MODULE 2.6.1. INTRODUCTION

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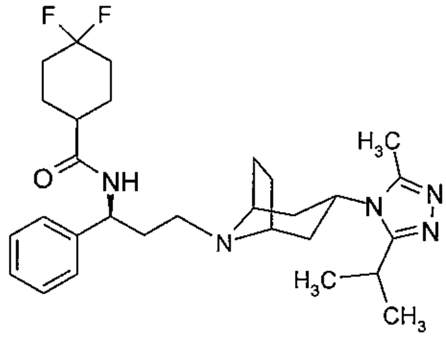
2.6.1. Introduction

Maraviroc (MVC, UK-427,857) is an inhibitor of HIV-1 entry and is intended for chronic administration to help prevent the development and progression of AIDS in individuals positive for HIV-1. Maraviroc selectively binds to the human chemokine receptor CCR5 and inhibits the interaction of the envelope glycoprotein (gp120) from CCR5-tropic HIV-1 strains with CCR5. Binding of gp120 to CCR5 is an essential step in the HIV-1 entry process for CCR5-tropic strains. The structure of maraviroc is presented in Table 1.

Table 1. Structure of Maraviroc

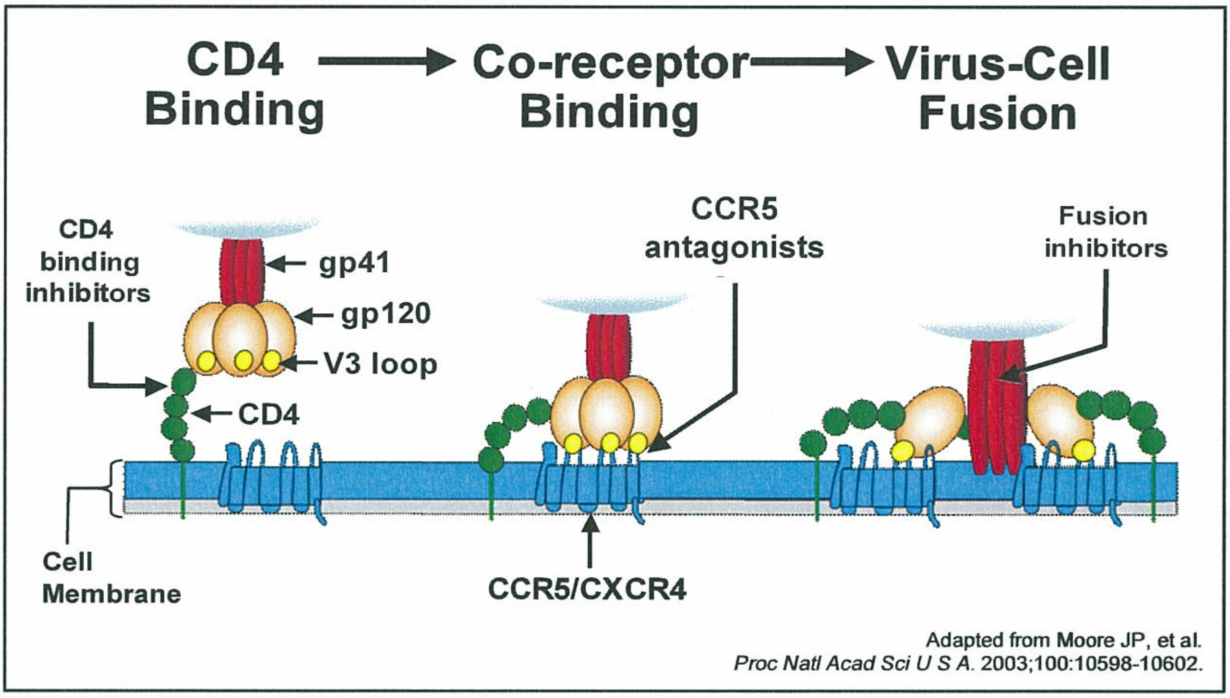
INN	MARAVIROC
Laboratory Code:	UK-427,857
Molecular Weight:	513.7
Molecular Structure:	
Chemical Name	4,4-difluoro-*N*-{(1*S*)-3-[*exo*-3-(3-isopropyl-5-methyl-4*H*-1,2,4-triazol-4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl}cyclohexanecarboxamide
Molecular Formula	$C_{29}H_{41}F_2N_5O$

Targeting a human protein in order to prevent viral entry is a new approach to HIV-1 therapy, which leads to novel methods for testing in vitro potency and pre-clinical safety, as will be described in this section. The entry of HIV-1 into CD4-expressing cells can be divided into 3 discrete sequential steps (Moore and Doms, 2003; figure 1). First, the attachment of the viral glycoprotein 120 (gp120) to the CD4 receptor leads to subtle conformational changes in gp120. These changes expose structural elements on the V3 loop that then bind to a co-receptor, either CCR5 or CXCR4. This in turn induces a structural rearrangement in the viral protein gp41, which is then able to insert a hydrophobic fusion peptide region into the target cell membrane. This brings the virus and cell membranes into close apposition to initiate fusion and ultimately entry of the viral core into the target cell. Compounds have been identified that selectively inhibit at each of these entry steps. The most advanced of these is the fusion inhibitor, enfuvirtide (Fuzeon, T-20), which is licensed for treatment of HIV-1 infection and validates the targeting of HIV-1 entry as a successful therapeutic approach. Maraviroc blocks viral entry by inhibiting the interaction of viral gp120 with the CCR5 co-receptor.

A potential advantage of targeting HIV-1 entry is that the site of inhibitory action is extracellular. An extracellular target is potentially more accessible and, unlike NRTIs, there is no requirement for intracellular processing of the agent. Therefore, there is no mechanistic reason for intracellular adverse effects such as mitochondrial toxicity. A further advantage of entry inhibitors is that there is no cross-resistance with existing agents that act on intracellular targets, as demonstrated by the potency of co-receptor antagonists AMD3100 (originally JM3100, Este et al., 1996) and aplaviroc (Maeda et al., 2001 & 2004), and the fusion inhibitors enfuvirtide and T-1249 (Sista et al., 2001) against viruses resistant to other classes of anti-HIV drugs.

However, there are potential disadvantages of entry inhibition. Firstly, gp120 is the most sequence-variable of the HIV-1 proteins (Griffin, 2003). Hence, differences in drug sensitivity between HIV-1 strains are possible, as has been described for the gp120 inhibitor, BMS-806, which has highly variable activity against panels of B-clade and non-B-clade viruses (Lin et al., 2003). Secondly, targeting host receptors may inhibit their natural function. If this interrupts essential host processes then compounds may have unwanted secondary pharmacological effects.

Figure 1: Schematic of the HIV entry process



The HIV-1 co-receptors, CXCR4 and CCR5, are inherently compelling targets for therapeutic intervention. They belong to the G protein-coupled receptor superfamily, which has historically been tractable to discovery of potent, selective, low-dose small molecule drugs. CCR5 is an attractive target for intervention in HIV-1 disease since the natural genetic absence of surface-expressed CCR5 in Δ32 homozygous genotype populations is highly protective against HIV-1 infection (Dean et al, 1996; Dragic et al, 1996; Liu et al, 1996; Samson et al, 1996). Moreover, reduced cell surface expression of CCR5 in CCR5Δ32 heterozygotes is associated with a slower rate of disease progression (Pasi et al, 2000; Taylor et al, 2000). Furthermore, the natural genetic absence of surface-expressed CCR5 in Δ32 homozygous genotype populations has little impact on their immune status or general health.

Such epidemiological evidence, together with experimental results from CCR5-ablated mice showing no discernible phenotype other than subtle changes in immune function (Zhou et al, 1998), support the hypothesis that antagonism of CCR5 may impose an antiviral effect without causing mechanism-related side effects. Most HIV-1 variants isolated from newly infected individuals utilize CCR5 in combination with CD4 to gain entry into host cells (Berger et al, 1999; Brumme et al, 2005; O'Brien and Moore 2000) and these CCR5-tropic (R5) viruses remain the dominant form in more than half of HIV-1-infected patients in late-stage disease (Owen et al, 2002; Moyle et al, 2004). HIV variants able to utilize CXCR4 emerge in about half of infected persons over the course of HIV disease; the ability to use CXCR4 as an entry co-receptor is frequently associated with at least 1 amino acid substitution at position 11 or 25 of the V3 loop of gp120 (de Jong et al, 1992). CXCR4-tropic (X4) virus is generally observed in patients with low CD4+ cell counts and is associated with accelerated CD4 depletion during advanced stages of HIV disease (Brumme et al, 2005; Richman and Bozzette, 1994). However, it is not known whether the X4 variants are inherently more pathogenic and therefore cause a more rapid disease progression or whether they simply emerge as a consequence of severe immune dysfunction (Shaheen and Collman, 2004). These considerations led to the establishment of a programme to identify a small molecule CCR5 ligand with antiviral properties, which led to the discovery of maraviroc (summarized in Wood and Armour, 2005).

Although maraviroc is an antagonist of CCR5 functional activity this is not the mechanism by which it exerts its antiviral activity. Therefore, the primary pharmacodynamics of the compound are discussed in two separate sections. The first of these, Section 2.6.2.2.1, covers the CCR5 primary pharmacodynamics related to the antiviral mode of action including the binding characteristics of maraviroc at the receptor and the effect that this has on the interaction of viral proteins with target cells. The second, Section 2.6.2.2.2, covers the CCR5 primary pharmacodynamics not related to the antiviral mode of action such as the blocking of chemokine binding and intracellular signaling.

An issue associated with targeting the human CCR5 receptor is the potential to affect the host immune system, and this is particularly true in patients with impaired immunity as a consequence of HIV/AIDS. In the current FDA Guidance for Industry document 'Immunotoxicology Evaluation of Investigational New Drugs' (October 2002) the following statement is made: 'If a drug is intended for treatment of HIV infection (e.g., nucleoside analogues, protease inhibitors), immune function studies should be conducted as part of the standard nonclinical assessment of safety, even when no signs of immunosuppression have been observed in the standard toxicology studies'. We have therefore profiled the activity of maraviroc in a variety of in vitro models of human immune system function, including the effect on binding and CCR5-mediated signaling of the endogenous ligands MIP-1α, MIP-1β and RANTES. This included an assessment of activity at related chemokine receptors and other critical functional activities. In addition, to assess the immunotoxicological consequences of interacting with the CCR5 receptor it was decided to identify a toxicology species in which the compound was pharmacologically active. We therefore carried out an assessment of the binding characteristics of maraviroc to cloned mouse, rat, dog and macaque receptors, which lead to the identification of the macaque as a pharmacologically appropriate toxicology species (Napier et al, 2005).

The general and safety pharmacology of maraviroc was assessed in a wide range of in vitro and in vivo assays. An issue faced by an early example of a CCR5 antagonist (SCH-351,125, SCH-C; 2001; Este, JA 2002) was the prolongation of the QT interval of the ECG, which for other pharmaceutical agents is associated with potentially fatal cardiac arrhythmias (Fermini B and Fossa AA, 2003). With this in mind, the screening sequence that resulted in the identification of maraviroc incorporated an assessment of interaction with the hERG ion channel that is implicated in QT prolongation (Barber, 2004; Wood and Armour, 2005). Further in vitro and in vivo analysis provided a detailed preclinical profile of the potential for maraviroc to influence the QT interval. Additionally, clinical data emerging from Phase 1 trials of maraviroc indicated that postural hypotension was the dose limiting adverse event. As a result we conducted investigative studies in human and animal tissues, recombinant cell lines and in vivo in an attempt to elucidate the mechanism for this outcome.

During early development the presence of two major human metabolites of maraviroc was identified. The antiviral activity and affinity for a wide range of physiological receptors, ion channels and enzymes was assessed for these compounds with the conclusion that they are unlikely to be of biological relevance at the likely clinical exposure.

The currently preferred treatment regime for HIV infection is Highly Active Antiretroviral Therapy (HART), which combines drugs of different therapeutic classes to suppress viral replication and so prevent the development of drug-resistant virus (Richman, 2001). It is therefore highly likely that maraviroc will be used in combination with a variety of drugs from the NRTI, NNRTI, PI and entry inhibitor classes. We therefore conducted an in vitro antiviral study to assess the pharmacodynamic interaction between maraviroc and 18 examples of marketed HIV drugs across the range of currently used therapeutic classes, in addition to a limited number of alternative CCR5 antagonists that have been, or still are, in development.

Development of resistance to antiviral drugs is a significant issue, not least for an example of a novel class of agents such as maraviroc. The nonclinical in vitro resistance studies that were conducted for maraviroc are described in Section 5.3.5.3 (Microbiology Review of Resistance/Tropism in Maraviroc Program).

The issues introduced above are addressed in this document in the following sections:

- Mechanism of action, antiviral activity and cytotoxicity will be discussed in Section 2.6.2.2.1
- CCR5 pharmacodynamics not related to the antiviral mode of action, including effects on endogenous CCR5 ligand binding and function, will be discussed in section 2.6.2.2.2
- Secondary pharmacodynamics, including the selectivity profile against immunologically relevant human targets and the affinity for CCR5 receptors in relevant toxicology species will be discussed in section 2.6.2.3

- Safety pharmacology studies, including an analysis of the effect of maraviroc on parameters related to the QT interval, potential mechanisms for clinically observed postural hypotension and the activity of metabolites will be discussed in section 2.6.2.4
- In vitro drug-drug interactions will be discussed in section 2.6.2.5
- Emergence of viral resistance in in vitro studies will be introduced in section 2.6.2..2.1.13 and discussed in detail in Section 5.3.5.3 (Microbiology Review of Resistance/Tropism in Maraviroc Program).

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MODULE 2.6.2 PHARMACOLOGY WRITTEN SUMMARY

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2.6.2. PHARMACOLOGY WRITTEN SUMMARY

2.6.2.1. Brief Summary

The objectives of the studies described in this Section were: to establish the mode of action of maraviroc, its in vitro efficacy and spectrum in a variety of viral infection models; its effect on endogenous signaling pathways; its secondary pharmacodynamics for key host defence mechanisms in vitro; the pharmacodynamic interaction between maraviroc and other antiviral agents. Efficacy was assessed on the basis of reductions in viral biomarkers released from human blood cells and in a recombinant model of viral infectivity. Although in vivo models of efficacy have been developed (Nakata et al, 2005; McCune et al, 1990; Mosier DE et al, 1991) we feel that no animal model can accurately reproduce the receptor-dependent dynamics of infection in humans. Instead, the in vitro IC_{90} value against a large panel of primary viral isolates in peripheral blood lymphocytes (PBL) was used to extrapolate to the efficacious clinical dose.

In addition to the above studies, the wider pharmacological properties of maraviroc were investigated in a variety of tests designed to evaluate its effects, if any, on other major physiological systems, in particular the central nervous, peripheral nervous, cardiovascular, renal and respiratory systems. These were all conducted in accordance with the ICH S7A guidelines for safety pharmacology studies. In vitro and in vivo studies were conducted on the effect of maraviroc on cardiac repolarisation in accordance with ICH S7B guidelines. Further, pharmacology studies were conducted to investigate the effect of maraviroc in a cardiovascular tilt model in the conscious dog and in rat, dog and human in vitro tissues and human recombinant cells to explore the mechanism of clinically observed postural hypotension.

Moreover, the maraviroc metabolites UK-408,027 and UK-463,977 have been evaluated for antiviral activity and for their wider pharmacological effects on a range of receptors, enzymes and ion channels.

2.6.2.1.1. CCR5 Primary Pharmacodynamcis Related to Antiviral Mode of Action

Three types of in vitro study have been conducted in order to confirm the mechanism of action of maraviroc. Firstly, studies were conducted that confirmed the specific binding of maraviroc to CCR5. Secondly, studies were conducted that investigated the ability of maraviroc to inhibit the HIV-1 envelope glycoprotein, gp120, from binding to CCR5. Finally, 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 R5 strains).

Tritiated maraviroc was used, in radioligand binding experiments, to confirm that maraviroc binds with high affinity and specificity to human CCR5.

The ability of maraviroc to inhibit the HIV-1 envelope glycoprotein, gp120, from binding to CCR5 was characterized by assessing the inhibition of soluble gp120 binding to CCR5-expressing cells. Maraviroc inhibited gp120-mediated fusion in a cell-cell fusion assay with CCR5-expressing cells.

The in vitro antiviral activity of maraviroc was profiled against the laboratory adapted strain Ba-L and against a range of CCR5 and CXCR4-tropic clinical primary isolates from various sub-types or "clades" of HIV-1 in mitogen stimulated peripheral blood lymphocytes (PBL) from pooled donors. These studies confirmed that maraviroc selectively inhibits CCR5 tropic viruses.

The effect of PBL donor on the antiviral activity of maraviroc was assessed in vitro using the lab adapted strain Ba-L to infect mitogen-stimulated PBL from individual donors. This is relevant because maraviroc binds to a human receptor rather than a viral target. The inter-donor variability in antiviral activity of maraviroc was comparable to that seen with the HIV-1 protease inhibitor, saquinavir, tested in the same assay.

The in vitro antiviral activity of maraviroc in primary human macrophages was assessed against the lab-adapted HIV-1 strain, Ba-L. Monocyte-derived human macrophages co-express CD4 and CCR5 and are a relevant cellular reservoir for HIV-1 replication in vivo. Maraviroc inhibited the infection of macrophages by Ba-L with an IC_{90} of 0.79 nM (0.40 ng/mL).

The effect of viral multiplicity of infection (MOI) on the antiviral activity of maraviroc was assessed in vitro using the lab-adapted strain, Ba-L, to infect PBL and PM-1 cells. There was no effect on IC_{90} of maraviroc across a wide range of virus inputs in either cell system.

Maraviroc was active against all 43 CCR5-tropic primary isolates tested with a geometric mean IC_{90} of 2.03 nM (1.04 ng/mL). In contrast, maraviroc was inactive against CXCR4-tropic and dual-tropic strains. When the extent of compound binding to protein present in the assay medium was taken into consideration the unbound concentration of maraviroc at the antiviral IC_{90} was approximately 1 nM (0.5 ng/mL).

Maraviroc was also tested against a panel of 200 recombinant viruses derived from naïve or drug-experienced clade B and non-clade B clinical samples. Maraviroc gave consistent inhibition of a broad range of viruses with only minor variation in drug susceptibility across clade type and protease/reverse transcriptase resistance genotype.

The above antiviral activity was observed in the absence of cytotoxicity since cells expressing the CCR5 receptor were viable in the presence of 10 μM maraviroc. Moreover, maraviroc did not induce CCR5 internalization so the antiviral activity was not a consequence of removal of the viral co-receptor from the target cell surface.

Using in vitro techniques, maraviroc-resistant (MVCres) variants have been selected following extended serial passage of two CCR5-tropic primary HIV-1 isolates in the presence of increasing amounts of the compound. The MVCres variants remain CCR5-tropic, are sensitive to other entry inhibitors and contain amino acid substitutions/deletions in the gp120 V3 loop. These studies are described in detail in Section 5.3.5.3. (Microbiology Review of Resistance/Tropism in Maraviroc Program).

2.6.2.1.2. CCR5 Pharmacodynamics Not Related To the Antiviral Mode of Action

In vitro pharmacology studies showed that maraviroc binds to the human chemokine receptor CCR5 and is a slowly reversible and selective antagonist of binding and intracellular signalling by the endogenous chemokines MIP-1α, MIP-1β and RANTES.

It was demonstrated that the affinity of maraviroc for the macaque CCR5 is very similar to that for the human receptor (K_D = 1.36 nM, 0.7 ng/mL and 0.86 nM, 0.44 ng/mL respectively). In addition, the association rate in both species is similar ($t_{1/2}$(on) = 8.37 minutes and 11.05 minutes respectively) whereas the dissociation rate is faster from the macaque receptor ($t_{1/2}$(off) = 1.54 hours vs 15.95 hours). The macaque therefore represents a functionally relevant species in which to study the immunotoxicology of maraviroc.

2.6.2.1.3. Secondary Pharmacodynamics

The selectivity profile in in vitro immune function assays suggests that maraviroc is unlikely to affect immune processes mediated by a range of alternative chemokine receptors and immune system mediators.

2.6.2.1.4. Safety Pharmacology (general pharmacology)

Results from studies examining the general pharmacologic properties of maraviroc indicate that it was well tolerated at 1000 mg/kg in conscious male rats where mild effects on appearance and behaviour were observed when compared with vehicle-treated animals. At 2000 mg/kg adverse effects were observed in the form of piloerection, salivation, and breathing changes (decreased, irregular and laboured). Posture, skin colour, grip strength, core temperature and body weight were normal throughout the experiment.

Maraviroc had little interaction with physiologically important receptors, binding sites, enzymes, or ion channels apart from weak functional activity at the human μ opioid receptor and a moderate affinity for the human α_{2A} adrenergic receptor. The compound had no effect in vitro on the electrically stimulated guinea pig ileum at 1 μM (514 ng/mL), a preparation that is sensitive to μ opioid active agents. Maraviroc was a weak competitive functional antagonist at alpha-adrenergic receptors in isolated canine saphenous vein and produced a transient suppression of reflex control of blood pressure in the conscious dog following postural challenge. However, in a rat vas deferens preparation no direct agonism of α_{2A} receptors and no dose related or biologically relevant antagonism of α_{2A} agonists was observed. In addition, in human α_{2A} expressing cells no direct agonist activity up to 100 μM and only a 3-fold shift in the noradrenaline dose response curve at 30 μM occurred, leading to the conclusion that maraviroc has minimal functional activity at the human receptor. Finally, there was no effect on phenylephrine pre-constricted, isolated human saphenous vein at 100 μM that could be differentiated from vehicle effects. Therefore, although maraviroc binds to the human α_{2A} receptor and acts as a weak vasodilator in canine vasculature, this does not provide definitive evidence for the mechanism of postural hypotension as observed in clinical studies.

Maraviroc had no effect on sodium pentobarbitone induced sleeping times, motor coordination or excretion of fluid and electrolytes. There was no effect on blood pH, pCO_2 or pO_2, indices of respiratory function in anaesthetised rats. There was a small, but statistically significant decrease in mean arterial blood pressure and heart rate in the rat dosed at 1 mg/kg IV.

Maraviroc was assessed in a variety of assays to determine the potential for effects on cardiac rhythm. Results from studies of dofetilide binding to the hERG channel, patch clamping of the hERG channel and cardiac action potential morphology in dog isolated Purkinje fibre suggest that maraviroc has the potential to block the I_{Kr} current. This could affect cardiac repolarisation in vivo at unbound plasma concentrations greater than 3 μM (1541 ng/mL), which is approximately 10-fold higher than the mean unbound C_{max} in HIV-positive patients at a dose of 300 mg BID. No effects were observed on haemodynamic or electrocardiogram parameters in the conscious dog at a dose of 1.5 mg/kg PO, which achieved a mean unbound C_{max} of approximately 0.17 μM. However, in a further study in the conscious dog a statistically significant 14.5 msec prolongation of the ECG QT interval was observed in the absence of an effect on heart rate at a mean unbound plasma concentration of approximately 1.2 μM (631 ng/mL), which is approximately 4-fold higher than the mean unbound C_{max} in HIV positive patients at a dose of 300 mg BID.

2.6.2.1.5. Pharmacodynamic Drug Interactions

There was no evidence of antagonistic interaction with any of the 22 marketed and developmental antiviral compounds tested. On the whole, the antiviral effect of adding maraviroc to any one other compound was additive, although some evidence of minor synergy was observed.

2.6.2.1.6. Metabolites UK-408,027 and UK-453,977

The maraviroc secondary amine and carboxylic acid metabolites (UK-408,027 and UK-463,977 respectively; Section 2.6.4.5.1.13) were shown not to be inhibitors of the HIV-1 gp160 and CCR5-dependent cell-cell fusion process, with IC_{50}s of >10μM. In this assay maraviroc had an IC_{50} of 0.22nM. On the basis of this data the metabolites of maraviroc are not expected to be inhibitors of CCR5-dependent HIV-1 replication or contribute to the antiviral efficacy of maraviroc in humans.

Both compounds were profiled in vitro against a range of 74 receptors, ion channels and enzymes and the only activities of note were inhibition of binding to endothelin ETB, 5HT2c and μ opioid receptors (21, 29 and 34% respectively) by UK-408,027 and to the μ opioid receptor (53%) by UK-463,977 at 10 μM. The binding of the metabolites to the μ opioid receptor was considerably weaker than that of the parent compound.

Neither compound had any direct effect in the electrically stimulated rat vas deferens preparation, indicating an absence of adrenergic agonist activity, and a less than 2-fold potentiation of the α2 adrenergic agonist UK-14,304, which is unlikely to be of biological relevance.

2.6.2.2. Primary Pharmacodynamics

This section is divided into two separate sections. The first of these, Section 2.6.2.2.1, covers the CCR5 primary pharmacodynamics related to the antiviral mode of action. The second, Section 2.6.2.2.2, covers the CCR5 primary pharmacodynamics not related to the antiviral mode of action such as the blocking of chemokine binding and intracellular signaling.

2.6.2.2.1. CCR5 Primary Pharmacodynamics Related to the Antiviral Mode of Action

The objectives of the in vitro studies described in this Section were threefold. Firstly, to confirm the mechanism of action of maraviroc as a selective inhibitor of CCR5-tropic HIV-1 replication mediated through its binding to the human chemokine receptor CCR5 and the consequent blockade of the interaction of viral envelope proteins with the receptor. The second objective was to derive the primary pharacological value for maraviroc, used to extrapolate the efficacious clinical dose. The third objective was to assess the activity of maraviroc against virus resistant to existing antiretrovirals.

2.6.2.2.1.1. Affinity of maraviroc for the human CCR5

Tritiated maraviroc was used, in radioligand binding experiments, to confirm that maraviroc binds with high affinity to human CCR5. The dissociation constant (K_D) for maraviroc binding to CCR5 was assessed in two separate studies. In the first (DI/012/1), the saturation binding of [3H]-maraviroc to HEK-293 membranes expressing human CCR5 was estimated in the presence of MIP-1β. In a second study (CG/015/02) saturation binding was conducted in the absence of MIP-1β and the resulting dissociation constant (K_D = 0.86 nM, Table 1) indicates a high affinity for the receptor. In this study ligand depletion was maintained below 10% and the unbound concentration of ligand was measured rather than estimated and therefore the latter K_D is considered to be the definitive value. In an earlier study (DI/011/1) it was demonstrated that maraviroc did not significantly bind to non-recombinant parental HEK-293 cells and membranes or to CHO-K1 cell membranes that do not contain the CCR5 receptor. Thus the binding reported here is CCR5 specific.

Table 1. Key Primary Pharmacologic Properties of Maraviroc Related to the Antiviral Mode of Action

In Vitro Findings	N	Maraviroc Activity	
		nM	ng/mL
Receptor Binding			
Saturation of [^{3}H]maraviroc binding (K_D value)		K_D ± SEM (95% CI)	
[^{3}H]maraviroc binding to cell membranes[a]	2	4.4±0.24 (3.92 to 5.04)	2.26±0.14 (2.02 to 2.59)
[^{3}H]maraviroc binding to cell membranes[b]	4	0.86±0.08 (0.65 to 1.14)	0.44±0.04 (0.33 to 0.59)
Inhibition of viral protein attachment and fusion		Geometric Mean IC_{50} (95% CI)	
gp120 binding to human CCR5[c]	8	43.4 (31.6 to 59.5)	22.3 (16.2 to 30.6)
gp120 binding to human CCR5[d]	18	11.0 (10.5 to 11.6)	5.7 (5.4 to 6.0)
HIV-1 envelope protein cell-cell fusion[e]	4	0.22 (0.13 to 0.39)	0.11 (0.07 to 0.20)

Study Codes: [a]DI/012/1 (derived data from original report), [b]CG/015/02, [c]DI/006/01, [d]DI/005/04, [e]DI/007/1.

Further kinetic characterisation of [^{3}H]-maraviroc binding to human CCR5 indicated that the compound dissociates slowly with a dissociation $t_{½}$ (off) of approximately 16 hours whereas the rate of association is relatively fast ($t_{½}$ (on) = 11 minutes, Table 2).

Table 2. Kinetic Constants for [^{3}H]-Maraviroc Binding to Human CCR5 Receptors at Room Temperature and at 37°C

Temp.	K_{obs}	$t_{½}$ (on) min	$t_{1/2}$ (off) h	K_{on} $min^{-1}.nM^{-1}$	K_{off} min^{-1}	K_{off}/K_{on} nM
RT	0.063 (0.044-0.089) (n = 5)	11.05 (7.83-15.59) (n = 5)	15.95 (9.36-27.16) (n = 3)	0.010 (0.0069-0.015) (n = 3)	0.00072 (0.00043-0.0012) (n = 3)	0.071 (0.055-0.091) (n = 3)
37°C	0.089 (n = 1)	7.78 (n = 1)	-	-	-	-

Values are the geometric mean (95% CI). K_{obs} = observed association rate constant, K_{off} = dissociation rate constant, K_{on} = association rate constant, RT = room temperature. Study Code CG/015/02 (this study extended the data obtained in an earlier report, Study Code DI/013/1, which led to similar conclusions).

2.6.2.2.1.2. Characterization of the maraviroc binding site on CCR5

The binding site for maraviroc on human CCR5 was further characterized by site directed mutagenesis of amino acids within the transmembrane domain of the receptor in order to identify the physical site of interaction of the compound with the receptor. The effect of these changes on the antagonistic activity of maraviroc against MIP-1β induced decreases in cAMP levels was determined in a cyclic AMP response element-dependent luciferase (cre-luc) reporter gene assay Table 3. In this table the first letter in each code represents the wild-type amino acid, the number represents the position of the amino acid in the protein sequence and the second letter indicates the introduced amino acid.

Table 3. The functional activity of maraviroc on site directed mutant variants of human CCR5

Virus	IC_{50} (nM)	Fold change with respect to wild type
Wild type	2.95	-
L104F	29.5	10
Y108A	≥1803	≥612
A159V	252	85
I164F	≤ 1.5	≤0.52
H181A	≤ 0.8	≤0.27
I198N	149	51
E283A	≥3,979	≥1349

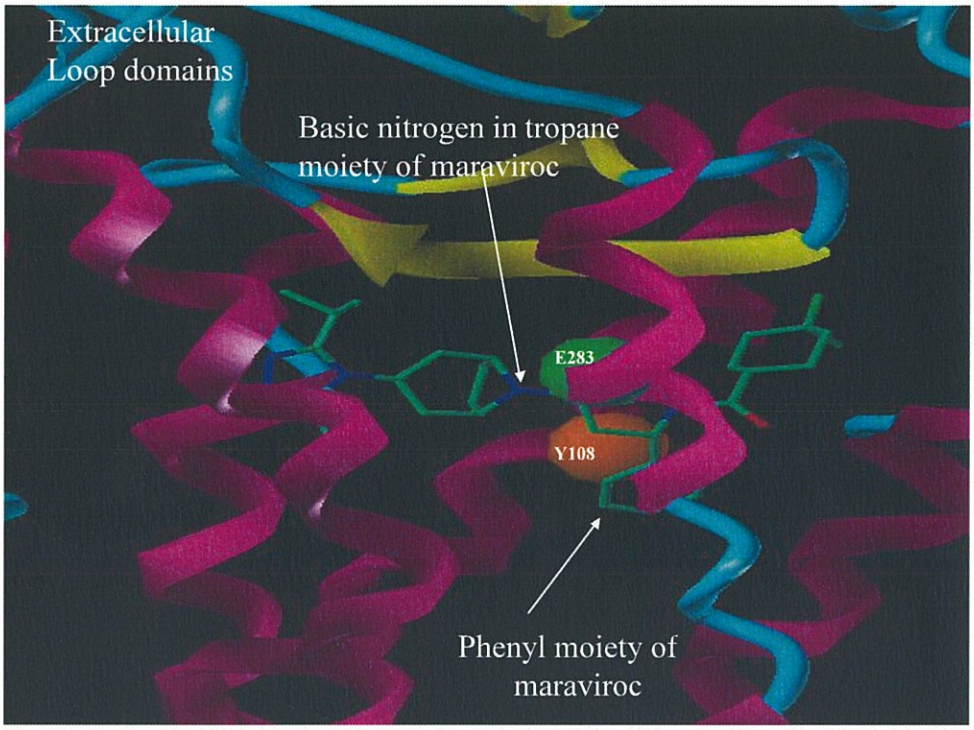
Study Code DI/102/05. Amino acid code used: A, alanine; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; L, leucine; N, asparagine; V, valine; Y, tyrosine.

These data indicate relatively large reduction in potency (fold-increase in IC_{50} values) compared with wild-type (estimated to be >500-fold) for the mutants E283A and Y108A, indicating that maraviroc interacts with these residues in a binding pocket within CCR5. The IC_{50} fold-increase values relative to wild-type CCR5 were lower for the L104F, A159V, I164F, H181A, I198N and F85L mutations, suggesting that these residues do not form part of the binding pocket.

These data were used to inform a computer-generated docking model of maraviroc in the putative binding pocket of a CCR5. The model highlights a strong ionic interaction between the basic nitrogen of the tropane moiety of maraviroc and the acidic carboxyl group of the E283 (glutamic acid) in the binding pocket residue (see Figure 1). A strong hydrophobic interaction is also apparent between the phenyl moiety of maraviroc and the aromatic group of the Y108 (tyrosine) residue.

It is conceivable that the different IC_{50} values against the various receptor isoforms are the result of differences in CCR5 expression levels, however this variation (approximately 10-fold) is relatively low compared with the reduction (approximately >500-fold) in IC_{50} observed for the E283A and Y108A mutated CCR5 isoforms. Variation in MIP-1β EC_{50} value between isoforms was deemed not be a critical factor in determination of maraviroc antagonist potency since the EC_{50} for each was approximately 1nM. An alternative explanation for the changes in potency of maraviroc at the various isoforms could be a gross change in tertiary structure of the receptors as a consequence of mutation. However, this is believed to be unlikely for Y108A and E283A since their modeled location in the binding pocket does not infringe on helical structure, and the mutant isoforms retain ability to signal following exposure to MIP-1β.

Figure 1. Docking of Maraviroc in the CCR5 binding pocket to highlight interactions between the antagonist and the pocket residues E283 and Y108



2.6.2.2.1.3. Inhibition of CCR5 Association with Viral Glycoproteins and Membrane Fusion

Entry of HIV-1 into host cells is a complex process involving the binding of viral envelope proteins to surface proteins on the host cell followed by fusion between the viral and host membranes. The first step in viral entry is the binding of the viral envelope protein gp120 to the host cell receptor CD4 protein. This induces a conformational change in gp120, which enables it to bind to the co-receptor CCR5. The ability of maraviroc to block attachment of gp120 to recombinant cells expressing the CCR5 co-receptor was demonstrated by two different methodologies. In the first, direct binding of gp120 to CCR5 receptors was assessed. In the second, the effect of maraviroc on the functional consequence of gp120/CCR5 interaction, i.e. membrane fusion, was determined.

To enable direct measurement of gp120 binding to human CCR5 in vitro, a truncated soluble form of CD4 (sCD4) was incubated with soluble gp120 (derived from the CCR5-tropic laboratory-adapted strain Ba-L) to form a gp120-sCD4 complex in solution. Binding of this complex to CCR5 expressed on the surface of HEK-293 cells was measured by time-resolved fluorescence immunoassay (TR-FIA) using a Europium (Eu^{3+})-labeled anti-gp120 antibody. Inhibition of gp120-sCD4 binding to CCR5 was subsequently determined over a range of maraviroc concentrations to determine the IC_{50} value.

In a first study (DI/012/1), with a limited concentration-response curve, an estimate of the IC_{50} produced a value of approximately 43 nM (22 ng/mL, Table 1). In a subsequent study (CG/015/02), with a broader concentration range and greater n-numbers, this value was refined to 11 nM (5.7 ng/mL). These data indicate that maraviroc inhibits attachment of the HIV-1 gp120 subunit to human CCR5 on cells and is therefore expected to inhibit HIV-1 entry into target cells to exert an antiviral effect. In a modification of this study, cells exposed to maraviroc were washed for 20 hours in the absence of compound prior to assessment of gp120 binding in order to determine the duration of the functional occupancy of CCR5 (DI/128/04). In this study maraviroc showed an approximately 2-fold loss in potency following the prolonged washing process indicating that the inhibitory activity of maraviroc against gp120-sCD4 binding to CCR5 can be retained following the removal of exogenous compound from the assay medium. This observation is consistent with the long duration of maraviroc binding to CCR5 (Table 2).

To further investigate the activity of maraviroc against viral envelope binding, the ability of the compound to inhibit HIV-1 envelope protein-dependent cell-cell fusion was determined (DI/007/1). Two separate cell lines were constructed to facilitate this. One consisted of CHO cells co-expressing recombinant viral envelope protein gp160 (from the R5 strain JR-FL) and the viral transactivation protein Tat. Cell-cell fusion occurs in the presence of functional, complete viral envelope protein (gp160), consisting of both soluble (gp120) and membrane-attached (gp41) sub-units. The other cell line consisted of HeLa cells co-expressing CCR5 and CD4 receptors and a β-galactosidase HIV-1-LTR reporter construct. Upon fusion, Tat present in the CHO cell is able to transactivate the HIV-1 LTR present in the HeLa cell, leading to the expression of β-galactosidase. The level of β-galactosidase expression is proportional to the degree of cellular fusion that has occurred and is determined using the fluorogenic substrate 4-methylumbelliferyl-galactopyranoside. Inhibition of cell-cell fusion reduces fluorescent signal levels in this assay relative to mock-treated control and a concentration-response curve for compound can be used to calculate the IC_{50}. Maraviroc was effective in this assay, giving an IC_{50} of 0.22 nM (0.11 ng/mL, Table 2), indicating that it blocks gp120/CCR5-mediated membrane fusion.

The potency difference for maraviroc in the gp120/CD4 binding assay (IC_{50} = 11 nM) and the in the cell fusion assay (IC_{50} = 0.22 nM) reflects the different characteristics of each assay. The binding assay used a relatively crude cell lysate preparation of gp120 containing factors that would non-specifically remove maraviroc from solution. There is also a significantly greater CCR5 expression level on the HEK-293 cells in comparison with that on the HeLa cells used for the fusion assay. The binding assay is generally unable to differentiate between compounds with potency below approximately 10 nM whereas the fusion assay is significantly more sensitive. The difference in the potency of maraviroc in these two assay systems may also reflect the fact that soluble monomeric gp120 is used in the ligand binding assays whilst membrane-expressed gp120/gp41 complexes exist in the cell fusion assay. In its native form gp120 assembles on viral surface as trimers, complexed with gp41 trimers via non-covalent linkages. Thus the gp120/CCR5 cell fusion assay more closely resembles true viral infection.

2.6.2.2.1.4. Metabolite Inhibition of CCR5 Mediated Membrane Fusion

The principal metabolites of maraviroc found in human plasma are the secondary amine product of N-dealkylation, UK-408,027, and the carboxylic acid UK-463,977 (Section 2.6.4.5.1.13).

In light of the structural overlap with maraviroc and significant plasma concentration achieved by these metabolites, it was considered that they could potentially be antagonists of CCR5 and thus could inhibit HIV-1 entry and replication. To investigate the inhibitory activity of UK-408,027 and UK-463,977 against HIV-1 the compounds were evaluated in the in vitro model of membrane fusion described in Section 2.6.2.2.1.1. It was shown that the mean IC_{50} for inhibition of membrane fusion by UK-408,027 and UK-463,977 was greater than 10 µM (3110 ng/mL and 4070 ng/mL respectively, N=3, UK463977/DI/001/05). Given the lack of activity in this in vitro model of HIV-1 cell entry in comparison with maraviroc (IC_{50} = 0.22nM, Table 2) UK-408,027 and UK-463,977 are not expected to be inhibitors of CCR5-dependent HIV-1 replication or contribute to the antiviral efficacy of maraviroc in humans.

Taken together, the protein-protein interaction, membrane fusion, binding and structural data indicate that maraviroc rapidly associates, with high affinity, to a central region of human CCR5. Once bound it dissociates slowly and this binding has the effect of blocking attachment of the viral envelope protein gp120 and consequent membrane fusion. These data indicate that maraviroc is likely to inhibit CCR5-mediated HIV-1 entry into target cells to exert an antiviral effect.

2.6.2.2.1.5. Maraviroc Antiviral Activity

The antiviral activity of maraviroc was assessed by determining its ability to inhibit the replication of a laboratory-adapted strain of HIV-1 (Ba-L), primary HIV-1 isolates and a range of recombinant viral isolates. These viruses represent a range of different clades and exposures to current therapeutic agents. The aims of these studies were: to confirm that maraviroc was a selective inhibitor of CCR5-tropic HIV-1 (to confirm its mechanism of action); to identify the primary pharmacological value for maraviroc (to enable extrapolation of the efficacous clinical dose); and to define its spectrum of antiviral activity against viruses resistant to existing drug classes (to assess its potential for treatment-naïve and treatment-experienced patients).

2.6.2.2.1.6. Antiviral activity of maraviroc against lab-adapted strains

The effect of maraviroc was assessed in vitro in a 96-hour viral replication assay (Table 4). PM-1 cells (an immortalized CD4+ T-cell line naturally co-expressing CCR5 and CXCR4) or peripheral blood lymphocytes (PBL) were infected with the CCR5-tropic laboratory-adapted HIV-1 strain Ba-L, prior to treatment with compound and the extent of viral replication was determined by measuring levels of reverse transcriptase activity in the culture supernatant (DI/019/1). Maraviroc inhibited the replication of HIV-1 Ba-L in PM-1 cells with a geometric mean IC_{90} of 1.08 nM (0.56 ng/mL). In contrast maraviroc was completely inactive against the CXCR4 lab-adapted HIV-1 strain IIIB, when tested in PM-1 cells at concentrations up to 10 μM (5137 ng/mL). Maraviroc also demonstrated consistent activity against Ba-L in primary PBL from pooled (2-4 donors per experiment) and single donors, where the receptor is expressed under its native conformation. This data indicates that maraviroc selectively inhibits CCR5-tropic HIV-1 in vitro. Furthermore the compound is able to inhibit replication of virus in PBL, which contain CD4+ T-cells and represent the major cellular reservoir for HIV-1 replication in vivo.

The effect of virus input (the multiplicity of infection, MOI) on the antiviral activity of maraviroc was assessed (DI/051/06). PM-1 cells and PBL were infected with serial dilutions of the laboratory-adapted HIV-1 strain Ba-L, prior to treatment with compound and the extent of viral replication was determined by measuring levels of reverse transcriptase activity. The potency of maraviroc (as defined as the IC_{50} or IC_{90}) was not significantly different across the input ranges of virus used. This data is consistent with maraviroc acting as a non-competitive inhibitor of HIV-1 entry.

Monocyte-derived human macrophages co-express CD4 and CCR5 and are susceptible to infection with CCR5-tropic HIV-1 strains in vitro and in vivo. The in vitro antiviral activity of maraviroc in primary human macrophages was therefore assessed against the lab-adapted HIV-1 strain, Ba-L (DI/006/06). Monocyte-derived human macrophages from single donors, differentiated by adherence to plastic, were pre-incubated with serial dilutions of maraviroc prior to infection with Ba-L. Virus replication was determined by measuring the reverse transcriptase activity in the culture supernatant after 7-10 days. Maraviroc inhibited the infection of macrophages by Ba-L with a geomean IC_{90} of 0.79 nM (0.40 ng/mL). This indicates that maraviroc binds to CCR5 expressed on macrophages and prevents HIV-1 infection of this cell type in vitro.

Table 4. Antiviral Properties of Maraviroc against lab adapted HIV-1 strains

In Vitro Finding	N	Maraviroc Activity	
		nM	ng/mL
Antiviral Activity		Geometric Mean IC_{90} (95% CI)	
HIV-1 Ba-L in PM-1[a]	16	1.08 (0.71 to 1.64)	0.56 (0.37 to 0.84)
HIV-1 IIIB in PM-1[a]	1	>10,000	>5,137
HIV-1 Ba-L in PBL – pooled donors[a]	11	2.64 (1.75 to 3.97)	1.36 (0.90 to 2.04)
HIV-1 Ba-L in PBL - single donors[a]	10	1.96 (1.21 to 3.15)	1.01 (0.62 to 1.62)
HIV-1 Ba-L in monocyte derived macrophages[b]	4	0.79 (0.36 to 1.72)	0.40 (0.19 to 0.88)

Ba-L = CCR5-tropic laboratory-adapted strain, IIIB = CXCR4-tropic laboratory-adapted strain, PBL = Peripheral Blood Lymphocytes. Study Codes: [a]DI/019/1; [b]DI/06/06

2.6.2.2.1.7. In vitro antiviral activity of maraviroc against primary HIV-1 isolates in PBL, derivation of the primary pharmacological value

The potency of maraviroc was also measured against a range of 43 CCR5-tropic HIV-1 primary isolates, from various subtypes or 'clades' (DI/18/01). The data from these experiments are summarised in Table 5. Maraviroc inhibited replication of all 43 CCR5-tropic primary isolates with a geometric mean IC_{90} of 2.03 nM (1.04 ng/mL, N = 190).

Primary HIV-1 isolates are more genetically diverse, have been through a limited number of passages in culture and therefore more closely resemble infections in patients than lab-adapted strains. This panel of isolates represented a wide geographical origin and diverse viral clades. PBL contain primary CD4+ T-cells, which represent the major cellular reservoir for HIV-1 replication in vivo. Furthermore, since maraviroc binds CCR5 rather than a viral target, PBL are the relevant cells in which to investigate the compound's antiviral activity. The geometric IC_{90} value of 2.03 nM (1.04 ng/mL) obtained in these studies is therefore taken as the primary pharmacological value representing the antiviral activity of maraviroc.

Maraviroc was also tested against CXCR4-tropic and dual-tropic HIV-1 isolates (N = 12) to confirm the specificity of antiviral activity (Table 5). When tested in parallel with CCR5-tropic isolates, no CXCR4-tropic or dual-tropic isolate was inhibited at concentrations up the highest tested (10 nM). In contrast, each of the CXCR4-tropic and dual-tropic primary isolates was sensitive to saquinavir, a protease inhibitor that is currently approved for treatment of HIV infection. This data confirms that maraviroc is inactive against HIV-1 isolates that can use CXCR4 as their entry co-receptor for infection of PBL.

Table 5. 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)			

PBLs from 4-pooled donors were infected with Ba-L, or primary clinical isolates previously shown to use exclusively CCR5 for entry. Infected cultures were treated with maraviroc, and mean IC_{50} and IC_{90} for each viral strain were derived from at least 2 independent experiments. Values for clades A to O are the geometric mean of the respective values for each strain. [a] Clade classification for all isolates used in the study was based on information provided by the source of the isolates, National Institute for Biological Standards and Control AIDS Reagent Programme. The E clade isolate used was listed as clade E when genotyped using both env & gag sequences. However NIBSC now state that all known viruses of what was initially described as clade E are in fact A/E recombinants, and have been reclassified as Circulating Recombinant Form CRF_AE. [b] excludes data generated with HIV-1 Ba-L. [c] number of independent assays. Study Code DI/18/01.

The antiviral activity of maraviroc was determined against a smaller panel of 20 primary isolates and the lab adapted strains, Ba-L and IIIB, at [redacted] USA (DI/004/3). Maraviroc inhibited all 16 of the CCR5-tropic primary isolates tested and the CCR5-tropic lab adapted strain, Ba-L. Two of the CCR5-tropic primary isolates were paediatric derived samples; maraviroc also inhibited a third paediatric primary isolate, which had no defined tropism at the time of the study. Overall, the antiviral activity of maraviroc against CCR5-tropic primary isolates tested at SRI was in broad agreement with the internal data (presented in Table 5), with the geometric mean IC_{50} and IC_{90} values at SRI being approximately 2- and 4-fold higher, respectively. No cytotoxicity was observed in these assays at 50 nM (26 ng/mL) maraviroc. Two dual-tropic primary isolates and the CXCR4-tropic HIV-1 IIIB strain were included in the panel. Maraviroc was inactive against one dual tropic virus whereas it was active against the other; this may be a result of preferential use of CCR5 versus CXCR4 by the maraviroc sensitive virus in the assay system, although this was not formally tested. Maraviroc was found to be marginally active to inactive against HIV-1 IIIB in a single experiment. Whilst this experiment met validation criteria it is considered by SRI to be an anomaly of unknown origin since in a further experiment maraviroc was found to be inactive against HIV-1 IIIB.

2.6.2.2.1.8. In vitro antiviral activity of maraviroc against antiretroviral drug sensitive and drug resistant clinical viruses.

To characterize the antiviral activity of maraviroc against relevant clinical strains of HIV-1, it was tested at Monogram Biosciences Inc., San Francisco, CA, USA (previously ViroLogic Inc.) 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 in the PhenoSense™ HIV Entry phenotypic drug susceptibility assay (CG/016/02). Replication defective pseudotyped viruses were generated using gp160 envelopes (Env) amplified from clinical samples. The assay measures the ability of compounds to inhibit infection of the pseudotyped viruses into an immortialised cell line (U87) that has been transformed to express high levels of CD4 and CCR5 on its cell surface. These data are summarized in Table 6.

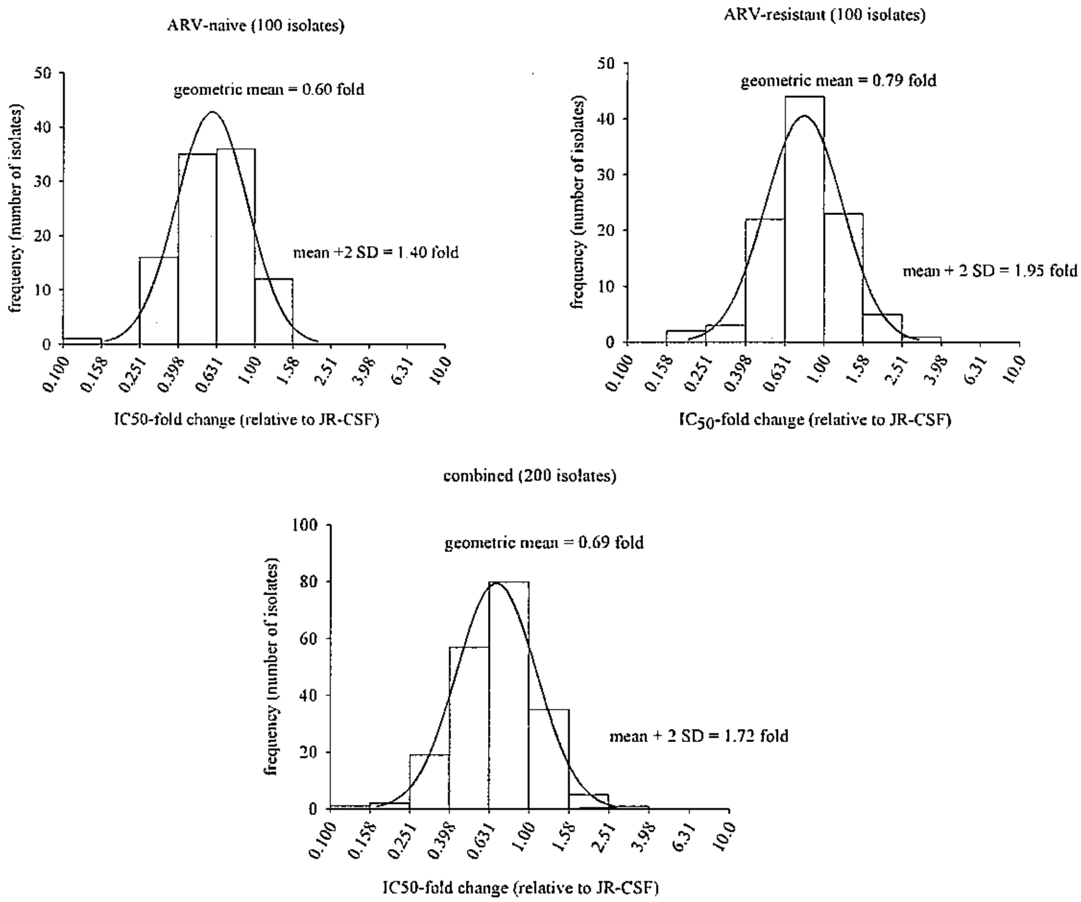
Table 6. Maraviroc Geometric Mean IC_{50} and IC_{90} Values Among 200 HIV-1 Clinical Isolates, and Among Virus Subgroups Defined by Clade (B vs Non-B Clade) and Drug Sensitivity to Protease and/or Reverse Transcriptase Inhibitor Drugs (Resistant vs Wild-Type)[a]

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

a Study Code CG/016/02

Maraviroc inhibited all 200 pseudotyped viruses with a geometric mean IC_{90} of 13.7 nM (95% CI, 12.3 to 15.1 nM) and a geometric mean IC_{50} *n*-fold change (defined as the clinical isolate IC_{50}/JRCSF reference virus IC_{50}) of 0.69-fold (95% CI, 0.64 to 0.73). The range of susceptibilities to maraviroc was narrow, as illustrated by an estimated biological cut-off (= geometric mean *n*-fold change + 2 standard deviations, Parkin et al, 2004) of 1.72 fold. There was no difference in the susceptibilities of clade B viruses and non-B viruses to maraviroc. There was a small but statistically significant difference between the drug-naïve and drug resistant groups, with geometric n-fold changes of 0.60 fold (95% CI, 0.55 to 0.65) and 0.79 fold (95% CI, 0.72 to 0.86) respectively ($p < 0.001$; Figure 2). This difference was not considered biologically relevant since it is less than the inter-assay variability previously reported. There was no correlation between maraviroc susceptibility and the number of drug-associated mutations in viruses from which the envelopes were derived, indicating that the compound will be active in patients harbouring virus resistant to existing antiretrovirals.

Figure 2. Range of susceptibilities of 200 pseudotyped viruses to maraviroc, as determined in the PhenoSense™ HIV Entry assay (Monogram Biosciences)



Maraviroc was active in vitro against viruses resistant to the HIV-1 fusion inhibitor, enfuvirtide (Fuzeon™). This has been assessed using patient-derived samples from the clinical phase of the program. These results are discussed in detail in Section 5.3.5.3. (Microbiology Review of Resistance/Tropism in Maraviroc Program).

2.6.2.2.1.9. Effect of Plasma Protein Binding on Antiviral Activity

The antiviral assays used to determine the IC_{90} value for maraviroc were conducted in a growth medium containing 10% (w/v) heat inactivated foetal calf serum (FCS). In light of this, the fraction of maraviroc that was bound to the protein present in the assay was determined by an equilibrium dialysis method in order to determine the unbound concentration of compound at the IC_{90}. In this study, the bound and unbound fractions of maraviroc in the PBL-antiviral medium were 45.2% and 54.8% respectively (DI/138/05). The geometric mean IC_{90} value for maraviroc as determined against 43 primary isolates in PBL culture was measured as 2.0nM (Section 2.6.2.2.2.2). As the unbound concentration of maraviroc is estimated to be 54.8%, the unbound in vitro antiviral IC_{90} for maraviroc is estimated to be approximately 1.0 nM (0.5 ng/mL).

2.6.2.2.1.10. Cytotoxicity

To demonstrate that the maraviroc-induced decrease in viral production in the above assays was not the result of toxic effects of the compound on host cell activity or growth, specific cytotoxicity assays were carried out. The absence of cytotoxicity could also be inferred from the normal behaviour of host cells, in comparison with controls, in the presence of high concentrations of maraviroc in a variety of assays.

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. Both cell types were grown in parallel with those used in the antiviral assays. The assay plates were incubated at 37°C in a humidified 5% CO_2 (v/v) incubator for 5 days prior to spectrophotometric analysis of cell proliferation and viability in an MTS assay (DI/019/1). No differences in the viability of cells treated with or without the compound were seen, thus confirming that at these concentrations maraviroc is not cytotoxic and that the observed antiviral activity occurs in the absence of effects on cell growth.

Various indirect lines of evidence point to an absence of maraviroc induced cytotoxicity. For example, at 10 μM maraviroc is inactive against the HIV-1 strain IIIB, (which utilises the alternative co-receptor CXCR4) and other CXCR4-tropic or dual-tropic strains (Table 6). This indicates that these virally infected cells are able to grow and produce virus at this compound concentration. Similarly, the IC_{50} 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 (Table 11). These cells were therefore functionally active in the presence of concentrations of maraviroc greater than 5,000-fold the antiviral IC_{90} for the compound. In human cell lines, TNFα production from THP-1 cells and chemotaxis of THP-1 and H9 cells were unaffected by 25 μM maraviroc. In addition, chromosomal damage was not observed in cultures of human lymphocytes when tested, up to cytotoxic concentrations, in either the presence or absence of metabolic activation. Chromosome damage was also absent in bone marrow of mice treated orally once daily for 3 days with up to 2000 mg/kg of maraviroc (Section 2.6.6.4.3, Toxicology Written Summary). Moreover, the macaque, which has a CCR5 receptor that functionally interacts with maraviroc, has been used in toxicology studies and no significant cytotoxicity could be observed in the wide range of tissues examined following histopathological analysis (Sections 2.4.4.3.4 and 2.4.4.7.1, Nonclinical Overview).

2.6.2.2.1.11. Receptor Internalisation

To demonstrate that the decrease in viral production in the above antiviral assays was not the result of maraviroc-induced receptor internalisation, the effect of the compound on levels of CCR5 at the cell surface was investigated (DI/009/03). Maraviroc is not expected to induce CCR5 cellular internalization, as this event is associated with functional agonists and not antagonists of GPCRs in general (Scott et al, 2004) and CCR5 specifically (Maeda et al, 2004; Dorr et al, 2003; Oppermann, 2004). However, the potential for maraviroc to induce receptor internalization was investigated since this process has been identified as the mechanism driving the antiviral activity of chemokines and chemokine analogues (Mack et al, 1998; Mosier et al, 1999). 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/R5) 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 to and exceed the IC_{50} values generated for maraviroc-dependent inhibition of:

a. chemokine binding to CCR5 ($IC_{50} \leq 8$nM against all chemokines – Table 7) and

b. chemokine-mediated signaling ($IC_{50} \leq 30$nM – Table 7).

Maraviroc did not induce CCR5 internalization in 300.19/R5 cells at 10 nM and 100 nM. Maraviroc effects on cell surface CCR5 were consistent with those observed with the negative control ligand SDF-1α. In contrast, the positive control RANTES, induced CCR5 internalization as shown by a reduction in CCR5-dependent cell population fluorescence. These observations for maraviroc are consistent with studies reported for other CCR5 antagonists (Maeda K et al, 2004; Dorr P et al, 2003) and observations from FACS analysis on blood samples taken from maraviroc-dosed volunteers run as part of the clinical development program on this compound (Fatkenheuer et al, 2003). In light of these data, the antiviral activity of maraviroc is believed to be a result of direct blockade of CCR5 rather than induction of co-receptor internalization in R5-HIV-1 susceptible cells.

2.6.2.2.1.12. Summary of mechanism of action and antiviral activity

Radioligand binding experiments, using [^{3}H]-maraviroc, confirm that maraviroc binds with high affinity to human CCR5. 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 receptor.

Maraviroc inhibits the binding of CD4-complexed, recombinant soluble gp120 to CCR5 (IC_{50} = 11 nM). Maraviroc is also able to inhibit cell-cell fusion between a cell line expressing recombinant viral envelope (gp120/gp41) and cells expressing recombinant CD4 and recombinant CCR5 IC_{50} of 0.22 nM). These assays confirm that maraviroc inhibits the interaction between the viral gp120 envelope protein and its co-receptor, CCR5.

Maraviroc selectively inhibits lab-adapted and primary CCR5-tropic HIV-1 isolates. This activity is distinct from any compound-induced cytotoxicity. Maraviroc is inactive against viruses that can use CXCR4 as their entry co-receptor, at concentrations up to 5,000-fold above the concentration required to inhibit replication of CCR5-tropic viruses to >90%.

CCR5 agonists, such as the natural chemokine ligands RANTES and MIP-1β, can inhibit virus replication by internalisation of the receptor. Maraviroc does not induce internalisation at concentrations above that required to inhibit replication of CCR5-tropic viruses to >90%.

Taken together these studies identify the mechanism of action of maraviroc relevant to the indication of preventing HIV-1 disease progression and AIDS. Maraviroc acts externally to the host cell and the virus. It binds to cell surface CCR5 and blocks the interaction of CCR5-tropic HIV-1 gp120 with its co-receptor and thus prevents fusion between the lipid membrane of the virus and the host cell membrane and subsequent viral entry. Site directed mutagenesis studies of human CCR5 indicate that maraviroc binds within the transmembrane region of the receptor. As a consequence of this binding, maraviroc is thought to alter the three dimensional structure of the surface-exposed regions of CCR5 such that the viral gp120 protein is unable to recognize and bind to the co-receptor. Further evidence in support of this model can be derived from studying the behaviour of CCR5-tropic maraviroc-resistant viruses (Section 5.3.5.3. Microbiology Review of Resistance/Tropism in Maraviroc Program).

Maraviroc inhibits CCR5-tropic HIV-1 primary isolates of clade B and non-B with similar potency. It also inhibits wild-type and drug-resistant viruses over a narrow concentration range. This indicates that the compound should be effective in patients harbouring virus resistant to existing antiretrovirals and from different geographical regions.

The pharmacologically relevant value for maraviroc is derived from the antiviral IC_{90}, which is 2.03nM (1.04 ng/mL), and the unbound assay concentration (estimated to be 54.8%) thus giving a value of approximately 1.0 nM (0.5 ng/mL). This was determined from experiments using primary isolates to infect PBL. Primary HIV-1 isolates are more genetically diverse, have been through a limited number of passages in culture and therefore more closely resemble infections in patients than lab-adapted strains. The panel of isolates selected covered a wide geographical origin and diverse viral clades. PBL contain primary CD4+ T-cells, which represent the major cellular reservoir for HIV-1 replication in vivo. Furthermore, since maraviroc binds CCR5 rather than a viral target, PBL are the relevant cells in which to investigate the compound's antiviral activity.

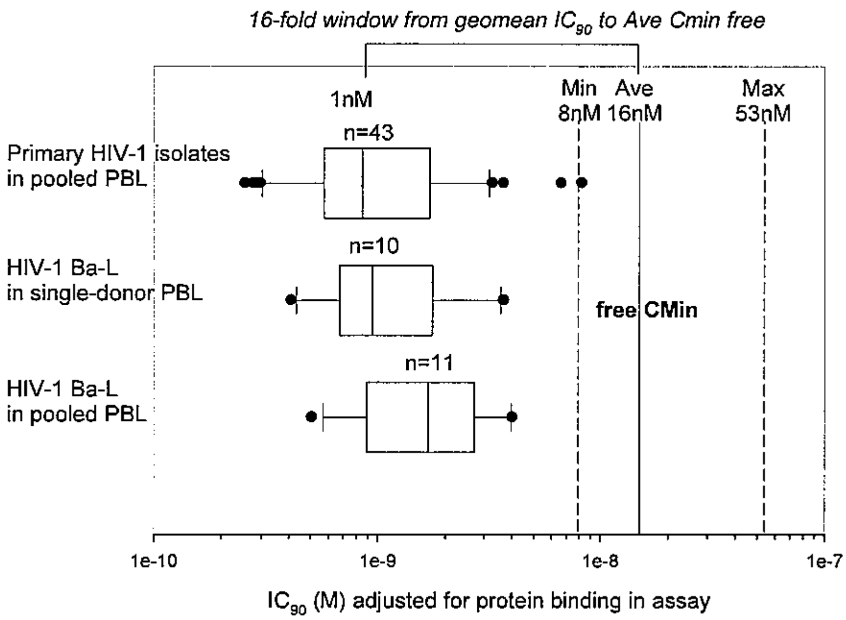
Inhibitory quotients (IQ) have been described in the development of antiretrovirals, particularly for the HIV protease inhibitor drug class (Back et al, 2006; Morse et al, 2006, Hickman et al, 2004). Having a high IQ is thought to indicate that an effective drug concentration can be achieved in a patient to inhibit the virus and minimise the development of drug resistance. Using the in vivo pharmacokinetic data from the Phase 2 clinical studies and the in vitro activity data against the 43 CCR5-tropic HIV-1 primary isolates an IQ for maraviroc can be approximated using the equation:

$$IQ = C_{min}/\text{serum adjusted } IC_{50} \text{ value (FDA Guidance for Industry, 2006).}$$

As shown in Figures 3 and 4, the average unbound C_{min} levels of maraviroc in plasma achieved in clinical study A4001007 at a dose of 300 mg BID (16 nM, 8 ng/mL, Module 5.3.4.2 Patient PD and PK/PD Study Reports) are in excess of the levels of maraviroc required to inhibit replication of HIV-1 primary isolates, representing clades A-J of Group M viruses and Group O. An IQ of 60 is estimated, using the average unbound C_{min} and the primary isolate geometric mean IC_{50}.

The dose selection for the phase 2b/3 studies was ultimately based on incorporation of maraviroc anti-viral data from 10 day monotherapy studies and pharmacokinetic data, into a fully integrated drug-disease model which was subsequently used to simulate the outcome of the phase 2b/3 trials. This data, in combination with the safety and toleration data generated in the phase 1/2a program lead to the selection of 300mg QD and BID doses (adjusted for drug interactions).

Figure 3: In vitro antiviral activity of maraviroc (unbound IC_{90}) against acute HIV-1 infection of PBL, in relation to unbound Cmin levels achieved at steady state in HIV-1 infected patients following monotherapy with maraviroc for 10 days.



Data obtained for primary HIV-1 isolates in pooled PBL were derived from results presented in DI/18/01, Table 3. Data obtained for HIV-1 Ba-L in pooled PBL were derived from results presented in DI/019/1, Table5. Data obtained for HIV-1 Ba-L in single-donor PBL were derived from results presented in DI/019/1, Table 7. In each case the unbound assay concentration used in calculation was 54.8%.

Figure 4: In vitro antiviral activity of maraviroc (unbound IC_{50}) against acute HIV-1 infection of PBL, in relation to unbound Cmin levels achieved at steady state in HIV-1 infected patients following monotherapy with maraviroc for 10 days.

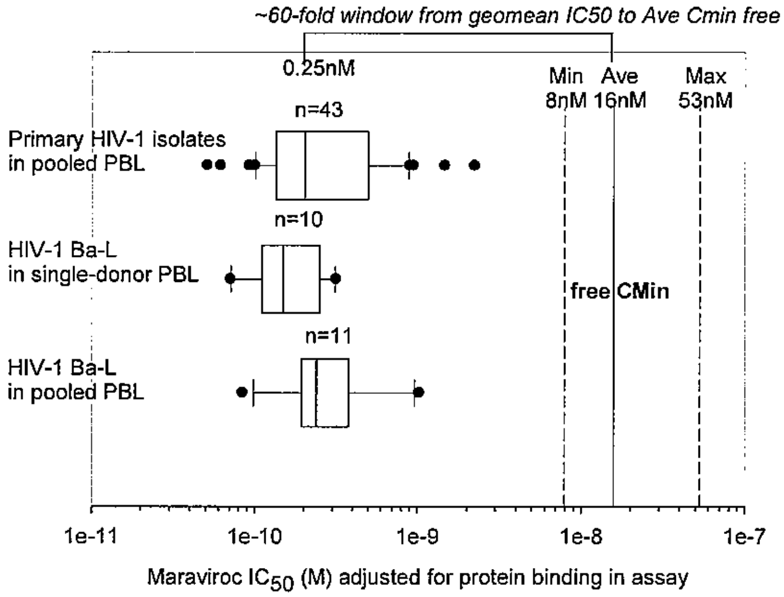


Figure legend as in Figure 3

2.6.2.2.1.13. In vitro emergence of resistance

Studies have been conducted in vitro to investigate the selection of maraviroc resistance from CCR5 tropic HIV-1 strains. The objectives of these studies were to investigate the phenotypic and genotypic correlates of maraviroc resistance. Specifically:

- Does resistance to maraviroc emerge rapidly during serial passage of virus in the presence of compound?
- Does the use of maraviroc promote the selection of variants that use CXCR4 (or other co-receptors) for virus entry?
- What fold changes in susceptibility are associated with maraviroc resistance?
- What amino acid changes in gp160 are associated with maraviroc resistance?
- Is resistance to maraviroc associated with cross-resistance to antiviral agents of the same drug class or other drug classes?
- Do maraviroc-resistant viruses revert to a wild type phenotype/genotype during growth in the absence of maraviroc?

The findings of these studies are discussed in detail in the resistance report in Section 5.3.5.3 (Microbiology Review of Resistance/Tropism in Maraviroc Program).

The main findings of these studies are:

- Resistance to maraviroc did not rapidly emerge during weekly serial passage of CCR5-tropic primary isolates through PBL for up to 20 weeks, or the lab adapted strain, Ba-L, through PBL and PM-1 cells for up to 32 weeks.
- Selection of maraviroc-resistant (MVCres) variants by serial passage of virus in the presence of maraviroc was not associated with a shift in co-receptor tropism, except where this also occurred in untreated control virus passaged in parallel.
- Resistance to maraviroc is associated with dose response curves with plateaus in maximal inhibition rather than shifts in IC_{50}. This type of dose response curve is frequently seen with allosteric inhibitors of receptors, and occurs when the receptor ligand is able to recognise both inhibitor-bound and inhibitor-free receptor molecules. Consistent with this, MVCres variants appear able to use both maraviroc-occupied and maraviroc-free CCR5 molecules to infect target cells.
- Resistance to maraviroc in vitro is associated with selection of variants containing amino acid substitutions/deletions in the V3 loop of the HIV-1 envelope (gp120).
- Maraviroc-resistant variants remained susceptible to other CCR5 antagonists and to enfuvirtide (HIV fusion inhibitor) and saquinavir (PI).
- Serial passage of the MVCres variants in the absence of maraviroc selects for variants with sensitivities increased towards wild type maraviroc susceptibility.

These in vitro findings were used to guide the design and execution of investigational virology studies of resistance in viruses from patients failing maraviroc-containing regimens in the clinical program.

2.6.2.2.2. CCR5 Primary Pharmacodynamics Not Related to the Antiviral Mode of Action

The objectives of the studies described in this Section were to establish the effects, if any, of maraviroc on endogenous chemokine binding and on immune processes mediated by a range of alternative chemokine receptors and immune system mediators. In addition, the interaction of maraviroc with homologous CCR5 receptors from various animal species was determined, or projected on the basis of sequence homology, in order to assess the likelihood that CCR5 mediated effects might be observed in toxicology studies.

2.6.2.2.2.1. Antagonism of Endogenous Ligands

The chemokine signaling network is a complex system in which cognate receptors may bind to more than one ligand and the ligands may bind to several receptors. This has led to a complexity of immune response that is both pathogen- and location-specific and allows a finely orchestrated control of immune cell proliferation, migration and maturation (Mantovani, 1999). In many instances, as a consequence of ligand/receptor promiscuity, genetic heterogeneity is tolerated without significant adverse effect on the individual. The human CCR5 interacts with three endogenous chemokines, MIP-1α, MIP-1β and RANTES (also known as CCL3, CCL4 and CCL5) all of which act as receptor agonists causing downstream cellular effects such as chemotaxis. The potential for maraviroc to block binding of the endogenous human CCR5 ligands and thereby to antagonise the functional activity of the receptor was assessed in radioligand binding assays and by inhibition of agonist-induced intracellular calcium release in whole cells (Table 7).

Table 7. CCR5 Primary Pharmacodynamic Properties of Maraviroc Not related to the Antiviral Mode of Action

In Vitro Finding	N	Maraviroc Activity	
		nM	ng/mL
Receptor Binding		Geometric Mean IC_{50} ± SEM (95% CI)	
Displacement of chemokine binding			
[^{125}I]MIP-1α binding to cell membranes[a]	3	3.30±0.43 (1.9 to 5.7)	1.70±0.22 (1.0 to 2.9)
[^{125}I]MIP-1α binding to whole cells[a]	3	3.43±0.22 (2.6 to 4.5)	1.76±0.11 (1.3 to 2.3)
[^{125}I]MIP-1β binding to cell membranes[a]	17	7.18±0.93 (5.5 to 9.5)	3.69±0.48 (2.8 to 4.9)
[^{125}I]MIP-1β binding to whole cells[a]	3	3.57±0.79 (1.4 to 9.0)	1.83±0.41 (0.7 to 4.6)
[^{125}I]RANTES binding to cell membranes[a]	3	5.22±1.10 (2.1 to 13.0)	2.68±0.57 (1.1 to 6.7)
[^{125}I]RANTES binding to whole cells[a]	3	6.30±0.63 (4.1 to 9.7)	3.24±0.32 (2.1 to 5.0)
[^{125}I]MIP-1α binding to cell membranes[b]	3/4	No effect on dissociation rate up to 1 µM (514ng/mL)	
Functional antagonism in CCR5 expressing HEK cells			
		Geometric Mean IC_{50} range	
MIP-1α-, MIP-1β- and RANTES-induced intracellular calcium release[c]	2 to 4	7 to 30	4 to 15
		Geometric Mean IC_{50} ± SEM (95% CI)	
MIP-1β-induced intracellular calcium release[d]	8	14±2 (10 to 20)	7±1 (5 to 10)
		pK_b (95% CI)	
MIP-1β-induced intracellular cAMP levels[e]	4	9.4 (9.01 – 9.75)	4.8 (4.6 – 5.0)

RANTES: Regulated on Activation of Normal T cell Expressed and Secreted.
Study Codes: [a]DI/002/1, [b]CG/005/04, [c]DI/005/1, [d]DI/056/04; [e]CG/001/05.

Maraviroc inhibited binding of iodinated MIP-1α, MIP-1β and RANTES ligands to recombinant human CCR5 receptors expressed in whole HEK-293 cells and membrane homogenates with IC_{50} values in the single figure nanomolar range (Table 7). In a separate experiment the IC_{50} for maraviroc was unaffected when the concentration of radiolabelled macrophage inflammatory protein-1β (MIP-1β) increased from 0.005 to 100 nM (IC_{50} range = 2.7 to 6.1 nM, 1.4 to 3.1 ng/mL) suggesting that the inhibition of chemokine binding was insurmountable under these conditions and that circulating chemokines are unlikely to affect the binding potency of maraviroc (DI/003/1). In addition, the observation that maraviroc had no effect on the dissociation rate of MIP-1β from CCR5 at concentrations up to 1 µM (513.68 ng/mL) indicates that the compound is not an allosteric inhibitor of chemokine binding (CG/005/04). Furthermore, in a single experiment in which the effect of a 2 h pre-incubation of maraviroc with cell membranes was assessed, the affinity of the compound for the human CCR5 was increased 2-fold (as indicated by a shift in the inhibition of MIP-1β binding from an IC_{50} value of 5.6 nM to a value of 2.8 nM (2.9 to 1.4 ng/mL, N=1; CG/010/04) with no change in the maximum response. This finding is consistent with a non-competitive mode of binding.

To demonstrate that maraviroc not only blocks chemokine binding but also prevents agonist-induced functional response the effect of the compound on intracellular calcium release from HEK-293 cells expressing the recombinant human CCR5 receptor was investigated (Table 7). The data indicate that maraviroc blocks functional signalling at similar concentrations to the inhibition of chemokine binding and is a functional antagonist at the human CCR5 receptor. In the same experiments, maraviroc alone did not trigger release of intracellular calcium at concentrations up to 10 μM (5137ng/mL), indicating that it is devoid of agonist activity at the human CCR5 receptor. Moreover, within this study it was shown that maraviroc did not inhibit signalling induced by 1 μM carbachol (N = 3), a non-selective muscarinic receptor agonist. This demonstrates that the inhibition of CCR5 ligand-induced calcium flux by maraviroc is specific to CCR5, and does not involve other signal transduction steps that may be common to G protein-coupled receptors (DI/056/04).

In a further study (DI/108/06), maraviroc did not increase basal levels of γ-S-GTP incorporation in a human epithelial kidney cell line, further confirming an absence of agonist activity; although an observed reduction of basal γ-S-GTP binding at higher concentrations may indicate that maraviroc can act as an inverse agonist to promote the formation of CCR5 in an inactive state.

The activity of maraviroc as an inhibitor of MIP-1β signalling was also determined in an assay that measures cellular cAMP levels (CG/001/05). Maraviroc at 1, 3 and 10 nM, caused a dose dependent rightward shift of the MIP-1β dose response curve, together with a marked suppression of the maximum response, indicative of insurmountable antagonism. The most likely explanation for this insurmountable profile relates to the slow rate of maraviroc dissociation from the human CCR5 receptor. Previous binding experiments have shown that maraviroc has a half-life of approximately 16 hours at the human CCR5 receptor (Section 2.6.2.2.1.1), compared to MIP-1β, which has a half-life of 16.6 minutes (CG/005/04). When an antagonist dissociates very slowly from a receptor, a state of hemi- (or quasi-) equilibrium results (Paton and Rang, 1966; Paton and Waud, 1967; Kenakin, 1984) and the antagonist appears to behave essentially as an irreversible blocker over the time course of the experiment. The hemi-equilibrium state has been well documented in the literature (Yamamoto and Koike, 2001; Keith et al, 1989; Christopoulos et al, 1999). The data for MIP-1β antagonism was therefore analyzed assuming hemi-equilibrium conditions, and a p*K*b value of 9.4 (95% CI 9.01 – 9.75) was calculated for maraviroc (Table 7).

Thus, we have demonstrated that maraviroc inhibits endogenous chemokine binding to CCR5 and, as a consequence prevents downstream signalling processes within the cell. It should be reiterated however that this is not thought to constitute the mechanism of antiviral activity of maraviroc. Instead the maraviroc-induced inhibition of CCR5 mediated chemokine signalling is probably a correlate of the structural changes within the receptor that also block viral attachment.

2.6.2.2.3. Interaction with CCR5 from Relevant Non-Human Species

To support subsequent toxicology testing, and in particular the assessment of potential immunotoxicology, the CCR5 sequence homology and interaction of maraviroc with various animal CCR5s was studied.

Following sequence analysis of the human, monkey, dog, rabbit, rat and mouse CCR5 it was shown that at the full sequence level the monkey protein is very similar to human CCR5 (98% identity), whereas the dog, rabbit, rat and mouse are all less similar to human but roughly by the same amount (i.e. 83%, 80%, 82% and 82% identity respectively, Table 8). However, this may not provide an accurate picture of the differences at the binding site level. Thus, percentage sequence identities for the putative ligand binding site have been calculated. At a radius of 2Å from the putative binding site the monkey receptor is very similar to human CCR5 (94 % identity), whereas the dog, rabbit, rat and mouse are all less similar than human but roughly by the same amount (i.e. 78%, 61%, 72% and 72% identity respectively).

Table 8. Percentage Sequence Identity of Full Length and Putative Ligand Binding Site Amino Acids in the CCR5 Receptor from Various Species

Human Sequence Comparison	Species				
	Macaque	Dog	Rabbit	Rat	Mouse
Full Length	97.7%	82.6%	79.8%	82.3%	82.3%
(352)	(344)	(291)	(281)	(290)	(290)
8 Å radius	97.7%	85.2%	79.5%	80.6%	84.9%
(88)	(86)	(75)	(70)	(76)	(75)
6 Å radius	98.1%	83.6%	76.3%	74.5%	80%
(55)	(54)	(46)	(42)	(41)	(44)
4 Å radius	97.1%	80%	65.7%	74.2%	77.1%
(35)	(34)	(28)	(23)	(26)	(27)
2Å radius	94.4%	77.8%	61.1%	72.2%	72.2%
(18)	(17)	(14)	(11)	(13)	(13)

The number of amino acid residues that are identical to the human sequence is shown in parentheses. The various sequence comparisons were made on either the full length protein or on the amino acids present within the given radii from the putative ligand binding site. Study Code DI/100/06

Direct maraviroc binding and/or functional experiments were conducted with mouse, rat, dog and macaque recombinant CCR5 (Table 9). It has been shown that maraviroc has no functional interaction with recombinant mouse CCR5 receptors at concentrations up to 1 μM (514 ng/mL; DI/092/06). To determine whether the cognate rat and dog receptors are likely to interact with maraviroc, the CCR5 receptors from those species were cloned and expressed in cell lines for functional analysis. At 10 μM maraviroc, the mean (± SEM) percentage inhibition of [^{125}I]MIP-1β binding to rat and dog CCR5 receptors was 33.3±4.4% (N = 3) and 38.7±3.8% (N = 3) respectively (CG/002/04). This contrasts to an IC_{50} of 7.2±0.9 nM (3.7±0.5 ng/mL) for the human receptor (Table 7) and demonstrates at least 1,400-fold lower affinity of maraviroc for the rat and dog CCR5 receptors. The macaque CCR5 is known to differ from the human sequence by a single residue (I198M) within 2 Angstroms of the binding pocket (DI/100/06). Therefore, the macaque receptor was also cloned and a stable HEK-293 cell line was produced with which to carry out functional and radioligand binding studies (Table 9).

Table 9. Evaluation of Maraviroc Interaction with Animal CCR5

Assay	Maraviroc Activity
Mouse CCR5 MIP-1α induced intracellular calcium release assayed by Fluorometric Imaging Plate Reader (FLIPR) analysis [a] (N = 1, 6 replicates)	No agonist activity up to 1000 nM. No antagonist activity up to 1000 nM
Rat CCR5 Inhibition of [^{125}I]-MIP-1β binding to membrane preparations derived from the rat CCR5 receptor cell line [b] (± SEM, N = 3)	33.3 ± 4.4 % inhibition at 10000 nM
Dog CCR5 Inhibition of [^{125}I]-MIP-1β binding to membrane preparations derived from the dog CCR5 receptor cell line [b] (± SEM, N = 3)	38.7 ± 3.8 % inhibition at 10000 nM
Macaque CCR5 Saturation binding of [3H]maraviroc to membrane preparations derived from the macaque CCR5 receptor cell line [c] (N = 3)	K_D = 1.36 nM (0.7 ng/mL) (95% CI = 1.07 to 1.71 nM)
Macaque CCR5 Inhibition of [^{125}I]-MIP-1β binding to membrane preparations derived from the macaque CCR5 receptor cell line [d] (N = 5)	IC_{50} = 17.5 nM (9.0 ng/mL) (95% CI = 14.1 to 21.0 nM)
Macaque CCR5 MIP-1β induced intracellular calcium release assayed by Fluorometric Imaging Plate Reader (FLIPR) analysis[e] (N = 4)	IC_{50} = 17.5 nM (9.0 ng/mL) (95% CI = 9.6 to 32.9 nM)

Study Codes: [a] DI/092/06, [b] CG/002/04, [c] CG/015/02, [d] CG/011/02, [e] CG/009/02 (data derived from original report)

The values obtained with the macaque CCR5 cell line compare closely with those obtained in previous studies with maraviroc on a recombinant human CCR5 receptor cell line (Tables 1 and 7). Maraviroc has a similar K_D at both receptors (0.86 nM for human and 1.36 nM for macaque); it had a similar IC_{50} for inhibition of MIP-1β binding (7.18 vs 17.5 nM respectively) and a similar inhibition of cellular signalling as determined by MIP-1β induced intracellular calcium release (14 nM vs 17.5 nM respectively).

Further kinetic studies (Table 10) indicated that maraviroc associated with macaque CCR5 at a similar rate to the human homolog ($t_{½}$(on) = 11.05 minutes vs 8.37 minutes respectively, human data from Table 2) whereas it dissociated at a markedly faster rate ($t_{½}$ (off) approximately 1.5 hours vs approximately 16 hours respectively).

Table 10. Kinetic Constants for [^{3}H]Maraviroc Binding to Macaque CCR5 Receptors at Room Temperature and at 37°C

Temp.	$t_{½}$ (on) min (95% CI)	$t_{½}$ (off) min (95% CI)	K_{on} $min^{-1}.nM^{-1}$ (95% CI)	K_{off} min^{-1} (95% CI)	K_{off}/K_{on} nM (95% CI)
RT	8.37 (6.98-10.02)	92.18 (75.26-112.89)	0.013 (0.010-0.018)	0.0075 (0.0001-0.0092)	0.51 (0.46-0.57)
37°C	3.92 (2.61-5.90)	39.13	0.032	0.018	0.56

Values are the geometric mean (95% CI), N=2-6 or 1 where no confidence interval is given. K_{off} = dissociation rate constant, K_{on} = association rate constant, RT = room temperature. Study Code CG/015/02

Therefore, the affinity of maraviroc for macaque CCR5 is similar to that of human CCR5, and the macaque is likely to represent a non-rodent species with a functionally relevant interaction with the compound.

Although no comparable affinity studies have been conducted in the rabbit, receptor homology analysis within the putative CCR5 binding site across species (Table 8) suggests that maraviroc would bind to CCR5 in the rabbit with an affinity approximately equivalent to that for the rat and dog receptor. In the rat and rabbit embryo-foetal toxicity studies, the free C_{max} concentrations at the high doses were 7.2 μM (3709 ng/mL) and 6.7 μM (3437 ng/mL,) respectively (Section 2.6.6.10, Table 6). Pregnant rats were therefore exposed to maraviroc concentrations that provide approximately 30% inhibition of MIP1-β binding (cf binding data in Table 9). This dose produced slight maternal toxicity in terms of reduced maternal body weight. Pregnant rabbits were therefore exposed to maraviroc concentrations that are predicted to achieve approximately 30% inhibition of MIP1-β binding in that species. The high dose produced severe maternal toxicity (including death).

2.6.2.3. Secondary Pharmacodynamics

2.6.2.3.1. Selectivity Profile against Relevant Human Targets

Since the chemokine receptors play an integral role in immune cell function and thus host immune responses it was important to assess the potential for maraviroc to inhibit functions mediated by chemokine and cytokine receptors other than CCR5. Therefore, the activity of maraviroc was evaluated in a number of in vitro human immune function and binding assays (Table 11). These assays included examples of specific interactions between a ligand and receptor (for example CCR2b mediated, MCP-3 induced intracellular Ca^{2+} release) as well as assays in which multiple signal transduction pathways might be operating (for example antigen-stimulated lymphocyte proliferation). No significant activity was observed in any of these assays, at concentrations in excess of 500-fold the IC_{50} value for maraviroc inhibition of chemokine binding to the human CCR5 receptor (maraviroc inhibition of MIP-1β binding to cell membranes expressing CCR5 has an IC_{50} of approximately 7 nM; Table 7, DI/002/1). This includes activity against CCR2b, which has the closest structural identity to CCR5. These experiments indicate both that maraviroc is selective for CCR5, and that inhibition of CCR5 function is unlikely to affect immune processes as modelled in these in vitro immune function assays.

Table 11. Evaluation of Maraviroc in In Vitro Models of Human Immune System Function

Selectivity Assay (N = 1 to 3)	Activity of Maraviroc
MCP-3 induced intracellular Ca^{2+} release (CCR2b)[a]	IC_{50} >10 μM
IL-2-stimulated T-cell proliferation[b]	IC_{50} >10 μM
Antigen-stimulated lymphocyte proliferation[c]	IC_{50} >10 μM
Superoxide production in neutrophils[d]	IC_{50} >10 μM
MIP-1α binding to CCR1[e]	IC_{50} >10 μM
Eotaxin binding to CCR3[f]	IC_{50} >10 μM
IL-4-stimulated IgE synthesis in lymphocytes[g]	IC_{50} ≥10 μM (2 donors) IC_{50} = 1 to 10 μM (1 donor)
LPS-stimulated TNF-α release by differentiated THP-1 cells[h]	IC_{50} >40 μM
rhMIP-3β binding to CCR7[i]	IC_{50} >10 μM
MIP-1α induced chemotaxis of THP-1 cells (CCR1)[j]	IC_{50} >25 μM
MCP-1 induced chemotaxis of THP-1 cells (CCR2)[j]	IC_{50} >25 μM
ITAC induced chemotaxis by H9 cells (CCR3)[j]	IC_{50} >25 μM
SLC induced chemotaxis by H9 cells (CCR7)[j]	IC_{50} >25 μM
IL-8 induced chemotaxis of neutrophils (CXCR1/CXCR2)[j]	IC_{50} >25 μM
Gro-α induced chemotaxis of neutrophils (CXCR2)[j]	IC_{50} >25 μM
TARC binding to CCR4[k]	IC_{50} >10 μM
I309 binding to CCR8[l]	IC_{50} >10 μM

MCP - monocyte chemoattractant protein; IL-2-interleukin-2, LPS-lipopolysaccharide; MIP-macrophage inflammatory protein; ITAC-interferon inducible T-cell and chemoattractant; SLC-secondary lympoid tissue chemokine; IL-8 - interleukin 8; Gro-α - growth chemokine-α; TARC-thymus and activation regulated chemokine; RhMIP-3β - macrophage inflammatory protein 3β. (>4 μM = >2054 ng/mL; >10 μM = ≥ 5137 ng/mL; >25 μM = > 12842 ng/mL; 1 to 10 μM = 514 to 5137 ng/mL)
Study Codes: [a]DI/008/1, [b]DI/020/1, [c]DI/021/1, [d]DI/022/1, [e]DI/023/1, [f]DI/024/1, [g]DI/025/1, [h]DI/026/1, [i]DI/027/1, [j]DI/028/1, [k]DI/029/1 & [l]DI/030/1.

2.6.2.4. Safety Pharmacology

The wider pharmacological properties of maraviroc were assessed in a variety of tests designed to evaluate its effects, if any, on the major physiological systems (Table 12). All studies in Table 12, except for the hERG patch clamp assay and the radioligand binding assays, were performed in compliance with International Good Laboratory Practice regulations. For in vitro studies the highest concentration tested was 1 and 100 μM (513.68 and 51368 ng/mL) in different assays. For in vivo studies maximum doses were predicted to achieve unbound plasma concentrations of approximately 300 nM (154 ng/mL) representing >100 times the antiviral potency of maraviroc (IC_{90} = 2.03 nM, 1.04 ng/mL). Exceptions included the dog haemodynamic study where the peak unbound C_{max} was 168 nM (86 ng/mL, based on plasma protein binding of 63.7% in dogs), and the rodent appearance and behaviour study where high doses were given to assess the toleration of maraviroc (Table 12). Where margins versus the clinically relevant mean unbound C_{max} are expressed, the latter value is derived from clinical study A4001007 at a dose of 300 mg BID and is 300 nM (155 ng/mL, Module 5.3.4.2 Patient PD and PK/PD Study Reports).

Table 12. Summary of Safety Pharmacology Profile of Maraviroc

Affinity for Other Receptors, Enzymes and Ion Channels and Effects on Isolated Tissue				
Experiment	**Species (N)**	**Concentration**	**Standard**	**Result/Observation**
Muscarinic nonselective [ab]	Rat brain (2)	100 nM & 10 µM (51.4 & 5137 ng/mL)	Atropine	100 nM: no effect, 10 µM: 39% & 47% inhibition
Opioid nonselective[a]	Rat brain (1)	100 nM & 10 µM (51.4 & 5137 ng/mL)	Naloxone	100 nM: no effect, 10 µM: 83% inhibition
µ opioid (Cerep)[a]	Human recombinant (1)	0.1 nM to 10 µM (0.05 to 5137 ng/mL)	Naloxone	K_i = 106 nM (54.5 ng/mL) (Cerep)
µ opioid[c]	Human recombinant (3)	0.03 nM to 100 µM (0.0154 to 51368 ng/mL)	Naloxone	$K_i = 208.8 \pm 0.3$ nM (107.3 ng/mL)
µ opioid[d]	Human recombinant (4)	3 nM to 100 µM (1.54 ng/mL to 51368 µg/mL)	DAMGO / Naloxone	Ki = 589.29 nM (95% CI, 430.1-807.4 nM) (302.7 ng/mL)
δ opioid[a]	Human recombinant (2)	0.1 nM to 10 µM (0.05 to 5137 ng/mL)	DPDPE	10 µM: 48% inhibition
Adrenergic α_{2A}[b]	Human recombinant (1)	100 nM & 10 µM (51.4 & 5137 ng/mL)	Yohimbine	100 nM: no effect; 10 µM: 52% inhibition K_i = 5.47 µM (2809.8 ng/mL)
Adrenergic α_{2B}[b]	Mouse neuroblastoma (1)	100 nM & 10 µM (51.4 & 5137 ng/mL)	Yohimbine	100 nM: no effect; 10 µM: 24% inhibition
Adrenergic α_{1A}[e]	Rat Salivary gland (2)	10 µM (5137 ng/mL)	WB4101	26% inhibition
Histamine H_2[e]	Guinea pig striatum (2)	10 µM (5137 ng/mL)	Cimetidine	22% inhibition
Norepinephrine transporter[e]	Human recombinant (2)	10 µM (5137 ng/mL)	Protryptiline	27% inhibition
HERG (I_{Kr})[f]	Human recombinant (6)	0.3 nM to 10 µM (0.154 to 5137 ng/mL)	Dofetilide	3 µM: 15±4% inhibition 10 µM: 43±3% inhibition

Study Codes: [a]CG/013/00; [b]CG/013/02; [c]CG/009/01; [d]CG/001/03; [e]CG/001/04; [f]IC/001/02

Table 12. Summary of Safety Pharmacology Profile of Maraviroc (Continued)

Affinity for Other Receptors (Transporters), Enzymes and Ion Channels and Effects on Isolated Tissue		
Maraviroc induced <20% inhibition at 10 μM (5136.8 ng/mL) for the following sites		
Receptors	**Receptors**	**Receptors**
Adenosine (nonselective, A_1) [a]	Glutamate (NMDA) [a]	Vasopressin (human V_{1A}) [h]
Adenosine (human A_3) [g]	Ghrelin (human GHS) [h]	5-HT transporter (human) [g]
Adrenergic (nonselective α_1, α_2, β_1 & β_2) [a]	Histamine (H_1) [a]	**Ion Channels**
Adrenergic (human α_{2C})[b]	Imidazoline I_2 (rat brain) [b]	Calcium channels:
Angiotensin-II (AT_1) [a]	Interleukin (IL-1β, IL-2, IL-4, human IL-6) [a]	L-type (verapamil, dihydropyridine & diltiazem sites) [a]
Atrial Naturietic Peptide (ANP) [h]	IL-8A (CXCR1, human) & IL-8B (CXCR2, human) [a]	**Enzymes**
Bradykinin (human B_2) [h]	CCR1 (human) [a]	Acetylcholinesterase (human) [a]
Choline transporter (rat striatum) [g]	Muscarinic (human M_1, M_2, M_3, M_4 & M_5) [i]	Angiotensin Converting Enzyme (human) [g]
Dopamine (human D_1, D_2) [a]	Nicotinic (neuronal, α-BGTX sensitive) [a]	Guanylyl cyclase [j]
Dopamine transporter (human) [g]	κ opioid (human) [a]	Monoamine oxidase (human A & B) [a]
Endothelin (human ET_A & ET_B) [h]	Serotonin (human $5HT_{1A}$, human $5HT_{2A}$ & $5HT_{1B}$ [a]	Na^+/K^+ ATPase [a]
$GABA_A$ (benzodiazepine central) [a]	Thromboxane (human TXA_2/PGH_2) [h]	Phosphodiesterase (human III & IV) & (human V) [j]
GABA transporter (rat striatum) [g]	Urotensin (human UT1) [h]	Tyrosine hydroxylase [a]

Study Codes: [a]CG/013/00; [b]CG/013/02; [g]CG/001/04; [h]CG/003/05; [i]CG/001/02; [j]CG/005/05

Table 12. Summary of Safety Pharmacology Profile of Maraviroc (Continued)

In Vitro Isolated Tissue / Whole Cell Studies :				
Experiment	**Species (n)**	**Concentration**	**Standard**	**Result/Observation**
GTP-γ-S binding to human μ receptor [k]	Human recombinant (5 to 8)	0.03 nM to 10 μM (0.015 to 5137 ng/mL)	DAMGO Naloxone	1 μM: no effect 10 μM: 22.2±3.7% stimulation, no antagonism of DAMGO response.
Electrically-stimulated guinea pig ileum [l]	Guinea pig, male, Dunkin-Hartley, (6 to 8 tissues)	20 nM to 1 μM (10.3 to 514 ng/mL)	DAMGO Naloxone	No effect.
Oxytocin-induced contraction of isolated uterine smooth muscle [m]	Rat, female, Sprague Dawley, (8 tissues)	10 nM, 100 nM & 1 μM (5.14, 51.4, 514 ng/mL)	Verapamil	No effect.
HERG potassium channels (patch clamp assay) [no]	Human cloned ion channel (4)	200 nM (103 ng/mL) 3, 10 μM (1541 & 5137 ng/mL)	Dofetilide	200 nM: No effect. 3 μM: 5.74±2.0% inhibition 10 μM: 19±3% inhibition
Action potentials recorded from dog isolated Purkinje fibres [pqr]	Dog, Beagle either sex (5)	200, 500 nM, & 1 μM (103, 257, 514 ng/mL) 1, 3, & 10 μM (514, 1541, 5137 ng/mL)	D-sotolol	No effect 1 μM: no effect 3 μM: 8.8±3.5% prolongation of APD50, 9.6±2.4% prolongation of APD90 10 μM 25.9±10.9% prolongation of APD50, 30.6±6.7% prolongation of APD90

APD – Action Potential duration.
Study Codes: [k]CG/011/01; [l]CG/006/00; [m]CG/007/01; [n]CG/001/00; [o]IC/005/02; [p]CG/002/00; [q]IC/006/01; [r]IC/003/02.

Table 12. Summary of Safety Pharmacology Profile of Maraviroc (Continued)

In Vivo Studies:

Experiment	Species (n)	Concentration	Standard	Result/Observation
Rat maximum tolerated dose [s]	Rat, male, (2) PO	1000, 2000 mg/kg	None	1000 mg/kg: Salivation, transient ↑ in respiration and a ↓ in activity (returning to normal by 30 min). Unbound C_{max} 5.0 µM (2543 ng/mL), Total C_{max} 10.1 µM (5190 ng/mL). 2000 mg/kg: Piloerection, transient ↑ in respiration and a ↓ in activity (returning to normal by 45 min). Marked salivation lasting 1 h after dosing. Respiratory distress lasting 1 h in 1 animal at 2000 mg/kg 2 h after dosing
Sodium pentobarbitone-induced sleeping times [t]	Male Mouse (20) PO	1, 3, 10 mg/kg	Chlorpromazine	No effect.
Motor coordination[u]	Male Mouse (12 to 20) PO	1, 3, 10 mg/kg	Chlorpromazine	No effect.
Excretion of fluid and electrolytes [v]	Rat, female, (12) PO	10, 20, 60 mg/kg	Frusemide	No effect.
Respiratory system [w]	Rat, male, (4) IV	1 mg/kg	Morphine	No effect on arterial blood pH, pO_2 and pCO_2. A small but statistically significant ↓ in mean arterial blood pressure and heart rate 10 min after dosing.
Haemodynamic and electrocardiogram (ECG) parameters [xy]	Dog, male, Beagle, (4) PO	0.05, 0.15, 0.5, 1.5 mg/kg	None	No effect. Mean unbound C_{max} = 168 nM (86 ng/mL) Total 464 nM (238 ng/mL)

Study Codes: [s]CG/009/00; [t]CG/005/01; [u]CG/002/01; [v]CG/004/01; [w]CG/001/01; [x]CG/003/00; [y]CG/008/00.

2.6.2.4.1. Affinity for Other Receptors, Enzymes and Ion Channels and Effects on Isolated Tissues

The affinity of maraviroc for a range of physiologic receptors, ion channels, and enzymes (studies conducted at [redacted] France) and the effects on isolated tissues is summarised in Table 12. There was no biologically relevant activity apart from moderate inhibition in the human μ opioid, rat non-selective muscarinic receptor, and the human α_{2A} adrenergic binding assays.

Maraviroc gave a range of moderate-to-weak binding affinities for the human μ opioid receptor depending on the assay system used. When assayed in buffer systems optimised for analysis of binding, K_i values of 106-209 nM (55-107 ng/mL) were obtained (CG/013/00 & CG/009/01). However, in the presence of a relatively physiological buffer system the binding affinity of maraviroc was weaker (K_i = 589 nM, 303 ng/mL, CG/001/03). The functional activity of maraviroc at the μ opioid receptor was determined by assessing the effect of the compound on agonist stimulated GTP-γ-S binding to human μ receptor in the latter buffer system (CG/011/01). There was no functional agonist or antagonist activity at 1 μM (514 ng/mL), and only a weak agonist effect at 10 μM (5137 ng/mL). Moreover, there was no μ opioid mediated agonist or antagonist activity in the electrically stimulated guinea pig ileum, an isolated tissue preparation in which the μ-specific agonist DAMGO and the antagonist naloxone were shown to be active (CG/006/00). Because the weak GTP-γ-S activity occurred at a concentration representing approximately 33-fold the mean unbound C_{max} in HIV-positive patients at a dose of 300 mg BID, it is unlikely that it will be of biological significance.

The activity of maraviroc in the rat muscarinic receptor assay (CG/013/00 & CG/013/02) is unlikely to be of biological relevance because maraviroc had no significant affinity (<12% inhibition) for the human muscarinic receptor subtypes M1-M5 in an additional study (CG/001/02) and was also inactive in the electrically stimulated guinea pig ileum preparation (CG/006/00), which is known to be sensitive to muscarinic antagonists (Clague R et al, 1985).

The effect of maraviroc and the standard agent verapamil on the contractile response to a sub-maximal concentration of oxytocin was determined in the isolated rat uterus, a recognized and reliable method to study the effects of compounds on the contraction of smooth muscle (Table 12, CG/007/01). The control compound verapamil (0.1, 1.0 and 10.0 mM) caused a concentration dependent reduction of the oxytocin-induced contraction of the isolated uterus, which reached statistical significance at all concentrations tested ($p<0.05$, $p<0.001$ and $p<0.001$ respectively). Maraviroc had no effect on the oxytocin-induced contraction in the isolated rat uterus when tested at 100 and 1000nM. It did however produce a statistically significant increase (approximately 17%) in the oxytocin-induced contraction at the lowest concentration tested (10 nM) when compared to the time-matched vehicle control ($p<0.05$). This effect was small and not concentration dependent, and so is unlikely to be biologically relevant. In the absence of oxytocin, maraviroc had no direct effect on the contractile activity of the isolated uterus.

Postural hypotension has been observed in the clinic at doses of 1200 mg (Study A4001001, following a single-oral dose) and 600 mg (Study A4001002, on Day 7 of multiple oral doses QD, Module 2.7.4, Section 2.7.4.2.1.6.2.1. Adverse Events Relating to Postural hypotension). To assess the activity of maraviroc at adrenergic receptors, and thus the significance of this activity as a potential mechanism for postural hypotension, the compound was tested in a variety of in vitro preparations (Table 13).

Maraviroc was shown to be a weak inhibitor of agonist binding to the human α_{2A} adrenergic receptor (CG/003/04 and CG/007/04), in agreement with data presented in Table 12. However, in two separate analyses maraviroc had no effect on the functional activity of recombinant human adrenergic receptors (CG/002/05 and CG/004/05). Moreover, in the α_{2A} responsive rat vas deferens preparation (CG/014/02 and CG/004/03) maraviroc had no direct effects on electrical field stimulated contractions and had only minor, and non-biologically relevant, effects on α_{2A} adrenergic agonist (UK-14,304 and clonidine) activity. Incubation with maraviroc led to dilation of isolated canine venous (but not arterial) tissue that had been pre-constricted with the adrenergic agonist phenylephrine, but was ineffective on tissue pre-constricted with the physiological agonist potassium chloride (CG/004/04 and CG/006/04). In addition, maraviroc was shown to be a weak competitive antagonist at alpha adrenergic receptors expressed in canine saphenous vein (CG/008/04 and CG/009/04). However, in an equivalent human venous preparation (CG/011/04) maraviroc produced no greater effect than vehicle treatment. The observations of binding to human α_{2A} adrenergic receptors and vasodilatation/adrenergic antagonism in canine venous preparations, at concentrations that lead to postural hypotension in clinical studies, may provide a mechanistic explanation for the clinical outcome. However, this is not fully supported by other in vitro rat or human data.

The potential for maraviroc to influence vascular tone and thereby produce symptoms of orthostatic hypotension via a CCR5 mediated mechanism was investigated in human vasculature (DI/073/06). The CCR5 chemokine MIP-1β, at concentrations up to 0.1 μM, produced a concentration dependent vasoconstriction of isolated, endothelium-denuded human saphenous vein tissues with an apparent pEC_{50} of 7.71 ± 0.17 (n=10 donors, 13 observations). Pre-incubation of the tissue with 300 nM maraviroc for 30 min had no effect on basal tension. However, in the presence of 300 nM maraviroc, MIP-1β failed to cause contraction of human isolated saphenous veins. Therefore MIP-1β can act as a potent, low efficacy vasoconstrictor of human venous tissue and this contractile response can be antagonised by 300 nM maraviroc; this is suggestive of a functional role for the CCR5 ligand-receptor system in vascular function in human saphenous veins.

Table 13. Summary of in Vitro Adrenergic Profile for Maraviroc

Experiment	Species (N)	Concentration	Standard	Result/Observation
[3H]UK-14,304 binding to recombinant α2A receptors [a]	Human (2)	0.3 nM to 10 μM (0.15 ng/mL to 5.14 μg/mL)	UK-14,304	10, 100 nM, & 1 μM: no effect on the Ki of UK-14,304 for α2A receptors. 10 μM: 37.5% inhibition of [3H]UK-14,304 binding.
[3H]UK-14,304 binding to recombinant α2A receptors [b]	Human (3-12)	3 nM to 100 μM (1.54 ng/mL to 51.4 μg/mL)	UK-14,304	1 μM: Statistically significant ($p<0.01$) shift in Kd value of [3H]UK-14,304 from 0.64 nM (95% CI, 0.49 to 0.84 nM) to 1.06 nM (95% CI, 0.71 to 1.57 nM). Ki = 8.04 μM (95% CI, 4.2 to 15.2 μM).
cAMP production in α2A recombinant cells [c]	Human (3-4)	1 nM to 100 μM (0.51 ng/mL to 51.4 μg/mL)	Noradrenaline yohimbine	No effect
Calcium release from recombinant α2A, α2B and α2C receptors [d]	Human (4)	1 nM to 100 μM (0.51 ng/mL to 51.4 μg/mL)	BRL44408 PF-266,414	No effect
Electrically stimulated vas deferens [e]	Rat (3-12)	0.1 nM to 300 μM (0.051 ng/mL to 154 μg/mL)	UK-14,304 / yohimbine	No direct effect on contraction. Statistically significant ($p<0.05$) potentiation of UK-14,304 potency at 10 and 100 nM only.
Electrically stimulated vas deferens [f]	Rat (6-9)	10 nM, 100nM & 1 μM (5.14, 51.4, & 514 ng/mL)	clonidine / yohimbine	Statistically significant ($p<0.05$) decrease in clonidine potency at 10 nM and 1 μM only.
Isolated carotid artery & saphenous vein [g]	Dog (3-11)	1 nM to 100 μM (0.51 ng/mL to 51.4 μg/mL)	Phenylephrine / SNAP	10 μM: No direct effect on basal tone in either vessel. Carotid artery: No effect on phenylephrine pre-constricted vessels. Saphenous vein: vasodilatation with threshold effect at 3 μM and Emax = 83% (95% CI, 63 to 103%).
Isolated saphenous vein – KCl pre-constriction [h]	Dog (6)	10 nM to 100 μM (5.14 ng/mL to 51.4 μg/mL)	KCl / SNAP	No effect on KCl pre-constricted vessels.
Isolated saphenous vein – effect on phenylephrine concentration response curve [i]	Dog (5)	1,10, and 30 μM (0.51, 5.14, and 15.4 μg/mL)		pKb = 5.72 (95% CI, 5.24 to 6.21 nM) (2.92 ng/mL) with no suppression of the phenylephrine Emax.
Isolated saphenous vein – effect on noradrenaline concentration response curve [j]	Dog (5)	1,10, and 30 μM (0.51, 5.14, and 15.4 μg/mL)		pKb = 5.21 (95% CI, 4.85 to 5.56 nM) (2.66 ng/mL) with no suppression of the noradrenaline Emax.
Isolated saphenous vein – phenylephrine pre-constriction [k]	Human (3)	1 nM to 100 μM (0.51 ng/mL to 51.4 μg/mL)	SNAP	No effect in comparison with vehicle.

SNAP = (S)-Nitroso-N-acetylpenicillamine. Study Codes: [a]CG/003/04, [b]CG/007/04, [c]CG/002/05, [d]CG/004/05, [e]CG/014/02; [f]CG/004/03; [g]CG/004/04; [h]CG/006/04; [i]CG/008/04; [j]CG/009/04; [k]CG/011/04

2.6.2.4.2. Effects on Central and Peripheral Nervous System

Following oral administration of maraviroc at 1000 mg/kg to conscious male rats, mild effects on appearance and behaviour were observed when compared with vehicle-treated animals (CG/009/00). At 2000 mg/kg adverse effects were observed in the form of piloerection, salivation, and breathing changes (increased, irregular and laboured). Posture, skin colour, grip strength, and core temperature were normal throughout the experiment. Body weights were not consistently affected by maraviroc. The peak unbound C_{max} of maraviroc following the maximum tolerated dose of 1000 mg/kg was 5 µM (2543 ng/mL) at 5 hours after dosing indicating that concentrations of approximately 17-fold the mean unbound C_{max} in HIV-1-positive patients at a dose of 300 mg BID did not induce marked effects on appearance and behaviour of rats.

Maraviroc up to 10 mg/kg p.o. had no effect on sodium pentobarbitone induced sleeping times or motor coordination in male mice (CG/005/01 & CG/002/01).

2.6.2.4.3. Effects on Cardiovascular System

2.6.2.4.3.1. Freely-moving dog

Vehicle and maraviroc at dose levels of 0.05, 0.15, 0.5 and 1.5 mg/kg, were administered orally by gavage to 4 conscious, freely moving dogs, on separate occasions, to study the effects upon haemodynamic parameters and the electrocardiogram (ECG) using telemetry techniques, which included a continuous measure of the animal's activity (Table 12, CG/003/00 and CG/008/00).

Vehicle treatment was not associated with any biologically relevant haemodynamic or ECG effects.

Maraviroc, at 0.05 to 1.5 mg/kg PO (N = 4) caused no biologically relevant, or statistically significant, haemodynamic or ECG changes in conscious freely moving dogs. The unbound C_{max} at 1.5 mg/kg was 168 nM (86 ng/mL) indicating that concentrations approximately 0.6-fold the mean unbound C_{max} in HIV-positive patients at a dose of 300 mg BID caused no meaningful effects upon haemodynamic or ECG parameters. The parameters were investigated further in the conscious dog at higher doses of maraviroc, as discussed in Section 2.6.2.4.3.2.

2.6.2.4.3.2. Restrained dog

To investigate potential cardiovascular effects of maraviric at higher exposures and to attempt to understand the mechanism responsible for maraviroc induced postural hypotension as observed in clinical studies A4001001 and A4001002 (Module 2.7.4, Section 2.7.4.2.1.6.2.1. Adverse Events Relating to Postural hypotension), the effect of intravenously administered maraviroc on blood pressure, heart rate, and ECG was studied in the dog (CG/007/02). This model has been shown to be sensitive to α adrenoceptor antagonists, which cause symptoms of hypotension in humans (Baum et al, 1981). Increasing the plasma concentration of α adrenoceptor antagonists in the dog results in a dose-related compensatory

tachycardia, both initially during the change to an upright posture and thereafter, to maintain blood pressure at normal levels during postural challenge. When vasodilatation produced by this class of compounds prevents adequate cardiac output, the compensatory tachycardia is not maintained, and severe decreases in heart rate and blood pressure occur.
In this study, with the dogs settled in the lying position, administration of maraviroc (at mean unbound plasma concentrations in the range of 779 nM, 399 ng/mL to 1823 nM, 935 ng/mL) produced small reductions in systolic (-3.3 mmHg), and increases in diastolic blood pressure (+1.9 mmHg), relative to vehicle which resulted in a slight, but statistically significant ($p<0.05$) reduction in pulse pressure, compared with vehicle. Maraviroc administration produced no effects on basal HR compared with vehicle. Maraviroc concentrations of approximately 3-6-fold the mean unbound C_{max} in HIV-positive patients at a dose of 300 mg BID did not cause any meaningful effects upon basal haemodynamic parameters.

During postural challenge, maraviroc (at the concentrations given above) produced effects on the initial response to change to the upright position. Thus the initial heart rate increase was less, and the initial fall in systolic blood pressure was greater, at each postural challenge during administration of maraviroc, than during vehicle administration. This effect resolved within less than 1 minute. This suggests that maraviroc produces some impairment of normal reflex control of blood pressure during the change to the upright position in the dog. However, once the upright position had been established, blood pressure control was maintained at a normal level and therefore the compound did not produce obvious postural hypotension in the conscious dog. However, the effects on the initial reflex response may be sufficient to cause symptoms of postural dizziness in humans.

When compared with vehicle, maraviroc administration caused no biologically relevant effects on basal ECG parameters, except for a statistically significant 14.5 msec prolongation of the ECG QT interval, in the absence of an effect on heart rate (51.4 bpm in vehicle compared with 51.8 bpm for maraviroc-treated animals). The greatest increase in QT interval compared with vehicle of 23.1 msec occurred at an unbound plasma concentration of maraviroc of 1310 nM (674 ng/mL).

The mean unbound plasma concentration of maraviroc in this study was 1227 nM (631 ng/mL).

2.6.2.4.3.3. In vitro studies of the effect of maraviroc on cardiac ion channel activity

Maraviroc has been examined in a range of in vitro tests designed to assess its potential to cause increases in the QT interval of the ECG. The hERG protein is known to form the channel underlying the rapidly activating delayed rectifier current I_{Kr} (Sanguinetti, 1995). Agents that block hERG delay repolarization, and thereby prolong the action potential, which can lead to arrhythmias and the potentially fatal clinical outcome, Torsades Des Pointes (Fermini and Fossa, 2003). Maraviroc was tested in assays of dofetilide binding to, and current flow through, hERG channels expressed in cultured human HEK-293 cells. Maraviroc was also tested on canine Purkinje tissue. The Purkinje fibre preparation is particularly sensitive to agents known to prolong the electrocardiogram QT interval in animals and man and is recommended as the in vitro preparation of choice by the European Medicines Evaluation Agency to determine the potential of compounds to prolong the QT interval in man. The procedure used conformed to that described in a points to consider document from the Committee for Proprietary Medicinal Products (CPMP) on 'The assessment of the potential for QT interval prolongation by non-cardiovascular medicinal products' (CPMP, 1997).

2.6.2.4.3.3.1. Effect of maraviroc on dofetilide binding to hERG

Maraviroc, at 1 μM (514 ng/mL), did not inhibit binding of [^{3}H]dofetilide to the hERG channel. However, at 3 and 10 μM (1541 and 5137 ng/mL) there was a concentration-dependent inhibition of [^{3}H]dofetilide binding (15 and 43% inhibition respectively, Table 12, IC/001/02).

2.6.2.4.3.3.2. Effect of maraviroc on hERG potassium channels

hERG currents were studied using standard patch clamp techniques in the whole-cell recording mode and a standard voltage protocol was initiated to evoke membrane currents. This protocol is designed to activate the hERG channel and allow the measurement of drug interaction at various voltages.

Maraviroc, at 3 and 10 μM (1541 and 5137 ng/mL) produced a concentration-dependent inhibition of hERG potassium current (6 and 19% inhibition respectively, Table 12, CG/001/00 & IC/005/02).

2.6.2.4.3.3.3. Effect of maraviroc on dog isolated Purkinje fibres

The action of maraviroc was assessed on the electrophysiology of canine isolated Purkinje cells.

Maraviroc, at 1 μM (514 ng/mL), was devoid of effects on the canine cardiac action potential morphology. However, at 3 and 10 μM (1541 and 5137 ng/mL) there was concentration-dependent prolongation of the action potential duration at both 50% (9% and 26% prolongation respectively) and 90% (10% and 31% prolongation respectively) of repolarisation (Table 14, CG/002/00, IC/006/01 & IC/003/02).

2.6.2.4.3.3.4. Summary of cardiac ion channel in vitro studies

These results show that maraviroc is active at the human cardiac hERG channel and suggest that the compound has the potential to block the I_{Kr} current and affect cardiac repolarisation in vivo at unbound plasma concentrations greater than 3 μM (1541 ng/mL, approximately 10-fold the mean unbound C_{max} in HIV-positive patients at a dose of 300 mg BID).

2.6.2.4.3.4. Summary of in vitro and in vivo cardiovascular studies

Maraviroc, at mean unbound plasma concentrations in the range 779 to 1823 nM (399 to 935 ng/mL, based on plasma protein binding of 63.7% in dogs) produced only subtle changes to control of blood pressure during postural challenge in the conscious dog. Small reductions in systolic and increases in diastolic blood pressures resulted in a slight, but statistically significant ($p<0.05$), reduction in pulse pressure compared with vehicle. Maraviroc administration produced no effects on basal HR compared with vehicle. It is unlikely that the effects of maraviroc on basal haemodynamics are of biological importance at this concentration range.

In vitro studies suggest that, in the dog at least, the minor and transient effect on the ability of the dog to maintain blood pressure and heart rate during postural challenge could be accounted for by antagonism of the alpha-adrenergic receptor. However, although there is evidence for binding of maraviroc to the human $\alpha 2_A$ adrenergic receptor this does not appear to translate to functional effects at a cellular or tissue level and cannot definitively be claimed as the mechanism for the clinically observed incidence of orthostatic hypotension. The affinity of maraviroc for a range of receptors responsible for binding vasoactive peptides was assessed as a potential mechanism for postural hypotension. These included receptors for angiotensin-II, atrial naturietic peptide, bradykinin-2, endothelins A and B, ghrelin, thromboxane, urotensin-1 and vasopressin-1A, with no evidence of any significant affinity found (CG/003/05). Similarly, there was no evidence for inhibition of angiotensin converting enzyme or phosphodiesterases (III, IV and V) and no stimulation of guanylyl cyclase (CG/001/04 & CG/005/05), which indicates that maraviroc does not affect the processing of angiotensin or cyclic nucleotides hence excluding these as hypotensive mechanisms.

An alternative hypothesis for the mechanism of clinically observed postural hypotension is the potential for CCR5 mediated effects on vascular tone. In support of this hypothesis it has been shown that the endogenous CCR5 agonist MIP-1β produces in vitro contraction of human isolated saphenous vein, which can be blocked by maraviroc (DI/073/06). An argument against this hypothesis is that there have been no reports of postural hypotension in clinical trials of CCR5 antagonists from other companies; however other non-specific effects may have limited the dose escalation for these compounds. It remains plausible that maraviroc could produce postural hypotensive symptoms mediated by antagonism of CCR5 where there is an underlying and perhaps localised vasoconstriction maintained to some degree by MIP-1β, or other endogenous CCR5 agonist.

Nonclinical in vitro effects of maraviroc on the hERG channel are summarized in Table 14. The threshold for inhibition of hERG is approximately 3 μM (1541 ng/mL) and the IC_{50} for this effect is at least >10 μM (5140 ng/mL). The clinically relevant mean unbound Cmax derived from study A4001007 at a dose of 300 mg BID is 300 nM (155 ng/mL). From these observations a value for the ratio hERG IC_{50}/free C_{max} of at least 33 can be calculated. According to Redfern et al (2003) 'A 30-fold margin between C_{max} and hERG IC_{50} may suffice for drugs currently undergoing clinical evaluation …'. Therefore, whilst it is recognised that at high concentrations maraviroc will interact with hERG and prolong the QT interval of ECG, the in vitro data predict an adequate exposure multiple.

Table 14. Maraviroc activity at cardiac ion channels

Assay	Activity at 1 μM	Activity at 3 μM	Activity at 10 μM
hERG dofetilide binding [a]	No Effect	15% inhibition	43% inhibition
hERG current (patch clamp) [bc]	No Effect	5.7% inhibition	19% inhibition
Purkinje Fibre [def]	No Effect	APD_{50} +9% APD_{90} +10%	APD_{50} +26% APD_{90} +31%

hERG, Human Ether-a-go-go Related Gene; APD, Action Potential Duration.
Study Codes: [a]IC/001/02; [b]CG/001/00; [c]IC/005/02; [d]CG/002/00; [e]IC/006/01; [f]IC/003/02

Maraviroc demonstrated less than 20% inhibition of L-type calcium channels at 10 μM (CG/013/00). Therefore, unlike the calcium channel blocker verapamil and agents such as amiodarone, sertindole and ranolazine that display electrophysiological polypharmacology, maraviroc is not expected to have a complex effect on torsadogenic potential. The behaviour of maraviroc in the Purkinje fibre assay is consistent with an agent that displays relatively straightforward I_{Kr} block. Moreover, in this model maraviroc did not cause early afterdepolarisations that have been linked to an increased risk for arrhythmogenicity (Hondeghem et al, 2001). A variety of other in vitro studies have been proposed in the literature to assess torsadogenic risk, including triangulation analyses (Hondeghem et al, 2001; Hondeghem, 2006). However, these in vitro models of arrhythmia have yet to be fully validated and the existing literature does not support a predictive methodology for assessing risk (Steidl-Nichols et al, submitted).

There is a good correspondence between the in vitro and in vivo effects of maraviroc on parameters associated with cardiac repolarization suggesting that inhibition of I_{Kr} is the likely mechanism of the QT interval changes observed in vivo. The weight of evidence from all the non-clinical assays suggests that unbound plasma concentrations in the region of 1-2 μM (514-1028 ng/mL) are necessary to consistently observe a modest effect on cardiac repolarization. A formal Phase 1 QT study (A4001016, Module 5.3.4.1 Healthy Subject PD and PK/PD Study Reports) did not show evidence of clinically significant QT interval prolongation at the doses studied (<4 msec increase vs placebo at single doses up to 900 mg, corresponding to 3 times the 300 mg unit dose used in the Phase 2b/3 studies, and achieving approximately 2-fold the mean unbound Cmax seen in HIV-positive patients receiving 300 mg BID in study A4001007 (Module 5.3.4.2 Patient PD and PK/PD Study Reports), i.e. approximately 600 nM, 300 ng/mL unbound compound). This indicates that QT prolongation in humans is not anticipated at concentrations expected in clinical use.

2.6.2.4.4. Effects on the Respiratory System

The effect of a single intravenous dose of maraviroc (1 mg/kg), on arterial blood pH, pCO_2 and pO_2 was investigated in urethane anaesthetized male rats. Whereas the control agent, morphine (4mg/kg i.v.) produced statistically significant reductions ($p<0.05$) in arterial blood pH and pO_2 and increases in pCO_2 compared with vehicle treated animals, maraviroc had no significant effect on any blood gas parameter in the rat (Table 12, CG/001/01).

2.6.2.4.5. Effects on the Renal System – Excretion of Fluid and Electrolytes

The effects of orally administered maraviroc (10, 20 and 60mg/kg) were investigated on the excretion of urine and electrolytes over a five hour period after dosing in conscious, normotensive female Sprague Dawley strain rats, given an oral saline load. Whereas the control agent, frusemide (20mg/kg p.o.) produced pronounced diuresis and increased ion excretion, maraviroc did not produce any significant differences in urine volume, pH or electrolyte excretion compared to vehicle treated animals (Table 12, CG/004/01).

2.6.2.4.6. Metabolite Affinity for Other Receptors, Enzymes and Ion Channels

The principal metabolite of maraviroc found in human plasma is a secondary amine product, UK-408,027 that accounted for 22% of the radioactivity in a metabolism study; the parent compound, maraviroc, constituted 42% of plasma radioactivity (Pharmacokinetics Written Summary, Section 2.6.4.5.1.13). In addition, a hydroxylated metabolite (11%) that arose by further metabolism of the amine and a carboxylic acid metabolite UK-463,977 (5%) were found to be present in human plasma. All of the human metabolites are observed in the toxicology species.

The pharmacologic profile of UK-408,027 against a range of 74 physiologic receptors, ion channels and enzymes was assessed (UK408027/CG/003/04). The only activities greater than 20% inhibition at 10 μM (4065 ng/mL) were against the recombinant human endothelin E_{TB}, $5HT_{2C}$ and μ opioid receptors (21, 29, and 34% respectively). Because UK-408,027 was present in human plasma at about 50% relative to the parent concentration it is unlikely that the observed activities will be of biological relevance.

Similarly, the only activity of note for UK-463,977 at 10 μM (3,394 ng/mL) was 53% inhibition of the μ opioid receptor (UK463977/CG/001/04). Because UK-463,977 was present in human plasma at about 12% relative to the parent concentration it is unlikely that the observed activities will be of biological relevance.

In the light of the alpha adrenergic affinity of maraviroc, and potential role of adrenergic receptors in postural hypotensive outcomes, the metabolites UK-408,027 and UK-463,977 were assessed for their ability to interact with α2 adrenergic receptors in an electrically stimulated rat vas deferens preparation (UK408027/CG/004/04). Neither compound had any direct effect on the tissue at concentrations up to 10 μM. However, there was a statistically significant ($p<0.05$) increase in potency of the α2 adrenergic agonist, UK-14,304 in the presence of either compound at 1 μM and of UK-408,027 at 100 nM. Since this increase was less than 2-fold it is unlikely to be biologically relevant.

2.6.2.4.7. Safety Pharmacology Summary

Results from studies examining the general pharmacologic properties of maraviroc indicate that maraviroc was well tolerated and had little interaction with physiologically important receptors, binding sites, enzymes, or ion channels apart from weak functional activity at the human μ opioid receptor and a moderate affinity for the human α_{2A} adrenergic receptor. Weak μ opioid functional activity occurs at concentrations approximately 33-fold the mean unbound C_{max} in HIV-positive patients at a dose of 300 mg BID, and it is therefore unlikely that this will be of biological significance. Maraviroc was shown to be a weak functional competitive antagonist at alpha adrenergic receptors expressed in canine saphenous vein. This activity may result in vasodilatation at concentrations that lead to postural hypotension in clinical studies; however this result could not be replicated in human vascular tissue. There were no effects on the central or peripheral nervous, renal, and respiratory systems at exposures that are relevant to the intended use. Results from dog isolated Purkinje fibre and hERG channel studies suggest that maraviroc has the potential to block the I_{Kr} current and affect cardiac repolarisation in vivo at unbound plasma concentrations greater than 3 μM (1541 ng/mL), which is approximately 10-fold the mean unbound C_{max} in HIV-positive patients at a dose of 300 mg BID.

2.6.2.5. Pharmacodynamic Drug Interactions

The in vitro antiviral activity of maraviroc was evaluated in combination with standard and developmental antiviral agents. An initial study was conducted to investigate the potential for interactions between maraviroc and an example of the NNRTI, NRTI, and PI drug classes in acute HIV-1 Ba-L-infected PM-1 cells in vitro (Table 14, DI/005/3). Maraviroc had slightly synergistic interaction with efavirenz and nelfinavir while displaying an additive interaction with lamivudine. The observed synergies were independent of drug concentration.

An additional study was then carried out to assess the interaction of maraviroc with a wider range of licensed antiviral drugs including fusion inhibitors, both marketed and currently in development. In total the antiviral activity of maraviroc was assessed in combination with 18 approved anti-HIV agents, in addition to three alternative CCR5 antagonists that have been, or still are, in development (Table 14, DI/002/04).

Additive antiviral interactions were observed for all compounds in combination with maraviroc, with the exception of the protease inhibitors atazanavir and indinavir, and the entry inhibitor enfuvirtide, where synergy was observed in one experiment and additivity in a second experiment. Minor synergy was observed with maraviroc in combination with the CCR5 antagonist SCH-D (Schering) in two out of three experiments and additivity in the third experiment. Maraviroc displayed additive drug interactions with two other CCR5 antagonists, SCH-C (Schering) and [redacted] a [redacted] compound in [redacted]

There was no evidence of antagonistic interaction with any of the compounds investigated. In addition, there was no evidence of synergistic cytotoxicity at the highest drug concentration tested (40 nM, 21 ng/mL, vs lamivudine, efavirenz and nelfinavir or 2 nM, 1 ng/mL, vs the remaining agents shown in Table 14).

Table 14. Antiviral Effects of Maraviroc in Combination with Different Classes of Antiviral Agent

Maraviroc in combination with:	N	Volume (μM^2 %) Synergy	Antagonism	Combined Effect
Lamivudine (NRTI) [a]	2	41	-34	Additive
Efavirenz (NNRTI) [a]	2	63	-18	Minor Synergy
Nelfinavir (PI) [a]	2	74	-23	Minor Synergy
Abacavir (NRTI) [b]	2	10.4±14.6	-0.2±0.3	Additive
Didanosine (NRTI) [b]	2	4.0±4.6	-0.9±1.3	Additive
Emtricitabine (NRTI) [b]	2	0.1±0.2	-1.2±1.7	Additive
Stavudine (NRTI) [b]	2	1.6±1.5	0.0	Additive
Tenofovir (NRTI) [b]	(3)	5.2±7.2	-8.0±13.9	Additive
Zalcitabine (NRTI) [b]	2	0.0	-4.3±1.2	Additive
Zidovudine (NRTI) [b]	2	0.1±0.1	-0.1±0.1	Additive
Delavirdine (NNRTI) [b]	2	7.1±3.4	-0.2±0.3	Additive
Efavirenz (NNRTI) [b]	(3)	0.0±0	-4.1±4.5	Additive
Nevirapine (NNRTI) [b]	2	5.7±8.0	-3.2±2.8	Additive
Amprenavir (PI) [b]	2	3.5±2.4	-1.6±1.4	Additive
Atazanavir (PI) [b]	2	35.4±43.2[d]	-0.2±0.3[d]	Additive/ Synergy
Indinavir (PI) [b]	2	19.6±27.7[e]	-6.5±4.0[e]	Additive/Synergy
Lopinavir (PI) [b]	2	12.5±17.6	-11.0±15.2	Additive
Ritonavir (PI) [b]	2	0.0	-0.3±0.4	Additive
Saquinavir (PI) [b]	(3)	10.7±12.6	-1.0±0.2	Additive
Enfuvirtide (Fusion) [b]	(2), 1	16.1±16.8[f], 11.2[c]	-1.3±1.8[f], -0.0[c]	Additive/Synergy
SCH-C (Entry R5) [b]	2	1.2±0.6	-7.2±0.8	Additive
SCH-D (Entry R5) [b]	3	40.0±40.8[g]	-3.8±5.6[g]	Additive/ Synergy
[redacted] (Entry R5) [b]	2	2.8±4.0	-4.0±4.2	Additive

NRTI = Nucleoside reverse transcriptase inhibitor, NNRTI = Non-nucleoside reverse transcriptase inhibitor, PI = Protease Inhibitor

[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 μM^2%, minor synergistic activity and highly synergistic activity are defined as yielding values of 50-100 μM^2%and > 100 μM^2% respectively. Additive drug interactions have values ranging between –50 μM^2% and 50 μM^2% while values <-50 μM^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 μM^2% indicate additive response; values >25 and <50 μM^2% indicate minor synergy /antagonism; values >50 and <100 μM^2% indicate moderate synergy/antagonism; and values >100 μM^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

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

2.6.2.6. Discussion and Conclusions

Maraviroc binds to human CCR5 (dissociation constant, K_D = 0.86 nM) and blocks the interaction of viral envelope protein gp120 with the receptor and thus inhibits fusion of the viral and cellular membranes and consequently blocks viral entry to the cell. Maraviroc therefore has an extracellular site of action. Maraviroc has been shown to be a broad-spectrum anti-HIV-1 agent with antiviral activity against cross-clade primary isolates of CCR5-tropic HIV-1 (unbound geometric mean IC_{90} = 1.0 nM, 0.5 ng/mL) and demonstrates activity against recombinant viruses derived from clinical isolates that have genotypic evidence of resistance to the protease inhibitor and reverse transcriptase inhibitor agents (geometric mean IC_{50} fold change = 0.60 for wild type viruses vs 0.79 for drug-resistant viruses). This activity occurred in the absence of cytotoxicity or receptor internalization and there was no evidence of antagonism of the antiviral activity of other HIV antiviral agents.

Binding, site directed mutagenesis and computer modeling studies indicate that maraviroc interacts with the transmembrane domain of CCR5, having a strong ionic interaction with glutamic acid E283 and strong hydrophobic interaction with tyrosine Y108 in the binding pocket. These interactions contribute to a prolonged CCR5 physical and functional occupancy at the receptor (dissociation $t_{½}$ (off) of approximately 16 hours). Watson et al. (2005) previously predicted a value of 136 hours. However, this finding was based upon an indirect measure of dissociation by following the association of $[^{125}I]$-MIP-1α to CCR5 at 4 °C after pre-treatment with maraviroc. The 16 hour receptor binding half life reported here is derived from an assessment of tritiated maraviroc binding to human CCR5 at room temperature and is more likely to represent the true physiological value.

Maraviroc blocks chemokine binding and CCR5-mediated signaling, as was demonstrated in Ca^{2+} mobilization and cAMP turnover assays. Although maraviroc behaves as a functional antagonist of CCR5 mediated cellular signaling, this is not the mechanism responsible for antiviral activity.

One of the attractive properties of a new class of inhibitors is the expectation that they will be effective against HIV-1 strains regardless of the previous drug experience of the patient. Consistent with this, maraviroc has been shown to be active against R5 virus strains isolated from patients with experience of one or more of the pre-existing classes of therapeutic agent as well as against virus from drug-naive subjects. Furthermore, there was a tight distribution of responses to the 200 viruses tested. The magnitude of the decrease in sensitivity of viruses resistant to other classes of antiviral agent compared with drug sensitive viruses was 1.7-fold, which is less than the expected assay-to-assay variation.

The analysis of antiviral activity has also been extended to strains from diverse geographic origins. HIV-1 is comprised of three subgroups based upon phylogenetic clustering, of which the M ("Main") group is responsible for the majority of global infections. This is further divided into nine recognized clades, clustering into independent branches based upon *env* amino acid sequences. While clade B is responsible for the majority of infections in the Western world, viruses from other clades predominantly cause the epidemic (clades A and C in particular; see UNAIDS 2002 AIDS epidemic update), and global spread of these other clades is increasing (Perrin et al, 2003). Encouragingly, maraviroc had potent cross-clade activity against all CCR5-tropic HIV-1 primary isolates tested, with a less than 10-fold difference between the most- and least-susceptible clades and a less than 30-fold difference between the most- and least-susceptible individual isolates (among more than 40 isolates tested). In particular, the two clade G strains tested are within threefold of the geometric mean IC_{90} for all primary isolates, with the RU570 isolate falling within six fold.

In general pharmacology studies maraviroc was well tolerated. The compound showed weak agonist activity at the human μ opioid receptor. However there is little evidence that this in vitro finding has translated to observed effects in general pharmacology, toxicology or clinical studies. The expected actions of a μ opioid agonist would include analgesia, respiratory depression, euphoria, pupil constriction, reduced GI motility, sedation and physical dependence. In the rat acute appearance and behaviour study (CG/009/00) increased respiratory rate was observed at a dose that achieved an unbound C_{max} of 5 μM (2543 ng/mL) and in a respiratory study (CG/001/01) pO_2 and pCO_2 were unaffected at an intravenous dose of 1 mg/kg. These data suggest that there were no signs of respiratory depression in these animals. In clinical studies there has been no evidence of abuse potential for maraviroc (see Summary of Clinical Safety, Section 2.7.4.5.6).

Maraviroc is a weak inhibitor of agonist binding to the human α_{2A} adrenergic receptor and is an antagonist at alpha adrenergic receptors in canine venous tissue giving rise, in vitro, to vasodilatation. Since postural hypotension has been observed in the clinic at doses of 1200 mg (Study A4001001, following a single-oral dose) and 600 mg (Study A4001002, on Day 7 of multiple oral doses QD) and drug induced orthostatic hypotension has been associated with adrenergic blockade (Schoenberger, 1991; Hugues et al, 1992; Mathias and Kimber, 1999; Meredith, 2001) an extensive profile of the interaction of maraviroc with these receptors was carried out. However in a variety of other preparations, including rat vas deferens, recombinant human adrenergic receptor cell lines and isolated human saphenous vein, there was no consistent adrenergic mediated activity. The observations of binding to human α_{2A} adrenergic receptors and vasodilatation/adrenergic antagonism in canine venous preparations, at concentrations that lead to postural hypotension in clinical studies, may provide a mechanistic explanation for the clinical outcome. However, this is not fully supported by in vitro rat or human data.

Results from hERG channel and dog isolated Purkinje fibre studies suggest that maraviroc has the potential to block the I_{Kr} current and affect cardiac repolarisation in vivo at unbound plasma concentrations greater than 3 μM (1541 ng/mL). hERG channel block and consequent potential for prolongation of the QT interval of the ECG was identified as an issue with examples of CCR5 antagonists such as SCH-C (SCH-351,125; Este, 2002), some analogues in the Merck series (Shu et al, 2004) and early examples in our own synthetic programme (Wood and Armour, 2005). The maraviroc nonclinical data however, indicates a margin of approximately 10-fold versus the mean unbound C_{max} in HIV-positive patients at a dose of 300 mg BID and therefore careful monitoring of ECG parameters was advised during early clinical trials. A single-dose clinical QT study was conducted and did not show evidence of clinically significant QT interval prolongation up to a dose of 900mg (Study A4001016, Module 5.3.4.1 Healthy Subject PD and PK/PD Study Reports).

Antiviral combination studies in vitro demonstrate that maraviroc is not antagonistic to existing antiretroviral agents. Since current HIV-1 treatment regimens use combinations of agents to minimize the emergence of resistance this data suggests that maraviroc will not adversely affect the efficacy of agents with which it may be co-administered. Moreover, clinical studies reveal a drug-drug interaction profile commensurate with a convenient dosing regime, without affecting the pharmacokinetics of potential co-medications (Abel et al 2005).

In conclusion, nonclinical pharmacology data suggest that maraviroc is a potent inhibitor of replication of a range of clinically relevant HIV-1 clades. It is active against viral isolates that show evidence of resistance to other classes of HIV-1 medications. The compound acts by binding to CCR5 and blocking attachment and subsequent entry of the virus to host cells. Maraviroc binds selectively to CCR5 and has minimal effect on in vitro immune system functions. Results from a wide range of general pharmacology studies suggest that the compound will be well tolerated and, in addition, will have minimal effect of the efficacy of currently administered HIV drugs.

2.6.2.7. Tables and Figures

No Further Tables or Figures

2.6.2.8. References

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2.6.3.1. PHARMACOLOGY Overview

Test Article: Maraviroc

Type of Study	Test System	Method of Admin.	Testing Facility	Study Number	Location Module	Location Volume
Primary Pharmacodynamics Related to the Antiviral Mode of Action						
In vitro receptor binding	[^{3}H]maraviroc binding to human recombinant CCR5 in HEK-293, HeLa P4 and CHO-K1 cells/cell membranes.	In vitro	[redacted] UK	DI/012/1, CG/015/02, (DI/011/1), (DI/013/1)	4.2	
Structural characterisation of the CCR5 binding site	CRE-Luc reporter assay on mutant variants of human recombinant CCR5 in HEK-293 cells	In vitro	[redacted] UK	DI/102/05	4.2	
Mode of action	Human recombinant CCR5 receptor binding in HEK-293 Cells	In vitro	[redacted] UK	DI/002/1, DI/003/1	4.2	
	Human recombinant CCR5 receptor induced calcium fluxes in HEK-293 cells	In vitro	[redacted] UK	DI/005/1, DI/056/04	4.2	
Inhibition of viral protein attachment and fusion	gp120 binding to recombinant human CCR5 in HEK-293 cells.	In vitro	[redacted] UK	DI/006/1, DI/005/04, DI/128/04	4.2	
	HIV-1 envelope protein cell-cell fusion	In vitro	[redacted] UK	DI/007/1,	4.2	
In vitro antiviral activity	HIV-1 Ba-L in PM-1 cells	In vitro	[redacted] UK	DI/019/1	4.2	
	HIV-1 Ba-L in PBL – pooled donors	In vitro	[redacted] UK	DI/019/1	4.2	
	HIV-1 Ba-L in PBL – single donors	In vitro	[redacted] UK	DI/019/1	4.2	
	HIV-1 primary isolates CXCR4 tropic	In vitro	[redacted] UK	DI/18/01	4.2	
	HIV-1 primary isolates dual tropic	In vitro	[redacted] UK	DI/18/01	4.2	
	HIV-1 primary isolates CCR5 tropic plus Ba-L	In vitro	[redacted] UK	DI/18/01	4.2	
	HIV-1 primary isolates	In vitro	[redacted] US	DI/004/3	4.2	
	HIV-1 recombinant isolates	In vitro	Monogram, US	CG/016/02	4.2	

Tabular Summary: 2.6.3.1 Pharmacology Overview

Type of Study	Test System	Method of Admin.	Testing Facility	Study Number	Location Module	Location Volume
In vitro antiviral activity continued	HIV-1 primary isolates in Macrophages	In vitro	[redacted] UK	DI/006/06	4.2	
	HIV-1 primary isolates (multiplicity of infection)	In vitro	[redacted] UK	DI/051/06	4.2	
Receptor internalisation	Recombinant human CCR5 in mouse pre-B cell line	In vitro	[redacted] UK	(DI/009/03)	4.2	
Plasma Protein Binding	Equilibrium dialysis in the presence of 10% Foetal Calf Serum	In vitro	[redacted] UK	(DI/138/05)	4.2	
Cytotoxicity	Cell proliferation (MTS assay)	In vitro	[redacted] UK	DI/019/1	4.2	
Primary Pharmacodynamics Not Related to the Antiviral Mode of Action						
In vitro receptor binding	(^{125}I)-MIP-1α, -MIP-1β and -RANTES binding to recombinant human CCR5 in HEK-293 cell membrane homogenates	In vitro	[redacted] UK	DI/002/1, CG/005/04, DI/003/1	4.2	
In vitro receptor binding	(^{125}I)-MIP-1β binding to human CCR5 in whole HEK-293 cells and membrane homogenates	In vitro	[redacted] UK	(CG/010/04)	4.2	
Functional receptor activity	Intracellular calcium release	In vitro	[redacted] UK	DI/005/1	4.2	
	Intracellular cAMP levels	In vitro	UK	CG/001/05		
	γ-S-GTP incorporation	In vitro	UK	DI/108/06		
Affinity for Relevant Non-Human Species' CCR5	Mouse CCR5-mediated intracellular calcium release	In vitro	[redacted] UK	DI/092/06	4.2	
	[^{125} I]-MIP-1β binding to rat CCR5	In vitro	[redacted] UK	CG/002/04	4.2	
	[^{125} I]-MIP-1β binding to dog CCR5	In vitro	UK	CG/002/04	4.2	
	[3H]-maraviroc binding to macaque CCR5	In vitro	UK	CG/015/02	4.2	
	[^{125} I]-MIP-1β binding to macaque CCR5	In vitro	[redacted] UK	CG/011/02	4.2	
	Macaque CCR5-mediated intracellular calcium release	In vitro	[redacted] UK	CG/009/02	4.2	
Computational analysis of cross species CCR5 homology	Mouse, rat, rabbit, dog, monkey, human amino acid sequences	N/A	[redacted] UK	DI/100/06	4.2	

Tabular Summary: 2.6.3.1 Pharmacology Overview

Type of Study	Test System	Method of Admin.	Testing Facility	Study Number	Location Module	Location Volume
Secondary Pharmacodynamics						
Functional selectivity assay	MCP-3 induced intracellular Ca_2^+ release in THP-1 cells	In vitro	[redacted] UK	DI/008/1	4.2	
Functional selectivity assay	IL-2 stimulated human T-cell proliferation	In vitro	[redacted] US	DI/020/1	4.2	
Functional selectivity assay	Antigen-stimulated peripheral blood lymphocyte proliferation	In vitro	[redacted] US	DI/021/1	4.2	
Functional selectivity assay	fMLP-stimulated superoxide production in human neutrophils	In vitro	[redacted] UK	DI/022/1	4.2	
Receptor binding selectivity assay	MIP-1α binding to human CCR1 in MIP34.10 cells	In vitro	[redacted] US	DI/023/1	4.2	
Receptor binding selectivity assay	Eotaxin binding to human CCR3 in murine pre-B cell line, 300-19	In vitro	[redacted] US	DI/024/1	4.2	
Functional selectivity assay	IL-4 stimulated IgE synthesis in human peripheral blood lymphocytes	In vitro	[redacted] UK	DI/025/1	4.2	
Functional selectivity assay	LPS-stimulated TNF-α release from THP-1 cells	In vitro	[redacted] UK	DI/026/01	4.2	
Receptor binding selectivity assay	MIP-3β binding to human CCR7 in human T-cell lymphoma H9 cells	In vitro	[redacted] US	DI/027/1	4.2	
Functional selectivity assay	MIP-1α & MCP-1 induced chemotaxis of THP-1 cells	In vitro	[redacted] US	DI/028/1	4.2	
Functional selectivity assay	SDF-1α, ITAC & SLC induced chemotaxis of human T-cell lymphoma H9 cells	In vitro	[redacted] US	DI/028/1	4.2	
Functional selectivity assay	IL-8 & Gro-α induced chemotaxis of human neutrophils	In vitro	[redacted] US	DI/028/1	4.2	
Receptor binding selectivity assay	TARC binding to human CCR4 in murine pre-B cell line, 300-19	In vitro	[redacted] US	DI/029/1	4.2	
Receptor binding selectivity assay	[^{125}I]-I309 binding to human CCR8 human leukaemic T-cells (PEER cells)	In vitro	[redacted] US	DI/030/1	4.2	

Tabular Summary: 2.6.3.1 Pharmacology Overview

Type of Study	Test System	Method of Admin.	Testing Facility	Study Number	Location Module	Location Volume
Safety Pharmacology						
Affinity for a broad range of receptors, enzymes and ion channels	Mouse, Rat, Guinea pig, recombinant Human	In vitro	[redacted] UK & [redacted] France	CG/013/00, CG/13/02, CG/009/01, CG/001/02 CG/001/04, G/003/05, CG/005/05	4.2	
Ligand binding competition assay	Recombinant human µ opioid receptor	In vitro	[redacted] UK	(CG/001/03)	4.2	
Functional µ opioid receptor activity	GTP-γ-S binding to recombinant human µ opioid receptor	In vitro	[redacted] UK	CG/011/01	4.2	
Functional µ opioid mediated tissue activity	Electrically-stimulated guinea pig isolated ileum	In vitro	[redacted] UK	CG/006/00	4.2	
Functional activity in isolated vascular tissue	Dog isolated carotid artery & saphenous vein	In vitro	[redacted] UK	CG/004/04	4.2	
Functional activity in isolated vascular tissue	Dog isolated saphenous vein	In vitro	[redacted] UK	CG/008/04	4.2	
Functional activity in isolated vascular tissue	Dog isolated saphenous vein	In vitro	[redacted] UK	CG/009/04	4.2	
Functional activity in isolated vascular tissue	Dog isolated saphenous vein	In vitro	[redacted] UK	CG/006/04	4.2	
Ligand binding competition assay	Radioligand binding to recombinant human α2A receptors	In vitro	[redacted] UK	CG/003/04	4.2	
Ligand binding competition assay	Radioligand binding to recombinant human α2A receptors	In vitro	[redacted] UK	CG/007/04	4.2	
Functional receptor activity	cAMP production in recombinant human α2A cells	In vitro	[redacted] UK	CG/002/05	4.2	
Functional receptor activity	Calcium release from recombinant human α2A, α2B and α2C cells	In vitro	[redacted] UK	CG/004/05	4.2	
Functional activity in isolated tissue	Electrically stimulated rat isolated vas deferens	In vitro	[redacted] UK	CG/014/02	4.2	
Functional activity in isolated tissue	Electrically stimulated rat isolated vas deferens	In vitro	[redacted] UK	CG/004/03	4.2	
Functional activity in isolated tissue	Human isolated saphenous vein	In vitro	[redacted] UK	CG/011/04	4.2	
Functional activity in isolated tissue	Canine isolated Purkinje fibre	In vitro	[redacted] UK	(CG/002/00)	4.2	

Tabular Summary: 2.6.3.1 Pharmacology Overview

Type of Study	Test System	Method of Admin.	Testing Facility	Study Number	Location Module	Location Volume
Functional activity in isolated tissue	Rat isolated uterus	In vitro	[redacted] UK	CG/007/01	4.2	
Functional activity in isolated tissue	Human isolated saphenous vein	In vitro	[redacted] UK	DI/073/06	4.2	
Effects on Central and Peripheral Nervous System						
Appearance & Behaviour (Maximum tolerated dose)	Rat	PO	[redacted] UK	CG/009/00	4.2	
Sleeping times	Mouse	PO	[redacted] UK	CG/005/01	4.2	
Motor coordination	Mouse	PO	[redacted] UK	CG/002/01	4.2	
Effects on Renal/Urinary System						
Urine volume, pH, electrolytes	Rat	PO	[redacted] UK	CG/004/01	4.2	
Effects on Respiratory System						
Arterial blood pH, pCO_2, pO_2	Rat	IV	[redacted] UK	CG/001/01	4.2	
Effects on cardiovascular system						
Haemodynamic & ECG	Dog	PO	[redacted] UK	CG/003/00, CG/008/00	4.2	
Haemodynamic, ECG & tilt response	Dog	IV	[redacted] UK	CG/007/02	4.2	
hERG dofetilide binding	Recombinant Human in HEK293 cells	In vitro	[redacted] UK	IC/001/02	4.2	
hERG current	Recombinant Human in HEK293 cells	In vitro	[redacted] UK	CG/001/00, IC/005/02	4.2	
Evoked action potentials	Dog isolated Purkinje fibres	In vitro	[redacted] UK	IC/006/01, IC/003/02	4.2	

Tabular Summary: 2.6.3.1 Pharmacology Overview

Type of Study	Test System	Method of Admin.	Testing Facility	Study Number	Location Module	Location Volume
Metabolite studies						
Inhibition of HIV envelope and CCR5-mediated cell-cell fusion by UK-463,977 and UK-408,027	Tat-induced β-galactosidase activity following fusion between HeLaP4 cells (expressing human CCR5, CD4 and b-galactosidase) and CHO cells expressing gp160 along with HIV Tat.	In vitro	[redacted] UK	(UK463977/DI/001/05, UK408027/DI/001/05)	4.2	
Pharmacology of UK-408,027						
Affinity for a broad range of receptors, enzymes and ion channels	Mouse, Rat, Guinea pig, recombinant Human	In vitro	[redacted] France	CG/003/04	4.2	
Effect on isolated smooth muscle	Rat	In vitro	[redacted] UK	UK408027/CG/004/04	4.2	
Pharmacology of UK-463,977						
Affinity for a broad range of receptors, enzymes and ion channels	Mouse, Rat, Guinea pig, recombinant Human	In vitro	[redacted] France	UK463977/CG/001/04	4.2	
Effect on isolated smooth muscle	Rat	In vitro	[redacted] UK	UK408027/CG/004/04	4.2	
Pharmacology Drug Interactions						
Antiviral activity in combination with other agents	HIV-1 Ba-L infected PM-1 cells	In vitro	[redacted] US	(DI/005/3)	4.2	
Antiviral activity in combination with other agents	HIV-1 Ba-L infected PM-1 cells	In vitro	[redacted] UK	DI/002/04	4.2	

Abbreviations: HEK, Human Embryonic Kidney; CRE-Luc, Cyclic Adenosine Monophosphate Response Element – Luciferase; gp, glycoprotein; PBL, Peripheral Blood Lymphocyte; [redacted] MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; MIP, Macrophage Inflammatory Protein; RANTES, Regulated on Activation of Normal T cell Expressed and Secreted; cAMP, Cyclic Adenosine Monophosphate; GTP, Guanosine Triphosphate; MCP - monocyte chemoattractant protein; THP, Human acute monocytic leukaemia cell line; IL, Interleukin; fMLP, formyl-Met-Leu-Phe peptide; IgE, Immunoglobulin E; LPS, Lipopolysaccharide; SDF-1α, Stromal Derived Factor; ITAC, Interferon inducible T-cell And Chemoattractant; SLC, Secondary Lympoid tissue Chemokine; Gro-α, Growth chemokine-α; TARC, Thymus and Activation Regulated Chemokine; I309, endogenous CCR8 chemokine ligand; PEER, human leukaemic T-cell line; PO, Oral; IV, Intravenous; ECG, Electrocardiogram; hERG, Human Ether-a-Go-Go Related Gene

Test Article: Maraviroc

2.6.3.2. Primary Pharmacodynamics

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results	GLP Compliance	Study Number
Receptor Binding					
Saturation of [^{3}H]maraviroc binding (K_D value)	Recombinant human CCR5 in HEK-293 cell membrane preparations (2)	0.002 – 100 nM (0.001 – 51 ng/mL)	K_D (95% CI) = 4.4 nM (3.92 to 5.04) [2.26 ng/mL (2.02 to 2.59)]	No	DI/012/1
Saturation of [^{3}H]maraviroc binding (K_D value)	Recombinant human CCR5 in HEK-293 cell membrane preparations (4)	0.45 - 45 nM (0.23 - 23 nM ng/mL)	K_D (95% CI) = 0.86 nM (0.65 to 1.14) [0.44 ng/mL (0.33 to 0.59)]	No	CG/015/02
[^{3}H]maraviroc binding	Non-recombinant parental HEK-293 cells and membranes & CHO-K1 cell membranes	0.002 – 100 nM (0.001 – 51 ng/mL)	No specific binding	No	DI/011/1
Inhibition of viral protein attachment and fusion					
gp120/CD4 binding to human CCR5	Recombinant human CCR5 expressed in whole HEK-293 cells (8)	0.46 – 1000 nM (0.24 – 514 ng/mL)	Geometric Mean IC_{50} (95% CI) 43.4 nM (31.6 to 59.5) [22.3 ng/mL (16.2 to 30.6)]	No	DI/006/01
gp120/CD4 binding to human CCR5	Recombinant human CCR5 expressed in whole HEK-293 cells (18)	0.78– 400 nM (0.4 – 206 ng/mL)	Geometric Mean IC_{50} (95% CI) 11.0 nM (10.5 to 11.6) [5.7 ng/mL (5.4 to 6.0)]	No	DI/005/04
gp120 binding to human CCR5	Recombinant human CCR5 expressed in whole HEK-293 cells (3)	0.05 – 10 µM ((0.026 – 5140 ng/mL)	Geometric Mean IC50 (95% CI) 8.6 nM (3.4 to 21.9) [4.4 ng/mL (1.7 to 11.3)] for standard protocol. 18.1 nM (11.9 to 27.7) [9.3 ng/mL (6.1 to 14.2)] following 20 hr wash.	No	DI/128/04
HIV-1 envelope protein mediated cell-cell fusion	Recombinant gp160 & Tat in CHO cells with recombinant CCR5 & CD4 in HeLa β-gal -actosidase reporter cells (4)	0.01 – 100 nM (0.005 – 51 ng/mL)	Geometric Mean IC_{50} (95% CI) 0.22 nM (0.13 to 0.39) [0.11 ng/mL (0.07 to 0.20)]	No	DI/007/01

Test Article: Maraviroc

2.6.3.2. Primary Pharmacodynamics

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results	GLP Compliance	Study Number
Kinetic constants for [^{3}H]maraviroc binding to human CCR5 receptors at room temperature	Recombinant human CCR5 in HEK-293 cell membrane preparations (3-5)	0.45 - 45 nM & 0.005 – 100 nM (0.23 - 23 nM ng/mL & 0.003 – 51 ng/mL)	Geometric mean (95% CI) K_{obs} 0.063 (0.044-0.089) $t_{½}$ (on) 11.05 min (7.83-15.59) $t_{½}$ (off) 15.95 h (9.36-27.16) K_{on} 0.010 $min^{-1}.nM^{-1}$ (0.0069-0.015 K_{off} 0.00072 min^{-1} (0.00043-0.0012) K_{off}/K_{on} 0.071 nM (0.055-0.091)	No	CG/015/02
Kinetic constants for [^{3}H]maraviroc binding to human CCR5 receptors at 37°C	Recombinant human CCR5 in HEK-293 cell membrane preparations (1)	0.45 - 45 nM & 0.005 – 100 nM (0.23 - 23 nM ng/mL & 0.003 – 51 ng/mL)	Geometric mean K_{obs} 0.089 $t_{½}$ (on) 7.78 min	No	CG/015/02
Kinetic constants for [^{3}H]maraviroc binding to human CCR5 receptors at room temperature and 37°C	Recombinant human CCR5 in HEK-293 cells and cell membrane preparations (1-2)	0.005 – 100 nM & 0.41-100nM (0.003 – 51 & 0.21 - 51 n/mL)	Mean (95% CI) on cells at RT K_{obs} 0.38, N=2 $t_{½}$ (on) 1.81 min (1.23-3.37), N=2 $t_{½}$ (off) 9.3 h, N= 1 Mean (95% CI) on membranes at RT K_{obs} 0.2, N=2 $t_{½}$ (on) 3.51 min (2.93-4.39), N=2 $t_{½}$ (off) 8.7 h, N=1 Mean (95% CI) on cells at 37°C K_{obs} 0.87, N=2 $t_{½}$ (on) 0.8 min (0.51-1.84), N=2 Mean (95% CI) on membranes at 37°C K_{obs} 0.26, N=2 $t_{½}$ (on) 2.71 min (2.02-4.07), N=2	No	DI/013/1

2.6.3.2. Primary Pharmacodynamics

Test Article: Maraviroc

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results	GLP Compliance	Study Number
Functional activity of maraviroc on site directed mutant variants of human CCR5	HEK-Gα15 cells (1-3)	0.1 nM - 10 µM (0.051 – 5140 ng/mL)	IC_{50} (nM) Wild type 2.95 L104F 29.5 Y108A ≥1803[a] A159V 252 I164F ≤ 1.5 H181A ≤ 0.8 I198N 149 E283A ≥3,980[a]	No	DI/102/05
Metabolite inhibition of CCR5 mediated membrane fusion					
HIV-1 envelope protein mediated cell-cell fusion	Recombinant gp160 & Tat in CHO cells with recombinant CCR5 & CD4 in HeLa β-gal -actosidase reporter cells (3)	0.51 nM – 10 µM (0.16–3113 ng/mL UK-463,977 and 0.21–4065 ng/mL UK-408,027)	IC_{50} nM (ng/mL) UK-408,027 >10 µM (3110 ng/mL) UK-463,977 >10 µM (4070 ng/mL)	No	UK463977/ DI/001/05
Antiviral activity					
In vitro viral replication assay with reverse transcriptase assay endpoint	HIV-1 Ba-L in PM-1 (16)	0.013 – 10 nM (0.007 – 5 ng/mL)	Geometric mean IC_{90} (95% CI) 1.08 nM (0.71 to 1.64) [0.56 ng/ml (0.37 to 0.84)]	No	DI/019/1
Ditto	HIV-1 IIIB in PM-1 (1)	0.013 nM – 10 µM (0.007 – 5140 ng/mL)	Geometric mean IC_{90} (95% CI) >10,000 nM [>5,137 ng/mL]	No	DI/019/1
Ditto	HIV-1 Ba-L in PBL - pooled donors (11)	0.013 – 10 nM (0.007 – 5 ng/mL)	Geometric mean IC_{90} (95% CI) 2.64 nM (1.75 to 3.97) [1.36 ng/mL (0.90 to 2.04)]	No	DI/019/1
Ditto	HIV-1 Ba-L in PBL - single donors (10)	0.013 – 10 nM (0.007 – 5 ng/mL)	Geometric mean IC_{90} (95% CI) 1.96 nM (1.21 to 3.15) [1.01ng/mL (0.62 to 1.62)]	No	DI/019/1

Test Article: Maraviroc

2.6.3.2. Primary Pharmacodynamics

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results	GLP Compliance	Study Number
Antiviral activity continued					
Multiplicity of Infection; In vitro viral replication assay with reverse transcriptase assay endpoint	HIV-1 Ba-L in PM-1 and PBL	0.013 - 10 nM (0.007 – 5 ng/mL)	No Effect on IC50 and IC90 with differing viral input	No	DI/051/06
In vitro viral replication assay with reverse transcriptase assay endpoint	HIV-1 Ba-L in monocyte derived macrophages (4)	0.015 – 100 nM (0.008 – 51 nM)	Geometric mean IC_{90} (95% CI) 0.79 nM (0.36 to 1.72) [0.40 ng/mL (0.19 to 0.88)]	No	DI/006/06
Ditto	43 CCR5-tropic primary isolates in PBL (190)	0.013 – 10 nM (0.007 – 5 ng/mL)	Geometric mean IC_{90} (95% CI) 2.03 nM (1.75 to 2.36) [1.04 ng/mL (0.90 to 1.22)]	No	DI/18/01
Ditto	4 CXCR4 tropic isolates (9)	0.013 – 10 nM (0.007 – 5 ng/mL)	No effect at 10 nM (5.13 ng/mL)	No	DI/18/01
Ditto	2 Dual tropic isolates (3)	0.013 – 10 nM (0.007 – 5 ng/mL)	No effect at 10 nM (5.13 ng/mL)	No	DI/18/01
Ditto	18 CCR5-tropic primary isolates in PBMC (triplicate assay)	0.005 - 50 nM (0.003 – 26 ng/mL)	Geometric mean IC_{90} (95% CI) 8.3 nM (5.7 to 12.2) [4.25 ng/mL (2.92 to 6.25)][a]	No	DI/004/3
Ditto	1 CXCR4 tropic isolate in PBMC (triplicate assay)	0.005 - 50 nM (0.003 – 26 ng/mL)	$IC_{90} > 50$ nM (26 ng/mL)	No	DI/004/3
Ditto	2 Dual tropic isolates in PBMC (triplicate assay)	0.005 - 50 nM (0.003 – 26 ng/mL)	$IC_{90} > 50$ nM (26 ng/mL) and 39.2 nM (20.1 ng/mL)	No	DI/004/3

Test Article: Maraviroc

2.6.3.2. Primary Pharmacodynamics

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results	GLP Compliance	Study Number
Antiviral activity continued					
In vitro viral replication assay with reverse transcriptase assay endpoint	1 Ba-L isolate in PBMC (triplicate assay)	0.005 - 50 nM (0.003 – 26 ng/mL)	IC_{90} =47.9 nM (24.5 ng/mL)	No	DI/004/3
Monogram Biosciences PhenoSense™ HIV Entry phenotypic drug susceptibility assay	Recombinant CCR5 & CD4 in U87 cells (200)	0.01 nM - 2.67 µM (0.005 – 1372 ng/mL)	Geometric mean IC_{90} (95% CI) 13.7 nM (12.3 to 15.1) [7.04 ng/mL (6.3 to 7.7)]	No	CG/016/02
Ditto	Recombinant CCR5 & CD4 in U87 cells (N = 160 B clade viruses)	0.01 nM - 2.67 µM (0.005 – 1372 ng/mL)	Geometric mean IC_{90} (95% CI) 14.2 nM (12.7 to 15.8) [7.29 ng/mL (6.5 to 8.1)]	No	CG/016/02
Ditto	Recombinant CCR5 & CD4 in U87 cells (N = 40 Non-B clade viruses)	0.01 nM - 2.67 µM (0.005 – 1372 ng/mL)	Geometric mean IC_{90} (95% CI) 11.7 nM (8.8 to 15.6) [6.01 ng/mL (4.5 to 8.0)]	No	CG/016/02
Ditto	Recombinant CCR5 & CD4 in U87 cells (N = 100 PI/RTI resistant viruses)	0.01 nM - 2.67 µM (0.005 – 1372 ng/mL)	Geometric mean IC_{90} (95% CI) 17.7 nM (15.3 to 20.5) [9.09 ng/mL (7.8 to 10.5)]	No	CG/016/02
Ditto	Recombinant CCR5 & CD4 in U87 cells (N = 100 Wild-type viruses)	0.01 nM - 2.67 µM (0.005 – 1372 ng/mL)	Geometric mean IC_{90} (95% CI) 10.5 nM (9.2 to 12.0) [5.39 ng/mL (4.7 to 6.1)]	No	CG/016/02
Protein binding determined by equilibrium dialysis	Non-cell/tissue based dialysis chamber (4)	389 nM (200 ng/ml)	Unbound concentration = 54.8% in antiviral assay medium	No	DI/138/05
CCR5 internalization using a specific monoclonal antibody and FACS	Recombinant human CCR5 expressed in mouse pre-B cell line (300.19/R5)	10 and 100 nM (5 and 51 ng/mL)	No receptor internalization	No	DI/009/03

Test Article: Maraviroc

2.6.3.2. Primary Pharmacodynamics

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results	GLP Compliance	Study Number
Displacement of chemokine binding					
[^{125}I]MIP-1α binding to CCR5	Recombinant human CCR5 expressed in HEK-293 cell membrane homogenates (3)	0.12 – 1000 nM (0.06 – 514 ng/mL)	Geometric mean IC_{50} (95% CI) 3.30 nM (1.9 to 5.7) [1.70 ng/mL (1.0 to 2.9)]	No	DI/002/1
[^{125}I]MIP-1α binding to CCR5	Recombinant human CCR5 expressed in whole HEK-293 cells (3)	0.12 – 1000 nM (0.06 – 514 ng/mL)	Geometric mean IC_{50} (95% CI) 3.43 nM (2.6 to 4.5) [1.76 ng/mL (1.3 to 2.3)]	No	DI/002/1
[^{125}I]MIP-1β binding to CCR5	Recombinant human CCR5 expressed in HEK-293 cell membrane homogenates (17)	0.12 – 1000 nM (0.06 – 514 ng/mL)	Geometric mean IC_{50} (95% CI) 7.18 nM (5.5 to 9.5) [3.69 ng/mL (2.8 to 4.9)]	No	DI/002/1
[^{125}I]MIP-1β binding to CCR5	Recombinant human CCR5 expressed in whole HEK-293 cells (3)	0.12 – 1000 nM (0.06 – 514 ng/mL)	Geometric mean IC_{50} (95% CI) 3.57 nM (1.4 to 9.0) [1.83 ng/mL (0.7 to 4.6)]	No	DI/002/1
[^{125}I]RANTES binding to CCR5	Recombinant human CCR5 expressed in HEK-293 cell membrane homogenates (3)	0.12 – 1000 nM (0.06 – 514 ng/mL)	Geometric mean IC_{50} (95% CI) 5.22 nM (2.1 to 13.0) [2.68 ng/mL (1.1 to 6.7)]	No	DI/002/1
[^{125}I]RANTES binding to CCR5	Recombinant human CCR5 expressed in whole HEK-293 cells (3)	0.12 – 1000 nM (0.06 – 514 ng/mL)	Geometric mean IC_{50} (95% CI) 6.30 nM (4.1 to 9.7) [3.24 ng/mL (2.1 to 5.0)]	No	DI/002/1
[^{125}I]MIP-1α binding to CCR5	Recombinant human CCR5 expressed in HEK-293 cell membrane homogenates (3/4)	10, 100 and 1000 nM (5, 51, 514 ng/mL)	No effect on maraviroc dissociation rate up to 1 μM (514ng/mL)	No	CG/005/04
Functional chemokine antagonism					
MIP-1α-, MIP-1β- and RANTES-induced intracellular calcium release	Recombinant human CCR5 expressed in HEK-293 cells (2-4)	0.5 – 500 nM (0.26 – 257 ng/mL)	Geometric Mean IC_{50} range 7 to 30 nM (4 to 15 ng/mL)	No	DI/005/01
MIP-1β-induced intracellular calcium release	Recombinant human CCR5 expressed in HEK-293 cells (8)	0.51 nM – 10 μM (0.26 – 5140 ng/mL)	Geometric Mean IC_{50} (95% CI) 14 nM (10 to 20) [7 ng/mL (5 to 10)]	No	DI/056/04

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2.6.3.2. Primary Pharmacodynamics

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results	GLP Compliance	Study Number
Functional chemokine antagonism continued					
MIP-1β-induced intracellular cAMP levels	Recombinant human CCR5 expressed in HEK-Gα15 cells (4)	1, 3 & 10 nM (0.5, 1.5 & 5.1 ng/mL)	pK_b (95% CI) 9.4 nM (9.01 – 9.75) [4.8 ng/mL (4.6 – 5.0)]	No	CG/001/05
Radioligand binding					
[^{125}I]MIP-1β binding to CCR5	Recombinant human CCR5 expressed in HEK-293 cell membrane homogenates (2)	0.05 – 1000 nM (0.026 – 514 ng/mL)	IC_{50} range = 2.7 to 6.1 nM (1.4 to 3.1 ng/mL) when the concentration of radiolabelled macrophage inflammatory protein-1β (MIP-1β) increased from 0.005 to 100 nM	No	DI/003/1
[^{125}I]MIP-1β binding to CCR5	Recombinant human CCR5 expressed in HEK-293 cell membrane homogenates (1)	0.3 nM – 10 µM (0.15 – 5140 ng/mL)	IC_{50} value shifts from 5.6 nM to 2.8 nM (2.9 to 1.4 ng/mL) following 2 h pre-incubation	No	CG/010/04
γ-S-GTP incorporation	Recombinant human CCR5 expressed in HEK-293 cell membrane homogenates	3nM – 1µM (1.5 – 514 ng/mL)	No effect on basal levels of γ-S-GTP incorporation until reduction at 100 nM	No	DI/108/06
Interaction with CCR5 from Relevant Non-Human Species					
Computational analysis of Sequence similarity	NA	NA	Full sequence homology to human CCR5 for monkey (98% identity), dog (83%), rabbit (80%), rat (82%) and mouse (82%). Sequence homology to putative human CCR5 binding site at 2Å radius for monkey (94% identity), dog (78%), rabbit (61%), rat (72%) and mouse (72%)	No	DI/100/06
Mouse CCR5 mediated, MIP-1α induced intracellular calcium release	Recombinant mouse CCR5 in HEK-293 cells (1, 6 replicates)	1-1000nM (0.5 – 514 ng/mL)	No agonist activity up to 1000 nM. No antagonist activity up to 1000 nM	No	DI/092/06

Test Article: Maraviroc

2.6.3.2. Primary Pharmacodynamics

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results	GLP Compliance	Study Number
Interaction with CCR5 from Relevant Non-Human Species continued					
[^{125}I]-MIP-1β binding to rat CCR5	Recombinant rat CCR5 in HEK-293 cell membrane preparations (3)	0.1, 1.0 & 10 µM (51, 514 & 5140 ng/mL)	33.3 ± 4.4 % inhibition at 10000 nM	No	CG/002/04
[^{125}I]-MIP-1β binding to dog CCR5	Recombinant dog CCR5 in HEK-293 cell membrane preparations (3)	0.1, 1.0 & 10 µM (51, 514 & 5140 ng/mL)	38.7 ± 3.8 % inhibition at 10000 nM	No	CG/002/04
[3H]maraviroc binding to macaque CCR5	Recombinant macaque CCR5 in HEK-293 cell membrane preparations (N = 3)	0.45 - 45 nM (0.23 – 23 ng/mL)	K_D = 1.36 nM (0.7 ng/mL) (95% CI = 1.07 to 1.71 nM)	No	CG/015/02
[^{125}I]-MIP-1β binding to macaque CCR5	Recombinant macaque CCR5 in HEK-293 cell membrane preparations (N = 5)	0.03 nM - 10 µM (0.015 – 5140 ng/mL)	IC_{50} = 17.5 nM (9.0 ng/mL) (95% CI = 14.1 to 21.0 nM)	No	CG/011/02
Macaque CCR5 mediated, MIP-1β induced intracellular calcium release	Recombinant macaque CCR5 in HEK-293 cells (N = 4)	0.78-800 nM (0.4 - 411 ng/ml)	IC_{50} = 17.5 nM (9.0 ng/mL)[a] (95% CI = 9.6 to 32.9 nM)	No	CG/009/02
Kinetic constants for [^{3}H]maraviroc binding to macaque CCR5 receptors at room temperature	Recombinant macaque CCR5 in HEK-293 cell membrane preparations (4-6)	0.45 - 45 nM (0.23 – 23 ng/mL)	Geometric Mean IC_{50} (95% CI) $t_{½}$ (on) 8.37 min (6.98-10.02) $t_{½}$ (off) 92.18 min (75.26-112.89) K_{on} 0.013 $min^{-1}.nM^{-1}$ (0.010-0.018) K_{off} 0.0075 min^{-1} (0.0001-0.0092) K_{off}/K_{on} 0.51 nM (0.46-0.57)	No	CG/015/02
Kinetic constants for [^{3}H]maraviroc binding to macaque CCR5 receptors at 37°C	Recombinant macaque CCR5 in HEK-293 cell membrane preparations (1-2)	0.45 - 45 nM (0.23 – 23 ng/mL)	Geometric mean (95% CI) $t_{½}$ (on) 3.92 min (2.61-5.90) $t_{½}$ (off) 39.13 min K_{on} 0.032 $min^{-1}.nM^{-1}$ (0.010-0.018) K_{off} 0.018 min^{-1} (0.0001-0.0092) K_{off}/K_{on} 0.56 nM	No	CG/015/02

2.6.3.2. Primary Pharmacodynamics

Test Article: Maraviroc

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results	GLP Compliance	Study Number
Pharmacodynamic Drug Interactions					
In vitro viral replication assay with reverse transcriptase assay endpoint	HIV-1 Ba-L in human PBMC(2)	0.16 – 40 nM (0.08 – 21 ng/mL)	Slightly synergistic interaction with efavirenz and nelfinavir with an additive interaction with lamivudine	No	DI/005/3
In vitro viral replication assay with reverse transcriptase assay endpoint	HIV-1 Ba-L in PM-1 (1-3)	0.03 – 2 nM (0.015 – 1 ng/mL)	Additive interaction with abacavir, didanosine, emtricitabine, stavudine, tenofovir, zalcitabine, zidovudine, delavirdine, efavirenz, nevirapine, lopinavir, ritonavir, saquinavir, SCH-C & [redacted] Additive/synergistic interaction with atazanavir, indinavir & SCH-D.	No	DI/002/04

HEK, Human Embryonic Kidney; CHO, Chinese Hamster Ovary; gp, glycoprotein; Tat, HIV transactivation protein; RT, Room Temperature; Ba-L = CCR5-tropic laboratory-adapted strain; PM-1, Immortalized human CD4+ T-cell line naturally co-expressing CCR5 and CXCR4; IIIB, CXCR4-tropic laboratory-adapted strain; PBL, Peripheral Blood Lymphocytes; PBMC, Peripheral Blood Mononuclear Cells; PI, Protease Inhibitor; RTI, Reverse Transcriptase Inhibitor; FACS, fluorescence-activated cell sorting; MIP, Macrophage Inflammatory Protein; RANTES, Regulated on Activation of Normal T cell Expressed and Secreted; cAMP, cyclic Adenosine Monophosphate; GTP, Guanosine Triphosphate; NA, Not Applicable; SCH-C, Schering CCR5 antagonist ; SCH-D, Schering CCR5 antagonist (vicriviroc); [redacted] CCR5 antagonist in [redacted]

[a] Data derived from original report

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2.6.3.3. Secondary Pharmacodynamics

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results	GLP Compliance	Study Number
MCP-3 induced intracellular Ca^{2+} release (CCR2b)	THP-1 cells (2)	20 nm - 10µM (10 – 5140 ng/mL)	IC_{50} >10 µM	No	DI/008/1
IL-2-stimulated T-cell proliferation	Human T lymphocytes (1)	0.156 – 10 µM (80 – 5140 ng/mL)	IC_{50} >10 µM	No	DI/020/1
Antigen-stimulated lymphocyte proliferation	Human PBL (3)	0.156 – 10 µM (80 – 5140 ng/mL)	IC_{50} >10 µM	No	DI/021/1
Superoxide production in neutrophils	Human neutrophils (2)	0.3 nM – 10 µM (1.5 - 5140 ng/mL)	IC_{50} >10 µM	No	DI/022/1
MIP-1α binding to CCR1	MIP34.10 cells - HEK cells expressing human CCR1 (1)	0.1 nM – 10 µM (0.05 – 5140 ng/mL)	IC_{50} >10 µM	No	DI/023/1
Eotaxin binding to CCR3	Murine pre-B cell line, 300-19 expressing human CCR3 (3)	0.1, 1, & 10 µM (51, 514 & 5140 ng/mL)	IC_{50} >10 µM	No	DI/024/1
IL-4-stimulated IgE synthesis in lymphocytes	Human Peripheral Blood Lymphocytes (3)	1, 10, 100, 1000 & 10,000 nM (0.5, 5, 51, 514 & 5140 ng/mL)	IC_{50} ≥10 µM (2 donors) IC_{50} = 1 to 10 µM (1 donor)	No	DI/025/1
LPS-stimulated TNF-α release by differentiated THP-1 cells	THP-1 cells (3)	2nM - 40µM (1 – 20,560 ng/mL	IC_{50} >40 µM	No	DI/026/1
rhMIP-3β binding to CCR7	Human CCR7-transfected human H-9 T-cells (3)	0.1, 1 & 10 µM (51, 514 & 5140 ng/mL)	IC_{50} >10 µM	No	DI/027/1

Test Article: Maraviroc

2.6.3.3. Secondary Pharmacodynamics

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results	GLP Compliance	Study Number
CCR1-mediated, MIP-1α induced chemotaxis	THP-1 Cells (3)	0.25, 2.5 & 25μM (129, 1290 & 12900 ng/mL)	IC_{50} >25 μM	No	DI/028/1
CCR2-mediated, MCP-1 induced chemotaxis	THP-1 Cells (3)	0.25, 2.5 & 25μM (129, 1290 & 12900 ng/mL)	IC_{50} >25 μM	No	DI/028/1
CCR3-mediated, ITAC induced chemotaxis	H9 cells (3)	0.25, 2.5 & 25μM (129, 1290 & 12900 ng/mL)	IC_{50} >25 μM	No	DI/028/1
CCR7-mediated, SLC induced chemotaxis	H9 cells (3)	0.25, 2.5 & 25μM (129, 1290 & 12900 ng/mL)	IC_{50} >25 μM	No	DI/028/1
CXCR1/CXCR2-mediated, IL-8 induced chemotaxis of neutrophils	Human neutrophils (3)	0.25, 2.5 & 25μM (129, 1290 & 12900 ng/mL)	IC_{50} >25 μM	No	DI/028/1
CXCR2-mediated, Gro-α induced chemotaxis	Human neutrophils (3)	0.25, 2.5 & 25μM (129, 1290 & 12900 ng/mL)	IC_{50} >25 μM	No	DI/028/1
TARC binding to CCR4	Murine pre-B cell line, 300-19 expressing human CCR4 (3)	0.1, 1 & 10 μM (51, 514 & 5140 ng/mL)	IC_{50} >10 μM	No	DI/029/1

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2.6.3.3. Secondary Pharmacodynamics

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results	GLP Compliance	Study Number
I-309 binding to CCR8	PEER cells (3)	0.01, 0.1, 1 & 10 µM (5, 51, 514 & 5140 ng/mL)	IC_{50}>10 µM	No	DI/030/1

GLP, Good Laboratory Practice; MCP, Monocyte Chemoattractant Protein; THP-1, human acute monocytic leukemia cell line; IL, Interleukin; PBL, Peripheral Blood Lymphocyte; MIP, macrophage inflammatory protein; HEK, Human Embryonic Kidney cells; IgE, Immunoglobulin E; LPS, lipopolysaccharide; TNF-α, Tissue necrosis factor-α ; ITAC, interferon inducible T-cell and chemoattractant; rh, recombinant human; H9, human CCR7-transfected T-cell lymphoma cell line; SLC, secondary lympoid tissue chemokine; IL-8, interleukin 8; Gro-α, growth chemokine-α; TARC, thymus and activation regulated chemokine; I-309, monocyte chemoattractant induced by apolipoprotein in human vascular endothelial cells;RhMIP-3β, macrophage inflammatory protein-3β; PEER cells, human leukaemic T-cells.

>4 µM = >2054 ng/mL; >10 µM = ≥ 5137 ng/mL; >25 µM = > 12842 ng/mL; 1 to 10 µM = 514 to 5137 ng/mL

2.6.3.4 Safety Pharmacology — Test Article: Maraviroc

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results[a]	GLP Compliance	Study Number
Muscarinic nonselective	Rat brain (2)	100 nM & 10 μM (51.4 & 5140 ng/mL)	100 nM: no effect, 10 μM: 39% & 47% inhibition	No	CG/013/00 CG/13/02
Opioid nonselective	Rat brain (1)	100 nM & 10 μM (51.4 & 5140 ng/mL)	100 nM: no effect, 10 μM: 83% inhibition	No	CG/013/00
μ opioid	Human recombinant (1)	0.1 nM to 10 μM (0.05 to 5140 ng/mL)	K_i = 106 nM (54.5 ng/mL)	No	CG/013/00
μ opioid	Human recombinant (3)	0.03 nM to 100 μM (0.0154 to 51368 ng/mL)	$K_i = 208.8 \pm 0.3$ nM (107.3 ng/mL)	No	CG/009/01
μ opioid	Human recombinant (4)	3 nM to 100 μM (1.54 ng/mL to 51368 μg/mL)	Ki = 589.29 nM (95% CI, 430.1-807.4 nM) (302.7 ng/mL)	No	CG/001/03
δ opioid	Human recombinant (2)	0.1 nM to 10 μM (0.05 to 5140 ng/mL)	10 μM: 48% inhibition	No	CG/013/00
Adrenergic α_{2A}	Human recombinant (1)	100 nM & 10 μM (51.4 & 5140 ng/mL)	100 nM: no effect; 10 μM: 52% inhibition K_i = 5.47 μM (2809.8 ng/mL)	No	CG/13/02
Adrenergic α_{2B}	Mouse neuroblastoma (1)	100 nM & 10 μM (51.4 & 5140 ng/mL)	100 nM: no effect; 10 μM: 24% inhibition	No	CG/13/02
Adrenergic α_{1A}	Rat Salivary gland (2)	10 μM (5140 ng/mL)	26% inhibition	No	CG/001/04
Histamine H_2	Guinea pig striatum (2)	10 μM (5140 ng/mL)	22% inhibition	No	CG/001/04
Norepinephrine transporter	Human recombinant (2)	10 μM (5140 ng/mL)	27% inhibition	No	CG/001/04
hERG (I_{Kr})	Human recombinant (6)	0.3 nM to 10 μM (0.154 to 5140 ng/mL)	3 μM: 15±4% inhibition 10 μM: 43±3% inhibition	No	IC/001/02

2.6.3.4 Safety Pharmacology

Test Article: Maraviroc

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results[a]	GLP Compliance	Study Number
Adenosine (A_1)	Human recombinant (2)	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Adenosine (A_3)	Human recombinant (2)	10 µM (5140 ng/mL)	No effect	No	CG/001/04
Adrenergic (nonselective α_1 & α_2)	Rat brain (2)	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Adrenergic (β_1 & β_2)	Human recombinant (2)	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Adrenergic (α_{2C})	Human recombinant (2)	10 µM (5140 ng/mL)	No effect	No	CG/13/02
Angiotensin-II (AT_1)	Rat lung (2)	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Atrial Naturietic Peptide	From guinea pig cerebellum (2)	10 µM (5140 ng/mL)	No effect	No	CG/003/05
Bradykinin (B_2)	Human recombinant (2)	10 µM (5140 ng/mL)	No effect	No	CG/003/05
Choline transporter	Rat striatum (2)	10 µM (5140 ng/mL)	No effect	No	CG/001/04
Dopamine (D_1, D_2)	Human recombinant (2)	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Dopamine transporter	Human recombinant (2)	10 µM (5140 ng/mL)	No effect	No	CG/001/04
Endothelin (ET_A & ET_B)	Human recombinant (2)	10 µM (5140 ng/mL)	No effect	No	CG/003/05
$GABA_A$ (benzodiazepine central)	Rat cerebral cortex (2)	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00

2.6.3.4 Safety Pharmacology — Test Article: Maraviroc

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results[a]	GLP Compliance	Study Number
GABA transporter	Rat striatum (2)	10 µM (5140 ng/mL)	No effect	No	CG/001/04
Glutamate (NMDA)	Rat cerebral cortex (2)	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Ghrelin (GHS)	Human recombinant (2)	10 µM (5140 ng/mL)	No effect	No	CG/003/05
Histamine (H_1)	Guinea pig cerebellum (2)	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Imidazoline I_2	Rat brain (2)	10 µM (5140 ng/mL)	No effect	No	CG/013/02
Interleukin (IL-1β, IL-2, IL-4, human IL-6)	Balb/c 3T3 cells CTLL-2 cells MLA 144 cells U-266 cells	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
IL-8A (CXCR1) & IL-8B (CXCR2)	Human recombinant (2)	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
CCR1	Human recombinant (2)	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Muscarinic (M_1, M_2, M_3, M_4 & M_5)	Human recombinant (4)	10 µM (5140 ng/mL)	No effect	No	CG/001/02
Nicotinic (neuronal, α-BGTX insensitive)	Rat cerebral cortex (2)	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
κ opioid	Human recombinant (2)	0.1 nM to 10 µM (0.05 to 5140 ng/mL)	No effect	No	CG/013/00
Serotonin ($5HT_{1A}$, $5HT_{2A}$ & $5HT_{1B}$)	Human recombinant (2)	100 nM & 10 µM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Thromboxane (TXA_2/PGH_2)	From human platelets (2)	10 µM (5140 ng/mL)	No effect	No	CG/003/05

2.6.3.4 Safety Pharmacology

Test Article: Maraviroc

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results[a]	GLP Compliance	Study Number
Urotensin (UT1)	Human recombinant (2)	10 μM (5140 ng/mL)	No effect	No	CG/003/05
Vasopressin (V_{1A})	Human recombinant (2)	10 μM (5140 ng/mL)	No effect	No	CG/003/05
5-HT transporter	Human recombinant (2)	10 μM (5140 ng/mL)	No effect	No	CG/001/04
L-type Calcium channels (verapamil, dihydropyridine & diltiazem sites)	Rat cerebral cortex (2)	100 nM & 10 μM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Acetyl-cholinesterase	Human recombinant (2)	100 nM & 10 μM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Angiotensin Converting Enzyme	Human recombinant (2)	10 μM (5140 ng/mL)	No effect	No	CG/001/04
Guanylyl cyclase	From bovine lung	10 μM (5140 ng/mL)	No effect	No	CG/005/05
Monoamine oxidase (A & B)	Human recombinant (2)	100 nM & 10 μM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Na^+/K^+ ATPase	Dog kidney (2)	100 nM & 10 μM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
Phosphodiesterase (III, IV&V)	Human recombinant (2)	10 μM (5140 ng/mL)	No effect	No	CG/013/00 CG/005/05
Tyrosine hydroxylase	Rat striatum (2)	100 nM & 10 μM (51.4 & 5140 ng/mL)	No effect	No	CG/013/00
GTP-γ-S binding to human μ receptor	Human recombinant (5 to 8)	0.03 nM to 10 μM (0.015 to 5140 ng/mL)	1 μM: no effect 10 μM: 22.2±3.7% stimulation, no antagonism of DAMGO response.	No	CG/011/01

2.6.3.4 Safety Pharmacology

Test Article: Maraviroc

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results[a]	GLP Compliance	Study Number
Electrically-stimulated smooth muscle	Guinea pig (male, Dunkin-Hartley) ileum (6 to 8 tissues)	20 nM to 1 μM (10.3 to 514 ng/mL)	No effect	**No**	CG/006/00
Oxytocin-induced smooth muscle contraction	Rat (female, Sprague Dawley) isolated uterine smooth muscle (8 tissues)	10 nM, 100 nM & 1 μM (5.14, 51.4, 514 ng/mL)	No effect	**Yes**	CG/007/01
hERG potassium channels (patch clamp assay)	Human recombinant ion channel (4)	200 nM (103 ng/mL) 3, 10 μM (1541 & 5140 ng/mL)	200 nM: No effect. 3 μM: 5.74±2.0% inhibition 10 μM: 19±3% inhibition	**No**	CG/001/00 IC/005/02
Action potentials	Dog (Beagle) isolated Purkinje fibres, either sex (5)	20, 100 & 200 nM (10, 51 & 103 ng/mL)	No significant effect on membrane potential, action potential amplitude, or Vmax. 100nM: $APD_{50} = 5.2 \pm 1.8\%$ (p=0.05) 200nM: $APD_{50} = 8.9 \pm 2.1\%$ (p=0.01) and $APD_{90} = 7.8 \pm 1.9\%$ (p=0.05) when compared to time matched vehicle control. This batch of maraviroc contained an impurity.	**Yes (for vehicle samples)**	CG/002/00
Action potentials	Dog (Beagle) isolated Purkinje fibres, either sex (5)	200, 500 nM, & 1 μM (103, 257, 514 ng/mL) 1, 3, & 10 μM (514, 1541, 5140 ng/mL)	0.2 - 1 μM: no significant effect 3 μM: 8.8±3.5% prolongation of APD50, 9.6±2.4% prolongation of APD90 10 μM 25.9±10.9% prolongation of APD50, 30.6±6.7% prolongation of APD90	**No**	IC/006/01 IC/003/02
[^{3}H]UK-14,304 binding to adrenergic α2A receptors	Human recombinant cells(2)	0.3 nM to 10 μM (0.15 ng/mL to 5.14 μg/mL)	10, 100 nM, & 1 μM: no effect on the Ki of UK-14,304 for α2A receptors. 10 μM: 37.5% inhibition of [3H]UK-14,304 binding.	**No**	CG/003/04

2.6.3.4 Safety Pharmacology — Test Article: Maraviroc

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results[a]	GLP Compliance	Study Number
[^{3}H]UK-14,304 binding to adrenergic α2A receptors	Human recombinant cells (3-12)	3 nM to 100 μM (1.54 ng/mL to 51.4 μg/mL)	1 μM: Statistically significant (p<0.01) shift in Kd value of [^{3}H]UK-14,304 from 0.64 nM (95% CI, 0.49 to 0.84 nM) to 1.06 nM (95% CI, 0.71 to 1.57 nM). Ki = 8.04 μM (95% CI, 4.2 to 15.2 μM).	No	CG/007/04
Adrenergic α2A cAMP production	Human recombinant cells (3-4)	1 nM to 100 μM (0.51 ng/mL to 51.4 μg/mL)	No effect	No	CG/002/05
Calcium release from adrenergic α2A, α2B and α2C receptors	Human recombinant cells (4)	1 nM to 100 μM (0.51 ng/mL to 51.4 μg/mL)	No effect	No	CG/004/05
α2 adrenoceptor mediated functional activity	Rat vas deferens (3-12)	0.1 nM to 300 μM (0.051 ng/mL to 54 μg/mL)	No direct effect on contraction. Statistically significant (p<0.05) potentiation of UK-14,304 potency at 10 and 100 nM only.	No	CG/014/02
α2 adrenoceptor mediated functional activity	Rat vas deferens (6-9)	10 nM, 100nM & 1 μM (5.14, 51.4, & 514 ng/mL)	Statistically significant (p<0.05) decrease in clonidine potency in presence of 10 nM and 1 μM maraviroc only.	No	CG/004/03
Vasoconstrictor / vasodilatory functional activity	Dog isolated carotid artery & saphenous vein (3-11)	1 nM to 100 μM (0.51 ng/mL to 51.4 μg/mL)	10 μM: No direct effect on basal tone in either vessel. Carotid artery: No effect on phenylephrine pre-constricted vessels. Saphenous vein: vasodilatation with threshold effect at 3 μM and E_{max} = 83% (95% CI, 63 to 103%).	No	CG/004/04
Vasodilatory functional activity	Dog isolated saphenous vein (6)	10 nM to 100 μM (5.14 ng/mL to 51.4 μg/mL)	No effect on KCl pre-constricted vessels.	No	CG/006/04
Vasodilatory functional activity	Dog isolated saphenous vein (5)	1,10, and 30 μM (0.51, 5.14, and 15.4 μg/mL)	pKb = 5.72 (95% CI, 5.24 to 6.21 nM) (2.92 ng/mL) with no suppression of the phenylephrine E_{max}.	No	CG/008/04

2.6.3.4 Safety Pharmacology

Test Article: Maraviroc

In Vitro

Type of Test	Test Cells / Tissues (N)	Test Concentrations	Results[a]	GLP Compliance	Study Number
Vasodilatory functional activity	Dog isolated saphenous vein (5)	1,10, and 30 µM (0.51, 5.14, and 15.4 µg/mL)	pKb = 5.21 (95% CI, 4.85 to 5.56 nM) (2.66 ng/mL) with no suppression of the noradrenaline E_{max}.	No	CG/009/04
Vasodilatory functional activity	Human isolated saphenous vein (3)	1 nM to 100 µM (0.51 ng/mL to 51.4 µg/mL)	No effect in comparison with vehicle.	No	CG/011/04
Vasodilatory functional activity	Human isolated saphenous vein (2)	300 nM (154 ng/mL)	Maraviroc blocked MIP-1b induced vasoconstriction	No	DI/073/06

In Vivo

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses[a] (mg/kg)	Number/Sex/Group	Noteworthy Findings	GLP Compliance	Study Number
Whole body - maximum tolerated dose	Rat / Sprague Dawley	Oral	1000, 2000	2/Male	<u>1000 mg/kg</u>: Salivation, transient ↑ in respiration and a ↓ in activity (returning to normal by 30 min). Unbound C_{max} 5.0 µM (2543 ng/mL), Total C_{max} 10.1 µM (5190 ng/mL). <u>2000 mg/kg</u>: Piloerection, transient ↑ in respiration and a ↓ in activity (returning to normal by 45 min). Marked salivation lasting 1 h after dosing. Respiratory distress lasting 1 h in 1 animal at 2000 mg/kg 2 h after dosing	Yes	CG/009/00

2.6.3.4 Safety Pharmacology

Test Article: Maraviroc

In Vivo

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses[a] (mg/kg)	Number/Sex/Group	Noteworthy Findings	GLP Compliance	Study Number
CNS - Sodium pentobarbitone-induced sleeping times	Mouse / CD-1	Oral	1, 3, 10	20 / Male	No effect	**Yes**	CG/005/01
CNS - Motor coordination	Mouse / CD-1	Oral	1, 3, 10	12 to 20 / Male	No effect	**Yes**	CG/002/01
Renal - Excretion of fluid and electrolytes	Rat / Sprague Dawley	Oral	10, 20, 60	12 / Female	No effect	**Yes**	CG/004/01
Respiratory	Rat / Sprague Dawley	Intravenous	1	4 / Male	No effect on arterial blood pH, pO_2 and pCO_2. A small but statistically significant ↓ in mean arterial blood pressure and heart rate 10 min after dosing.	**Yes**	CG/001/01
Haemodynamic and electrocardiogram (ECG) parameters	Dog / Beagle	Oral	0.05, 0.15, 0.5, 1.5	4 / Male	No effect. Mean unbound C_{max} = 168 nM (86 ng/mL) Total 464 nM (238 ng/mL)	**Yes**	CG/003/00 CG/008/00

2.6.3.4 Safety Pharmacology — Test Article: Maraviroc

In Vivo

Organ Systems Evaluated	Species/Strain	Method of Administration	Doses[a] (mg/kg)	Number/Sex/Group	Noteworthy Findings	GLP Compliance	Study Number
Haemodynamic and electrocardiogram (ECG) parameters during postural challenge	Dog / Beagle	Intravenous	10.5 mg/kg over 180 min (& 1 dog at 7 mg/kg over 120 min)	4 / Male	Reduction in systolic (-3.3 mmHg) and increase in diastolic blood pressure (+1.9 mmHg), slight, but statistically significant ($p<0.05$) reduction in pulse pressure. No effect on heart rate. Transient (1 min) amplification of initial heart rate increase and fall in systolic blood pressure upon postural challenge. 14.5 msec QT prolongation in the absence of an effect on heart rate; mean unbound plasma concentration of 1.2 µM (631 ng/ml).	Yes	CG/007/02

GLP, Good Laboratory Practice; hERG, Human Ether-a-Go-go Related Gene; I_{Kr}, hERG current; GABA, γ-Aminobutyric Acid ; NMDA, N-Methyl-D-Aspartic Acid; GHS, Growth Hormone Secretion; IL, Interleukin; BGTX, Bungarotoxin; 5HT, 5-Hydroxytryptamine; TXA2, Thromboxane A2; PGH2, Prostaglandin H2; ATP, Adenosine Triphosphate; GTP, Guanosine Triphosphate; DAMGO, [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-Enkephalin acetate; APD50, Action Potential Duration at 50% repolarisation; APD90, Action Potential Duration at 90% repolarisation; cAMP; Cyclic Adenosine Monophosphate; ↑, increase; ↓, decrease; CNS, Central Nervous System; ECG, Electrocardiogram.

[a] <20% inhibition at 10 µM is recorded as 'No effect'.