



The Mark of
Individualized Medicine

Key Presentations on HIV Drug Resistance, Replication Capacity, Co-Receptor Tropism and Related Topics





Executive Summary

Monogram Biosciences' presentations at the 14th Conference on Retroviruses and Opportunistic Infections covered many important issues and topics. Overall, Monogram's science was involved in 17 presentations, including 3 orals and 14 posters pertaining to Monogram's HIV patient selection and resistance testing technologies.

Abstracts include information on the following:

- Comparative studies comparing MT-2 assays and Trofile™
- Longitudinal co-receptor switches in individuals with persistent viremia
- Antiviral resistance, HIV-1 tropism and HIV-1 subtypes of recently infected men who have sex with men (EXPLORE study)
- Effects of tropism on mortality of infants in Uganda
- Dual tropic HIV-1 viruses ability to use CCR5 and CXCR4 co-receptors
- Tropism during early and late stage HIV-1 infection
- AMD 3100 (CXCR4) antagonist has the ability to inhibit X4-tropic and certain dual tropic variants
- AMD11070 (CXCR4 antagonist) phase Ib/IIA proof of concept study
- CXCR4 antagonism with AMD11070
- Upper and lower phenotypic cut-offs for Darunavir/ritonavir by the phenosense assay
- Darunavir-amprenavir cross resistance
- Resistance profiles of GS-8374
- Continued evolution of pol after interruption of reverse transcriptase inhibitors in patients with advanced HIV disease
- Superinfection susceptibility
- Complexity of viral populations in acute and chronic HIV-1 infection
- Utility of pol replication capacity in predicting immunologic course among HIV-1 infected patients with drug resistant viremia

Abstract 181b

Assessing HIV-1 tropism in ACTG A5211: a comparison of assays using replication-competent virus from peripheral blood mononuclear cells (PBMC) versus plasma-derived pseudotyped virions

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Background: Pseudotyped viruses are commonly used to determine the chemokine receptor tropism of HIV-1 (Trofile™; Monogram Biosciences). In ACTG A5211, a phase 2b trial of the CCR5 inhibitor vicriviroc (VCV), 10% of subjects with R5 virus at screening showed dual-mixed (D/M) virus at entry (prior to receiving VCV). To determine whether assays using infectious virus gives similar results, we compared the Trofile™ assay to a traditional MT-2 cell assay using patient-derived isolates.

Methods: Cryopreserved PBMC obtained at study entry were co-cultured with PHA-stimulated PBMC from seronegative donors. MT-2 cells were inoculated with HIV-1 isolates and inspected every 3-4 days (d) for syncytium induction (SI); virus also was inoculated onto U87-CD4-CCR5 and U87-CD4-CXCR4 cells and tested every 3-4 d for p24 antigen. Plasma virus was tested by the Trofile™ assay, in which luciferase activity measured as relative light units (RLU) on U87-CD4-CCR5 or U87-CD4-CXCR4 cells, or both, was used to classify pseudotyped viruses as R5, X4, or D/M, respectively.

Results: Infectious HIV-1 was recovered from 55/106 cultures (52%). Virus from 5/55 (9%) subjects was SI by MT-2 cell assay; all 5 also replicated on U87-CD4-CXCR4 cells, confirming that these viruses were CXCR4-tropic. Samples from 3 of these subjects were among those that tested as D/M by the Trofile™ assay; the other 2 subjects had R5 virus by Trofile™ at entry but showed early emergence of X4 virus on study. Conversely, entry samples from 8/55 (15%) subjects were D/M by Trofile™ assay; 7 of these were confirmed in samples from later time points. Four of 7 confirmed D/M samples were non-SI by MT-2 cell assay. Five other subjects who developed D/M virus on study had no evidence of X4 or SI virus at entry by either assay. Of note, the geometric mean RLU on CXCR4+ cells for samples from the 12 subjects with D/M at entry (but R5 at screening) was significantly lower than that of the 184 subjects with D/M virus at screening (295 vs 25,704 respectively; $p < 0.0001$), suggesting that a low fraction of dual-tropic or X4 viruses in the population near the threshold of detection account for fluctuations in Trofile™ assay results.

Conclusions: Variable sensitivity for detecting CXCR4-using virus in subjects with a low proportion of D/M or X4 virus was noted for both the Trofile™ and MT-2 cell assays. Improved assay sensitivity may identify additional patients at risk of developing D/M or X4 virus while receiving a CCR5 inhibitor.

Comments about Abstract 181b

- In a phase IIb clinical trial of vicriviroc (ACTG 5211), 10 % of subjects with R5 virus at screening showed dual-mixed virus at entry (prior to VCV). These results were generated using the Trofile™ assay. To determine whether assays using infectious virus gave similar results, the Trofile™ assay was compared to a traditional MT-2 assay using patient derived isolates.
- Cyopreserved peripheral blood mononuclear cells (PBMC) obtained at study entry were co-cultured with PHA-PBMC from seronegative donors. HIV-1 isolates obtained from the PBMC’s were allowed to infect MT-2 cells. The HIV-1 isolates were also inoculated on U87/CD4/CCR5 and U87/CD4/CXCR4. Plasma virus was tested by Trofile™.
- Infectious HIV-1 was recoverable in only 55/106 cultures.
- Of these the following results were generated:

MT-2 Assay			
Trofile™	SI	NSI	Total
D/M	3	5	8 (15 %)
R5	2	45	47 (85 %)
Total	5 (10 %)	50 (90 %)	55 (100 %)

- Of the discordant results. The 2 samples identified as R5 by Trofile™ but SI by MT-2 assay were later identified as D/M. The 5 samples identified as NSI by the MT-2 assay, 4 were later identified as D/M.

Key Points of Abstract 181b

- Only 52 % of cultures (55/106) yielded infectious HIV-1 for MT-2 assays.
- Data suggests that a low fraction of dual-tropic or X4 viruses in the population near the threshold of detection accounting in the fluctuations in MT-2 and Trofile™ assay results.
- Variable sensitivity in detecting CXCR4-using viruses with low proportions of DM or X4 viruses was noted in both Trofile and MT-2 assays.
- Improving assay sensitivity may identify patients at risk of developing DM or X4 viruses while receiving CCR5 antagonists.

Abstract 619

Longitudinal Evaluation of Viral Co-receptor Tropism Switches among HIV-infected Patients with Drug-resistant Viremia

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Background: CCR5 inhibitors are being developed for the management of drug-resistant HIV. These drugs have limited virologic activity in patients with measurable levels of CXCR4-using virus (X4), and incomplete viral suppression on these drugs may select for pre-existing X4. Defining the rate of emergence of X4 in treated patients with drug-resistant viremia in the absence of CCR5 inhibitors may help define the “cost” of deferring their use for future salvage regimens and clarify the degree to which CCR5 inhibitors cause expansion of X4.

Methods: HIV-infected patients with drug-resistant viremia >1,000 copies/ml on a stable antiretroviral regimen for >4 months were sampled from a clinic-based cohort study. Viral co-receptor tropism was measured with a phenotypic recombinant pseudo-virus assay every 4 months until treatment change. To distinguish true co-receptor-mediated entry vs. background luciferase activity, entry into CCR5- or CXCR4-expressing cells was confirmed by reductions in relative light units (rlus) with co-receptor inhibitors.

Results: In 76 patients with drug-resistant viremia, median baseline values were: plasma HIV RNA level, 3.8 log₁₀ copies/ml and CD4 count, 241 cells/mm³. At baseline, 52 (68%) had R5-tropic virus (R5), 22 (29%) had dual/mixed-tropic viruses (DM), and 2 (3%) had pure X4. Patients contributed a median of 3 tropism observations over a median of 9 months. In those with baseline R5, 12% (95% CI: 6%-26%) switched to DM by 1 year. Of 7 patients experiencing R5->DM switches, 3 (43%) had switches driven by very low-level CXCR4 entry around the assay's limit of detection (≤255 rlus) and one experienced repeated oscillation between R5 and DM. In those with baseline DM, 11% (95% CI: 3%-37%) experienced “reversion” to R5 by 1 year and 8% (95% CI: 1%-43%) “progressed” to X4, but there was no evidence for changes in CXCR4 rlus in those without tropism switches (P=0.39). There was no evidence for a change in CD4 trajectory during tropism switch.

Conclusions: Among stably treated patients with drug-resistant viremia, the incidence of new tropism changes is relatively low, occurs in both directions, and is often associated with small changes in CXCR4 entry, suggesting natural oscillations in X4 replication at or near the level of detection. Some treated patients with apparent pure R5 may have harbored DM in the recent past. Deferring treatment change may carry a small risk of losing CCR5 inhibitors as an effective future treatment option.

Commentary on Abstract 619

- The Abstract aimed to define the rate of emergence of CXCR4 using viruses in treated patients with persistent viremia in the absence of CCR5 antagonists.
- 76 Patients were monitored. At baseline 52 had R5-tropic virus, 22 had dual-mixed tropic virus and 2 had pure X4-tropic virus. Patients allowed 3 tropism observations over a median of 9 months.
- Those with R5-tropic viruses, 12% switched to dual/mixed tropic viruses by 1 year. Of 7 patients with this switch, 3 were driven by low expression of CXCR4-tropic viruses and these accounted for 50 % of all observed switches.
- Those with DM-tropic viruses, 11% switched from DM-tropic to R5 tropic in 1 year. Eight percent progressed from DM-tropic to X4 tropic during the same time period.

Key Points on Abstract 619

- In patients with persistent viremia show a relatively low rate of new tropism changes.
- Tropism changes can occur in both directions.
- Some patients that seem to have pure CCR5-tropic viruses may have harbored CXCR4 in the past or have very low levels of CXCR4.
- Deferring use of CCR5 antagonists may carry a small risk of losing CCR5 inhibitors as an effective future treatment option.

Abstract 650

Antiretroviral drug resistance, HIV-1 tropism, and HIV-1 subtype among men who have sex with men recently infected with HIV-1 in the United States: The EXPLORE Study

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Background: Antiretroviral drug (ARV) treatment may be complicated in individuals infected with ARV-resistant or non-subtype B HIV-1 strains. HIV-1 tropism may also affect disease progression. We analyzed ARV resistance, HIV-1 subtype, and HIV-1 tropism among 195 men who have sex with men (MSM) from six major cities in the United States, using samples collected within six months of HIV-1 seroconversion (1999-2003).

Methods: HIV-1 genotyping was performed using the ViroSeq HIV-1 Genotyping System. HIV-1 tropism was determined using a commercial assay. HIV-1 subtyping was performed by phylogenetic analysis of *pol* region sequences.

Results: Thirty-one (15.9%) of the men had evidence of ARV resistance. Seven (3.6%) men had multi-class resistance, including three (1.5%) with resistance to all three ARV classes. We found no statistically significant association of ARV resistance with demographic factors, sexual practices, self-reported sexually transmitted infections, use of recreational drugs, or use of ARV post-exposure prophylaxis. All samples were HIV-1 subtype B. Four men had CXCR4-using HIV-1 strains. One man with a CXCR4-using strain also had ARV resistance.

Conclusions: ARV resistance is relatively common among recently infected MSM in the United States (U.S.). CXCR4-using strains were detected in a small number of these infections, which were all subtype B HIV-1.

Comments on Abstract 650

- Study identified 195 men who had sex with men who had recently undergone HIV-1 seroconversion. ARV (antiretroviral)-resistance, HIV-1 subtype, and HIV-1 tropism was investigated in these subjects
- Results: 15.9% had evidence of ARV resistance, 3.6 % had evidence of multiclass drug resistance and 1.5 % had resistance to all three classes of ARV's. All samples of HIV-1 were subtype B. Two percent of the subjects were CXCR4 using and one subject was CXCR4 using that also had ARV resistance.

Key points on Abstract 650

- ARV resistance is relatively common among recently infected MSM in the United States.
- CXCR4-using strains were detected in only a small number of these subtype B infections.

Abstract 704

Analysis of HIV tropism in HIV infected Ugandan infants

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Background: HIV-1 may utilize the CCR4 co-receptor, the CCR5 co-receptor or both (dual tropic virus). HIV-1 infection usually initiated with CCR5-using virus. Evolution to CXCR4-using virus is associated with more rapid progression to AIDS. We analyzed HIV-1 tropism in samples from HIV-1 infected Ugandan infants.

Methods: Plasma or serum samples (100uL) collected at 6-14 weeks of age were available from 75 out of 106 HIV-1 infected Ugandan infants in the HIVNET012 trial who were HIV-infected by 14 weeks of age. HIV tropism was analyzed using a commercial co-receptor tropism assay, Trofile™ (Monogram Biosciences). Additional samples were tested from infants who had evidence of CXCR4-using strains, and from their mothers.

Results: Fifty-seven (76%) of 75 samples were successfully analyzed. Inability to obtain results on the other 18 samples most likely reflected the low sample volumes available for testing. Fifty-two (91.2%) of the 57 samples had the CCR5-using (R5) phenotype. Five (8.8%) of the 57 infants had evidence of CXCR4-using (X4) virus, including one infant with predominantly X4 tropic virus and four infants with dual or mixed tropism (DM). Four of the five infants were diagnosed with HIV infection at birth, and one at 6-8 weeks of age. Results from maternal samples collected at delivery, infant samples collected at the time of birth, follow-up samples (available for 2/5 infants), and the age at death are shown in the Table.

	Mother	Infant				
	Delivery	Birth	6-8 wks	14 wks	12-18 months	Age at death
197	X4	DM	X4	X4		14.5 months
185	DM	DM	NA	DM ^a		10.5 months
223	DM	NA ^b	DM	DM	R5	2.5 years
827	DM	DM	DM		DM	2 years
632	R5 ^c	DM	DM			4.7 years

^a This sample had a high efficiency of replication in cells with CXCR4.

^b HIV infection was diagnosed in this infant at 6-8 weeks of age.

^c A minor population of dual tropic clones was identified in this sample.

The median survival of these five infants (24.3 months) was not significantly different from the median survival of the 52 infants who had CCR5-using virus at 6-14 weeks of age (23.6 months, log-rank $p=0.293$). The two infants with CXCR4-using virus or DM tropic virus that used CXCR4 efficiently died within 14.5 months.

Conclusions: CCR5 tropic virus was detected in the majority of HIV-infected infants at 6-14 weeks of age. However, five (8.8%) of 57 infants had evidence of infection with either CXCR4-using virus or DM tropic virus. Further studies are needed to define the tropism of strains in newly infected infants and its effect on disease progression in infants.

Comments on Abstract 704

- Study looked at the progression to death in HIV infected Ugandan infants based on their tropism status.
- Of the 57 successfully analyzed samples (by 6-8 wks after birth), 52 were of the CCR5 phenotype. Five were CXCR4 using (1 was CXCR4 exclusive, 4 were dual mixed (one of which used CXCR4 efficiently)).
- Median survival of infants with CCR5 using HIV was 23.6 months, Median survival of infants with CXCR4 using HIV was 24.3 months. However, in infants (n=2) that had exclusively CXCR4 or a dual mixed virus that efficiently used CXCR4 had all succumbed to HIV by 14.5 months.

Key points on Abstract 704

- No significant differences were seen between the median survival of infants infected with the CCR5 using strain of HIV versus the median survival of infants infected with dual mixed using strains of HIV.
- The survival of infants infected with exclusively CXCR4 using HIV or a dual mixed strain of HIV that efficiently used CXCR4 seemed to have a reduced survival of 14.5 months but the numbers are too small and further research is needed.

Abstract 251

Dual-tropic Human Immunodeficiency Viruses display a range of ability to use the CXCR4 or CCR5 co-receptors

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Background: Many CXCR4-using human immunodeficiency viruses are classified as dual-tropic (DUAL) because they have the ability to use both CCR5 and CXCR4 co-receptors to enter target cells *in vitro*. The *in vivo* target cell specificity of DUAL viruses remains poorly defined. In this study, we analyzed a panel of DUAL envelope clones for their ability to use CCR5 and CXCR4 on cells expressing both co-receptors.

Methods: gp160 envelope genes derived from different patient samples were cloned into an expression vector. Tropism of pseudotyped virus was determined by measuring luciferase activity (relative light units, RLU) following infection of U87/CD4+CCR5+ or U87/CD4+CXCR4+ cells (Trofile assay, Monogram Biosciences). Inhibition of infection by small molecule CCR5- (Merck) and CXCR4- (AnorMED) inhibitors was examined on U87/CD4+CCR5+CXCR4+ cells. Envelope genes were also inserted into an NL43 infectious clone and evaluated for their ability to promote syncytia in MT2 cells.

Results: 20 DUAL Env clones were identified that exhibited a broad range of infectivity on U87/CD4+CCR5+ (range $10^3 - 10^6$ RLU) and/or U87/CD4+CXCR4+ (range $10^3 - 10^6$ RLU) cells and were loosely grouped into three categories ('R5>>X4', 'R5~X4' and 'X4>>R5'). We used inhibition of infection on U87/CD4+/CCR5+/CXCR4+ cells by specific co-receptor inhibitors to test whether DUAL clones use co-receptors preferentially when both CCR5 and CXCR4 were present on the cell surface. DUAL clones that efficiently use both CCR5 and CXCR4 ('R5~X4') were not inhibited well by either CCR5- or CXCR4-inhibitors, indicating that infection by these viruses is not restricted to only CCR5 or only CXCR4. Infection by 'X4>>R5' DUAL clones was blocked by a CXCR4 inhibitor, but not a CCR5 inhibitor; conversely, infection by 'R5>>X4' DUAL clones was blocked by a CCR5-inhibitor, but not a CXCR4-inhibitor. Use of the CXCR4 co-receptor by 'R5>>X4' DUAL viruses was confirmed by syncytia formation on MT2 cells when the envelopes were transferred into an NL43 infectious clone.

Conclusions: Dual-tropic variants display a broad range of ability to use CCR5 or CXCR4 for infection *in vitro*. The variation in relative use of CCR5 and/or CXCR4 observed among dual-tropic variants suggests distinct entry pathways that might have an impact on virus pathogenesis and patient treatment response to regimens including co-receptor inhibitors.

Commentary on Abstract 251

- Many HIV viruses are classified as dual-tropic phenotype. A panel of dual-tropic *env* clones was analyzed for their ability to use CCR5 and CXCR4 on cells expressing single or both co-receptors.
- The relative contribution of each co-receptor mediated virus entry was evaluated by using specific CCR5 (Merck) and CXCR4 (AnorMED) antagonists to block virus infection on the cell line expressing both co-receptors and PBMC.
- Dual-tropic clones that efficiently use both CCR5 and CXCR4 were not inhibited well by either CCR5- or CXCR4-inhibitors. Infection by dual-tropic clones that efficiently use CXCR4 was blocked by a CXCR4 inhibitor, but not a CCR5 inhibitor. Conversely, infection by dual-tropic clones that efficiently use CCR5 was blocked by a CCR5-inhibitor, but not a CXCR4-inhibitor.

Key points on Abstract 251

- Dual-tropic variants display a broad range of ability to use CCR5 and CXCR4 for infection in cell line expressing CXCR4 or/and CCR5 co-receptors and PBMC.
- This variation in use of CCR5 and CXCR4 seen with these dual-tropic clones may impact pathogenesis and patient treatment response to co-receptor inhibitors.

Abstract 122

HIV-1 Envelopes from Acute and Chronic Infection Differ in Their Levels of Fusogenicity and Sensitivity to Inhibition with sCD4

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Background: During early infection neutralizing antibody (NAb) responses to autologous virus drives successive waves of evolution in envelope. Virus NAb escape is associated with changes in variable loop length, potential N-linked glycosylation sites (PNGS), and amino acid sequence. Here we characterize the viral envelope during the first 1-3 years post infection by measuring cell-to-cell fusogenicity, co-receptor tropism usage, and sensitivity to sCD4.

Methods: Longitudinal samples from 38 untreated individuals with recent (within 6 months) of infection) HIV clade B infection were assessed for co-receptor tropism usage (Trofile™ assay, Monogram Biosciences), envelope-mediated cell-to-cell fusogenicity using an in-house assay, and autologous and heterologous NAb and sensitivity to sCD4 utilizing a recombinant pseudovirus assay that evaluates the pool of envelopes present in the patient plasma.

Results: Thirty-five of the 38 early infection viruses were CCR5-utilizing (R5) throughout the first 3 years of infection; one was CCR5/CXCR4-tropic (dual/mixed, DM) from the earliest timepoint; one virus switched tropism from DM to exclusively CXCR4 tropic during the first 6 months; and one switched from R5 to DM at 23 months post-infection. In 6 cases the first sample had very high plasma viral RNA levels (6.0 – 7.7 log copies/mL), usually associated with acute viremia. Five of these cases were characterized by high cell-cell fusion efficiency, and significantly increased sensitivity to sCD4 when compared with envelopes from samples with lower viral loads. Longitudinal isolates progressively lost fusogenicity and sCD4 sensitive as the viral load decreased. Increases in the amount of CXCR4-usage in the viral population was associated with significant increases in viral load, greater cell-cell fusogenicity and greater sensitivity to sCD4.

Conclusions: Early HIV infection is characterized by viruses that are predominantly CCR5 using; however, utilization of CXCR4 is associated with increased viral loads. Envelopes from acute viremia viruses are more fusogenic and have greater sensitivity to sCD4. This result suggests that the viruses present early in infection are able to spread rapidly through efficient use of cellular CD4 and efficient membrane fusion. This characteristic is lost quickly after the acute phase of infection, possibly due to the influence of the emerging NAb response.

Commentary on Abstract 122

- During early HIV infection neutralizing antibody responses drives waves of successive evolution of the HIV envelope. This study characterized the HIV envelope mediated cell-to-cell fusogenicity, tropism and inhibition by sCD4 to examine phenotypic changes in the HIV envelope region during the first 1-3 years of infection.
- Most HIV remained R5-tropic during the study period (35/38).
- During the period of acute infection period characterized by very high viremia, most of the HIV isolates had high cell-to-cell fusogenicity and increased sensitivity to sCD4 as compared to individuals with lower viral loads. In those cases as the acute viremia peak viral loads decreased, cell-to cell-fusogenicity and sCD4 sensitivity also decreased.

Key points on Abstract 122

- Early HIV infection is characterized by viruses that predominantly use CCR5.
- Utilization of CXCR4 is associated with increasing viral loads over time.
- Many envelopes from peak acute viremia are more fusogenic with increased sensitivity to sCD4 suggesting that viruses early in infection can efficiently and rapidly spread throughout the body. However, this characteristic is lost after the acute phase of infection, perhaps due to selection of a less fusogenic envelope by the developing immune response.

Abstract 91

Suppression of dual-tropic HIV-1 variants by the CXCR4 inhibitor AMD3100 is associated with efficiency of CXCR4 use and clonal composition of the baseline virus population.

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Background: In a phase I/II evaluation of the CXCR4 inhibitor AMD3100, HIV RNA was significantly reduced (0.9 log₁₀) in the single study subject that harbored CXCR4-tropic virus, but not in the remaining subjects that harbored either dual/mixed (DM)-tropic or R5-tropic virus. In this study, we further evaluated the targeted antiviral activity of AMD3100 *in vivo* by characterizing the tropism composition of the baseline and on treatment DM virus populations.

Methods: Subjects from the AMD3100 phase I/II study that were determined to have DM virus at baseline were included in this study (n=15). Co-receptor tropism of virus population and individual envelope clones was evaluated using a single replication cycle envelope pseudovirus assay (Trofile™) and patient-derived gp160 env clones were sequenced.

Results: By comparing the infectivity of U87/CD4/CXCR4 cells of paired baseline and day 11 virus populations, two groups were identified: the suppressor group (n=11) displayed a reduction in CXCR4 infectivity at day 11 relative to baseline. Most subjects in this group experienced >5 fold reductions in CXCR4 infectivity and 8 subjects switched from DM- to R5-tropic. In contrast, no changes in the CXCR4 infectivity of paired baseline and day 11 samples were observed in the non-suppressor group (n=4). No significant viral load reductions were observed between the baseline and day 11 samples in any subjects from either group. Furthermore the suppressor group showed a much lower level of luciferase activity on CXCR4 cells (10² - 10⁴ RLU) than that of the non-suppressor group (10⁵ - 10⁶ RLU) at baseline. Clonal analysis of baseline populations indicated that the non-suppressor group was enriched for dual-tropic variants while the suppressor group contained a higher proportion of R5-tropic variants. The mean percentage of CXCR4-using clones in non-suppressor group was 95% compared to only 27% in the suppressor group.

Conclusions: This study indicates that AMD3100 has the ability to inhibit both X4-tropic and certain dual-tropic variants *in vivo*. Dual-tropic viruses exhibit considerable variation in their efficiency of CXCR4 and CCR5 co-receptor use. The efficiency of CXCR4 usage and the tropism composition of the baseline virus population appears to influence the suppression of dual-tropic variants by AMD3100. Further characterization of dual-tropic variants is needed to fully appreciate the susceptibility of dual tropic viruses to CXCR4 and CCR5 targeted therapies.

Commentary on Abstract 91

- In previous study the CXCR4 inhibitor AMD3100 significantly reduced HIV RNA in a patient with CXCR4-tropic virus but not in dual/mixed tropic viruses. This was further investigation on inhibition of dual/mixed tropic virus population to AMD3100.
- X4-using variants in patient samples with dual/mixed-tropic phenotype showed different response to AMD3100. Some of patient X4-using variants were inhibited by AMD3100 (suppressor) as noted by decreased CXCR4 mediated virus entry and others did not (non-suppressor). However, no changes in viral loads were detected in either group.
- Envelope clonal analysis of these dual/mixed virus populations at baseline noted that the X4- and dual-tropic variants were inhibited by AMD3100 in suppressor virus populations with enriched R5-tropic variants. The non-suppressor virus populations were enriched with dual-tropic variants that were not inhibited by AMD3100.

Key points on Abstract 91

- AMD3100 has the ability to inhibit both X4-tropic and certain dual tropic variants *in vivo*.
- Dual-tropic variants exhibit considerable variation in their efficiency of CXCR4 and CCR5 co-receptor use.
- The efficiency of CXCR4 usage and the tropism composition of the baseline virus population appeared to influence the suppression of dual-tropic variants by AMD3100.

Commentary of Abstract 512

- AMD11070 exhibits potent and selective inhibition of HIV-1 replication by binding to the chemokine receptor CXCR4.
- A5210 is a phase IB/IIA proof of concept dose escalating study to determine safety and antiviral activity of AMD11070.
- Subjects were accepted with a HIV-1 viral load ≥ 5000 and who had been off antiviral drugs for at least 14 days before entry. Subjects were screened for the presence of X4 or X4/R5 virus. Primary end point was $\geq 1 \log_{10}$ reduction in X4-tropic virus rlu (relative luminescence units).
- At screening 92% failed as most patients were R5. Eight percent ($n = 6$) enrolled in the study all of which were dual tropic (X4/R5 virus).
- After 10 day treatment, 3 patients had a greater than $1 \log_{10}$ drop in X4 virus rlu (-3.3, -1.8, and -1.0 \log_{10}). Three patients saw less than $\pm 0.05 \log_{10}$ drop in X4.
- No significant changes in HIV RNA or CD4+ count was noted.
- No toxicities graded as 3 or above were noted during the 10 day treatment.

Key Points on Abstract 512

- AMD11070 can selectively inhibit X4-tropic virus in HIV-1 infected patients.
- Reductions of greater than $\geq 1 \log_{10}$ in X4 tropic virus was seen in 3/6 patients at 10 days.
- AMD11070 is on a FDA clinical hold due to animal toxicity studies.
- Further studies on CXCR4 antagonists are warranted.



Poster 511

CXCR4 Antagonism: Proof of Activity with AMD11070

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Abstract

Background: CXCR4 is a chemokine receptor that is highly expressed on T cells and is a key component of the HIV-1 coreceptor. CXCR4 antagonists have been shown to inhibit HIV-1 infection and to improve CD4 counts in HIV-1 infected individuals. CXCR4 antagonists have also been shown to improve outcomes in patients with HIV-1 associated neurocognitive disorders (HAND).
Methods: The CXCR4 antagonist AMD11070 was evaluated in a phase 2b study in HIV-1 infected individuals. The study was a randomized, double-blind, placebo-controlled trial. The primary endpoint was the change in CD4 count from baseline to week 48. Secondary endpoints included the change in HIV-1 RNA viral load, the change in HAND score, and the change in quality of life (QoL).
Results: At baseline, the mean CD4 count was 350 cells/mm³ (SD 100). At week 48, the mean CD4 count was 450 cells/mm³ (SD 120) in the AMD11070 group and 380 cells/mm³ (SD 110) in the placebo group. The difference in CD4 count between the two groups was statistically significant (p < 0.001). The change in HIV-1 RNA viral load, the change in HAND score, and the change in QoL were also statistically significant (p < 0.001).
Conclusion: CXCR4 antagonism with AMD11070 improves CD4 counts, HIV-1 RNA viral load, HAND score, and QoL in HIV-1 infected individuals.

Demographics and Baseline Characteristics

Table 1: Demographics and Baseline Characteristics

Characteristic	AMD11070 (n=100)	Placebo (n=100)
Age (mean, SD)	42 (10)	41 (9)
Sex (Male/Female)	85/15	80/20
Race (White/Black/Hispanic/Asian)	75/15/5/5	70/15/5/5
Time since HIV-1 diagnosis (mean, SD)	10 (5)	11 (6)
CD4 count at baseline (mean, SD)	350 (100)	350 (100)
HIV-1 RNA viral load at baseline (mean, SD)	1000 (100)	1000 (100)

Table 2: Baseline Characteristics by HIV-1 RNA Viral Load

Characteristic	AMD11070 (n=100)	Placebo (n=100)
Age (mean, SD)	42 (10)	41 (9)
Sex (Male/Female)	85/15	80/20
Race (White/Black/Hispanic/Asian)	75/15/5/5	70/15/5/5
Time since HIV-1 diagnosis (mean, SD)	10 (5)	11 (6)
CD4 count at baseline (mean, SD)	350 (100)	350 (100)
HIV-1 RNA viral load at baseline (mean, SD)	1000 (100)	1000 (100)

Safety Results

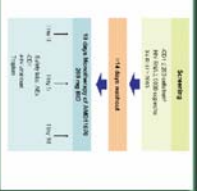
Table 3: Safety Results

Adverse Event	AMD11070 (n=100)	Placebo (n=100)
Headache	15	10
Nausea	10	8
Dizziness	8	5
Fatigue	5	3
Diarrhea	3	2
Upper respiratory tract infection	2	1
Other	1	1

Introduction

CXCR4 is a chemokine receptor that is highly expressed on T cells and is a key component of the HIV-1 coreceptor. CXCR4 antagonists have been shown to inhibit HIV-1 infection and to improve CD4 counts in HIV-1 infected individuals. CXCR4 antagonists have also been shown to improve outcomes in patients with HIV-1 associated neurocognitive disorders (HAND).
 The CXCR4 antagonist AMD11070 was evaluated in a phase 2b study in HIV-1 infected individuals. The study was a randomized, double-blind, placebo-controlled trial. The primary endpoint was the change in CD4 count from baseline to week 48. Secondary endpoints included the change in HIV-1 RNA viral load, the change in HAND score, and the change in quality of life (QoL).
 At baseline, the mean CD4 count was 350 cells/mm³ (SD 100). At week 48, the mean CD4 count was 450 cells/mm³ (SD 120) in the AMD11070 group and 380 cells/mm³ (SD 110) in the placebo group. The difference in CD4 count between the two groups was statistically significant (p < 0.001). The change in HIV-1 RNA viral load, the change in HAND score, and the change in QoL were also statistically significant (p < 0.001).
 CXCR4 antagonism with AMD11070 improves CD4 counts, HIV-1 RNA viral load, HAND score, and QoL in HIV-1 infected individuals.

Methods - Study Design



Study Endpoints

- Primary Endpoint: Change in CD4 count from baseline to week 48.
- Secondary Endpoints: Change in HIV-1 RNA viral load, change in HAND score, change in QoL.

Figure 1: AMD11070 Inhibition



Efficacy Results

Table 4: Efficacy Results - CD4 Count

Time Point	AMD11070 (n=100)	Placebo (n=100)
Baseline	350	350
Week 4	380	360
Week 8	400	370
Week 12	420	380
Week 16	430	385
Week 20	440	390
Week 24	445	395
Week 28	450	395
Week 32	450	395
Week 36	450	395
Week 40	450	395
Week 44	450	395
Week 48	450	395

Table 5: Efficacy Results - HIV-1 RNA Viral Load

Time Point	AMD11070 (n=100)	Placebo (n=100)
Baseline	1000	1000
Week 4	800	900
Week 8	700	850
Week 12	600	800
Week 16	550	750
Week 20	500	700
Week 24	480	680
Week 28	460	660
Week 32	450	650
Week 36	440	640
Week 40	430	630
Week 44	420	620
Week 48	410	610

Table 6: Efficacy Results - HAND Score

Time Point	AMD11070 (n=100)	Placebo (n=100)
Baseline	10	10
Week 4	8	9
Week 8	7	8
Week 12	6	7
Week 16	5	6
Week 20	4	5
Week 24	4	4
Week 28	4	4
Week 32	4	4
Week 36	4	4
Week 40	4	4
Week 44	4	4
Week 48	4	4

Table 7: Efficacy Results - QoL

Time Point	AMD11070 (n=100)	Placebo (n=100)
Baseline	50	50
Week 4	55	52
Week 8	60	53
Week 12	65	54
Week 16	70	55
Week 20	75	56
Week 24	80	57
Week 28	85	58
Week 32	90	59
Week 36	95	60
Week 40	100	61
Week 44	105	62
Week 48	110	63

Conclusion

- CXCR4 antagonism with AMD11070 improves CD4 counts, HIV-1 RNA viral load, HAND score, and QoL in HIV-1 infected individuals.
- AMD11070 is well-tolerated and has a favorable safety profile.
- AMD11070 is a promising treatment for HIV-1 infection and associated neurocognitive disorders.

Commentary on Abstract 511

- AMD11070 is an orally available CXCR4 antagonist. This study (XACT) was to investigate safety and activity of AMD11070 on 10 monotherapy.
- Patients were enrolled in they were treatment naïve or had been off ART treatment for 2 weeks. Patients had to have ≥ 2000 X4 related RLU's and have a viral load ≥ 2000 . The primary end point was $\geq 1 \log_{10}$ in X4 related RLU's. CD4 and viral loads were also noted.
- 10 patients were enrolled and were dosed twice daily (n = 8 at 200 mg and 2 at 100mg). Nine of these patients were dual mixed and one was X4.
- Four of the 9 individuals saw $\geq 1 \log_{10}$ reduction in X4 related RLU's (mean – 1.56). Two of the responders saw a switch from DM to R5 at day 5 and one switched from DM to R5 at day 10.
- No changes in CD4 nor viral load were noted.
- No serious adverse events or lab abnormalities were noted.

Key Points on Abstract 511

- AMD11070 was generally well tolerated.
- AMD 11070 was able to significantly reduced X4 RLU's (greater than $\geq 1 \log_{10}$ X4 related RLU's) in four out of the 9 enrolled patients.

Abstract 610

Defining the Upper and Lower Phenotypic Clinical Cut-offs for Darunavir/r (DRV/r) by the PhenoSense Assay

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3 Tibotec BVBA, Mechelen, Belgium

Background: The POWER 1, 2 and 3 trials demonstrated high efficacy of darunavir/ritonavir (DRV/r) in treatment experienced patients. We defined the upper (UCCO) and lower (LCCO) clinical cut-offs for DRV/r within these trials datasets by evaluating week 4 (W4) HIV RNA outcomes.

Methods: Phenotyping of a set (n=184) of baseline samples was by PhenoSense HIV (Monogram Biosciences). This dataset was restricted to patients in whom the on-study regimen did not include enfuvirtide. The LCCO was defined as the FC where the HIV RNA response was first observed to decline relative to the wildtype reference population. The UCCO was defined as the FC above which the attributable HIV RNA change from baseline was less than $-0.3 \log_{10}$ copies/mL. The impact of the on-study background therapy was explored by deriving PhenoSense specific continuous phenotypic susceptibility scores (cPSS) for the drugs in each regimen. Linear regression and local linear fitting by the function *lowess* were used to define the optimal correlation between the baseline DRV fold change (FC) and the W4 change in HIV RNA (\log_{10} copies(c)/mL).

Results: Among the 184 analyzed subjects 87 received functional DRV/r monotherapy, i.e. their optimized background regimen was scored as cPSS=0. Within this subset the baseline DRV FC correlated with the W4 HIV RNA change from baseline ($R=0.42$, $p<0.0001$). From the lowest DRV FC (0.42) to FC 10 the W4 HIV RNA reductions were constant (median $-1.99 \log_{10}$ c/mL). Thus, the LCCO was defined at a FC of 10. For DRV FC >10 a gradual reduction in HIV RNA response was observed as FC increased ($R=0.37$, $p=0.015$, median W4 HIV RNA change $-1.11 \log_{10}$ c/mL). Within this distribution the UCCO for DRV was defined at a FC of 90. The median HIV RNA change for samples with FC 10-<40 and 40-<90 was -1.34 (-2.66 to 0.15) and 0.39 (-2.93 to 0.28) \log_{10} c/ml, respectively. Analyses at study weeks 2 and 8 were concordant with the W4 findings. Applying these CCOs to the 184 phenotyped baseline samples, the proportions fully susceptible were: DRV/r, 53.8%; TPV/r, 33.7%; APV/r, 18.5%; ATV/r, 14.6%; LPV/r, 11.4% and SQV/r, 9.8%. By contrast the proportions above the UCCO were: DRV/r, 3%; TPV/r, 31%; APV/r, 66.3%; LPV/r, 70% and SQV/r, 71.7%.

Conclusions: In the POWER trials, DRV/r demonstrated optimal anti-HIV activity with a FC 10 and reduced anti-HIV activity with FC >10. These analyses defined the phenotypic LCCO and UCCO for DRV/r at 10 FC and 90 FC, respectively.

Commentary on Abstract 610

- The Power 1, 2, 3 trials demonstrated high efficacy of darunavir/ritonavir in treatment experienced patients. Using the HIV RNA outcome data sets at week 4 from these trials, both upper (UCCO) and lower (LCCO) clinical cutoffs for darunavir/ritonavir were defined.
- Three different questions were addressed. 1) LCCO was defined as a fold change (FC) where the HIV RNA response was first observed to decline relative to the wild type reference population. 2) The UCCO was defined as the FC above which the attributable HIV RNA change from baseline was less than $-0.3 \log_{10}$ copies/ml. 3) The impact of the on study background therapy was explored using phenotypic susceptibility scores (cPSS) for the drugs in each regimen.

Key points on Abstract 610

- The lower clinical cutoff (LCCO) for Darunavir/Ritonavir is at 10 FC and the upper clinical cutoff (UCCO) for Darunavir/Ritonavir is 90 FC.

Abstract 607

Darunavir-amprenavir cross-resistance in clinical samples submitted for phenotype-genotype combination resistance testing.

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Monogram Biosciences, South San Francisco, CA, USA.

Background: Darunavir (DRV, TMC-114) is the most recently approved protease inhibitor (PI). Mutations correlated with reduced clinical response to DRV are similar to those known to confer reduced susceptibility to amprenavir (APV). We examined PI cross-resistance patterns and predictive accuracy of a simple DRV mutation score genotype interpretation among samples tested in the Monogram Clinical Reference Laboratory.

Methods: Fold-change in IC_{50} (FC) data from 2682 samples tested since May 2006 (PhenoSense HIV, Monogram Biosciences) with at least one major PI mutation were analyzed. A DRV mutation score was calculated as the number of mutations present among V11I, V32I, L33F, I47V, I50V, I54L or M, G73S, L76V, I84V, and L89V. Samples with mixtures at any of these positions were excluded. The presence of 3 or more of these mutations is considered to indicate reduced susceptibility. Samples were classified as drug sensitive (S), partially sensitive (PS), or resistant (R) based on lower and upper clinical cutoffs (CCO). Mutations in gag or protease associated with reduced susceptibility (RS, $FC > 2$) were determined using Fisher's Exact test.

Results: The coefficient of correlation R^2 for log-transformed PI FC data was highest between DRV and APV (0.90), intermediate for lopinavir (0.60), and relatively low for other PIs (range 0.25-0.51). However, all 305 APV PS samples retained DRV susceptibility, and only 11% of 301 APV R samples were DRV R; 52% were PS to DRV. The percentage of samples with 1 (n=400) or 2 (n=355) DRV mutations which were not DRV S was 12.5% and 50%, respectively. Conversely, 32% of samples with 3 mutations (n=165) were DRV S. When present as the only major PI mutation, D30N (n=9), I50L (n=5), and N88S (n=5) were associated with increased susceptibility to DRV (median FC 0.52, 0.36, 0.25, respectively; corresponding median APV FC 0.79, 0.67, 0.13). There was extensive overlap between mutations associated with RS to both APV and DRV (38 of 65 mutations shared), including all DRV mutations.

Conclusions: DRV and APV *in vitro* susceptibility patterns are very similar. Predicted incidence of clinically meaningful cross-resistance is low, due to differences in CCO which are higher for DRV. The expected increased efficacy of DRV compared to APV in PI-experienced patients is most likely a result of higher potency (16-fold lower IC_{50} in the PhenoSense assay) and ~2-fold higher free drug levels in plasma, rather than a unique cross-resistance profile.

Commentary on Abstract 607

- Darunavir is the most recently approved protease inhibitor (PI). Protease inhibitor cross-resistance patterns and predictive accuracy of a simple darunavir mutation score genotype interpretation was examined.
- Fold changes in IC₅₀ were tested from 2862 samples with at least one mutation and no mixtures at positions in the DRV mutation score were analyzed. The darunavir mutation score was calculated as the number of mutations present among V11I, V32I, L33F, I47V, I50V, I54L or M, G73S, L76V, I84V, and L89V.
- The pattern of resistance is similar to amprenavir (correlation co-efficient $R^2 = 0.9$), intermediate for Lopranivir ($R^2 = 0.6$) and low for other PI's.
- All samples that were partially sensitive to amprenavir were darunavir sensitive and only 11 % of amprenavir resistant samples were darunavir resistant.

Key Points on Abstract 607

- Darunavir and amprenavir have very similar susceptibility patterns *in vitro*.
- Clinical cross-resistance is low, due to differences to clinical cut offs which are higher for darunavir.
- The increased efficacy of darunavir as compared to amprenavir in PI-experienced patients is most likely due to a higher potency (16-fold lower IC₅₀ for the WT reference virus) and ~2-fold higher free drug levels in the plasma.

Abstract 491

Resistance Profile of GS-8374, a Phosphonate-Containing HIV Protease Inhibitor.

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Background: GS-8374 is a novel protease inhibitor (PI) containing diethyl-phosphonate substituent off its P1 benzyl residue. Initial biophysical and structural analysis has shown that the phosphonate moiety of GS-8374 is involved in a novel type of interaction termed solvent anchoring that allows the inhibitor to maintain its activity against PI resistant mutants (Cihlar *et al.*, J. Mol. Biol. 2006). Here we report on the activity of GS-8374 against a panel of patient-derived HIV-1 variants with a high level resistance to multiple licensed PIs and assess its genetic barrier for *in vitro* resistance selection.

Methods: Resistance profile of GS-8374 was assessed using the PhenoSense assay (Monogram Biosciences) against a panel of 24 PI-resistant viruses containing an average of 10 resistance mutations (range 7-14). Standard drug resistance selection experiments were conducted in MT-2 cells infected with HIV-1 (IIIb).

Results: The average EC₅₀ value of GS-8374 against the whole panel of tested mutant viruses was 7 nM, representing a mean EC₅₀ fold change (FC) of 6.2 (range 0.6-26) relative to the wild-type control virus. In comparison, darunavir and brexanavir had a mean FC of 29.8 (range 1.0-157) and 23.6 (range 1.2-121), respectively. Only 3/24 tested viruses were >10-fold resistant to GS-8374 while 16/24 and 17/24 viruses were >10-fold resistant to darunavir and brexanavir, respectively. Although tipranavir exhibited similar FC as GS-8374 (mean 5.9-fold, range 0.5-27), its mean EC₅₀ value against the 24 tested viruses was 90-fold higher than that of GS-8374. All the other marketed PIs showed a mean FC ranging from 60 to >200. The exposure of wild-type HIV-1 to increasing concentrations of lopinavir, atazanavir, and darunavir for 5 months resulted in the selection of L10F/M46I/I54V/V82A, I50L/A71V/V77I/N88S, and R41T/K70E mutations in protease, respectively. In contrast, parallel selection with GS-8374 did not induce any genotypic changes in protease as determined by extensive clonal sequencing.

Conclusions: Results of this study demonstrate that the *in vitro* resistance profile of GS-8374 is superior to all tested PIs including the most recently licensed darunavir. Direct comparison with major marketed PIs suggests a high genetic barrier of GS-8374 against the selection of drug resistance. Collectively, these data support the unique role of the phosphonate moiety in the ability of GS-8374 to inhibit HIV-1 strains with high-level PI resistance.

Commentary on Abstract 491

- GS-8374 is a novel protease inhibitor that has been shown to maintain its activity against PI resistant mutants. GS-8374 was tested against a panel of 24 PI-resistant viruses that on average had 10 resistant mutations.
- The average EC50 value of GS-8374 against the whole panel of tested mutant viruses was 7 nM and represented an EC50 fold change (FC) of 6.2 (range 0.6-26) as compared to wild type virus.
- In comparison, darunavir and brexnavir had relative to wild type control virus FC of 29.8 (range 1-157) and 23.6 (range 1.2-121) respectively.
- Three of the 24 viruses tested were >10 fold resistant to GS-8374, whereas 16/24 and 17/24 were >10 fold resistant to darunavir and brexnavir respectively.

Key Points on Abstract 491

- *In vitro* GS-8374 is superior in its resistance profile to all PI's including darunavir.
- GS-8374 has a high genetic barrier for selection of drug resistance.
- GS-8374 novel structure seems to be able to inhibit HIV-1 strains with high level PI resistance.

Abstract 588

Continued Evolution of pol after Interruption of Reverse Transcriptase Inhibitors in Patients with Advanced HIV Disease.

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Background: Using allele-specific PCR (ASPCR), we have previously shown that the M184V mutation decays slowly in subjects with multidrug-resistant (MDR) HIV-1 infection who interrupt the reverse transcriptase inhibitor (RTI) component of their antiretroviral therapy (ART). To understand the mechanism of wildtype (WT) emergence in this context, we analyzed the evolution of the M184V variants before and after the interruption of RTIs.

Methods: We examined 4 HIV-infected individuals with documented MDR HIV-1 who were receiving ART including lamivudine (3TC). All subjects interrupted RTI therapy and 3 continued with protease inhibitor (PI) treatment. An average of 24 clones encompassing the first 1174 nucleotides of pol, were sequenced before RTI interruption (T0) and at the first time point after interrupting RTI therapy when M184V became undetectable by ASPCR (T1). Consensus sequence data before the initiation of 3TC treatment was available for 1 subject. Sequences from the T0 and T1 time points were assessed using maximum likelihood, neighbor joining and phylogenetic network approaches after ruling out recombination and hypermutation.

Results: Sequences from different subjects clustered separately. In 3 individuals, sequences from T0 and T1 clustered together, indicating a common most recent ancestor (MRCA). In each of these subjects, there was an increase in sequence diversity at T1 relative to T0. In the fourth subject, sequences from the 2 timepoints clustered separately, showing distinct MRCAs and had similar diversity. In this subject, however, T1 sequences were more closely related to sequences at T0 (genetic distance=0.019) than to the pre-3TC consensus sequence (genetic distance=0.034).

Conclusions: These results suggest that, in patients with MDR HIV who interrupt RTIs, reverse transcriptase WT variants arise because of continued evolution and back mutation rather than by reemergence of archived variants. The lack of rebound in an archived WT virus may reflect either infrequent release of such variants from latent reservoir and/or the fact that these pre-existing variants remained partially susceptible to the non-interrupted drugs. The fact that M184V emerges rapidly in presence of selective pressure (days to weeks) but takes months to revert in absence of pressure illustrates that the impact of this mutation on drug susceptibility is far stronger than the impact of this mutation on replicative capacity.

Commentary on Abstract 588

- Evidence exists that mutation M184V decays slowly in subjects with multidrug resistant (MDR) HIV-1 who interrupt their reverse transcriptase inhibitor (RTI) component of their antiviral therapy (ART).
- This study analyzed the emergence of M184V variants before and after the interruption of RTI's.
- Four patients with documented MDR HIV-1 interrupted RTI (3 continued on PI treatment). Twenty 24 clones encompassing the first 1174 nucleotides of *pol* before RTI interruption were sequenced (T0). When the M184V mutation became undetectable as measured by allele-specific PCR (ASPCR), clones were sequenced again (T1).
- In 3 of the four identified subject sequences from T0 and T1 were clustered together indicating recent ancestry although there was more sequence diversity at T1 rather than T0. The 4th patient however showed that the T0 and T1 time points were clustered separately showing distinct ancestry.

Key Points on Abstract 588

- In multiple drug resistant HIV-1 patients who interrupt reverse transcriptase inhibitors there is evidence that reverse transcriptase wild type variants arise because of continued evolution and back mutation rather than re-emergence of archived variants.
- The fact that M184V emerges rapidly in presence of selective pressure (days to weeks) but takes months to revert in absence of pressure indicates that mutation on drug susceptibility is far stronger than the impact of this mutation on replicative capacity.

Abstract 21

Superinfection Susceptibility and Low Neutralizing Serum Responses in Treated Persons with Suppressed Plasma Viral RNA Levels

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Methods: We evaluated viral populations and serum neutralization in chronically infected seroconcordant couples with no evidence of systemic superinfection despite high levels of exposure, recently infected individuals with apparent superinfections, and multiply infected persons with suppressed plasma RNA levels on therapy. A drug resistant RT/PR was cloned into a genomic test vector derived from a highly exposed individuals isolate. Drug resistant PR/RT is used in the test pseudotype viruses to evaluate antibody neutralization in the presence of PR/RT inhibitors in serum of treated individuals. HIV envelope sequences isolated from plasma samples of local controls and sexual partners are inserted into the vector to provide a panel of test vectors. Neutralizing antibody titers, reported as the inverse of the dilution giving 50% inhibition, were determined using a modification of the PhenoSense Entry assay (Monogram).

Results: Viral clonal analysis revealed multiple infections in blood cell DNA populations in 28% (7/25) of persons with suppressed plasma RNA levels. Serum neutralization titers were evaluated in these 7, and 18 highly exposed and viremic individuals. Neutralizing antibody titers against the test vectors were evident in 79.6% of serum/virus pairs among those with no evidence of superinfection, but only 22.9% of those with multiple infections. Responses to a laboratory strain in treated multiply infected individuals and 5 apparent superinfection cases among recently infected individuals were similar, and lower than observed in exposed persons without superinfection.

Conclusions: Multiple infections suggestive of superinfection were observed frequently in groups having negligible serum neutralizing levels, including those with suppressed viral load on therapy and those in the first 3 years of primary infection. Broad serum neutralization including partner-derived viruses may block superinfection or prevent systemic spread of additional infections.

Commentary on Abstract 21

- This study studied viral populations and serum neutralization in chronically infected seroconcordant couples with no evidence of systemic superinfection despite high levels of exposure.
- Drug resistant RT/PR clones were generated to evaluate antibody neutralization in the presence of PR/RT inhibitors in serum of treated individuals.
- Viral clone analysis revealed multiple infections in blood cell DNA populations in 28 % of persons with suppressed plasma RNA levels.
- Neutralizing antibody titers against test vectors were evident in 79.6 % of serum/virus among those with no evidence of superinfection but only in 22.9 % of those with multiple infection.

Key Points of Abstract 21

- Multiple infections suggestive of superinfection were observed frequently in groups having negligible serum neutralizing levels.
- Broad serum neutralization including partner derived viruses may block superinfection or block systemic spread of additional infections

Abstract 324

A Comprehensive Study of the Complexity of Viral Populations in Acute and Chronic HIV-1 Infection

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Background: The pool of envelope genes is much less diverse in acute compared to chronic HIV infection. The limited diversity in early infection is thought to be the result of a transmission bottleneck and/or selected growth kinetics in the newly infected host. The increased diversity in chronic infection is due to the accumulation of random mutations and continuous antigenic drift. We provide evidence that envelope clones derived from the same infected individual can display a wide range of characteristics, making it difficult to describe any one clone as representative of the entire population.

Methods: Plasma samples were collected from 5 acutely and 10 chronically infected subjects. HIV *env* genes were amplified and then transferred to an expression vector. Pseudotyped viruses were generated by co-transfection of the *env* expression vector with an *env*-defective genomic vector containing a luciferase reporter gene. Forty-eight *env* clones were picked from each patient sample, and then screened for viability in the tropism assay. 10-12 viable clones were selected from each patient for sequencing and neutralization sensitivity testing.

Results: We confirm that the overall genotypic and phenotypic diversity of *env* clones in chronic infection is much greater than in acute infection. We found that each of the acutely infected patients harbored only R5 tropic clones, whereas 2/10 chronic patients also harbored dual tropic clones (89% and 9% dual, respectively). Despite the low sequence diversity in the acute *env* populations, distinct differences in relative light units (RLU) were observed amongst clones from the same infectious pool. In addition, we found that the average RLU of acute clones was more than 3 fold greater than the average RLU of the chronic clones, suggesting a possible change in envelope function over time.

Conclusions: Population pools of *env* genes derived from acutely infected patients are more homogenous than those derived from chronically infected patients. Despite the reduced diversity, many acute clones from the same infectious pool display sequence variations and phenotypic differences that make it difficult to describe one clone as being representative of the entire population. We conclude that multiple viral envelope clones must be analyzed in order to get a true representation of the genotypic and phenotypic diversity present in the envelope population of an infected individual.

Commentary on Abstract 324

- It is thought that the pool of envelope genes is far less diverse in the acute phase of HIV-1 infection as compared to the envelope genes seen in the chronic phase of HIV-1 infection.
- Five acutely infected and 10 chronically HIV-1 infected individuals gave plasma samples. HIV *env* genes were amplified and then transferred to an expression vector. Pseudotyped viruses were generated by co-transfection with an *env*-defective genomic vector containing a luciferase reporter gene. Forty eight *env*-clones were picked from each patient and screened for viability on the tropism assay. Twelve viable clones were selected from each patient for sequencing and neutralization sensitivity testing.
- All acute phase HIV-1 infection, patients were R5 tropic, whereas 2/10 chronic phase HIV-1 infection, patients harbored dual tropic viruses.
- Despite the low sequence diversity in *env* genes in the acute phase patients, distinct changes in RLU's (relative light units) were seen amongst clones from the same infectious pools
- Average RLU's of acute clones were more than 3 fold greater than average RLU of chronic clones.

Key Points on Abstract 324

- *env* genes derived from acutely infected individuals are more homologous than those derived from chronically infected patients.
- Many acute clones display variations and phenotypic differences making it difficult for one clone as being representative of the entire population.
- The results of acute versus chronic average RLU's suggest a possible change in envelope function over time.
- Multiple viral envelope clones must be analyzed to get a true representation of the genotypic and phenotypic diversity present in the envelope population of an infected individual.

Abstract 636

Utility of *pol* Replication Capacity in Predicting Immunologic Course Among HIV-Infected Patients with Drug-Resistant Viremia

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Background: The ability to predict immunologic course among HIV-infected patients with drug-resistant viremia would greatly aid in decisions as to whether to maintain or change antiretroviral therapy. We hypothesized that, in this patient population, assessment of *pol* replication capacity (RC) -- one of few functional assays of HIV available in high throughput -- could predict subsequent CD4 count changes.

Methods: We examined subjects in SCOPE, a clinic-based cohort of HIV-infected adults, who had a stable antiretroviral regimen for ≥ 120 days, plasma HIV RNA >500 copies/ml, and genotypic resistance to ≥ 1 drug class. *pol* RC was assessed by modification of the PhenoSense assay (Monogram Biosciences). A phenotypic susceptibility score (PSS) was generated for each isolate by summing susceptibility (0=resistant; 0.5=intermediate; 1=susceptible) to all current drugs. Prediction of subsequent CD4 counts was assessed by mixed effects linear models. Observation was censored upon any change in therapy.

Results: Among 92 patients with 117 episodes of drug-resistant viremia, the median (IQR) baseline values were: plasma HIV RNA 3.8 (3.3-4.5) \log_{10} copies/ml, CD4+ cells 232/ mm^3 (151-320), PSS 1 (0.5-1.5), and RC 35% (18-61). Over a median of 8.1 months of observation and a total of 688 CD4 counts, the median CD4 slope was -1.6 cells/ mm^3 per month (IQR -2.8 to +0.017; absolute range -11.7 to +11.9). Baseline RC was not strongly associated with subsequent CD4 count change, regardless of adjustment for baseline HIV RNA, CD4, or PSS. Each 10% absolute increase in RC was associated with 0.13 more CD4 cells/ mm^3 lost per month (95% CI: 0.69 cells lost to 0.43 cells gained; $p=0.51$). Various dichotomizations of RC or contrasts of lowest vs. highest values also failed to strongly predict CD4 changes. Among 60 patients with ≥ 1 follow-up RC value (median of 4 months), the median RC change was +0.2% (IQR -11 to +10). There was not strong evidence for an effect of RC change on subsequent CD4 count slope ($p=0.60$).

Conclusions: Among patients with drug-resistant viremia, neither a single RC value nor a short-term within-person change in RC was strongly associated with subsequent CD4 count change. Larger studies will be needed to assess for smaller effects of RC than could be observed with the available cohort. Also, whether a change in RC over a longer period, or from a pre-therapy isolate to the drug-resistant variant, can predict subsequent CD4 count changes merits investigation.

Commentary on Abstract 636

- It has been hypothesized that assessment of *pol* replication capacity (RC) could predict subsequent CD4 count changes among HIV-infected patients with drug resistant viremia. This study set out to examine this question.
- Subjects entered the SCOPE cohort with stable antiviral treatment for ≥ 120 days, plasma HIV RNA >500 copies/ml and genotypic resistance to ≥ 1 drug class. *pol* replication capacity was measured and a phenotypic susceptibility score (PSS) was generated for each isolate by summing susceptibility to all current drugs in these subjects (0=resistant; 0.5=intermediate; 1=susceptible).
- 92 patients identified with median HIV RNA of 3.8 (IQR 3.3-4.5) \log_{10} copies/ml, CD4+ cells of $232/\text{mm}^3$, PSS of 1 (IQR 0.5-1.5) and RC of 35% (IQR 18-61). Patients were observed over an 8.1 month period.
- Median loss of CD4+ cells per month was $-1.6 \text{ cells}/\text{mm}^3$. Baseline RC was not strongly associated with subsequent CD4+ count regardless of adjustment for baseline HIV RNA, CD4+ or PSS.

Key points on Abstract 636

- There was not strong evidence for an effect of RC change on subsequent CD4+ count slope.
- Patients in SCOPE were already very stable (virologically and immunologically) prior to study entry, and patients were censored when changes to antiretroviral regimen were made. Median CD4 change over the follow-up period was only $-1.6 \text{ cells}/\text{mm}^3$. Thus, it is likely we would have needed a much larger sample size in order to show any differences in RC effects in this group.

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