CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-128

MICROBIOLOGY REVIEW

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 22-128 **SN:** 000 **DATE REVIEWED:** 6/01/07
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

NDA#: 22128 **Serial #:** 000
Reviewer's Name(s): Lisa K. Naeger, Ph.D.

Sponsor's Name and Address:
Pfizer, Inc.
50 Pequot Avenue
New London, CT 06320

Initial Submission Dates:
Correspondence Date: December 19, 2006
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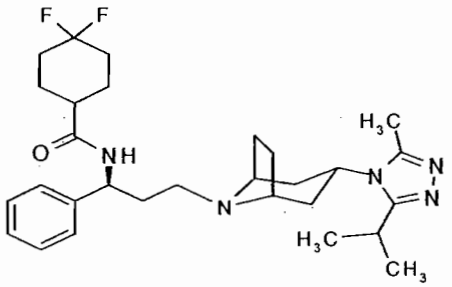
Amendments:
Related/Supporting Documents:
ViroLogic (now Monogram, Inc.) Drug Master File and submitted to the Division on January 18, 2005

Product Name(s)
Proprietary: Selzentry
Non-Proprietary/USAN: Maraviroc,
Code Name/Number: UK-427,857

Chemical Name: 4,4-difluoro-*N*-{(1*S*)-3-[*exo*-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl}cyclohexanecarboxamide
Molecular Formula: $C_{29}H_{41}F_2N_5O$
Structural Formula:



Dosage Form(s): 150 or 300 mg film-coated tablets
Route(s) of Administration: Oral
Indication(s): The treatment of HIV-1 in combination with other antiretroviral agents; for treatment-experienced adult patients infected with CCR5-tropic HIV-1 who have HIV-1 strains resistant to multiple antiretroviral agents.

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Dispensed: Rx X **OTC**_____

Abbreviations:
CCR5, chemokine receptor CCR5; CXCR4, chemokine receptor CXCR4; EC_{50}, effective concentration at 50%; ENF, enfuvirtide; GSS, genotypic sensitivity score; MPI, maximum percentage inhibition; IC_{50}, inhibitory concentration at 50%; MVC, maraviroc; NNRTI, non-nucleoside analogue reverse transcriptase inhibitor; NRTI, nucleoside analogue reverse transcriptase inhibitor; NR/NP, no replication or non-productive virus; OBT, optimized background therapy; OSS, overall sensitivity score; PBL, peripheral blood lymphocytes; PDTF, protocol-defined treatment failure; PI, protease inhibitor; PR, protease; PSS, phenotypic sensitivity score; RT, reverse transcriptase;

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Executive Summary

Mechanism of Action
Maraviroc (MVC) is a unique anti-HIV drug because it targets a host protein rather than a viral target. MVC binds to the cell membrane chemokine receptor CCR5 and blocks the interaction of CCR5-tropic HIV-1 gp120 with the CCR5 co-receptor, an essential step in the viral entry process. MVC inhibits the binding of CD4-complexed, recombinant soluble gp120 to CCR5 with an IC_{50} value of 11 nM. Site-directed mutagenesis and computer modeling studies located the likely binding site of MVC to a pocket within the transmembrane region of CCR5. As a consequence of this binding, MVC is thought to alter the three dimensional structure of CCR5 such that the viral envelope glycoprotein, gp120, is unable to recognize and bind to the co-receptor.

In vitro pharmacology studies showed that MVC is a selective antagonist of binding and intracellular signaling by the endogenous chemokines MIP-1α, MIP-1β and RANTES. MVC prevents agonist-induced intracellular calcium release from cells expressing the recombinant human CCR5 receptor indicating that it is a functional antagonist of the human CCR5 receptor. It did not act as an agonist of the human CCR5 receptor as it did not trigger release of intracellular calcium at concentrations up to 10 μM. CCR5 ligand-induced calcium flux inhibition by MVC is specific to CCR5 because there was no muscarinic (non-selective) signaling by carbochol at 1 μM on HEK-293 cells.

Anti-HIV Activity in Cell Culture
MVC showed inhibitory activity against HIV-1 grown in PM-1 cells with an EC_{50} value of 0.21 nM and similar activity was seen against HIV-1 in pooled PBLs from multiple donors with an EC_{50} value of 0.54 nM and for HIV-1 in individual donor PBLs (mean EC_{50} value = 0.28 nM, n=10). The activity of MVC was measured against a range of CCR5-tropic clinical primary isolates from various clades A to O and the mean EC_{50} value for all clades was 0.51 nM (range 0.40 – 1.11).

Clinical Studies A4001027 and A4001028
Baseline Resistance Assessment
Studies 1027 and 1028 were randomized (2:2:1), double-blind and placebo-controlled, designed to compare the safety and antiviral activity of maraviroc at two different doses versus placebo, each in combination with optimized background therapy (OBT). Investigators were to optimize therapy, with 3-6 (excluding low-dose ritonavir) branded, open-label agents, on the basis of resistance testing, treatment history and safety/adverse event considerations.

Susceptibility testing was used to calculate the genotypic susceptibility score (GSS) and phenotypic susceptibility score (PSS). The GSS is the sum of the number of active drugs in OBT based on PR, RT and gp41 amino acid sequences. The PSS is the sum of the number of active drugs in the OBT based on drug susceptibility testing in cell culture

using the Monogram Biosciences PhenoSense GT assay. A higher GSS or PSS score signifies that there are more active drugs in the OBT. The distribution of genotypic sensitivity scores (GSS) and phenotypic sensitivity scores (PSS) was balanced across the three treatment groups in studies 1027 and 1028 with median GSS=1, PSS=2 and overall susceptibility score (OSS) =2 for each treatment group in both studies. Combining both studies, 67% of subjects had overall susceptibility scores $\leq$2. Thirty percent had one potentially active drug in their OBT and fourteen percent had no potentially active drug in their OBT. These virologic assessments are consistent with a heavily treatment-experienced population.

At baseline in studies A4001027 and A4001028, 90% (940/1049) of the subjects had CCR5-tropic virus with 4% having dual/mixed tropic virus and 5% have non-phenotypable virus. It is noteworthy that subjects were screened as having CCR5-tropic virus as entry criteria into the trials. Therefore, about 10% of viruses changed from CCR5-tropic to dual/mixed or non-phenotypable in the time period between screening and baseline. Throughout these trials, it appears that the percentage of isolates which are non-phenotypable using the Monogram assay ranges from 5 to 15%.

Tropism and Resistance
A potential concern with using a CCR5 co-receptor antagonist is that it will increase the chances of HIV-1 switching to the use of the CXCR4 co-receptor through mutation and selection. The evolution to a CXCR4-utilizing HIV-1 has been proposed to result in a more virulent virus and more rapid progression to AIDS. The potential for co-receptor switching as a mechanism of escape from CCR5 co-receptor antagonists concerned the Division and thus we requested sponsors of CCR5 inhibitors to vigilantly monitor co-receptor tropism changes and resistance in clinical trials. We requested tropism reports on a monthly basis to monitor tropism switching, viral load and $CD4^+$ cell counts in all clinical trials. In addition, a comprehensive analysis of subjects who experienced treatment failure in clinical studies was requested.

Reasons for Failure on MVC could include:

- Co-receptor switch from CCR5-tropic to CXCR4-tropic virus by mutation in the virus and outgrowth in the presence of maraviroc
- Outgrowth of CXCR4-tropic viruses present at baseline but not detected with the standard tropism assay (one of the limitations of the assay is that it is not able to detect with 100% sensitivity CXCR4 when present below 10% in a viral mixture)
- Outgrowth of CCR5-tropic viruses that are resistant to MVC
- Resistance to the OBT
- Host CCR5 genotype – although not known at this point, it might be possible that MVC may not bind efficiently to some host CCR5 receptor polymorphisms or CCR5 expression may vary in different individuals

An analysis of the percentage of virologic failures by tropism at time of failure found that ~50-60% of subjects failed with CXCR4- or dual/mixed-tropic virus in the MVC arms,

whereas <20% of the subjects in the placebo arm failed with CXCR4- or dual/mixed-tropic virus. A high percentage of treatment failure on MVC appears to be driven by tropism change from CCR5-tropic to CXCR4- or dual/mixed-tropic virus. These results support the mechanism of action of MVC and suggest emergence of CXCR4-tropic virus is a prominent reason for failure on MVC.

A comprehensive analysis was requested for subjects who had experienced treatment failure and/or had changes in their co-receptor tropism. However, given the complexity and exploratory nature of the tropism and resistance analyses, sub-studies from Clinical Studies A4001027 and A4001028 were proposed by the applicant. A subset of 20 subjects (16 MVC; 4 placebo) failing with CXCR4-tropic virus were analyzed to ascertain whether the CXCR4-tropic virus emerged from undetected CXCR4-tropic virus at screening or as a result of mutations in a CCR5 tropic virus causing a tropism switch while on MVC. The evaluation included clonal evaluation of virus at baseline and on-treatment samples to determine the relative number of CXCR4-tropic and CCR5-tropic viral isolates, nucleotide sequence analysis of the gp120 region to identify amino acid changes that may contribute to a co-receptor switch, phylogenetic analysis to determine the relationship of emerging CXCR4-tropic virus to the CCR5-tropic virus at baseline and nucleotide sequence analysis of PR and RT to assess resistance to the OBT.

In 14 subjects, CXCR4-tropic clones in the 'on-treatment' samples shared a common ancestor with a pre-treatment CXCR4-tropic virus indicating outgrowth of CXCR4-tropic virus. In the remaining 6 subjects, CXCR4-using *env* clones identified in the 'on-treatment' samples did not have a pretreatment CXCR4 ancestor but they were also genetically distinct from both the 'pre-treatment' and 'on-treatment' CCR5 population. The V3 loop sequences of the these on-treatment CXCR4-tropic clones differed by 7-17 amino acid residues from the V3 loop of the nearest CCR5 sequence on the phylogenetic tree. A tropism switch whereby CXCR4-using virus emerged on MVC resulting from these 7-17 amino acid substitutions in the CCR5 precursor cannot be ruled out from the review of the data submitted. However, from the amino acid sequence differences and phylogenetic tree data, the most likely explanation is the CXCR4-using clones in these 6 subjects emerged from a pre-existing CXCR4-using virus not detected by the tropism assay at baseline.

A subset of 38 subjects (13 MVC; 25 placebo) failing with CCR5-tropic virus were analyzed to identify possible phenotypic and genotypic markers associated with MVC resistance in vivo including determining MVC susceptibility in cell culture, nucleotide sequence analysis of gp120 to identify amino acid substitutions that might contribute to MVC resistance, and nucleotide sequence analysis of PR and RT to identify resistance to drugs in the OBT.

Viruses from 5 subjects failing MVC treatment with CCR5 tropic virus showed evidence of a lower plateau in maximum percentage inhibition rather than fold changes in EC_{50} values. These results support previous findings from selection of MVC-resistant virus in

cell culture and isolates from Phase 2 MVC studies that subjects failing a MVC-containing regimen had lower plateaus in the maximum percentage inhibition (MPI) rather than changes in EC_{50} values. All 5 subjects had amino acid changes in the V3 loop of gp120 which were present at failure timepoints but were not present at baseline. All the failure clones had multiple different V3 loop amino acid changes which reflected the heterogeneity associated with the V3 region of gp120. However, changes at either amino position 13 or 26 (gp120 amino acid positions 303 and 328 (HXB2 numbering)) were seen in the V3 loop of gp120 in all five of the subjects with MVC-associated lower plateaus in maximal percentage inhibition. The role of the V3 loop amino acid substitutions in MVC resistance was confirmed by site-directed mutagenesis. These 5 subjects also had lower MVC concentrations with C_{min} values <75 ng/mL.

Not all subjects failing MVC treatment with CCR5-tropic virus had phenotypic markers of MVC resistance. Seven of the subjects receiving MVC did not show phenotypic markers of MVC resistance in the Monogram assay. However, the majority of these subjects (5/7) had evidence of reduced susceptibility to one or more drugs within their OBT at screening and/or at failure. The CCR5 receptor genotype should be examined from these subjects to assess the contribution of CCR5 genotype to MVC treatment failure.

In summary, the data from the virology sub-studies suggest that the most prominent reason for failure in these studies was outgrowth of CXCR4-using viruses not detected at screening. Treatment failure on MVC with CCR5-tropic virus also occurred and resulted from phenotypic and genotypic resistance to MVC and resistance to OBT. It remains to be determined if host CCR5 genotype also plays a role in MVC treatment failure.

Follow-Up on Treatment Failures with CXCR4-Tropic Virus

An examination of $CD4^+$ cell counts by tropism at treatment failure showed that the mean and median change in $CD4^+$ cell counts from baseline using last observation carried forward was lower in subjects with CXCR4 and dual/mixed tropic virus than those with CCR5-tropic virus. Long term follow-up including viral loads, $CD4^+$ cell counts, HIV-1 co-receptor usage and AIDS defining events was requested for the subjects who failed with CXCR4-tropic virus in studies 1027 and 1028. Of the 28 subjects who failed with CXCR4 virus and were followed by the sponsor, 20 had at least one follow-up visit. At follow-up, 35% of these subjects still had CXCR4-tropic virus but the remaining had changed tropism back to CCR5 or dual/mixed. For the subjects with CCR5- or dual/mixed-tropic virus at the end of follow-up, the median time to last follow-up was approximately 5 months (range 18 days to 8 months). In contrast, the follow-up time for the subjects who remained CXCR4-tropic at the last follow-up visit was one month or less (median time was approximately 11 days). This difference suggests that over a longer period of follow-up, CCR5 viruses may outgrow CXCR4 viruses in these subjects. In half of the subjects, $CD4^+$ cell counts also declined (mean change -21, median change -3) consistent with the ongoing viremia. No new category C AIDS-defining events were reported for any of the 20 subjects. Viral loads remained similar to the value at treatment

failure. If subjects went on a new ARV treatment, viral loads decreased and $CD4^+$ cell increases were seen concomitant with the reduction in viral load.

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1. Recommendations

1.1. Recommendation and Conclusion on Approvability

This NDA is approvable with respect to microbiology for the treatment of HIV-1 in combination with other antiretroviral agents, and is indicated for treatment-experienced adult patients infected with only CCR5-tropic HIV-1 who have HIV-1 strains resistant to multiple antiretroviral agents. Maraviroc should not be used in subjects with dual/mixed tropic or CXCR4 tropic HIV-1.

1.2. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable.

#7. Perform cell culture combination activity or maraviroc with darunavir and tipranavir, and submit complete study report of these assessments by May 2008.

Final report submission: May 2008

[redacted]

In addition to the post-marketing commitment requests, we also sent the following to Pfizer: We are requesting the maraviroc phenotype and genotype for all subjects who fail on maraviroc with CCR5-tropic virus in studies A4001026, A4001027, and A4001028 to be included with the traditional approval application.

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2. Summary of OND Microbiology Assessments

2.1. Non-clinical Microbiology

Maraviroc (MVC) is a member of a therapeutic class called CCR5 co-receptor antagonists. MVC inhibits viral entry by binding to the cell surface chemokine receptor CCR5. Specifically, MVC blocks the interaction of CCR5-tropic HIV-1 gp120 envelope (env) with the CCR5 co-receptor, an essential step in the entry process. CXCR4-tropic and dual-tropic HIV-1 entry is not inhibited by MVC. MVC binds to human CCR5 with a K_D of 0.86 nM and at room temperature has a dissociation half-life of approximately 16 hours. Site-directed mutagenesis and computer modeling studies locate the likely binding site of MVC to a pocket within the transmembrane region of CCR5. As a consequence of this binding, MVC is thought to alter the three dimensional structure of CCR5 such that the viral envelope glycoprotein, gp120, is unable to recognize and bind to the co-receptor. Consistent with this, MVC blocks the soluble form of gp120 binding to CCR5 with an IC_{50} value of 11 nM and inhibits gp120/CCR5-mediated membrane fusion with an IC_{50} value of 0.22 nM.

treatment failure 1) the protocol-defined treatment failure (PDTF) definition and 2) subjects with PDTF plus subjects with >400 copies/mL plasma HIV RNA at Week 24. Regardless of the definition of treatment failure, more subjects (~50-60%) failed with CXCR4- or dual/mixed-tropic virus in the MVC arms, whereas <20% of the subjects in the placebo arm failed with CXCR4-using virus. A high percentage of treatment failure on MVC appears to be driven by tropism change from CCR5-tropic to CXCR4- or dual/mixed-tropic virus. This supports the mechanism of action of MVC and suggests emergence of CXCR4-tropic virus is a prominent reason for failure on MVC. For the subjects who switched from CCR5-tropic to CXCR4- or dual/mixed tropic between Baseline and Week 24, the mean and median time to tropism change for each treatment group were similar with a mean of 47-51 days and median of 28-30 days.

Change in $CD4^+$ Cell Counts Corresponding with Tropism Changes

Overall, there was a greater increase in $CD4^+$ cell count from baseline to Week 24 for both MVC arms (106-109 cells/μL) compared to placebo (57 cells/μL). The change in $CD4^+$ cell counts (from baseline to Week 24 using the last observation carried forward at Week 24 (LOCF24)) was examined in the treatment failures (PDTF+>400 copies/mL serum HIV RNA at Week 24) by tropism at the failure timepoint. Subjects in the MVC arms failing with CXCR4- or dual/mixed tropic virus had less of an increase in $CD4^+$ cell counts than those subjects failing with CCR5-tropic virus. This difference was not seen in the placebo arm.

Failure of Optimized Background Therapy

Another reason for treatment failure could be resistance to the other drugs in the OBT. Most subjects typically had low GSS/PSS scores at screening, indicating reduced susceptibility to their OBT. In Studies 1027 and 1028, the mean GSS and PSS scores (baseline susceptibility to the OBT) in subjects who responded were higher than the scores in subjects who failed therapy. As the number of susceptible drugs in the OBT increased, reflected in an increased OSS, the percent of subjects who achieved <400 copies/mL serum HIV RNA increased in the MVC arms to 70% if subjects had 3 or more susceptible drugs in the OBT.

The susceptibility to drugs in the OBT at baseline and treatment failure was analyzed in detail in the subjects who failed treatment (PDTF + >400 copies/mL Week 24). Twenty-eight percent of treatment failure subjects had no susceptible drugs in the OBT at baseline and 43% of treatment failure subjects lost susceptibility to drugs in their OBT on treatment. There was no difference between the MVC and placebo treatment arms. There were significantly fewer ENF mutations that developed on ENF treatment in the MVC BID arm than in the placebo arm ($p=0.01$) or the MVC QD arm ($p=0.02$). Interestingly, the data suggest that more subjects who failed in the MVC arms with CXCR4-tropic virus developed resistance to ENF and drugs in the OBT. An examination of OSS of the treatment failures by tropism shows that 80% of subjects who failed with CXCR4-tropic virus had OSS of 0-1 compared to only 3% who had 3 or more active

drugs in OBT. Whereas, 50-60% of subjects who failed with CCR5 or dual/mixed tropic virus had OSS of 0-1 compared to less than 20% with susceptibility scores of 3 or more.

Δ32 Deletion/WT or CCR5 Promoter Haplotype

The percentage of subjects heterozygous for the Δ32 genotype (wildtype CCR5/Δ32 deletion) at baseline ranged from 5% to 9% in studies 1027 and 1028 and was comparable between arms. An examination of subjects who failed treatment showed that there was no difference between treatment arms in the percentage of subjects who were heterozygous for the Δ32 Deletion or had CCR5 promoter haplotypes P1, P4 and P1/P4. The majority of treatment failures (>80%) were wildtype CCR5. Approximately 40% of treatment failures had the P1 promoter haplotype and a third had the P1/P4 haplotype.

Virology Sub-studies

Virology sub-studies from Clinical Studies 1027 and 1028 were conducted. Subjects were selected on a blinded basis for more detailed analysis from a pool of 267 subjects from clinical studies A4001027 and A4001028 who had the potential to reach Week 24 by December 1, 2005. Two hundred thirteen of these subjects received MVC and 54 received placebo. Viruses from 20 subjects in whom CXCR4-using virus emerged during the blinded phase of treatment were analyzed. The objective of this study was to understand whether the CXCR4-using virus emerges from a pre-treatment CXCR4-using reservoir or as a result of mutation from a CCR5-tropic progenitor ("tropism switch"). In a second virology sub-study, 38 subjects were identified as failing blinded therapy and having CCR5-tropic virus. Viral isolates from these subjects were examined to identify possible phenotypic and genotypic markers associated with MVC resistance in vivo.

Origin of CXCR4 or Dual-tropic Virus that Emerged on Treatment

Of the 20 subjects selected in whom CXCR4-using virus was detected during treatment, 16 were in a MVC treatment group and 4 in the placebo group. The nucleotide sequence of approximately 290 bases from the envelope gene encompassing the V3 loop was determined from 192 pre-treatment and 48 on-treatment clones from each of the 20 subjects. Phylogenetic trees were generated using these sequences in order to investigate possible ancestry of the CXCR4-using clones. Finally, twelve clones from each timepoint (*i.e.* 24 clones per subject) were selected and tropism was confirmed in the validated format of the PhenoSense™ HIV tropism Assay (Trofile™).

CXCR4-using *env* clones in the 'on-treatment' samples from 14 subjects (11 MVC: 3 PLC) shared a common ancestor with a pre-treatment virus that was phenotypically and/or genotypically classified as CXCR4-using. The applicant states that the CXCR4-using *env* clones identified in the 'on-treatment' samples from the remaining 6 subjects (30%) (5 MVC: 1 PLC) were genetically distinct from both the 'pre-treatment' and 'on-treatment' CCR5 population based on phylogenetic tree analysis. The V3 loop sequences of the on-treatment CXCR4-using clones differed by 7-17 amino acid residues from the V3 loop of the nearest CCR5 sequence on the phylogenetic tree. Although a tropism switch whereby CXCR4-using virus emerged on MVC resulting from these 7-17 amino

acid substitutions in the CCR5 precursor cannot be ruled out from the review of the data submitted for these 6 subjects, the data indicate that the CXCR4-tropic virus originated from a pre-existing CXCR4-using virus not detected by the assay at day 1.
The primary findings from this sub-study were that CXCR4-using virus, detected during blinded treatment of these subjects, originated from a pre-existing CXCR4-using virus reservoir, regardless of treatment arm (MVC or placebo) or time of onset of virology failure. In addition, the baseline samples from subjects whose virus was classified as CCR5-tropic at baseline had a low (≤ 7%) frequency of CXCR4-using *env* clones. These results support the 10% sensitivity performance assessment reported for the Monogram tropism assay.

Subjects Who Failed Treatment with CCR5-Tropic Virus
The susceptibility to MVC and ENF of Env recombinant pseudoviruses was analyzed from 38 subjects who failed blinded therapy with a CCR5-tropic virus; 13 subjects were randomized to receive MVC (6 BID, 7 QD) and 25 subjects were on placebo. The results support previous findings from selection of MVC-resistant virus in cell culture and isolates from Phase 2 MVC studies that lower plateaus in the maximum percentage inhibition (MPI) to MVC were associated with subjects failing a MVC-containing regimen rather than changes in EC_{50} values. MVC dose response curves demonstrating lower plateaus in MPI (<95%) were observed in 4 subjects following failure of a MVC-containing regimen during the blinded study period and from a fifth subject who failed in the placebo arm and developed a lower plateau in MPI following open-label MVC (Table F). Lower plateaus in MPI in the PhenoSense™ HIV Entry assay correlated with data obtained with Env clones and in PBL assays.

The entire Env (gp160) sequence was obtained for all clones and timepoints from 10 subjects in an attempt to determine genotypes associated with decreased MVC susceptibility. Genotypic analysis focused primarily on the V3 region (amino acids 300-350) of gp160 based on characterization of MVC-resistant virus selected in cell culture. Changes in the V3 loop region of the viral Env appeared to correlate with the presence of lower plateaus in MPI and MVC resistance. Although there is heterogeneity of the envelope protein and likely multiple pathways to MVC resistance, substitutions at amino acid positions P13 or V26 occurred in the V3 loop of the five MVC treatment failure isolates that demonstrated lower plateaus in MPI. Additionally, a P13S amino acid substitution was also seen in Subject 10050022 who had a shift in MVC EC_{50} value from baseline. Changes outside the V3 loop were observed in some subject viruses and the impact of these is not understood.

Maraviroc treatment failure was generally not associated with fold changes in EC_{50} values to maraviroc. However, virus from two subjects had approximately a 3-fold shift in MVC susceptibility between baseline and treatment failure. Each of these subject's failure isolates had one V3 loop amino acid change: K18R in Subject 10800003 and P13S in Subject 10050022. All other subjects on maraviroc had EC_{50} FC values <2-fold within the normal range of the Monogram assay (0.32-1.95). In addition, there was no

significant difference between the maraviroc (0.84 -1.0) and placebo (**0.65 – 0.67**) treatment arms for EC_{50} FC values to MVC after treatment failure (p=0.13).
Not all subjects failing treatment on MVC treatment with CCR5-tropic virus had phenotypic markers of MVC. Seven subjects receiving MVC during their blinded phase of treatment did not show phenotypic markers of MVC resistance in the PhenoSense assay. However, the majority of subjects (5/7) had evidence of reduced susceptibility to one or more drugs within their OBT at screening and/or failure.
Site-directed mutagenesis (SDM) was performed on representative Env clones from baseline and failure timepoints from four of the subjects who had MVC resistance-associated plateaus in MPI and confirmed the role of the V3 loop amino acid substitutions in contributing to MVC resistance. For isolates from Subjects 10070008, 10460014, and 10950001, mutating the V3 loop amino acids of the Day 1 clones resulted in a MVC-resistant phenotype (i.e., <95% MPI), and, back-mutation of the amino acid changes in the V3 loop of the failure clones resulted in a MVC-sensitive phenotype (>95% MPI). In the isolates from Subject 10290004, the V3 amino acids (S11G and V26I were necessary, but not sufficient for the MVC-resistant phenotype. Perhaps the other substitutions (T2I, V33A and R48Q) that developed in the V3 loop on MVC treatment and were not examined in this study played a role in MVC resistance.

Enfuvirtide-resistant viruses were not cross-resistant to MVC and MVC-resistance did not result in cross-resistance to enfuvirtide.

Summary of Maraviroc Treatment Failure in Studies 1027 and 1028
Given the novel mechanism of action of this new drug, the reasons for treatment failure could include a co-receptor "tropism switch" from CCR5-using virus to dual/mixed CXCR4-using virus, outgrowth of minor populations of CXCR4-using virus not detected at screening, resistance of CCR5-tropic virus to MVC, and/or emergence of resistance to OBT. Most (~50-60%) subjects failed with CXCR4- or dual/mixed-tropic virus in the MVC arms. The most prominent reason for failure in these studies was outgrowth of CXCR4-using viruses not detected at screening. Treatment failure on MVC with CCR5-tropic virus also occurred and resulted from phenotypic and genotypic resistance to MVC and resistance to OBT.

The results of Study A4001027 and A4001028 were further supported by results from studies A4001026 and A4001029.

Study A4001026
Analysis of three subjects who failed on 300 mg MVC QD with CXCR4-tropic virus from this treatment-naïve study showed that the CXCR4-tropic virus clones were present at baseline. The proportion of CCR5-tropic viruses was reduced during MVC treatment and the reduction was reversed after MVC treatment was stopped. In all three subjects, the LAM-resistance-associated M184V substitution was detected at failure and the viruses at failure were dual/mixed tropic. In one subject, the AZT-resistance-associated

RT substitutions M41M/L and K70R/K were also detected. When treatment with MVC and AZT/LAM was stopped, CCR5-tropic virus was again detected and the RT mutations were not detected in the post-treatment samples. These results suggest that the RT mutations are on the CXCR4-tropic virus, not the CCR5-tropic virus, although this cannot be proven with the existing PhenoSense^{GT} and Trofile assays because they separately amplify regions of the virus.

Study A4001029

The main findings of a post-hoc analysis of viral tropism and $CD4^+$ cell counts at Week 24 in this study of treatment-experienced subjects with non-CCR5 tropic virus were:

- Approximately 50% of the subjects discontinued (failed) treatment by Week 24 and about 25% had ≤ 500 copies/mL serum HIV RNA at Week 24. There was no difference between the MVC and placebo arms.
- Subjects who experienced treatment failure or had >500 copies/mL serum HIV RNA at Week 24 on MVC were more likely to have CXCR4-tropic virus at failure than subjects failing on placebo. Specifically, 45% (24/53) subjects had a CXCR4-tropic virus at failure in MVC arms compared to 9% (2/22) subjects in placebo.
- Increases in $CD4^+$ and $CD8^+$ cell counts were higher for both MVC treatment arms compared to placebo ($p<0.05$).
- For subjects on MVC whose virus was dual/mixed at screening, changes from baseline in $CD4^+$ cell counts were greater for those responding to MVC treatment at Week 24 (median +80-98) than those who experienced treatment failure (median +15-19).
- Changes in $CD4^+$ cell counts from baseline to time of treatment failure were similar for subjects on MVC whose virus was CXCR4-tropic at failure to those whose virus remained dual/mixed tropic at failure.

3. Administrative

3.1. Reviewer's Signature(s)

_____
[Lisa K. Naeger, Ph.D.]
Sr. Microbiologist, HFD-530

3.2. Concurrence

HFD-530/Signatory Authority _____ Signature _____ Date

HFD-530/Micro TL _____ Signature _____ Date

4. OND Microbiology Review

4.1 Important Milestones in Product Development

The potential for co-receptor switching as a mechanism for reduced susceptibility to CCR5 co-receptor antagonists concerned the Division and thus we requested sponsors of CCR5 inhibitors to vigilantly monitor co-receptor tropism changes and resistance in clinical trials. We requested tropism reports of all clinical trials on a monthly basis to monitor tropism switching, viral load and $CD4^+$ counts.

The Division requested a comprehensive analysis for subjects who had experienced treatment failure and/or had changes in their co-receptor tropism. However, given the complexity and exploratory nature of the tropism and resistance analyses, Pfizer proposed virology sub-studies from Clinical Studies A4001027 and A4001028.

4.2 Methodology

Genotypic and Phenotypic Methods
Phenotypic and genotypic resistance to NNRTIs, NRTIs and PIs were evaluated using the Monogram Biosciences PhenoSense GT (PSGT) assay at screening, Weeks 24 and 48, time of treatment failure and early termination visit. A lower viral load limit of 500 copies/mL was used. The results were used to calculate susceptibility scores for the OBT. Genotypic resistance to enfuvirtide was determined by the British Columbia Centre for Excellence in HIV using gp41 sequencing and identification of specific mutations in the HR1 domain at screening, Weeks 24 and 48, time of treatment failure or early termination visit.

Susceptibility testing was used to calculate the genotypic susceptibility score (GSS), phenotypic susceptibility score (PSS), and overall susceptibility score (OSS). The GSS is the sum of the number of active drugs in OBT based on PR, RT and gp41 amino acid sequences. The genotype of gp41 was used to score enfuvirtide for all the scores. The PSS is the sum of the number of active drugs in OBT based on drug susceptibility testing in cell culture (EC_{50} values). Therefore, a higher GSS or PSS score signifies that there are more active drugs in the OBT. The OSS is the sum of active drugs in OBT based on combined information from genotypic and phenotypic testing. When the GSS and the PSS tests above are in agreement then the net assessment of OSS will be the same. When the tests are not in agreement then a proprietary algorithm was used to determine whether the subject isolate was sensitive or resistant.

For each drug in the OBT, a score of "1" was given if resistance mutations were not detected or if the virus was susceptible to drug in the PSGT report. A score of "0" was given if one or more resistance mutations were detected or if the virus had reduced susceptibility to drug in the PSGT report. Samples were collected for retrospective analysis of virus susceptibility to maraviroc by gp160 sequencing as well as CCR5-medicated phenotypic susceptibility assays.

Assessment of CCR5 Δ32 Genotype and Promoter Haplotype

With appropriate regulatory permission and subject informed consent, blood samples for genetic analysis were collected prior to treatment with either maraviroc or placebo. Subject's DNA samples were analyzed in the Sponsor's laboratories to establish CCR5 Δ32 status and additional CCR5 locus polymorphisms. Combinations of ten of the selected promoter polymorphisms (SNPs) specify ten CCR5 promoter region haplotype alleles, designated P1 through to P10 (Martin et al., 1998; Gonzolez et al., 1999). Haplotypes P1 and P4 occur with highest frequency in various populations and were used as the basis to classify individual haplotype pairs (P1 and P4, P1 and other allele, P4 and other allele, neither P1 nor P4). In addition to CCR5 Δ32 status and additional CCR5 locus polymorphisms, pharmacogenomic studies incorporated analysis of clinical endpoints by classification of likely promoter haplotype as assigned by EM algorithm.

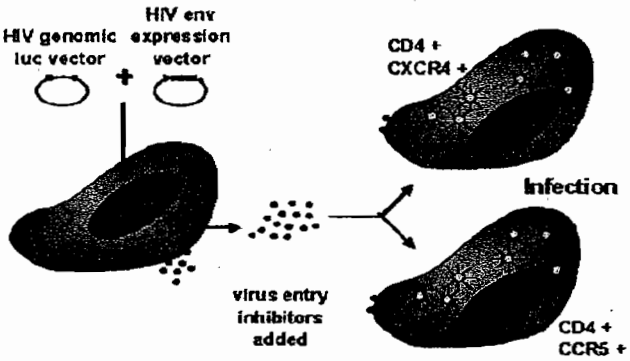
Viral Tropism Assessment

HIV-1 co-receptor tropism was carried out by Monogram Biosciences using a cell culture phenotypic assay (Trofile™ HIV Entry Tropism assay) (Fig. 1). The assay uses subject-derived virus envelope (gp160) sequences amplified from plasma to infect cell lines expressing the HIV receptor CD4 and either CXCR4 or CCR5 co-receptor. Co-receptor usage is determined by the presence of viral replication in these cell lines, as indicated by the expression of a reporter gene (firefly luciferase). To confirm the phenotype, CCR5-specific or CXCR4-specific entry inhibitors can be added prior to, or at the time of infection of target cells.

The tropism assay determines the relative size of the CCR5- and CXCR4-using populations and is not quantitative or absolute. In mixing experiments conducted in cell culture, a 10% minority population of CXCR4-using clones could be detected with 100% sensitivity, while the presence of a 5% CXCR4-using population could be detected with 83% sensitivity. The assay has a lower limit of sensitivity for reliable amplification of 1,000 copies of HIV-1 RNA/mL for amplification. In these studies, a lower limit of >500 copies/mL was used in order to increase the number of samples with low viral load for which tropism was determined. However, if viral load was ≤500 copies/mL, tropism was not determined at that timepoint or the result was censored.

Individual HIV-1 strains are categorized in cell culture as CCR5-tropic, CXCR4-tropic or dual-tropic. However, a single subject may harbor a heterogeneous population of viruses with different tropism. The Trofile™ HIV Entry Tropism assay cannot discern between true dual-tropic virus and a mixture of CCR5 and CXCR4 mono-tropic viruses. Subject samples that show any detectable replication in the CCR5-expressing and CXCR4-expressing cells are thus all collectively scored as "dual/mixed tropic".

Figure 1.



The Trofile™ assay has been validated pre-clinically by Monogram Biosciences (Whitcomb et al., 2007). The parameters investigated in this validation and key performance conclusions are listed in Table 1 below:

Table 1. Validation of the PhenoSense™ HIV Entry Tropism Assay (Monogram Biosciences)

Parameter	Overview	Key Performance Finding
Accuracy	Comparison to other methods using characterised and commercially available viruses	100% correlation for the viruses tested
Precision	Intra-assay variability	100% over ~1,000 pair-wise comparisons using 3 viruses
Reproducibility	Inter-assay variability	100% for 184 pair-wise comparisons using 36 patient samples
Sensitivity to amplify	Assay performance to report tropism using patient virus with low HIV RNA copies	Assay can be reliably performed on plasma samples with viral loads ≥1,000
Sensitivity to detect minor variants	CCR5-tropic and CXCR4-tropic envelope clones from the same patient mixed in defined ratios	100% of minor variants detected at the 10% mixture level. 83% of minor variants were detected at the 5% mixture level.
Linearity	Effect of virus load on tropism assignment (Tested in the range from ~3,000 to ~600,000 RNA copies/mL).	Variations in virus concentration in plasma do not significantly affect assay results

Genotypic and Phenotypic Analyses to Characterize Subjects who Failed in MVC Studies with CCR5-Tropic Viruses

For 38 subjects selected from studies 1027 and 1028 who failed blinded therapy with CCR5-tropic virus, paired pre- and on-treatment samples of frozen plasma were selected. Additional samples were analyzed from 4 subjects who subsequently received open-label (OL) MVC. HIV-1 envelope (Env) genes were amplified from all samples and these were used to generate Env pseudoviruses which were tested in the PhenoSense™ Entry assay (Monogram Biosciences) for susceptibility to MVC. Three additional compounds (ENF, the CCR5 co-receptor antagonist aplaviroc (GlaxoSmithKline), and EFV) were included as controls. Three criteria were used to identify viruses which had reduced phenotypic susceptibility to MVC:

- Dose response curves which had a plateau defined as a maximum percent inhibition, (MPI) <95%.
- Dose response curves with an EC_{50} value fold change outside the "normal range" (i.e. >1.9 EC_{50} value fold change to the reference virus JRCSF). The normal range of EC_{50} value FC (0.32-1.95)
- Subjects whose virus showed a >2-fold change in EC_{50} value between the protocol-defined treatment failure sample and Day 1

Susceptibility testing, gp160 sequencing and tropism confirmation were then performed on 12 Env clones from Day 1 and treatment failure/OL samples for each of the subjects meeting above criteria. In each case the clonal results agreed with the susceptibility testing performed on the original sample. In addition, amino acid changes were identified in the V3 loops of the clones of subjects whose virus showed a lower plateau in maximum percentage inhibition. Envelope genes from 5 of these subjects (together with samples from a subject whose virus could not be phenotypically characterized in the PhenoSense™ assay) were cloned into the NL4-3 background. These replication competent, recombinant viruses were then assayed in peripheral blood lymphocytes (PBL). In all cases, these results confirmed the phenotype observed in the PhenoSense™ Entry assay. All practical work described in this study was conducted at Monogram Biosciences Inc. (formerly ViroLogic Inc., South San Francisco) and Pfizer Sandwich Laboratories (UK).

Genotypic and Phenotypic Analyses to Characterize Subjects who Failed in MVC Studies with CXCR4 and Dual-Tropic Viruses

For each of the 20 subjects in this analysis, paired pre- and on-treatment samples were analyzed using frozen plasma samples stored at Monogram Biosciences. The Day 1 plasma sample was used as the "pre-treatment" sample and the first plasma sample available after Day 1 that was reported as dual/mixed or CXCR4 in the PhenoSense™ HIV Tropism assay was selected as the "on-treatment" sample. Published information and a simple probability mathematical model were used to decide how many env clones to pre-screen for tropism. Using this model and doubling the number of clones to allow for a proportion of non-functional envelope clones (20-80%), it was estimated that approximately 180 env clones per subject would need to be screened for co-receptor tropism in order to be able to detect minor variants present at a 5% incidence with 99% probability.

Thus, Monogram isolated and pre-screened for co-receptor tropism 192 env clones from the baseline sample and 48 clones from the on-treatment sample for each of the 20 subjects. The purpose of the pre-screen was to identify any CXCR4-using env clones in the baseline sample in a rapid screening format of the Monogram PhenoSense™ HIV tropism assay. Each clone was tested in a single well of two 96-well plates: one containing CCR5-expressing U87CD4 cells and the other CXCR4-expressing U87CD4 cells.

The nucleotide sequence of the V3 loop of gp160 was determined for all 192 baseline and 48 on-treatment *env* clones from each of the 20 subjects and a phylogenetic analysis was performed to investigate possible ancestry of the CXCR4-using clones. Nucleotide sequences were first aligned in the ClustalX program using seven reference sequences to root the trees and confirm that all viruses in a single subject were related to one another. The alignment was submitted to ClustalX to compute a neighbor-joining and maximum parsimony tree

In addition, twelve clones from each time-point (*i.e.*, 24 clones per subject) were selected and tropism was confirmed in the validated format of the PhenoSense™ HIV tropism Assay (Trofile™). The blind was broken for the 20 subjects described in this study report on 19th July 2006. All practical work was conducted at Monogram Biosciences, Inc. (South San Francisco).

4.3 Major microbiological issues that arose during product development

A potential concern with using a CCR5 inhibitor is that it will force HIV-1 to switch to using the CXCR4 co-receptor. The evolution to a CXCR4-utilizing HIV-1 has been proposed to result in a more virulent virus. The potential for co-receptor switching as a mechanism of reduced susceptibility to CCR5 co-receptor antagonists concerned the Division and thus we requested sponsors of CCR5 inhibitors to vigilantly monitor co-receptor tropism changes and resistance in clinical trials. We requested tropism reports of all clinical trials on a monthly basis to monitor tropism switching, viral load and $CD4^+$ cell counts.

In Clinical Studies 1027 and 1028, a comprehensive analysis of subjects who had a change in their HIV-1 co-receptor usage and/or increased viral load over the course of the study was requested in order to determine if

a. HIV-1 mutated to be able to use the CXCR4 co-receptor rather than the CCR5 co-receptor (co-receptor switch)
b. There was outgrowth of CXCR4-tropic HIV-1 not detected at screening
c. HIV-1 is CCR5-tropic and resistant to Maraviroc
d. HIV-1 is resistant to the OBT

The analyses were extensive and included

If virus was CCR5-tropic at failure:

- Determining the viral isolates's susceptibility to MVC in cell culture
- Nucleotide sequence analysis of the gp120 region to identify amino acid changes that may contribute to resistance to MVC (Potential amino acid changes should be verified, *e.g.* site-directed mutagenesis)
- Nucleotide sequence analysis of PR and RT

If virus was CXCR4-tropic at failure:

- A clonal evaluation of virus at screening or baseline and subsequent timepoints to determine the relative number of CXCR4-tropic and CCR5-tropic viral isolates.
- Nucleotide sequence analysis of the gp120 region to identify amino acid changes that may contribute to co-receptor switch to CXCR4
- Phylogenetic analysis to determine the relationship of emerging CXCR4-tropic virus to the CCR5-tropic virus at baseline
- Long term follow-up on the subjects viral loads, $CD4^+$ cell counts, HIV co-receptor usage and AIDS defining events

Given the complexity and exploratory nature of the tropism and resistance analyses, the applicant proposed virology sub-studies from Clinical Studies A4001027 and A4001028. A subset of 38 subjects (13 MVC; 25 placebo) failing with CCR5-tropic virus were analyzed to identify possible phenotypic and genotypic markers associated with MVC resistance in vivo including determining MVC susceptibility in cell culture, nucleotide sequence analysis of gp120 to identify amino acid substitutions that might contribute to MVC resistance, and nucleotide sequence analysis of protease and RT to identify resistance to drugs in the OBT.

In addition, the data for the assay performance characteristics for the PhenoSense™ HIV Entry Tropism assay were requested and these data were submitted as a Data Master File (DMF) by ViroLogic (now Monogram, Inc.).

Genetic variation in the CCR5 locus has been documented. Since the activity of CCR5 antagonists may vary depending on the individual's CCR5 genotype, we requested that a baseline sample be stored for retrospective analysis of CCR5 genotype for subjects with CCR5-tropic virus who did not respond to MVC in order to determine if MVC is not effective against certain CCR5 genotypes.

4.4 State of antimicrobials used for the indication (s) sought:

An estimated 40 million people worldwide were infected with HIV in 2001 and 3 million died from AIDS. Since HAART regimens have been introduced, the number of AIDS cases has decreased dramatically. HAART does not eradicate HIV from subjects and even though the number of HIV RNA copies is reduced to undetectable levels, HIV re-emerges quickly after discontinuation of HAART. Therefore, with the currently available regimens, it is likely that most HIV-infected subjects will require antiretroviral therapy throughout their lives.

There are currently over 20 FDA-approved anti-HIV drugs including three NNRTIs (delavirdine, efavirenz, nevirapine), eight NRTIs (abacavir, didanosine, emtricitabine, lamivudine, stavudine, tenofovir, zalcitabine, zidovudine), nine PIs (amprenavir/fosamprenavir, atazanavir, darunavir, indinavir, lopinavir, nelfinavir,

ritonavir, saquinavir, tipranavir), and the fusion inhibitor enfuvirtide. NNRTIs inhibit HIV-1 RT by binding near the catalytic site of RT and acting as noncompetitive inhibitors. NRTIs mimic nucleosides and target HIV-1 RT by competing with natural deoxynucleoside triphosphates for binding to RT and by incorporating into newly synthesized viral DNA resulting in chain-termination. PIs work at the late stage of viral replication to prevent virus production from infected cells. They block the HIV protease enzyme, which is necessary for the production of mature virions, resulting in defective particles that are unable to infect new cells. Enfuvirtide (T-20) is a gp41 fusion inhibitor preventing the joining of the viral and cellular membranes necessary for virus entry.

Unfortunately, HIV develops resistance to antiretroviral drugs over time usually from the accumulation of multiple mutations. HAART regimens are also associated with acute toxicities such as diarrhea, kidney stones, rash, CNS toxicities and hepatotoxicity. Long-term toxicities from antiretroviral therapies include mitochondrial toxicities associated with NRTIs (lactic acidosis, myopathy, neuropathy, pancreatitis), and disorders of lipid metabolism (dyslipidemia) and glucose metabolism (lipodystophy, hypercholesterolemia, hypertriglyceridemia) associated with PIs. These tolerability issues make compliance to therapy more challenging. Compliance is an important determinant of successful virologic suppression for subjects on HAART. Regimens that are well-tolerated and easy to administer with a few pills once daily are likely to aid in patient compliance and improve clinical outcomes. There is a need for new anti-HIV drugs that are well-tolerated and easy to use with new modes of action and low likelihood of viral resistance development. Additionally, drugs that are effective against viruses resistant to all currently approved drugs are needed for the heavily treatment-experienced population.

4.5 Non-clinical Microbiology

Maraviroc is unique in antiviral drugs because it targets a host membrane protein CCR5 rather than a viral target. The CCR5 chemokine receptor binds MIP-1α, MIP-1β, and RANTES, and is the only known receptor for MIP-1β.

Mechanism of Action

Maraviroc blocks the interaction of CCR5-tropic HIV-1 gp120 with the CCR5 co-receptor, an essential step in the entry process. Maraviroc binds to human CCR5 with a K_D of 0.86 nM and at room temperature has a dissociation half-life of approximately 16 hours. Site-directed mutagenesis and computer modeling studies indicate that binding of maraviroc to CCR5 is mediated principally by strong molecular interactions with two amino acid residues (Y108 and E283) that are thought to be located within the transmembrane domain of the CCR5 receptor. As a consequence of this binding, maraviroc is thought to alter the three dimensional structure of CCR5 such that the viral envelope glycoprotein, gp120, is unable to recognize and bind to the co-receptor. Consistent with this, maraviroc blocks the soluble form of gp120 binding to CCR5 with

an IC_{50} value of 11 nM and inhibits gp120/CCR5-mediated membrane fusion with an IC_{50} value of 0.22 nM.

Three types of in vitro studies were conducted by the applicant in order to confirm the mechanism of action of maraviroc.

1. Studies were conducted that confirmed the specific binding of maraviroc to CCR5. Radioligand binding experiments, using [^{3}H]-maraviroc, confirm that maraviroc binds with high affinity to human CCR5 and was shown to dissociate slowly from the human CCR5 receptor. In radioligand binding competition assays, MVC inhibited binding and intracellular signaling of endogenous chemokines MIP-1α, MIP-1β and RANTES to recombinant human CCR5 receptors expressed in whole human embryonic kidney HEK-293 cells and membrane homogenates. The inhibition of binding is non-competitive and circulating chemokines are unlikely to affect the binding activity of MVC.

2. Studies were conducted that investigated the ability of maraviroc to inhibit the HIV-1 envelope glycoprotein, gp120, from binding to CCR5. Binding competition experiments against the soluble subunit of the viral envelope glycoprotein (gp120) from the CCR5-tropic laboratory-adapted BaL strain showed that MVC inhibits virus attachment. In a cell-based assay using CHO cells expressing recombinant viral envelope protein (from strain JR-FL) and HeLa cells expressing CCR5 and CD4 receptors, cell-cell fusion occurs in the presence of functional, complete gp160 consisting of gp120 and gp41. MVC was effective at inhibiting cell-cell fusion in this assay which is believed to closely mimic the viral entry process.

3. Viral replication assays were used to confirm that blocking the interaction between gp120 and CCR5 selectively inhibits HIV-1 replication of viruses that are obligate users of CCR5 as their entry co-receptor (i.e. CCR5-tropic or CCR5 strains). The antiviral activity of MVC in cell culture was profiled against the laboratory adapted strain Ba-L and against a range of CCR5 and CXCR4-tropic clinical primary isolates in mitogen stimulated peripheral blood lymphocytes (PBL) from pooled donors. These studies confirmed that MVC selectively inhibits CCR5-tropic viruses.

In addition, in vitro pharmacology studies showed that MVC prevents agonist-induced intracellular calcium release from cells expressing the recombinant human CCR5 receptor indicating that it is a functional antagonist of the human CCR5 receptor. MVC did not act as an agonist of the human CCR5 receptor as it did not trigger release of intracellular calcium at concentrations up to 10 μM. CCR5 ligand-induced calcium flux inhibition by MVC is specific to CCR5 because there was no muscarinic (non-selective) signaling by carbochol at 1 μM on HEK-293 cells. This suggests that MVC inhibition

does not involve other signal transduction steps that may be common to G protein-coupled receptors.

To demonstrate that the decrease in viral production in the above antiviral assays was not the result of MVC-induced receptor internalization, the effect of the compound on levels of CCR5 at the cell surface was investigated. Because of the low and variable levels of CCR5 expression on primary cells, an internalization-competent recombinant human CCR5 expressing mouse pre-B cell line (300.19/CCR5) was used for this study. Cell surface CCR5 levels were measured using a CCR5-specific monoclonal antibody and fluorescence-activated cell sorting (FACS) technology. The endogenous CCR5 agonist RANTES, and endogenous CXCR4 agonist Stromal Derived Factor 1α (SDF-1α) were used as positive and negative CCR5 internalization controls respectively. Maraviroc was tested at 10 and 100 nM (5 and 51 ng/mL, respectively) in this study as these concentrations respectively approximate and exceed the EC_{50} values generated for MVC-dependent inhibition. MVC did not induce CCR5 internalization in 300.19/CCR5 cells at 10 nM and 100 nM. MVC effects on cell surface CCR5 were consistent with those observed with the negative control CXCR4 ligand SDF-1α. In contrast, the positive control RANTES, induced CCR5 internalization as shown by a reduction in CCR5-dependent cell population fluorescence.

Anti-HIV Activity in Cell Culture

The antiviral activity of maraviroc was assessed in a 96-hour viral replication assay by RT activity or p24 antigen levels in PM-1 or PBLs that were infected with laboratory strain HIV-1_{BaL} prior to treatment with compound. MVC showed inhibitory activity against HIV-1 in PM-1 cells with an EC_{50} value of 0.21 nM and EC_{90} value = 1.08 nM (n=16). Similar activity was seen against HIV-1 in pooled PBLs from multiple donors with an EC_{50} value of 0.54 nM (n=11), and for HIV-1 in individual donor PBLs (EC_{50} value = 0.28 nM, n=10). MVC was inactive up to 10 μM (highest concentration) against CXCR4-tropic HIV-1_{IIIB} and other CXCR4-tropic and dual-tropic strains. Anti-HIV activity was shown not to be due to non-specific cytotoxicity, as MVC did not show any cytotoxicity against PM-1 (n=5) or PBL (n=3) cells at concentrations up to 10 μM.

The activity of MVC was measured against a range of CCR5-tropic clinical primary isolates from various clades A to O and the mean EC_{50} value for all clades was 0.51 nM (range 0.40 – 1.11) (Table 2).

The antiviral activity of MVC against a panel of 200 well-characterized drug sensitive (N = 100) and drug (protease/reverse transcriptase)-resistant (N = 100) viruses of clade B and non-clade B HIV-1 was tested at Monogram Biosciences Inc.(San Francisco, CA, USA) using the PhenoSense™ HIV Entry phenotypic drug susceptibility assay (Table 3). Maraviroc inhibited all 200 pseudotyped viruses with a geometric mean EC_{50} value of 1.6 nM and a geometric mean 0.69-fold change. The geometric mean EC_{50} value and fold change was 1.3 nM and 0.60-fold, respectively, for the drug-naïve group and 2.1 nM and

0.79-fold, respectively, for the PI/RTI drug-resistant groups. This difference is probably not biologically relevant since it is less than the inter-assay variability previously reported. The data suggests that MVC will be active in subjects with virus resistant to existing antiretrovirals.

Table 2. Antiviral Activity of Maraviroc against Various Primary HIV-1 Isolates

Clade[a](No. of isolates)	N[c]	Mean IC_{50} (nM) (95% CI)	Mean IC_{50} (ng/mL) (95% CI)	Mean IC_{90} (nM) (95% CI)	Mean IC_{90} (ng/mL) (95% CI)
Ba-L	20	1.25 (0.81 to 1.93)	0.64 (0.42 to 0.99)	5.57 (3.98 to 7.78)	2.85 (2.04 to 4.0)
A (4)	16	0.40 (0.23 to 0.70)	0.21 (0.12 to 0.36)	1.09 (0.66 to 1.80)	0.56 (0.34 to 0.92)
B (21)	94	0.42 (0.36 to 0.50)	0.22 (0.18 to 0.26)	1.83 (1.53 to 2.18)	0.94 (0.78 to 1.12)
C (4)	20	0.69 (0.37 to 1.28)	0.37 (0.17 to 0.82)	3.02 (1.67 to 5.48)	1.54 (0.85 to 2.79)
D (4)	10	0.68 (0.39 to 1.19)	0.35 (0.20 to 0.61)	2.65 (1.72 to 4.07)	1.36 (0.88 to 2.09)
E (1)	4	0.10 (0.08 to 0.12)	0.05 (0.04 to 0.06)	0.51 (0.37 to 0.71)	0.26 (0.19 to 0.36)
F (1)	2	1.11 (0.50 to 2.46)	0.57 (0.26 to 1.26)	2.95 (0.92 to 9.46)	1.52 (0.47 to 4.86)
G (2)	14	1.11 (0.50 to 2.46)	0.57 (0.26 to 1.26)	4.44 (2.18 to 9.06)	2.28 (1.12 to 4.65)
J (2)	10	0.52 (0.18 to 1.47)	0.27 (0.09 to 0.75)	3.51 (1.25 to 9.85)	1.24 (0.40 to 3.91)
O (4)	20	0.73 (0.47 to 1.13)	0.37 (0.24 to 0.58)	1.80 (1.14 to 2.85)	0.92 (0.59 to 1.46)
ALL	210	0.56 (0.48 to 0.65)	0.29 (0.25 to 0.33)	2.24 (1.94 to 2.58)	1.15 (0.99 to 1.32
All primary[b] (43)	**190**	**0.51 (0.44 to 0.60)**	**0.26 (0.23 to 0.31)**	**2.03 (1.75 to 2.36)**	**1.04 (0.90 to 1.22)**
CXCR4 tropic (4)	9	No effect at 10 nM (5.13 ng/mL)			
Dual tropic (2)	3	No effect at 10 nM (5.13 ng/mL)			

Table 3. Maraviroc Geometric Mean EC_{50} and EC_{90} Values among 200 HIV-1 Clinical Isolates, and Virus Subgroups Defined by Clade (B vs Non-B Clade) and Drug Sensitivity to Protease and/or Reverse Transcriptase Inhibitor Drugs

Viral Isolate		IC_{50}	IC_{90}
All viruses (N = 200)	Geo. mean, nM (ng/mL)	1.6 (0.82)	13.7 (7.04)
	95% CI of mean	1.5 to 1.7	12.3 to 15.1
	Range	0.3 to 8.9	1.0 to 95.5
B clade viruses (N = 160)	Geo. mean, nM (ng/mL)	1.6 (0.82)	14.2 (7.29)
	95% CI of mean	1.5 to 1.8	12.7 to 15.8
	Range	0.5 to 6.9	2.5 to 95.0
Non-B clade viruses (N = 40)	Geo. mean, nM (ng/mL)	1.6 (0.82)	11.7 (6.01)
	95% CI of mean	1.3 to 1.9	8.8 to 15.6
	Range	0.3 to 8.9	1.0 to 95.5
PI/RTI resistant viruses (N = 100)	Geo. mean, nM (ng/mL)	2.1 (1.08)	17.7 (9.09)
	95% CI of mean	1.9 to 2.3	15.3 to 20.5
	Range	0.7 to 8.9	2.5 to 95.5
Wild-type viruses (N = 100)	Geo. mean, nM (ng/mL)	1.3 (0.67)	10.5 (5.39)
	95% CI of mean	1.2 to 1.4	9.2 to 12.0
	Range	0.3 to 3.0	1.0 to 39.9

Effect of Plasma Protein Binding on Antiviral Activity

Antiviral assays were conducted in a growth medium containing 10% (w/v) heat inactivated fetal calf serum (FCS). Using the equilibrium dialysis method, the bound and unbound fractions of MVC in the PBL-antiviral medium were determined to be 45.2% and 54.8%, respectively. The geometric mean EC_{90} value for MVC against 43 primary isolates in PBL culture was measured as 2.0 nM. Since the unbound concentration of

MVC is estimated to be 54.8%, the unbound antiviral EC_{90} value for MVC is estimated to be approximately 1.0 nM (0.5 ng/mL).

Cytotoxicity

The direct cytotoxic potential of maraviroc was tested at concentrations up to 10 μM (5137 ng/mL) in cultures of uninfected, actively dividing human PBLs and PM-1 cells that express the complement of chemokine receptors, including CCR5 and CXCR4. Cytotoxicity assays were done in parallel with antiviral assays for 5 days prior to spectrophotometric analysis of cell proliferation and viability in an MTS assay. No differences in the viability of cells treated with or without MVC were seen, thus confirming that at these concentrations, MVC is not cytotoxic and that the observed antiviral activity occurs in the absence of effects on cell growth.

In addition, 10 μM MVC is inactive against HIV-1_{IIIB}, which utilizes the alternative co-receptor CXCR4, and other CXCR4-tropic or dual-tropic strains. This result indicates that these virally infected cells are able to grow and produce virus at this concentration. Similarly, the EC_{50} value of maraviroc was greater than 10 to 25 μM in a variety of functional assays using ex vivo human white blood cells, including T-cell proliferation, neutrophil chemotaxis and superoxide production, and lymphocyte IgE synthesis. These cells were functionally active in the presence of concentrations of maraviroc greater than 5,000-fold the antiviral EC_{90} value for the compound. Furthermore, chromosomal damage was not observed in cultures of human lymphocytes when tested up to cytotoxic concentrations. Chromosome damage was also absent in bone marrow of mice treated orally once daily for 3 days with up to 2,000 mg/kg of MVC. Moreover, the macaque, which has a CCR5 receptor that functionally interacts with MVC, has been used in toxicology studies and no significant cytotoxicity could be observed in the wide range of tissues examined following histopathological analysis.

Cell Culture Drug Combination Activity Assessments

A study was conducted [redacted] to investigate interactions between MVC and EFV, NFV, and lamivudine. MVC exhibited an additive interaction with lamivudine and additive to synergistic interactions with EFV and NFV. The antiviral activity of MVC was assessed in in-house studies in combination with 17 approved anti-HIV agents in HIV-1_{Ba-L}-infected PM-1 cells in cell culture (Table 4). There was no evidence of antagonism of MVC with any of these compounds and no evidence of cytotoxicity at the highest drug combination test (40 nM). Additive to synergistic interactions were observed when MVC was tested in combination with three other CCR5 antagonists, SCH-D, SCH-C, [redacted].

Table 4. Antiviral Effects of MVC in Combination with Different Classes of Anti-HIV Agents

UK-427,857 in combination with:	n	Volume ($\mu M^2\%$) Synergy	Volume ($\mu M^2\%$) Antagonism	Combined Effect
Study Conducted [a]				
Lamivudine (NRTI)	2	41	-34	Additive
Efavirenz (NNRTI)	2	63	-18	Minor Synergy
Nelfinavir (PI)	2	74	-23	Minor Synergy
Study Conducted at Pfizer Global Research & Development [b]				
Abacavir (NRTI)	2	10.4 ±14.6	-0.2 ± 0.3	Additive
Didanosine (NRTI)	2	4.0 ± 4.6	-0.9 ± 1.3	Additive
Emtricitabine (NRTI)	2	0.1 ± 0.2	-1.2 ± 1.7	Additive
Stavudine (NRTI)	2	1.6 ± 1.5	0.0	Additive
Tenofovir (NRTI)	(3)	5.2 ± 7.2	-8.0 ± 13.9	Additive
Zalcitabine (NRTI)	2	0.0	-4.3 ± 1.2	Additive
Zidovudine (NRTI)	2	0.1 ± 0.1	-0.1 ± 0.1	Additive
Delavirdine (NNRTI)	2	7.1 ± 3.4	-0.2 ± 0.3	Additive
Efavirenz (NNRTI)	(3)	0.0 ± 0	-4.1 ± 4.5	Additive
Nevirapine (NNRTI)	2	5.7 ± 8.0	-3.2 ± 2.8	Additive
Amprenavir (PI)	2	3.5 ± 2.4	-1.6 ± 1.4	Additive
Atazanavir (PI)	2	35.4 ± 43.2[d]	-0.2 ± 0.3[d]	Additive/ Synergy
Indinavir (PI)	2	19.6 ± 27.7[e]	-6.5 ± 4.0[e]	Additive/Synergy
Lopinavir (PI)	2	12.5 ± 17.6	-11.0 ± 15.2	Additive
Ritonavir (PI)	2	0.0	-0.3 ± 0.4	Additive
Saquinavir (PI)	(3)	10.7 ± 12.6	-1.0 ± 0.2	Additive
Enfuvirtide (Fusion)	(2),1	16.1± 16.8[f], 11.2[c]	-1.3 ± 1.8[f], -0.0[c]	Additive/Synergy
SCH-C (Entry R5)	2	1.2 ± 0.6	-7.2 ± 0.8	Additive
SCH-D (Entry R5)	3	40.0 ± 40.8[g]	-3.8 ± 5.6[g]	Additive/ Synergy

NRTI = Nucleoside reverse transcriptase inhibitor, NNRTI = Non-nucleoside reverse transcriptase inhibitor, PI = Protease Inhibitor, SRI =Southern Research Institute Ltd.

[a] Study Code DI/005/3, Volume of synergy and antagonism was computed at the 95% confidence interval using the method of Pritchard and Shipman, 1990. For these studies synergy is defined as drug combinations yielding values >50$\mu M^2\%$, minor synergistic activity and highly synergistic activity are defined as yielding values of 50-100 $\mu M^2\%$ and >100$\mu M^2\%$ respectively. Additive drug interactions have values ranging between –50$\mu M^2\%$ and 50 $\mu M^2\%$ while values <-50 $\mu M^2\%$ are considered antagonistic.

[b] Study Code DI/002/04, Volumes (mean ± standard deviation) were calculated by MacSynergy™II software using 95% confidence intervals and are defined by the program as follows: values <25 $\mu M^2\%$ indicate additive response; values >25 and <50 $\mu M^2\%$ indicate minor synergy /antagonism; values >50 and <100 $\mu M^2\%$ indicate moderate synergy/antagonism; and values >100 $\mu M^2\%$ indicate strong synergy/antagonism.

[c] Values obtained from different compound dose ranges.

[d] Individual MacSynergy™II volumes for atazanavir: 65.9, 4.9 synergy; -0.4, -0.0 antagonism

[e] Individual MacSynergy™II volumes for indinavir: 39.2, 0.0 synergy; -9.3, -3.6 antagonism

[f] Individual MacSynergy™II volumes for enfuvirtide: 27.9, 4.2 synergy; -2.5, 0.0, antagonism

[g] Individual MacSynergy™II volumes for SCH-D: 81.5, 38.5, 0.0 synergy; -0.1, -10.2, -1.0 antagonism

Values in parenthesis indicate that one result includes a mean value from up to 3 single replicate plate experiments

All values were obtained from five replicate plates per assay except those in parentheses for which one result includes a mean value from up to 3 single replicate plate experiments.

Selection of MVC-Resistant Virus in Cell Culture

Initially, maraviroc-resistant virus was not obtained by passaging HIV-1_{Ba-L} in PBL or PM1 cell cultures in the presence of increasing concentrations of MVC for 30 and 32

weeks, respectively. Subsequently, six HIV-1 isolates were passaged through PBLs in the presence or absence of increasing concentrations of maraviroc for up to 20 weeks. By week 14, (passage 14) three of the viruses, CC 1/85, RU570, and SF162 were able to replicate in the presence of high levels of MVC (ranging from 4 μM to 16 μM). Addition of compound at higher concentrations failed to suppress viral replication any further, suggesting these viruses had become highly resistant to MVC. The remaining 3 viruses (92BR017, 92BR018 and 92BR023) failed to replicate in the presence of increased concentrations of MVC, suggesting that these viruses were unable to develop resistance to the compound in this study. In addition, virus stocks were prepared from virus passaged in the absence of compound (passaged control) to identify any 'natural' evolution of virus in the absence of selective pressure. The sensitivity of these viruses to MVC (and saquinavir and the CXCR4 antagonist AMD100 as controls) was determined in a six-day antiviral assay in PBLs, using HIV-1 reverse transcriptase activity to quantify viral replication.

RU570 virus passaged in the presence of MVC showed high levels of resistance with >20,000-fold shift in EC_{50} values compared with the starting virus. CC 1/85 virus passaged in the presence of MVC gave >5,000-fold shift compared with the starting virus. Both RU570 and CC 1/85 viruses remained sensitive to the HIV protease inhibitor saquinavir. Importantly, neither RU570 nor CC 1/85 showed sensitivity to the CXCR4 antagonist AMD3100, suggesting that they did not use CXCR4 as a co-receptor for viral entry. The passaged viruses were not inhibited by MVC and AMD3100 in combination confirming that the resistance observed with CC 1/85 and RU570-derived strains was not a consequence of the virus being able to use either CCR5 or CXCR4 ("dual-tropism"). MVC-resistant RU570 and CC 1/85 viruses were sensitive to inhibition by monoclonal antibody 2D7 (which binds to the 2nd extracellular loop of CCR5), indicating that both viruses still used the CCR5 receptor. Finally, neither the RU570- nor CC 1/85-derived MVC-resistant viruses were able to grow in PBL from a CCR5 Δ32/Δ32 blood donor, suggesting that the viruses remained obligate CCR5-tropic and had not evolved to use CXCR4 or any of the other co-receptors expressed on PBL.

SF162 virus developed complete resistance to MVC after 12 weeks of passaging, with a >20,000-fold shift compared with the starting virus both in the presence and absence of increasing concentrations of the compound. All MVC-resistant SF162-derived viruses were sensitive to inhibition by the CXCR4 antagonist AMD3100 indicating that they had acquired the ability to use CXCR4 as a co-receptor. This tropism shift occurred independently of MVC, and is possibly due to selective pressures inherent to passaging this particular strain in cell culture. All SF162 viruses remained sensitive to the protease inhibitor saquinavir.

Virus supernatants from MVC-resistant cultures of CC 1/85, RU570 and SF162 were sent to ViroLogic to confirm co-receptor tropism using the recombinant PhenoSense Entry Assay. The *env*-recombinant pseudotyped viruses that were generated from MVC-resistant cultures of CC 1/85 and RU570 infected $CD4^+CCR5^+$ U87 cells but not

$CD4^+CXCR4^+$ U87 cells indicating that they retained their CCR5-tropic co-receptor phenotype. However, *env*-recombinant pseudotyped viruses generated from MVC-resistant SF162 virus and SF162 passaged control virus were able to infect both $CD4^+CCR5^+$ and $CD4^+CXCR4^+$ U87 cells indicating a shift in both viruses to a dual-tropic co-receptor phenotype. The initial virus stocks for CC 1/85, RU570 and SF162 were all shown to be CCR5-tropic in the PhenoSense Entry assay consistent with the data obtained in PBLs with replication competent virus.

Although there were no changes in EC_{50} values, the viruses were not inhibited by 100% even at very high concentrations of drug. These viruses produced atypical dose-response curves that were characterized by a lower plateau in maximal percentage inhibition. This type of dose response curve is frequently seen with allosteric inhibitors of receptors, and occurs when the receptor ligand is able to recognize both inhibitor-bound and inhibitor-free receptor molecules. MVC-resistant variants appeared able to use both maraviroc-occupied and maraviroc-free CCR5 molecules to infect target cells.

Clonal analysis of the *env*-recombinant pseudotyped viruses was performed and amino acids changes in the env gene assessed. Resistance to maraviroc in cell culture is associated with selection of variants containing amino acid substitutions/deletions in the V3 loop of the HIV-1 envelope. Nucleotide sequence analysis of the Env regions of the CC1/85 and RU570 viruses identified distinct amino acid changes in resistant viruses when compared to the parental virus indicating the amino acid changes had been selected during the serial passage in cell culture.

[redacted]

. Analysis of functional viral clones showed that related but genetically distinct virus variants were identified within these clones. Furthermore, many of the clones gave a plateau in their dose responses to MVC when analyzed in the PhenoSense Entry Assay. Serial passage of the MVC-resistant virus variants in the absence of maraviroc selects for variants with increased sensitivity to MVC and loss of some of the V3 loop mutations contributing to decreased maraviroc susceptibility.

Cross-Resistance in Cell Culture

The sensitivity of the *env*-recombinant pseudotyped viruses to a range of entry inhibitors [MVC, [redacted]), SCH-C (Schering CCR5 antagonist), ENF (Roche/Trimeris gp41 fusion inhibitor), AMD3100 (a CXCR4 specific antagonist) and 2D7 (a CCR5-specific monoclonal antibody)] was

investigated using the PhenoSense Entry assay. In contrast to the data obtained in PBLs which showed >1,000-fold loss in sensitivity of MVC-resistant CC 1/85 and RU570 viruses to MVC, the *env*-recombinant pseudotyped viruses generated from these cultures did not show any significant shift in their EC_{50} values, when compared to the drug-free passaged or starting virus derived samples. No change in susceptibility to enfuvirtide was observed between the "start" and resistant strains of CC1/85 or RU570. The RU570 viruses were approximately 10-fold more sensitive to enfuvirtide than the CC1/85 viruses. Enfuvirtide-resistant viruses were not cross-resistant to MVC and MVC-resistance did not result in cross-resistance to ENF.

4.6 Clinical Studies

A4001027 and A4001028 Studies Overview

The two studies were randomized (2:2:1), double-blind and placebo-controlled, designed to compare the safety and antiviral activity of maraviroc at two different doses versus placebo, each in combination with optimized background therapy. Investigators were to optimize therapy, with 3-6 (excluding low-dose ritonavir) branded, open-label agents, on the basis of resistance testing, treatment history and safety/adverse event considerations.

For studies 1027 and 1028, 2560 subjects were screened for HIV-1 tropism and 56% were determined to CCR5-tropic virus, 41% had dual/mixed tropic virus, and 2.6% had CXCR4-tropic virus. Note that for the treatment-naïve study 1026, 85% screened as having CCR5-tropic virus with 14.7% having dual/mixed tropic and 0.3% having CXCR4-tropic virus.

Treatment Groups:

- Optimized Background Therapy + maraviroc once daily
- Optimized Background Therapy + maraviroc twice daily
- Optimized Background Therapy + maraviroc placebo

Randomization was stratified by screening HIV-1 RNA levels (<100,000 versus ≥ 100,000 copies/mL) and by inclusion of enfuvirtide in the background regimen. The primary efficacy endpoint for both studies is the mean reduction in serum HIV-1 RNA from baseline to Week 48. However, a planned interim analysis at Week 24 has been conducted for both studies for the purpose of this submission. Investigators and subjects will remain blinded until after the last subject reaches Week 48.

In studies A4001027 and A4001028, the populations randomized were comparable demographically. Subjects receiving a PI, with or without ritonavir boosting, or delavirdine as part of the OBT (other than tipranavir) received 150 mg unit dose of maraviroc (either QD or BID). Subjects on all other regimens, including those taking tipranavir/ritonavir, received 300 mg unit dose of maraviroc (QD or BID). More than

75% of the subjects in each group received an optimized background regimen containing a PI other than tipranavir and/or delavirdine.

Efficacy

Studies 1027 and 1028 demonstrated similar clinically significant reductions in viral load from baseline to Week 24 for maraviroc QD and BID compared with placebo (Table 5; Summary of Clinical Efficacy). In addition, all secondary efficacy virologic endpoints (<400 or <50 copies/mL; ≥1 $\log_{10}$ reduction in serum HIV-1 RNA from baseline) were substantially greater for both maraviroc treatment groups compared to the placebo group. There was a slight trend in favor of the pooled maraviroc BID treatment groups for all of these endpoints. Pooling the primary efficacy endpoint data from studies A4001027 and A4001028, the mean reduction in HIV-1 RNA from baseline through Week 24 was -1.876 $\log_{10}$ copies/mL for maraviroc QD, -1.960 $\log_{10}$ copies/mL for maraviroc BID and -0.987 $\log_{10}$ copies/mL for placebo. The treatment difference between maraviroc QD and placebo was -0.888 $\log_{10}$ copies/mL and the treatment difference between maraviroc BID and placebo was -0.973 $\log_{10}$ copies/mL. The 2-sided 97.5% CIs were completely to the left side of zero, indicating the superiority of maraviroc QD and maraviroc BID over placebo. There was a greater increase in $CD4^+$ and $CD8^+$ cell counts from baseline to Week 24 for both maraviroc treatment groups compared with placebo. For the complete safety and efficacy analyses, see Scott Proestel's (Medical Officer) clinical review and Susan Zhou's (statistician) review.

Table 5. Efficacy Endpoints at Week 24 (Studies A4001027 and A400128)

	Study A4001027			Study A4001028		
	Maraviroc QD	Maraviroc BID	Placebo	Maraviroc QD	Maraviroc BID	Placebo
Mean (se) Δ HIV-1 RNA[a] (copies/mL)	N= 232 -1.818 (0.092)	N= 235 -1.952 (0.091)	N= 118 -1.030 (0.129)	N= 182 -1.950 (0.105)	N= 191 -1.971 (0.103)	N= 91 -0.929 (0.147)
Patients <400 copies/mL, n (%)	N= 232 127 (54.7)	N= 235 142 (60.4)	N= 118 37 (31.4)	N= 182 101 (55.5)	N= 191 118 (61.8)	N= 91 21 (23.1)
Patients <50 copies/mL, n (%)	N= 232 98 (42.2)	N= 235 114 (48.5)	N= 118 29 (24.6)	N= 182 84 (46.2)	N= 191 79 (41.4)	N= 91 19 (20.9)
Patients >1 $\log_{10}$ Reduction in HIV-1 RNA, n (%)	N= 232 151 (65.1)	N= 235 161 (68.5)	N= 118 46 (39.0)	N= 182 121 (66.5)	N= 191 134 (70.2)	N= 91 29 (31.9)
Mean (se) Δ CD4 Count[a] (cells/μL)	N= 227 106.6 (7.3)	N= 233 111.1 (7.2)	N= 116 52.1 (10.1)	N= 180 111.7 (7.8)	N= 185 101.9 (7.7)	N= 90 63.8 (10.9)
Mean (se) Δ CD8 Count[a] (cells/μL)	N= 227 283.5 (30.2)	N= 233 302.3 (29.8)	N= 116 -0.7 (42.2)	N= 180 340.7 (41.6)	N= 185 255.4 (40.9)	N= 90 122.2 (58.2)

Source: A4001027 and A4001028 Clinical Study Reports.
[a] Change from Baseline to Week 24.
QD = Once daily dosing; BID = Twice daily dosing; se = Standard error.

Effect of Clade B versus Non-Clade B Virus

Virus subtype (clade) varies geographically. Although Clade B virus is not the most common subtype worldwide, it the predominant subtype found in North America and

Western Europe. The majority of subjects recruited into Studies A4001027 and A4001028 were from North America and Western Europe and 94% of those for whom virus subtype could be determined were infected with Clade B virus. The change in HIV-1 RNA from baseline analyzed by Clade is shown in Table 6.

Table 6. Summary of Change from Baseline to Week 24 in HIV-1 RNA by Clade (Combined Studies A4001027 and A4001028)

Virus Subtype		Change in HIV-1 RNA from Baseline (log_{10} copies/mL)		
		Maraviroc QD (N= 414)[a]	Maraviroc BID (N= 426)[a]	Placebo (N= 209)[a]
Clade B	N[b]	385	400	197
	Mean (SD)	-2.081 (1.277)	-2.154 (1.281)	-1.170 (1.294)
	Median (Range)	-2.388 (-4.492, 2.039)	-2.467 (-4.547, 1.317)	-0.629 (-4.148, 0.965)
Non-Clade B	N[b]	19	15	7
	Mean (SD)	-2.264 (1.301)	-2.305 (1.213)	-1.279 (1.469)
	Median (Range)	-2.268 (-4.231, 0.259)	-2.557 (-3.890, -0.218)	-0.346 (-3.398, 0.218)
Undetermined	N[b]	4	4	3
	Mean (SD)	-2.318 (0.486)	-1.999 (1.655)	-0.181 (0.459)
	Median (Range)	-2.240 (-2.974, -1.819)	-2.554 (-3.199, 0.313)	-0.361 (-0.521, 0.341)

Source: Table 13.4.9.1.15 Summary of Clinical Efficacy.
[a] Number of patients in treatment group.
[b] Number of patients contributing to summary statistics, which were patients with valid values for baseline and on treatment. Last Observation Carried Forward (LOCF) values were used to impute missing values.
QD = Once daily dosing; BID = Twice daily dosing; SD = Standard deviation.

4.7 Clinical Microbiology

BASELINE ANALYSIS OF STUDIES 1027 AND 1028

The distribution of genotypic sensitivity score (GSS), phenotypic sensitivity score (PSS) and overall sensitivity score (OSS) was balanced across the three treatment groups in studies 1027 and 1028 with median GSS=1, PSS=2 and OSS=2 for each treatment group in both studies (Appendix A). The median number of resistance-associated mutations at screening in the MVC BID arm and placebo arm was the same with a median of 1 NNRTI-resistance associated mutation, 6 NRTI-resistance associated mutations, and 6 PI-resistance associated mutations. The percentage of subjects with enfuvirtide-resistance mutations at screening was 21% in all arms.

A higher GSS or PSS score signifies that there are more active drugs in the OBT. In Study A4001027, 75%, 65% and 71% of subjects had GSS, PSS and OSS of $\leq$2, respectively (Table 7). The baseline resistance for subjects in Study A4001028 was similar with 70%, 58% and 63% of subjects having GSS, PSS and OSS of $\leq$2, respectively (Table 7). These percentages are consistent with a heavily treatment-experienced population. In Studies A4001027 and A4001028, 55% (581/1049) of subjects had a GSS score of $\leq$1 and 37% (393/1049) of subjects had a PSS score of $\leq$1 indicating that a third to half of the subjects had none or only one potentially active drug in their OBT.

Table 7. Baseline Resistance: n (%)

		Study 1027			Study 1028		
		QD (n=232)	BID (n=235)	Placebo (n=118)	QD (n=182)	BID (n=191)	Placebo (n=91)
GSS	0	52 (22%)	59 (25%)	31 (26%)	39 (21%)	42 (22%)	21 (23%)
	1	82 (35%)	80 (34%)	29 (25%)	64 (35%)	59 (31%)	23 (25%)
	2	38 (16%)	48 (20%)	21 (18%)	25 (14%)	32 (17%)	20 (22%)
	≥3	57 (25%)	47 (20%)	34 (29%)	52 (29%)	57 (30%)	25 (27%)
PSS	0	25 (11%)	24 (10%)	17 (14%)	20 (11%)	26 (14%)	12 (13%)
	1	70 (30%)	73 (31%)	18 (15%)	46 (25%)	42 (22%)	20 (22%)
	2	51 (22%)	69 (29%)	35 (30%)	42 (23%)	38 (20%)	23 (25%)
	≥3	83 (36%)	66 (28%)	45 (38%)	71 (39%)	84 (44%)	34 (37%)
OSS	0	30 (13%)	27 (11%)	19 (16%)	22 (12%)	29 (15%)	17 (19%)
	1	78 (34%)	86 (37%)	21 (18%)	55 (30%)	51 (27%)	22 (24%)
	2	51 (22%)	65 (28%)	38 (32%)	37 (20%)	39 (20%)	21 (23%)
	≥3	69 (30%)	54 (23%)	37 (31%)	65 (36%)	71 (37%)	29 (32%)

At baseline in studies 1027 and 1028, 90% (940/1049) of the subjects had CCR5-tropic virus with 4% having dual/mixed tropic virus and 5% have non-phenotypable virus (Table 8 and 9). It is noteworthy that subjects were screened as having CCR5-tropic virus as entry criteria into the trials. Therefore, about 10% of viruses changed from CCR5-tropic to dual-mixed or non-phenotypable in the time period from screening until baseline. Throughout these trials, it appears that the percentage of isolates which are non-phenotypable using the Monogram assay ranges from 5 to 15%.

Table 8. Tropism at Baseline by Treatment Arm in Study 1027 (n=585)

	QD (n=232)	BID (n=235)*	Placebo (n=118)
CCR5	210 (91%)	212 (91%)	107 (91%)
Dual/mixed	3 (1.2%)	3 (1.2%)	2 (1.6%)
NR/NP	19 (8%)	17 (7%)	9 (7.6%)
CXCR4		1	

*2 missing tropism data

Table 9. Tropism at Baseline by Treatment Arm in Study 1028 (n=464)

	QD (n=182)	BID (n=191)	Placebo (n=91)
CCR5	158 (88%)	171 (90%)	82 (90%)
Dual/mixed	14	16	8
NR/NP	5	3	1
CXCR4			
Missing tropism data	5	1	

CENSORED DATASETS

Subjects were censored from the analysis if they discontinued with ≤400 copies/mL serum HIV-1 RNA or if they discontinued with >400 copies/mL between Baseline and Week 4 or if they discontinued between Baseline and Week 8 with at least 0.5 $\log_{10}$ decrease and no rebound (previous ≥2 $\log_{10}$ decrease with 1 $\log_{10}$ increase). Forty-nine and thirty-nine subjects were censored from the analysis of studies 1027 and 1028, respectively (Appendix B) (Tables 10 and 11).

Table 10. Outcome of Subjects in Study 1027 Resistance Dataset

Overall number of subjects in 1027 Resistance dataset	**585**
Responders <400 copies/mL serum HIV RNA	288
Responders >400 copies/mL serum HIV RNA	60
Treatment Failures	164
D/C While Suppressed (Censored)	21
Blank - no timepoints after screening (Censored)	7
D/C Before achieve viral suppression	24
D/C Before achieve viral suppression - Censored	21
Number of subjects in Censored Dataset	**536**

Table 11. Outcome of Subjects in Study 1028 Resistance Dataset

Overall number of subjects in 1028 Resistance dataset	**465**
Responders <400 copies/mL serum HIV RNA	233
Responders >400 copies/mL serum HIV RNA	54
Treatment Failures	114
D/C While Suppressed (Censored)	19
Blank - no timepoints after screening (Censored)	8
D/C Before achieve viral suppression	25
D/C Before achieve viral suppression - Censored	12
Number of subjects in Censored Dataset	**426**

Definitions of Treatment Failure:
For all of the Phase 2b/3 maraviroc clinical studies subjects were defined as treatment failures if they met any one of the following virological endpoints:

- An increase to at least 3 times the baseline (mean of all 3 values before start of dosing) plasma HIV-1 RNA level at the Week 2 visit or thereafter (confirmed by a second measurement taken no more than 14 days after the first measurement);
- HIV-1 RNA <0.5 $\log_{10}$ decrease from baseline (mean of all 3 values before start of dosing) on two consecutive measurements starting at Week 8 (second measurement taken no more than 14 days after the first measurement);
- HIV-1 RNA <1.0 $\log_{10}$ decrease from baseline (mean of all 3 values before start of dosing) on two consecutive measurements starting at Week 8 (second measurement taken no more than 14 days after the first measurement), in a subject who had previously achieved a ≥2.0 $\log_{10}$ decrease from baseline; or

- An increase in HIV-1 RNA to ≥5,000 copies/mL on two consecutive measurements taken no more than 14 days apart, in subjects previously confirmed to have undetectable levels of <400 copies/mL on 2 consecutive visits.

TROPISM OF TREATMENT FAILURES

In an as-treated outcome analysis using the censored dataset and the protocol-defined definition of treatment failure (above), the treatment failure rate was 20-26% in the MVC arms of Studies 1027 and 1028 compared to 54-57% in the placebo arm (Table 12 and 13).

Table 12. Outcome by Treatment Arm in Study 1027 Censored (n=536)

	QD (n=209)	BID (n=218)	Placebo (n=109)
Treatment failure	49 (23%)	56 (26%)	59 (54%)
No failure and VL>400 copies/mL	30 (14%)	16 (7%)	14 (13%)
No failure and VL≤400 copies/mL	119 (57%)	139 (64%)	30 (28%)
DC VL>400 copies/mL	11 (5%)	7 (3%)	6 (6%)

Table 13. Outcome by Treatment Arm in Study 1028 Censored (n=426)

	QD (n=164)	BID (n=178)	Placebo (n=84)
Treatment failure	32 (20%)	35 (20%)	48 (57%)
No failure and VL>400 copies/mL	23 (14%)	19 (11%)	11 (13%)
No failure and VL≤400 copies/mL	99 (60%)	114 (64%)	20 (24%)
DC VL>400 copies/mL	10 (6%)	10 (6%)	5 (6%)

Given the novel mechanism of action of this new drug, the reasons for treatment failure could include a co-receptor switch from CCR5-using virus to dual/mixed CXCR4-using virus, outgrowth of minor populations of CXCR4-using virus not detected at screening, resistance of CCR5-using virus to MVC, or resistance to the OBT. Therefore, we first determined the percentage of virologic failures that had CCR5-tropic and CXCR4-tropic virus at time of failure. The analysis was done using two definitions of treatment failure 1) the protocol-defined treatment failure (PDTF) definition (Table 14) and 2) subjects with PDTF plus subjects with >400 copies/mL at Week 24 (Table 15). See also Appendix D. Regardless of the definition of treatment failure, more subjects (~50-60%) failed with CXCR4- or dual/mixed-tropic virus in the MVC arms, whereas >80% of the subjects in the placebo arm failed with CCR5-tropic virus. A high percentage of treatment failure on MVC appears to be driven by tropism change from CCR5-tropic to CXCR4- or dual/mixed-tropic virus. This supports the mechanism of action of MVC and suggests emergence of CXCR4-tropic virus is a prominent reason for failure on MVC.

Table 14. Study 1027 and 1028: Tropism of Protocol-Defined Treatment Failures (n=281)

	QD (n=81)	BID (n=91)	Placebo (n=109)
CCR5	25 (31%)	24 (26%)	96 (88%)
CXCR4	10 (12%)	14 (15%)	1 (1%)
Dual/mixed	35 (43%)	42 (46%)	6 (5.5%)
NR/NP	11 (14%)	11 (12%)	6 (5.5%)

Table 15. Study 1027 and 1028: Tropism of Treatment Failures PDTF +>400 copies/mL (n=443)

	QD (n=154)	BID (n=143)	Placebo (n=146)
CCR5	72 (47%)	48 (34%)	122 (84%)
CXCR4	18 (12%)	20 (14%)	1 (0.7%)
Dual/mixed	48 (31%)	61 (43%)	11 (7.5%)
NR/NP*	16 (10%)	14 (10%)	12 (8%)

NR/NP = no replication or non-productive virus

TROPISM CHANGES ON TREATMENT

Of the 204 treatment failure subjects who were CCR5 at baseline, 67 (33%) had a change in tropism to CXCR4 or dual/mixed at time of treatment failure. All but four of these subjects were in the MVC treatment arms. The percentage of subjects with a change in tropism from CCR5 at baseline to CXCR4 or dual/mixed at time of treatment failure was 54% (31/57), 55% (32/58), and 4.5% (4/89) for the MVC QD, MVC BID, and placebo arms, respectively.

In the placebo arm, 82% of treatment failures had HIV-1 that remained CCR5-tropic compared to 22-26% of treatment failures in the MVC arms (Table 16). In contrast, 9-12% of MVC treatment failures had HIV-1 that changed tropism to CXCR4-using compared to 0% in the placebo arm. Approximately 30% of MVC treatment failures had HIV-1 that changed from CCR5-tropic to dual/mixed tropic compared to only 4% of treatment failures in the placebo arm. A high percentage of treatment failure on MVC appears to be driven by tropism change.

Table 16. Tropism Change of Treatment Failures on Treatment in Studies 1027 and 1028 (n=242)

	QD (n=68)	BID (n=77)	Placebo (n=97)
CCR5 to CCR5	26% (18)	22% (17)	82% (80)
CCR5 to Dual/mixed	34% (23)	32% (25)	4% (4)
CCR5 to CXCR4	12% (8)	9% (7)	0
CCR5 to NR/NP/BLQ/missing	12% (8)	12% (9)	5% (5)
Non-CCR5 to all	16% (11)	25% (19)	8% (8)

Seventy percent of subjects who had dual/mixed tropic virus at baseline (changing from CCR5-tropic virus at screening) were treatment failures (>400 copies/mL at Week 24). Tropism remained dual/mixed in the majority of the MVC failures who had dual/mixed tropic virus at baseline and approximately 30% changed to CXCR4-tropic virus (Table 17). The majority of failures in the Placebo grouped had CCR5-using virus at failure reflecting either normal changes in tropism from CCR5 to dual/mixed or sensitivity shortfalls of the tropism assay.

Table 17. Subjects with Dual/mixed Tropic Virus at Baseline (n=81)

	QD (n=33)	BID (n=32)	Placebo (n=16)
Failures (PDTF+ >400 copies/mL)	70% (21)	72% (23)	69% (11)
Tropism at Failure			
CCR5	5% (1)	0%	64% (7)
Dual/Mixed	62% (13)	57% (13)	27% (3)
CXCR4	24% (5)	30% (7)	9% (1)
NR/NP	9% (2)	13% (3)	

CHANGE IN $CD4^+$ CELL COUNTS

Overall, there was a greater increase in $CD4^+$ cell count from baseline to Week 24 for both MVC arms (106-109 cells/μl) compared to placebo (57 cells/μl). Not surprisingly, the increase in $CD4^+$ cell counts was also greater in the responders compared to failures with >400 copies/mL at Week 24 in all treatment groups (Table 18). There was a higher mean change in $CD4^+$ cell count for patients who failed therapy with maraviroc, compared to placebo (+49.35 and +71.06 cells/mm^3 for maraviroc once daily and twice daily, respectively, compared to +13.78 cells/mm^3 for patients receiving placebo). However, the change in $CD4^+$ cell counts (from baseline to Week 24 using the last observation carried forward at Week 24 (LOCF24)) was examined in the treatment failures (>400 copies/mL) by tropism at failure timepoint. Subjects in the MVC arms failing with CXCR4- or dual/mixed tropic virus had less of an increase in $CD4^+$ cell counts than those subjects failing with CCR5-tropic virus (Table 19). This difference was not seen in the Placebo arm. Subjects in the maraviroc arms failing with CXCR4- or dual/mixed tropic virus (n=101) had less of a median increase in $CD4^+$ cell counts from baseline (+27 cells/mm^3) (mean = 45 cells/mm^3) than those subjects failing with CCR5-tropic virus (n=49) (+88 cells/mm^3) (mean =119 cells/mm^3).

Table 18. Mean Change in $CD4^+$ Cell Counts of Treatment Failures and Responders (Median)

	QD (N=377)	BID (n=399)	Placebo (n=196)
Responders	129 (113)	118 (107)	108 (80)
Failures >400 copies/mL	87 (56)	87 (67)	39 (13)

Table 19. Mean (median) Change in $CD4^+$ Cell Counts from Baseline to LOCF24 by Tropism at Failure (>400 copies/mL)

Tropism at Failure	QD N=154	BID N=143	Placebo N=146
CCR5	123 (93) N=72	128 (110) N=48	38 (11) N=122
CXCR4	60 (33) N=18	52 (31) N=20	76 N=1
Dual/mixed	47 (25) N=48	63 (58) N=61	43 (14) N=11
NR/NP	70 (70) N=16	99 (103) N=14	63 (29) N=12

Subjects failing maraviroc BID with CXCR4-using virus had a lower median increase in $CD4^+$ cell counts from baseline (+27 cells/mm^3; n=56) than those subjects failing with CCR5-tropic virus (+147 cells/mm^3; n=24). The median increase in CD4 count in subjects failing in the placebo arm (n=109) was +6 cells/mm^3. The sponsor had similar results - subjects failing maraviroc BID with CXCR4-using virus had a +22 cells/mm^3 median increase in $CD4^+$ cell counts from baseline compared to +149 cells/mm^3 median increase in $CD4^+$ cell counts from baseline for subjects failing with CCR5-tropic virus and the median increase in $CD4^+$ cell counts in subjects failing in the placebo arm was +5 cells/mm^3.

Follow-up data was requested on subjects (n=42) who failed with CXCR4-tropic virus to try and determine if there were any negative consequences from the tropism change to CXCR4 tropism (See Appendix C for listing of subjects). Specifically, the data requested included tropism, $CD4^+$ cell cell counts, viral load, AIDS defining events, and new ARV treatments at all follow-up timepoints. The applicant informed us that subjects in the list who had plasma HIV RNA >400 copies/mL and did not meet the protocol-defined definition of treatment failure were not discontinued from MVC therapy at Week 24. Therefore, data was sent only for the remaining 28 subjects. Of these 28 subjects, 20 had at least one follow-up visit. Data from the 13 subjects who had multiple follow-up visits are shown in Table 20. The viruses from 65% of the subjects who had available follow-up data had changed tropism back to CCR5 or dual/mixed (Table 21). Viruses from the remaining 7 subjects remained CXCR4-tropic at the last follow-up visit, but the follow-up time for these subjects was one month or less (median time was approximately 11 days). In contrast, for the subjects whose virus was CCR5- or dual/mixed-tropic at end of follow-up, the median time to last follow-up was approximately 5 months (range 18 days to 8 months). For these subjects, viral loads remained similar to the value at treatment failure unless the subjects went on a new ARV treatment, in which case viral loads decreased. Consistent with the ongoing viremia, $CD4^+$ cell counts also declined (mean change -21, median change -3). In all 4 subjects who responded virologically to a new ARV regimen (PIDs 10050038, 10230068, 10990015 and 11090001), $CD4^+$ cell increases were seen concomitant with the reduction in viral load (Table 20). The

applicant states that no new category C AIDS-defining events were reported for any of the 28 subjects in studies 1027 ad 1028.

Table 20. Follow-up Data from Subjects Who Failed with CXCR4-tropic Virus

PID	Arm	BTRP	Date of Follow-up*	Tropism	Viral Load	$CD4^+$ cell count	New ARV
1027 10050038	QD	CCR5	21Nov2005	CXCR4	28,900	66	
			21Dec2005	CXCR4	15,300	35	
			16Feb2006	CCR5	120,000	35	
			17Apr2006	CCR5	96,300	22	
			15Jun2006	CCR5	11,400	21	TMC114
			07Aug2006		<400	67	
1027 10230068	BID	CCR5	30Jan2006	CXCR4	60,600	55	
			07Feb2006	CXCR4	40,800	85	
			13Mar2006	DM	57,100	26	
			11May2006	CCR5	76,300	19	TMC114
			25Aug2006	CCR5	5,590	101	
1027 10480028	QD	DM	26Apr2006	CXCR4	51,700	60	
			04May2006	DM	83,000	60	
			13Jun2006	DM	61,000	38	
1027 10500012	BID	CCR5	09Oct2005	CXCR4	96,200	147	
			09Nov2005	CXCR4	66,000	111	
			11Jan2006	CCR5	76,400		
			06Mar2006	DM	60,200	42	
			24Apr2006	DM	81,100	52	
			05Jun2006	CCR5	74,300	34	
1027 10680006	BID	CCR5	10May2005	CXCR4	59,900	25	
			09Jun2005	DM	79,400	35	
			05Aug2005	CCR5	146,000	5	
			28Sep2005	CCR5	129,000	7	LPV
1027 10990015	BID	CCR5	09Jul2006	CXCR4	6,610	254	
			10Jul2006	CXCR4	8,280	231	ATV
			03Aug2006		342	292	
1027 11010002	BID	DM	11Aug2005	CXCR4	48,700	6	
			01Sep2005	CXCR4	53,600	4	
			27Sep2005	DM	37,600	5	
			21Nov2005	CCR5	61,700	6	
			13Mar2006	DM	81,800	8	
			11Apr2006	CCR5	85,800	6	
1027 11090001	QD	CCR5	11Aug2005	CXCR4	8,630	238	
			21Sep2005	DM	3,350	229	

			19Dec2005	CCR5	10,400	174	
			13Feb2006	CCR5	7,940	177	FTC/TDF/ LPV
			05Jun2006		<50	224	
1027 11300004	QD	CCR5	30Dec2005	CXCR4	17,700	165	
			18Jan2006	CCR5	15,400	127	
1028 10440004	BID	CCR5	08Aug2005	CXCR4	215,000	42	
			19Sep2005	CCR5	124,000	15	
			10Nov2005	CCR5	259,000	16	
			05Jan2006	CCR5	395,000	6	
1028 10510008	BID	DM	07Nov2005	CXCR4	522,000	12	
			02Dec2005	DM	1,460,000	12	
1028 10890001	BID	DM	17Feb2006	CXCR4	60,800	103	
			10Mar2006	CXCR4	34,200	140	
1028 11130002	QD	CCR5	23Feb2006	CXCR4	27,000	514	
			20Mar2006	DM	28,400	493	
			16May2006	CCR5	40,700	364	AZT

***First date is failure date.**

Table 21. Summary Data for Subjects Failing MVC Treatment with CXCR4-tropic Virus

Arm	#Subjects With Follow-up Data	Median Time From Failure to Follow-up	# Subjects who had CXCR4-tropic Virus at Follow-up	# Subjects who had decreased CD4+ cells	Mean change CD4+ cells	Median change CD4+ cells	# Subjects with AIDs defining event
All	**20**	**33**	**35% (7)**	**50% (10)**	**-21**	**-3**	**0**
QD	**6**	**41**	**33% (2)**	**83% (5)**	**-53**	**-38**	**0**
BID	**14**	**37**	**36% (5)**	**36% (5)**	**-11**	**0**	**0**

FAILURE OF OPTIMIZED BACKGROUND THERAPY

As indicated above, another reason for treatment failure could be resistance to the other drugs in the OBT. Most subjects typically had low PSS/GSS scores at screening, indicating reduced susceptibility to their OBT. The mean GSS and PSS scores (baseline susceptibility to OBT) in subjects who responded was higher than the scores in subjects who failed therapy (Table 22).

Table 22. Mean GSS and PSS Scores of Treatment Failures vs. Responders (N)

		QD	BID	Placebo
Failures -	GSS	1.25 (155)	1.10 (143)	1.19 (146)
	PSS	1.65 (155)	1.56 (143)	1.73 (146)
Responders -	GSS	1.86 (222)	1.75 (256)	2.80 (50)
	PSS	2.3 (222)	2.21 (256)	2.98 (50)

As the number of susceptible drugs in the OBT increased, reflected in an increased OSS score, the percent of subjects who achieved <400 copies/mL increased. The percent of subjects who achieved <400 copies/mL increased in the MVC arms to 70% if 3 or more susceptible drugs in OBT. In the placebo arm, response rates were <20% with less than 2 active drugs, but increased to 61% when subjects had 3 or more susceptible drugs in OBT (Table 23).

Table 23. Percent Responders (<400 copies/mL) by OSS score at Baseline

OSS	QD	BID	Placebo
0	25% (13/51)	40% (39/53)	6% (2/35)
1	54% (63/116)	56% (72/129)	7% (3/44)
2	67% (53/79)	72% (74/102)	16% (9/55)
≥3	72% (89/124)	78% (87/111)	61% (35/57)

The susceptibility to drugs in the OBT at baseline and treatment failure was analyzed in the subjects who failed treatment (PDTF +>400 copies/mL Week 24). Twenty-eight percent of treatment failure subjects had no susceptible drugs in the OBT at baseline. See Appendix E. Forty-three percent of treatment failure subjects lost susceptibility to drugs in their OBT on treatment. There was no difference in the number of susceptible drugs in the OBT at baseline or loss of susceptible drugs in OBT on treatment between the arms (Table 24).

Table 24. OBT of Failures in Studies 1027 and 1028

	QD (N=154)	BID (N=143)	Placebo (n=143)
Subjects with No Susc drugs at BL	**29% (45/154)**	**29% (41/143)**	**27% (39/143)**
Subjects with Changes in OBT on Therapy	**43% (55/128)**	**42% (55/132)**	**46% (60/131)**
ENF Use	**45% (69/154)**	**45% (65/143)**	**45% (65/143)**
ENF Mutations at Failure	**71% (49/69)**	**52% (34/65)***	**74% (48/65)***

*2-sided p-value = 0.01

Enfuvirtide (ENF) use was examined separately from other drugs in the OBT. Overall ENF use was comparable between arms at 45% (Table 24) with more use of ENF in Study 1027 (approximately 50% in Study 1027 and 40% in Study 1028) (Appendix E). There were significantly fewer ENF mutations that developed on ENF treatment in the MVC BID arm than in the placebo arm (p=0.01) or the MVC QD arm (p =0.02) (Table 24). Interestingly, the data in Table 25 suggests that more subjects who failed on MVC with CXCR4-tropic virus developed resistance to drugs in the OBT and ENF.

Table 25. Loss of Susceptibility to the OBT of Treatment Failures (>400 copies/mL) on Treatment

	QD		BID		Placebo	
Tropism at Failure	**Resistance to OBT on Treatment**	**ENF mutations at Failure**	**Resistance to OBT on Treatment**	**ENF mutations at Failure**	**Resistance to OBT on Treatment**	**ENF mutations at Failure**
CCR5	31% (16/52)	72% (23/32)	41% (17/41)	38% (9/24)	44% (49/111)	75% (41/55)
CXCR4	47% (8/17)	100% (7/7)	53% (10/19)	83% (10/12)	1/1	
Dual/mixed	57% (25/44)	58% (14/24)	37% (22/60)	55% (11/20)	56% (5/9)	80% (4/5)

Analysis of the number of susceptible drugs in the OBT at baseline by tropism shows that in general in the MVC arms (especially the BID arm), as the number of susceptible drugs in the OBT increased, the percentage of failures who failed with CCR5-tropic virus increased (Table 26). Many of the subjects who failed with CCR5-tropic virus and ≥3 baseline susceptible drugs in the OBT had low viral loads (<1,000 copies/mL) at Week 24. In the placebo arm, the percentage of failures who failed with CCR5-tropic virus remained constant at around 85%.

Table 26. Tropism at Failure by Number of Susceptible Drugs in OBT at Baseline

	QD				BID				Placebo			
# Susceptible Drugs in OBT at Baseline	**0 n=45**	**1 n=44**	**2 n=32**	**≥3 n=31**	**0 n=41**	**1 n=49**	**2 n=21**	**≥3 n=32**	**0 n=39**	**1 n=37**	**2 n=33**	**≥3 n=34**
CCR5	50%	34%	44%	65%	29%	24%	38%	50%	85%	86%	79%	85%
CXCR4	14%	11%	16%	6%	12%	22%	19%				3%	
Dual/mixed	23%	50%	25%	23%	56%	39%	33%	38%	5%	11%	3%	9%
CXCR4/Dual/ mixed Combined	37%	61%	41%	29%	68%	61%	52%	38%	5%	11%	6%	9%
NR/NP	14%	5%	16%	6%	2%	14%	10%	12%	10%	3%	15%	6%

An examination of OSS of the treatment failures by tropism shows that 80% of subjects who failed with CXCR4-tropic virus had OSS of 0-1 compared to only 3% that had 3 or more active drugs in OBT (Table 27). Whereas, 50-60% of subjects who failed with CCR5 or dual/mixed tropic virus had OSS of 0-1 compared to less than 20% with susceptibility scores of 3 or more.

Table 27. Overall Susceptibility Scores of Treatment Failures by Tropism

OSS	CXCR4-tropic n=29	CCR5-tropic n=163	Dual/Mixed n=94	All n=320
0	21% (6)	27% (44)	24% (23)	25% (79)
1	59% (17)	28% (46)	39% (37)	35% (113)
2	17% (5)	24% (39)	23% (22)	23% (74)
3	3% (1)	12% (20)	7% (7)	10% (31)
4		6% (10)	2% (2)	4% (14)
5		1% (2)	1% (1)	1% (4)
6			1% (1)	0.3% (1)

THERAPEUTIC CONCENTRATIONS

PK/PD analyses indicated that 75 ng/mL MVC might be a good target MVC concentration to achieve a response of HIV-1 RNA <400 or <50 copies/mL on MVC treatment (See Pravin Jadhav's review). Using this value, 41% (190/464) of subjects with <75 ng/mL MVC C_{min} concentrations were treatment failures (>400 copies/mL). Conversely, 64% (190/297) of treatment failures (>400 copies/mL) had <75 ng/mL MVC C_{min} concentrations.

The percentage of subjects (<75 ng/mL MVC C_{min} concentrations and treatment failures >400 copies/mL) with change in the OBT susceptibility at end of treatment was 45% (72/161). The percentage of subjects (<75 ng/mL MVC C_{min} concentrations and treatment failures >400 copies/mL) with no susceptible drugs in OBT at Baseline was 26% (49/190). These values were similar to the results from the studies overall (See Table 24 above). An analysis of the treatment failure subjects with <75 ng/mL MVC C_{min} concentrations by tropism showed similar results to the overall results of failure in MVC QD dosing (Table 28).

Table 28. Tropism of Treatment Failure Subjects with <75 ng/mL MVC C_{min} Concentrations

Tropism	% of Failures with <75 ng/mL MVC (n)
CCR5	46% (87)
CXCR4	13% (24)
Dual/mixed	32% (61)
NR/NP	9% (18)

Interestingly, the 5 subjects from the sub-study analysis who failed with CCR5 virus and had evidence of MVC resistance through lower plateaus in maximum percentage inhibition also had MVC C_{min} concentrations <75 ng/mL (Table 29). One of the treatment failure subjects with CCR5-tropic virus and a 3-fold shift in EC_{50} value in MVC susceptibility also had <75 ng/mL C_{min} concentrations (Table 30).

Table 29. Subjects with Lower plateaus in MIP had C_{min} concentrations of <75 ng/mL

Study	PID	C_{min}	Baseline OSS	TRP at failure
1027	10460014	12.40773	1	CCR5
1027	10950001	34.47289	0	CCR5
1028	10290004	49.33684	0	CCR5
1027	10680001	PLC	2	CCR5
1027	10070008	34		CCR5

Table 30. MVC Treatment Failure Subjects with 3-fold Shifts in MVC Susceptibility (EC_{50} value)

Study	PID	MVC C_{min}	Baseline OSS	TRP at failure
1027	10050022	0.080208	3	CCR5
1028	10800003	131	0	5

RESISTANCE AND TROPISM

Virology Sub-studies

An investigational, interim cell culture analysis of virus selected from a pool of 267 subjects from clinical studies A4001027 and A4001028, who initiated treatment on blinded therapy before 1st June 2005 and therefore had the potential to reach Week 24 by December 1, 2005, was submitted in the NDA. Two hundred thirteen of these subjects received maraviroc and 54 received placebo. Virus isolates from 38 of these subjects were identified as failing blinded therapy and having CCR5-tropic virus and were selected for further analysis. The objective was to identify possible phenotypic and genotypic markers associated with maraviroc resistance in vivo.

Fifty subjects comprising responders and non-responders whose virus was categorized as CXCR4 or dual/mixed tropic on treatment were identified. A second cell culture study analyzed virus from 20 of these subjects in whom CXCR4-using virus emerged during the blinded phase of treatment. The objective of this study was to understand whether the CXCR4-using virus emerges from a pre-treatment CXCR4-using reservoir (i.e., not detected at baseline) or as a result of **mutation from a CCR5-tropic progenitor ("tropism** switch").

FAILURE OF SUBJECTS WITH CCR5-TROPIC VIRUS

Of the 38 subjects who failed blinded therapy with a CCR5-tropic virus, 13 subjects were randomized to receive maraviroc. Of these, 6 received an MVC 300 mg BID dose equivalent and 7 received an MVC 300 mg QD dose equivalent.

Phenotypic susceptibility testing of viruses from subjects failing therapy with a CCR5 tropic virus in the PhenoSense™ HIV Entry assay

The susceptibility to MVC and ENF of Env recombinant pseudoviruses, generated from 37 of the 38 subjects who failed therapy, were analyzed. Thirteen of these subjects were randomized to MVC arms. The susceptibility data for one subject who received MVC 300 mg BID dose equivalent (PID #10050022, study A4001027) was found to be unreliable by the Monogram assay following repeat testing but was analyzed in PBLs.

Of the 79 plasma-derived viruses analyzed for susceptibility to maraviroc, 7 viruses (from 5 subjects) showed evidence of a lower plateau in maximum percentage inhibition (MPI) (Table 31). All of these lower plateaus in MPI were observed following failure of a maraviroc-containing regimen. Four subjects had virus which gave a lower plateau following treatment failure in the blinded study period (PID #10070008, #10460014, #10950001 and #10290004). One of these subjects subsequently received open-label maraviroc and virus from this subject continued to demonstrate a lower plateau (PID #10290004). Virus from the fifth subject, who failed the OBT + placebo arm, developed a lower plateau during the open-label maraviroc treatment phase (PID #10680001). No lower plateaus in MPI to MVC were observed in any of the baseline samples from the 37 subjects studied.

However, shifts in EC_{50} value fold change (FC) to maraviroc were generally not associated with failure to a maraviroc containing regimen. Virus from two subjects had approximately a 3-fold shift in MVC susceptibility between baseline and treatment failure (PID #10800003 and PID #10050022). All other subjects on maraviroc had EC_{50} FC values <2-fold within the normal range of the Monogram assay (0.32-1.95). In addition, there was no significant difference between the maraviroc (0.84 -1.0) and placebo (0.65 – 0.67) treatment arms for EC_{50} FC values to MVC after treatment failure (p=0.13).

Table 31. Phenotypic and Genotypic Analysis of Subjects Failing MVC with CCR5-tropic Virus

PID	Study	MVC	WK	MPI Monogram assay (MPI PBL data)	EC_{50} FC from BL	ENF (y/n)	gp160 Substitutions	OBT
10070008	1027	QD	E term	30% (-27%)	ND	y	**P13S**/A16ins/S42G	No susc drugs in OBT at BL ENF^R
10460014	1027	QD	WK24	84% (75%)	1.21	y	I20F/A25D/**I26V**	
10950001	1027	QD	WK8	80% (70%)	1.05	y	**N13H**/T22A	Change in OBT ENF^R
10290004	1028	BID MVC +OL	WK32	57% (46%)	0.59	y	T2I/**G11S**/**I26V**/ V33A/R48Q	No susc drugs in OBT at BL ENF^R

10680001	1027	PLC +OL MVC	WK48	41%	ND	n	**S13H/A19T/I26V** and P16A/A19S [1]	
10800003	1028	BID	WK24	100%	**3.1**	n	K18R	ENF^R
10050022	1027	BID	WK16	(92%)	**3.22**		**P13S**	No change
10350010 rebound wk16	1027	BID	WK24	97%	0.79	n		Change in OBT
10470002 rebound wk8	1027	BID	WK20	99%	0.91	n		No change
10930011 never suppressed	1027	BID	WK32	99%	1.34	y		No susc drugs in OBT at BL ENF^R
10220001 rebound wk2	1027	QD	WK8	100%	0.81	n		No change
10300002 rebound wk20	1027	QD	WK20	98%	1.92	y		ENF^R
10690003 rebound wk4	1028	QD	E term	100%	1.28	y		No susc drugs in OBT at BL ENF^R
11000002 never suppressed	1028	QD	WK8	96%	0.89	y		Change in OBT ENF^R
10350001	1027	PLC	WK12	98%	0.82	n	L4P/Q13H/M14I/ D25E/I26V/K32Q	
10460003 [2]	1027	PLC	E term	98%	1.06	n	No changes	
10460013	1027	PLC	WK8	97%	1.02	n	No changes	

MPI = maximum percentage inhibition
ND = EC_{50} values could not be determined because MPI did not reach 50%
OL = open-label
[1]two pathways to MVC resistances
[2]EC_{50} values 2.65 and 2.82 at baseline and E term, respectively.

Genotypic (gp160) Analysis for Clones Selected from Baseline and Treatment Failure for the Subgroup of 10 Subjects

The entire Env (gp160) nucleotide sequence was obtained for all clones and timepoints from 10 subjects in an attempt to determine genotypes associated with decreased MVC susceptibility. Genotypic analysis focused primarily on the V3 region (amino acids 300-350) of gp160. The 5 subjects on MVC whose virus showed lower plateaus in maximum percentage inhibition with maraviroc in the **PhenoSense™ assay all had amino acid** changes in the V3 loop which were present at failure timepoints but were not present at Day 1 (Table 31). The V3 loop sequences reflected the heterogeneity associated with the V3 region of gp120. At the early termination timepoint, the isolate from Subject 10070008 had the amino acid substitutions P13S and S42G and an insertion of alanine at position 16 that were not present at baseline. At Week 24, the isolate from Subject

10460014 developed amino acid substitutions I20F/A25D/I26V and at Week 8 the isolate from Subject 10950001 developed substitutions N13H/T22A.

In the isolate from Subject #10290004, amino acid substitutions G11S and I26V were observed at failure (Week 8) but not at Day 1. Following discontinuation of the blinded treatment phase, only 7 clones from this subject carried the double mutation at Week 16 and evidence of a lower plateau in MPI. The subject subsequently went on to receive open-label MVC which resulted in the re-appearance of the 2 amino acid substitutions G11S and I26V in all clones by Week 32 and MVC-associated lower plateaus in MPI in the PhenoSense™ assay.

In the isolate from Subject #10680001, who was on the placebo arm of the trial, lower plateaus in all 12 clones were only seen at the Week 48 timepoint following open-label access to MVC. Two V3 genotypes were identified in this subject's isolates at Week 48: 10 clones had the substitutions S13H/A19T/I26V and 2 clones had substitutions P16A/A19S. This finding suggests that there were 2 pathways to MVC resistance in this subject.

One V3 amino acid change each was seen in the 2 MVC subject isolates which had a shift in EC_{50} fold change value at treatment failure: K18R in Subject 10800003's isolate and P13S in Subject 10050022's isolate. In isolates from the 3 subjects who had received placebo as part of their blinded treatment regimen, PIDs #10460003 and #10460013, no amino acid changes from screening were found in the V3 loop. Isolates from PID # #10350001 had numerous amino acid changes in the V3 loop (L4P/Q13H/M14I/D25E/I26V/K32Q) including changes at amino acid positions 13, 25 and 26 which were also changed in the subjects who demonstrated lower plateaus in MPI. No specific V3 amino acid changes were consistently observed in all clones at failure. However, changes at either position 13 or 26 were seen in the V3 loop of all five of the subjects with MVC-associated lower plateaus in MPI. A P13S amino acid substitution was also seen in Subject 10050022's isolates which had a shift in MVC EC_{50} value from baseline.

Amino acid changes were also identified outside of the V3 region in gp160 of some subject isolates. Of the five subject isolates in whom lower plateaus in MPI were observed, three (PID #10680001, #10460014, #10070008) had changes outside the V3 loop. The relevance of these substitutions in association with MVC resistance is not known.

Confirmation of the Role of the V3 Loop Amino Acid Substitutions in MVC Resistance by Site-Directed Mutagenesis

Site-directed mutagenesis (SDM) was performed on representative env clones from baseline and failure timepoints from four of the subjects who had lower MVC resistance-associated plateaus in MPI (Table 32; Report UK427857/DI/154/06, page 11). The aim of these experiments was to confirm the role of the V3 loop amino acid substitutions in

conferring MVC resistance. In isolates from all four subjects, the V3 loop amino acid substitutions played a role in MVC resistance. In isolates from two of the four subjects (10070008 and 10460014), mutating the V3 loop amino acids to P13S/A16$_{insertion}$ and I20F/A25D/I26V, respectively of the Day 1 clones resulted in a MVC-resistant phenotype (i.e., <95% MPI) and back-mutation of the amino acid changes in the V3 loop of the failure clones resulted in a MVC-sensitive phenotype. Therefore, these substitutions were both necessary and sufficient for MVC resistance, i.e. the MVC resistance phenotype of <95% MPI could be recovered by mutation of the codons for these residues.

In the isolate from Subject 10950001, mutation of the V3 amino acid N13H was sufficient to alter the MVC-sensitive phenotype of >95% MPI to a MVC-resistant MPI of 66%. The reverse mutation of this amino acid in the MVC-resistant *env* clone with an MPI of 50% increased the susceptibility of the SDM clone to MVC with an MPI of 91%. However, this change was insufficient to restore sensitivity to >95% MPI.

In the isolate from Subject 10290004, mutation of the V3 amino acids (S11G and V26I) in the MVC-sensitive *env* clone with an MPI of >95% had no effect on the phenotype (MPI 100%). However, mutation of these amino acids in the MVC-resistant *env* clone with an MPI of 55% did restore the MVC-sensitive phenotype of clone to MPI >95%. Therefore, these mutations were necessary, but not sufficient for the MVC resistant phenotype. Perhaps the other substitutions (T2I, V33A and R48Q) that developed in the V3 loop on MVC treatment and were not examined in this study played a role in MVC resistance.

Table 32. MVC Susceptibility of Parental and Site-Directed Mutagenesis *env* Clones

Patient PID (Study code)	Clone ID	Patient time-point[d]	Clone type	MPI MVC	V3 sequence[e]
10070008 (A4001027)	119186-START	Day 1	WT[c]	100[a]	
	119186-START(SDM V3 Fail)		SDM[c]	41[a]	
	149672-FAIL	E_TERM[d]	WT[c]	51[a]	
	149672-FAIL(SDM V3 Start)		SDM[c]	98[a]	
10290004 (A4001028)	117153-START	Day 1	WT[c]	100[a]	
	117153-START(SDM V3 Fail)		SDM[c]	100[a]	
	132441-FAIL	Week 8	WT[c]	55[a]	
	132441-FAIL(SDM V3 Start)		SDM[c]	99[a]	
10460014 (A4001027)	123762-START	Day 1	WT[c]	100[a]	
	123762-START(SDM V3 Fail)		SDM[c]	85[a]	
	154141-FAIL	Week 24	WT[c]	63[a]	
	154141-FAIL(SDM V3 Start)		SDM[c]	99[a]	
10950001 (A4001027)	119228-START	Day 1	WT[c]	96[a]	
	119228-START(SDM V3 Fail)		SDM[c]	66[a]	
	128538-FAIL	Week 8	WT[c]	50[a]	
	128538-FAIL(SDM V3 Start)		SDM[c]	91[a]	

Susceptibility to MVC of Env Clones is in Agreement with the Data Obtained on the Pooled Virus

A total of 12 clones from day 1, treatment failure and, where available, open-label treatment were evaluated in the PhenoSense™ Entry assay against MVC and ENF. The drug susceptibility data obtained with the clones was in agreement with the pooled samples. Lower plateaus in MPI were observed in Env clones from the treatment failure time-points of all 5 subjects who failed maraviroc-containing regimens and showed evidence of lower plateaus in the pooled sample (PIDs #10070008, #10290004, #10460014, #10680001 and #10950001). While individual Env clones derived from a given subject sample varied in plateau height, the frequency and average MPI among the 12 env clones was consistent with the MPI seen in the pooled sample. MVC susceptibility data obtained for clones of the 2 subjects with 3-fold shifts in EC_{50} values to MVC (#10800003 and #10460003) concurred with data from the pooled sample. Furthermore, the phenotypic susceptibility data to ENF of env clones was in agreement with susceptibility data obtained for the pooled sample.

Phenotypic Susceptibility of Env Recombinant NL4-3 Viruses to MVC in the PBL Antiviral Assay

In order to confirm that the lower plateaus and EC_{50} value shifts seen in the PhenoSense™ HIV Entry assay could be reproduced in PBLs (the host target cell for HIV-1 in vivo), a cloning strategy was employed to transfer the entire gp160 envelope genes into an infectious HIV-1 clone. The Env from four subject viruses that showed lower MVC resistance-associated plateaus at failure (PIDs #10070008, #10290004, #10460014 and #10950001) and from two subject isolates which demonstrated MVC EC_{50} value shifts (PIDs #10050022 and #10800003) were cloned into the pNL4-3 background. These subjects had all been selected prior to unblinding, all were subsequently found to be on the MVC arm of the study. Phenotypic results for maraviroc resistance were qualitatively similar in the PBL and PhenoSense™ HIV Entry assays. Lower plateaus in MPI were observed for the 4 subjects who failed a maraviroc-containing regimen (PIDs #10070008, #10290004, #10460014 and #10950001) in both assays, while virus from subject #10800003 showed a shift in EC_{50} value from baseline to MVC at failure in both assays (Table above).

Not All Subjects Failing Treatment on MVC had Phenotypic Markers of MVC Resistance

Seven subjects receiving MVC during their blinded phase of treatment did not show phenotypic markers of MVC resistance in the entry assay (Table above). The majority of subjects (5/7) had evidence of reduced susceptibility to one or more drugs within their OBT at screening and/or failure.

Summary

These clinical results are consistent previous findings from cell culture studies of MVC resistant virus that lower plateaus in maximum percentage inhibition (MPI) to maraviroc were associated with resistance rather than EC_{50} fold changes. Changes in the V3 loop

region of the viral envelope (gp120) appeared to correlate with the presence of lower plateaus and maraviroc resistance. Although there is heterogeneity of the envelope protein and likely multiple pathways to MVC resistance, substitutions at amino acid positions P13 and V26 occurred in the V3 loop of the five examined MVC treatment failure isolates that demonstrated lower plateaus in MPI. Additionally, a P13S amino acid substitution was also seen in Subject **10050022's isolates which had a shift in MVC** EC_{50} value from baseline.

- Lower plateaus were detected in viruses from some subjects failing maraviroc regimens. Maraviroc dose response curves demonstrating lower plateaus in MPI (<95%) were observed in 4 subjects following failure of a maraviroc-containing regimen during the blinded study period and from a fifth subject who failed in the placebo arm and developed a lower plateau following open-label MVC.
- Lower plateaus in the PhenoSense™ HIV Entry assay correlated with the data obtained with Env clones and in PBL assays.
- Changes in the V3 sequence of the Env gp160 in clones at treatment failure correlated with the presence of lower plateaus and Maraviroc resistance.
- Although there is heterogeneity of the envelope protein and likely multiple pathways to MVC resistance, substitutions at amino acid positions 13 and 26 occurred in the V3 loop of the five examined MVC treatment failure isolates that demonstrated lower plateaus in MPI. [Additionally, a P13S amino acid substitution was also seen in Subject 10050022 who had a shift in MVC EC_{50} value from baseline.]
- Changes outside the V3 loop were observed in some subject viruses and the impact of these is not understood.
- Fold changes in EC_{50} values to maraviroc were not generally associated with failure to a maraviroc containing regimen.
- Resistance to the fusion inhibitor enfuvirtide was not cross-resistant to Maraviroc.
- Maraviroc resistance did not result in cross-resistance to the fusion inhibitor Enfuvirtide.

CROSS-RESISTANCE

Viruses with Reduced Susceptibility to Enfuvirtide are Susceptible to MVC in Cell Culture

In the subgroup analysis, genotype data for gp41 were available for 33 subjects at study screening. Of these, 16 were identified as having mutations in gp41 associated with ENF resistance and 17 subjects had no specific mutations present and were classified as ENF sensitive. Geometric mean values representing EC_{50} value fold changes to reference (JRCSF) for MVC showed no difference between the ENF-resistant and ENF-sensitive genotypes (0.76-fold and 0.68-fold respectively; $p>0.05$). In contrast, geometric mean values for ENF susceptibility correlated with the presence or absence of the gp41 specific mutations (78.7 and 1.59, respectively; $p\leq0.01$). This was further confirmed when

individual gp41 mutations (G36D, N42S/T, N43D, Q40H, V38A) were studied for MVC and ENF susceptibility and ENF EC_{50} value fold changes values ranged from 2- to 883-fold while MVC fold changes values ranged from 0.32- to 1.35-fold.

The response rates of subjects with ENF-resistance mutations at screening were examined (Table 33). The percentage of treatment failures increased and the percentage of responders decreased in the MVC arms for subjects with ENF resistance at screening. These results are most likely a reflection of the need for two or more active drugs for effective response rates rather than cross-resistance between MVC and ENF. Moreover, 35-40% of subjects with ENF-resistant mutations responded on MVC treatment compared to only 4% in the placebo arm.

Table 33. Response Rates of Subjects with ENF-Resistance Mutations at Screening

	QD N=79	BID N=92	Placebo N=45
Treatment Failures	34% (26/77)	44% (40/91)	93% (42/45)
Responders (<400 copies/mL)	35% (27/77)	43% (39/91)	4% (2/45)

Treatment Failure on a Maraviroc-containing Regimen and Resistance to Enfuvirtide

Viruses from subjects following failure of a maraviroc-containing regimen showed no reduced susceptibility to ENF unless ENF was also included in their OBT (Table 34). A similar response was seen for subjects in the placebo arm. All four subjects who received MVC and developed lower plateaus in maximum percentage inhibition (MPI) to MVC following treatment failure, also received ENF as part of their OBT. Three of these had virus with reduced susceptibility to ENF at baseline and this did not significantly change following treatment failure. The fourth subject (PID #10070008) had a virus which was sensitive to ENF at baseline but highly resistant following treatment failure. This indicates that the virus became less susceptible to both MVC and ENF while being treated with both of these drugs.

Susceptibility to Aplaviroc Measured by Plateaus in MPI

Drug susceptibility data were obtained for 79 viruses using another investigational CCR5 antagonist aplaviroc (APL, GlaxoSmithKline) in the PhenoSense™ Entry assay with efavirenz (EFV) as a control. Viruses from 11 subjects showed a MPI <95% at baseline and/or treatment failure to either APL or MVC (Table 35). Since APL resistance has not been characterized and it is not known if measuring a plateau in maximal percentage inhibition is the best way to determine susceptibility to APL, it is unclear if APL and MVC are cross-resistant. All 79 viruses were susceptible to EFV.

Table 34. Changes in ENF Susceptibility for Subjects Who Failed a Maraviroc-containing Regimen

ENF in OBT		MVC BID + QD Group			Placebo Group			p-value (mean fold difference, 95% CI)
		IC_{50} Δ Resist. FC JRCSF		IC_{50} Δ Resist. FC Day 1	IC_{50} Δ Resist. FC Day 1		IC_{50} Δ Resist. FC Day 1	
		BL	Fail		BL	Fail		
YES	Geo. Mean (nM)	12.5[a]	90[a]	7.18[a]	13.3	64.1	4.83	0.57[b] (0.69, 0.18-2.60)
	Range (Min-Max)	0.91-291	32-311	0.77-343	0.39-156	0.60-964	0.64-184	
	n		7			13		
NO	Geo. Mean (nM)	5.68	5.16	0.91	8.03	3.67	0.46	0.53[b] (0.60, 0.12-3.06)
	Range (Min-Max)	1.49-121	1.52-133	0.77-1.10	0.38-883	0.36-341	0.015-1.44	
	n		4			12		
p-value (mean fold difference, 95% CI)		<0.01[b] (0.09, 0.01-0.51)			<0.01[b] (0.07, 0.02-0.23)			

Table 35. Summary of Viruses MPI Tested against Aplaviroc (APL) and Maraviroc (MVC) in the PhenoSense assay

MVC arm	Study	PID	APL (MPI)		MVC (MPI)	
			BL	Fail	BL	Fail
BID	A4001028	10290004	98	99	100	85
		11000002	85	88	95	96
QD	A4001027	10070008	99	90	100	30
		10460014	100	99	100	84
		10470002	95[a]	95	98	99
		10950001	99	99	100	80
Placebo	A4001027	10070012	94	93	97	97
		10350001	89	96	96	98
		10460003	94	94	98	98
		10460013	86	89	96	97
		10680001	93	95	96	98

BL= Day 1; MPI, maximum percent inhibition
[a]MPI = 94.7%
MPI ≤ 95% were also reported for the virus reference strain JRCSF tested against APL (but not MVC) in the same assay (with exception of PID #11000002 where the MPI values for JRCSF were 98 and 97).
Data extracted from Monogram Biosciences report PJ01636 (Appendix A3)

DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 22-128 **SN:** 000 **DATE REVIEWED:** 6/01/07
Microbiology Reviewer: Lisa K. Naeger, Ph.D.

ORIGIN OF CXCR4 OR DUAL/MIXED TROPIC VIRUS THAT EMERGED ON TREATMENT

A total of 50 subjects enrolled in clinical studies A4001027 and A4001028 were identified in whom CXCR4-using virus was detected during treatment. Fifteen (30%) of the subjects were classified as having CCR5-tropic virus at screening but dual/mixed tropic virus at Day 1, while the remaining 35 had CXCR4-tropic virus emerge on therapy. Of these 50 subjects, a representative set of 20 were chosen and viruses from these 20 subjects were analyzed in cell culture. The objective was to understand whether the CXCR4-using virus emerged from a pre-treatment CXCR4-using reservoir (*i.e.* not detected at baseline) or as a result of mutation from a CCR5-tropic progenitor ('tropism switch'). The 20 subjects were selected to include the following: 'early failures' who reached a protocol defined treatment failure by Week 8 (n=9); 'late failures' who reached protocol defined treatment failure after Week 8 (n=5) and 'responders' who did not fail by Week 24 (n=6). The 'responders' only contained subjects for whom a valid tropism result was obtained at their Week 24 visit (*i.e.*, viral load >500 copies/mL). The 20 subjects included those whose virus was classified as CCR5-tropic at screening but dual/mixed (DM) at day 1 (pre-dose, n=5), and those subjects in whom CXCR4-using virus only emerged after receiving blinded study drug (n=15) (Table 36). Of the 20 subjects selected, 16 were in a maraviroc treatment group and 4 in the placebo group (Table 36). The blind was broken for the 20 subjects described in this study report on 19th July 2006.

The nucleotide sequence of approximately 290 bases from the envelope gene encompassing the V3 loop was determined from 192 pre-treatment and 48 on-treatment clones from each of the 20 subjects. Phylogenetic trees were generated using these sequences in order to investigate possible ancestry of the CXCR4-using clones. Finally, twelve clones from each time-point (*i.e.* 24 clones per subject) were selected and tropism was confirmed in the validated format of the PhenoSense™ HIV tropism Assay (Trofile™).

Table 36. Origin of CRCXCR4 Virus that Emerged on Treatment in Studies 1027 and 1028

Subject No.	PID	Study	Treatment	Tropism at Baseline	Origin of CXCR4 virus	Protocol Defined Treatment Failure	Change OBT Susc at End (susc drugs)	C_{min} <75 ng/mL
1	10130001	1027	MVC bid	**DM**	R4 virus on-TRT was detected at BL	Early failure By Week 8	No (TDF)	yes
2	10230014	1027	placebo	**DM**	R4 virus on-TRT was detected at BL	Early failure By Week 8	No (TDF) Lost ENF	-

3	10360004	1027	MVC bid	CCR5	R4 virus on-TRT was detected at BL	Early failure By Week 8	Yes	no
4	10410002	1027	MVC qd	CCR5	R4 virus on-TRT was detected at BL	Early failure By Week 8	Yes (ENF)	yes
5	10460006	1027	MVC qd	CCR5	R4 virus on-TRT was detected at BL	Early failure By Week 8	No	yes
6	10510003	1027	MVC bid	CCR5	R4 virus on-TRT was detected at BL	Early failure By Week 8	Yes (TDF) Lost ENF	no
7	10680006	1027	MVC bid	CCR5	R4 virus on-TRT was detected at BL	Early failure By Week 8	No (TDF) Lost ENF	yes
8	10710020	1027	MVC qd	CCR5	**R4 virus distinct from CCR5 virus at BL**	Early failure By Week 8	No Lost ENF	yes
9	10200001	1028	MVC bid	CCR5	**R4 virus distinct from CCR5 virus at BL**	Early failure By Week 8	No Lost ENF	no
10	10450003	1027	MVC bid	**DM**	R4 virus on-TRT was detected at BL	Failure after Week 8	No Lost ENF	no
11	10050009	1027	MVC qd	CCR5	R4 virus on-TRT was detected at BL	Failure after Week 8	Yes Lost ENF	yes
12	10050015	1027	MVC bid	CCR5	R4 virus on-TRT was detected at BL	Failure after Week 8	Yes	no
13	10120001	1027	Placebo	CCR5	**R4 virus distinct from CCR5 virus at BL**	Failure after Week 8	No (ABC ATV LAM TDF AZT)	no
14	10370005	1027	MVC qd	CCR5	**R4 virus distinct from CCR5 virus at BL**	Failure after Week 8	No	no
15	10050010	1027	MVC qd	**DM**	R4 virus on-TRT was detected at BL	>400 copies/mL	Yes Lost ENF	yes

16	10710016	1027	placebo	**DM**	R4 virus on-TRT was detected at BL	>400 copies/mL	Yes (DDI) Lost ENF	no
17	10010016	1027	placebo	CCR5	R4 virus on-TRT was detected at BL	>400 copies/mL	Yes Lost ENF	no
18	10640004	1027	MVC bid	CCR5	R4 virus on-TRT was detected at BL	>400 copies/mL	No (ENF)	yes
19	10740001	1027	MVC qd	CCR5	**R4 virus distinct from CCR5 virus at BL**	>400 copies/mL	No	yes
20	11000003	1028	MVC bid	CCR5	**R4 virus distinct from CCR5 virus at BL**	>400 copies/mL	No	yes

CXCR4-using *env* clones in the 'on-treatment' samples from 14 subjects shared a common ancestor with a pre-treatment virus that was phenotypically and/or genotypically classified as CXCR4-using. CXCR4-using *env* clones identified in the 'on-treatment' samples from the remaining 6 subjects (30%) were genetically distinct from both the 'pre-treatment' and 'on-treatment' CCR5 population. The V3 loop sequences of the on-treatment CXCR4-using clones differed by 7-17 amino acid residues from the V3 loop of the nearest CCR5 sequence on the phylogenetic tree. There did not appear to be intermediate sequences between the CCR5 and CXCR4-using clones, which would be expected if the CXCR4-using clones had evolved from the pre-treatment CCR5 clones during treatment. The most likely explanation therefore is that the CXCR4-using clones in the 'on-treatment' samples of these 6 subjects emerged from a pre-treatment CXCR4-using reservoir that was not detected at Day 1.

CXCR4-using virus detected on-treatment shared a common ancestry with CXCR4-using clones detected at day 1 in 77% (7/9) 'early' failures and 60% (3/5) 'late' failures. Four of the 6 subjects who had CXCR4 virus detected on study drug and that were considered protocol defined responders at Week 24 were in the MVC arms. Clonal analysis identified CXCR4 virus in the day 1 samples from 3 of these 4 subjects.

PID10460006 from Study 1027 was described as having CXCR4 virus on-TRT that was detected at BL when according to the clonal analysis there are no CXCR4 or DM tropic clones at Day 1. However, there is one non-functional baseline clone that had 9 amino acid changes from the CCR5 clones including a positive charge at amino acid position 11 which is a genotypic predictor of CXCR4-tropism. At the Week 4 timepoint, the dual/mixed CXCR4 clones could be divided into two groups based on their V3 loop amino acid sequences and both groups differed from CCR5 sequences by 9 different

amino acids. The majority of clones (33/46) had V3 loop sequences identical to the non-functional clone's sequence at day 1.

Phenotypic and genotypic tropism results of env clones were in agreement. The majority of env clones classified phenotypically as 'CXCR4-using' had a V3 loop that was different from the CCR5-classified clones from the same subject. The CXCR4-using clones generally had V3 loops containing one or more genetic markers previously associated with CXCR4 usage: i.e. a higher net positive charge, loss of an N-linked glycosylation site and a basic amino acid residue at positions 11/25. Most apparent discordances between genotype and phenotype were accounted for by low/borderline RLU values in the tropism pre-screen. In these cases, the original pre-screening tropism call was not confirmed when the validated PhenoSense™ HIV Entry tropism assay was performed on representative clones from these virus populations.

Two genotypic algorithms were used in this analysis: the 11/25 rule (where lysine and/or arginine residues at amino acid positions 11 and/or 25 in the V3 loop predict CXCR4 use) and the Position-Specific Score Matrix (PSSM) 12. Both algorithms shared >80% agreement in tropism assignments with the PhenoSense HIV Tropism assay (Table 37; Report UK427857/DI/116/06, page 48).

Table 37. Concordance between PhenoSense Tropism Assay and V3 Sequence Algorithms (11/25 rule and PSSM)

PhenoSense	11/25 rule		PSSM	
Tropism assay	R5	CXCR4-using	R5 (%)	CXCR4-using
R5	96%	4%	99%	1%
(n=72)	(n=69)	(n=3)	(n=71)	(n=1)
DM/X4	34%	66%	19%	81%
(n=74)	(n=25)	(n=49)	(n=14)	(n=60)
Overall concordance	81%		90%	

Table assembled from data in Appendix A6.2. The PhenoSense HIV Tropism Assay was performed on 477 Env clones submitted for confirmatory tropism. These clones comprised 161 unique V3 sequences of which 146 were used to build this table. Clones representing 11 sequences failed the tropism test (no result) and were excluded from this analysis. Clones representing 9 sequences had more than one tropism assignment (see Appendix A8): in 5 cases this was due to a single clone and in these cases the majority tropism assignment was used in this analysis. The remaining 4 sequences were excluded as no single phenotypic assignment could be made.

The clonal tropism assay results generally support the original PhenoSense™ Entry tropism assignment made in the clinical studies. Of the 15 subjects with a virus categorized as 'CCR5' at baseline, the frequency of CXCR4-using env clones detected in the baseline sample was 7% in 14 subjects. In contrast, of the 5 subjects with a virus categorized as dual/mixed (DM) at baseline, the frequency of CXCR4-using env clones was 15% in 4 subjects. For the one subject (subject 20) with a CCR5 tropism assignment, the frequency of CXCR4-using env clones was 29% in the baseline sample, but all of the CXCR4-using clones in this sample had a very weak RLU score on the CXCR4-using cells. Conversely, one subject with a dual/mixed assignment in the

clinical study (subject 15) had 2% CXCR4-using env clones in their baseline sample, but all the CXCR4-using clones had a strong RLU score on the CXCR4-using cells and one-third of the CCR5 virus had a weak signal score. There was good agreement between the phenotypic assay (PhenoSense tropism pre-screen) and the V3 sequence (using the 11/25 algorithm and including functional and non-functional Env clones) when estimating the frequency of CXCR4-using clones in the baseline samples.

While not a validated assay format, the clonal tropism pre-screen analysis proved to be a useful method to approximate the frequency of CXCR4-using and CCR5-tropic env clones in a subject sample. For example, of 480 functional env clones assigned a tropism call in the pre-screen and subsequently re-tested in the validated PhenoSense™ HIV Tropism assay (Trofile), 388 (80%) had the same tropism call in both assay formats. If dual/mixed and CXCR4 tropism calls are grouped together as "CXCR4-using", then the percentage concordance between pre-screen and tropism confirmation rises to 86%. The majority of discordant tropism assignments between the pre-screen and validated tropism formats of the PhenoSense™ HIV tropism assay were the result of low/borderline RLU values in the tropism pre-screen. The strategy to screen approximately 180 env clones based on probability modeling was able to identify low frequency CXCR4-using env clones in the baseline samples of most subjects.

Evidence of a switch in co-receptor usage of a CCR5-tropic virus during the course of the study appears unlikely. The on-treatment CXCR4-using virus emerged from a subsequently-identified pre-treatment CXCR4-using clone(s) or was shown to be phylogenetically distant from the CCR5-tropic baseline virus. Therefore, emergence of pre-treatment archived CXCR4 virus was by far the most likely origin of CXCR4-using virus which emerged in 20 subjects enrolled in the Phase 2b/3 clinical studies of maraviroc (16 subjects on MVC arms and 4 subjects on placebo).

Summary

The primary findings were that CXCR4-using virus, detected during blinded treatment of these subjects, originated from a pre-existing CXCR4-using virus reservoir, regardless of treatment arm (maraviroc or placebo) or time of onset of virology failure. Generally, the baseline (Day 1) samples from subjects whose virus was classified as CCR5-tropic at baseline (n=14) had a low (≤7%) frequency of CXCR4-using *env* clones. This supports the 10% sensitivity performance assessment of the assay.

The results of Study A4001027 and A4001028 were further supported by results from studies A4001026 and A4001029.

STUDY A4001029

Study A4001029 is an ongoing multi-center, randomized, double-blind, placebo-controlled trial of MVC in combination with OBT vs. OBT alone for the treatment of ARV-experienced non-CCR5 tropic (CXCR4- or dual/mixed-tropic) HIV-1 infected

subjects. One of the main objectives of this study was to investigate if there was a negative effect in subjects with CXCR4- or dual/mixed-tropic virus receiving MVC because of MVC-driven changes in tropism.

Baseline analysis of Study 1029 determined that baseline genotypic and phenotypic measures of resistance were comparable between arms (Tables 38 and 39). Tropism assessments showed that 80% of subjects had dual/mixed tropic virus at baseline and 9% of subjects had changed tropism assignment to CCR5-tropic from screening to baseline (consistent with what was seen in studies 1027 and 1028) (Table 40).

Table 38. Mean (Median) Baseline Resistance in Study 1029 (n=184)

	QD (n=63)	BID (n=60)	Placebo (n=61)
GSS	1.55 (1)	1.83 (2)	2.25 (2)
PSS	2.26 (2)	2.56 (2)	2.55 (3)
OSS	1.97 (2)	2.27 (2)	2.37 (2)

Table 39. Baseline Resistance in Study 1029, N (%)

		Study 1029		
		QD (n=63)	BID (n=60)	Placebo (n=61)
GSS	0	6 (10%)	6 (10%)	3 (5%)
	1	30 (48%)	23 (38%)	20 (33%)
	2	16 (25%)	15 (25%)	8 (13%)
	≥3	10 (16%)	15 (25%)	29 (48%)
PSS	0	0	2 (3%)	1 (2%)
	1	13 (21%)	7 (12%)	14 (23%)
	2	25 (40%)	21 (35%)	11 (18%)
	≥3	24 (38%)	29 (48%)	34 (56%)
OSS	0	1 (2%)	2 (3%)	1 (2%)
	1	20 (32%)	13 (22%)	16 (26%)
	2	24 (38%)	22 (37%)	14 (23%)
	≥3	17 (27%)	22 (37%)	29 (48%)

Table 40. Tropism at Baseline by Treatment Arm in Study 1029 (n=184)

	QD (n=63)	BID (n=60)	Placebo (n=61)
CCR5	4	6	7 (11%)
Dual/mixed	55	45	48 (79%)
NR/NP	3	6	3 (5%)
CXCR4		2	3 (5%)

Greater than 50% of the subjects in this study experienced treatment failure or had >400 copies/mL HIV RNA at Week 24 and there was no difference between MVC arms and

placebo (Table 41). About 30% of the subjects achieved ≤400 copies/mL HIV RNA at Week 24.

Table 41. Outcome by Treatment Arm in Study 1029 (n=184)

	QD (n=63)	BID (n=60)	Placebo (n=61)*
Treatment failure	35 (56%)	24 (40%)	24 (41%)
No failure and VL>400	7 (11%)	7 (12%)	10 (17%)
No failure and VL≤400	18 (29%)	21 (35%)	14 (24%)
DC VL>400	2 (3%)	8 (13%)	9 (16%)
DC VL≤400	1 (1.5%)		1 (2%)

*3 protocol violations

The main findings of a post-hoc analysis of viral tropism and $CD4^+$ cell counts at Week 24 were:

- Approximately 50% of the subjects discontinued (failed) treatment by Week 24 and about 25% had <500 copies/mL HIV RNA and there was no difference between the MVC and placebo arms.
- Subjects who experienced treatment failure or had >500 HIV RNA copies/mL at Week 24 on MVC in Study 1029 were more likely to have CXCR4-tropic virus at failure than subjects failing on placebo. Specifically, 45% (24/53) subjects had a CXCR4-tropic virus at failure in MVC arms compared to 9% (2/22) subjects in placebo.
- Increases in $CD4^+$ and $CD8^+$ cell counts were higher for both MVC treatment arms compared to placebo ($p<0.05$).
- For subjects on MVC who virus was dual/mixed at screening, changes from baseline in $CD4^+$ cell counts were greater for those responding to MVC treatment at Week 24 (median +80-98) than those who experienced treatment failure (median +15-19).
- Changes in $CD4^+$ cell counts from baseline to time of treatment failure were the same for subjects on MVC whose virus was CXCR4-tropic at failure to those whose virus remained dual/mixed tropic virus at failure.

STUDY A4001026

Subjects Failing Treatment with CXCR4-Tropic virus in the MVC QD arm of Study A4001026

Study A4001026 is a phase 2/3, 96-week, non-inferiority study to compare MVC 300 mg QD or BID vs. EFV, each in combination with AZT/LAM for the treatment of ARV-naïve subjects infected with HIV-1. A formal interim efficacy analysis was performed was planned when 205 subjects had been treated for 16 weeks. The DSMB upon reviewing the data recommended discontinuation of the MVC QD arm because it failed to meet pre-specified criteria for establishing non-inferiority to EFV. Following

termination of the MVC QD arm of Study 1026, 6% of subjects (11/174) had been discontinued due to lack of efficacy. Of these, 3 subjects were identified as having failed with a CXCR4-tropic virus (PIDs 10610018, 11490016, and 12140005).

All 3 subjects had 8-37% detectable CXCR4-tropic clones in their baseline sample, which confirms that these subjects had CXCR4-tropic virus that pre-existed treatment with MVC (Table 42; Report UK427857/DI/140/06 page 5). The relative frequency of CCR5-tropic clones was reduced in samples from the subjects on MVC treatment, which is consistent with MVC selectively inhibiting CCR5-tropic clones in the subjects. After these three subjects went off MVC treatment, only CCR5-tropic functional clones were detected in the first post-treatment sample.

Table 42. Subjects Failing Treatment with CXCR4-Tropic virus in the MVC QD arm of Study A4001026

Sample Details			Tropism result (Trofile™, Clinical program)			Functional Env clones (Investigational analyses, this report) among functional clones:		
PID	CPE	ACC	Tropism Assignment	R5 RLU	X4 RLU	R5 n (%)	X4 n (%)	DM n (%)
10610018	SCREENING	05-121076	R5	276958	74	20 (**100**)	0 (0)	0 (0)
	DAY1	05-128936	DM	258946	903	17 (**63**)	0 (0)	[redacted]
	WEEK8	05-140085	DM	346605	269066	1 (3)	1 (3)	28 (**93**)
	WEEK24	05-158714	DM	33413	27496	0 (0)	0 (0)	11(**100**)
	E_TERM	06-104335	R5	247816	87	18 (**100**)	0 (0)	0 (0)
11490016	SCREENING	05-114155	R5	1160999	109	25 (**100**)	0 (0)	0 (0)
	DAY1	05-121063	DM	1874416	612	23 (**92**)	0 (0)	[redacted]
	WEEK16	05-141488	DM	943147	258437	0 (0)	0 (0)	12 (**100**)
	E_TERM	05-155749	DM	794288	399967	4 (24)	0 (0)	13 (**76**)
	WEEK32	05-160934	R5	750036	51	25 (**100**)	0 (0)	0 (0)
12140005	SCREENING	04-154412	R5	1462932	97	14 (74)	1 (5)	4 (21)
	DAY1	05-105431	DM	473250	2060	23 (**85**)	0 (0)	[redacted]
	WEEK2	05-107773	DM	122487	23498	13 (43)	1(3)	15(**52**)
	WEEK12	05-119959	R5	368663	102	21 (**100**)	0 (0)	0 (0)
	WEEK16	05-125290	DM	486759	3813	8 (**57**)	0 (0)	6 (43)

Figures shown in bold indicate the majority clones at that timepoint. Figures shown in [redacted] indicate that DM clones were identified in the Day 1 (pre-dose) sample, confirming that DM virus pre-existed the active treatment phase in these patients. The dotted line indicates when blinded MVC was initiated; the dashed line indicates when blinded MVC was stopped. PID =patient ID, CPE= study visit, ACC=Monogram accession ID; RLU=relative light units. Source of data for this table: Appendix A2 (using corrected data for functional clones, rightmost columns) and Appendix A3.

The M184V LAM-resistance mutation in RT was detected in all three subjects at the time of treatment failure. In subject 10610018, AZT-resistance RT mutations M41M/L and K70R/K were also detected. However, these mutations were not detected in the post-treatment samples (26 days after treatment cessations), which were also CCR5-tropic. These results suggest that the RT mutations are on the CXCR4-tropic virus, not the CCR5-tropic virus. This linkage cannot be proven with the existing PhenoSense^{GT} and Trofile assays because they separately amplify regions of the virus from plasma.

Subjects 10610018 and 11490016 had 1-3 log_{10} decreases in viral load and reached protocol defined treatment failure at Week 24. These two subjects also had increases in $CD4^+$ cell counts. After discontinuation of MVC, the viral load returned to almost baseline levels and only CCR5-tropic virus was detected. Subject 1214005 had an initial drop in viral load at Week 2, but rebounded to baseline by Week 4 at which time he discontinued treatment. The low $CD4^+$ cell count at baseline of 29 cells/mm^3 increased to 72 cells/mm^3 by Week 4. After discontinuation of MVC, only CCR5-tropic virus was detected at follow-up Week 12. The subject started on FosAPV/rtv, TDF, and FTC. After an initial response to <50 copies/mL, he failed treatment and again had dual/mixed tropic virus at the Week 40 off study drug visit.

Summary

Analysis of three subjects who failed on 300 mg MVC QD with CXCR4-tropic virus from treatment naïve study 1026 showed that the CXCR4-tropic virus clones were present at baseline. The proportion of CCR5-tropic viruses was reduced during MVC treatment and was reversed after MVC treatment was stopped. In all three subjects, the LAM-resistance M184V mutation was detected at failure and the viruses at failure were dual/mixed tropic. In one subject, the AZT-resistance RT mutations M41M/L and K70R/K were also detected. When treatment with MVC and AZT/LAM was stopped, CCR5-tropic virus was again detected and the RT mutations were not detected in the post-treatment samples. These results suggest that the RT mutations are on the CXCR4-tropic virus, not the CCR5-tropic virus, although this cannot be proven with the existing PhenoSense^{GT} and Trofile assays because they separately amplify regions of the virus.

PHARMACOGENOMICS

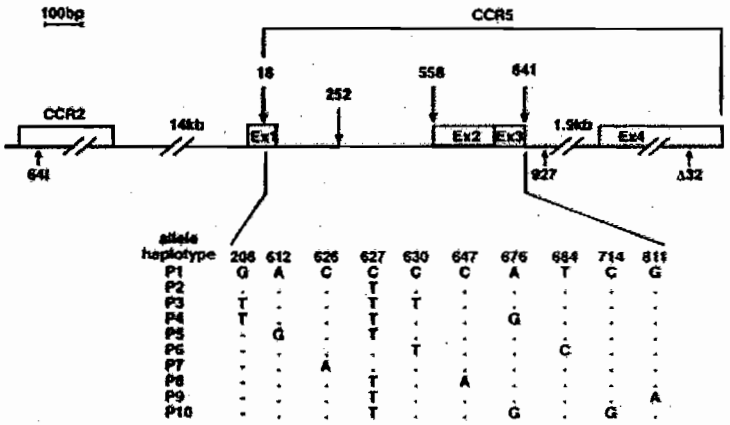
Sponsors were requested to obtain and store a baseline sample for potential retrospective analysis of CCR5 genotype for subjects with CCR5-tropic virus who did not respond to MVC in order to determine if MVC is not effective against certain CCR5 genotypes. There is documented genetic variation in the CCR5 promoter and coding sequences and the activity of CCR5 antagonists may vary depending on an individual combination CCR5 genes.

The best characterized host genetic factor known to affect susceptibility to HIV infection and disease progression is a 32 base pair deletion in the CCR5 gene (CCR5Δ32). There does not appear to be any detectable phenotypic consequences of lacking a functional CCR5 protein in individuals homozygous for the CCR5Δ32 deletion, apart from being almost resistant to HIV-1 infection. The Δ32 deletion results in the production of a non-functional protein that is not expressed on the cell surface. In addition to the association with resistance to infection, the deletion is also associated with slower progression to AIDS and death in HIV-1 infected patients. Individuals heterozygous for the deletion have been found to have lower viral loads compared to wild-type individuals. The frequency of the CCR5Δ32 polymorphism varies in different populations, ranging from approximately 10% in Europeans to 2-5% in Middle Eastern and Asian Indian

populations and is rare <1% in Africans. In Europeans, the highest frequencies are in Northern and Northeastern regions (16% in Finnish and Mordvinian populations investigated) and the lowest in the Mediterranean area (4% in Sardinian individuals). A number of less frequent polymorphisms have also been identified in the coding sequence of CCR5 and several have been shown to have functional consequences *in vitro*. However, no significant associations with HIV pathogenesis have been identified for these mutations because they are rare in the studied HIV cohorts. One exception, the nonsense mutation (m303) that truncates the CCR5 protein, resulting in a non-functional receptor, has been associated with resistance to HIV infection in some studies. This polymorphism was found to have an allele frequency of approximately 0.2-0.7% in European (ethnic origin unspecified) individuals and 1.4% in African Americans.

Several polymorphisms that are associated with rapid progression to AIDS have been identified in the promoter region of CCR5. These promoter polymorphisms are more common than the CCR5 Δ32 mutation with the allele frequencies of the five most common promoter polymorphisms ranging from 18-44%. Martin *et al.* (1998) identified four common haplotypes and six less frequent haplotypes across the CCR5 promoter region. There are 10 SNPs that describe these 10 CCR5 promoter haplotypes, as shown in Figure 2. The CCR5 P1 promoter haplotype is the most common in Caucasians and African Americans, with a reported frequency of 56% and 43% respectively. A functional effect of an A/G polymorphism, pCCR5-59029, in the promoter has been identified. The *in vitro* promoter activity of the A allele, which is associated with rapid progression to AIDS, is significantly higher than the G variant. One small study also showed a significant increase in numbers of $CD4^+$ cells expressing CCR5 in samples isolated from A/A homozygotes compared to heterozygous individuals at the pCCR5-59029 locus.

Figure 2: Map of CCR2, CCR5 & CCR5 promoter region and haplotype alleles



The applicant selected [redacted] polymorphisms for genotyping studies. These polymorphisms included [redacted] variants in the CCR5 coding region, [redacted] in the CCR5 promoter region and [redacted] in CCR2 coding region. In addition, [redacted] haplotypes derived from the

promoter variants have also been selected since these variants are in strong linkage equilibrium with each other.

Subjects heterozygous at the promoter locus [redacted] (n=15) and for the CCR5Δ32 genotype (n=14) had lower CCR5 expression than individuals with two common alleles. In addition, subjects who were heterozygous at the promoter locus [redacted] also had lower CCR5 expression than those with two common alleles.

Subjects with the P1 haplotype pair (31/481 [6.4%] had a slightly lower frequency of dual tropism reported at baseline compared to subjects with the P1/P4 haplotype pair (32/338 [9.5%]). The P1 haplotype pair subjects were also slightly more likely to have lower viral load compared with all the other haplotypes and the 'other' haplotype group tended to have higher viral load. When stratified by CCR5Δ32, the mean baseline HIV-1 RNA levels were similar across treatment week and the distribution of change in mean viral load from baseline at week 24 was similar in both groups. There was no evidence of a treatment-genotype interaction.

Δ32 Deletion/WT or CCR5 Promoter Mutations

The percentage of subjects with the Δ32 genotype (wildtype/deletion) ranged from 5% to 9% in study 1027 [7% (17/232) in MVC QD arm, 5.5% (13/235) in the MVC BID arm, and 9% (11/118) in the placebo arm]. The percentage of Δ32 genotypes was similar in study 1028 with 8% (15/182) in MVC QD arm, 7.9% (15/191) in the MVC BID arm, and 5.5% (5/91) in the placebo arm. Overall in studies 1027 and 1028, the percentage of subjects heterozygous for the Δ32 genotype ($CCR5^+$/Δ32 deletion) at baseline ranged from 5% to 9% and was comparable between arms. An examination of subjects who failed treatment showed that there was no difference between treatment arms in the percentage of subjects who were heterozygous for the Δ32 Deletion or had CCR5 promoter haplotype pairs P1, P4, and P1/P4 (Table 43). The majority of treatment failures (>80%) were homozygous wildtype CCR5. Approximately 40% of treatment failures had the P1/P1 promoter haplotype and a third had the P1/P4 haplotype.

Table 43. Percentage of Failures with $CCR5^+$/Δ32 Deletion or CCR5 Promoter Haplotype Pairs

	QD	BID	Placebo
Δ32/ $CCR5^+$	8% (13/154)	3% (5/143)	6% (9/143)
$CCR5^+$/ $CCR5^+$	84% (130/154)	89% (127/143)	85% (122/143)
P1	46% (71/154)	41% (59/143)	46% (31/143)
P4	11% (17/154)	12% (17/143)	12% (17/143)
P1/P4	33% (51/143)	34% (49/143)	31% (45/143)

Frequency of Δ32 CCR5 and Promoter Haplotypes in Blacks

There was a significant difference between CCR5 promoter haplotype and ethnicity in studies 1027 and 1028; the P1/P4 haplotype pair being more common in Caucasians and the 'other' (i.e. non P1, P1/P4 or P4) haplotype pairs being more common in Black subjects (Table 44).

Table 44. Percentage of Subjects with Δ32 Deletion/WT or CCR5 Promoter Haplotypes

	Blacks	Caucasian	Overall
Δ32/WT	0%		7% (76/1049)
WT/WT	100% (136)		86% (906/1049)
P1	64%* (87/136)	47% (386/823)	46%* (481/1049)
P4	10% (14/136)	14% (116/823)	13% (135/1049)
P1/P4	11% (15/136)	38% (313/823)	32% (338/1049)
Other	15% (20/136)	1% (8/823)	

The remaining were missing
*P=0.002

Blacks did not respond as well as the overall group to MVC treatment in Studies 1027 and 1028: 33% of Blacks achieved <50 copies/mL at Week 24 compared with 46% of Caucasians in the MVC QD arm; 29% of Blacks achieved <50 copies/mL at Week 24 compared with 48% of Caucasians in the MVC BID arm; 23% of Blacks achieved <50 copies/mL at Week 24 compared with 24% of Caucasians in the Placebo arm.

Fifty-three percent (65/123) of Blacks had >400 copies/mL at Week 24. The frequencies of promoter haplotype pairs in the virologic failures who were Black were similar to the frequencies seen overall in Blacks (Table 45). However, the specific promoter haplotype and coding sequence haplotypes paired with the common promoter haplotyes were not identified.

Table 45. Frequencies of Promoter Haplotypes in Virologic Failures who were Black

	Blacks
P1	57% (37/65)
P4	9% (6/65)
P1/P4	11% (7/65)
Other	15% (10/65)

Missing =5

Additional SNPs in CCR5 reported to be relevant in Black and Asian population should be studied. For example, C101X variant in Black population (allele frequency, 1.4%) which results in non-functional CCR5 similar to CCR5 WT/△32 seen in Caucasian population and C269F variant in Asians that results in poor expression of CCR5 with impaired function. In addition, A29S variant in Blacks (allele frequency 1.5%) and R60S variant in Blacks (allele frequency, 1.3%), have been reported to affect the pharmacology

of CCR5 receptor function; G106R variation in Asians has been shown to cause deficiency in binding chemokines and in allowing entry of HIV (Arenzana-Seisdedos and Parmentier, 2006). See also Shashi Amur's review.

5. CONCLUSION

This NDA is approvable with respect to microbiology for the treatment of HIV-1 in combination with other antiretroviral agents, and is indicated for treatment-experienced adult patients infected with CCR5-tropic HIV-1 who have HIV-1 strains resistant to multiple antiretroviral agents.

This indication is based on analyses of plasma HIV-1 RNA levels in two controlled studies of Maraviroc of 24 weeks duration. Both studies were conducted in clinically advanced, 3-class antiretroviral (NRTI, NNRTI, PI, or enfuvirtide) treatment-experienced adults with evidence of HIV-1 replication despite ongoing antiretroviral therapy.

The following points should be considered when initiating therapy with Maraviroc:

- Antiviral efficacy has not been demonstrated in patients with dual/mixed or CXCR4-tropic HIV-1. Patients with evidence of dual or mixed CXCR4-tropic HIV should not be started on Maraviroc.
- Tropism testing and treatment history should guide the use of Maraviroc.
- The safety and efficacy of Maraviroc have not been established in treatment-naïve adult patients or pediatric patients.

A study in antiretroviral-naïve subjects is ongoing; the benefit-risk assessment for this population is therefore not yet known.

4 Page(s) Withheld

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Withheld Track Number: Microbiology-_____

7. References

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Gonzolez E et al., Race-specific HIV-1 disease-modifying effects associated with *CCR5* haplotypes. 1999 *PNAS* 96: 12004-12009

Martin MP, M Dean, MW Smith, et al. Genetic acceleration of AIDS progression by a promoter variant of CCR5. 1998 *Science* 282: 1907-1911.

8. APPENDICES

APPENDIX A

Mean (Median) Baseline Resistance in Study 1027 (n=585)

	QD (n=232)	BID (n=235)	Placebo (n=118)
GSS	1.6 (1)	1.46 (1)	1.6 (1)
PSS	2.01 (2)	1.91 (2)	2.15 (2)
OSS	1.86 (2)	1.74 (2)	1.97 (2)

Mean (Median) Baseline Resistance in Study 1028 (n=464)

	QD (n=182)	BID (n=191)	Placebo (n=91)
GSS	1.6 (1)	1.67 (1)	1.69 (2)
PSS	2.06 (2)	2.13 (2)	2.07 (2)
OSS	1.92 (2)	1.96 (2)	1.8 (2)

APPENDIX B

Censored:

- D/C While Suppressed
- Blank
- D/C Before Achieve Viral Suppression:
  - Subjects D/C between Week 0-4
  - Subjects with HIV RNA data only through week 8 censored if achieve at least 0.5 log decrease and no rebound (previous ≥2 log decrease with 1 log increase)

Subjects Censored from Study 1027: D/C Before Achieve Viral Suppression:

PID	TRT
A4001027 10010017	bid
A4001027 10130004	plc
A4001027 10170005	bid
A4001027 10200018	bid
A4001027 10270005	qd
A4001027 10370009	bid
A4001027 10410012	bid
A4001027 10480045	qd
A4001027 10530008	qd
A4001027 10580014	qd
A4001027 10630003	qd
A4001027 10680019	plc
A4001027 10680020	qd
A4001027 10810014	bid
A4001027 10900025	bid
A4001027 11010015	qd
A4001027 11060006	qd
A4001027 11090016	qd
A4001027 11110018	qd
A4001027 11310010	bid
A4001027 10220015	plc

Subjects Censored from Study 1028: D/C Before Achieve Viral Suppression:

PID	TRT
A4001028 10440012	bid
A4001028 10510034	plc
A4001028 10820032	plc
A4001028 10890014	qd
A4001028 11140006	plc
A4001028 11230001	bid
A4001028 11400019	qd
A4001028 11610005	plc
A4001028 11710003	qd
A4001028 11740005	qd
A4001028 11940011	qd
A4001028 11980001	plc

APPENDIX C
Treatment Failures with CXCR4-TropicVirus at Failure

Treatment Failures from Study 1027 with CXCR4 Tropic Virus at Failure (n=31)

PID	Arm	Outcome	BTRP	TRP Fail
A4001027 10050010	QD	VL > 400	Dual Mixed	CXCR4
A4001027 10050038	BID	Treatment failure	CCR5	CXCR4
A4001027 10070018	BID	Treatment failure	CCR5	CXCR4
A4001027 10120016	QD	VL > 400	Dual Mixed	CXCR4
A4001027 10130001	BID	Treatment failure	Dual Mixed	CXCR4
A4001027 10190006	QD	VL > 400	Dual Mixed	CXCR4
A4001027 10220008	QD	Treatment failure	CCR5	CXCR4
A4001027 10230012	QD	VL > 400	CCR5	CXCR4
A4001027 10230068	BID	Treatment failure	CCR5	CXCR4
A4001027 10240019	QD	VL > 400	CCR5	CXCR4
A4001027 10240024	QD	Treatment failure	CCR5	CXCR4
A4001027 10240029	BID	Treatment failure	Dual Mixed	CXCR4
A4001027 10370005	QD	Treatment failure	CCR5	CXCR4
A4001027 10430022	QD	VL > 400	CCR5	CXCR4
A4001027 10480025	BID	Treatment failure	Dual Mixed	CXCR4
A4001027 10480028	QD	Treatment failure	Dual Mixed	CXCR4
A4001027 10500012	BID	Treatment failure	CCR5	CXCR4
A4001027 10520021	Placebo	Treatment failure	Dual Mixed	CXCR4
A4001027 10530016	BID	VL > 400	CCR5	CXCR4
A4001027 10670006	QD	VL > 400	CCR5	CXCR4
A4001027 10670008	QD	Treatment failure	CCR5	CXCR4
A4001027 10680006	BID	Treatment failure	CCR5	CXCR4
A4001027 10720009	BID	DC and VL > 400	Dual Mixed	CXCR4
A4001027 10740001	QD	VL > 400	CCR5	CXCR4
A4001027 10990015	BID	Treatment failure	CCR5	CXCR4
A4001027 11010002	BID	Treatment failure	Dual Mixed	CXCR4
A4001027 11090001	QD	Treatment failure	CCR5	CXCR4
A4001027 11110011	QD	Treatment failure	Dual Mixed	CXCR4
A4001027 11300004	QD	DC VL > 400	CCR5	CXCR4
A4001027 11480001	QD	Treatment failure	CCR5	CXCR4
A4001027 11480004	BID	VL > 400	CCR5	CXCR4

Treatment Failures from Study 1028 with CXCR4 Tropic Virus at Failure (n=11)

A4001028 10020007	BID	Treatment failure	CCR5 CXCR4
A4001028 10440002	QD	DC VL > 400	CCR5 CXCR4
A4001028 10440004	BID	Treatment failure	CCR5 CXCR4

A4001028 10510008	**BID**	**Treatment failure**	**Dual Mixed**	**CXCR4**
A4001028 10880018	**QD**	**VL > 400**	**Dual Mixed**	**CXCR4**
A4001028 10890001	**BID**	**Treatment failure**	**Dual Mixed**	**CXCR4**
A4001028 10890013	**BID**	**>400**	**CCR5 CXCR4**	
A4001028 10920002	**Placebo DC VL > 400**		**CCR5 CXCR4**	
A4001028 11130002	**QD**	**Treatment failure**	**CCR5 CXCR4**	
A4001028 11540001	**QD**	**VL > 400**	**CCR5 CXCR4**	
A4001028 11580007	**QD**	**Treatment failure**	**CCR5 CXCR4**	

APPENDIX D

Percentage of virologic failures with CCR5-using and CXCR4-using virus at time of failure in both treatment arms of each study.

Table. Study 1027 Tropism of Treatment Failures (n=163)

	QD (n=49)	BID (n=56)	Placebo (n=58)
CCR5	13 (27%)	14 (25%)	51 (88%)
CXCR4	8 (16%)	10 (18%)	1 (1.7%)
Dual Mixed	23 (47%)	28 (50%)	2 (3%)
NR/NP	4	4	4
BLQ	1		

Table. Study 1027 Tropism of Treatment Failures >400 copies/mL (n=247)

	QD (n=90)	BID (n=79)	Placebo (n=78)
CCR5	37 (41%)	22 (28%)	65 (83%)
CXCR4	17 (19%)	13 (16%)	1 (1.2%)
Dual/mixed	26 (29%)	35 (44%)	5 (6.4%)
NR/NP	7	7	7
BLQ	3	2	

Table. Study 1027 Tropism of Responders <400 copies/mL (n=)

	QD (n=150)	BID (n=124)	Placebo (n=33)
CCR5	5	3	
CXCR4			
Dual/mixed	1		
BLQ	139 (93%)	117 (94%)	32 (97%)
missing	5	4	1

Table. Study 1028 Tropism of Treatment Failures (n=114)

	QD (n=32)	BID (n=35)	Placebo (n=47)
CCR5	12 (38%)	10 (29%)	42 (89%)
CXCR4	2 (6%)	4 (11%)	
Dual/mixed	11 (34%)	14 (40%)	3 (6%)
NR/NP	7 (22%)	7 (20%)	2 (4%)

Table. Study 1028 Tropism of Treatment Failures >400 copies/mL (n=193)

	QD (n=65)	BID (n=63)	Placebo (n=65)
CCR5	28 (43%)	19 (30%)	53 (82%)
CXCR4	5 (8%)	5 (8%)	1 (1.5%)
Dual/mixed	18 (28%)	23 (37%)	6 (9%)
NR/NP	10 (15%)	8 (13%)	3 (5%)
BLQ	4 (6%)	8 (13%)	2 (3%)

APPENDIX E
Optimized Background Therapy

OBT of Failures in Study 1027

	QD (N=90)	BID (N=79)	Placebo (n=79)
ENF Use	49% (44/90)	51% (40/79)	47% (37/79)
Subjects with No Susc drugs at BL	34% (31/90)	24% (19/79)	27% (21/79)
Subjects with Changes in OBT on Therapy	47% (36/76)	47% (34/73)	46% (33/72)
Endpt of failure (WK)	WEEK12 (16%) WEEK16 (7%) WEEK24 (19%) WEEK48 (18%)	WEEK12 (24%) WEEK16 (13%) WEEK24 (13%) WEEK48 (13%)	WEEK12 (30%) WEEK16 (6%) WEEK24 (18%) WEEK48 (11%)

OBT of Failures in Study 1028

	QD (N=64)	BID (N=64)	Placebo (n=64)
ENF Use	39% (25/64)	39% (25/64)	44% (28/64)
Subjects with No Susc drugs at BL	22% (14/64)	34% (22/64)	28% (18/64)
Subjects with Changes in OBT on Therapy	37% (19/52)	36% (21/59)	46% (27/59)
Endpt of failure (WK)	WK12 (17%) WK16 (17%) WK24 (17%) WK48 (14%)	WK12 (27%) WK16 (13%) WK24 (17%) WK48 (13%)	WK8 (17%) WK12 (22%) WK16 (13%) WK24 (16%) WK48 (8%)

Table. Percentage of Failures in Studies 1027 and 1028 with No Susceptible Drugs in OBT at Baseline by Tropism at Failure

	QD (N=45)	BID (N=41)	Placebo (n=39)
CCR5	50% (22)	29% (12)	85% (33)
CXCR4	14% (6)	12% (5)	
Dual/mixed	23% (10)	56% (23)	5% (2)
NR/NP	14% (6)	2% (1)	10% (4)

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