



The Mark of
Individualized Medicine

Key Presentations on HIV Drug Resistance and Co-Receptor Tropism

February 3rd-6th, 2008

Boston, Massachusetts



Executive Summary

Monogram Biosciences presentations at the 15th Conference on Retroviruses and Opportunistic Infections covered numerous important topics. Overall, Monogram Biosciences was involved in 13 presentations.

Abstracts include information on the following:

- Virological correlates associated with treatment failure at week 48 in the phase 3 study of maraviroc in treatment naïve individuals (MERIT).
- An enhanced version of Trofile™ co-receptor tropism assay predicts emergence of CXCR4 use in ACTG5211 vicriviroc trial samples.
- Detection of viral co-receptor tropism switches with an enhanced version of Trofile™ co-receptor tropism assay among HIV –infected patients with drug resistant viremia
- Characterization of maraviroc resistance in patients failing treatment with CCR5-tropic virus in MOTIVATE 1 & 2..
- Emergence *in vivo* of Vic riviroc Resistance in HIV-1 Subtype C: Role of V3 Loop and Susceptibility to Other CCR5 Antagonists
- Co-receptor tropism ENV genotype and *in vitro* susceptibility to CCR5 antagonists during a 14-day monotherapy study with INCB9471.
- The gp41 transmembrane subunit of HIV-1 envelope influences CD4 receptor-dependent virus entry.
- Measurement of maturation inhibitor susceptibility using the PhenoSense® assay.
- Prevalence, mutational patterns and phenotypic correlates of the 76V protease mutation in relation to LPV/r therapy.
- Associations of HIV-1 infectivity on CCR5-bearing cells with survival in vertically-infected Ugandan infants.
- Examination of HIV-1 Subtype and Response to ART in Ugandan Children
- Analysis of NVP resistance in Ugandan infants who were HIV-infected despite receiving single dose (SD) nevirapine (NVP) vs. SD NVP plus up to 6-weeks of daily NVP to prevent HIV vertical transmission.
- Heterosexually Transmitted Viruses in Uganda Possess Signature Envelope Genotypes.

Abstract 40LB

Virological Correlates associated with Treatment Failure at Week 48 in the Phase 3 Study of Maraviroc in Treatment-Naïve Patients (MERIT)

J Heera¹, M Saag², P Ive³, J Whitcomb⁴, M Lewis⁵, L McFadyen⁵, J Goodrich¹, H Mayer¹, E van der Ryst⁵, and MikeWestby*⁵

¹Pfizer Global R&D, New London, CT, US; ²Univ of Alabama at Birmingham, US; ³Univ of the Witwatersrand, Johannesburg, South Africa; ⁴Monogram Biosci, South San Francisco, CA, US; and ⁵Pfizer Global R&D, Sandwich, UK

Background: MERIT was designed to compare the safety and efficacy of maraviroc (MVC) 300mg BID versus efavirenz (EFV) 600mg QD, each administered with Combivir, in antiretroviral-naïve patients. Analysis of Week 48 data demonstrated that 65.3% of patients receiving MVC achieved an HIV-1 RNA of <50 copies/ml compared to 69.3% for EFV. More patients discontinued from MVC for lack of efficacy compared with EFV (11.9% versus 4.2%), whereas fewer patients discontinued MVC due to adverse events (4.1% versus 13.6%). The aim of this analysis was to understand the virological correlates of failure.

Methods: For all patients, tropism was measured at pre-determined study visits throughout the study using the Trofile™ Assay (Monogram Biosciences). Resistance to the NRTIs and EFV was evaluated by the PhenoSense GT assay at screening and time of failure. Resistance to MVC was evaluated using the PhenoSense HIV Entry assay, with plateaus in dose response as a marker of resistance. MVC plasma concentrations were determined using sparse PK sampling at protocol specified time points and combined with compliance data obtained from the study database.

Results: Of 721 patients, 24 (3%) changed from R5 at screening to D/M at baseline. The response in this patient group was lower for both EFV and MVC groups, with only 54.6% and 7.1% patients achieving <50 RNA copies/ml at week 48. CXCR4-using virus was detected at failure in 10/32 (31.3%) of MVC patients with R5 virus at baseline. NRTI resistance was selected at failure in all of these patients. For the 13 patients who failed with R5 virus, MVC-resistant virus was selected in 2 patients and resistance to NRTI in 7. In contrast, NNRTI resistance was detected in 6/7 patients failing EFV who had a valid tropism result at failure. Race, clade, geographic region and gender did not appear to be associated with increases in virological failure to MVC, but poor treatment adherence was a significant contributor to failure. Mean CD4+ cell increases from baseline were greater in MVC failures (101 cells/uL) compared with EFV failures (44 cells/uL), regardless of tropism at time of failure.

Conclusions: CXCR4-using virus at baseline was an important predictor of failure on MVC in this study. As with treatment-experienced patients, failure with CXCR4-using virus is an important mechanism associated with virological failure. In these patients viral replication in the presence of NRTIs only occurred, resulting in selection of NRTI mutations.

Comments about Abstract 40LB

- MERIT compared the efficacy and safety of maraviroc (300 mg BID) versus efavirenz (600 mg QD) on a combivir backbone in antiretroviral-naïve patients.
- This study aimed to investigate the virological correlates' in subjects who failed MERIT.
- **Method:**
 - Tropism was measured at pre-determined study visits throughout the study using the Trofile™ assay (Monogram Biosciences).
 - Resistance to NRTIs and efavirenz (EFV) was evaluated by the PhenoSense GT® assay at screening and at time of failure.
 - Resistance to maraviroc (MVC) was evaluated by PhenoSense Entry™ Resistance to maraviroc was noted as plateau's in the MVC dose response curve.
 - MVC plasma concentrations were determined using sparse PK sampling at protocol specified time points and combined with compliance data obtained from the study database.
- **Results**
 - Of 721 patients, 24 (3%) changed from R5 at screening to D/M at baseline. The response in this patient group was lower for both EFV and MVC groups:
 - Response EFV: 54.6% <50 RNA copies/ml at week 48.
 - Response MVC: 7.1% <50 RNA copies/ml at week 48.
 - CXCR4-using virus was detected at failure in 10/32 (31.3%) of MVC subjects with R5 virus at baseline. NRTI resistance was selected at failure in all of these patients.
 - 13 patients failed MVC with R5 virus of which 2 has resistance to MVC and 7 had resistance to NRTI.
 - NNRTI resistance was detected in 6 of 7 patients failing EFV who still had R5 virus.
 - Race, clade, geographic region or gender did not seem to be associated with increases of virologic failures to MVC.
 - Poor treatment adherence was a significant contributor to failure.
 - Mean CD4 + cell increases as compared to baseline :
 - At MVC failure, 101 cells/uL (regardless of final tropism).
 - At EFV failure. 44 cells/uL (regardless of final tropism).

Key points about Abstract 40LB

- CXCR4-using virus at baseline was an important predictor of failure on MVC in this study.
- Failure with CXCR4-using virus is an important mechanism associated with virological failure.
- Viral replication in the presence of NRTIs only occurred and resulted in selection of NRTI mutations.

Abstract 869

An Enhanced Version of the Trofile HIV Co-receptor Tropism Assay Predicts Emergence of CXCR4 Use in ACTG5211 Vicriviroc Trial Samples

Jacqueline Reeves*¹, Dong Han¹, Timothy Wilkin², Terri Wrin¹, Daniel Kuritzkes³, Christos Petropoulos¹, Jeannette Whitcomb¹, Neil Parkin¹, Roy Gulick², and Eoin Coakley¹

Monogram Biosciences, South San Francisco, CA, US, Medical College of Cornell University, New York, NY, US, Harvard School of Public Health, Boston, MA, US

Background: HIV entry inhibitors which block infection via CCR5 have shown efficacy in suppressing CCR5- (R5) but not CXCR4- (X4) or dual/mixed-tropic (DM) HIV. ACTG5211 was a phase 2b study of the CCR5 antagonist vicriviroc (VCV) in treatment-experienced subjects with R5 virus at study screen. Reduced virologic response was observed among subjects with R5 virus at screen but DM at baseline, and in subjects with CXCR4-using virus on study as determined by a standard tropism assay (Trofile, Monogram Biosciences, South San Francisco). An enhanced version of this assay has been developed with improved ability to detect low levels of CXCR4-using variants. We hypothesized these assay enhancements might better identify CXCR4-using virus in the screening samples from subjects enrolled into ACTG 5211.

Methods: The co-receptor tropism of virus populations obtained at screen and baseline from 116 subjects was determined with the enhanced assay and compared to original tropism results. The number of subjects reclassified with DM virus at screen and who would have been excluded from the study by the enhanced assay was determined and compared to the number of patients with CXCR4-using virus on-study by the standard tropism assay.

Results: Enhanced tropism results are available for 116 subjects. 25 subjects previously defined as having R5 virus in the screening sample by the standard assay were reclassified as having DM virus by enhanced assay. On-study, 15 of these 25 reclassified subjects received VCV with 12/15 demonstrating DM virus by the standard assay during follow-up. Conversely, 3/15 reclassified VCV recipients had R5 virus at all follow-up time points by the standard assay. 12 subjects were previously defined as having R5 virus in the screening sample and DM virus at baseline by the standard assay. The enhanced assay detected DM virus in the screening sample in 7 of these subjects.

Conclusions: The enhanced sensitivity tropism assay identified CXCR4-use at screen in 12/15 VCV recipients with emergence of CXCR4-using variants on-study as determined by the standard tropism assay. This suggests that the enhanced assay may be an improved screening tool for determining eligibility for CCR5 antagonist therapy. Reanalysis of virologic response to VCV in light of enhanced tropism assay calls will determine if the enhanced assay may further optimize selection of patients who may benefit from CCR5 inhibitors.

Comments about Abstract 869

- ACTG5211 was a phase 2b study of the CCR5 antagonist vicriviroc in treatment experienced individuals with R5 virus at screen (identified using Trofile™).
- In those individuals (n = 25) who had R5 virus at screen but DM (dual/ mixed-tropic) at baseline and those with CXCR4 virus on study (as determined by Trofile) had reduced virological responses on drug as compared to those with R5 virus.
- The Trofile assay was enhanced to allow for the improved ability to measure CXCR4 minor variants.
- Hypothesis: To better identify CXCR4-using virus in screening samples enrolled in ACTG5211.

- **Method:**
 - The co-receptor tropism of virus populations obtained at screen and baseline from 116 subjects was determined with the enhanced assay and compared to original tropism results.

- **Results**
 - Enhanced Trofile was available on 116 patients.
 - 25 subjects previously defined as have R5 virus by standard Trofile were reclassified as having DM virus by enhanced Trofile.
 - On study, 15 of these 25 of these reclassified individuals received vicriviroc.
 - 12 of these 15 individuals had DM virus by standard Trofile during follow up.
 - 3 of these 15 individuals remained R5 throughout the study.
 - Twelve subjects who were identified with R5 virus at screen and DM at baseline using standard Trofile were reassessed using enhanced Trofile. Seven of these 12 samples were reclassified with DM virus.

Key points about Abstract 869

- Data suggests that enhanced Trofile may be an improved screening tool for determining eligibility for CCR5 antagonist therapy.

Abstract 864

Detection of Viral Co-receptor Tropism Switches with a High Sensitivity Phenotypic Assay among HIV-infected Patients with Drug-resistant Viremia

*Peter Hunt¹, Jeffrey Martin¹, Wei Huang², Eoin Coakley², Christos Petropoulos², Michael Bates², Rebecca Hoh¹, Steven Deeks¹, and Jackie Reeves²

¹ *University of California and San Francisco General Hospital, San Francisco, California, USA;*

² *Monogram Biosciences, Inc., South San Francisco, California, USA*

Background: Approximately 10% of treated patients with drug-resistant viremia experience apparent switches from CCR5-tropic virus (R5) to dual/mixed-tropic virus (DM) when followed on a stable antiretroviral regimen for 1 year. These apparent tropism switches have been associated with subsequent virologic failure of CCR5 inhibitor-based therapy. It is unclear whether these tropism “switches” simply reflect low assay sensitivity for a clinically significant minority CXCR4-using virus population (X4) at baseline.

Methods: HIV-infected patients with drug-resistant viremia >1,000 copies/ml on a stable CCR5 inhibitor-sparing antiretroviral regimen for >4 months were sampled from a clinic-based cohort study. Viral co-receptor tropism was measured every 4 months until treatment change with both the currently available Trofile assay and a new modification of the Trofile assay with enhanced sensitivity for X4-using viruses (ES-Trofile).

Results: In 57 patients with drug-resistant viremia, median baseline values were: plasma HIV RNA level, 3.9 log₁₀ copies/ml and CD4 count, 236 cells/mm³. Using the Trofile assay, 40 (70%) had R5, 15 (26%) had DM, and 2 (4%) had pure X4 at baseline. However, 6/40 (15%) of those apparently harboring pure R5 at baseline according to the Trofile assay actually had DM according to the ES-Trofile assay. There was a median of 3 tropism observations/patient over a median of 8 months. Using the Trofile assay, 15% of those harboring pure R5 at baseline (95% CI: 5%-28%) had apparently switched to DM at 1 year. Of these 5 apparent early R5 to DM switches, 3 (60%) were actually persistently DM by the ES-Trofile assay. Using the ES-Trofile assay, 15% of those harboring R5 at baseline (95% CI: 7%-32%) switched to DM and 15% of those harboring DM at baseline (95% CI: 5%-41%) experienced “reversion” to R5 by 1 year. One of 2 patients with pure X4 at baseline by the ES-Trofile assay switched to DM after 4 months of observation.

Conclusions: The majority of apparent R5 to DM switches detected with the currently available Trofile assay in among stably treated patients with drug-resistant viremia are due to pre-existing minority X4 populations, detectable by the new ES-Trofile assay. However, R5 to DM tropism switches remain common in this population and a substantial number of patients with apparently pure R5 on the new assay may have harbored DM in the recent past. The clinical significance of low-level X4 remains to be established in clinical trials.

Comments about Abstract 864

- Approximately 10% of treated patients with drug-resistant viremia experience apparent switches from CCR5-tropic virus (R5) to dual/ mixed-tropic virus (DM) when followed on a stable antiretroviral regimen for a year.
- This study aimed to investigate whether these tropism switches were a reflection of low assay sensitivity in detecting clinically significant minority CXCR4-using viruses (X4) at baseline.
- **Method:**
 - HIV-infected patients with drug resistant viremia > 1000 copies/ml on a stable CCR5 inhibitor sparing regimen for > 4 months were sampled in a clinic based cohort.
 - Viral co-receptor tropism was measured every 4 months until treatment change. Measurements were made with both Trofile and Trofile ES.
- **Results**
 - 57 patients with drug resistant viremia (average baseline plasma HIV RNA, 3.9 log₁₀ copies ml, and CD4 count, 236 cells/ mm³) had their tropism identified by Trofile™.
 - 40 individuals had R5 virus (70 %), 15 had DM virus (26 %) and 2 had pure X4 virus (4 %) by standard Trofile.
 - Using Trofile-ES (enhanced), 6 of the 40 previously identified R5 patients (15 %) were identified as DM.
 - Over a median of 8 months a median of 3 tropism observations were made in this patient population.
 - Using the Trofile assay, 15% [n = 5] (95 % CI: 5%-28%) of those identified as having R5 virus at baseline apparently switched to DM at 1 year. Of these R5 to DM patients, 3 (60%) were persistently DM by Trofile ES
 - Using the Trofile ES, 15 % of those harboring R5 virus (95% CI: 7%-32%) switched to DM and 15 % of those harboring DM switched to R5 (95% CI: 5%-41%)

Key points about Abstract 864

- The majority of apparent R5 to DM switches detected with Trofile in stably treated patients with drug-resistant viremia are due to pre-existing minority X4 populations. These minority populations are detectable by the new ES-Trofile assay.
- However, these R5 to DM tropism switches remain common in this population but the clinical significance of low level X4 remains to be established in clinical trials.

Abstract 871

Changes in V3 Loop Sequence Associated with Failure of Maraviroc (MVC) Treatment in Patients Enrolled in the MOTIVATE 1 and 2 Trials

M Lewis¹, J Mori¹, P Simpson¹, J Whitcomb², X Li³, DL Robertson³, and M Westby¹
¹Pfizer Global Research and Development, Sandwich Laboratories, Kent, UK; ²Monogram Biosciences, California, USA; and ³Faculty of Life Sciences, University of Manchester, UK

Background: Preliminary virologic analyses have identified two phenotypic pathways associated with virologic failure in the Phase 3 studies of maraviroc (MVC) (MOTIVATE 1 and 2). Either CXCR4-using virus was detected on MVC treatment (tropism) or virus was selected that was able to infect cells using MVC-bound CCR5 (resistance). In each case, changes in V3 loop sequence could be associated with the phenotypic pathway selected. The aims of this genotypic analysis were firstly to characterize the V3 sequences of the CXCR4-using virus selected in some patients on MVC and secondly to determine the V3 sequences for all patients with phenotypic evidence of MVC resistance at Week 24.

Methods: Paired baseline and on-treatment samples from 35 patients were studied by clonal analysis. *Env* (gp160) sequencing and tropism confirmation were performed on 12 *env* clones from each time point. Clones from patients failing with R5 virus were analyzed for evidence of phenotypic resistance. Dual-tropic clones were further categorized as “dual-R” or “dual-X” based on V3 loop similarity to R5 or X4 clones isolated from the same patient. The V3 loops were analyzed using 8 freely available genotypic algorithms.

Results: Clonal analyses confirmed results for the original population samples. *Resistance:* amino acid changes were identified in the V3 loops of all patients with evidence of phenotypic resistance. The amino acid changes differed between patients, reflecting the heterogeneity of the gp160 sequence and multiple genetic pathways to resistance. *Tropism:* dual-tropic clones were identified in samples from 18 out of 20 patients. Twelve patients who received MVC had “dual-X” virus on-treatment; in 7 of these the virus was identified at baseline; in 5 MVC-treated patients and 2 placebo-treated patients, the “dual-X” virus emerged during treatment. Six patients had “dual-R” clones at baseline; 4 received MVC. The “dual-R” virus was not identified during treatment in 3 of the MVC patients but persisted in both placebo patients. An analysis of the infectivity values from the Trofile™ assay showed a different profile between the two types of dual-tropic virus.

Conclusions: Mutations in the V3 loop appear to play a key role in conferring the MVC CCR5-tropic resistant phenotype. This limited dataset suggests that some dual-tropic clones are responsive to MVC *in vivo*. However, in the patients studied, these clones always coexisted with non-responsive “dual-X” clones.

Comments about Abstract 871

- Preliminary virologic analysis have identified 2 phenotypic pathways associated with virologic failure in the phase 3 studies of maraviroc (MVC) (Motivate 1 & 2):
 - 1. CXCR4 using virus on MVC treatment (tropism).
 - 2. Virus was able to infect cells using MVC-bound CCR5 (Resistance).
- Both of these phenotypic pathways are thought to involve changes in the viruses V3 loop region on gp160.
- This study aimed to:
 - Genotypically characterize the V3 sequences of the CXCR4-using viruses selected by MVC.
 - Genetically characterize the V3 sequences of viruses with phenotypic evidence of MVC resistance at week 24.
- **Method:**
 - Paired baseline and on treatment samples from 35 patients failing treatment were studied using clonal analysis.
 - *Env* (gp160) sequencing and tropism confirmation were performed on 12 *env* clones from each time point.
 - Clones from patients failing with R5 virus were analyzed for evidence of phenotypic resistance.
 - Dual-tropic clones were further categorized as “dual-R” or “dual-X” based on V3 loop similarity to R5 or X4 clones isolated from the same patient.
 - The V3 loops were analyzed using 8 freely available genotypic algorithms.
- **Results**
 - Resistance:
 - Amino acid changes were identified in the V3 loops of all patients with evidence of phenotypic resistance.
 - Amino acid changes differed between patients, reflecting the heterogeneity of the gp160.
 - Tropism:
 - Dual-tropic clones were identified in samples from 18 out of 20 patients.
 - 12 who received MVC had “dual-X” virus on-treatment;; in 7 of these the virus was identified at baseline.
 - 5 MVC-treated patients and 2 placebo-treated patients, the “dual-X” virus emerged during treatment.
 - 6 patients had “dual-R” clones at baseline; 4 received MVC.
 - The “dual-R” virus was not identified during treatment in 3 of the MVC patients but persisted in both placebo patients.
 - Analysis of the infectivity values from the Trofile™ assay showed a different profile between the two types of dual-tropic virus.

Key points about Abstract 871

- Mutations in the V3 loop appear to play a key role in conferring the MVC.
- This limited dataset suggests that some dual-tropic clones are responsive to MVC *in vivo*. However, in the patients studied, these clones always coexisted with non-responsive “dual-X” clones.

Abstract 870

Emergence *in vivo* of Vicriviroc Resistance in HIV-1 Subtype C: Role of V3 Loop and Susceptibility to Other CCR5 Antagonists

Athe Tsibris^{1,2}, M Sagar^{2,3}, Z Su⁴, C Flexner⁵, W Greaves⁶, P Skolnik⁷, E Coakley⁸, M Subramanian⁹, R Gulick¹⁰, and D Kuritzkes^{2,3}

¹Massachusetts Gen Hosp, Boston, US; ²Harvard Med Sch, Boston, MA, US; ³Brigham and Women's Hosp, Boston, MA, US; ⁴Harvard Sch of Publ Hlth, Boston, MA, US; ⁵Johns Hopkins Univ, Baltimore, MD, US; ⁶Schering-Plough Res Inst, Kenilworth, NJ, US; ⁷Boston Med Ctr, MA, US; ⁸Monogram Biosci, South San Francisco, CA, US; ⁹Human Genome Sci, Rockville, MD, US; and ¹⁰Weill Med Coll of Cornell Univ, New York, NY, US

Background: Few data are available regarding *in vivo* emergence of resistance to the CCR5 antagonist vicriviroc (VCV). We determined co-receptor usage, VCV susceptibility, and *env* sequences of HIV-1 from subjects with virologic failure (VF) in ACTG A5211, a phase IIb trial of VCV.

Methods: Samples from eight subjects with VF at week 16 were randomly selected for clonal sequence analysis (two subjects each from the placebo, 5-, 10-, and 15-mg VCV arms, respectively). VCV susceptibility (Entry Susceptibility assay), co-receptor usage (Trofile), and *env* cloning and sequencing were performed on samples obtained at entry, confirmation of virologic failure and week 24. Sequencing was performed on full-length molecular clones of *env* obtained from plasma virus by RT-PCR. *Env* sequencing was also performed on samples from one additional subject from the 10-mg arm after susceptibility testing showed progressive loss of inhibition by VCV.

Results: No consistent increase in VCV IC₅₀ was observed in samples obtained from the 8 randomly selected subjects through week 24 (maximum increase compared to control = 2.83-fold). HIV-1 variants from four of four subjects enrolled in the VCV 5- and 10-mg arms had changes in the V3 loop stem that became fixed in the population following VF; such changes were not found in samples from subjects in the placebo or 15-mg arms. Observed sequence changes involved different amino acid positions and substitutions in samples from each subject. Serial samples from the subject with phenotypic evidence of resistance showed emergence of multiple changes in the V3 loop sequence. Tropism assays on samples from the same time points showed a modest capacity to infect CXCR4-expressing cells, although the virus remained predominantly R5.

Conclusions: VF in subjects receiving a VCV-containing regimen was associated with changes in the V3 loop in samples from 5 of 9 subjects tested. Virus from the one subject with phenotypic evidence of VCV resistance also showed a modest capacity to use CXCR4, suggesting that some changes in gp120 that allow CCR5-mediated entry in the presence of VCV may also allow binding to CXCR4.

Comments about Abstract 870

- To investigate *in vivo* emergence of HIV-1 resistance to CCR5 antagonist vicriviroc (VCV) in the phase IIb ACTG 5211 clinical trial.
- **Method:**
 - 8 subjects with virologic failure (VF) at week 16 were randomly identified for clonal sequence analysis.
 - The 16 subjects were equally derived from each arm of the trial (2 subjects from placebo, 5 – 10, and 15 mg VCV).
 - Sequencing was performed on full length molecular clones of Env which were derived from plasma virus using RT-PCR.
 - Env sequences were derived from a single patient in the 10 mg arm after susceptibility testing showed a progressive loss of inhibition to VCV.
- **Results**
 - No consistent rise in IC50 was noted in the 8 subjects at wk 24.
 - All HIV-1 variants in the 5 and 10 mg arms (4 out of 4) had mutations in the V3 loop stem that became fixed in the viral population after VF.
 - No changes were noted in the placebo or 15 mg arms of the trial.
 - Observed sequence changes involved different amino acid positions and substitutions in samples from each subject.
 - Serial samples from a subject developing progressive VCV resistance showed emergence of multiple changes in V3 loop sequence.
 - Tropism testing at the same time points showed viruses with a modest capacity to infect CXCR4 expressing cells although the virus was predominant R5.

Key points about Abstract 870

- Virologic failure to VCV was associated with V3 loop mutations in 5 out 9 patients studied (including the 1 individual with progressive loss of VCV susceptibility).
- Virus from the one patient with phenotypic evidence of resistance to VCV showed a modest capacity to use CXCR4. This suggested that alterations occurred within the gp 120 that allowed CCR5-mediated entry in the presence of VCV and may also allow binding to CXCR4.

Abstract 862

Co-receptor Tropism, ENV Genotype, and *in vitro* Susceptibility to CCR5 Antagonists during a 14-Day Monotherapy Study with INCB9471

Susan Erickson-Viitanen¹, K Abremski¹, K Solomon¹, R Levy¹, E Lam²,
J Whitcomb², R Lloyd³, R Mathis³, J Reeves², and D Burn^{s3}

¹*Incyte Corp, Wilmington, DE, US*; ²*Monogram Biosci, South San Francisco, CA, US*; and ³*Res Think Tank, Buford, GA, US*

Background: Study INCB 9471-201 was a 14-day monotherapy study to examine once daily doses of the CCR5 antagonist INCB009471 in naïve or therapy-experienced subjects who were R5-tropic by the Trofile assay at Screening. Forty-nine subjects were enrolled to receive placebo (N=9), 100 mg (N=9), 200 mg (N=19) or 300 mg of a sustained release formulation (N=12).

Methods: Co-receptor tropism (Trofile assay), *in vitro* susceptibility to INCB9471 (PhenoSense Entry Assay) and ENV genotype (HIV-1 GeneTanker® C2V3-gp41^{HR1} domain Select assay) were determined at Screening, Day 1 (Baseline), Day 14 and Day 28.

Results: Virus present at Screening and end of study was highly susceptible to inhibition by INCB9471 (Screening IC₅₀ = 12.1 ± 1.5 nM, N=49, Day 28 IC₅₀ = 8.0 ± 1.5 nM, N=32), indicating a lack of emergence of resistance in this short term study. Plasma derived viral RNA genotypes were obtained and compared to V3 polarity 11/25 basic prediction rules. Additionally modified prediction analysis, MuTanker™, was performed and compared. Thirty of 39 subjects receiving 14 days of INCB9471 treatment achieved viral load declines > 1.5 log₁₀ relative to baseline: 5/9 receiving 100 mg, 16/19 receiving 200 mg, 9 of 11 receiving 300 mg of INCB9471. Circulating virus remained R5 tropic in 35 of 39 INCB9471-treated subjects. Dual/mixed tropic virus was observed on Day 14 and/or Day 28 for 2 subjects each, who received 200 mg or 300 mg INCB9471. These 4 subjects exhibited attenuated viral load response to INCB9471; 3 of 4 had previous therapy experience. A clonal analysis has been completed on 2 of these subjects. In subject A, receiving 200 mg, dual/mixed tropic virus was present in ~13% of clones examined at Baseline; the corresponding V3 loop sequence revealed lysine at position 25 relative to other viruses in the swarm with glutamic acid. Circulating virus was again R5 tropic at a visit subsequent to study completion. In subject B, receiving 200 mg, circulating virus was dual/mixed at Day 14, returning to R5 tropic at Day 28. At Day 14, the X4-utilizing viruses formed a monophyletic group and were distinct from R5 viruses. This phylogenetic analysis suggested that the dual/mixed virus present at Day 14 likely arose via outgrowth from an unidentified ancestral virus present at Baseline.

Conclusions: As shown with other CCR5 antagonists, the data from a 14-day monotherapy study with once-daily INCB9471 suggests that emergence of X4-utilizing viral variants in a minority of the treated patients reflects outgrowth from pretreatment X4-utilizing viral reservoirs.

Comments about Abstract 862

- The study examined a 14-day monotherapy trial on once daily dosing of INCB009471, a CCR5 antagonist in naïve or therapy-experienced subjects who were R5 by screening (by Trofile™).
- **Method:**
 - 49 subjects were enrolled and randomized to placebo (n=9), 100 mg (n=9), 200 mg (n=19) or 300 mg (n=12).
 - Co-receptor tropism (Trofile), *in vitro* susceptibility to INCB9471 (PhenoSense Entry™) and ENV genotyping (HIV-1 GeneTanker® C2V3-gp41^{HR1 domain} Select assay) were determined at screening, day 1 (baseline), day 14 and day 28.
- **Results**
 - Virus present at screening and the termination of the study were highly susceptible to inhibition by INCB9471 (Screening IC₅₀ = 12.1 ± 1.5 nM, N=49, Day 28 IC₅₀ = 8.0 ± 1.5 nM, N=32).
 - No emergence of resistance was seen in this short study.
 - 30 of 39 patients receiving 14 days monotherapy of INCB9471 saw viral load reductions > 1.5 log₁₀ relative to baseline, 5/9 receiving 100 mg, 16/19 receiving 200 mg and 9 of 11 receiving 300 mg of INCB9471.
 - Circulating virus remained R5 in 35 of 39 patients treated with INCB9471. However 4 subjects (2 in the 200 mg arm and 2 in the 300 mg arm) had evidence of DM-tropic viruses at days 14 and day 28.
 - Two of these of these individuals have undergone clonal analysis:
 - Subject A: 200 mg arm. At baseline, a DM virus was present at ~13% of clones. Analysis of this DM clone revealed that the V3 loop sequence had a lysine at position 25 relative to other viruses in the swarm with glutamic acid. The subject had circulation R5 virus at subsequent time points.
 - Subject B: 200 mg arm. At day 14 the subject was identified with dual/mixed virus. However, by day 28 the circulating virus had reverted to R5. The identified X4-using viruses formed a monophyletic group that was distinct from R5 viruses. This phylogenetic analysis suggested that the DM virus at day 14 probably arose via outgrowth from an unidentified ancestral virus at Baseline.

Key points about Abstract 862

- Once-daily INCB9471 suggests that emergence of X4-utilizing viral variants in a minority of the treated patients reflects outgrowth from pretreatment X4-utilizing viral reservoirs.

Abstract 251

The gp41 Transmembrane Subunit of HIV-1 Envelope Influences CD4 Receptor-dependent Virus Entry

Jonathan Toma, Signe Fransen, Terri Wrin, Jeannette M, Whitcomb, Neil Parkin, Christos J. Petropoulos and Wei Huang
Monogram Biosciences, South San Francisco, CA, USA.

Background: Binding of the surface (SU) subunit of HIV-1 envelope protein to the CD4 receptor is required for virus infection. However, large differences in sensitivity of different HIV strains to soluble CD4 (sCD4) suggest that CD4 dependence is variable. The primary CD4 binding site is located in SU. In this study we show that the transmembrane (TM) subunit can significantly impact the ability of SU to utilize CD4 for viral entry.

Methods: Multiple gp160 *env* clones from 3 HIV-1 positive patient plasma samples were isolated. Sensitivity to sCD4 and neutralizing antisera was measured using the PhenoSense HIV Entry assay. Cell-cell membrane fusion was assessed using a GFP/luciferase reporter assay. The determinants of sCD4 sensitivity were mapped for one of the patients by constructing and evaluating a series of *env* chimeras containing different regions of two clones displaying different sCD4 susceptibility phenotypes. Additional chimeras containing TM derived from the patient *env* clones and SU from JRCSF were also tested.

Results: In one patient, two *env* clones (S and R) with identical SU amino acid sequences were identified. Clone S was 100-fold more sensitive to sCD4 than clone R, induced higher membrane fusion (14-fold), and was more sensitive to neutralizing antiserum (27-fold). A clone S chimera containing six substitutions located in and near the heptad repeat region 2 (HR2) in TM from clone R had 10-fold reduced sCD4 sensitivity. Introduction of M536T (near the fusion peptide) and L592F (in HR1) from clone R into clone S decreased sCD4 susceptibility 100-fold. sCD4 sensitivity correlated with susceptibility to heterologous patient sera, and with *env* mediated fusion. Env chimeras containing SU from JRCSF and TM regions derived from S or R clones from 2 other patients varied by up to 10-fold in their sensitivity to sCD4, confirming the potential for TM from unrelated viruses to impact sCD4 sensitivity.

Conclusions: The study demonstrates the ability of the TM envelope subunit to influence CD4-dependent entry of HIV-1 and reinforces the complexity of determinants involved in Env/CD4 interactions. The ability of residues in TM to influence conformational changes in SU that affect CD4 interactions, or membrane fusion following CD4 binding, will require further study.

Comments about Abstract 251

- Binding of the surface (SU) subunit of the HIV-1 envelope to the CD4 receptor is required for HIV-1 infection.
- Different sensitivity to HIV strains to soluble (sCD4) indicated that CD4 dependence was variable.
- This study aimed to show that the transmembrane (TM) subunit can significantly alter the ability of SU to utilize CD4 for viral entry.

- **Method:**
 - Multiple gp 160 *env* clones from 3 HIV- positive patient plasma issues were isolated.
 - Sensitivity to sCD4 and neutralizing antisera was measured using PhenoSenseGT Entry™.
 - Cell-cell membrane fusion was accessed using a GFP/luciferase reporter assay.
 - Determinants of sCD4 sensitivity were mapped by constructing and evaluating a series of *env* chimera's containing different regions of two clones displaying different sCD4 susceptibility phenotypes.
 - Chimera's containing TM from patient *env* clones and SU from JRCSF were also tested.

- **Results**
 - In 1 patient 2 *env* clones were identified that had identical SU amino acid sequences. These clones were termed S and R.
 - Clone S:
 - Was 100-fold more sensitive to sCD4 than clone R.
 - Clone S induced higher membrane fusion (14-fold)
 - Clone S was more sensitive to neutralizing antiserum (27-fold)
 - Clone S containing six substitutions located in and near the heptad repeat region 2 (HR2) in TM from clone R had 10-fold reduced sCD4 sensitivity.
 - Introduction of M536T (near the fusion peptide) and L592F (in HR1) from clone R into clone S decreased sCD4 susceptibility 100-fold.
 - sCD4 sensitivity correlated with susceptibility to heterologous patient sera, and with *env* mediated fusion.

Key points about Abstract 251

- The study demonstrates the ability of the TM envelope subunit to influence CD4-dependent entry of HIV-1 and reinforces the complexity of determinants involved in Env/CD4 interactions.

Measurement of Maturation Inhibitor Susceptibility using the PhenoSenseHIV Assay

Sunny S Choe¹, Yanhua Feng¹, Kay Limoli¹, Karl Salzwedel², Scott McCallister², Wei Huang¹, and Neil T Parkin¹.

¹Monogram Biosciences, South San Francisco, CA, USA

²Panacos Pharmaceuticals, Inc., Gaithersburg, MD, USA

Background: Maturation inhibitors that target the HIV-1 Gag protein represent a new class of antiretroviral agents. Bevirimat (BVM, PA-457), currently in Phase 2 development, inhibits replication by blocking cleavage of the capsid/SP1 junction in Gag, leading to impaired maturation of the viral core. Viruses selected *in vitro* with reduced BVM sensitivity contain mutations at the CA/SP1 cleavage site (CS). Here we describe adaptation of the PhenoSenseHIV susceptibility assay to enable testing of maturation inhibitors.

Methods: The NL4-3-based PhenoSense resistance test vector (RTV) was modified to allow the capture of HIV Gag, PR and RT sequences. CA/SP1 CS mutations known to reduce sensitivity to BVM *in vitro* were introduced into the RTV using site-directed mutagenesis. BVM was added to transfected cells prior to virus production. Susceptibility (fold change in IC₅₀, FC) and replication capacity (RC) were measured using the single-cycle PhenoSense assay. Immunoblot analysis using a p24 antibody was performed to evaluate Gag processing in transfected cells and virus supernatants.

Results: CA/SP1 CS mutations H358Y, V362L, L363F, A364L, A366T and A366V conferred significantly reduced RC, ranging from 0.02% to 22% of wild-type; L363M and A364V had slightly decreased RC. The A366T mutant RTV did not replicate well enough to generate susceptibility data. V362L, L363F and L363M mutant RTVs had BVM FC values of 28, 12, and 20, respectively. H358Y, A364L, A364V and A366V had FC > 100. All of the mutants retained sensitivity to PR inhibitor, with FC values of 0.24-1.4. These results are consistent with published data. In the presence of intermediate drug concentrations, the H358Y mutant RTV was stimulated by up to 3-4 fold. A PR and RT inhibitor-resistant RTV demonstrated hypersusceptibility to BVM (FC = 0.17). In the absence of drug, all of the virions contained only p24, except V362L, which had both p24 and p25 due to impaired CA/SP1 cleavage.

Conclusions: The single-cycle, luciferase-based PhenoSenseHIV assay can measure susceptibility to BVM, a maturation inhibitor. Introduction of mutations associated with reduced BVM susceptibility yielded expected shifts over a range of sensitivities. Mutations in the CA/SP1 cleavage site also resulted in significant RC defects. As well as testing maturation inhibitor susceptibility, this Gag RTV system may be used to examine viral fitness and CTL escape mutations.

Comments about Abstract 880

- Maturation inhibitors that target the HIV-1 Gag protein represent a new class of antiretrovirals. Bevirimat, (BVM) a drug in this class is currently undergoing phase 2 clinical development.
- This study describes the adaptation of PhenoSense® susceptibility assay to enable testing of maturation inhibitors.
- **Methods**
 - The NL4-3 based PhenoSense resistance test vector (RTV) was modified to allow the capture of the Gag, PR and RT sequences.
 - CA/SP1 CS mutations known to reduce sensitivity to BVM *in vitro* were introduced into the RTV using site-directed mutagenesis.
 - BVM was added to transfected cells prior to virus production.
 - Susceptibility (fold change in IC₅₀) and replication capacity (RC) was measured using PhenoSense.
 - Immunoblot analysis using a p24 antibody was used to check Gag processing in transfected cells and virus supernatants.
- **Results**
 - CA/SP1 CS mutations H358Y, V362L, L363F, A364L, A366T and A366V conferred significantly reduced RC, ranging from 0.02% to 22% of wild-type. L363M and A364V had minimally decreased RC.
 - The A366T mutant RTV had impaired replication capacity and no susceptibility data could be collected.
 - In the presence of BVM, V362L, L363F and L363M mutant RTVs had FC values of 28, 12, and 20, respectively.
 - All of the mutants retained sensitivity to PR inhibitor, with FC values of 0.24-1.4.
 - In presence of intermediate concentrations of BVM the H358Y mutant RTV was stimulated by up to 3-4 fold.
 - Using an RT and PR resistant RTV, a hypersusceptibility to BVM was noted (FC 0.17).
 - In absence of drugs all virions contained p24 except mutation V362L which expressed p25 and p25 due to impaired CA / SPI cleavage.

Key points about Abstract 880

- The single-cycle, luciferase-based PhenoSenseHIV assay can measure HIV susceptibility to BVM, a maturation inhibitor.

Abstract 854

Prevalence, mutational patterns, and phenotypic correlates of the L76V protease mutation in relation to LPV/r therapy

Norton M¹, Young T¹, Parkin N², Tokimoto D¹, Liangiun L¹, Matias TP¹, Stawiski E², Stewart K¹, Kempf D¹, and Rahim S¹

¹Abbott, Abbott Park, Illinois, USA, ² Monogram Biosciences, South San Francisco, CA

Background: Recently the L76V mutation in HIV-1 protease has been reported in patients with detectable viral load while receiving lopinavir/ritonavir. L76V has also been identified as an important mutation contributing to darunavir + ritonavir resistance.

Methods: A database containing protease inhibitor susceptibility and sequence information (Monogram Biosciences) was queried to identify isolates containing at least 1 PI resistance associated mutation (PRAM, defined as L23I, L24I, D30N, V32A/I, M46I/L/V, I47A/V, G48A/M/V, I50L/V, I54A/L/M/S/T/V, L76V, V82A/C/F/G/L/M/S/T, I84A/C/V, N88S/T, L90M). The frequency of L76V, and its effect on susceptibility to currently marketed protease inhibitors either alone or in combination with other PI RAMs, was determined. Mixtures were included in frequency estimates but excluded for phenotypic profiling.

Results: Out of 30,276 clinical sequences with at least one PRAM. 948 (3.1%) contained L76V. Only 11 samples (0.03%) existed with L76V as the sole PI RAM. The majority (757/948, 80%) of L76V-containing sequences also had ≥ 3 PI PRAMS. The most common partner mutations in descending order of prevalence occurred at positions M46, I54, V82, and I84. Among isolates which also had phenotypic data available, the presence of L76V further decreased the susceptibility to LPV, DRV, APV and IDV by 2- to 6-fold compared to samples with similar PI RAMs but without L76V. In contrast, susceptibility to ATV, SQV, TPV, and NFV was either similar or increased in the presence of L76V.

Conclusion: Phenotypic data indicates that the L76V mutation, in combination with other PRAMS leads to a decrease in susceptibility to LPV, DRV, IDV, and APV in the context of multiple PI resistant backgrounds. L76V mutation should be considered an important mutation contributing to resistance to these PIs.

Comments about Abstract 854

- Recent reports have indicated the presence of a L76V mutation in HIV-1 protease in patients with detectable viral load while receiving Loppanivir/ritonavir. L76V has also been identified as an important mutation contributing to darunivir and lopanivir resistance.
- The study aimed to investigate the frequency of L76V and its effect on susceptibility on currently marketed protease inhibitors by using a large database of paired large paired genotype phenotype database.
- **Methods**
 - The Monogram Biosciences database which contains protease inhibitor susceptibility and sequence information was utilized.
 - The database was queried to identify isolates containing at least 1 PI resistance associated mutation (PRAM, defined as L23I, L24I, D30N, V32A/I, M46I/L/V, I47A/V, G48A/M/V, I50L/V, I54A/L/M/S/T/V, L76V, V82A/C/F/G/L/M/S/T, I84A/C/V, N88S/T, L90M).
 - The frequency of L76V, and its effect on susceptibility to currently marketed protease inhibitors either alone or in combination with other PI RAMs, was determined.
 - Mixtures were included in frequency estimates but excluded for phenotypic profiling.
 - Site-directed mutants with or without L76V were tested for PI susceptibility and replication capacity (Abbott)
- **Results**
 - In 30,276 clinical sequences with at least 1 PRAM, 948 (3.1%) contained L76V.
 - 11 samples (0.03%) existed as L76V.
 - 757/948, (80%) of L76V-containing sequences also had ≥ 3 PI PRAMS.
 - The most common partner mutations in descending order of prevalence occurred at positions M46, I54, V82, and I84.
 - With isolates with phenotypic data, the presence of L76V further decreased the susceptibility to LPV, DRV, APV and IDV by 2- to 6-fold compared to samples with similar PI RAMs but without L76V.
 - The susceptibility to ATV, SQV, TPV, and NFV was either similar or increased in the presence of L76V.

Key points about Abstract 854

- The L76V mutation, in combination with other PRAMs leads to a decrease in susceptibility to LPV, DRV, IDV, and APV in the context of multiple PI resistant backgrounds. L76V.

Abstract 620

Association of HIV-1 Infectivity on CCR5-bearing Cells with Survival in Vertically-Infected Ugandan Infants

Jessica D. Church¹, Wei Huang², Anthony Mwatha³, Jonathan Toma², Deborah Donnell³, Laura A. Guay¹, Francis Mmiro⁴, Philippa Musoke⁵, J. Brooks Jackson¹, and Susan H. Eshleman¹

¹ Johns Hopkins Univ., Baltimore, MD, USA, ² Monogram Biosciences, South San Francisco, CA, USA, ³ Statistical Center for HIV/AIDS Research & Prevention, Fred Hutchinson Cancer Res. Ctr., Seattle, WA, ⁴ MUJHU Research Collaboration, Kampala, Uganda, ⁵ Makerere University, Kampala, Uganda

Background: HIV-1 uses CD4 and a co-receptor to infect cells (CXCR4=X4-tropic, CCR5=R5-tropic, or both=dual/mixed or DM-tropic). In HIV-infected adults, co-receptor usage is related to disease progression. However, this may not be the case in HIV-infected infants. We previously analyzed tropism in HIV-infected infants, and found no association between the presence of X4-tropic virus and decreased infant survival. In this study, we analyzed HIV infectivity on CCR5-bearing cells and its relationship to infant survival.

Methods: Plasma was collected from infants in the nevirapine arm of the HIVNET 012 trial at 6-14 weeks of age. Samples were analyzed using a commercial co-receptor tropism assay (Trofile, Monogram Biosciences). The level of infectivity on CCR5-bearing cells was measured in relative light units (R5-RLU). HIV *env* subtype was determined by phylogenetic methods.

Results: Samples from 57 (75%) of 75 infants were successfully analyzed using the Trofile assay: (1 X4-tropic, 52 R5-tropic, 4 DM-tropic). Among the 56 infants with R5-using virus in this study, the median R5-RLU was 159,291. We used this median value to define two groups of infants: those with high R5-RLU (R5-RLU \geq median) and those with low R5-RLU (R5-RLU $<$ median). Among 28 infants with viral load results, the median \log_{10} HIV viral load at 6-8 weeks of age was similar for infants in these two groups. HIV *env* subtypes in the 56 infants were: 31 A, 19 D, 2 C, and 4 recombinant. High level infectivity in CCR5-bearing cells was more common in infants with subtype D than subtype A HIV (15/19=79% vs. 9/31=29%, $p=0.0006$). Twenty-four (85.7%) of 28 infants with high R5-RLU died by 5 years of age, vs. 10 (35.7%) of 28 infants with low R5-RLU ($p=0.001$). In univariate analyses, both high R5-RLU and high baseline maternal HIV viral load were associated with decreased infant survival, but maternal baseline CD4 cell count, infant HIV status at birth, and infant HIV subtype were not. High R5-RLU was still associated with decreased infant survival in a Cox proportional hazards model, after adjusting for maternal HIV viral load.

Conclusions: High-level infectivity of HIV in CCR5-bearing cells was associated with subtype D infection and with decreased infant survival. More studies are needed to evaluate the impact of HIV tropism and infectivity on morbidity and mortality in HIV-infected infants.

Comments about Abstract 620

- In adult HIV infected individuals, HIV's use of co-receptors CXCR4, and CCR5 has been linked disease progression.
- Previous data published by this group indicates that the effects seen in adults may not be reflected in HIV infected children (no association was found between the presence of X4-tropic virus and decreased infant survival).
- This study investigated HIV infectivity on CCR5-bearing cells and its relationship to infant survival.
- **Methods**
 - Plasma samples were collected from infants in the nevirapine arm of HIVNET 012 trial at 6-14 weeks of age.
 - The samples were analyzed using the commercial co-receptor tropism assay (Trofile™). HIV infectivity on CCR5 expressing cells was measured in relative light units (R5-RLU).
 - HIV *env* subtype was determined by phylogenetic methods.
- **Results**
 - 57 of 75 children were successfully tested using the Trofile assay:
 - 1 X4-tropic.
 - 52 R5-tropic.
 - 4 DM-tropic.
 - Among the 56 children with R5 using virus the median R5-RLU was 159,291.
 - Two patient populations were defined, 1 population with high R5-RLU (R5-RLU \geq median) and individuals with low R5-RLU (R5-RLU $<$ median).
 - 28 infants had known viral loads (VL) at week 6-8. No difference in VL was detectable between the patient populations.
 - HIV *env* subtypes in the 56 patients were: 31 A, 19 D, 2 C, and 4 recombinant.
 - High R5 infectivity was more often seen in subtype D than subtype A HIV (15/19=79% vs. 9/31=29%, $p=0.0006$).
 - 24 out of 28 (87.5%) infants in the high R5 RLU group died by 5 years.
 - 10 out of 28 (37.5%) infants in the low R5 RLU group died by year 5.
 - The 5 year mortality between the populations was significantly different $p = 0.001$.
 - In univariate analysis, both high R5-LU and high maternal baseline viral load were associated with increased infant mortality. Maternal baseline CD4 count, infant HIV status at birth and infant HIV subtype were not associated.
 - In a Cox proportional Hazards model, High R5-RLU was still associated with decreased infant survival after adjusting for maternal HIV viral load.

Key points about Abstract 620

- High levels of HIV infectivity in CCR5 expressing cells was associated with subtype D and decreased infant survival.

Abstract 585

Examination of HIV-1 Subtype and Response to ART in Ugandan Children

Addy Kekitiinwa¹, P Kasirye¹, D Friedman², E Coakley³, Y Lie³, and F Graziano²

1Mulago Hosp, Kampala Uganda; 2Univ of Wisconsin Hosp and Clin, Madison, US; and 3Monogram Biosci, South San Francisco, CA, US

Introduction: ARVs were developed by primarily studying the response of subtype B virus to the drugs. This HIV subtype predominates in North America, Western Europe and the rest of the industrialized world. However, worldwide, subtype B makes up a much smaller number of the HIV infection. In Uganda, HIV-1 subtypes A and D predominate. Although there is evidence that the ARV's developed against subtype B virus have activity against non-subtype B virus, as the demand grows for ARV's in resource-poor settings, it is uncertain whether viruses of non-B subtypes or isolated from a particular region will respond robustly to the ARV's available in this region of the world. With the advent of PEPFAR (Presidents Emergency Plan for AIDS Relief) in Uganda, more Ugandans receive ARV therapy for HIV-1 infection. With this in mind, the objective of this study is to examine the relationship of HIV-1 subtype to success or failure of ARV and the resistance patterns and adverse events in Uganda treaters with ARV's.

Methods: A retrospective evaluation was performed with 50 PIDC children who attend the Pediatric Infectious Disease Clinic (PIDC, Mulago Hospital, Kampala) and failed ARV therapy. The presenting clinical symptoms of children at ARV failure were recorded and blood samples stored at -70°C. Blood samples were evaluated for viral load, CD4 count, subtype, genetic resistant pattern, phenotype, replication capacity, and viral tropism (Monogram).

Results: 41% of the children had subtype A virus, 36% had subtype D virus and 6% had subtype C virus. There were also recombinant subtype A/D, A/C and C/D (1 of each). In general, the virus replicative capacity (RC) was greater for subtype D virus (>90). Genotype at failure was represented by K103N, G190A, Y188L, V106V/I for NNRTIs, M184V for Lamivudine, and all AZT/d4T major mutations but that at position 41. Cellular tropism assay revealed dual mixed, R5 and X4 representation.

Conclusions: The relationship between subtype and resistance/RC and viral tropism and those treated with ARVs will be important to further analyze treatment of children for HIV disease.

Comments about Abstract 585

- Antiretrovirals (ARVs) were developed by studying the response of subtype B HIV virus to the drug.
- While this subtype is prevalent in most industrial nations, it makes up only a proportion of total HIV infections.
- While evidence exists that ARVs developed against subtype B show activity against non B subtypes, its robustness of drug effect in these non B subtypes is unclear.
- In Uganda HIV-1 subtypes A and D predominate.
- This study aimed to examine the relationship of HIV-1 subtype to success or failure of ARV.
- The study also examined the resistance patterns and adverse events in patients on ARVs.

- **Methods**
 - This retrospective analysis identified 50 children who had failed ARV.
 - The presenting clinical symptoms of the children at ARV failure were recorded. Blood was drawn and stored at -70°C.
 - The blood samples were evaluated for viral load, CD4 count, subtype, genetic resistant pattern, phenotype, replication capacity and viral tropism (Monogram Biosciences).

- **Results**
 - 41% of children had subtype A virus..
 - 36% of children had subtype D virus..
 - 6% of children had subtype C..
 - Recombinant subtypes A/D, A/C and C/D were also noted (1 child each).
 - Higher replicative capacity was generally seen in subtype D (>90).
 - NNRTI mutations included K103N, G190A, Y188L, V106V/I..
 - The M184V mutation was seen with Lamivudine exposure.
 - All AZT / d4T major mutations except for position 41 were present.
 - R5, X4 and dual / mixed tropic viruses were identified in the study population.

Key points about Abstract 585

- The relationship between subtype and successful or failing ARV therapy in treated children is important and should be further studied.

Abstract 635b

Analysis of NVP resistance in Ugandan infants who were HIV-infected despite receiving single dose (SD) nevirapine (NVP) vs. SD NVP plus up to 6-weeks of daily NVP to prevent HIV vertical transmission

Jessica D. Church¹, Saad B. Omer², Laura A. Guay¹, Wei Huang³, Philippa Musoke⁴, Francis Mmiro⁵, J. Brooks Jackson¹, Susan H. Eshleman¹

¹Johns Hopkins Univ. School of Medicine, Baltimore, MD, USA ²Johns Hopkins Univ. School of Public Health, Baltimore, MD, USA, ³Monogram Biosciences, South San Francisco, CA USA, ⁴Makerere University, Kampala, Uganda⁵ MUJHU Research Collaboration, Kampala, Uganda

Background: The SWEN Study in Uganda, Ethiopia, and India compared a regimen of SD NVP (the HIVNET 012 regimen) to a regimen of SD NVP plus up to 6 weeks of daily NVP to the infant for prevention of HIV transmission in breastfeeding infants. We analyzed HIV genotypic resistance, phenotypic resistance, and replication capacity (RC) in Ugandan infants in this study.

Methods: Plasma samples were available from 49 (71%) of 69 infants diagnosed with HIV infection by 6 weeks of age (24 in the SD NVP arm; 25 in the extended NVP arm). In the extended NVP arm, the median number of NVP doses received was 14 (range 3-33) in infants diagnosed with HIV infection at birth (n=17), 14 (range 7-26) in infants diagnosed at 2 weeks of age (n=5), and 34 (range 21-35) in infants diagnosed with HIV infection by 6 weeks of age (n=3). Samples were analyzed using the ViroSeq system, the PhenoSense GT assay (Monogram Biosciences), and quantitative point mutation assay (LigAmp, for K103N, Y181C, and G190A). If NVP resistance was detected at 6 weeks, a 6-month sample was tested.

Results: Maternal CD4 cell count, infant viral load, and HIV subtypes were similar in the two study arms. Using ViroSeq, NVP resistance was detected at 6 weeks in a higher portion of infants in the extended NVP arm compared to the SD NVP arm (21/25=84% vs. 12/24=50%, p=0.01). A higher portion of infants in the extended NVP arm also had at least one NVP resistance mutation detected using the LigAmp assay (19/25=79% vs. 7/24=35%, p=0.004). In the extended NVP arm, detection of NVP resistance was not associated with the number of NVP doses received or the HIV status at birth. Among infants with resistance at 6 weeks, only one of six infants in the SD NVP arm had NVP resistance detected by ViroSeq at 6 months. In contrast, all seven infants in the extended NVP arm still had NVP resistance detected. Phenotypic resistance results were available for 42 (85.7%) of 49 infants analyzed at 6 weeks. The portion of infants with phenotypic resistance was higher in the extended NVP arm than in the SD NVP arm (19/22=86.3% vs. 9/20=45%, p=0.005). Phenotypic results were consistent with genotypic resistance results. The median HIV RC was similar among infants in the two study arms, and among infants with and without NVP resistance.

Conclusions: HIV-infected Ugandan infants who received extended NVP prophylaxis were more likely to have genotypic and phenotypic NVP resistance than those who received only SD NVP.

Comments about Abstract 635b

- Comparisons of a regimen of SD NVP (the HIVNET 012 regimen) to a regimen of SD nevirapine (NVP) plus up to 6 weeks of daily NVP to the infant for prevention of HIV transmission in breastfeeding infants was attempted.
- This study was carried out in newly borne Ugandan infants (approx 6 weeks).
- HIV genotypic resistance phenotypic resistance, and replication capacity (RC) was analyzed in this patient population to compare between the nevirapine regimens.
- **Methods**
 - Plasma samples were obtained from 49 infants diagnosed with HIV within 6 weeks of birth (24 in the SD NVP arm; 25 in the extended NVP arm).
 - Samples were analyzed using the ViroSeq system, the PhenoSense GT assay (Monogram Biosciences), and quantitative point mutation assay (LigAmp, for K103N), Y181C, and G190A). If NVP resistance was detected at 6 weeks, a 6-month sample was tested.
- **Results**
 - In the NVP extended arm:
 - The median number of NVP doses received was 14 (range 3-33) in infants diagnosed with HIV infection at birth (n=17), 14 (range 7-26) in infants diagnosed at 2 weeks of age (n=5), and 34 (range 21-35) in infants diagnosed with HIV infection by 6 weeks of age (n=3).
 - Maternal CD4 cell count, infant viral load, and HIV subtypes were similar in the two study arms.
 - NVP resistance was detected at 6 weeks in a higher portion of infants in the extended NVP arm compared to the SD NVP arm (21/25=84% vs. 12/24=50%, p=0.01. This group also had at least 1 VDV mutation to VDV as compared to the SD VCV group assay (19/25=79% vs. 7/24=35%, p=0.004).
 - NVP resistance was not associated with the number of NVP doses received or the HIV status at birth.
 - Genotypic NVP resistance results:
 - 1 of 6 of the SD NVP had evidence of NVP resistance by ViroSeq at 6 months.
 - 7 of 7 infants of the daily NVP had evidence of NVP resistance by ViroSeq at 6 months.
 - Phenotypic NVP resistance results:
 - The proportion of phenotypic resistance (Monogram Biosciences) was higher in the extended NVP versus SD NVP (19/22=86.3% vs. 9/20=45%, p=0.005).

Key points about Abstract 635h

- HIV-infected Ugandan infants who received extended NVP prophylaxis were more likely to have genotypic and phenotypic NVP resistance than those who received only SD NVP.

Abstract 682

Heterosexually transmitted viruses in Uganda possess signature envelope genotypes

Manish Sagar¹, Oliver Laeyendecker^{2,3}, Jordyn Gamiel³, Maria Wawer⁴, Ronald Gray⁴, David Serwadda⁵, Nelson K. Sewankambo⁵, Jonathan Toma⁶, Wei Huang⁶, Thomas Quinn^{2,3}

¹Brigham and Women's Hospital, Cambridge, MA, USA, ²Laboratory of Immunoregulation, NIAID, NIH, Baltimore, MD, USA, ³Johns Hopkins University School of Medicine, Baltimore, MD, USA, ⁴Johns Hopkins University School of Medicine, Baltimore, MD, USA, ⁵Makerere University, Kampala, Uganda, ⁶Monogram Inc., South San Francisco, CA, USA
Group: Rakai Health Sciences Program.

Background: Previous studies suggest that heterosexually transmitted subtype A and subtype C HIVs, but not non-heterosexually transmitted subtype B HIVs, possess signature genotypes of compact and less glycosylated envelope variable loops. To understand whether other heterosexually transmitted HIVs also display similar or other signature sequence characteristics, we examined envelope sequences from 13 donor-recipient pairs in Uganda.

Methods: From a trial of STD treatment to prevent HIV-1 acquisition in the Rakai district of Uganda, newly infected subjects with documented HIV-1 seroconversion over prospective follow up and their epidemiologically identified monogamous heterosexual partner were examined in this study. A minimum of 8 full-length envelope sequences were isolated and examined from the earliest available plasma sample after HIV-1 acquisition from the newly infected subject and the corresponding collection day matching plasma sample from the transmitting partner.

Results: Samples from the newly infected partner were collected a median of 189 days after estimated HIV-1 seroconversion (range 142 – 359 days). Each recipient's sequences clustered with the corresponding donor's sequences in neighbor joining phylogenetic analysis which confirmed the epidemiological linkage. Ten of the 13 couples were infected with subtype D HIV-1 and the remaining 3 pairs had subtype A HIV-1. Sequences in the recipients showed lower diversity ($p < 0.001$) and divergence ($p < 0.001$) compared to the donor sequences. Recipient envelope V1-V2 ($p = 0.002$) and V1-V4 ($p < 0.001$) sequences were significantly shorter compared to donor sequences (Wilcoxon rank-sum test stratified by couple). There was no significant difference in the number of potential N-linked glycosylation sites (PNGS) between recipient and donor sequences within the V1-V2 ($p = 0.91$) or V1-V4 ($p = 0.40$) segments. Within each pair, signature amino acids differentiated the donor and recipient sequences although these differences were not consistent among all couples.

Conclusions: Our results suggest that a small subset of HIVs closely related to ancestral viruses are selected during heterosexual transmission of predominantly subtype D HIV-1 in Uganda. Similar to heterosexually transmitted subtype A and subtype C HIVs, viruses in newly infected Ugandan subjects also have shorter envelope variable loops but they do not have a significantly lower number of PNGS.

Comments about Abstract 682

- Studies suggest that heterosexually transmitted subtype A and subtype C HIVs, but not non-heterosexually transmitted subtype B HIVs, possess signature genotypes of compact and less glycosylated envelope variable loops.
- This study investigated whether other heterosexually transmitted HIVs also display similar or other signature sequence characteristics.
- Envelope sequences from 13 donor-recipient pairs in Uganda were examined.

- **Methods**
 - Newly infected subjects with documented HIV-1 seroconversion over prospective follow up and their epidemiologically identified monogamous heterosexual partner were identified.
 - A minimum of 8 full-length envelope sequences were isolated and examined from the earliest available plasma sample after HIV-1 acquisition from the newly infected subject.
 - A corresponding (same collection day) matching plasma sample from the transmitting partner was also collected.

- **Results**
 - Samples from the newly infected partner were collected a median of 189 days after estimated HIV-1 seroconversion (range 142 – 359 days).
 - Each recipient's sequences clustered with the corresponding donor's sequences in neighbor joining phylogenetic analysis (indicating epidemiological linkage).
 - 10 of 13 couples were infected with subtype D HIV-1. 3 couples were infected with subtype A HIV-1.
 - The envelope sequences in the recipients showed lower diversity ($p < 0.001$) and divergence ($p < 0.001$) compared to the donor envelope sequences.
 - Recipient envelope V1-V2 ($p = 0.002$) and V1-V4 ($p < 0.001$) sequences were significantly shorter compared to donor sequences (Wilcoxon rank-sum test stratified by couple).
 - No significant difference in the number of potential N-linked glycosylation sites (PNGS) between recipient and donor sequences within the V1-V2 ($p = 0.91$) or V1-V4 ($p = 0.40$) segments.
 - Differentiating amino acids were seen between recipient and donor envelope sequences but this was not seen consistently between couples.

Key points about Abstract 682

- The study suggests that a small subset of HIVs closely related to ancestral viruses are selected during heterosexual transmission of predominantly subtype D HIV-1 in Uganda.
- Heterosexually transmitted subtype A and subtype C HIVs, viruses in newly infected Ugandan subjects also have shorter envelope variable loops but they do not have a significantly lower number of PNGS.