CCTG 590 & 590s

The Impact of CCR5 Inhibitor Treatment Intensification on CD4+ T-cell Recovery and Gene Expression Profiles in HIV-Infected Patients with Viral Suppression (Main Study)

Maraviroc Pharmacokinetics and Immunomodulatory Effects within the Central Nervous System (CSF Sub-Study)

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SCHEMA

The Impact of CCR5 Inhibitor Treatment Intensification on CD4+ T-cell Recovery and Gene Expression Profiles in HIV-Infected Patients with Viral Suppression (Main Study)

Maraviroc Pharmacokinetics and Immunomodulatory Effects within the Central Nervous System (CSF Sub-Study)

Design:

CCTG 590 is a single-arm, open-label study to evaluate the impact of therapy intensification of Maraviroc (MVC) (a CCR5 inhibitor) to a stable suppressive HIV antiretroviral regimen on the rate of CD4+ T-cell recovery and gene expression profiles. Subjects on a stable first-line HIV regimen with continued viral suppression and sub-optimal CD4+ T-cell counts will be eligible for this study. Those who are found to be eligible will have MVC (dose-adjusted to background HIV regimen) added to their current HIV regimen for 24 weeks. After the 24 week intensification, the MVC will be discontinued, the original antiretroviral regimen will be continued and the subjects will be followed for an additional 12 weeks.

CCTG 590s is a central nervous system (CNS) sub-study that will evaluate MVC cerebral spinal fluid (CSF) pharmacokinetics and immunomodulatory effects within the CNS. The primary eligibility criteria will be enrollment into the MVC parent study, ability to give informed consent to lumbar puncture (LPs). Pre-treatment LPs will be performed, followed by a LP at week 4 of MVC treatment intensification (i.e., “steady state”). For subjects willing to undergo more than one LP, an additional LP will be performed at week 24 of MVC treatment intensification.

Duration:

Total study duration is 36 weeks: 24 weeks of MVC treatment intensification, followed by an additional 12 weeks of continued monitoring on baseline antiviral therapy without MVC.

Sample Size: 30 subjects (17 subjects for the CSF Sub-Study)

Study Population:

HIV-infected patients receiving an initial stable antiretroviral regimen with 12 months or more of viral suppression (defined by HIV RNA below detection limits) will be offered enrollment into this study. Eligible patients must have

documented dates of initiation of first HIV regimen and have been on continuous treatment for at least 15 months. In addition, available laboratory data must include at least 3 measurements of CD4 count and plasma HIV RNA loads obtained within the 12 months prior to screening.

Regimen:

Maraviroc treatment intensification will be provided to all subjects dose-adjusted to each participant's background antiretroviral therapy:

A. with strong CYP3A4 inhibitors, including:
- Protease inhibitors (except tipranavir/ ritonavir)
- Delavirdine
- ketoconazole, itraconazole, clarithromycin, nefazadone, telithromycin
- Darunavir/r + etravirine

MVC 150 mg twice daily

B. with non-inducers/ non-inhibitors of CYP3A4, including:
- Tipranavir/ ritonavir
- Nevirapine
- All NRTIs
- Enfuvirtide

MVC 300 mg twice daily

C. with strong CYP3A4 inducers, including:
- Efavirenz, etravirine
- rifampin

MVC 600 mg twice daily

1.0 STUDY OBJECTIVES

1.1 Primary Objectives

1.1.1 To characterize changes in gene expression profiles associated with early (week 4) and late (week 24) MVC therapy intensification.

1.1.2 To evaluate the association of gene expression profiles induced by MVC on the rate of CD4+ T-cell recovery during MVC therapy intensification.

1.2 Secondary Objectives

1.2.1 To compare the rates of phase 2 CD4+ T-cell recovery prior to and during 24 weeks of MVC therapy intensification.

1.2.2 To evaluate the impact of MVC therapy intensification on persistent immune activation and maturation phenotype (as measured by flow cytometry) during viral suppression.

1.2.3 To characterize differences in the gene expression profiles associated with MVC therapy intensification in individuals receiving a PI/r- vs. NNRTI-based regimen.

1.2.4 To evaluate the safety and tolerability of adding MVC to a stable antiretroviral regimen as assessed by the rate of > grade 2 adverse clinical or laboratory events.

1.2.5 To characterize cerebral spinal fluid concentrations of MVC compared to plasma concentrations.

1.2.6 To evaluate the impact of MVC therapy intensification on persistent immune activation (as measured by soluble and cellular markers) in the central nervous system.

2.0 INTRODUCTION

2.1 Background (Main study)

Suboptimal CD4+ T-cell recovery during potent antiretroviral therapy (ART) is a common clinical dilemma with an incidence as high as 40% [1-4]. Reconstitution of CD4+ T-cells during viral suppression follows a biphasic pattern [5]. In the first three months of ART, CD4+ T-cells typically increase by 50 to 120 cells/mm^3 [6-8] - primarily as a result of redistribution of T-cells into peripheral circulation [9, 10]. The magnitude of this early recovery is likely a function of prior T-cell destruction as lower CD4+ T-cell nadirs have been associated with limited immune recovery during therapy [2, 11]. This initial burst is followed by a second, slower, phase of T-cell repopulation (average rate of 2 to 10 cell/mm^3 increase per month [6-8, 12]) which may continue for as long as 6 years [13]. This second phase is often termed the ‘regenerative phase’ as de novo T-cells are produced from the thymus and by peripheral expansion. However, in some individuals, the phase 2 CD4+ T-cell recovery plateaus and these patients fail to recover ‘normal range’ T-cells despite optimal viral suppression [3, 13].

We propose to evaluate the potential beneficial immunomodulatory-effects of MVC on CD4+ T-cell recovery during optimal viral suppression. Individuals with well-controlled HIV-infection on ART will undergo therapy intensification with MVC for 6 months followed by a 3 month post-intensification visit. The goals of this study include:

1. To determine the impact of MVC treatment intensification on host genomic responses. Whole genome (~38,000 genes) analysis on purified CD4+/CD8+ lymphocytes will be used to evaluate changes in gene expression patterns, in particular genes associated with proliferation, trafficking and activation, before and during MVC treatment intensification.

2. To evaluate differences in the rate of phase 2 CD4+ T-cell recovery before and during 6 months of MVC treatment intensification.

3. To characterize changes in immune T-cell subsets (activation, maturation and apoptosis) before and during MVC treatment intensification.

4. To develop a gene classifier of MVC treatment-responsive genes and propose a model of the impact of CCR5-inhibition on immune reconstitution during suppressive ART.

2.2 Background (CSF Sub-Study)

The central nervous system (CNS) is a frequent site of HIV replication [14] and HIV-induced neuronal injury leading to a spectrum of cognitive disorders, ranging from aymptomatic neuropsychological impairment to severe HIV-associated neurocognitive disorders (HAND) [15, 16]. While the incidence of HAND has declined in the potent antiretroviral (ARV) era, minor neurocognitive disorders (MND) remains a significant source of morbidity among treated HIV-individuals with a prevalence as high as 50% [17]. HIV neuropathology during ARV therapy may result from at least two interdependent mechanisms. First, persistent HIV replication in the CNS has been associated with neurocognitive syndromes in cross-sectional studies [3, 4] and predicts their future development in longitudinal studies [18]. Although ARV therapy reduces HIV replication in both the plasma and CSF in early-stage HIV disease, the rate of HIV decline in CSF is slower than in plasma among individuals with advanced HIV disease and may lead to on-going neuronal injury. Second, HIV infection of perivascular macrophages and microglia results in cellular activation of these cells. This inflammatory response within the CNS is thought to be an important mediator of indirect damage of uninfected neuronal cells leading to the neurodegeneration observed in HAND [19] and may persist even after HIV replication is suppressed [20].

In this sub-study we propose to evaluate the pharmacokinetics and therapeutic effects of MVC within the CNS among subjects with sub-optimal CD4 recovery despite plasma viral suppression. Paired plasma-CSF measures of MVC concentrations, HIV RNA and markers of immune activation will be obtained pre- and weeks 4 and 24 of MVC-treatment intensification.

2.2.1 Maraviroc

Maraviroc selectively binds to the human chemokine receptor CCR5 present on the cell membrane, preventing the interaction of HIV-1 gp120 and CCR5 necessary for CCR5-tropic HIV-1 to enter cells [21]. CXCR4-tropic and dual-tropic HIV-1 entry is not inhibited by MVC.

2.1.1.1 Safety and Tolerability

The safety profile of MVC is primarily based on 840 HIV-infected subjects who received at least one dose of MVC during two Phase 3 trials (MOTIVATE 1 and MOTIVATE 2) [22]. Treatment-experienced, triple-class drug resistant subjects with R5 tropic virus only and plasma viral loads above 5000 copies/mL were randomized in a 1:2:2 fashion to placebo, MVC once-daily or MVC twice-daily plus optimized background therapy (OBT) for 48 weeks. The most common adverse events reported with frequency rates higher than placebo, regardless of causality, were cough, pyrexia, upper respiratory tract infections, rash, musculoskeletal symptoms, abdominal pain and dizziness. Additional adverse events that occurred were diarrhea, edema, influenza, esophageal candidiasis, sleep disorders, rhinitis, parasomnias, and urinary abnormalities. In both studies, the rates of discontinuation due to adverse events were 4.9% in subjects receiving MVC twice daily + optimized background therapy (OBT) compared to 5.3% in those receiving OBT + placebo. Most of the adverse events reported were judged to be mild to moderate in severity.

Specific potential adverse events associated with MVC have been included in the package insert as a warning and precaution for use [23]. And these include:

Hepatotoxicity Events: One case of possible MVC-induced hepatotoxicity with allergic features has been reported in a study of healthy volunteers. Although an increase in hepatic adverse events was observed during studies of treatment-experienced subjects with HIV infection, there was no overall increase in ACTG Grade 3/4 liver function test abnormalities. In pooled analysis of A4001027 and A4001028 the percent of subjects experiencing grade 3/4 laboratory abnormalities (without regard to baseline values) receiving MVC (N=421) were similar to those that received placebo (N=207) in addition to OBT, including: AST 5xULN: 4.5% vs. 2.9%; ALT 5xULN: 2.4% vs. 3.4%; and Total Bili: 5.7% vs. 5.3%.

Cardiovascular Events: When MVC was administered to healthy volunteers at doses higher than the recommended dose, symptomatic postural hypotension was seen at a greater frequency than in placebo. At lower doses used in HIV subjects in Phase 3 studies, postural hypotension was seen at a rate similar to placebo (approximately 0.5%). However, eleven HIV subjects (1.3%) who received MVC had cardiovascular events including myocardial ischemia and/or infarction during the Phase 3 studies (total exposure 267 patient-years), while no subjects who received placebo had such events (total exposure 99 patient-years). These subjects generally had cardiac disease or cardiac risk

factors prior to MVC use, and the relative contribution of MV to these events is not known.

Potential Risk of Infection: Maraviroc antagonizes the CCR5 co-receptor located on some immune cells, and therefore could potentially increase the risk of developing infections. The overall incidence and severity of infection, as well as AIDS-defining category C infections, was comparable in the treatment groups during the Phase 3 studies. While there was a higher rate of certain upper respiratory tract infections reported in the MVC arm compared to placebo (20.0% versus 11.5%), there was a lower rate of pneumonia (2.1 % vs. 4.8%). In addition, a higher incidence of Herpes virus infections (11.4 per 100 patient-years) was also reported in the MVC arm when adjusted for exposure compared to placebo (8.2 per 100 patient-years).

Potential Risk of Malignancy: Blockade of the CCR5 inhibitor by MVC represents a theoretical risk for decreased immune surveillance and potential increased risk of malignancy. However, no increase in malignancy has been observed with MVC.

2.2.1.2 Drug Interactions and Special Populations

In vitro studies demonstrated that MVC is principally metabolized by the cytochrome P450 CYP3A and to a lesser extent CYP2C9. As a substrate of CYP3A and P-gp MVC pharmacokinetics are modulated by inhibitors and inducers of these enzymes/transporters and a dose adjustments are required when co-administered with those drugs (see section 5.4 Concomitant Medications).

Pregnancy: There are no adequate and well-controlled studies in pregnant women. The incidence of fetal variations and malformations was not increased in embryofetal toxicity studies in rats at exposures (AUC) approximately 20-fold higher and in rabbits at approximately 5-fold higher than human exposures at the recommended daily dose. Maraviroc is a Category B drug for pregnancy (see section 4.0 Selection and Enrollment of Subjects).

Renal Impairment: The safety and efficacy of MVC have not been specifically studied in patients with renal impairment. In the absence of metabolic inhibitors, renal clearance accounts for approximately 25% of total clearance of MVC. Patients with a creatinine clearance of less than 50 mL/min who receive MVC and a CYP3A inhibitor may be at an increased risk of adverse effects related to increased MVC concentrations, such as dizziness and postural hypotension; these subjects will be excluded (see section 4.0 Selection and Enrollment of Subjects).

Hepatic Impairment: The pharmacokinetics of MVC has not been sufficiently studied in patients with hepatic impairment. Because MVC is metabolized by the liver, concentrations are likely to be increased in these patients (see section 4.0 Selection and Enrollment of Subjects).

2.2.1.2 Impact on CD4 Recovery

There are no study results demonstrating the effect of MVC on clinical progression of HIV-1. However, the peripheral CD4 count is an accepted prognostic marker for HIV-related morbidity and mortality both among untreated and treated patients with viral suppression [24]. Interestingly, the use of CCR5 antagonists may increase CD4 recovery independent of their effect on viral replication.

The Merit study was a phase 3, prospective study of MVC in antiretroviral-naïve patients with R5 virus [25]. Subjects were randomized to either efavirenz (EFV) 600mg once daily (n=361) or MVC 300mg twice-daily (n=360) in combination zidovudine/ lamivudine for 48 weeks. Baseline characteristics were similar between the EFV and MVC treatment groups (median CD4: 254 vs. 241 cells/mL; median HIV RNA: 4.88 vs. 4.86 log10 copies/mL). Despite a greater proportion of subjects in the EFV arm achieving viral suppression at 48 weeks (HIV RNA < 50 copies/mL: 69% vs. 64.4%), those receiving MVC experienced significantly greater CD4 recovery than the EFV group (mean CD4 gain: 169 vs. 142 cells/mm^3).

The potential independent benefit in CD4 recovery with MVC has also been observed in treatment experienced patients with ongoing viral replication. Study A4001029 was prospective study in treatment-experienced with non-R5 tropic virus (i.e. X4, dual/mix or indeterminate) and plasma viral loads above 5000 copies/mL that were randomized to placebo, MVC once-daily or MRV twice-daily plus optimized background therapy (OBT) for 48 weeks [26]. In a planned interim analysis at week 24, a similar decrease in viral load was observed in all 3 treatment groups. However, even among subjects with dual or mixed tropic virus as baseline those receiving MVC twice-daily (N=52) had significantly greater CD4 recovery than the placebo arm (N=54) at week 24 (change in CD4: +62.4 vs. +35.7 cells/mL; $P<0.05$).

These results are consistent with a recent meta-analysis that evaluated difference in CD4 recovery during viral suppression among recent Phase 2 and Phase 3 HIV trials of new HIV agents [27]. This analysis included 37 treatment arms from 16 clinical trials, including the above mentioned trials and two additional trials with a different CCR5 inhibitor, vicroviroc. In analyses that adjusted for baseline HIV RNA and degree of viral suppression, the use of a CCR5 inhibitor was associated with a greater CD4 count increase (mean difference: +32 cells/mm^3) after 24 weeks of treatment compared to non-CCR5-containing regimens. Together, these data suggest a favorable, independent effect of MVC on CD4 recovery during viral suppressive therapy with ART and that MVC is safe in subjects with CXCR4 or dual/mixed tropic HIV isolates.

2.3 Rationale (Main Study)

Blunted CD4+ T-cell responses during viral control may be a consequence of on-going T-cell destruction in the regenerative phase of CD4 recovery from activation-induced apoptosis [4, 28] and/or reduced production from decreased thymic output [29, 30]. Occult HIV replication may contribute to both processes. R5 HIV envelop glycoprotein (gp) 120 interacts with CCR5 receptors which induce a distinct gene expression profile of numerous activation and transcription factors, including; nuclear factor of activated T-cells (NFAT), NF-kB and mitogen-activated protein kinase (MAPK)-related genes [31]. This receptor interaction by gp120 likely creates a replication permissive state in the cell and promotes viral replication from quiescent naïve T-cells with very low CCR5 expression. Further, R5 envelope protein engagement of CCR5 also induces caspase gene activity [31] and may consequently increase T-cell apoptosis rates of uninfected cells in the peripheral circulation as well as within the thymus itself.

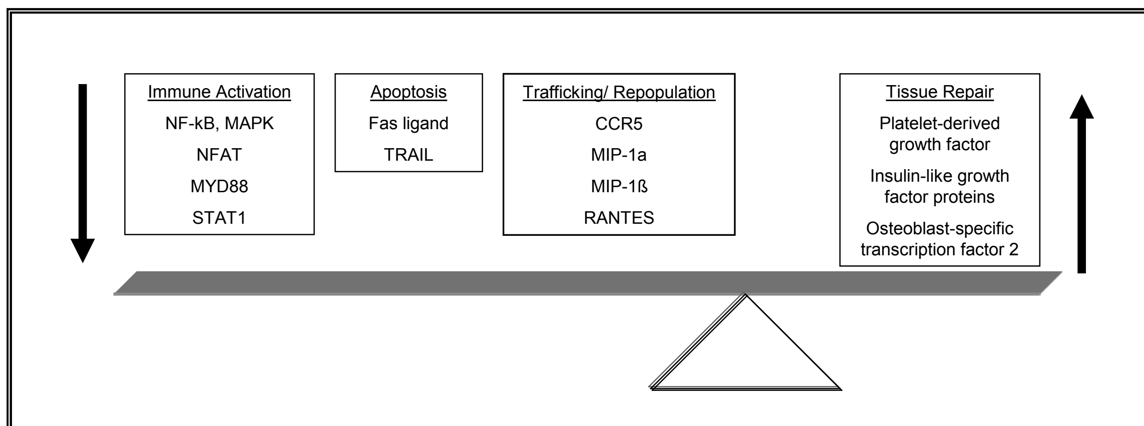
CCR5 inhibitors can improve the clinical status of HIV-infected by two distinct mechanisms. First, by blocking HIV entry into CD4+ T-cells, CCR5 inhibitors have direct antiviral activity. Second, as the pro-inflammatory state of HIV infection up-regulates CCR5 ligands and receptors [32], these receptor antagonists may abrogate immune activation and resultant T-cell apoptosis. Importantly, MVC binds CCR5 receptors without inducing intracellular signaling or altering cell-surface expression [21]. Potentially, MVC intensification during viral suppression with ART may further decrease persistent activation-induced apoptosis and improve repair and remodeling of lymphoid tissue leading to increased CD4+ T-cell recovery and function.

It is well-recognized that the chemosensitivity and prognosis of some cancers is correlated with specific gene-expression profiles [33, 34], however, our understanding of host gene interactions with HIV during antiretroviral therapy and the impact on CD4+ T-cell recovery is at an early stage. Li et al. performed functional genomic analysis to identify treatment-responsive genes in HIV-infected patients initiating ART [35]. Genomic chip arrays were used to screen approximately 12,000 human genes of which ~ 200 genes' expression appeared to be modified in response to HIV therapy. Genes involved in T-cell apoptosis, immune activation and some chemokines and cognate receptors (i.e. CCR5, MIP-1β, RANTES and others) were down-regulated, while genes involved in tissue repair and remodeling were up-regulated.

Based upon the biology of CCR5 and its role in HIV infection [36] and ART-responsive gene expression profiles observed by Li et al. [35], we have selected candidate genes involved in immune function whose expression may be further altered during CCR5 intensification. We hypothesize that expression will <u>decrease</u> among genes involved in immune activation (NF-kB, MAPK, nuclear factor of activated T-cells, MYD88 and STAT1), apoptosis (Fas ligand and TRAIL) and trafficking/repopulation of T-cells (CCR5, MIP-1α, MIP-1β and RANTES) and <u>increase</u> among genes involved in tissue repair (platelet-derived growth factor,

insulin-like growth proteins and osteoblast-specific transcription factor) (**Figure 1**).

Figure 1. Model of gene expression due to anti-inflammatory affects of Maraviroc in combination with suppressive antiretroviral therapy



Chemokine receptors and their ligands are both redundant and pleiotropic in function and may have a varied spectrum of biologic activities in vivo which may not be represented by a small set of selected gene candidates. Further gene expression profiles of peripheral blood mononuclear cells differ from T-lymphocyte subsets [37]. Therefore, whole genome expression (38,000 genes) will initially be done on purified CD4+/CD8+ T-cells at baseline, week 4 and at week 24 of MVC intensification.

Use of MVC is indicated in combination with other antiretroviral agents for treatment-experienced adult patients infected with detectable CCR5-tropic HIV-1 virus and evidence of multi-drug resistant virus. The manufactures of MVC recommend that the following points should be considered when initiating therapy with MVC:

- Consider baseline tropism testing.

- Avoid in patients with dual/mixed or CXCR4-tropic HIV-1 as antiviral efficacy was not demonstrated in a phase 2 study of this patient group.

- The safety and efficacy have not been established in treatment-naïve adult patients or pediatric patients. [*although trials of MVC in naïve patients have shown comparable (though not strictly non-inferior) virologic efficacy to and EFV-containing regimen in this population*]

The current study proposes to use MVC for treatment-experienced patients with on-going viral suppression and without evidence of multi-drug resistant virus. Since the patients will have undetectable HIV RNA levels, baseline tropism

testing is not possible. The use of MVC in this patient population should be safe and offers potential therapeutic benefit for the following reasons:

First, in phase 3 studies, treatment failure on MVC was associated with detection of CXCR4-using (i.e., CXCR4- or dual/mixed-tropic) [23]. This CXCR4-using HIV virus could have appeared in two ways: 1. due to *de novo* mutations in viral envelope or 2. due to emergence of pre-existing, low frequency variants that were not detected on baseline tropism testing. In both scenarios, as consistent with development of drug resistance with other HIV medications, MVC-associated drug resistance emerged in the setting of on-going HIV replication (i.e. detectable plasma HIV loads). An important difference in the treatment population of this study is that subjects would have already initiated ARV therapy and achieved viral suppression for at least 12 months prior to study enrollment. In the setting of on-going viral suppression it is very unlikely that CXCR4 tropic virus will be selected during the 24 weeks of MVC treatment intensification.

Second, the clinical and laboratory safety and tolerance of MVC is comparable to standard therapy in both treatment-experienced [26] and treatment-naïve patients [25]. Among treatment-naïve patients, the rate of discontinuation due to adverse events was less in the MVC arm (4.2%) than among efavirenz-treated subjects (13.6%) [25].

Finally, suboptimal CD4+ T-cell recovery during ART is a common clinical dilemma and may affect as much as 40% of viral suppressed patients [4].This suboptimal CD4+ T-cell response during therapy, or immunologic discordance, can have detrimental clinical consequences [38], including an increased rate of HIV associated infections, malignancies, and cardiovascular morbidity/mortality. In contrast, mortality rates for HIV-infected individuals during the ear of potent HAART (2004 to 2006) have life expectancy rates comparable to matched HIV-negative controls within the first 5 years of seroconversion [39].

The aim of this study is to evaluate a potentially therapeutic immunomodulatory effect of MVC. Several measures of immune homeostasis will be determined in this study, including functional genomic analysis and extended T-cell phenotyping. Genes responsive to MVC therapy will be identified and categorized into functional groups. Based upon existing literature of the identified genes and observed immune responses (change in CD4/CD8 subsets) during MVC therapy, a model of CCR5 responsive-genes and potential impact on immune recovery will be outlined. Potentially, individuals experiencing immune discordance during suppressive ART may be better treated by MVC antiretroviral intensification.

2.3.1. Hypotheses

1. We hypothesize that expression will decrease among genes involved in immune activation (NF-kB, MAPK, nuclear factor of activated T-cells, MYD88 and STAT1), apoptosis (Fas ligand and TRAIL) and

trafficking/repopulation of T-cells (CCR5, MIP-1α, MIP-1β and RANTES) and increase among genes involved in tissue repair (platelet-derived growth factor, insulin-like growth proteins and osteoblast-specific transcription factor).

a. The gene expression profiles induced by MVC will be associated with a favorable increase in the rate of CD4+ T-cell recovery.

b. The rate of CD4 recovery (cells/month) will be greater during MVC compared to before.

c. The proportion of cells expressing activation/ apoptosis markers will decrease from baseline and this decrease will be associated with improved CD4 recovery.

d. The proportion of naïve cells will increase from baseline and this increase will be associated with improved CD4 recovery.

e. The rate of CD4 recovery will be greater among those subjects receiving PI-containing treatment regimens compared to those reciving NNRTI-containing treatment regimen.

2.4 Rationale (CSF – Sub-Study)

HIV replication in the CNS primarily originates from brain macrophages or microglia [40]. Considering those HIV strains that infect macrophages primarily utilize CCR5 (R5 virus) [41], it is not surprising that most HIV strains isolated from individuals with HAD have been R5 tropic [42, 43]. However, paired plasma-CSF samplings from neurologically-asymptomatic HIV-infected individuals across a range of CD4 counts also demonstrate R5 virus, suggesting R5-tropic virus is the predominant stain in the CNS [44]. Potentially, co-receptor utilization may actually mediate neuronal injury. HIV-1 R5 strains isolated from AIDS patients with HAD were highly fusogenic and demonstrated enhanced macrophage tropism despite low of CD4 and CCR5 expression [42, 43]. Although these strains showed reduced sensitivity to anti-CD4-antibodies, the CCR5 inhibitor (TAK-779) appeared to be equally effective as with plasma isolates.

Although markers of immune activation decrease during treated HIV infection, levels can remain abnormally high even after several years of HIV suppression within the plasma and CSF compartments [45, 46]. One explanation is that an inflammatory response, once established, gives rise to a self-sustaining state of macrophage and microglia activation [19]. Activated macrophages produce pro-inflammatory cytokines that both propagate activation-induced apoptosis of uninfected cells and increase blood-brain-barrier (BBB) permeability resulting in enhanced migration of activated T-cells into the CNS. Potentially, the 'motor' for neurodegeneration continues after suppression of HIV replication and it is

unknown if MCMD, or other mild HIV cognitive disorders will progress despite potent ARV therapy. In a recent report, the neuropsychological performance of 60% of individuals who had HAND did not normalize despite suppressive HIV therapy [47].

Better penetration of ARVs into the CNS (brain and CSF) is associated with improved neuropsychological performance [8, 9]. Some indirect evidence suggests MVC may achieve therapeutic concentrations in the CNS. In a pilot-study of HIV uninfected women (n=12), MVC 300 mg twice-daily for 7 days achieved exposure levels in cervicovaginal fluid and vaginal tissue comparable or exceeding plasma concentrations [48], suggesting MVC is able to penetrate pharmacokinetic sanctuary sites. In addition, MVC is a substrate for P-glycoprotein (Pgp). Co-administration of ritonavir, a P-gp inhibitor, increased CSF exposure of indinavir by inhibition of P-gp-mediated efflux across the BBB [49, 50]. Potentially, co-administration of a ritonavir-boosted protease-inhibitor (PI/r) will enhance the CNS penetration of MVC.

2.4.1 Hypotheses

1. Maraviroc (MVC) concentrations in CSF will approximate 24% of the plasma Cmin and will vary across the dosing interval.

   a. Subjects receiving background HIV therapy containing a PI/r will achieve higher CSF MVC concentrations than those receiving not receiving a PI/r

2. MVC treatment-intensification will decrease immune activation (activated T-cells and pro-inflammatory cytokines) in the CSF.

3. Among subjects with CSF viral replication above 2 copies/mL at baseline, all will be suppressed below 2 copies/mL after 4 weeks of MVC treatment-intensification.

3.0 STUDY DESIGN

3.1 Study Design (Main Study)

Thirty patients will be enrolled to evaluate the impact of adding the CCR5 inhibitor, MVC, to a stable suppressive HIV regimen on the rate of CD4+ T-cell recovery and gene expression profiles. At baseline whole genome expression (38,000 genes) profiles and extended flow cytometry will be performed on purified CD4+/CD8+ T-cells in all subjects. In addition, pre-MVC phase 2 CD4+ T-cell recovery rate will be estimated for each subject from historic laboratory data obtained during the 12 months prior to the baseline visit. All subjects will receive treatment intensification with MVC dose-adjusted to their background HIV regimen for 24 weeks. Regular visits will occur on weeks 2, 4, 8, 12 and 24 to assess efficacy, safety and medication tolerance. Additional microarray and

extended flow cytometric analyses will be performed at weeks 4 and 24 of MVC therapy. After 24 weeks of MVC treatment intensification, MVC will be discontinued, the original antiretroviral regimen will be maintained and subjects will return for a 12 week follow-up visit to evaluate on-going CD4+ T-cell recovery, continued HIV RNA suppression and any potential adverse events.

3.2 Study Design (CSF Sub-Study)

This is a sub-study of the MVC treatment-intensification protocol (i.e., a "piggyback" protocol). The primary eligibility criteria will be enrollment into the MVC parent study, ability to give informed consent to lumbar puncture (LPs), and the absence of medications or conditions that would contraindicate lumbar puncture (e.g., coagulopathy). Pre-treatment LPs will be performed, followed by a LP at week 4 of MVC treatment intensification (i.e., "steady state"). All LPs will be performed within one hour of a parent protocol-dictated blood draw, reducing the need for separate visits or additional phlebotomy. For subjects willing to undergo more than one LP, an additional LP will be performed at week 24 of MVC treatment intensification. The primary study outcomes will be measurement of MVC concentrations and reductions in markers of immune activation levels in CSF at week 4.

The study will aim to obtain 3 LPs in as many subjects as possible with the goal being at least two LPs separated by at least 4 weeks in up to 17 participants. After informed consent is obtained, subjects will undergo a brief screening to ensure the absence of contraindications to LP. If eligible subjects choose to enroll, subsequent visits will occur at major visits of the parent protocol (baseline, week 4 and 24). Based on prior experience we conservatively estimate that approximately 60% of subjects in the parent protocol will be willing to consent to lumbar puncture an average of 2.5 LPs per consenting subject. In total, we estimate that this CSF sub-study will yield approximately 43 LPs from individuals taking MVC. Post-dose timing of the lumbar punctures will be limited by the parent protocols but we will strive to vary the post-dose lumbar puncture interval in order to have approximately equal representation from two intervals.

- Interval 1: 2 - 6 hours post dose
- Interval 2: 6 - 12 hours post dose

If successful, this variation across the dosing interval will improve the accuracy of population pharmacokinetic modeling.

3.3 PBMC isolation

Viable PBMCs will be collected at pre-entry, baseline (day 0), weeks 4 and 24.

3.4 Flow Cytometry for CD4 and CD8 T-cells

Measurements for CD4+/CD8+ subsets will be determined at screening, weeks 2, 4, 8, 12, 24 and 12 weeks post CCR5-intensification. In addition, extended phenotype characterization will be performed at baseline, pre-entry and weeks 4 and 24 to determine the proportion of CD4+ and CD8+ lymphocytes that are naive (CD45RA+), activated (CD38+/HLA-DR+) or, among CD4+ cells, regulatory (CD25+CD127-).

3.5 Microarray analysis

Whole genome expression (38,000 genes) will initially be done on purified CD4+/CD8+ T-cells at baseline, week 4 and at week 24 of MVC intensification. However, considering that other immune cells, such as monocytes, also express CCR5 these cells will be stored for future microarray analyses.

3.6 Lumbar Puncture

Standard lumbar puncture-related adverse events will be monitored following all visits. Lumbar punctures will be performed under aseptic conditions at the clinical facilities of the HNRC by a neurologist, an internist, or by a trained registered nurse working under the supervision of the neurologist. Standard risks associated with HNRC medical procedures include 1) venipuncture: the risk of pain, bruising, and a very low risk infection; and 2) lumbar puncture: the risk of pain, bruising and a very low risk of infection as well as a risk of post-dural puncture cephalgia which can range from mild to severe.

Routine clinical lab assays (cell counts, glucose, total protein) will be performed on all CSF. HIV RNA will be measured with a modified version of the Biomerieux assay validated to quantify HIV as low as 2 copies/mL in CSF [51]. MVC concentrations will be measured in plasma and CSF. Remaining specimens will be aliquoted and then stored at –70C at the HIV Neurobehavioral Research Center (HNRC) Specimen Bank.

Local back pain will be minimized by injection of an anesthetic, 1% lidocaine into the subcutaneous tissue surrounding the needle puncture site. Oral analgesics and anti-inflammatory medications will be provided when necessary. Following LP, patients will be observed for 10-15 minutes. Over 3,000 LPs have been performed at the HNRC using non-cutting or "pencil point" spinal needles that reduce the risk of post-LP cephalgia. A site clinician will be available by pager 24 hours-per-day to respond to problems, should they occur. If post-LP headache occurs, initial conservative management will include recumbency, hydration, and oral analgesics. When headaches are prolonged, a blood patch, involving an epidural injection of a sample of the subject's own blood, may be performed by consulting anesthesiologists. This is extremely effective in aborting post-LP headache, but is only necessary in fewer than 1% of HNRC subjects. Palliative procedures will be performed at no cost to the participant.

Lumbar punctures will not be performed if any medical contraindication to the procedure is identified. LPs will be deferred when factors associated with increased risk of bleeding are present, such as a clinical history of bleeding diathesis, ongoing treatment with anticoagulants or platelet counts <50,000/mm3. LPs also will be deferred when signs of untreated systemic or local infection are present. If a subject becomes uncomfortable, the procedure will be discontinued.

4.0 SELECTION AND ENROLLMENT OF SUBJECTS

4.1 Inclusion Criteria (Main Study)

4.1.1 HIV-1 infection documented by a rapid HIV test or any licensed ELISA test kit and confirmed by a different method ELISA, Western blot, rapid HIV test, or plasma HIV-1 RNA assay at any time prior to study entry. Alternatively, if a licensed ELISA is not available, two HIV-1 RNA values >2000 copies/mL at least 24 hours apart by any CLIA certified laboratory may be used to document HIV-1 infection at any time prior to study entry.

4.1.2 All available CD4+ T cell counts within the last 12 months of screening below 350 cells/mm^3 (minimum of 3 values obtained $\geq$ 30 days apart).

4.1.3 CD4+ cell count below than 350 cells/ mm^3 determined by site clinical laboratory within 30 days of screening.

4.1.4 Subject receiving a stable (for at least 6 months) antiretroviral regimen consisting of at least 2 NRTIs and either a protease inhibitor boosted with low dose ritonavir or an NNRTI. A stable regimen is defined as no additions or deletions for more than 14 cumulative days.

4.1.5 Subject considered to be receiving initial HIV regimen (history of medication substitution for toxicity is allowed).

4.1.6 All available plasma HIV RNA levels within the last 12 months are below the level of detection. Isolated values that are detectable but < 1000 copies will be allowed as long as the plasma HIV RNA levels before and after this detectable time point are undetectable – The subject should have a minimum of 3 values obtained $\geq$ 30 days apart.

4.1.7 Screening plasma HIV RNA levels below level of detection (< 50 copies RNA/mL using Roche Amplicor or < 75 copies/mL using Bayer bDNA) obtained by site clinical laboratory within 30 days of screening.

4.1.8 Greater than 90% adherence to HIV therapy within the preceding 30 days, as determined by self-report.

4.1.9 Laboratory values obtained by screening laboratories within 30 days of entry:

- Absolute neutrophil count (ANC) ≥ $750/mm^3$.
- Hemoglobin ≥ 8.0 g/dL.
- Platelet count ≥ $50{,}000/mm^3$.
- Calculated creatinine clearance (CrCl) ≥ 50 mL/min as estimated by the Cockcroft-Gault equation:
  - * For men, (140 - age in years) x (body weight in kg) ÷ (serum creatinine in mg/dL x 72) = CrCl (mL/min)
  - * For women, multiply the result by 0.85 = CrCl (mL/min)
- AST (SGOT), ALT (SGPT), and alkaline phosphatase ≤ 5 x ULN.
- Total bilirubin ≤ 2.5 x ULN (unless subject receiving atazanavir).

4.1.8 Females of childbearing potential must have a negative serum pregnancy test at screening and agree to use a double-barrier method of contraception throughout the study period.

4.1.9 Karnofsky performance score ≥ 70.

4.1.10 Men and women age ≥ 18 years.

4.1.11 Ability and willingness of subject or legal guardian/representative to give written informed consent.

4.2 Inclusion Criteria (CSF Sub-Study)

4.2.1 Enrollment in the MVC parent protocol.

4.2.2 Willingness to undergo lumbar puncture.

4.3 Exclusion Criteria (Main Study)

4.3.1 Current antiretroviral regimen contains tenofovir disoproxil fumarate AND didanosine in combination.

4.3.2 History of chronic hepatitis C (defined as HCV antibody positive and HCV RNA detectable).

4.3.3 History of chronic active hepatitis B (defined as surface antibody negative, surface antigen positive and HBV DNA detectable).

4.3.4 Concurrent use of G-CSF or GM-CSF.

4.3.4 Prior or concurrent use of IL-2.

4.3.5 Prior or concurrent use of a CCR5 inhibitor.

4.3.6 Active drug or alcohol use or dependence that, in the opinion of the investigator, would interfere with adherence to study requirements.

4.3.7 Use of any immunomodulator, HIV vaccine, or investigational therapy within 30 days of study entry.

4.3.8 Use of human growth hormone within 30 days prior to study entry.

4.3.9 Initiation of testosterone or anabolic steroids within 30 days prior to study entry. (Exception: Chronic replacement dosages in patient's with diagnosed hypogonadism is allowed).

4.3.10 Evidence of splenic sequestration or suppressed bone marrow function:
- Clinical or radiographic evidence of significant splenomegaly.
- History of leukemia or lymphoma.
- History of myelosuppressive chemotherapy or irradiation

4.4 Exclusion Criteria (CSF Sub-Study)

4.4.1 Relative or absolute contraindication to lumbar puncture, such as current coagulopathy, thrombocytopenia (platelets<50,000/µL), hemophilia, or use of anticoagulant medication.

4.5 Study Enrollment Procedures

Prior to implementation of this protocol, sites must have the protocol and consent form approved by their local institutional review board (IRB). Sites must be registered with and approved by the CCTG Data Unit. Site registration must occur before any subjects can be enrolled in this study.

Once a candidate for study entry has been identified, details will be carefully discussed with the subject. The subject will be asked to read and sign the consent form that was approved by both the local IRB and the CCTG Data Center.

A patient identification number (PID) will be assigned to each patient screened for the study. PIDs will include a site code and three-digit extension. PIDs should not be reassigned even if the patient fails to enter the study. The PID must be included on every CRF and patient blood sample. Each site must maintain a master list of PIDs in a central location. The patient registration and inclusion/exclusion CRF will be entered into the online CCTG data system

4.6 Co-enrollment Guidelines

Co-enrollment in this study and other studies will be discussed with the protocol team and will be decided on a case by case basis.

5.0 STUDY TREATMENT

5.1 Dosing, Administration, and Duration

NOTE: Maraviroc will be dose-adjusted for concomitantly administered HIV medications according to the manufacture's recommendations:

5.1.1 Dosing

5.1.1.1 with strong CYP3A4 inhibitors, including:

- Protease inhibitors (except tipranavir/ ritonavir)
- Delavirdine
- ketoconazole, itraconazole, clarithromycin, nefazadone, telithromycin
- Darunavir/r + etravirine

MVC 150 mg twice daily

5.1.1.2 with non-inducers/ non-inhibitors of CYP3A4, including:

- Tipranavir/ ritonavir
- Nevirapine
- All NRTIs
- Enfuvirtide

MVC 300 mg twice daily

5.1.1.3 with strong CYP3A4 inducers, including:

- Efavirenz, etravirine
- rifampin

MVC 600 mg twice daily

5.1.4 Administration

NOTE: Maraviroc should be co-administered at the same time as the subject's other HIV medications. All other HIV medications should be administered according to each medication's recommended dosing.

5.1.4.1 MVC will be administered as a single 150 or 300 mg tablet or two 300 mg tablets PO twice daily with or without food

5.1.3 Duration

5.1.3.1 Maraviroc Therapy Intensification: Each subject will receive 24 weeks of therapy.

5.1.3.2 Post-Therapy Intensification: After 24 weeks, MVC will be discontinued and each subject will continue on baseline background HIV regimen, without Maraviroc, for an additional 12 weeks.

5.2 Product Formulation and Preparation

5.2.1 Maraviroc (Selzentry™, MVC): 150 or 300 mg tablets. Store at room temperature; 15º - 30º C (59º – 86º F).

5.3 Product Supply, Distribution, and Pharmacy

5.3.1 Study Product Acquisition

Study medications will be provided by Pfizer. All other HIV medications will not be supplied by the study, but will be made available to patients via prescription at cost to the patient and/or patient's health insurance.

5.3.2 Investigational Agent Accountability

The clinical site pharmacist is required to maintain complete records of all study products received from this study. All unused study products must be returned to the sponsors after the study is completed or terminated.

5.4 Concomitant Medications

5.4.1 Required Medications

5.4.1.1 Study medications (Maraviroc)

5.4.2 Prohibited Medications

NOTE: This list applies only to MVC. Refer to manufacture's recommendations for potential drug interactions/ contraindications for background HIV medications.

5.4.2.1 All investigational drugs and HIV vaccines

5.4.2.2 The combination of efavirenz + rifampin

5.4.2.3 Any immunomodulators

5.4.2.4 Systemic cytotoxic chemotherapy

5.4.2.5 All herbal products, including St John's wort, should be avoided because of the unknown drug interactions between herbal products and the antiretroviral drugs used in this study.

5.4.3 Precautionary Medications

NOTE: Refer to the individual package inserts for additional information regarding potential drug interactions that may require therapeutic drug monitoring and/or adjustment of concomitant medications. Competition for primary CYP3A metabolism or other mechanisms by study drugs could result in inhibition or stimulation of the metabolism of these drugs and create the potential for serious and/or life-threatening reactions such as cardiac arrhythmias, prolonged or increased sedation, and respiratory depression.

5.4.3.1 Systemic medications that interact at CYP3A as substrates, inhibitors, or inducers of the enzyme must be used with caution. These include but are not limited to:

Agent by Class	Precautionary Concomitant Medications
Alternative/complementary	Milk Thistle
Analgesics	Opioids, including:
	Codeine
	Methadone
	Meperidine
	Morphine
	Pentazocine (Talwin™)
Anesthetics	Propofol (Diprivan™)
Anti-arrhythmics	Amiodarone (Cordarone®)
	Bepridil (Vascor®)
	Disopyramide (Norpace™)
	Encainide (Enkaid™)

Agent by Class	Precautionary Concomitant Medications
	Lidocaine (Xylocaine™)
	Mexiletine (Mexitil™)
	Quinidine (Quinidex®)
Anti-convulsants	Carbamazepine (Tegretol™)
	Lamotrigine (Lamictal™)
	Phenobarbital (Luminal™)
	Phenytoin (Dilantin™)
	Valproic acid (Depakene™)
Anti-histamines	Chlorpheniramine (Chlor-Trimetron™ and others)
	Diphenhydramine (Benadryl™ and others)
Anti-infectives	Acyclovir (Zovirax™)
	Aminoglycosides
	Amphotericin B (Amphocin™, Fungizone™)
	Cidofovir (Vistide™)
	Clarithromycin (Biaxin™)
	Dapsone
	Erythromycin (E-mycin™ and others)
	Fluconazole (Diflucan™)
	Ganciclovir (Cytovene™)
	Isoniazid
	Systemic Itraconazole (Sporonox®)
	Systemic Ketoconazole (Nizoral®)
	Ribavirin (Virazole™)
	Rifabutin (Mycobutin®)
	Vancomycin (Vancocin™, Vancoled™)
	Voriconazole (Vfend)
Beta blockers (selected agents listed, caution should be exercised for the entire class)	Atenolol (Tenormin™)
	Metoprolol (Lopressor™, Toprol™)
	Propranolol (Inderal™, Inderide™,

Agent by Class	Precautionary Concomitant Medications
	and others)
Calcium channel blockers (selected agents listed, caution should be exercised for the entire class)	Amlodipine (Norvasc ®)
	Diltiazem (Cardizem® and others)
	Felodipine (Plendil®)
	Isradipine (DynaCirc®)
	Lacidipine (Lacipil™)
	Nicardipine (Cardene®)
	Nifedipine (Adalat™ and Procardia®)
	Nimodipine (Nimotop®)
	Nisoldipine (Sular®)
	Nitrendipine (Nitrendipine™)
	Verapamil (Calan™ and Isoptin®)
Hormonal agents	Estrogens and Progesterones
	Glucocorticoids
Hypoglycemics	Pioglitazone (Actos™)
HMG Co Reductase Inhibitors	Atorvastatin (Lipitor®)
	Fluvastin (Lescol®)
Psychiatric medications	Bupropion (Wellbutrin®, Zyban®)
	Chlorpromazine (Thorazine™)
	Clozapine (Clozaril®)
	Fluoxetine (Prozac™ and others)
	Nefazodone (Serzone®)
	Paroxetine (Paxil™)
	Risperidone (Risperdal®)
	Venlafexine (Effexor®)
Tricyclic Antidepressants (including but not limited to)	Amitriptyline (Elavil™ and others)
	Desipramine (Norpramin™ and others)
	Imipramine (Tofranil™ and others)
	Nortriptyline (Pamelor™ and others)

Agent by Class	Precautionary Concomitant Medications
Sedative/Hypnotics	All benzodiazepines
	Alprazolam (Xanax™)
	Clorazepate (Tranxene™)
	Diazepam (Valium™)
	Estazolam (ProSom™)
	Flurazepam (Dalmane™)
	Oxazepam (Serax™)
	Temazepam (Restoril™)
	Busprione (Buspar™)
	Zaleplon (Sonata™)
	Zolpidem (Ambien™)
Other agents	Chlorzoxazone (Parafon Forte™)
	Cimetidine (Tagamet™)
	Naloxone (Narcan™)
	Piroxicam
	Promethazine (Mepergan™, Phenergan™)
	Sildenafil (Viagra®)
	Warfarin (Coumadin™)

NOTE: For additional information and information updates please refer to:

http://www.hivdruginteractions.org/new/Uploaded_Attachment/52_Maraviroc%20Oct07.pdf

5.5 Adherence Assessment

Throughout the study, the documentation of adherence to study drugs and concomitant HIV medications is essential. During each visit an evaluation of adherence to study medications will be done by study personal and the clinical importance of strict adherence will be reinforced with subjects. An evaluation of study medication adherence will be done during each study visit at weeks 2, 4, 8, 12, 24 and 36.

6.0 CLINICAL AND LABORATORY EVALUATIONS

6.1 Schedule of Events – Clinical Evaluations

	Screening	Pre-Entry	Entry	Maraviroc Therapy Intensification (24 weeks)					Post-Maraviroc Intensificatoin	
	Within 30 days of entry	> 48 hrs before entry	Day 0	Week 2	Week 4	Week 8	Week 12	Week 24	Week 36	Treatment Failure, Tox or Premature D/C
Informed Consent	X									
Medical/Medication History	X									
Documentation of HIV	X									
Clinical Assessment / AE			X	X	X	X	X	X	X	X
Documentation of within criteria HIV RNA and CD4 prior to screen labs	X									
Complete Physical Exam			X							X
Targeted Physical Exam							X	X	X	
Start and Dispense Drug			X		X		X			
Stop and Collect Drug								X		X
Concomitant Medications	X		X	X	X	X	X	X	X	X
Adherence Assessment	X		X	X	X	X	X	X	X	X

Schedule of Events – Laboratory Evaluations

	Screening	Pre-Entry	Entry	Maraviroc Therapy Intensification (24 weeks)					Post-Maraviroc Intensificatoin	
	Within 30 days of entry	> 48 hrs before entry	Day 0	Week 2 (± 1 wk)	Week 4 (± 1 wk)	Week 8 (± 2 wk)	Week 12 (± 2 wk)	Week 24 (± 2 wk)	Week 36 (± 2 wk)	Treatment Failure, Tox or Premature D/C
Hematology (local lab)	X		X	X	X	X	X	X	X	X
Prothrombin time (local lab) †	X									
Chemistry / LFT (local lab)	X				X		X	X	X	X
Pregnancy test (local lab)	X	And anytime when suspected								
CD4+/CD8+ (local lab)	X		X	X	X	X	X	X	X	X
§ CD4 ≤ 350; HIV RNA ≤ 50	X									
Plasma HIV-1 RNA PCR (local lab)	X		X		X			X	X	X
Plasma for MVC levels †			X		X			X		
Extended flow cytometry & PBMC collection (UCSD lab)		X	X		X			X		
Microarray - PBMCs collection (local processing in real-time)			X		X			X		
Lumbar puncture†			X		X			X		
Plasma for storage			X		X			X	X	
Serum for storage			X		X			X	X	
Ship ambient sample for extended flow in real-time		X	X		X			X		
Ship frozen all stored PBMCs, plasma and serum									X	
HIV Resistance & Tropism testing										X

§ If available within 30 days of screen then do not need to repeat laboratory test and can use value obtained within 90 days as screen value
† Only for subjects co-enrolled in the central nervous system sub-study

6.2 Definitions for Schedule of Events – Timing of Evaluations

6.2.1 Evaluations Prior to Study Entry

Occur prior to the subject taking any study medications, treatments, or interventions.

Patient Registration

A patient identification number (PID) will be assigned to each patient screened for the study. PIDs will include a site code and three-digit extension. PIDs should not be reassigned even if the patient fails to enter the study. The PID must be included on every CRF and patient blood sample. Each site must maintain a master list of PIDs in a central location. The patient registration and inclusion/exclusion must be entered on-line at www.cfar.ucsd.edu/intranet

Screening Site Clinical Labs

If plasma HIV-1 RNA and CD4+ T-cell counts were obtained at a CLIA-approved lab no more than 30 days prior to screening and results are available then these labs do not need to be repeated. Otherwise, a plasma HIV-1 RNA level and a CD4 cell count measurement need to be done at screening. Please record actual dates laboratories were done in the CRF.

All other study screening laboratories must be determined within 30 days of study entry (Day 0). Screening and entry evaluations must be separated by at least 48 hours. Hematology, chemistry and pregnancy testing will be coordinated through the site's local laboratory and results will be tracked by the CCTG Data Center.

Once the screening laboratories have been done the patient will be evaluated for study eligibility if all other entry criteria are satisfied.

The total blood volume for screening labs is 20 mL.

If the HIV RNA or other laboratory values are outside the eligible range, the site may re-screen a patient on one occasion. However, the study will not pay for additional re-screening laboratories.

Pre-Entry

Specimens for extended flow cytometry and PBMC storage need to be shipped by each site in real-time to the central laboratory (UCSD).

The total blood volume for pre-entry labs is 20 mL.

Entry

The entry evaluation should be scheduled within 30 days of the screening visit. Plasma and serum for storage should be obtained, however, specimens will be stored then shipped in batch on week 36 (end of study) along with other plasma, PBMC and serum specimens. The first dose of study medication should be given and witnessed by the study coordinator **after** drawing the entry laboratories.

The total blood volume for entry labs is 73 mL.

Lumbar puncture – 25 mL cerebral spinal fluid

6.2.2 On-Study Evaluations

Evaluations during the MVC treatment intensification period will occur on weeks 2, 4, 8, 12, and 24. An addition visit will occur after MVC treatment intensification on week 36. Study visits at week 2 and 4 will have a visit window period of 1 week. For study visits at week 8, 12, 24 and 36 there will be a 2 week visit window period. In instances of virologic failure and/or premature treatment discontinuation evaluations would also need to occur as soon as possible.

Total blood volume for laboratory evaluations during MVC treatment intensification (week 2 to week 24) will be approximately 200 mL.

Lumbar puncture – 25 mL cerebral spinal fluid (week 4 and 24)

Total blood volume for laboratory evaluations during post-MVC treatment intensification (week 36) will be approximately 40 mL.

6.2.3 Evaluations and plan for registered subjects that do not complete week 4 of the study:

a. Those subjects that do not start treatment will be replaced and will have no follow-up evaluations performed.

b. Subjects that discontinue antiretroviral therapy and/or MVC before week 4 will be replaced and will have no follow-up evaluations performed.

6.2.4 Evaluations and plan for randomized or registered subjects that complete week 4 but not the remainder of the study:

a. Subjects who complete week 4 of the study but then discontinue their antiretroviral regimen and/or MVC will not be replaced and will not be discontinued from the study.

6.2.6 Change in Background Antiretrovial Regimen

a. Subjects who change their background treatment regimen before week 4 will be discontinued from the study. Those subjects that change their background treatment regimen after week 4 and who choose to continue MVC treatment intensification will be followed on the study.

b. If the regimen change is due to toxicity, then the subject would be followed per criteria of a Change of Therapy Due to Toxicity visit (section 6.1).

6.2.7 Virologic Failure during the MVC treatment intensification portion of study

Virologic failure will be decided on a case by case basis by the local site's study investigator in collaboration with the protocol team. Suggested criteria include: two consecutive HIV RNA loads > 50 copies/mL. For subjects who develop a plasma HIV-1 RNA > 50 copies/mL, a confirmatory plasma HIV-1 RNA test must be performed within 4 weeks of the initial HIV-1 RNA sample. Subjects who meet criteria for confirmed virologic failure will undergo real-time genotypic resistance and tropism testing (testing not provided by study) and alternative regimen will be chosen by the site investigator with recommendations of the protocol team. Follow-up intervals will continue per study protocol, but the site investigator may schedule additional non-study, safety visits as necessary. The patient will be followed off study medication but on study for the remainder of the study.

6.2.8 Post-Treatment Evaluations

All randomized subjects who complete the 24 weeks of MVC intensification will complete the week 36 post-MVC intensification visit.

6.2.9 Pregnancy

Women who become pregnant during the study will be required to permanently discontinue their MVC treatment intensification. They should be advised to seek best available medical care for their pregnancy according to USPH Guidelines

6.3 Special Instructions and Definitions of Evaluations

6.3.1 Documentation of HIV

HIV-1 infection documented by a rapid HIV test or any licensed ELISA test kit and confirmed by a different method ELISA, Western blot, rapid HIV test, or plasma HIV-1 RNA assay at any time prior to study entry. Alternatively, if a licensed ELISA is not available, two HIV-1 RNA values >2000 copies/mL at least 24 hours apart by any CLIA certified laboratory may be used to document HIV-1 infection at any time prior to study entry.

6.3.2 Medical and Laboratory History

At screening a medical history will be obtained and must be recorded in the source documents. The medical history should include any previous HIV-related diagnoses and AIDS-defining events.

In addition, prior to obtaining screening laboratories, documentation of all historic plasma HIV-1 RNA load and CD4+ T-cell counts since initiation of first ARV regimen must be recorded in the source documents and on-line CRF form (www.cfar.ucsd.edu/intranet). **Of note**: a minimum of 3 CD4+ T-cell (below 350 cells/mm^3) and HIV RNA values (below 50 copies/mL) obtained $\geq$ 30 days apart must be obtained and recorded.

6.3.3 Medication History

At screening, a complete history or all prior antiretroviral medications will be recorded in the CRF. For all other medications, a medication history (**only of those taken within the last 30 days prior to entry**) with actual or estimated start and stop dates should be obtained and recorded in the source documents and the concomitant medication CRF, including:

- All prescription medications. Including medications taken for the treatment or prophylaxis of opportunistic infections.
- Non-prescription medications.
- Alternative therapies and/or dietary supplements.
- Allergies to any medications and their formulations must be documented.

6.3.4 Concomitant Medications

During study visits (See section 6.1 for specific dates) all concomitant medications taken since the last visit will be recorded in the source documentation and entered into the concomitant medication log CRF.

6.3.5 Study Treatment Modifications

All modifications to study drug(s) including initial doses, patient-initiated and/or protocol-mandated interruptions, modifications, and permanent discontinuation of HIV antiretrovirals need be recorded on the CRFs at each visit. The same needs to be done for all background antiretroviral agents.

6.3.6 Clinical Assessments

Complete Physical Exam

A complete physical examination will be performed at entry. Documentation must include a complete review of systems and any HIV-related, toxicity-related, or AIDS-defining events.

Targeted Physical Exam

A targeted physical examination will be based on any signs or symptoms previously identified that the subject has experienced within 30 days of entry or since the last visit. This examination will be performed at entry, weeks12, 24 and 36 and in instances of virologic failure or premature treatment discontinuation if they should occur. Documentation must include any HIV-related, toxicity-related, or AIDS-defining events.

Height and Weight

Height and weight should be measured at study entry.

Signs and Symptoms

All signs, symptoms, deaths, and toxicities must be documented in the subject's record. At entry, record all signs/symptoms experienced within 30 days of entry on the CRFs. For all other visits including the time of confirmation of virologic failure, record all Grade $\geq$ 2 signs and symptoms, HIV-related and AIDS-defining events and deaths on the CRFs that have occurred since the last visit. Any signs or symptoms that lead to a change in treatment, regardless of Grade, must be recorded on the CRF. The source document must include date of onset and date of resolution, but the CRF will only record prevalence of a given adverse event since the previous study visit.

Refer to the Division of AIDS Table for Grading Adult Adverse Experiences, which can be found on the CCTG website: www.cctg.ucsd.edu

Diagnoses

The following should be recorded on the CRFs: HIV-related diagnoses, HIV-related malignancies, non-HIV related malignancies, myocardial infarctions,

hepatic failures, AIDS-defining events and death. Any other diagnosis that is, in the opinion of the site investigator, associated with study medications, should be recorded on the adverse event CRF. The source document must include date of diagnosis and date of resolution.

Karnofsky Performance Status

A Karnofsky performance status must be completed within 30 days before study entry.

Vital Signs

Temperature, pulse, and blood pressure collected at all visits and kept as a part of the source document.

6.3.7 Laboratory Evaluations

For all visits record all Grade ≥ 2 laboratory toxicities on the CRFs throughout the course of the study. All values, regardless of toxicity, of specific laboratories will also be recorded on the laboratory CRF; including: wbc, neutrophil count, hemoglobin, platelets, blood urea nitrogen, creatinine, glucose, AST/ALT, alkaline phosphatase, and total bilirubin.

Any laboratory toxicities that lead to a change in treatment, regardless of Grade, must be recorded on the adverse event CRF.

Refer to the Division of AIDS Table for Grading Adult Adverse Experiences, which can be found on the CCTG website: www.cctg.ucsd.edu

Hematology:

Hemoglobin, hematocrit, white blood cell count (WBC), differential WBC, absolute neutrophil count (ANC), and platelet count will be performed in real time at the site's local laboratory.

Blood volume: 3 mL

Coagulation tests

For subjects enrolled in the CSF Sub-Study: Prothrombin time, partial prothrombin time and calculation International Ratio (INR) will be performed in real time at the site's local laboratory.

Blood volume: 3 mL

Liver & Kidney Function Tests

Total bilirubin, AST (SGOT), ALT (SGPT), and alkaline phosphatase, BUN, creatinine, electrolytes (sodium, potassium, chloride, and bicarbonate) will be performed in real time at the site's local laboratory.

Blood volume: 7 mL

Pregnancy Test

For women with reproductive potential: Urine β-HCG (urine test must have a sensitivity of ≤ 50 mIU/mL) should be done at the site's local laboratory during screening, at study entry and whenever clinically suspected.

5 ml urine

6.3.8 Immunologic Studies

Nadir CD4+

The subject's prior nadir CD4+ cell count (absolute value and date) should be documented during screening and, when possible, a copy of the nadir CD4+ cell count report should be included in the source document. If this documentation is not available, then subject recollection will suffice. For subjects who do not know the exact nadir value and for whom there is no source documentation, then recall of the categorical nadir (e.g., <50, <100, <200 cells/mm^3) will suffice.

CD4/CD8

A screening CD4/CD8 count and percentage must be performed at the site's local laboratory within 30 days prior to study entry unless a CD4 value is available from a CLIA-certified laboratory within 30 days of screening.

Because of the diurnal variation in CD4+ and CD8+ cell counts; all determinations should be obtained consistently in either the morning or the afternoon throughout the study, if possible.

Blood volume: 5 mL

Extended Flow Cytometry Markers

Extended flow markers for activation, maturation and regulatory function will be evaluated on CD4+/CD8+ T-cells. Samples will be collected at pre-entry, entry and on weeks 4 and 24. Pre-entry and entry laboratories must be separated by a minimum of 48 hours. These analyses will be done on fresh samples in real-time. **Specimens will need to be shipped ambient, overnight and in real-time to the UCSD central laboratory**.

Blood volume: 10 mL

6.3.9 Virologic Studies

Plasma HIV-1 RNA

A screening HIV-1 RNA must be performed at the site's local laboratory within 30 days prior to study entry unless a HIV-1 RNA value is available from a CLIA-certified laboratory within 30 days of screening.

Plasma HIV-1 RNA should be performed at the same local laboratory during the study and collected at entry, weeks 4, 24, 36 and in instances of virologic failure.

Genotype Resistance and Tropism Assay

Samples for genotypic and viral tropic analysis obtained at the time of virologic failure will be tested in real time for subject management by the site's local laboratory. All resistance testing results should be scanned and uploaded on the on-line data collection system for review by the protocol team (www.cfar.ucsd.edu/intranet). The study does not provide resistance testing.

Stored Plasma

Stored Plasma will be collected at pre-entry, study entry, and weeks 4, 24 and 36.

* Specimens will be stored at the site's local laboratory and batched shipped to the central laboratory (UCSD) after completion of the study.

Blood volume for combined HIV RNA and stored plasma: 15 mL

6.3.10 Specimens for Serum Lipopolysaccharide (LPS) measurements

Stored Serum

Stored Serum will be collected at pre-entry, study entry, and weeks 4, 24 and 36.

* Specimens will be stored at the site's local laboratory and batched shipped to the central laboratory (UCSD) after completion of the study.

Blood volume: 10 mL

6.2.11 Microarray Studies

Blood for PBMCs for microarray studies will be collected at entry and weeks 4 and 24. Specimens will need immediate on-site processing and storage. All

specimens must be **shipped overnight, frozen to the central laboratory (UCSD)** on week 36.

Blood volume: 30 mL

6.3.12 PBMCs for storage

Blood for PBMCs will be collected and **shipped ambient, overnight and in real-time to the central laboratory (UCSD)** at pre-entry, entry and weeks 4 and 24.

Blood volume: 10 mL

6.3.13 Lumbar Puncture

For subjects enrolled in the CSF Sub-Study: CSF will be collected at baseline, weeks 4 and 24 for cell counts, protein, glucose, HIV RNA and markers of immune activation.

Cerebral Spinal Fluid volume: 25 mL

6.3.14 Plasma MVC plasma concentration

For subjects enrolled in the CSF Sub-Study: Blood for plasma MVC concentrations will be collected at baseline and weeks 4 and 24.

Blood volume: 20 mL

6.3.15 Adherence Assessment

Adherence assessment will be preformed by the study coordinator using standard ACTG adherence format on weeks 2, 4, 8, 12, 24, and 36 of therapy and in instances of virologic failure and/or premature treatment discontinuation.

6.4 Off-Drug Requirements

Additional safety monitoring and reporting of serious adverse experiences (SAEs) continues to be required upon completion or discontinuation of study treatment regardless of whether a protocol follow-up period is scheduled to occur. Adverse experiences occurring during the immediate 8-week period after the last dose of study treatment which meet SAE reporting requirements must be reported to the **CCTG Data Center**. Additionally, after 8 weeks OFF study treatment, there are four types of events that must be reported to the **CCTG Data Center** if the relationship to the study drug is assessed by the site physician as definitely, possibly, or unable to judge: DEATHS, NEW ONSET CANCERS, CONGENITAL ANOMALIES, AND PERMANENT DISABILITIES.

7 TOXICITY MANAGEMENT

Specific management of toxicity will be discussed only for the study-provided medication, MVC. The management of medication related toxicities should be undertaken by the local investigators, with guidance available from the protocol team, protocol pharmacist and pharmaceutical sponsor, to insure the optimal safety and efficacy for the individual subject.

7.1 Commonly occurring adverse events

7.1.1 Grade 1 or 2

Subjects who develop a Grade 1 or 2 adverse event or toxicity may continue MVC without alteration of the dosage. Those subjects experiencing Grades 1 or 2 adverse events which results in discontinuation of the MVC should be removed from the study.

7.1.2 Grade 3

Management of Grade 3 toxicities should be discussed with the protocol team via email. Please refer to the subsequent sections for management of specific events.

In the event that a subject develops a **symptomatic** Grade 3 reaction considered to be **MVC-related**, the study drug should be discontinued and the subject should be followed weekly until resolution of the adverse event. Once Grade 3 is resolved the subject should be removed from study.

7.2.3 Grade 4

Management of Grade 4 toxicities should be discussed with the protocol team via email.

Subjects who develop a Grade 4 adverse event or toxicity judged to be **MVC related** will have the study drug permanently discontinued. Exemptions include: asymptomatic elevations of CPK, cholesterol or triglycerides. For these laboratories, medication may be continued at the discretion of the site investigator and laboratories repeated within 2 weeks. For other Grade 4 events, if the toxicity or laboratory elevation is thought not to be due to MVC, MVC may be continued and laboratories repeated within 2 weeks. If it is not possible for the investigator to discern the causative agent or MVC is possible/ probably the causative agent, then all MVC must be discontinued. A new regimen may be chosen at the discretion of the local investigator. Subjects experiencing Grade 4 adverse events requiring permanent discontinuation of MVC therapy should be followed weekly until resolution of the adverse event. Once Grade 4 is resolved the subject should be removed from study.

7.2.4 Rash

Grade 1 or 2

MVC may be continued without interruption. Subjects with a Grade 1 or 2 rash may be treated symptomatically with permitted antipyretic, antihistamine, and/or non-steroidal anti-inflammatory medications, but should be monitored closely by the local investigator.

Grade 3 or 4

A rash of Grade 3 or 4 necessitates that all MVC should be held for any Grade 3 or 4 rash unless the rash is determined to be unrelated to MVC. Upon resolution to Grade ≤ 1, the subject should be removed from study.

7.2.5 Nausea and Vomiting

Grade 1 or 2

MVC may be continued without interruption. Subjects with Grade 1 and 2 nausea or vomiting may be treated symptomatically with permitted oral antiemetic therapies or antiemetic suppositories. Subjects should be instructed to take medications with food.

Grade 3 or 4

Subjects with Grade 3 or 4 MVC-related nausea and vomiting should interrupt MVC until the toxicity grade returns to Grade ≤ 2 or to baseline and be treated symptomatically. If Grade ≥ 3 nausea and vomiting recurs upon the resumption of MVC despite symptomatic treatment, MVC should again be interrupted and the protocol team notified for possible study discontinuation.

7.2.6 Diarrhea

Grade 1 or 2

MVC should be continued without interruption. Subjects with diarrhea of any toxicity grade may be treated symptomatically with permitted antimotility agents.

Grade 3 or 4

For diarrhea that is unresponsive to antimotility agents and for which an alternative etiology (e.g., infectious diarrhea) is not established, MVC should be interrupted until resolution of diarrhea to Grade ≤ 2 or baseline. If Grade ≥ 3 diarrhea recurs upon the resumption of study medications, MVC should be

interrupted and the protocol team should be notified for possible study discontinuation.

7.2.7 Hyperglycemia

Fasting hyperglycemia of > 110 to 125 mg/dL is considered evidence of impaired glucose tolerance. A fasting blood glucose level above 126 mg/dL is highly suggestive of diabetes mellitus. Subjects with fasting hyperglycemia may continue MVC at the discretion of the investigator, but should be discussed with the protocol team via email. A confirmatory fasting glucose must be obtained within 4 weeks and prior to the institution of medical therapy. Hyperglycemia may be treated with oral hypoglycemic agents or insulin according to standard guidelines.

7.2.8 AST/ALT Elevations

Grade 1 or 2

MVC may be continued.

Grade 3

All grade 3 or 4 elevations of AST/ALT should be discussed immediately with the protocol team via email. MVC may be continued for Grade 3 AST/ALT elevations at the discretion of the site investigator after discussion with the protocol team. Careful assessments should be done to rule out the use of alcohol, non-study medication-related drug toxicity, or viral hepatitis as the cause of the Grade 3 elevation. The possibility of lactic acidosis syndrome should also be explored.

Grade 4

MVC should be held for AST or ALT Grade 4 elevations until the toxicity returns to Grade $\leq$ 2. If the Grade 4 elevation in AST or ALT recurs, MVC should again be interrupted and the protocol team notified for possible study discontinuation.

7.2.9 Creatinine Elevations

Discontinue MVC if confirmed creatinine clearance becomes < 50 mL/min. Subjects should be followed as medically indicated until the creatinine returns to Grade < 2. The protocol team should be notified within 48 hours of any permanent therapy discontinuations.

7.2.10 Anemia/Neutropenia

Subjects with Grade 3 or 4 anemia or neutropenia attributed to any of the NRTI's should have all study treatment interrupted until the abnormality returns to Grade ≤ 2. Therapy may be resumed after the anemia or neutropenia has returned to Grade ≤ 2 using the same dual-NRTI regimen. If Grade 3 or 4 anemia or neutropenia recurs, MVC should again be interrupted and the protocol team notified for possible study discontinuation.

Subjects with anemia or neutropenia may be treated off-study with blood transfusion and/or erythropoietin (Epogen®, Procrit®) or G-CSF (Neupogen®) at the discretion of the site investigator.

7.2.11 CK Elevations

Asymptomatic elevations in CK or elevations due to exercise **should be repeated (within 2-4 weeks) to assure that they are transient or due to exercise and** will not require a change in study treatment. Elevations in CK > 20 X ULN should be repeated after the subject has abstained from exercise for ≥ 24 hours. For persistent CK elevations >20 x ULN should prompt a permanent discontinuation of MVC and subjects should be discontinued from study and the protocol team should be notified.

8 CRITERIA FOR TREATMENT DISCONTINUATION

8.1 Criteria for Treatment Discontinuation

- MVC drug-related toxicity (see section 7, Toxicity Management).
- Active drug or alcohol use or dependence that, in the opinion of the investigator, would interfere with adherence to study requirements.
- Requirement for prohibited concomitant medications (see section 5.4.2)
- Pregnancy or breast-feeding.

8.2 Criteria for Discontinuation from the Study

- Failure to complete week 4 whether due to toxicity, poor adherence or need to stop drug.
- Request by the subject to withdraw.
- Request of the primary care provider if s/he thinks the study is no longer in the best interest of the subject.
- Clinical reasons believed life threatening by the physician, even if not addressed in the toxicity management of the protocol.
- Subject judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm to self or seriously interfere with the validity of the study results.

- At the discretion of the CCTG, FDA, investigator, or pharmaceutical sponsors.

9.0 STATISTICAL CONSIDERATIONS

9.1 General Design Issues

This is a pilot study to investigate associations between MVC treatment intensification and 1) changes in gene expression as identified by microarray analysis and 2) change in the slope of the CD4 recovery. The study is powered to detect a difference in gene expression before and after MVC. Secondary analysis will investigate whether a difference in CD4 recovery (measured by CD4 slope) is observed before and after MVC treatment intensification.

9.2 Endpoints

9.2.1 Primary

1. Differences in gene expression profiles obtained at baseline and weeks 4 and 24.

9.2.2 Secondary

1. CD4+ T-cell absolute count and percentage at baseline, weeks 4 and 24.
2. The proportion of CD4+/CD8+ T-cell immune activation, maturation, regulatory and apoptosis markers at baseline and weeks 4 and 24.
3. Grades $\geq$ 2 laboratory abnormalities and signs and symptoms.
4. Paired plasma to CSF ratios of MVC concentration at week 4.
5. Soluble immune activation markers in CSF at baseline and week 4.
6. HIV RNA loads in CSF at baseline and week 4.

9.3 Sample Size and Accrual

Sample size calculations are based on within-patient comparisons of gene expression profiles from baseline to week 24. The calculations required to make such comparisons will depend on several experimental parameters including biological variability of the samples within each class, the expected fold change to be detected, technical sensitivity of the microarray technology, and acceptable error rates of the results. As researchers in this field have shown [52], one way to ensure acceptable error rates is to control the false discovery rate (FDR), which is

equivalent to setting a minimum specificity. The FDR is the expected fraction of significant genes, which in practice, do not differ in reality. This differs from the false positive rate (FPR), which is the proportion of non-differentially expressed genes that, just by chance, appear to differ.

Determining the biological variability of the system is a difficult task, especially when there is no pre-existing data available. The ranges of conditions that can contribute to biological variability include environmental conditions, cell types, and genotypic variation [53]. Based on an extensive literature review, Zien et al. [53] conclude that a value of 0.4 provides a conservative estimate of the biological standard deviation (variability between and within classes of biological samples). We will consider biological standard deviation values of 0.4 for our power calculations. Further, we will consider a technical variation of 0.3 and 0.4.

As a conservative approach, we base the sample size calculations on the Wilcoxon Rank Sum test. This is a non-parametric procedure, in which no distributional assumptions are placed on the data. Using the on-line power calculator developed by Zien et al. [53] which computes sample sizes for microarray experiments, Table 1 provides the approximate statistical power for various biological variation, technical variations and signal-to-noise ratios. Here, we consider a Type 1 error of 0.01 and a fold change of 2. We assumed 500 hits from the 38,000 genes. Notice that these power calculations are based on the number of samples allocated to the training set (either 20 or 25 per group). The sample size calculations indicate fair power (60-80%) to find a difference in gene expression before and after maraviroc treatment.

TABLE 1. Power Calculations (Type 1 error=0.01, Fold change=2.0)

Baseline (N)	Week 24 (N)	Biological Variation	Technical Variation	Signal-to-noise	Power
20	20	0.4	0.3	3	77%
20	20	0.4	0.4	3	68%
20	20	0.4	0.3	2	61%
25	25	0.4	0.3	3	88%
25	25	0.4	0.4	3	80%
25	25	0.4	0.3	2	75%

9.4 Methods of Analysis

9.4.1 Analysis of Primary Objective

RNA from a set of 60 samples (30 at baseline and 30 at week 24) will be isolated, amplified, labeled and hybridized on the Sentrix Human-6 Expression BeadChip containing approximately 38,000 probes per chip. The Illumina platform will be used which is a one-color platform using cye 3. Initially, filtering and pre-processing methods will be performed on the 38,000 genes in order to eliminate genes that are poorly measured or that exhibit aberrant values.

9.4.1.1 Pre-processing

Microarray data will be pre-processed via the Robust Multichip Average (RMA) method [54], as implemented in Bioconductor [55], a suite of programs, for the R statistical programming language [56]. RMA pre-processing consists of three steps: background adjustment, normalization, and summarization [54]. The RMA background noise adjustment corrects for processing effects and cross-hybridization by using a convolution model [54]. The purpose of normalization is to reduce the non-biological variation across the chips in an experiment. Non-biological variation includes technical variability, which is related to the experimental procedure of the assay. RMA uses the quantile normalization technique to adjust the gene expression intensities such that the distributions of the probe intensities are the same across all chips [57]. The last step, summarization, is a method to calculate a single gene expression value by mathematically combining the probe intensities from each probe set. RMA employs the robust multi-chip linear model fitted on a log scale [58].

9.4.1.2 Statistical Analysis

We expect to eliminate approximately 2-3% of genes in through pre-processing and filtering. The data will be randomly divided into a separate training set and a test set. 20 Baseline and 20 Week 24 samples will be used for training. The remaining 10 baseline and 10 week 24 samples will be used for testing. Using criteria such as 2-fold differences in gene expression between the two measured time points within the group of patients and a false discovery rate (the percentage of genes that will be identified by chance) of less than 2%, Significance Analysis of Microarrays (SAM) [59]) will be applied to the training set to rank order genes that best can predict differences in gene expression profiles between the two time points. The SAM method identifies genes with statistically significant changes in expression by computing a score for each gene relative to the gene specific standard deviation. Genes with scores greater than a certain (adjustable) threshold will be declared significant.

The set of highest ranking genes identified with the SAM procedure will then be clustered using unsupervised hierarchical clustering analysis [60]. The purpose of this clustering analysis is to confirm the results from SAM and to further explore the microarray data in order to possibly reveal unknown subclasses. In unsupervised hierarchical clustering, no predefined reference vectors are used and the data is not partitioned into a particular cluster in a single step. Instead, a series of partitions takes place. Next, we will use methods such as prediction analysis of microarrays (PAM) based on nearest shrunken centroids [61] to devise a cross-validated gene expression predictor for these cluster-defined classes. The PAM method is a modification of the more standard nearest-centroid method. Each class centroid is shrunk toward the overall centroids after standardizing by the within-class standard deviation. The test set will then be used for validation of our

predictor. The genes that best predict the classes will be examined for similarities such as possibly participating in common genetic pathways.

9.4.3.1. Analysis of Secondary Objectives

Secondary analyses will include descriptive statistics and tests of significance between pre- and post-MVC intensification. All significance tests will be two-sided and no adjustment for multiple comparisons will be made. If necessary due to assumption failures, appropriate non-parametric statistical tests will be applied.

1. Differences in the change in CD4+ T-cell absolute count and percentage from baseline to week 4 and baseline to week 24 will be compared descriptively via separate paired Student t-tests. Repeated CD4+ T-cell absolute count measured at different time points (3 historic time-points, baseline, week 4, and week 24) will be treated as the outcome variable in a linear mixed-effects model. The primary fixed effects will include time, period (MVC vs. pre-MVC), and interaction between time and period; random effects will include both intercept and slope allowing each subject to have individual starting CD4+ T-cell counts and rate of change. Baseline covariate adjustment will be included if necessary. A significant interaction term between time and period with positive coefficient will indicate if the rate of CD4 recovery is greater during MVC compared to pre-MVC.

2. Differences in the rate of CD4+ T-cell recovery (absolute count and percentage) during MVC intensification in subjects receiving a PI/r- vs. NNRTI-based regimen will be compared via the linear mixed effects model, The fixed effects will include time, regimen (PI/r vs NNRTI), and regimen-by-time interaction; random effects will include both intercept and slope. The regimen-by-time interaction term in the model will indicate the difference in CD4+ T cell recovery rates between the two regimen groups. Baseline covariate adjustment will be included if necessary.

3. Differences in gene expression profiles between subjects reciving a PI/r vs. NNRTI-based regimen at baseline and weeks 4 and 24 will be examined by comparing the gene lists generated in the primary analysis by regimen type. If necessary, statistical modeling will include a dichotomous variable to indicate regimen type.

4. Differences in the proportion of CD4+/CD8+ T-cell immune activation, maturation, regulatory and apoptosis markers between baseline and weeks 4 and 24 will be compared cross-sectionally via McNemar’s test or paired t-tests as appropriate.

5. Association between change in early immune subsets and phase 2 CD4 recovery will be assessed via Pearson’s correlation analysis.

6. MVC plasma to CSF ratios at weeks 4 and 24 will be reported via descriptive statistics. Differences in MVC concentration ratios between subjects receiving a PI/r- vs. NNRTI-based background regimen will be compared via McNemar's test or paired t-tests as appropriate.

7. Descriptive statistics of CNS markers of immune activation will be reported via descriptive statistics. Differences in CNS markers of immune activation between baseline and weeks 4 and 24 will be compared cross-sectionally via McNemar's test or paired t-tests as appropriate.

8. Association between change in CNS markers of immune activation and MVC plasma to CSF ratios will be assessed via Pearson's correlation analysis.

9. Descriptive statistics of toxicity/intolerance during MVC treatment intensification will be reported via descriptive statistics. Differences in proportions of grades > 2 laboratory abnormalities and signs and symptoms will be compared via McNemar's test between time points.

10. Virologic failure during MVC treatment intensification will be reported via descriptive statistics.

9.5 Monitoring

The safety and tolerability of the study medications will be monitored by means of adverse event reports (AER) and monthly toxicity reports that will include laboratory and clinical data. These reports will be discussed by the protocol team on monthly conference calls.

Data and Safety Monitoring Board, it is the responsibility of the Principal Investigator and the CCTG protocol team to interpret the toxicity data and make any decisions needed to protect patients from undue risk. Accrual and toxicity summaries will be provided to the Protocol Chairs and CCTG Investigators by the Data Manager and Biostatistician.

If more than **five** patients develop virologic failure or discontinue MVC due to an adverse event or laboratory toxicity, an independent interim review committee will be established to review the study results to date. The interim review committee will be composed of at least 3 people (including one statistician) outside the protocol team. The committee will make specific recommendations about changes to the study protocol.

10.0 DATA COLLECTION AND MONITORING AND ADVERSE EXPERIENCE REPORTING

10.1 Records to Be Kept

Case report forms (CRF) will be provided for each subject. Subjects must not be identified by name on any CRFs. Subjects will be identified by the patient identification number (PID) provided by the **C**CTG Data Unit upon registration.

10.2 Role of Data Management

10.2.1 Instructions concerning the recording of study data on CRFs will be provided by the **C**CTG Data Unit.

10.2.2 It is the responsibility of the **C**CTG Data Unit to assure the quality of computerized data for this study.

10.3 Clinical Site Monitoring and Record Availability

10.3.1 Site monitors provided by the CCTG will visit participating clinical research sites to review the individual subject records, including consent forms, CRFs, supporting data, laboratory specimen records, and medical records (physicians' progress notes, nurses' notes, individuals' hospital charts), to ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect sites' regulatory files to ensure that regulatory requirements are being followed and sites' pharmacies to review product storage and management.

10.3.2 The investigator will make study documents (e.g., consent forms, drug distribution forms, CRFs) and pertinent hospital or clinic records readily available for inspection by the local IRB, the site monitors, the Food and Drug Administration (FDA), the pharmaceutical sponsor(s), or the sponsor's designee for confirmation of the study data.

10.4 Serious Adverse Experience (SAE) Reporting

Serious adverse experiences must be documented on the Serious Adverse Experience (SAE) Reporting Form and submitted to the CCTG Data Unit.

11.0 <u>HUMAN SUBJECTS</u>

11.1 Institutional Review Board (IRB) Review and Informed Consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB or ethics committee responsible for oversight of the study. A signed consent form will be obtained from the subject. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the subject.

11.2 Subject Confidentiality

All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by coded number only to maintain subject confidentiality. All records will be kept locked. All computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the FDA, the OHRP, the pharmaceutical supporter(s), or the supporter's designee.

11.3 Study Discontinuation

The study may be discontinued at any time by the IRB, the pharmaceutical supporter(s), the FDA, or other government agencies as part of their duties to ensure that research subjects are protected.

12.0 PUBLICATION OF RESEARCH FINDINGS

Publication of the results of this trial will be governed by CCTG policies. Any presentation, abstract, or manuscript will be made available for review by the pharmaceutical supporters prior to submission.

13.0 BIOHAZARD CONTAINMENT

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health.

All infectious specimens will be transported using packaging mandated in the Federal Code of Regulations, CDC 42 CFR Part 72. Please also refer to individual carrier guidelines, e.g., FedEx, Airborne, for specific instructions.

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