



**16th Conference on Retroviruses and
Opportunistic Infections
(CROI 2009)**
Abstract Booklet



Montréal, Canada
February 8 – 11, 2009



Executive Summary

Monogram Biosciences and its collaborators presented the following abstracts at the 16th Conference on Retroviruses and Opportunistic Infections (CROI 2009) in Montréal, Canada:

- **Longitudinal Phenotypic Analysis of Raltegravir-Resistant Viruses during Virologic Failure**
- **Long-Term Evolution of Integrase Resistance During Failure of Integrase Inhibitor-Based Antiretroviral Therapy**
- **HIV-1 Mutations at Positions 143, 148 and 155 of Integrase Define Different Genetic Barriers to Raltegravir Resistance *in vivo***
- **CD4 Depletion During Multi-Tropic SIV Infection of Sooty Mangabeys Does Not Induce Immune Activation or Simian AIDS**
- **Cerebrospinal Fluid Compartmentalization of HIV-1 Replication Capacity and Coreceptor Tropism Differ between Early and Chronic Infection**
- **Antiretroviral Drug Susceptibility Among Drug Naïve Adults with Recent HVI Infection in Rakai, Uganda**
- **CXCR4-Mediated Infectivity is Correlated with the Proportion of Efficient CXCR4-Utilizing Variants in Dual/Mixed-Tropic HIV-1 Populations**
- **Combinations of Primary NNRTI and INI Resistance Mutations do not Alter HIV-1 Drug Susceptibility but Impair Replication Capacity**

Poster: 620

Longitudinal Phenotypic Analysis of Raltegravir-Resistant Viruses during Virologic Failure

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Background: Amino acid substitutions at HIV-1 integrase (IN) codons 148 and 155 have been identified as primary resistance mutations to raltegravir (RAL) and define independent genetic pathways. Limited data are available on phenotypic characteristics of plasma viruses collected longitudinally during RAL-treatment failure.

Methods: We characterized viruses from the plasma of two heavily pre-treated patients (pt), receiving a RAL-based salvage therapy, at baseline and multiple time-points during virologic failure on continued RAL treatment (pt 1: week 4, 7, 11, 22 and 39; pt 2: week 16 and 21). IN coding regions of viral populations and molecular clones were sequenced and RAL susceptibility and IN replication capacity (RC) were determined using the PhenoSense[®] Integrase assay.

Results: In pt 1, clonal analysis at W4 revealed 3 distinct RAL-resistant variants harboring Q148K, Q148R or N155H at 5, 26 and 63% of the population, respectively. All 3 mutants displayed reduced susceptibility to RAL (mean FC, N155H=14 (n=6 clones); mean FC Q148R/K=38 (n=4 clones)) and decreased IN RC (mean RC N155H=45%; mean RC Q148R/K=18%). By W11 the virus population was comprised predominantly of G140S-Q148H (74%) variants, which were not detected at W4. Both Q148R and N155H were detected at lower proportions (7% each) and Q148K variants were not found. The major G140S-Q148H mutants exhibited both the highest level of RAL resistance (FC>150) and IN RC (60%). Conversely, minority N155H variants displayed a dramatic decrease in IN RC (<1%). The G140S-Q148H was the unique mutant further found at W22 and W39. Pt 2 harbored virus with an uncommon genetic profile under RAL (T66A-T74I/M-E92Q) at W16. No significant reduction in RAL susceptibility was detected (FC=2.3) and IN RC was 60%. By W21, mutations E138K/N/D and G140R were both added without impact on RAL susceptibility (FC=1.3), whereas a further reduction in RC (24%) was observed. Interestingly, this patient exhibited a prolonged low viremia (200-400 copies/mL) over one year of follow-up associated with an increase in CD4 cells.

Conclusions: We observed that RAL drug pressure can serve as a major driving force for viral evolution; initially generating heterogeneous RAL-resistant subpopulations that, upon subsequent selection, resolve to more homogeneous populations of highly resistant variants. Our data also suggest that RAL drug pressure may select for a RAL-susceptible virus exhibiting low IN RC associated with a low viremia.

Session Information / Title: Session 110 / Resistance to Integrase Inhibitors

Session Type: Poster Session

Location: Poster Hall

Presentation Time: 1 – 2:30pm

Session Date: Monday, February 9, 2009

Poster: 621

Long-Term Evolution of Integrase Resistance During Failure of Integrase Inhibitor-Based Antiretroviral Therapy

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Background: Although integrase inhibitors are highly effective in the management of drug-resistant HIV, some patients fail to achieve durable viral suppression. The long-term consequences of integrase inhibitor failure have not been well defined.

Methods: We identified 29 individuals who exhibited evidence of incomplete viral suppression on a regimen containing an integrase inhibitor (24 raltegravir, 5 elvitegravir). Genotypic and phenotypic resistance testing was performed at regular intervals in patients with detectable viremia (> 50 copies RNA/mL).

Results: Prior to initiating the integrase inhibitor containing regimen, baseline CD4+ T cell count and plasma HIV RNA levels were 46 cells/mm³ and 4.61 log₁₀ copies/mL, respectively. Subjects had been on 13 prior antiretroviral drugs (4 prior drug classes). Subjects were followed for a median 13.2 months. At first failure, the most common resistance pattern was wild-type (no known mutations), followed in order of frequency by G140S+Q148H/K/R, N155H, and Y143R/C. The G140S+Q148H/K/R resistance pattern typically occurred early, was associated with high-level phenotypic resistance, and remained stable over time. N155H was associated with lower levels of resistance and tended to wane during long-term failure. Y143R/C occurred in the absence of other mutations, was associated with high-level resistance, and tended to be observed after long-term failure. Wild-type failure was common in patients with known partial adherence or in patients with low, intermittent viremia (< 1000 copies/mL). Despite evidence of integrase inhibitor failure, patients appeared to have a persistent immunologic benefit, with a median change in CD4+ T cell count of +65, +75, +96, and +31 cells/mm³ at months 3, 6, 9, and 12 of documented failure, respectively. However, there was evidence of continued evolution of resistance over time in subjects who remained on a failing integrase inhibitor containing regimen.

Conclusions: Long-term evolution of integrase resistance is complex, with various patterns emerging and waning over time. In this cohort of treatment-experienced subjects failing an integrase inhibitor containing regimen, wild-type failure was not uncommon, especially in the context of partial adherence or low-level viremia. Collectively, these data suggest that the genetic barrier to resistance to integrase inhibitors may be higher than previously assumed.

Session Information / Title: Session 110 / Resistance to Integrase Inhibitors

Session Type: Poster Session

Location: Poster Hall

Presentation Time: 1 – 2:30pm

Session Date: Monday, February 9, 2009

Abstract Number: M-274

Poster Board: 69

HIV-1 Mutations at Positions 143, 148 and 155 of Integrase Define Different Genetic Barriers to Raltegravir Resistance *in vivo*

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Background: Mutations at amino acids 155, 148 and 143 of HIV-1 integrase (IN) define primary pathways of resistance in subjects failing raltegravir (RAL). Although each pathway appears to be genetically distinct and non-overlapping, shifts in the predominant resistant virus subpopulation have been reported under continued drug pressure. To better understand this dynamic, we characterized the susceptibility and replication capacity (IN RC) of viruses containing Y143R or C and how they compare to viruses containing mutations at position 155 or 148.

Methods: Ninety-three subjects failing RAL were included in this study. Molecularly cloned integrase sequences and a series of site-directed mutants (SDMs) containing mutations at positions 143, 155, 148, alone or in combination with other IN resistance associated mutations were evaluated. Susceptibility to RAL and IN RC was assessed using PhenoSense Integrase. The nucleotide sequences of all IN coding regions were determined.

Results: A small number of subjects failing RAL with mutations at position 143 were observed compared to failures through the 155 or 148 pathway. Most subjects failing via the 143 pathway also contained the mutation T97A. Comparison of subjects failing with Y143R/C to N155H or Q148R/H showed that failures via the 143 and 148 pathways consistently displayed high level resistance to RAL, while reductions in susceptibility (fold change in IC₅₀, FC) varied for N155H containing viruses. Based on SDM data, susceptibility to RAL varied depending on the amino acid at position 143 (Y143R FC = 20; Y143C FC = 3.5). The reduction in susceptibility conveyed by the Y143R mutation is consistent with SDMs containing N155H, Q148R and Q148H. In all cases the addition of secondary mutations caused further reductions in RAL susceptibility. IN RC of the Y143R SDM was greater than Y143C, N155H, Q148R or H alone (95 vs 54, 69, 59, 43% respectively). Additional mutations resulted in further reductions in IN RC in all SDMs except Q148H, which increased with the addition of G140S. Virus clones containing N155H exhibited lower IN RC and were present in lower proportions than clones bearing 143 or 148 mutations within the same virus populations.

Conclusions: Mutations at position 143 or 148 appear to have less of an impact on RC and cause higher levels of resistance to RAL than 155 mutations, which may explain reported shifts from the N155H pathway to either the 143 or 148 pathways under continued drug pressure.

Session Information / Title: Session 18 / ART and HIV Drug Resistance

Session Type: Oral Session

Location: Room 517b-d

Presentation Time: 5:00pm

Session Date: Monday, February 9, 2009

Oral: 79

CD4 Depletion During Multi-Tropic SIV Infection of Sooty Mangabeys Does Not Induce Immune Activation or Simian AIDS

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Background: We previously reported a severe and sustained CD4+ T cell decline (<80/ul blood) in two SIV+ sooty mangabeys that was associated with the ability of SIV to utilize multiple coreceptors for entry (R5/X4/R8/R2) (Milush et al.). These two mangabeys have maintained low levels of immune activation and remained free of simian AIDS for more than eight years.

Methods: To assess the viral contribution to CD4+ T cell decline, plasma from one CD4-low mangabey was utilized to inoculate 3 additional animals. Flow cytometry was utilized to assess phenotypic and functional changes in the T cell subsets within these three CD4-low passaged mangabeys.

Results: CD4 T cells decline to between 20 and 100 cells/ul of blood occurred within 4 weeks of SIV plasma transfer in all 3 mangabeys. This CD4 depletion was observed in blood, lymph nodes, and gut associated lymphoid tissue from both naïve and memory CD4 subsets. Irrespective of CD4+ levels, proliferation/activation markers remained low in CD8+ T cells, indicating the maintenance of the low levels of immune activation generally observed in SIV natural host species. Moreover, plasma lipopolysaccharide (LPS) levels remained normal in all five CD4 low mangabeys indicating that depleting gut CD4 T cells was not sufficient to induce the bacterial translocation observed during pathogenic SIV/HIV infections. Functionality of the adaptive immune response (SIV specific antibodies and CTL) was maintained in all CD4-low mangabeys, even when CD4 T cells declined rapidly following infection.

Conclusion: We demonstrated that the multi-tropic SIV previously characterized in two mangabeys could be passaged to three additional mangabeys inducing severe, systemic CD4 T cell loss without any signs simian AIDS. Furthermore, we observed that mucosal integrity and adaptive immune responses in SIV+ sooty mangabeys are maintained through immune mechanisms that do not require high levels of CD4+ T cells.

Session Information / Title: Session 21 / HIV-Host Interaction

Session Type: Oral Session

Location: Room 511

Presentation Time: 11:00am

Session Date: Tuesday, February 10, 2009

Poster: 469

Cerebrospinal Fluid Compartmentalization of HIV-1 Replication Capacity and Coreceptor Tropism Differ between Early and Chronic Infection

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Background: Cerebrospinal fluid (CSF) HIV-1 quasiespecies diverge from those in blood in a portion of patients with chronic infection. HIV-1 invades the central nervous system (CNS) during primary infection, however neither the time course nor functional consequences of CNS viral compartmentalization during early stages of infection have been defined. We investigated the development of phenotypic viral compartmentalization through comparison of CSF and plasma pol replication capacity (RC) and coreceptor tropism in early and chronic infection subjects.

Methods: Concurrent blood and CSF samples were obtained from 16 subjects with early HIV-1 infection (within one year post exposure); at least one longitudinal pair was obtained in nine subjects. RC and coreceptor tropism were assessed using PhenoSense and Trofile assays, and compared with previously obtained paired cross-sectional CSF and plasma data from subjects with chronic (greater than three years) infection.

Results: RC in paired samples from ten early infection subjects demonstrated similar values in CSF (median 56%, IQR 37-79) and plasma (44%, 28-70), $p=.33$ (Wilcoxon rank sum test). In contrast, in chronic infection ($n=18$), RC was higher in plasma (121%, 109-156) than in CSF (100%, 60-124), $p=.0017$. RC was higher in both compartments in chronic as compared to early infection ($p<.0001$ for plasma, $p=.035$ for CSF, Mann-Whitney test). All 11 CSF viruses (from six subjects) and 36 plasma viruses from the 16 early infection subjects were CCR5-utilizing (R5). No tropism switches were observed during the longitudinal follow-up (up to 530 days post infection). In an analysis of cross-sectional paired samples, six early infection subjects with CXCR4-using viruses had similar infectivity (measured as luciferase readout) on CXCR4- and CCR5-expressing cells in each compartment, in contrast to 46 previously studied chronic infection subjects who showed higher infectivity on CXCR4-expressing cells in plasma as compared to CSF ($p=.022$)

Conclusions: In patients with early HIV-1 infection, CSF and plasma viruses had similar RC and were both R5-tropic, suggesting a lack of phenotypic compartmentalization. In contrast, during chronic infection, RC and coreceptor tropism assays indicate CNS compartmentalization. Strategies for HIV-1 treatment based on systemic viral phenotypes might be effective in suppression of CSF viral burden and establishment of CNS compartmentalization if initiated during the early stages of infection.

Session Information / Title: Session 87 / Viral Infection of CNS Compartments

Session Type: Poster Session

Location: Poster Hall

Presentation Time: 1 – 2:30pm

Session Date: Tuesday, February 10, 2009

Poster: 666

Antiretroviral Drug Susceptibility Among Drug Naïve Adults with Recent HVI Infection in Rakai, Uganda

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Background: Antiretroviral (ARV) drugs are becoming increasingly available in Uganda and other resource-poor countries. We analyzed ARV drug susceptibility in HIV isolates collected at the time of HIV seroconversion from individuals in Rakai, Uganda prior to the availability of ARV treatment, using both genotypic and phenotypic assays.

Methods: Serum samples were obtained from a longitudinal study of HIV infection; study subjects were tested for HIV infection annually. We tested samples collected at the time of HIV seroconversion (1998-2003) using the GeneSeq HIV and PhenoSense HIV assays (Monogram Biosciences).

Results: GeneSeq HIV and PhenoSense HIV results were obtained for 104 samples (subtypes: 26A, 1C, 66D, 9A/D, 1C/D, 1 intersubtype recombinant). GeneSeq HIV results: 7 (6.7%) samples had a mutation associated with reduced nucleoside reverse transcriptase inhibitor (NRTI) susceptibility: (M41L, E44D, V118V/I, K219K/R). One sample had a mutation associated with reduced non-nucleoside reverse transcriptase inhibitor (NNRTI) susceptibility (E138A). All 104 samples had ≥ 1 mutation associated with reduced protease inhibitor (PI) susceptibility. None of the samples had a sufficient number of mutations to predict reduced ARV susceptibility. Phenosense HIV results: 10 (9.6%) samples had reduced phenotypic susceptibility to ≥ 1 ARV drug (2 subtype A, 8 subtype D), including 1 with partial susceptibility to didanosine (ddl), 1 with resistance to nevirapine (NVP), and 8 with resistance and/or partial susceptibility to ≥ 1 PI. Hypersusceptibility (HS) to ≥ 1 ARV drug ($IC_{50} < 0.4$) was noted in 53 (51.0%) of those tested [19 (73.1%) for subtype A, 28 (42.4%) for subtype D]. Seven (6.7%) had HS to zidovudine, 28 (26.9%) had HS to ≥ 1 NNRTI, and 34 (32.7%) had HS to ≥ 1 PI. In subtype D, efavirenz (EFV) HS was associated with substitutions at RT codon 11 in this cohort ($p < 0.001$), and also in data from clinical samples (282 samples tested at Monogram Biosciences, $p < 0.0002$).

Conclusions: Among 104 ARV-naïve Ugandan adults tested at the time of HIV seroconversion, we found no evidence of reduced ARV susceptibility using a genotypic assay. However, 9.6% of those tested had evidence of reduced ARV susceptibility to ≥ 1 ARV drug using a phenotypic assay. Furthermore, 51% of samples had evidence of NRTI, NNRTI, or PI HS. In subtype D, EFV HS was associated with amino acid substitutions at codon 11 in HIV RT.

Session Information / Title: Session 117 / Circulating Drug Resistance in HIV-infected Populations

Session Type: Poster Session

Location: Poster Hall

Presentation Time: 1 – 2:30pm

Session Date: Tuesday, February 10, 2009

Poster: 654

CXCR4-Mediated Infectivity is Correlated with the Proportion of Efficient CXCR4-Utilizing Variants in Dual/Mixed-Tropic HIV-1 Populations

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Background: Dual/mixed (DM) viruses represent the majority of CXCR4-utilizing HIV isolates and display a broad range of infectivity in CXCR4+ target cells. Recent studies have shown that pre-existing minor subpopulations of CXCR4-using viruses can emerge to replace R5 virus populations shortly after initiation of CCR5 antagonist treatment. To better understand the characteristics and composition of DM populations, we conducted clonal analyses for a panel of DM viruses reflecting the full spectrum of infectivity observed for CXCR4-using viruses.

Methods: DM virus populations were selected from routine Trofile patient testing samples. Numerous full-length *env* clones were isolated from each virus population and evaluated for co-receptor usage. *Env* clones were classified as R5, X4 and dual tropic; dual clones were further classified as dual-X or dual-R based on their ability to infect CXCR4+ and CCR5+ target cells. *Env* sequences were determined by using conventional methods.

Results: The infectivity of 20 DM virus populations in CCR5+ and CXCR4+ target cells varied over a 3-4 log₁₀ range. Clonal analyses revealed the presence of both dual and X4 clones in DM viral populations, but the proportion of these CXCR4-using clones and R5 clones varied widely in different patient samples. DM viral populations with higher CXCR4 infectivity contained predominantly X4 and dual-X clones that efficiently use CXCR4. Conversely, viral populations with low CXCR4 infectivity consisted of predominately R5 clones with either minor subpopulations of dual-X clones, or dual-R clones that use CXCR4 inefficiently. Dual-X clones were identified in 19/20 samples analyzed. Tropism predictive algorithms based on substitutions in V3 sequences (codons 11/25, 6-8) accurately assigned X4 tropism but misclassified some dual-X and many dual-R clones as R5.

Conclusions: This study indicates that patient DM virus populations are comprised of either homogenous dual-tropic variants or more complex mixtures of dual, X4 and R5 variants. The ability of DM virus populations to infect CXCR4+ cells is associated with the proportion of *env* clones that efficiently use CXCR4. These data give more insight into the composition of DM virus populations and the potential for escape during treatment with CCR5 antagonists.

Session Information / Title: Session 115 / Drug Resistance and Viral Fitness

Session Type: Poster Session

Location: Poster Hall

Presentation Time: 1 – 2:30pm

Session Date: Wednesday, February 11, 2009

Poster: 652

Combinations of Primary NNRTI and INI Resistance Mutations do not Alter HIV-1 Drug Susceptibility but Impair Replication Capacity

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Background: In light of the approval of Raltegravir (RAL), there is great interest in understanding the phenotypic and genotypic profiles of resistance to integrase inhibitors (INIs). Recent data suggest that mutations at positions 155, 148 and 143 within IN constitute primary pathways of resistance in subjects failing RAL. However, the combined effect of NNRTI and INI mutations on NNRTI and RAL susceptibility, as well as replication capacity (RC), is unknown.

Methods: NNRTI mutations K103N, Y181C, G190A and G190S were constructed as single site-directed mutants (SDMs), or in combination with INI resistance mutations, N155H, E92Q, N155H+E92Q and Q148R/H/K. RC and susceptibility of these combined IN/NNRTI SDMs to NNRTIs and RAL were compared to NNRTI or IN SDMs alone, using MGRM PhenoSense assays.

Results: NNRTI mutations, when present together with INI resistance mutations, did not affect susceptibility to RAL. Similarly, INI resistance mutations, when present in combination with NNRTI mutations, did not affect susceptibility to NNRTIs. RC of SDMs containing NNRTI and INI mutations varied depending on the presence of INI mutations at positions 148 or 155. Viruses containing Q148R/H/K and any NNRTI mutation (K103N, Y181C and G190A/S) exhibited very low RC (<10%), with G190S-containing viruses being the least fit (RC<1%). This represented greater than 10-fold reduction in RC when compared to the RC of Q148R/H/K alone (%RC: 64/41/47 respectively). On the other hand, RC of viruses containing E92Q or N155H and NNRTI mutations were dependant on the particular combination of mutations present. While the addition of K103N to N155H only caused the RC of N155H to drop slightly (from 68% to 52%), viruses containing E92Q and K103N (E92Q+K103N and E92Q+N155H+K103N) completely lost infectivity. As the RC of N155H alone and E92Q alone are comparable (68% and 67% respectively), this implies that, in the absence of compensatory mutations, viruses containing K103N and E92Q are highly unfit. The addition of Y181C or G190A/S to viruses containing E92Q or N155H caused a similar decrease in RC in either IN backbone. For both E92Q and N155H, Y181C caused the lowest reduction (from 67% to 46% for E92Q, and 68% to 53% for N155H) while G190S caused the greatest reduction (from 67% to 1% for E92Q, and from 68% to 5% for N155H) in RC.

Conclusions: SDMs containing NNRTI and INI mutations display a reduction in RC, with the most dramatic loss in RC being exhibited by viruses containing mutations K103N in combination with E92Q. Further studies utilizing clinical samples are required to understand evolution of IN and RT mutations under selection pressure of HIV inhibitors (in the absence or presence of pre-existing NNRTI resistance).

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Session Type: Poster Session

Location: Poster Hall

Presentation Time: 1 – 2:30pm

Session Date: Wednesday, February 11, 2009