starting ATV/r, resistance testing revealed no phenotypic or genotypic PI resistance. He commenced ATV, TDF, ABC and 3TC. At 3 months the VL had fallen to 118 c/mL and RTV was added to dose adjusted ATV. The subsequent VL was <50 c/mL but gradually rose to 7,535 c/mL 7 months later. Comparisons were made to the phenotypic profiles of clinical isolates within the ViroLogic database with either N88S or I50L as the only major PI mutation (t-test). Temporal surveillance of N88S and I50L was also performed; mixtures were counted as mutant for this analysis.

Results: The PR genotype after rebound was K20T, M36I/V, L63P, A71T, and N88S. The susceptibility (fold change) to ATV, APV, IDV, LPV, NFV, and SQV was 56, 0.3, 13, 4.3, 68, and 4.2 respectively. The replication capacity was 14% (compared to 96% prior to ATV). Phenotypic profiles of samples in the ViroLogic database with N88S, or I50L for comparison, but no other major PI mutations, are shown in Table 1. Among samples with at least one primary PI mutation the prevalence of clinical samples with N88S has increased from 1% to 2.5% in 12 months (9/03-9/04), coincident with an increase in prevalence of I50L from 0 to 2%.

Table 1.

	N	Mean fold change						RC (%)
		ATV	APV	IDV	LPV	NFV	SQV	
N88S	96	12.0	0.2	5.4	1.2	20.2	1.8	35.3
I50L	7	9.6	0.9	0.5	0.3	0.8	0.4	11.7

p<0.01 for all drugs between groups

Conclusions: To our knowledge this is the first case of primary resistance to ATV following ATV/r therapy. High-level ATV resistance and low RC were associated with the N88S Mutation in the absence of other primary resistance mutations. In the ViroLogic database isolates with N88S as the only major mutation had cross-resistance to ATV, NFV and IDV, and APV hypersusceptibility (HS). These findings contrast with isolates bearing the I50L mutation which is associated with ATV-specific resistance and HS to IDV, LPV and SQV. These data highlight the potential clinical relevance of the N88S mutation as a mechanism of resistance to ATV inclusive therapies.

Poster Presentation

Friday, February 25, 1:30pm – 3:30pm





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Contribution of Non-Thymidine Analog Nucleoside RT Inhibitor Associated Mutations (non-TA NAMs) to Phenotypic Hypersusceptibility (HS) to Efavirenz (EFV)

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Background: EFV HS has been associated with TAMs and superior clinical outcomes in subjects treated with EFV. Recent studies have highlighted the activity and resistance profiles of a variety of thymidine analog (TA)-sparing nucleoside analog combinations. Therefore we evaluated the impact of non-TA NAMs on EFV susceptibility within the ViroLogic database.

Methods: EFV HS was defined as fold-change (FC) < 0.4 using the PhenoSense assay. Isolates with unmixed NRTI mutations (K65R, T69X, L74IV, V75X, M184IV; X=any non-wt amino acid) but no TAMs (M41L, D67N, K70R, L210W, T215FY, K219X), Q151M, T69 insertions or NNRTI mutations (A98G, L100I, K101EP, K103NS, V106AM, Y181X, Y188X, G190X, P225X, F227X, M230L, P236L) were identified. Isolates without NRTI, NNRTI or PI mutations served as a wild type (WT) reference group.

Results:

NAMS	N	% with EFV FC <0.4	Mean EFV FC	P value vs. WT
Wild-type	9120	4.1	0.98	-
1 mutation	1991	19	0.70	<0.0001
2 mutations	177	36	0.58	<0.0001
3 mutations	46	30	0.82	0.061*
K65R only	31	52	0.56	<0.0001
T69X only	103	19	0.78	0.0004
M184IV only	1799	21	0.70	<0.0001
K65R + M184IV only	76	53	0.46	<0.0001

*p=0.03 excluding a single outlier with EFV FC=13.

Mean EFV FC was lower for isolates with 1 NAM vs. WT (p<0.0001) and 2 vs. 1 NAM (p=0.0006). When analyzed by specific mutation, samples with only K65R, T69X or M184IV demonstrated significantly reduced EFV FC compared to WT. Further, the effect was greater for K65R compared to T69X (p=0.041) but not to M184IV (p=0.1). Isolates with K65R+M184IV had lower mean EFV FC than M184IV alone (p<0.0001) but not lower than K65R alone (p=0.12). NNRTI susceptibilities were measured in site-directed mutants (SDMs) bearing K65R in an NL4-3 background; FC to EFV, NVP and DLV were 0.56 +/- 0.02, 0.53 +/- 0.05 and 0.54 +/- 0.03, respectively (mean +/- SD of 18 replicates).

Conclusions: Groups of viruses containing the specific non-TA NAMs K65R, T69X, M184IV and K65R+M184IV demonstrate enhanced EFV susceptibility. An NL4-3/K65R SDM demonstrated significantly lower mean FC to EFV, NPV and DLV. Thus EFV HS is associated with both nonTA NAMs in addition to TAMs. These observations may have relevance to the use of NNRTIs in non-TA-NRTI combination regimens and may help explain the superior virological efficacy of these regimens.

Poster Presentation

Wednesday, February 23, 1:30pm – 3:30pm





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Phenotypic Hypersusceptibility to Multiple Protease Inhibitors and Low Replicative Capacity in Chronically HIV-1-Infected Patients

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Background: Increased susceptibility to the protease inhibitors (PI) saquinavir and amprenavir has been observed in human immunodeficiency virus type-1 (HIV-1) with specific mutations in protease (V82T and N88S, respectively). Increased susceptibility to ritonavir has also been described in some viruses from antiretroviral nave-patients with primary HIV-1 infection in association with combinations of amino acid changes at polymorphic sites in the protease. Many of the viruses displaying increased susceptibility to PIs also had low replication capacity (RC).

Methods: In this retrospective study, we analyze the drug susceptibility phenotype and the replication capacity of virus isolates obtained at the peaks of viremia during five consecutive structured treatment interruptions in 12 chronically HIV-1-infected patients.

Results: Ten out of 12 patients had at least sample with PI hypersusceptibility (HS, fold change 0.4) to 1 or more PI. HS to all PIs was observed at variable frequency, ranging from 38% to amprenavir to 11% to nelfinavir. Pairwise comparisons between susceptibilities for the PIs showed a consistent correlation among all pairs. There was also a significant relationship between susceptibility to PIs and RC in all patients. RC remained stable over the course of repetitive cycles of structured treatment interruptions. We could find no association between *in vitro* RC and *in vivo* plasma viral load doubling time, CD4+ and CD8+ T cell counts at each treatment interruption. Several mutations were associated with HS to each PI, however a multivariate model failed to detect independent effects indicating that these sites may not act individually.

Conclusions: This study extends the association between HS to PIs and low replication RC to virus isolated from chronically infected patients and highlights the complexity of determining the genetic basis of this phenomenon. The potential clinical relevance of PI HS and low RC to virologic response to PI-based therapies deserves to be further investigated.

Poster Presentation

Thursday, February 24, 1:30pm – 3:30pm



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Comparison of Phenotypic Effects of NRTI Mutations on Emtricitabine (FTC) and Lamivudine (3TC)

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Background: FTC (emtricitabine) and 3TC (lamivudine) are structurally related nucleoside inhibitors of HIV-1 reverse transcriptase. The phenotypic susceptibility to FTC of viruses containing common NRTI mutations is not well characterized. A large database was used to examine the resistance profiles of both drugs and the potential cross-resistance conferred by NRTI resistance-associated mutations.

Methods: The ViroLogic database was queried to identify samples with M184I/V only and those without M184I/V but containing: 2-3 TAMs from 41/210/215, 2-3 TAMs from 67/70/219, any 3-4 TAMs, any 5-6 TAMs, K65R, or T69 insertions (with TAMs allowed). The median fold change in resistance (MFC) and the percentage of isolates above assay cutoff were determined for both FTC and 3TC. Mutations/patterns which were represented by fewer than 10 samples were not included in this analysis.

Results: MFC to FTC and 3TC in the presence of the M184I/V mutation only was always above the maximum measurable level. The MFC and % samples over cutoff for the other mutations are presented. More samples had an FTC than 3TC MFC over the cutoff in every TAM category examined. For 2 or 3 TAMs from 67/70/219, 3-fold more samples had FTC than 3TC MFC over the cutoff.

mutations	FTC			3TC		
	N	MFC	% over cutoff	N	MFC	% over cutoff
65R only	31	6.7	93.5%	161	8.8	98.1%
T69ins (TAMs allowed)	10	14.7	90.0%	96	9.2	89.6%
2 or 3 TAMs from 41/210/215	61	2.3	9.8%	450	1.9	6.2%
2 or 3 TAMs from 67/70/219	27	2.2	18.5%	227	1.9	6.2%
any 3-4 TAMs	89	3.3	49.4%	645	3.0	38.8%
any 5-6 TAMs	22	6.8	100.0%	183	4.9	79.2%

The MFC in all groups were significantly different (P<0.001, t-test comparison of means)

Conclusion: Differences in FTC and 3TC resistance for M184I/V containing-samples could not be assessed as both exceeded upper assay limits. Slightly more resistance was observed for FTC than 3TC in samples containing TAMs or T69 insertions, and a greater percentage of isolates retained phenotypic susceptibility to 3TC than FTC in the presence of TAMs for each TAMs subgroup analyzed. With respect to K65R, the MFC for FTC was lower than for 3TC. These data highlight small but statistically significant differences in the MFC for FTC as compared to 3TC. It is unclear whether any of these differences are clinically significant.

Poster Presentation
Friday, February 25, 1:30pm – 3:30pm



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Cross-Resistance of Clinical Samples with K65R, L74V, and M184V MutationsM Underwood¹, M St Clair¹, L Ross¹, P Gerondelis¹, N Parkin², R Lanier¹¹GlaxoSmithKline, RTP, NC, ²ViroLogic, Inc., South San Francisco, CA

Background: Considerations for initial drug selection in HIV therapy should include the effects resistance will have on subsequent therapy options. Thymidine analog sparing NRTI backbones or all-NRTI regimens do not select for thymidine analog mutations, but may still result in multi-nucleoside resistance patterns, typically involving combinations of K65R, L74V and M184V. We evaluated phenotypic effects of these mutations in a large database of clinical samples to help define the range of cross-resistance, and potential drug sequencing implications.

Methods: Phenotypic susceptibilities (mean, median, range, and % above cutoff) to NRTIs of viruses in the ViroLogic database containing K65R, L74V, M184I, and M184V were determined. Samples with mixtures at the positions of interest or other nucleotide analogue mutations (NAMs) (41L, 67N, 69X, 70R, 75X, 115F, 151M, 210W, 215FY, 219X; X = any non-wt amino acid) were excluded. Data is presented as median fold resistance change (MFC) vs. reference, and as % above assay cutoff (AC). Clinical cutoffs for ABC=4.5, ddI=1.7, 3TC=3.5, d4T=1.7 and TDF=1.4; biological cutoffs for ddC=1.7 and ZDV=1.9.

Results:

drug	Mutations (N):					
	65R (82)	65R/184V (54)	74V (22)	74V/184V (74)	184V (1720)	184I (27)
ZDV	0.5 [2.4]*	0.4 [0]	0.3 [0]	0.3 [0]	0.4 [0.1]	0.2 [0]
3TC	9.3 [98]	200 [100]	1.4 [0]	200 [100]	200 [100]	200 [100]
ddI	1.7 [60]	2.9 [96]	1.2 [23]	2.2 [91]	1.4 [11]	1.4 [26]
ddC	2.3 [91]	3.9 [100]	1.2 [9.1]	2.3 [86]	1.6 [41]	2.0 [70]
d4T	1.3 [12]	1.0 [0]	0.9 [0]	0.8 [0]	0.7 [0.1]	0.8 [0]
ABC	2.5 [1.2]	6.9 [98]	1.6 [0]	5.4 [78]	2.8 [1.6]	1.6 [0]
TDF	1.8 [83]	1.1 [19]	0.5 [0]	0.3 [0]	0.5 [0]	0.4 [0]

*MFC [%AC]; **bold** indicates median fold resistance change > assay cutoff

For K65R MFC>AC for 3TC, ddI, ddC, and TDF. With L74V MFC was not reached for any drug, although 23% of samples had ddI FC > 1.7. For M184I MFC>AC for 3TC and ddC, and for M184V MFC>AC for 3TC. The double mutants K65R/M184V and L74V/M184V are similar, although the L74V/M184V variant is hypersusceptible to TDF.

Conclusions: These data suggest the most detrimental single and double mutations are respectively K65R>M184V>L74V and K65R/M184V>L74V/M184V. These in vitro results predict that selection of L74V or M184V alone, versus K65R, or the combinations K65R/M184V or L74V/M184V, will provide for more downstream NRTI therapy options.

Poster Presentation

Friday, February 25, 1:30pm – 3:30pm



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HIV-1 Replication Capacity as an Independent Predictor of Pre-Treatment CD4 Lymphocyte Count

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Background: Interactions between host and pathogen determine the natural history of infectious diseases. Characteristics of HIV may impact rates of CD4 lymphocyte decline over time and influence the potential for CD4 regeneration with antiretroviral therapy (ARV).

Methods: Patients who initiated highly active antiretroviral therapy (HAART) and achieved long-term viral suppression (HIV RNA < 400 copies/mL) after 12 months were identified from clinical databases at Duke University Medical Center, the University of North Carolina, and the University of Texas-Southwestern. The study population consisted of 109 ARV-naïve patients initiating antiretroviral therapy: 71 males, 37 females (gender not recorded for one patient); 66 African Americans, 28 whites, 12 Hispanics, and 3 other. The mean age was 40 years (range 19 to 69). Pretreatment baseline (BL)-stored serum samples collected 0 to 3 months prior to HAART initiation were analyzed for the presence of phenotypic resistance and HIV replication capacity (RC) using a single-cycle pseudo-typed virus construct (ViroLogic modified PhenoSense assay). Multiple linear regression (SAS v8.2) was used to examine relationships between demographics, viral replication, and CD4 counts. HAART regimens used were non-nucleoside reverse transcriptase (RT) inhibitor, 56%; protease inhibitor, 37%; and triple nucleoside RT inhibitor, 7%. The median BL CD4 count was 186 cells/mm³ (range 3 to 1066), and the median BL VL was 75,729 copies RNA/mL (range 400 to >750,000)

Results: All patients achieved suppression of HIV RNA to below 400 copies RNA/mL after 12 months of therapy. BL RC and viral load were independently associated with a lower BL CD4 count. In a model adjusting for both variables, for every increase of 1log₁₀ in HIV RNA viral load, BL CD4 tended to be 97 cells/mm³ lower ($p = 0.001$), and for every increase of 1 unit (percentage) in the RC, BL CD4 count tended to be 1.13 cells/mm³ lower ($p = 0.021$).

Conclusions: These data suggest that more advanced HIV is associated with both the quantity of viral replication and the fitness of the virus, as measured by the RC assay. RC appears to measure an intrinsic viral characteristic influencing HIV-1 disease progression independently of the magnitude of viral load.

Poster Presentation

Thursday, February 24, 1:30pm – 3:30pm



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HIV-1 Replication Capacity in HAART-Failing Patients Predicts Virologic and Immunologic Responses when Accounting for Viral Susceptibility to the Salvage Regimen: Results from the Argenta TrialA De Luca¹, M Bates², S Di Giambenedetto¹, A Cingolani¹, E Coakley², C Petropoulos², R Cauda¹, and J Schapiro^{3,4}¹Catholic Univ, Rome, Italy; ²ViroLogic, Inc, South San Francisco, CA; ³Sheba Med Ctr, Tel Aviv, Israel; and ⁴Stanford Univ Med Ctr, CA

Background: Whether HIV-1 replication capacity (RC) in HAART-failing patients predicts response to salvage therapy is incompletely determined. Our study objective was to determine whether RC at failure predicted subsequent treatment outcomes.

Methods: Patients from the prospective Argenta trial with available baseline plasma were analyzed for phenotypic susceptibility and RC by recombinant assays (ViroLogic). CD4, viral load, and clinical progression were monitored for 36 months. Linear regression was used to determine associations with changes from baseline viral load and CD4.

Results: We analyzed 139 patients: 72% male, 33% injecting drug users, median age 38 years, CD4 284 cells/ μ L, viral load = 4.31 log copies/mL, 37% CDC class C, median experienced HAART regimens 2 (range 1 to 5), time on HAART of 18 months (4 to 48). Baseline virus was susceptible to a median 10 of 17 drugs (0 to 17), median RC was 65% (1 to 185%). Median PSS of the first salvage regimen after baseline was 2 (0 to 4). RC correlated with n of phenotypically susceptible drugs ($r = 0.32$, $p < 0.001$). Higher PSS predicted better virological responses at months 3 ($p = 0.003$), 6 ($p = 0.005$), 12 ($p = 0.015$) and 36 ($p = 0.04$). Overall, higher log₁₀ RC showed no correlation with viral load responses. In the subset of 85 patients not suppressing viremia (viral load > 500 at 3 months), higher log RC predicted less profound 3-month viral load changes (for 1 log higher RC, mean +0.34 log; $p = 0.042$). In patients with PSS = 3 in first salvage regimen ($n = 25$), higher log RC significantly predicted worse viral load responses at 3 months (1 log higher RC, mean viral load change +1.52 log, $p = 0.019$). After adjusting for baseline CD4, viral load and phenotypically susceptible drugs, higher RC predicted worse CD4 recovery at 3 months: each 1 log RC higher, mean CD4 recovery -67 cells (95% CI -125 to -9), $p = 0.025$. In patients with viral load < 50 at 3 months ($n = 16$), log RC predicted CD4 gains at 3 months: (for 1 log higher RC, CD4 -320 cells, $p = 0.006$). In persistently viremic patients (viral load > 500 at different time points) after adjusting for salvage regimen's PSS, higher log RC was an independent predictor of less CD4 cell gains at months 3 (mean -58 cells, $p = 0.04$), 9 (-147 cells, $p = 0.003$), 12 (-134 cells, $p = 0.01$), and 24 (-125 cells, $p = 0.047$). No association was found of RC with clinical progression (Cox's models).

Conclusions: Overall, RC was not significantly associated with treatment responses. However, in persistently viremic patients, higher RC predicted worse 3-month virologic and as long as 24-month CD4 outcomes, after normalizing for the associated drug susceptibility. In patients achieving complete suppression, higher RC predicted less robust CD4 recovery.

Poster Presentation

Thursday, February 24, 1:30pm – 3:30pm



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Fitness Interactions with Positive Epistasis Prevail among Drug Resistance-Associated Mutations

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Background: Population genetic theory suggests that the effect of recombination depends on the form of interaction between mutations affecting fitness (epistasis). Recombination accelerates the fixation of beneficial mutation or the elimination of deleterious mutations only if mutations have negative epistasis (i.e. if beneficial mutations act synergistically and increase fitness less than multiplicatively or if detrimental mutations act antagonistically and decrease fitness less than multiplicatively). To test for the presence of epistasis we analyze fitness values of 9466 virus samples derived from HIV-1 infected patients for routine drug-resistance testing.

Methods: Viral fitness was measured using an adaptation of the PhenoSense HIV assay that quantifies the replicative capacity (RC) of a viral population during a single round of infection. Viral fitness was measured in absence of drugs. Therefore most drug resistance mutations reduce replicative capacity. We tested for epistasis using two methods. Method 1 determines whether log fitness decreases faster or slower than linear in as a function of the number of amino acid mutations and Method II quantifies the deviation from multiplicativity of fitness effects between pairs of alternative amino acids. Positive (negative) epistasis between deleterious mutations implies that fitness decreases slower (faster) than multiplicatively.

Results: Method I revealed a less than linear decrease of log fitness for large numbers of amino acid changes indicating positive epistasis. Analyzing 103,286 pairs of alternative amino acids by Method 2 revealed a highly significant predominance of fitness interactions with positive epistasis. Restricting the analysis to positions at which we were able to detect amino acid variants that significantly affect fitness, we also observed the predominance of positive epistasis.

Conclusions: Our finding of the predominance of interactions with positive epistasis in mutations in HIV-1 has fundamental implications both for applied and basic research. First, it challenges leading hypotheses for the evolutionary benefit of recombination that are based on negative epistasis. Second, the identification of antagonistic fitness interactions among drug resistance mutations suggests that contrary to the prevailing view in the field recombination may not generally facilitate the evolution of multi-drug resistance.

Poster Presentation

Thursday, February 24, 1:30pm – 3:30pm



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Resistance to HIV Chemokine Receptor Antagonists

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HIV-1 entry inhibitors represent a diverse new class of antiretroviral agents. Virus entry is a multi-step process involving several virus envelope proteins (gp120SU, gp41TM) and host cell receptors (CD4, CCR5, CXCR4). The cascade of protein-protein interactions and conformational changes that mediate virus entry represent novel targets that are functionally distinct from conventional enzymatic targets. Consequently, resistance to entry inhibitors can differ significantly from that of protease and reverse transcriptase inhibitors, and may emerge via alternative mechanisms depending on the specific molecular interaction that is targeted. Viruses with reduced susceptibility to fusion inhibitors (enfuvirtide) display log-sigmoid inhibition curves that typically reach 100% inhibition at high drug concentrations, consistent with a competitive mechanism of inhibition and escape. Resistance to fusion inhibitors is best described by increases in the IC₅₀. In contrast, viruses with reduced susceptibility to inhibitors that antagonize envelope-co-receptor interactions often exhibit incomplete inhibition even at elevated drug concentrations. The inability to inhibit 100% of virus replication at high drug concentrations is consistent with an allosteric mechanism of inhibition and escape. Viruses that develop resistance to co-receptor antagonists likely acquire the ability to bind and utilize receptor-inhibitor complexes. Consequently, resistance to allosteric co-receptor inhibitors is best described by the extent of incomplete inhibition observed at high drug concentrations.

Symposium

Wednesday, February 23, 4:00pm – 6:00pm



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Rapid Selection of High-Level Resistance to Enfuvirtide (Fuzeon)L. Maroldo^{1,2}, E. Coakley², C. Chappey², W. Huang², CJ Petropoulos²¹West Midtown Medical Group, New York, NY, ²ViroLogic, Inc., South San Francisco, CA

Background: Reductions in enfuvirtide (ENF) susceptibility are conferred by various amino acid substitutions at positions 36-45 located within the ENF binding site (heptad repeat 1) of gp41. Here we describe the rapid selection of a virus variant bearing a rare single mutation that confers high level resistance to ENF. The subject was a highly treatment experienced, ENF naïve, 52 y/o male with AIDS and multi-drug resistant HIV. Based on the results of a combined phenotype-genotype resistance assay he initiated a salvage regimen of TDF, 3TC, SQV/r and ENF. The pre-therapy HIV-1 RNA was 20,000 copies/mL and CD4 T-cell count was 15/mm³. Response to therapy was poor with a paradoxical rise in viral load and lack of CD4 T-cell count improvement.

Methods: Baseline and on-treatment ENF susceptibilities were measured using a single cycle HIV-1 *env* pseudovirion infectivity assay. gp160 *env* sequences were determined at multiple time points by population and clonal methods using chain terminator reaction chemistry.

Results: ENF susceptibilities (fold-change in IC₅₀ compared to the JRCSF reference) at baseline and days 9 and 30 were 0.4, 7.6 and 400, respectively. High-level ENF resistance was maintained through month 27 of ENF therapy and the virus remained R5-tropic throughout this time. Population genotyping of the day 9 virus revealed a fairly common G36D mutation along with a rare V38E mutation, both as mixtures with the wild-type sequence. On day 30, and all subsequent time points, only the V38E mutation was detected. Phylogenetic analysis of the baseline and on-treatment gp160 sequences confirmed the relatedness of all samples. Clonal analysis of the day 9 sample identified viruses with single G36D and V38E mutations. No double mutant was observed in the 48 clones analyzed. Reductions in infectivity of the on-treatment viruses were not observed.

Day	Tropism	Normalized T20 IC ₅₀ (µg/ml)	Substitution at codons 36 to 45 in gp41
Baseline	R5	0.01	GIVQQQNNLL
Day 9	R5	0.22	(G/D)(V/E)QQQNNLL
Day 30	R5	11.43	GIEQQQNNLL
Day 40	R5	11.22	GIEQQQNNLL
Day 58	R5	8.71	GIEQQQNNLL
Day 70	R5	10.55	GIEQQQNNLL
Day 74	R5	9.34	GIEQQQNNLL
Day 580	R5	5.97	GIEQQQKNLL

Conclusion: Rapid selection of high-level ENF resistance may occur in clinical practice, albeit uncommon. Phenotypic resistance was associated with the emergence of a virus variant bearing the V38E mutation within the ENF binding site. In a recent report, this rare mutation (in combination with the N42S polymorphism) was associated with high level ENF resistance (395-fold) and reduced fitness in the absence of ENF (J. Lu et al., J Virol. 78:4628, 2004). The rapid outgrowth and maintenance of the V38E mutant on ENF therapy is consistent with a high selective advantage of this mutation.

Poster Presentation

Friday, February 25, 1:30pm – 3:30pm



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Randomized Pilot Study of Immediate Enfuvirtide-Based Therapy vs. a Treatment Interruption Followed by Enfuvirtide-Based Therapy in Highly Treatment-Experienced PatientsG Beatty^{1*}, J Lu², P Hunt¹, W Huang³, J Martin¹, D Kuritzkes² SG Deeks¹*1University of California, San Francisco, San Francisco, CA, 2Partners AIDS Res Ctr, Boston, MA, 3ViroLogic, Inc. South San Francisco, CA*

Background: Enfuvirtide (ENF) has limited long-term effectiveness when the drug is the only fully active drug in a treatment regimen. The level of drug-resistant HIV-1 in plasma decreases dramatically after interruption of therapy (STI). We hypothesized that the suppression of drug-resistant virus with a regimen containing a single new therapeutic class would be more likely after an STI.

Methods: Thirty three-class experienced ENF-naïve individuals were randomized to either immediate therapy with ENF/optimized background (OB) or a 16-week STI followed by ENF/OB. Eligible subjects had a VL > 500 copies/mL and genotypic or historical evidence of resistance to ≥ 2 PIs, ≥ 2 NRTIs and ≥ 1 NNRTI. A segment of the gp41-coding region encompassing the HR-1 and HR-2 domains was amplified at baseline and weeks 16 and 24, and multiple independent clones sequenced to assess resistance mutations. The study was powered based on observing a 30% success rate in the control arm.

Results: The median CD4 and viral load at study entry were 47 cells/mm and 4.72 log₁₀ copies RNA/mL, respectively. As a consequence of the STI, CD4 decreased by a median of 27 cells/mm (IQR – 59 to –7) and HIV RNA increased by 0.40 (IQR +0.13 to +0.57) log copies/mL. At 24 weeks of therapy, 8 out of 15 (53%) subjects in the immediate therapy group vs. 5 of 14 (36%) of patients in the STI group had a VL < 75 copies/mL (p=NS). In multivariate analysis, only baseline phenotypic susceptibility score was predictive of treatment response at week 24 (P = 0.03). Baseline susceptibility to ENF, despite a 10-fold variation, did not predict outcome. Analysis of HR-1 sequences showed rapid emergence of ENF-resistance mutations within 2-4 weeks of treatment in most patients with virologic failure.

Conclusions: Interrupting therapy prior to initiating salvage therapy with ENF did not result in an improved virologic response at 24 weeks. The collective predictive activity of an ENF containing regimen, but not ENF baseline susceptibility, was important in predicting treatment response.

*Poster Presentation**Friday, February 25, 1:30pm – 3:30pm*



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Interruption of Enfuvirtide in Patients with Enfuvirtide Resistance

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Background: Antiretroviral drugs often exert a persistent benefit despite the presence of drug resistance-associated mutations. Several mechanisms may account for this benefit, including partial anti-HIV activity of the drug against the resistant variant and/or the selective maintenance of mutations that reduce replicative capacity. The objective of this study is to define the degree to which enfuvirtide (ENF) has residual benefit during virologic failure, and to define the mechanism for this benefit.

Methods: This is a prospective study of ENF-treated subjects with detectable viremia (> 400). ENF was interrupted while background drugs were continued. ENF phenotypic susceptibility and tropism were measured using single cycle replication entry assay in which patient-derived *env* genes was co-transfected with a luciferase-containing *env*-defective HIV genomic vector; infectivity is assessed by measuring the production of relative light units (RLUs) in cell lines expressing CCR5 or CXCR4. A segment of the gp41-coding region was also amplified, and multiple independent clones sequenced to assess resistance mutations.

Results: Twenty subjects were studied. The median baseline viral load was 5.12 log copies/ml (IQR 4.65-5.47) and the median CD4 cell count was 89 cells/mm (46 to 158). At week 2 off ENF, the median change in viral load was +0.19 (-0.07 to 0.36) (P = 0.05) and the median change in CD4 cells was +2 (-10 to +15) (P = 0.30). At week 16, the median change in viral load at week 16 was +0.14 (+0.04 to +0.28) (P=0.04), while the change in CD4 cell count was -4 (-25 to +1) (P=0.14). ENF susceptible virus emerged by week 16 in the absence of therapy in most individuals (P=0.003); this shift was associated with an increase in RLUs (as measured in absence of the inhibitor)(median 0.26 log₁₀ RLUs increase, P=0.01). Clonal analysis of HR-1 sequences revealed that ENF-resistance mutations could no longer be detected within 16 weeks of ENF cessation in most subjects.

Conclusions: Interrupting ENF therapy in patients with ENF resistant HIV was associated with a modest but measurable increase in viremia, indicating persistent low-level activity of the drug. Enfuvirtide resistance waned rapidly in the absence of drug pressure. This observation is consistent with a fitness cost (as measured in the absence of drug) associated with ENF resistance-associated mutations.

Poster Presentation
Wednesday, February 23, 1:30pm – 3:30pm



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R5 and X4 Tropic HIV-1 Envelope-Mediated Membrane Fusion Is Associated with Disease Stage

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Background: Membrane fusion events have been shown to contribute to HIV-1 cytopathic effects in vitro. The relationship between the level of fusion activity and disease progression is not clear. In this study, we characterized envelope mediated cell-cell fusion, and also evaluated the fusogenicity of viruses from recent and chronic infection patients.

Methods: The gp160 env gene was amplified from patient HIV-1 plasma samples and cloned into an expression vector. Membrane fusion was measured using a cell-cell fusion assay. Co-receptor tropism and entry inhibitor susceptibility were determined by using a single-cycle HIV envelope-pseudotyping assay. Expression vectors containing chimeric envelope sequences (gp120 and gp41) were used to map fusion determinants. Fusion activity of 400 primary viruses was evaluated and included 150 viruses from recently infected patients in the AIEDRP cohort, 150 from chronically infected patients in SCOPE and 70 PBMC-derived isolates obtained from the ARRRP.

Results: A broad range of membrane fusion activity was observed in patient HIV-1 isolates, which was independent of virus co-receptor tropism. PBMC-derived viruses were more fusogenic than primary isolates ($p < 0.001$). The determinants of fusion were mapped to gp120 using chimeric envelope constructs. Compared to low fusion viruses, high fusion viruses were more susceptible to CD4 blocking agents and less susceptible to anti-CD4 antibodies. To investigate the effect of membrane fusion on disease progression, viruses from recently and chronically infected patients were studied. R5-tropic viruses predominated in recent infection (R5=97%, X4/R5=3%) and X4/dual/mixed-tropic viruses were more common in chronic infection (R5=66%, X4/R5 and X4=32%). R5-tropic viruses from chronic infection were more fusogenic than R5-tropic viruses from recent infection ($P < 0.001$), but were not distinguishable from X4/dual-tropic viruses from chronic infection ($P > 0.5$).

Conclusions: Patient viruses exhibit broad differences in env-mediated membrane fusion. The envelope gp120 surface protein is a strong determinant of membrane fusion activity. Highly fusogenic viruses, including PBMC-derived viruses, were associated with increased susceptibility to CD4 inhibition. A comparison of plasma derived viruses indicated that both X4- and R5-tropic viruses from chronic infection are more fusogenic than R5-tropic viruses from recent infection.

Poster Presentation

Thursday, February 24, 1:30pm – 3:30pm



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Persistence of Archived Viruses with a Unique Tropism in Antiretroviral Treated Individuals with Drug-Resistant HIV

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Background: A complex latent reservoir of previously selected HIV variants persists during combination antiretroviral therapy. Interruption of therapy in patients with drug-resistant HIV is often associated with the emergence of these archived viruses as well as rapid losses in CD4+ T cell counts. The degree to which these dynamic virologic and immunologic changes reflect changes in HIV co-receptor tropism (R5 vs. X4-tropic) has not been defined.

Methods: We analyzed longitudinally collected samples from 20 individuals who had detectable drug-resistant HIV on a stable regimen and who experienced a shift to drug-susceptible HIV during a structured treatment interruption. Samples were selected which corresponded to a change in drug-resistant phenotype. Envelope of HIV-1 was amplified from patient plasma samples and recombinant viruses were generated and used to infect cells expressing either CCR5 or CXCR4. Tropism was determined by measuring luciferase activity in the presence of R5 or X4 inhibitors. Gp160 envelopes were sequenced as virus populations and individual envelope clones.

Results: Prior to therapy interruption, the dominant plasma virus was exclusively R5-tropic in 8 subjects, dual/mixed or X4-tropic in 7 subjects. Concurrent with emergence of drug-susceptible “wild-type” HIV, the virus population in plasma shifted co-receptor utilization in 5 subjects. There were no consistent trends in the direction of this shift (2 shifted from dual topic to either R5 or X4; two from R5 to dual tropic and one X4 to dual tropic). We assessed the impact of baseline tropism (R5 vs. R5/X4) on change in CD4+ T cell count. After controlling for baseline CD4 and change in viral load, we observed no evidence of difference in CD4 declines between those with R5 vs. those with dual tropic virus (P=0.20). Genetic changes during the tropism switch were found in the V3 loop.

Conclusions: Approximately half of patient viruses from treatment failure patients were able to use X4 as a co-receptor. Therapy interruption could result in the emergence of R5 or X4 variants suggested that antiviral treatment is able to suppress drug susceptible viruses with different tropisms. There is no evidence that a specific tropism is associated with an increase in CD4 T cell loss or an increased viral load over time during STI. The results imply that CD4 T cell loss during STI is related to the re-emergence of drug susceptible viruses with higher fitness rather than to a switch in virus tropism.

Poster Presentation

Thursday, February 24, 1:30pm – 3:30pm



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Multi-Drug Resistant HIV-1 Is Sensitive to Inhibition by Chemokine Receptor Antagonists

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Background: In a retrospective analysis of a Phase I/II study of the CXCR4 antagonist AMD3100, several subjects with X4/R5 tropic virus at baseline developed R5-tropic virus upon treatment. The total viral RNA in some patients remained high over the course of treatment despite receiving concomitant antiretroviral medications, suggesting that the virus in these patients was multi-drug resistant but sensitive to inhibition by chemokine receptor antagonists.

Methods: Virus populations at baseline (day 0), on treatment (day 11) and off treatment (day 18, 39) were characterized by determining the co-receptor tropism of 30-40 envelope clones per time point using an envelope pseudo-virus infection assay. Gp160 sequences were determined for at least 10 clones per time point. Sequence analysis of individual clones distinguished R5 viruses from X4 and dual-tropic viruses, largely based on differences in the V3 region. Viral stocks from patient samples at baseline were prepared by mixing patient lymphocytes with PHA-stimulated PBMC from uninfected donors. The viruses were tested for their sensitivity to inhibition by the concomitant antiviral medications they were receiving during AMD3100 treatment both in MT-4 cells and PBMC's, and the 50% inhibitory concentrations were compared to reference X4 and R5 strains, respectively. Viruses were also tested for sensitivity to inhibition by a combination of the CXCR4 antagonist AMD070 and CCR5 antagonists in PBMC.

Results: In one subject, the co-receptor utilization was 62.5%-R5, 7.5%-X4 and 30%-dual clones at baseline and predominantly R5-clones at day 11 (85%) and day 18 (92%). This patient was receiving concomitant treatment with Amprenavir, Lopinavir, ddI and d4T. Compared to the inhibition of NL4.3 in MT-4 cells, the EC₅₀'s for inhibition of patient virus (from baseline) with Amprenavir and Lopinavir were ca. 80 and 130-fold higher, suggesting that the virus was resistance to these medications. In contrast, NL4.3 and patient virus were equally sensitive to inhibition by ddI and d4T. Patient virus was completely inhibited by a combination of the CXCR4 antagonist AMD070 and CCR5 antagonists in PBMC. Several patient examples will be presented.

Conclusion: 10-day treatment with AMD3100 suppressed replication of X4 and dual-tropic variants, resulting in a predominance of R5-tropic variants. The X4 virus in these patients was in some cases, resistant to their concomitant antiretroviral medications but remained sensitive to inhibition with CXCR4 antagonists.

Poster Presentation

Wednesday, February 23, 1:30pm – 3:30pm



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The Selection of HIV-1 During Sexual Transmission, Differences in gp160 Diversity in Male to Female vs. Female to Male Transmission

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Background: To help determine the selection of HIV-1 that occurs during sexual transmission, sequence analysis was performed on multiple clones of the entire envelope gene from both the incident and prevalent partners at the time of transmission.

Methods: Ten transmission pairs, five male to female and five female to male, from the Rakai cohort who were in a monogamous partnership had samples analyzed from the transmitting time point that had been previously established as linked by direct sequencing of PCR products from the gag and gp41 regions. All samples had viral loads greater than 10,000 copies/ml. Stored sera from the transmission time point were extracted from 20 subjects, and the gp160 gene was amplified by RT-PCR. The PCR product was cloned and eight clones were sequenced. Gap stripped unweighted nucleotide distances were calculated for all fragments (V1 to V5 and C1 to C5 and gp41) and for the entire gp160. Additionally these distances were determined between transmission pairs. Phylogenetic analysis was performed using PHYLIP on the entire gp160 using envelope data from previously described full length HIV-1 sequences from the Rakai Cohort. Statistical analysis comparing the differences in diversity in the incident time point samples and the distance between transmitting pairs was performed using a student's t test incorporating a Bonferoni correction.

Results: Biologic linkage was established between each transmission pair, however in none of the cases was the majority clone transmitted. The amount variation between transmission pairs was greatest for female to male pairs in all regions except C4 and V5. The region with the least subject variation for the transmission pairs was C5, while the region with greatest variation was V4. The incident female subjects had a four fold greater variation in V3 region than the incidentally infected males (0.49%vs. 2.00%, $p < 0.05$).

Conclusions: These results demonstrate a potential difference in the selection of viral variants that occurs during male to female vs. female to male transmission of HIV.

Poster Presentation
Thursday, February 24, 1:30pm – 3:30pm



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Integrase (IN) Inhibitor Susceptibility Can Be Measured Using Recombinant Viruses that Express Patient Virus IN Alone, or in Combination with Protease (PR) and Reverse Transcriptase (RT)

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Background: Inhibitors of HIV-1 Integrase (IN) are in clinical development. Phenotypic and genotypic resistance assays will be required to monitor the emergence of IN inhibitor resistance during clinical trials. This abstract describes our efforts to adapt a single cycle replication assay (PhenoSense) to measure IN inhibitor susceptibility.

Methods: To evaluate IN inhibitor susceptibility a unique restriction site was introduced into the PhenoSense vector downstream of the *pol* gene. Recombinant test vectors containing patient derived RNaseH and IN (RHIN), or alternatively the entire *pol* gene were constructed. The IN inhibitor susceptibility of 45 IN inhibitor naïve patient viruses and 30 site-directed mutant viruses containing previously described IN inhibitor resistance mutations was determined using the standard PhenoSense methodology. IN Replication Capacity (INRC) was expressed as a percentage of luciferase production in infected cells relative to a reference virus control (NL4-3).

Results: RHIN sequences from 45 patient viruses were successfully amplified and tested for susceptibility to the naphthyridine carboxamide integrase inhibitor L-870,810 (Merck). The distribution of L-870,810 susceptibility (IC50) for all viruses was narrow (mean IC50 5 nM; range 2-9); differences between viruses sensitive or resistant to PR and/or RT inhibitors were not observed. No significant differences in L-870,810 susceptibility and no known resistant mutations were observed when comparing vectors containing either RHIN or *pol* fragments from the same virus. Consistent with published data, the site-directed mutants N155S, T125K/F125Y, and T66I/L74M/V151I displayed reduced L-870,810 susceptibility (fold change of 11, 12 and 15 respectively) relative to a reference virus control (NL43). Based on luciferase activity in the absence of drug, we observed variation in the IN RC of the 45 patient viruses (median 68%, range 5.2%-135%). The impaired replication of the N155S mutant (50%) was also confirmed.

Conclusions: Recombinant virus assays can be used to evaluate IN inhibitor susceptibility/resistance, analogous to existing PR and RT assay systems. Two approaches we evaluated rely on the assembly of recombinant viruses that express patient derived RHIN in the absence or presence of patient derived PR and RT fragments. Concordant measurements were obtained when both approaches were used to evaluate primary patient virus isolates.

Poster Presentation
Friday, February 25, 1:30pm – 3:30pm