MODULE 2.4. NONCLINICAL OVERVIEW

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

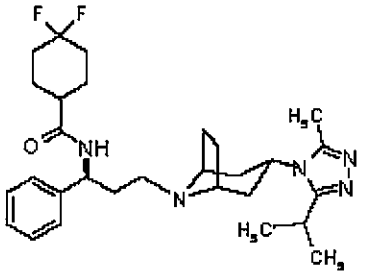
AIDS	Acquired immune deficiency syndrome
ALT	Alanine aminotransferase
APPT	Activated Partial Thromboplastin Time
AST	Aspartate aminotransferase
AUC	Area under the plasma concentration-time curve
AUC24h	Area under the plasma concentration-time curve over 24 hours
BID	Twice daily treatment
bpm	Beats per minute
caco	Human colonic adenocarcinoma cells
cAMP	Cyclic Adenosine Monophosphate
CCL	Chemokine (C-C motif) Ligand
CCR5	CC Chemokine Receptor 5
CCR5Δ32	CCR5 gene with 32 base pair deletion
CHMP	Committee for Medicinal Products for Human Use
CI	Confidence interval
Cmax	Maximum plasma concentration
Cmin	Minimum plasma concentration
CSF	Cerebrospinal Fluid
CXCR	CX Chemokine Receptor
CYP	Cytochrome P450 Enzyme
E	Extraction
ECG	Electrocardiogram
FDA	United States Food and Drug Administration
GALT	Gut-Associated Lymphoid Tissue
GGT	Gamma-glutamyltransferase
GLP	Good Laboratory Practice
gp120	Glycoprotein 120
GTP-γ-S	Guanosine 5'-[γ-thio]triphosphate
HEK	Human Embryonic Kidney
hERG	Human Ether a-go-go-Related Gene
HIV-1	Human immunodeficiency virus subtype 1
IC_{50}	The molar concentration at which in vitro activity is inhibited by 50%
IC_{90}	The molar concentration at which in vitro activity is inhibited by 90%
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
Ig	Immunoglubulin
I_{Kr}	hERG current
IL	Interleukin
IU	International Units
IV	Intravenous
K_D	Equilibrium constant for dissociation
K_i	Inhibition constant
KLH	Keyhole Limpet Haemocyanin

K_m	Michaelis-Menten Constant: the concentration of substrate that permits half maximal rate of reaction
MIP	Macrophage Inflammatory Protein
MNU	N-methyl-N-nitrosourea
MVC	Maraviroc
MVCres	Maraviroc resistant
NK	Natural Killer
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NOAEL	No-observed-adverse-effect level
NONS	Notification of New Substance
NRTI/NtRTI	Nucleoside/nucleotide reverse transcriptase inhibitor
NMR	Nuclear Magnetic Resonance
PBL	Peripheral blood leucocytes
PCNA	Proliferating Cell Nuclear Antigen
PD	Pharmacodynamics
P-gp	P-glycoprotein
Ph. Eur	European Pharmacopoeia
PI	Protease inhibitor
PK	Pharmacokinetics
pK_b	Negative log of the dissociation constant, K_b, the molar concentration which at equilibrium occupies 50% of the receptors
PO	Oral
QD	Once daily treatment
QT	Time from the beginning of the QRS complex to the end of the T wave in the electrocardiogram
QTc	QT interval, corrected for heart rate
QTcI	QTc interval, calculated using an individual correction factor
R5	CCR5 tropic virus
RANTES	Regulated on Activation of Normal T cell Expressed and Secreted
RITA	Registry of Industrial Toxicology for Animals
RT	Reverse Transcriptase
SCID	Severe Combined Immunodeficiency
SD	Standard Deviation
SEM	Standard Error of the Mean
T-Cell	Thymus derived T-lymphocyte
Tg	Transgenic
TSH	Thyroid Stimulating Hormone
UDPGT	Uridine 5-diphosphate glucuronyl transferase
V3	Third variable loop region on gp120

2.4.1. Overview of the Nonclinical Testing Strategy

Maraviroc (international non-proprietary name) or UK-427,857 (Pfizer code number), 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 (chemical name) is an inhibitor of HIV-1 entry. This new chemical entity acts by selectively binding to the human chemokine receptor CCR5 and inhibiting 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. Targeting a human protein in order to prevent viral entry is a new approach to HIV-1 therapy.

Figure 1. Structure of Maraviroc



Molecular Formula: C29H41F2 N5O
Molecular Weight: 513.7

The drug has been developed for chronic oral administration to help prevent the development and progression of AIDS in individuals positive for HIV-1.

The maximum well tolerated therapeutic dose was established at 300 mg BID. Total doses of 200mg daily and above ensure complete CCR5 receptor blockade over 24 hours. In treatment-experienced patients receiving CYP3A4 inhibitors such as boosted protease inhibitors the maximum well tolerated and recommended dose is 150 mg BID. The safety of maraviroc has been assessed in the toxicology species using doses and plasma exposures (Cmax and AUC24h) higher than those administered to human patients.

Maraviroc has been formulated as film-coated tablets (150 mg and 300 mg) for oral administration. The following well-established, compendial (Ph.Eur), inactive excipients are present in the tablet formulation: microcrystalline cellulose, dibasic calcium phosphate, sodium starch glycolate, and magnesium stearate. The tablet cores are film-coated with [redacted] The components of the [redacted] film-coats comply with Ph.Eur. requirements and are acceptable for worldwide use.

Primary pharmacodynamic studies were conducted to investigate the antiviral mode of action of maraviroc. The objectives of these in vitro studies were to characterize the binding of maraviroc to CCR5 and the consequent inhibition of virus entry. Cell based assays of acute viral infection were used to project an efficacious dose in order to guide subsequent non-

clinical and clinical testing. The ability of maraviroc to inhibit viruses of wide geographical origin, diverse clade and with reduced susceptibility to existing drug classes was also investigated. Studies were conducted to investigate the antiviral activity of maraviroc in combination with antiretroviral compounds representative of other drug classes indicated for HIV therapy. To investigate the effects of maraviroc on CCR5 that are not related to its antiviral activity, studies on chemokine binding and cellular signaling were conducted. Secondary pharmacodynamic studies were conducted to determine the effects of maraviroc unrelated to its desired therapeutic target. Safety pharmacology studies were conducted to investigate the potential undesirable pharmacodynamic effects of maraviroc on physiological functions in relation to exposure in the therapeutic range and above.

The nonclinical pharmacokinetic programme for maraviroc comprised detailed single dose studies in mice, rats and dogs to define basic pharmacokinetic parameters. This programme was supplemented by sampling during repeat dose toxicology studies in mice, rats, dogs and monkeys to confirm the pharmacokinetics of maraviroc under the actual conditions of safety evaluation. Tissue distribution, metabolism and excretion studies were conducted using three radiolabelled forms of maraviroc - tritium (3H) and both single and dual carbon-14 labelled material. In vitro metabolism studies were used to support in vivo studies, to characterise the enzymes involved in maraviroc metabolism and to determine the potential for interactions with co-administered drugs.

The nonclinical safety evaluation programme was carried out predominantly in rats and macaque monkeys and included an assessment of carcinogenicity potential using a 6-month study in transgenic mice and a 24-month study in rats. This programme served to underwrite oral therapy with maraviroc. Additional studies were conducted to assess the involvement of hepatic enzyme induction in thyroid changes observed in rats and to assess the effect of maraviroc on immune function in monkeys. The studies were conducted in line with current guidelines from the European Union, Organisation for Economic Co-operation and Development, US Food and Dug Administration and International Conference on Harmonisation and were carried out in compliance with Good Laboratory Practice Regulations.

The molecular weight of maraviroc is 513.7. Concentration data from pharmacology experiments are described in terms of nanomolar or micromolar values whereas pharmacokinetic and toxicology data is described in terms of nanograms per milliliter (1 nM = 0.514 ng/mL).

Exposure multiples have been calculated by comparing unbound plasma concentrations (Cmax or AUC24h) in the toxicology species with those at the maximum therapeutic dose in humans (300 mg BID: Cmax 155 ng/mL; AUC24h 1275 ng.h/mL; Clinical study A4001007, Module 5.3.4.2 Patient PD and PK/PD Study Reports). Concentration multiples in in vitro studies have been calculated by comparing total concentration in the assay with the mean unbound Cmax at the maximum therapeutic dose in humans (300 mg BID: Cmax 300 nM, 155 ng/mL).

The nonclinical programme was intended to generate data to support the use of maraviroc in patients as detailed in the proposed labeling documents (Module 1).

2.4.2. Pharmacology

2.4.2.1. Programme design

The objectives of the primary pharmacology studies were two-fold. Firstly, to establish the CCR5 pharmacology related to the antiviral mode of action. Secondly, to establish the CCR5 pharmacology not related to the antiviral mode of action; that is, effects on binding of endogenous CCR5 chemokines and subsequent signaling processes. Secondary pharmacology studies were conducted to establish the effects on host defence mechanisms in vitro. In addition, a programme of safety pharmacology studies has been conducted in order to underwrite the safety of maraviroc in clinical studies. All safety pharmacology studies were performed in accordance with ICH S7A guidelines on Safety Pharmacology Studies for Human Pharmaceuticals and were in compliance with international Good Laboratory Practice (GLP) regulations (except for a hERG path clamp study and some radioligand binding assays duly noted in 2.6.2 Pharmacology Written Summary, Section 2.6.2.4). Studies to investigate the effects of maraviroc on cardiac repolarisation in vitro were conducted in accordance with ICH S7B guidelines, and were compliant with GLP regulations except for the hERG patch clamp assay, which was conducted according to the principles of GLP.

An additional pharmacology study was conducted to investigate the effect of maraviroc on haemodynamic changes in the conscious dog as a result of postural challenge. Further antiviral studies were conducted to assess the pharmacodynamic drug interaction between maraviroc and other antiviral agents. Furthermore, UK-408,027 (a secondary amine metabolite) and UK-463,977 (a carboxylic acid metabolite) have been evaluated for antiviral activity and for their wider pharmacological effects on a range of receptors, enzymes and ion channels.

2.4.2.2. CCR5 Primary Pharmacodynamics Related to Antiviral Mode of Action

Maraviroc inhibits viral entry by binding to cell surface CCR5. Specifically, maraviroc blocks the interaction of R5 HIV-1 gp120 with its co-receptor (CCR5), the latter being an essential step in the entry process. Maraviroc binds to human CCR5 with a K_D of 0.86 nM and at room temperature has a dissociation half-life of approximately 16 hours (DI/012/1 & CG/015/02). Site directed mutagenesis and computer modeling studies locate the likely binding site of maraviroc to a pocket within the transmembrane region of CCR5 (DI/102/05). As a consequence of this binding, maraviroc is thought to alter the three dimensional structure of CCR5 such that the viral envelope glycoprotein, gp120, is unable to recognize and bind to the co-receptor. Consistent with this, maraviroc blocks the soluble form of gp120 binding to CCR5 with an IC_{50} of 11 nM (DI/005/04) and inhibits gp120/CCR5-mediated membrane fusion with an IC_{50} of 0.22 nM (DI/007/1).

2.4.2.2.1. Antiviral Activity In Vitro

Maraviroc selectively inhibits CCR5-tropic HIV-1 replication in vitro. The compound is active in antiviral assays using immortalised cell lines, peripheral blood lymphocytes (PBL) and monocyte-derived macrophages. Maraviroc also inhibits the infection by pseudotyped viruses of a recombinant cell line transformed to express high levels of CCR5 and CD4. Maraviroc inhibits viral replication in vitro in a non-competitive manner with respect to virus input. This is consistent with the compound binding to a human drug target (CCR5) rather than a viral target. The antiviral activity of maraviroc occurs in the absence of any compound cytotoxicity in all these assay systems.

In vivo models of HIV infection have been described (Nakata et al, 2005; McCune et al, 1990; Mosier et al, 1991). Indeed, studies in macaques and SCID-huPBL mice have validated CCR5 antagonists as a potential new class of antiretrovirals (Strizki et al, 2001; Veazey et al, 2003; Wolinsky et al, 2004). However, these in vivo models typically use single virus isolates or lab adapted strains that do not reflect the diversity of clinically relevant infections. This is of particular relevance given the heterogeneity of the viral envelope protein, gp120. Furthermore, no animal model can accurately reproduce the receptor-dependent dynamics of infection in humans. Therefore, cell based assays of acute viral infection using primary HIV-1 isolates were used to project an efficacious dose in order to guide subsequent non-clinical and clinical testing.

The primary, pharmacologically relevant value for maraviroc is derived from the geometric mean antiviral IC_{90} against a large panel of CCR5-tropic primary HIV-1 isolates following acute infection of PBL (2.03nM, 1.04 ng/ml), and the unbound assay concentration (estimated to be 54.8% of total compound) thus giving a value of approximately 1.0 nM (0.5 ng/mL). Data were obtained using primary HIV-1 isolates since these 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 (subtypes). 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 primary pharmacological value was used to plan and assess pre-clinical and clinical studies with the aim of targeting concentrations/doses where the free Cmin exceeded this value. The rationale for selecting IC_{90} to derive this value is that this represents a clinically relevant 1 log drop in virus replication. When exposure in patients treated with maraviroc (mean unbound Cmin at steady state at the therapeutic dose in humans; 300 mg BID, Module 5.3.4.2 Patient PD and PK/PD Study Reports) is compared to the in vitro unbound drug IC_{90}, the patient exposure is 16-fold higher than the in vitro antiviral values. When consideration of the IC_{50} is made, the patient exposure is 60-fold higher than the in vitro antiviral value.

Maraviroc inhibits replication in vitro of viruses resistant to existing antiretroviral agents that target viral enzymes. This has been evaluated in vitro with a large panel of pseudotyped viruses (200 viruses of which 100 were derived from protease and reverse transcriptase

inhibitor resistant viruses), containing envelope genes (gp120/gp41) derived from well-characterized clinical samples of clade-B and non-B genetic background (CG/016/02). The geometric IC_{50} *n*-fold change (defined as the clinical isolate IC_{50}/JRCSF reference virus IC_{50}) against this panel is 0.69-fold (95% CI, 0.64 to 0.73). The range of susceptibilities to maraviroc is narrow, as illustrated by an estimated biological cut-off (=geometric mean *n*-fold change + 2 standard deviations [SD]) of 1.72 fold. This value compares well with biological cutoffs for existing antiretroviral drugs tested in a similar pseudotyped-virus assay, using recombinant RT/protease derived from patient plasma (Parkin et al, 2004). The distribution of the response to maraviroc is also tighter than that reported for the fusion inhibitor, enfuvirtide, which displayed a biological cutoff of 7.5 in the same assay when tested against baseline samples of patients enrolled in the "TORO" phase 3 clinical trials (Greenberg, 2004; Melby, 2006).

There is no difference in the susceptibilities of clade B viruses and non-B viruses to maraviroc. An earlier reported CCR5 antagonist, SCH-C, showed potent antiviral activity against most R5 strains but was poorly active against representatives from clade G (Strizki et al, 2001). The difference in spectrum between maraviroc and SCH-C may indicate that different CCR5 antagonists have distinct binding patterns for CCR5, presenting the co-receptor to HIV-1 in diverse structural states. There is 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 ($p < 0.001$; Module 2.6.2. Pharmacology Written Summary, Figure 2, Section 2.6.2.2.1.8). Given the large window between in vitro efficacy and steady-state unbound Cmin levels achieved in HIV-1 infected patients, this small difference is unlikely to be of clinical relevance, supporting the use of maraviroc in patients harbouring virus strains resistant to existing antiretrovirals.

Maraviroc is also active in vitro against virus strains resistant to the fusion inhibitor, enfuvirtide. This was demonstrated during the clinical phase of the programme, when 38 patient-derived viruses (half of which harboured resistance to enfuvirtide) were all shown to be susceptible to maraviroc. This study is described in detail in Section 5.3.5.3 (Microbiology Review of Resistance/Tropism in Maraviroc Program).

Maraviroc-resistant (MVCres) virus has been selected by serial passage. The characteristics of these MVCres variants are that they remain CCR5-tropic, are sensitive to other entry inhibitors and contained 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).

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

As a consequence of binding to CCR5, maraviroc has pharmacological effects that are not related to its antiviral activity. Thus maraviroc blocks binding of endogenous human CCR5 chemokines and acts as an antagonist of subsequent signaling processes. The interaction of maraviroc with homologous CCR5 receptors from various animal species was assessed in order to determine the likelihood that CCR5 mediated effects might be observed in

toxicology studies. The potential implications for the safe human use of maraviroc as a result of these antagonistic effects are largely theoretical and a review of relevant literature is included (Section 1.8.2 Risk Management Plan).

2.4.2.3.1. Antagonism of Endogenous Ligand Binding

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. 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 (DI/002/01). In addition, the IC_{50} for maraviroc was unaffected when the concentration of radiolabelled MIP-1β was increased from 0.005 to 100 nM (IC_{50} = 2.7 to 6.1 nM) suggesting that the inhibition of chemokine binding was insurmountable under these conditions and that circulating chemokines are unlikely to affect the binding of maraviroc to CCR5 (DI/003/1).

2.4.2.3.2. Effect of Maraviroc on CCR5 Mediated Signalling

The antagonism by maraviroc of CCR5 mediated signalling was investigated by two different functional outcomes. Maraviroc was shown to inhibit chemokine induced intracellular calcium release (IC_{50} range 7-30 nM; DI/005/01) and changes in intracellular cAMP levels (IC_{50} 9.4 nM; CG/001/05) in human cells. In the latter assay maraviroc showed evidence of insurmountable MIP-1β antagonism. Insurmountable antagonism can occur by a variety of mechanisms, which include irreversible antagonism, non-competitive antagonism, and antagonism with a very slow rate of receptor dissociation. When an antagonist dissociates very slowly from a receptor, a state of hemi-equilibrium results (Kenakin, 1984) and the antagonist can appear to behave as an irreversible blocker. The data shown here lead us to conclude that the most likely explanation for this insurmountable profile relates to the slow rate of maraviroc dissociation from the human CCR5 receptor and the state of hemi-equilibrium that results. 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 (CG/001/05).

Both intrinsic antiviral potency and prolonged receptor occupancy are believed to be important factors that contribute to the antiviral efficacy of CCR5 antagonists in clinical trials.

Maraviroc is not itself a CCR5 agonist; the observed reduction of basal GTP-γ-S binding (DI/108/06) may indicate that maraviroc acts as an inverse agonist to promote the formation of CCR5 in an inactive state. However, this effect was not readily apparent in the cAMP reporter assay, except at the highest concentration of maraviroc tested. The consequences of partial or altered CCR5-mediated signaling cannot be reliably predicted from any known CCR5 genetic variations and therefore the outcomes of such an effect will only become evident in toxicology and clinical trials.

The considerable structural differentiation between maraviroc and previously reported CCR5 antagonists underpins their different pharmacological and pharmacokinetic behaviours. Maraviroc is a basic compound (tropane), which shows a pan-CCR5-chemokine blockade for both binding and signaling inhibition. This is in contrast to aplaviroc (a zwitterionic spirodiketopiperazine, Maeda et al, 2004), which does not inhibit RANTES binding to CCR5. Nevertheless, aplaviroc did inhibit RANTES induced calcium responses (Watson et al, 2005) and chemotaxis and receptor internalization (Maeda et al, 2004, figure 7).

2.4.2.3.3. Affinity for Relevant Non-Human Species' CCR5

To support subsequent toxicology testing, and in particular the assessment of potential immunotoxicology, the interaction of maraviroc with various animal CCR5s was studied. Direct maraviroc binding and / or functional experiments were conducted with mouse, rat, dog and macaque recombinant CCR5. It has been shown that the compound has no functional interaction with recombinant mouse CCR5 receptors at concentrations up to 1 μM (DI/092/06). 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 for the human receptor (DI/002/01) and demonstrates at least 1,400-fold lower affinity of maraviroc for the rat and dog CCR5 receptors.

Maraviroc has a similar K_D at both the human and macaque CCR5 (0.86 nM and 1.36 nM respectively; CG/015/02); it had a similar IC_{50} for inhibition of MIP-1β binding (7.18 vs 17.5 nM respectively; DI/002/01 & CG/011/02) and a similar inhibition of cellular signalling as determined by MIP-1β induced intracellular calcium release (14 nM vs 17.5 nM respectively; DI/056/04 & CG/009/02). Further kinetic studies indicated that maraviroc associated with the macaque CCR5 at a similar rate to the human homolog ($t_{½}$(on) = 8.37 minutes at the macaque receptor vs 11.05 minutes at the human receptor; CG/015/02). However, maraviroc dissociated from the macaque receptor at a markedly faster rate. The $t_{½}$ (off) for the macaque receptor was found to be approximately 1.5 hours whereas for the human receptor it was approximately 16 hours (CG/015/02). Therefore, overall the affinity of maraviroc for macaque CCR5 is similar, although not identical, to that of human CCR5, and the macaque is likely to represent a non-rodent species with a functionally relevant interaction with the compound.

2.4.2.3.4. Potential consequences of CCR5 antagonism derived from the literature

The potential consequences of the functional inhibition of CCR5 mediated chemokine signaling can, in part, be predicted by an analysis of the phenotype of both human individuals with a natural genetic absence of surface-expressed CCR5 and CCR5-knockout mice. Data from the literature, with particular reference to the potential for malignancy and altered susceptibility to infection is discussed in the Risk Management Plan and Module 5.3.5.3, Reports of Analyses of Data from More Than One Study: Special Safety Review of the Immunotoxic Potential of Maraviroc.

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2.4.2.4. Secondary Pharmacodynamics

The following studies were conducted to determine the effects of maraviroc not related to its desired therapeutic target.

2.4.2.4.1. Selectivity Profile against Relevant Human Targets

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.

Maraviroc has no significant inhibitory activity in a range of in vitro assays of immune function, including specific activities mediated by the chemokine receptors CCR1, 2, 2b, 3, 4, 7, 8, CXCR1 and 2, cytokines IL-2, IL-8 and IL-4 and non-specific activities such as antigen-stimulated lymphocyte proliferation (DI/008/01, DI/020/01, DI/021/01, DI/022/01, DI/023/01, DI/024/01, DI/025/01, DI/026/01, DI/027/01, DI/028/01, DI/029/01, DI/030/01). These experiments lead to two main conclusions. Firstly, maraviroc is selective for CCR5 over a wide range of chemokine and cytokine receptors. Secondly, in an immune function assay using CCR5-expressing cells maraviroc had no effect in lymphocyte proliferation in response to exogenous antigen.

2.4.2.5. Safety Pharmacology

The following studies were conducted to investigate the potential undesirable effects of maraviroc on physiological functions in relation to exposure in the therapeutic range and above.

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

Maraviroc up to 10 μM did not display biologically relevant affinity for a range of physiologic receptors, ion channels, and enzymes (CG/013/00, CG/13/02, CG/001/02, CG/009/01, CG/001/03, CG/001/04, IC/001/02, CG/003/05, CG/001/02 and CG/005/05). The exceptions to this were moderate inhibition in the human μ opioid, rat non-selective muscarinic receptor, and the human α_{2A} adrenergic binding assays as discussed below.

2.4.2.5.1.1. Affinity for the μ opioid receptor

Receptor binding studies for maraviroc interaction with the μ opioid receptor gave K_i values of 106-209 nM (CG/013/00, CG/009/01) and a further K_i value of 589 nM in a study in which a relatively physiological buffer system was used (CG/001/03). These observations were followed up by determination of the functional activity of maraviroc in a GTP-γ-S binding assay at the human μ opioid receptor in which a weak agonist effect (22% stimulation) at 10 μM was observed (CG/011/01). However, there was no μ opioid mediated agonist or antagonist activity in the electrically stimulated guinea pig ileum at 1 μM (CG/006/00). Because the weak μ opioid agonist activity occurred at a concentration

representing approximately 34-fold the mean unbound Cmax in HIV-positive patients at a dose of 300 mg BID, it is not considered to be of biological significance.

The literature indicates that there is an interaction between opioid and chemokine receptors, in particular the μ opioid receptor and CCR5. Opiate agonists can induce heterologous desensitization of chemokine receptor mediated leukocyte responses such as chemotaxis (Grimm et al, 1998; Szabo et al, 2003) by receptor heterodimerisation and phosphorylation (Chen et al, 2004). It has also been shown that opiates can up-regulate expression of CCR5 on human astrocytes (Mahajan et al, 2002). These opposing effects were linked to either a decreased or increased propensity for cellular infection by HIV-1 respectively. It is unlikely that clinical usage of maraviroc will influence the interaction between the μ opioid receptor and CCR5, or have any other opioid mediated effect because the weak functional agonist activity at the μ receptor occurred at a concentration representing approximately 34-fold the mean unbound Cmax in HIV-positive patients at a dose of 300 mg BID. In addition, it is unlikely that maraviroc will influence the interaction of exogenous opiates such as morphine or methadone with the μ opioid receptor since the K_i for maraviroc (589 nM; CG/001/03) is higher than for either morphine or methadone (0.6-6 nM; Valenzano et al, 2004; Gillen et al, 2000; Raynor et al, 1995). The absence of a μ opioid receptor mediated activity is reflected in the lack of μ opioid responses in animal studies (Section 2.6.6.9, Toxicology Written Summary).

2.4.2.5.1.2. Affinity for Muscarinic Receptors

The observation that maraviroc binds to the rat muscarinic receptor (CG/013/00, CG/13/02) was followed up with assays of binding to recombinant human muscarinic receptor subtypes M1-M5 (CG/001/02) and with an assay of functional activity in the electrically stimulated guinea pig ileum (CG/006/00). At concentrations up to 10 μM, maraviroc had no significant affinity for the human receptor and was inactive at 1 μM in the guinea pig ileum and it is therefore unlikely that the original finding will have any biological significance.

2.4.2.5.1.3. Affinity for Adrenergic Receptors

Maraviroc was shown to have a Ki of 5.47 μM at the human adrenergic $\alpha 2_A$ receptor (CG/013/02). Maraviroc was also shown to be an antagonist at alpha adrenergic receptors in canine venous tissue (pK_b 5.72 nM vs phenylephrine and pK_b 5.21 nM vs noradrenaline; CG/008/04 & CG/009/04 respectively) giving rise, in vitro, to relaxation of phenylephrine pre-constricted vessels (threshold effect level = 3 μM; CG/004/04). There was no relaxation in vessels pre-constricted with KCl (CG/006/04), indicating that the effect seen with phenylephrine was likely to have been mediated by an alpha adrenergic receptor mechanism.

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 adrenergic receptors was carried out. However, in a variety of preparations, including rat vas deferens,

recombinant human adrenergic receptor cell lines and isolated human saphenous vein, there was no consistent adrenergic mediated activity (CG/003/04, CG/007/04, CG/002/05, CG/004/05, CG/014/02, CG/004/03, CG/004/04, CG/006/04, CG/008/04, CG/009/04 &CG/011/04). Further studies that explore hypotensive observations and potential mechanisms for postural hypotension in vivo are discussed in sections 2.4.2.5.5.1, 2.4.4.3.3 and 2.4.4.3.4. The observations of binding to human α_{2A} adrenergic receptors and relaxation/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.

2.4.2.5.1.4. Effects on Isolated Tissues

In addition to the absence of effects of the isolated guinea pig ileum noted above, maraviroc had no effect on the contraction of smooth muscle when tested at concentrations up to 1μM in the isolated rat uterus (CG/007/01).

2.4.2.5.2. Effects on Central and Peripheral Nervous System

In a rat appearance and behaviour study, orally administered maraviroc caused adverse effects in the form of piloerection, salivation, and increased, irregular and laboured breathing at an oral dose of 2000 mg/kg. A lower dose (1000 mg/kg) produced only mild effects (salivation and transient increase in respiration and decrease in activity). The peak unbound Cmax of maraviroc following the maximum tolerated dose of 1000 mg/kg was 5 μM indicating that concentrations of approximately 17-fold the mean unbound Cmax in HIV-positive patients at a dose of 300 mg BID did not induce marked effects on appearance and behaviour of rats (CG/009/00).

Oral doses of 10 mg/kg had no effect on sodium pentobarbitone induced sleeping times or motor coordination in male mice (CG/005/01 & CG/002/01).

2.4.2.5.3. Effects on Renal/Urinary System

Maraviroc at oral doses up to 60 mg/kg had no effect on the excretion of fluid and electrolytes in saline loaded female rats (CG/004/01).

2.4.2.5.4. Effects on Respiratory System

Following an intravenous dose of 1 mg/kg in male rats there was no effect on respiratory function, however there was a small but statistically significant decrease in mean arterial blood pressure and heart rate shortly after dosing; however, given the magnitude of the changes, this was of doubtful biological relevance (CG/001/01).

2.4.2.5.5. Effects on the Cardiovascular System

The nonclinical safety pharmacology assessment of maraviroc has involved 2 haemodynamic studies in conscious dogs over a range of oral and intravenous doses and three in vitro preparations to assess the potential for causing delayed ventricular repolarization.

2.4.2.5.5.1. Effect on haemodynamic parameters

Oral (unbound Cmax at highest dose investigated = 168 nM; CG/003/00 & CG/008/00) and intravenous (unbound Cmax at highest dose investigated = 1823 nM; CG/007/02) administration of maraviroc to conscious dogs produced a slight but statistically significant ($p<0.05$) reduction in pulse pressure and no effects on basal heart rate compared with vehicle. Therefore, maraviroc concentrations of approximately 6-fold higher than the mean unbound Cmax in HIV-positive patients at a dose of 300 mg BID had no meaningful effects upon basal haemodynamic parameters in dogs.

To attempt to understand the mechanism responsible for maraviroc induced postural hypotension as observed in the clinic, the effect of intravenously administered maraviroc on blood pressure, heart rate, and ECG was studied in a conscious dog postural change model (CG/007/02). In a series of 2-minute postural challenges, maraviroc (at mean unbound plasma concentration of 1227 nM) produced a statistically significant greater fall in mean blood pressure (6.1 mmHg, $p<0.05$) and a non-statistically significant reduction in the initial heart rate increase (reduction of 9.5 bpm) over the first 12 seconds of challenge compared with vehicle treated animals. Although maraviroc did not produce overt postural hypotension in the conscious dog the effects on the initial reflex response may be indicative of the symptoms of postural dizziness seen in humans.

2.4.2.5.5.2. Effects on QT interval

Early compounds in the discovery pathway that led to the identification of maraviroc were potent inhibitors of the human ether a-go-go-related gene (hERG) potassium channel (Wood and Armour, 2005). The function of hERG channels is to conduct the rapidly activating delayed rectifier potassium current (I_{Kr}), which has a key role in the control of cardiac repolarisation (Sanguinetti et al, 1995; Keating and Sanguinetti, 2001). hERG channel inhibition is the predominant cause of acquired long QT interval and has been linked to Torsades de Pointes, a specific type of malignant ventricular arrhythmia that can be fatal and can be manifested as sudden death (Fermini B and Fossa A, 2003). Other CCR5 receptor antagonists such as SCH-351,125 have been shown to prolong the QT interval in man (Esté JA, 2002). hERG channel inhibition has also been reported for some analogues in the Merck series (Shu M et al, 2004). With the need to maintain a high plasma concentration of drug above the viral IC_{90} it is important to establish a significant margin between activity against hERG and systemic exposure.

Maraviroc, at 1 μM, did not inhibit binding of [^{3}H]dofetilide to the hERG channel and was devoid of effects on the canine Purkinje fibre action potential morphology. However, at 3 - 10 μM maraviroc there was a concentration-dependent inhibition of [^{3}H]dofetilide binding (43% inhibition at 10 μM) and of the hERG potassium current (19% inhibition at 10 μM) and prolongation of the Purkinje fibre action potential duration (up to 31% prolongation at 90% repolarisation; IC/001/02, CG/001/00, IC/005/02, IC/006/01, IC/003/02). These results indicate that maraviroc is active at the human cardiac hERG channel with an in vitro threshold for inhibition of the I_{Kr} current and an affect on cardiac repolarisation in vivo at unbound plasma concentrations greater than 3 μM, which is approximately 10-fold higher

than the mean unbound Cmax in HIV-positive patients at a dose of 300 mg BID. The integrated IC_{50} for these effects is >10 μM. From these observations a value for the ratio hERG IC_{50}/free Cmax of at least 33 can be calculated. According to Redfern et al (2003) 'A 30-fold margin between Cmax and hERG IC_{50} may suffice for drugs currently undergoing clinical evaluation ...'. Therefore, whilst at high concentrations (>10 μM) maraviroc will interact with hERG and prolong the QT interval, in vitro data predict an adequate exposure multiple.

Maraviroc caused no statistically significant changes to the QT interval following oral administration to conscious freely moving dogs (CG/003/00 & CG/008/00) at oral doses (1.5 mg/kg) that achieved an unbound Cmax of 168 nM. A further study was conducted in the conscious dog at higher doses of maraviroc (CG/007/02). In this study, intravenous administration of maraviroc (unbound Cmax 1823 nM) produced no biologically relevant effects on basal ECG parameters, except for a modest and non-dose related prolongation of the QT interval with an average increase of 14.5 ms and a maximum increase of 23.1 ms in the absence of any effects on heart rate. Therefore at concentrations approximately 6-fold the mean unbound Cmax in HIV-positive patients at a dose of 300 mg BID, modest prolongation in the QT interval was observed. This finding is consistent with toxicology studies in which maraviroc induced increases in the QTc interval in dogs and monkeys at unbound plasma concentrations of 899 and 1815 ng/mL, which represent exposure multiples of 6- and 12-fold, respectively. It is also consistent with clinical study A4001016 in which the mean maximum difference from placebo in QTcI was less than 4 msec at single doses up to 900 mg.

2.4.2.5.6. Metabolites (UK-408,027 and UK-463,977)

The principal metabolite of maraviroc found in human plasma following administration of radiolabelled parent compound is a secondary amine product UK-408,027 that accounted for 22% of the circulating radioactivity; the parent compound, maraviroc, constituted 42% of plasma radioactivity (This Module, Section 2.4.3.4.2). In addition, a hydroxylated metabolite (11%) which 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.

Since the two structurally diverse metabolites were significantly present in human plasma they were profiled for their affinity for various pharmacologically relevant receptors, enzymes and ion channels. The only UK-408,027 activities greater than 20% inhibition at 10 μM (4065 ng/mL) were against the recombinant human endothelin ETB, 5HT2c and μ opioid receptors (21, 29, and 34% respectively; UK408027/CG/003/04). These effects were observed at a concentration 34-fold higher than the mean unbound Cmax for maraviroc in HIV-positive patients at a dose of 300 mg BID. Because UK-408,027 was present in 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 plasma at about 12% relative

to the parent concentration it is unlikely that this observed activity 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 biologically relevant effects on the tissue at concentrations up to 10 μM.

2.4.2.6. Combination Studies In Vitro

Additive or slightly synergistic interactions are observed when maraviroc is tested in combination with HIV-1 protease inhibitors (PI), nucleoside/nucleotide reverse transcription inhibitors (NRTI/NtRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI) or fusion inhibitors. There is no evidence that maraviroc antagonises the antiviral activity of the currently licensed anti-retroviral agents tested to date. These data support the use of maraviroc in multi-drug regimens for the treatment of HIV-1 infection.

2.4.3. Pharmacokinetics

2.4.3.1. Programme design and methodologies

The nonclinical pharmacokinetic programme for maraviroc comprised detailed single dose studies in mouse, rat and dog to define basic pharmacokinetic parameters. This programme was supplemented by sampling during repeat dose toxicology studies in mouse, rat, cynomolgus monkey and dog to confirm the pharmacokinetics of maraviroc under the actual conditions of safety evaluation.

Bioanalysis of maraviroc was conducted using specific hplc methods with mass spectrometric detection. The sample isolation procedures differed somewhat during the development programme but since all assays were validated in terms of accuracy and precision this does not compromise any comparisons made across species. Specific validated hplc assays with mass spectrometric detection were also developed for measurement of the metabolites UK-408,027 and UK-463,977 across species in plasma and urine.

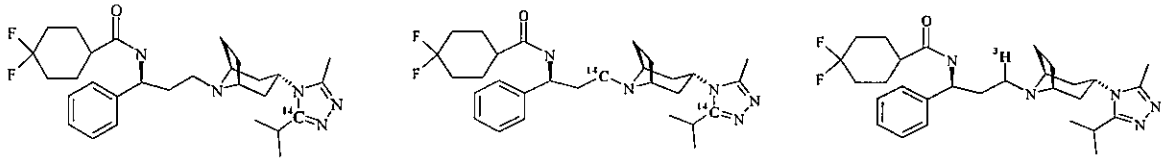
Tissue distribution, metabolism and excretion studies were conducted using three radiolabelled forms of maraviroc - tritium (^{3}H) and both single and dual carbon-14 labelled material (Figure 2). Initial metabolism studies performed with [^{3}H]-labelled compound were ultimately considered incomplete due to the liberation of tritium as a result of N-dealkylation adjacent to the tropane ring. This resulted in a proportion of the radiolabel tracer being unavailable for tracking the metabolic fate of maraviroc. Metabolism studies in vivo were therefore conducted in all toxicology species and human using [^{14}C]-labelled compound (single label). These studies confirmed the N-dealkylation pathway of metabolism resulting in two relatively large product fragments. Radiolabel was, however, only retained in the fragment containing the quinuclidine ring and triazole function. The identity of the non-labelled fragment was investigated by specific analysis and also by in vitro studies utilizing a second [^{14}C]-labelled compound (dual label; Figure 2) where radiolabel was retained in all

fragment products. In vitro metabolism studies were used to support in vivo studies, to characterise the enzymes involved in maraviroc metabolism and to determine the potential for interactions with co-administered drugs.

The human data quoted in this section are derived from a number of clinical studies which are defined in the text.

Figure 2. Structure of [^{14}C]-labelled and [^{3}H]-labelled Maraviroc

[^{14}C]-Maraviroc Single label	[^{14}C]-Maraviroc Dual label	[^{3}H]-Maraviroc



2.4.3.2. Absorption and bioavailability

Following oral administration maraviroc was absorbed rapidly with a Tmax of 2h or less in toxicology species, and 4h or less in human. In the rat, bioavailability was relatively low at 5%. Absorption in this species was judged to be incomplete (20-30%) from hepatic portal vein concentration data. Bioavailability was 40-42% in the dog and absorption was judged to be high based on anticipated first-pass extraction with respect to dog liver blood flow. Unchanged drug was observed in the faeces of all species (ranging from 19% in female dog to 77% in male rat) and may have resulted from biliary excretion and/or incomplete absorption. Similarly, after a 300 mg oral dose unchanged drug in human faeces accounted for 25% of the dose (Study 1010). Absolute bioavailability in human after a 100 mg dose was 23% (Study 1009).

Administration of maraviroc to Mdr1a/b double knockout mice (deficient in both Mdr1a and Mdr1b genes which encode murine P-glycoprotein) gave systemic exposure three-fold higher than that observed in wild-type mice indicating that P-glycoprotein (P-gp) limits absorption. Experiments in Caco-2 cells indicated that maraviroc had limited permeability and showed polarised transport across the cell monolayer. The degree of polarised transport was inhibited by verapamil and CP-100,356, compounds known to inhibit P-gp. The affinity of maraviroc for human recombinant P-gp was confirmed in vitro (K_m=37μM). Escalating dose studies in humans (Study A4001001) showed non-proportionality in terms of Cmax and AUC at all doses studied (1 to 1200 mg). The degree of non-proportionality was highest at doses up to 100 mg and is considered to be as a result of saturation of P-gp-mediated efflux, whereby a greater proportion of the administered dose is able to be absorbed when the concentration in the gut lumen is higher thus leading to saturation of the efflux mechanism. In the same clinical study at doses from 300 to 1200 mg non-proportionality of exposure was

evident but to a lesser degree, indicating that P-gp-mediated efflux had become fully saturated at these higher doses. Based on an estimated fluid volume of 500 ml in the small intestine, an oral dose of 10 mg maraviroc would provide a concentration in the gut lumen similar to the K_m determined in vitro using recombinant P-gp (37 μM). Hence P-gp saturation may be expected to affect absorption at clinical doses greater than 10 mg, in keeping with the observed clinical exposure data. This pattern of non-proportionality was not observed in the toxicology species indicating that P-gp (and potentially other transport proteins) does not restrict maraviroc absorption in the same way in the animal species. It is considered likely that multiple factors contribute to the species differences that are observed, including the generally higher permeability of dog intestinal tract relative to other species and the higher doses (on a weight normalized basis) employed in toxicology compared to clinical studies (Walker, 2005).

Multiple dose pharmacokinetics derived within the toxicology programme indicated minimal change in kinetics upon repeated administration of maraviroc at most doses, thus showing an absence of accumulation or induction. Some modest accumulation (up to 2-fold) was observed at high doses in rat and cynomolgus monkey, probably reflecting saturation of absorption or clearance.

2.4.3.3. Distribution

Maraviroc showed moderate plasma protein binding in all species with an approximate two-fold range in unbound fraction from 0.25 (human) to 0.52 (cynomolgus monkey) (DM23). Binding was independent of sex in the species studied (mouse, dog, cynomolgus monkey) and concentration over the range studied. Maraviroc was shown to bind moderately to both albumin and α1-acid glycoprotein (DM18).

Maraviroc shows some partitioning into red blood cells, with whole blood to plasma ratios of 1.1, 0.9 and 0.7 for rat, dog and human, respectively (DM16). Low partitioning of maraviroc into human red blood cells was confirmed in a set of six blood samples from different donors which provided a mean ratio of 0.6 (DM32). Lower partitioning into red blood cells in humans reflects the higher plasma protein binding relative to other species, which will act to reduce distribution into tissue.

In keeping with its moderate plasma protein binding and basic and lipophilic nature, the volumes of distribution of maraviroc are significantly above total body water (>0.7 L/kg) indicating extensive tissue distribution. Following single intravenous administration of tritium-labelled maraviroc to male pigmented rats, distribution of radioactivity was rapid (DM6). At early time points, most tissues contained concentrations that were several times higher than the blood. At 24 hours after dosing, levels of radioactivity remaining in the tissues were low (<0.6μg equivalents/g tissue), and by 96 hours most tissues were below the limit of quantification. Radioactivity was slowly eliminated from the eye and pigmented skin. This indicates that maraviroc and/or its metabolites have an affinity for melanin which is found in these tissues. This binding affinity is reversible and levels of radioactivity in the eye declined with a half-life of approximately 7 days. At 96 hours, the concentration of radioactivity in the whole eye (0.38 μg equivalents/g tissue) represented 0.07% of the total

administered dose per gram of tissue. Affinity for melanin is a common property of lipophilic bases and has no toxicological significance per se (Leblanc, 1998).

The tissue distribution study indicated low brain penetration of maraviroc, and this was confirmed in a separate study where rats were administered an intravenous infusion of the drug (DM3). In this study, the cerebrospinal fluid (CSF) concentrations of maraviroc were approximately 10% of unbound plasma levels. Poor brain penetration may be a result of P-glycoprotein mediated efflux at the blood brain barrier since maraviroc has been shown to be a substrate of this transporter (DM25).

In a separate study, the distribution of [^{14}C]-maraviroc to gut-associated lymphoid tissues (GALT) was determined in male rats following intravenous administration (DM46). At the two time points studied (1 and 4 h), radioactivity was present in all lymph nodes regardless of anatomical location. Given that the major circulating component in all species is maraviroc (this module, Section 2.3.4.2.) this indicates that drug is likely to be penetrating pharmacologically relevant GALT.

2.4.3.4. Metabolism

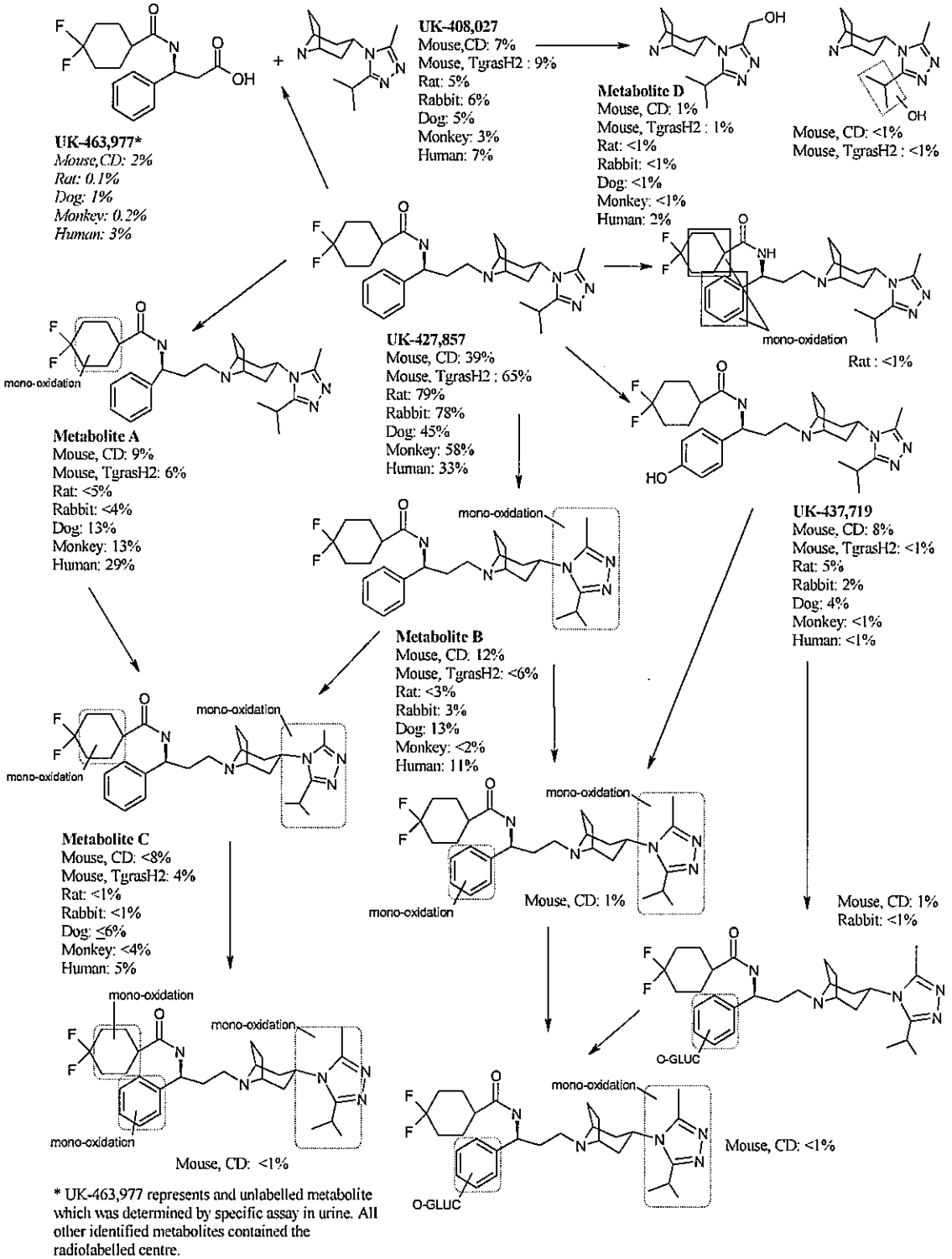
2.4.3.4.1. In vivo metabolism

The metabolism of maraviroc in humans (DM25, DM29, DM43) and toxicology species (DM36, DM37, DM38, DM39, DM40, DM41) has been studied following oral administration of [^{14}C]-labelled drug. Preliminary studies were also performed using [^{3}H]-maraviroc in mouse, rat and dog (DM13, DM14, DM15), but the carbon-14 data are considered to be definitive since they provided a more comprehensive metabolism profile due to some metabolic loss of the [^{3}H] label.

A summary of the proposed human excreted metabolites of maraviroc are shown in this module, Figure 3. In order to simplify the summary scheme, several metabolites have been combined and indicated by boxed structures within the figure (metabolites A, B & C). However, the actual site of oxidation has been fully elucidated using a combination of mass spectrometry and NMR analysis (DM25, DM43).

Unchanged drug was the major excreted component in all species, with combined urine and faecal excretion ranging from 33% of total administered dose in humans to 79% in rat. Metabolism was responsible for the remaining clearance of maraviroc with a high degree of commonality observed across species. The major metabolic pathways in human were oxidation of the methyl group of the triazole moiety (10% of the dose), oxidation in the difluorocyclohexyl ring (four metabolites together accounting for 29% of the dose) and N-dealkylation adjacent to the tropane ring yielding UK-408,027 (7% of the dose). All of these, as well as further minor metabolites (each representing <5% of the dose), were also identified in the excreta of toxicology species (this module, Figure 3).

Figure 3. Excreted metabolites of maraviroc (as% of the dose) following administration of [14C]-maraviroc to mouse, rat, rabbit, dog, cynomolgus monkey and human.



Note: Metabolites A, B & C – the actual site of oxidation has been determined in human samples by a combination of mass spectrometry and NMR (DM25, DM43).

N-dealkylation of maraviroc at the tertiary nitrogen splits the molecule into two relatively large fragments (Module 2.6.4. Pharmacokinetics Written Summary, Figure 3). The secondary amine UK-408,027 contains the radiolabelled centre and was quantified by radiochromatography. Further metabolism of the non-radiolabelled portion could proceed, *via* an aldehyde intermediate, to a carboxylic acid or an alcohol. The carboxylic acid UK-463,977 was quantified in human urine using a specific assay (DM29) and was shown to account for a minor proportion of the total dose (3%). Only trace levels of the alcohol UK-453,465 were detected in human urine. UK-463,977 was also measured in mouse, rat, dog and cynomolgus monkey (DM26, DM27, DM28, DM30) and accounted for <0.1% (rat) to 2% (mouse) of the dose in urine (this module, Table 1). These data demonstrate that a higher proportion of the dose was metabolised to the acid metabolite in human compared with animal species (in common with the amine cleavage product UK-408,027). However, overall exposure (in terms of urinary excretion) at the NOAEL doses in rat and dog was similar to man at the therapeutic dose (300 mg BID), and around 2.5-fold higher in cynomolgus monkey and considerably greater in the mouse.

Table 1. Urinary Excretion of the Acid Metabolite UK-463,977

Species (n)	Dose (mg/kg)	UK-463,977 Urine % dose	Total dose in urine (µg/kg)	Total dose in urine at NOAEL dose (µg/kg)
Mouse (3) DM27	200	1.6	3200	12000*
Rat (2) DM26	100	0.1	100	100
Dog (2) DM30	5	1.2	60	60
Monkey (2) DM28	50	0.2	100	240*
Human (3) DM25**	300 mg	3.1	133	NA

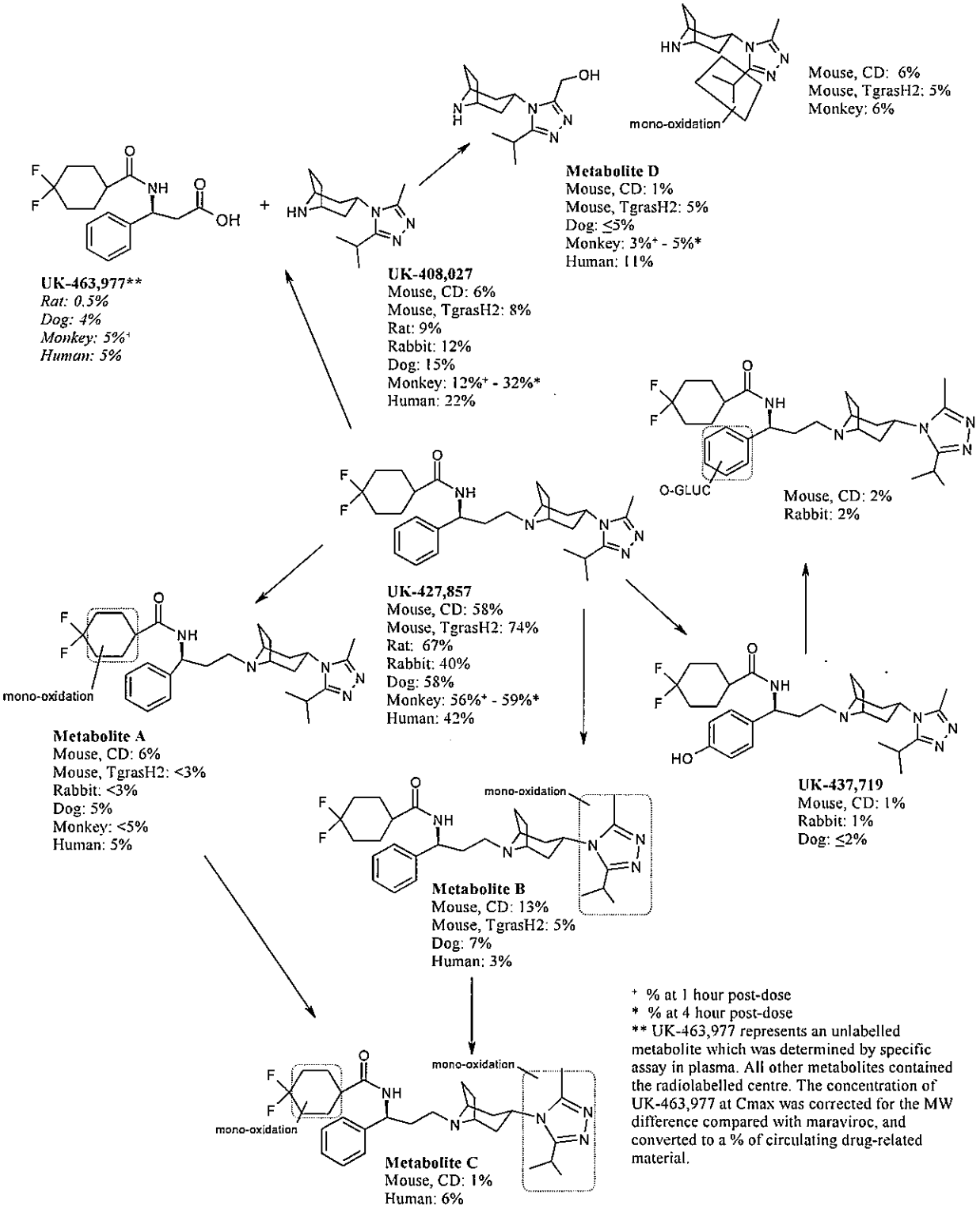
* Extrapolated to NOAEL doses in mouse (750 mg/kg) and cynomolgus monkey (120 mg/kg)
** Clinical Study No 1010
NA = not applicable

2.4.3.4.2. Circulating metabolites

Following oral administration of [^{14}C]-labelled maraviroc, the plasma concentrations of total radioactivity were higher than those of unchanged drug indicating the presence of circulating metabolites.

Analysis of human plasma showed that the major circulating component (as a percentage of the total radioactivity AUC) was unchanged drug which accounted for 42% of the total radioactivity (this module, Figure 4). The only other significant circulating radiolabelled components were two products of N-dealkylation, the amine metabolite UK-408,027 (22%) and a hydroxylated metabolite (11%) which arose by further metabolism of the amine (labelled as metabolite D in this module, Figure 4). Maraviroc remains the most abundant radiolabelled component in plasma even after dialysis, when only unbound concentrations are considered and the effects of plasma protein binding are removed. Unchanged maraviroc was also the major circulating component in the animal species (ranging from 40% in the rabbit to 74% in the TgrasH2 mouse) and UK-408,027 was present in all species at levels >5% circulating radioactivity. Metabolite D was present in mouse, dog and cynomolgus monkey at 1-5% of circulating radioactivity.

Figure 4. Circulating metabolites of maraviroc following administration of [14C]-maraviroc to mouse, rat, rabbit, dog, cynomolgus monkey and human



Note: Metabolites A, B & C – the actual site of oxidation has been determined in human samples by a combination of mass spectrometry and NMR (DM25, DM43).

UK-408,027 does not possess any relevant pharmacological activity based on in vitro pharmacological assessments performed (this module, Section 2.2.6.) and the same is likely to be true for metabolite D which is structurally similar. All other minor human circulating metabolites represented less than 5% of circulating radioactivity and these were present in one or more of the toxicology species (this module, Figure 4).

2.4.3.4.3. In vitro metabolism

Although unchanged drug was the major excreted and circulating component in all species, metabolism is responsible for a proportion of maraviroc clearance. Therefore the in vitro metabolism of maraviroc has been studied using human liver microsomes and recombinant cytochrome P450 enzymes (DM5, DM24). Maraviroc had a moderate clearance in these in vitro systems, with a range of measured half-life values. Further investigations showed that this metabolism was inhibited by the CYP3A4 inhibitor ketoconazole but not by sulphaphenazole or quinidine (CYP2C9 and CYP2D6 inhibitors, respectively). The use of recombinant enzyme systems confirmed a role for CYP3A4 (and its orthologue, CYP3A5) in the metabolism of maraviroc, and showed that neither of the polymorphic P450 enzymes CYP2C19 or CYP2D6 contribute significantly to its metabolism. Furthermore, the formation of the circulating N-dealkylated metabolite UK-408,027 has been shown to be mediated by CYP3A4 (DM35). CYP3A4 is therefore responsible for a large proportion of the metabolism of maraviroc and as a consequence its pharmacokinetics could be altered by co-administration of drugs that inhibit this enzyme. A series of clinical interaction studies have been performed to investigate this possibility (Section 2.7.2.2.6 Summary of Clinical Pharmacology).

The major pathway identified in vivo in humans was N-dealkylation adjacent to the tropane ring. The metabolism of [^{14}C]-labelled maraviroc has been studied in human liver microsomes and hepatocytes (DM42). Dual-labelled material was used in order to investigate the fate of the previously unlabelled portion of the molecule. The in vitro data showed that the N-dealkylation pathway did occur in both human hepatic microsomes and hepatocytes. In microsomes, the previously unlabelled portion of the molecule formed an alcohol, whilst the presence of cytosolic enzymes (such as aldehyde dehydrogenase) in hepatocytes also promoted formation of the carboxylic acid UK-463,977. No other significant metabolites were detected that would have arisen from the previously unlabelled portion of the molecule. In hepatocytes, the alcohol and acid metabolites together accounted for approximately the same amount of radioactivity as UK-408,027, also suggesting that no other metabolic components are formed from the N-dealkylation pathway.

The potential for maraviroc to inhibit the activity of the seven major cytochrome P450 enzymes CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 has been investigated in human liver microsomes, with and without preincubation (DM7, DM22, DM33). Maraviroc did not inhibit any of these enzymes at clinically relevant concentrations (IC_{50} > 30μM against probe substrates for each enzyme). Maraviroc is therefore unlikely to inhibit the metabolism of other cytochrome P450 substrates at clinical doses. A lack of clinical interaction between maraviroc (300 mg BID) and the CYP2D6 substrate

debrisoquine has been confirmed (Section 2.7.2.2.3 Summary of Clinical Pharmacology, Study A4001002).

The ability of maraviroc to induce CYP3A4 metabolism has been investigated in the clinic (Study A4001002) by measurement of the 6β-hydroxycortisol/cortisol ratio. This analysis showed no apparent effect of maraviroc administration and therefore no evidence for CYP3A4 induction at any dose (up to 600 mg) of maraviroc.

2.4.3.5. Elimination and excretion

The systemic pharmacokinetics of maraviroc have been studied in rat and dog following single intravenous administration. In these species, maraviroc has a moderate to high plasma clearance (21 and 74 ml/min/kg, in dog and rat respectively) combined with a moderate volume of distribution (4.3 and 6.5 L/kg for dog and rat). This results in a short elimination half-life of 0.9-2 hours. In contrast the systemic pharmacokinetics in humans after intravenous administration (Study 1009) indicated moderate clearance (11 ml/min/kg) combined with a lower volume of distribution (2.8 L/kg) compared with rat and dog. The reduced volume of distribution in human reflects higher plasma protein binding (76%) compared with rat and dog (51% and 64%, respectively). The elimination half-life of intravenous maraviroc in humans was 12 hours (Study 1009) which compares well with a value of approximately 15 hours estimated from population pharmacokinetic modelling (Section 2.7.2.3.1. , Summary of Clinical Pharmacology, Population Pharmacokinetics of Maraviroc after Oral Tablet Administration – A Pooled Analysis of Phase 1/2a Data). Maraviroc intravenous pharmacokinetics were approximately dose proportional in humans, with systemic clearance being independent of dose.

The excretion of [^{14}C]-maraviroc has been investigated in the toxicology species at doses within the range used during safety evaluation (DM26, DM27, DM28, DM30, DM31, DM34), and in male human volunteers (DM25). Although [^{3}H]-maraviroc was also administered to mouse, rat and dog (DM9, DM10, DM11), the carbon-14 data is considered to be definitive since it provided a more comprehensive metabolism profile. Overall there were no major differences in the qualitative pattern of excretion between species with the major route of elimination being faecal (≥72% of the administered dose). Elimination was also rapid and most of the radioactivity was recovered within 48 hours.

The predominance of faecal excretion across species probably reflects extensive biliary elimination of maraviroc and its metabolites. In an isolated perfused rat liver preparation (DM8), maraviroc showed moderate hepatic extraction (E = 0.4) with significant quantities of unchanged drug excreted in the bile (34% of the administered dose over 90 minutes). In a further study performed in male bile duct cannulated rats (DM17), 64% of intravenously-dosed radioactivity ([^{3}H]-maraviroc) was excreted into the bile over the 6 hour duration of the experiment. The majority of this was unchanged maraviroc. In addition, 15% of the administered dose was secreted directly into the gastrointestinal tract as parent compound. Since maraviroc has been shown to be a substrate of the efflux transporter P-gp (this module, section 2.4.3.3.) this may be mediating secretion of maraviroc into the intestine.

Following oral administration of [^{14}C]-maraviroc to lactating female rats, radioactivity could be measured in the milk for up to 24 hours after dosing at concentrations more than 2-fold higher than those in plasma (DM45). The major drug-related component identified in the milk was maraviroc, which accounted for 86% of total radioactivity. The only other identified minor component was the secondary amine UK-408,027 which represented <5% of total radioactivity. Thus maraviroc is extensively secreted into rat milk and the same is likely to be true for humans.

2.4.3.6. Toxicokinetics

Toxicokinetic data for mouse, rat, dog and cynomolgus monkey are presented as total and unbound maraviroc concentrations in Module 2.6.4.10.2. Pharmacokinetics Written Summary. There was generally no significant difference in the plasma concentrations in male and female animals so these values were combined, although a slightly larger gender difference was noted in TgrasH2 mice. Plasma exposure (defined by Cmax and AUC) increased with dose in all species.

In mice, the increase in exposure was approximately proportional with the increment in dose over the 200 to 750 mg/kg dose range (Study 03012). In rats (Study 911/092), Cmax increased approximately proportionally with dose whereas AUC increased super-proportionally and plasma concentrations were approximately 1.4 to 2.2-fold higher on Day 181 compared with Day 0 (first day of dosing). There was a proportional increase in Cmax and AUC in dogs with increasing dose although systemic exposure was similar on Days 1 and 176 (Study 02073). In cynomolgus monkeys (Study 911/102), the increase in mean AUC and Cmax values was greater than the increment in dose over the 30 to 400 mg/kg/day dose range (as twice daily doses). Systemic exposure was similar over the duration of treatment except for the highest dose group in which the AUC values were 1.5-fold higher from Day 133 and Day 270 compared with day 0.

2.4.3.7. Comparison of exposure in humans vs. animals

The plasma exposure of maraviroc at the maximum tested therapeutic dose (300 mg BID) can be compared to reference doses in animal studies to provide an assessment of safety. Reference doses were defined as the no observed adverse effect level (NOAEL) in mouse (750 mg/kg), rat (100 mg/kg), dog (5 mg/kg) and cynomolgus monkey (120 mg/kg).

The exposure in animals in toxicology studies at the reference dose is compared to the human clinical dose (4.3 mg/kg) in terms of dose, Cmax and AUC24h for both total and unbound maraviroc (this module, Table 2). Since there is a 2-fold variation in unbound fraction of maraviroc across species (0.25 in human to 0.52 in cynomolgus monkey), unbound exposure is considered to be a more relevant comparison (Walker, 2004). At the reference dose, all toxicology species have been exposed to similar or several-fold higher unbound concentrations of maraviroc than humans at the maximum expected clinical dose.

The major pathways of maraviroc metabolism were all represented in the toxicology species (this module, Section 3.5.). Thus the choice of mouse, rat, dog and cynomolgus monkey

were appropriate for the evaluation of the toxicology of maraviroc and are relevant to human safety.

Table 2. Exposure Multiples Based on Dose and NOAEL Plasma Concentrations in Toxicology Species

Maraviroc Toxicology Study	Reference dose (mg/kg)	Multiples over the 300 mg (BID), (total dose 8.6mg/kg) (Study A4001007[a])				
Species and Gender		Dose	Cmax Total	Cmax Unbound	AUC Total	AUC Unbound
Mouse (M + F)	750	87	27	45	41	68
Rat (M + F)	100	12	4.8	9	3.8	8
Dog (M + F)	5	0.6	1.4	2	0.5	1
Monkey (M + F)	120[b]	14	1.9	4	1.3	3

Studies used in the calculation of these multiples were mouse (03012, Day 83), rat (911/092, Day 181), dog (02073, Day 176) and cynomolgus monkey (911/102, Day 270)

[a] Human (Study A4001007, Day 10) mean Cmax=618 ng/mL and AUC_{24h}=5100 ng.h/mL.

[b] 60 mg/kg BID, total daily dose 120 mg/kg.

Table 3 in this module gives a comparison of the measured and estimated plasma concentration (in nM) of all human circulating metabolites with those in animal species at doses with no toxicological consequences. These values have been calculated from the % contribution that each metabolite makes to the overall radioactive plasma profile, and its relative proportion compared with maraviroc in each species. This calculation was performed for doses at which no toxicological events were observed in animals compared with the highest expected clinical dose and shows that at least one other species has been exposed to every human circulating metabolite at higher concentrations.

Table 3. Comparison of Estimated Plasma Concentrations of Maraviroc and Circulating Metabolites Detected in Humans and Animal Species at NOAEL Doses

	Total concentration (nM)*				
Species	Human	Mouse	Rat	Dog	Monkey
Dose Level	300 mg	750 mg/kg	100 mg/kg	5 mg/kg	120 mg/kg
Maraviroc	1203	32204	5803	1709	2245
Metabolite					
UK-408,027	630	3331	780	442	1218
Metabolite D**	315	555	-	147	190
UK-463,977	120	-	8	35	350
Metabolite A**	143	3331	-	147	190
Metabolite B**	86	7218	-	206	-
Metabolite C**	172	555	-	-	-

* Plasma Cmax nM concentration calculated by specific analysis in plasma for maraviroc and UK-463,977. Other metabolites estimated by analysis of the radiolabelled profile for each species after administration of [^{14}C]-maraviroc and the relative proportion of each metabolite (% AUC) compared with maraviroc (assuming dose proportionality with NOAEL dose).

** Human metabolite nomenclature as labelled in this module, Figure 4.

- not detected

The N-dealkylation pathway which gave rise to UK-408,027 in all species also resulted in an unlabelled portion of the molecule (this module, Figure 4). The carboxylic acid, UK-463,977, was measured by specific assay in plasma from rat, dog, cynomolgus monkey and human. The results are summarised in Table 3. UK-463,977 was measured in the circulation of rat, dog and cynomolgus monkey. Plasma samples from mouse were unavailable for assessment, but UK-463,977 was excreted in mouse urine and comprised 2% of the administered dose (this module, Table 1). This value equates to 12000 µg equivalents UK-463,977 per kg following a 750 mg/kg dose of maraviroc, and indicates significant exposure to this metabolite in the mouse at a dose level which showed no toxicological events.

Overall in toxicity studies at doses of maraviroc which caused no toxicological consequences, animals were exposed to concentrations of maraviroc and its metabolites which were similar to or exceeded those which were encountered at the maximum expected clinical dose.

2.4.4. Toxicology

2.4.4.1. Programme Design

The nonclinical safety evaluation programme was carried out predominantly in rats and cynomolgus monkeys and included an assessment of carcinogenicity potential using a 6-month study in transgenic mice and a 24-month study in rats. This programme served to underwrite oral therapy with maraviroc. Additional studies were conducted to assess the involvement of hepatic enzyme induction in thyroid changes observed in rats and to assess the effect of maraviroc on immune function in cynomolgus macaque monkeys. The compound was administered as the base throughout the toxicology programme, and all maraviroc dose levels in this report are expressed as mg/kg of body weight/day. While macaque monkeys in some studies received maraviroc by BID dosing regimen, the dose is expressed as a daily dose. All studies were conducted in accordance with Good Laboratory Practice Regulations.

The bulk lots of maraviroc used in the toxicology studies had impurity profiles that generally embraced those of the compound tested clinically and the proposed commercialized product.

Initially, the mouse was chosen as the rodent toxicology species as this was considered most appropriate in the assessment of immunological function. As maraviroc was known to act on CD4 lymphocytes and was intended for the treatment of immunocompromised patients, immunotoxicology testing was considered important in the safety assessment of the compound, as outlined in the Immunotoxicology Evaluation guidance issued by CHMP and FDA. Sporadic increases in liver enzymes identified during a Phase 1 study (Study A4001002) were not mirrored in early studies in mice, where only modest changes in plasma/serum transaminases occurred at very high doses. In contrast, rats responded with increases in plasma transaminase levels, hepatocellular necrosis and bile duct vacuolation. This data suggested that the rat was a better model for liver toxicity assessment than the mouse and consequently was adopted as the rodent species in the toxicology programme.

After initiating the non rodent toxicology programme in dogs, maraviroc was found to have a higher affinity for CCR5 receptors of macaque cynomolgus monkeys than those of rodents or dogs (Section 2.4.2.3.3). Importantly, receptor affinity in macaque cynomolgus monkeys was similar to that of humans, albeit with a shorter dissociation half-life. The macaque cynomolgus monkey was therefore chosen as the most appropriate non-rodent species in the toxicology programme for evaluating chronic effects and assessing immunological function.

Maraviroc plasma levels were measured in most toxicology studies and are presented in the study reports and tabulated summaries in terms of total concentrations. When comparing plasma exposures across animal species or to humans, unbound plasma exposures are provided, by taking into account plasma protein binding for each species (Section 2.6.4.10.2). Following the publication of CHMP recommendations, control plasma samples from the two carcinogenicity studies were analysed for the presence of maraviroc (CHMP/SWP/1094/04). Although control samples in earlier studies were not assessed for maraviroc, there were no unusual findings in the control groups of these studies to suggest inadvertent administration of maraviroc.

2.4.4.2. Single-Dose Toxicology Studies

Single oral administration of maraviroc to mice and rats produced no effects of treatment at 2000 mg/kg.

Intravenous administration to mice and rats at the dose of 200 mg/kg produced death within 5 minutes of dosing, accompanied by convulsions in mice and dyspnoea in rats. There were no effects of treatment at the lower dose of 20 mg/kg.

These data are broadly consistent with those observed in safety pharmacology studies in rats, in which there were mild effects on appearance and behaviour at the oral dose of 1000 mg/kg and adverse effects characterized by decreased activity, respiratory changes and vocalization at 2000 mg/kg. Changes in respiratory pattern were also seen in the acute intravenous toxicology study. The risk from acute toxicity of maraviroc is judged to be minimal, given the 233-fold multiple of the oral clinical dose (300 mg BID, i.e. 8.6 mg/kg, assuming a 70 kg subject) in toxicology studies, or the 116-fold multiple from safety pharmacology studies.

Table 4. Single-Dose Exposure Multiples in Terms of Oral Dose and Cmax

Species	NOAEL (mg/kg)	Dose multiple	Cmax multiple	Adverse effect	Study
Mice	2000	233	-	None	01-2120-04
Rat	2000	233	-	None	01-2120-06
Rat	1000	116	17	Respiratory changes, decreased activity, vocalisation	CG/009/00[a]
Dog	50	6	5	QT interval increase	01-2120-05
Monkey	400	47	23	Prostration, decreased activity	911/097

-: Plasma drug concentrations were not measured in the single dose toxicology studies

[a] See section 2.4.2.5.2

Information on acute toxicity of maraviroc in non-rodents is available from findings on the first treatment day during repeat-dose studies in dogs and cynomolgus monkeys.

In dogs, the effects observed on the first day of dosing were emesis from 5 mg/kg, skin reddening from 10 mg/kg, protruding nictitating membrane from 15 mg/kg, and red conjunctiva, partially closed eyes, and lacrimation at 250 mg/kg. Cardiovascular measurements on Day 1 indicate systolic blood pressure decreases of up to 60 and 80 mm Hg, respectively, at 50 mg/kg (1/6 dogs) and 250 mg/kg (2/6 dogs) and slight increases (16 msec) in mean QTc interval at 250 mg/kg (Study No. 01-2120-05). The dose of 50 mg/kg represents a 5-fold multiple (in terms of unbound Cmax) over the maximum clinical dose (300 mg BID).

In cynomolgus monkeys, the dose of 800 mg/kg (400 mg/kg BID) produced severe clinical signs appearing 2 or 3 hours after the first or second dose, consisting of reduced activity, prostration, loss of balance and vomiting. Due to their moribund status, the animals were euthanised on treatment Day 2. The cardiovascular changes (recorded 3 hours after the first dose on treatment Day 2) may have contributed to the moribund state. There were decreases in diastolic blood pressure (39 mmHg) and heart rate (139 bpm) and increases in QTc interval (148 msec). The dose of 400 mg/kg (200 mg/kg BID) was not associated with clinical signs or adverse effects of treatment on the first day of dosing. This dose represents a 23-fold multiple (in terms of unbound Cmax) over the maximum clinical dose (300 mg BID).

2.4.4.3. Repeat-Dose Toxicology Studies

Table 5. Repeat-Dose Toxicology Studies

Study No.	Duration	Route	Doses (mg/kg/day)			
Mice						
01017	5-day	PO	500	1000	2000	
01-2120-03	2-week	PO	20	200	1000	2000
02002	1-month	PO	200	500	750	
03012	3-month	PO	200	500	750	
Rat						
02072	1-month	PO	100	300	1500	
911/092	6-month [a]	PO	30	100	300	900
02146	7-day	IV	0.6	2	10	
Dog						
00095	4-day	PO	25	50	100	250 500
01-2120-05	2-week	PO	10	50	250	
02003	1-month	PO	5	50	150	
02073	6-month	PO	5	15	40	
Monkey						
911/089	7 or 8-day	PO	5	15	50	150
911/097	1-month	PO (BID)	100	200	400	800
911/102	9-month	PO (BID)	30	120	400	

[a] with 3-month reversibility

2.4.4.3.1. Mice

The selection of doses in mouse studies was based on toxicity in the 2-week study (mortality and caecal pathology) seen at 1000 and 2000 mg/kg.

In the 2-week study, mice died or were euthanised as moribund at 1000 mg/kg (2/10 mice) and 2000 mg/kg (3/10 mice). These deaths were associated with agonal clinical signs (decreased activity, hunched posture, pale skin or coldness to touch). In the 1- and 3-month studies, there were 2 deaths in each study that could not be attributed to gavage error or handling trauma. In the 1-month study, the deaths (1/sex) occurred at 500 mg/kg (the mid dose), while in the 3-month study, 2 males died at 750 and 200 mg/kg (low and high doses), the latter being from the PK satellite group. The deaths in the two longer term studies were not considered to be treatment-related, given the low incidence, the absence of clinical or post mortem findings and the lack of dose-relationship.

The only treatment-related clinical signs recorded in mice were decreased activity in one 1000 mg/kg female in the 2-week study and partially closed eyes seen occasionally in a few animals at 500 and 750 mg/kg in the 1-month study. Using similar observation time-points, there were no clinical signs in the 3-month study, at higher plasma exposures.

In mice treated for 2 weeks at a maraviroc dose of 2000 mg/kg, there were increases in serum cholesterol in males (36%) and in serum transaminases in 2/10 males (ALT up to 5.2-fold and AST up to 2.1-fold, compared to mean control values). A third male had a 2-fold increase in serum triglyceride. As these changes were not associated with liver histopathology, and as similar increases were absent in the 1- and 3-month studies at higher plasma exposures, these isolated changes were not considered to be treatment-related.

In the 2-week study, there were degenerative changes to the superficial epithelium of the caecum in 4/20 mice at both 1000 and 2000 mg/kg, accompanied in two mice at 2000 mg/kg by increases in leucoytes and fibrinogen. These changes were not observed at 750 mg/kg after either 1- or 3-months treatment (at AUC plasma exposures up to 68 times the expected therapeutic dose). Although the pathogenesis of caecal changes is unclear, degenerative changes affecting only the superficial epithelium are probably a local effect rather than systemic toxicity.

Maraviroc produced no significant alterations in haematological parameters, serum globulins, changes to organ weights or histology of the bone marrow, lymph nodes, spleen or thymus. Similarly there was no increase in the incidence of infections during these studies to suggest impairment of the immune system.

Plasma exposure of maraviroc increased with dose level, was similar in males and females and did not appear to vary greatly in studies up to 3 months duration. The maximum concentrations were observed between 1 and 7 hours after dosing and were still present 24 hours after dosing at doses of 20 mg/kg and above. At the NOAEL of 750 mg/kg, the free plasma Cmax and AUC24h were 45- and 68-fold higher, respectively, than those seen in humans at the maximum therapeutic dose.

2.4.4.3.2. Rats

The selection of doses in rat studies was based on adverse effects identified in shorter-term studies. The high dose of 1500 mg/kg in the 1-month study was chosen following slight decreases in maternal body weight observed in the embryofoetal development study at 1000 mg/kg. Doses in the 6-month study were limited to 900 mg/kg, given the liver changes (centrilobular necrosis and raised plasma transaminases) and decreased body weight found at 1500 mg/kg in the 1-month study.

Maraviroc was well tolerated in rats over a 6-month treatment period and was not associated with mortality. Excessive salivation, which generally began before dosing and continued up to 1 hour after dosing, is probably related to the poor taste of the compound identified in human volunteers (Study A4001032). The sign was rarely seen at 30 mg/kg but increased in incidence at 300 and 900 mg/kg, affecting most animals throughout the treatment period. Salivation did not occur during the 3-month recovery period.

Convulsions occurred in the 6-month study in 2 rats treated with 100 mg/kg and 2 rats treated with 900 mg/kg, as well as in 1 control animal during the reversibility phase. Given the lack of a dose relationship, their relation to treatment is considered unlikely. The absence of convulsions in the single oral dose study at 2000 mg/kg, and at 900 mg/kg in the 24 month carcinogenicity study further suggests that these findings were not associated with maraviroc.

The dose of 900 mg/kg was associated with stained fur in the urogenital region, suggesting a lack of grooming. Hair loss was also observed at this dose, and in a few animals at necropsy, and was considered to be a non-specific sign of stress. During the reversibility phase, hair loss partially recovered and was no longer apparent at necropsy.

Maraviroc produced a toxicologically significant, dose-related decline in body weight in male rats at doses of 900 and 1500 mg/kg. Body weight was reduced by about 11% at 1500 mg/kg from the first week of treatment. In the 6-month study, the dose of 900 mg/kg produced a more gradual reduction in body weight (4% at week 4, with a maximum reduction of 8%), which continued to the end of the recovery period (10%). Doses of 900 and 1500 mg/kg were also associated with a dose-related decrease in food consumption during the first week of treatment. Water consumption was increased at 300 and 900 mg/kg, and was associated with a corresponding increase in urinary volume at 900 mg/kg.

The liver was identified as the principal target organ in rats. In the 1-month study, the dose of 1500 mg/kg was associated with increases in plasma cholesterol (66%) and bile duct vacuolation (minimal to mild). In addition, 2/5 rats had increased plasma transaminase concentrations (ALT up to 7.9-fold and AST up to 3.9-fold, compared to mean control values), accompanied in one rat by mild multifocal hepatocellular necrosis and slight increases in GGT and alkaline phosphatase. In the 6-month study, the dose of 900 mg/kg produced mild increases in plasma cholesterol (45%), ALT (1.6-fold), GGT (3 IU/L) and decreases in triglycerides (51%) and bilirubin (35%); these changes were reversible. Relative liver weight was increased by up to 27% at the end of the treatment period and 14% at the end of the 3-month reversibility period. Histopathology changes in the liver included

altered cell foci (2/30) and multinucleated hepatocytes (13/30), although PCNA staining indicated no evidence of hepatocyte proliferation. Bile duct hyperplasia (minimal) was seen at 300 mg/kg and above, accompanied by decreases in bilirubin, and bile duct vacuolation (minimal to mild) was noted at 100 mg/kg and above. No adverse liver findings were seen at 100 mg/kg (8x the AUC exposure at 300 mg BID). At the end of a 3-month recovery period, bile duct hyperplasia and multinucleated hepatocytes were still present in male rats at 300 and 900 mg/kg, but were completely reversed in female rats.

Thyroid follicular cell hypertrophy was observed in the 6-month study at 300 and 900 mg/kg and was associated with increased TSH and decreased T4 hormone levels. The thyroid changes were reversible 3 months after withdrawing the treatment. No observable effects were seen at 100 mg/kg, at plasma AUC exposures 8-fold higher than found at the maximum therapeutic dose (300 mg BID). Pituitary vacuolation in the pars distalis occurred in 2/5 rats at 1500 mg/kg in the 1-month study. The mechanism responsible for thyroid and pituitary changes in rats is considered to be liver enzyme induction, as demonstrated in an investigative study in which rats were treated at 900 mg/kg for 1 month (Study No. 03165).

Maraviroc produced histopathological evidence of dilated caecum at doses of 300 mg/kg (25x the AUC exposure at 300 mg BID) and above in the 6-month study. Similar findings in the colon and caecum were observed at 1500 mg/kg in the 1-month study and accompanied by diarrhea.

The adrenal glands of male rats at 300 mg/kg and above showed a minimal increase in incidence and severity of cortical vacuolation (zona fasciculata), associated at 900 mg/kg with an increase in organ weight. These changes were reversible after a 3-month recovery period and are considered to represent an exacerbation of a background change present in control rats.

Minimal to mild inflammation of the stifle joint was noted in 1 or 2 animals at 300 and 1500 mg/kg in the 1-month study and also in the fertility study. However, given the absence of this finding in the 6-month or 24-month studies at 900 mg/kg, it is not considered to be treatment-related.

Maraviroc produced no noteworthy alterations in hematological parameters, serum globulins, changes to organ weights or histology of the bone marrow, lymph nodes, spleen or thymus. Similarly there was no increase in the incidence of infections during these studies to suggest impairment of the immune system.

In the 6-month study, plasma concentrations of maraviroc were similar in male and female rats, increased with dose level and were slightly higher at the end of the 6-month study than on the first day of dosing. At doses of 100 mg/kg and above, plasma concentrations were still detected 24 hours after dosing. At the NOAEL of 100 mg/kg, the free plasma Cmax and AUC24h were 9- and 8-fold higher, respectively, than those seen in humans at the maximum therapeutic dose.

2.4.4.3.3. Dogs

The selection of doses in dog studies was based on adverse findings in shorter-term studies. The high-dose level in the 2-week study was limited to 250 mg/kg, given the body weight loss and repeated emesis seen at 500 mg/kg in the 4-day escalating dose study. The repeated emesis at 250 mg/kg necessitated reducing the high dose in the 1-month study to 150 mg/kg. In the 6-month study, the high dose was further lowered to 40 mg/kg (given the multiple bouts of emesis and body weight loss observed at 150 mg/kg in the 1-month study, while 5 mg/kg was retained as the low dose, as it produced only minor clinical signs in the 1-month study.

Emesis occurred from 5 mg/kg, the lowest dose examined and may be associated with the poor taste of the compound. Additional signs were salivation, mydriasis and reddening of the skin and conjunctiva from 10 mg/kg, protruding nictitating membrane and lacrimation from 10 mg/kg and partially closed eyes from 40 mg/kg. Multiple bouts of emesis and body weight loss were observed at 150 mg/kg and are considered to set the maximum tolerated dose in dogs at plasma AUC exposures 28-fold those seen at the maximum therapeutic dose.

Emesis occurred from 15 minutes of dosing and was dose-related in terms of frequency, progressing to multiple bouts of emesis at 15 mg/kg and above. The number of episodes/day tended to decline with continued treatment. Reflux of the dose occurred immediately after dosing in some animals at 50 and 250 mg/kg, with a possible impact on plasma drug exposure.

Salivation was seen in single animals at 5 and 10 mg/kg, and more frequently at doses of 15 mg/kg and above. This was no longer seen 5 hours after dosing and did not appear to correlate with peak plasma drug concentrations. Similar findings were seen shortly after dosing in rats and were probably associated with the poor taste of the compound.

Ocular signs in the dog were dose-related and consisted of reddened conjunctiva, protruding nictitating membrane, lacrimation, mydriasis and partial eye closure (often associated with mydriasis). Reddened conjunctiva may reflect vasodilatation in the conjunctiva. Although the mechanisms responsible for mydriasis, partially-closed eyes and protruding nictitating membrane are unknown, maraviroc has weak binding affinity for human alpha adrenoreceptors (α_{2A} Ki 5.47 μM, 2809 ng/mL), rat muscarinic receptors (39% at 10 μM, 5136 μg/mL) and human μ opioid (Ki 589 nM, 303 ng/mL). However, the absence of pupillary constriction suggests that μ opioid activity is not a contributory factor.

Skin reddening was seen in the 2-week study in all drug-treated dogs on most days and is likely to reflect local vasodilation. The effect was noted within 1 hour of dosing and usually resolved by the end of the day. This observation was not noted in subsequent 1- and 6-month studies.

Lacrimation occurred occasionally from 15 mg/kg, predominantly in the 6-month study. The lack of dose-relationship, and the isolated occurrence suggest that this sign was not related to treatment.

Reductions in systolic blood pressure were noted in the 2-week study, at 50 mg/kg (1/6 dogs) and 250 mg/kg (2/6 dogs); diastolic pressure was not measured. Similar changes did not occur in the 1- and 6-month studies in dogs, where both systolic and diastolic pressures were recorded. Moreover, dogs in the 1-month study were exposed to higher plasma maraviroc concentrations than in the 2-week study. Although the changes in blood pressure lacked consistency, they may reflect a weak signal for hypotension in dogs.

Maraviroc produced no changes in heart rate at 250 mg/kg (2-week) or 150 mg/kg (1-month). Slight increases in heart rate were observed in the 6-month study at doses of 15 mg/kg (up to 19 bpm) and at 40 mg/kg (up to 27 bpm). The reason for these changes is unclear, although they appear unrelated to variations in blood pressure.

A prolongation of QT interval, corrected for heart rate using formulas derived from historical data (Hanton et al 2001), was recorded from doses of 50 mg/kg in the 1-month study (up to 24 msec) and from 15 mg/kg in the 6-month study (13 msec). The increases in QTc interval were consistent throughout the 6-month study and achieved statistical significance at 15 mg/kg and above. In both the 1- and 6-month studies, the dose of 5 mg/kg produced smaller or no changes in QTc interval, which were not statistically significant and not considered to be treatment-related. There was no evidence of cardiac arrhythmias up to doses of 250 mg/kg. In dogs, changes in QTc interval occurred at Cmax exposures 8-fold those seen at the therapeutic dose (300 mg BID), while no QTc effects were noted at exposures 2-fold those of the therapeutic dose.

Maraviroc produced no alterations in hematological parameters, serum globulins, changes to organ weights or histology of the bone marrow, lymph nodes, spleen or thymus. Similarly there was no increase in the incidence of infections during these studies to suggest impairment of the immune system.

In these studies, dogs were exposed to plasma concentrations of maraviroc that increased with dose, showed no gender difference and generally remained stable over the dosing period. At the NOAEL of 5 mg/kg (based on changes on QTc interval at 15 mg/kg), the free plasma Cmax was 2-fold higher and AUC24h was equal to those seen in humans at the maximum therapeutic dose.

2.4.4.3.4. Monkeys

The selection of doses in monkey studies was based on findings in shorter-term studies. The high dose (150 mg/kg) of the 7-day range-finding study produced no adverse effects. Consequently in the 1-month study the high dose was raised to 800 mg/kg/day, given as two daily administrations about 7 hours apart. The BID-dosing regimen was considered necessary as in vitro receptor binding offset is faster in Cynomolgus monkeys (half-life 1.5 hours) than in humans (half-life 16 hours). However pharmacokinetic analysis in the 9-month study at doses of 120 and 400 mg/kg showed that plasma maraviroc concentrations remained above the saturation of in vitro monkey CCR5 receptors (0.7 ng/mL) throughout the 24-hour period. A daily dose of 800 mg/kg was not tolerated, and so in this study 400 mg/kg became the

effective high dose. In the 9-month study, the daily high dose level of 400 mg/kg was retained from the 1-month study.

The daily dose of 800 mg/kg in the 1-month study provoked severe clinical signs (prostration, loss of balance, reduced activity and vomiting). For ethical reasons, the animals of this group were euthanised on the second day of dosing. At the dose of 400 mg/kg, moderate treatment reactions were observed throughout the 9-month study comprising subdued behaviour/reduced activity (29% of treatment days), prostration (32% of treatment days), and half-closed eyes (44% of treatment days). The absence of these findings at 400 mg/kg in the 1-month study was probably due to a slightly lower (25%) plasma Cmax exposure at steady state, reflecting inter-animal batch variation in pharmacokinetics. Animals given 120 mg/kg/day appeared subdued or had reduced activity and/or half-closed eyes on only a few occasions (1% of treatment days). These signs occurred rarely in animals treated with 30 mg/kg/day (≤0.5%). Liquid feces and vomiting were noted in animals from 30 mg/kg/day. Overall, the clinical signs in the 9-month study were considered toxicologically significant at 400 mg/kg/day only.

From month 4 of treatment, body weights were noticeably lower in 120 and 400 mg/kg males, and at the end of the study were 8% and 11% lower, respectively, than control animals. The effect on body weight is considered toxicologically significant at 400 mg/kg. Food consumption was not affected by treatment.

Maraviroc produced a dose-related reduction in systolic and diastolic blood pressure, when comparing measurements before and after dosing. In the 1-month study, daily doses of 100, 200, 400 and 800 mg/kg resulted in maximal decreases in diastolic pressure of 0, 22, 32 and 39 mmHg, respectively (mean values). The numbers of animals in each group of 4 monkeys having a reduction in blood pressure of ≥25 mmHg also increased with dose (0, 2, 4, 4, compared to 1 control animal). These findings were consistent with those in the 9-month study. The dose of 400 mg/kg produced decreases in systolic and diastolic pressure throughout the 9-month study (maximal changes of 28 and 24 mmHg, respectively) that attained statistical significance on 2/4 occasions. At the dose of 120 mg/kg, changes in systolic and diastolic pressures were neither consistent nor statistically significant (maximal reductions of 25 mmHg for each parameter). The blood pressure reduction on this occasion was considered artefactual, and attributed to two animals with unusually high systolic and diastolic pressures prior to dosing (compared to concurrent controls). No treatment effects on blood pressure were evident at 100 and 120 mg/kg in the 1- and 9-month studies, respectively. These doses were associated with Cmax values (716 and 719 ng/mL, respectively) which were 5-fold higher than that at the maximum therapeutic dose in humans.

Maraviroc reduced heart rate at daily doses of 400 mg/kg or higher (about 11-fold greater than the Cmax exposure in humans at 300 mg BID), but produced no changes in heart rate at doses of 200 mg/kg (1-month) or 120 mg/kg (9-month). The dose of 400 mg/kg, produced maximal reductions in heart rate of 107 and 84 bpm in the 1- and 9-month studies, respectively. The higher dose of 800 mg/kg reduced heart rate by 139 bpm.

QT interval prolongation (corrected for heart rate using Fridericia's formula) was recorded at daily doses of 400 and 800 mg/kg. The dose of 400 mg/kg resulted in mean QTc increases of 23 and 95 msec at the start and end of the 1-month study. Smaller increases (34, 18, 10 and 7 msec) occurred at the start and after 3, 6 and 9 months treatment in the 9-month study. An increase in QTc interval (46 msec) was also noted in one animal at 200 mg/kg (1-month study). There was no evidence of QTc changes at the dose of 120 mg/kg (9-month study). None of the maraviroc doses studied was associated with cardiac arrhythmias.

In the 9-month study, red blood cell parameters (red blood cell count, haemoglobin and packed cell volume) were slightly reduced (11-14%) in animals receiving 400 mg/kg/day at 3 months treatment. Smaller, occasional changes were also seen in males after 6 and 9 months of treatment.

Serum triglycerides were increased in males at 400 mg/kg by about 4-fold. There was also a trend towards a marginally elevated ALT in treated animals at 400 mg/kg, although the values remained similar to those of untreated animals and were not considered to be biologically important.

Urinary proteins were detected at trace-to-moderate levels (i.e. 0.3-1 g/L) in urine samples from animals treated with 400 mg/kg/day (and in one animal at 200 mg/kg/day), using a semi-quantitative analysis (Multistix strips). However a quantitative assessment of urinary protein using a colorimetric method with Pyrogallol red indicated no effect of treatment. A follow-up in vitro study was conducted in which maraviroc was added to urine samples to clarify this discrepancy. The results showed direct interference of maraviroc with the evaluation of urinary protein when using the semi-quantitative method. A similar artifactual positive protein response may arise during human urine testing. Although it is unlikely that the drug concentrations in the urine will be sufficiently high in man for this to occur, it is recommended that a quantitative assessment is used to confirm any positive urinary protein findings before instigating further assessments.

Maraviroc produced no alterations in circulating white blood cell parameters, serum globulins, changes to organ weights or histology of the bone marrow, lymph nodes, spleen or thymus. Similarly there was no increase in the incidence of infections during these studies to suggest impairment of the immune system.

There were no consistent differences in plasma drug concentrations between male and females. Highest plasma concentrations of maraviroc generally occurred 1-hour post dose up to 200 mg/kg, generally at 3 hours post dose at 400 mg/kg and at 7 hours post dose at 800 mg/kg. For this reason, cardiovascular measurements were taken 1 hour after dosing at daily doses below 400 mg/kg, and 3 hours after dosing at daily doses of 400 mg/kg and above. The exposure (AUC24h) increased with dose level in a superproportional manner and remained stable throughout the treatment period, except at 400 mg/kg where AUC exposure was about 50% higher at months 4 and 9 than at the start of the study. At the NOAEL of 120 mg/kg, free plasma Cmax and AUC24h were 5- and 3-fold higher, respectively, than those seen in humans at the maximum therapeutic dose.

2.4.4.4. Genotoxicity

Maraviroc has been evaluated in in vitro and in vivo tests designed to detect genotoxic activity. Maraviroc did not display mutagenic activity in a bacterial test system when tested up to cytotoxic concentrations in either the absence or presence of exogenous metabolic activation.

Chromosomal damage was not observed in human lymphocytes when maraviroc was tested up to cytotoxic concentrations in both the absence and presence of metabolic activation. Chromosomal damage was also absent in the bone marrow of male and female mice treated orally with maraviroc at a maximum practical dose of 2000 mg/kg/day for 3 days.

Thus, maraviroc did not display mutagenic activity in bacterial and mammalian cells in vitro or clastogenic activity in vitro or in vivo.

2.4.4.5. Carcinogenicity

Table 6. Range-Finding and Definitive Carcinogenicity Studies

Study No.	Duration	Route	Doses (mg/kg/day)			
Rat						
2003-0446	104-week	PO	50	100	500	900
Mice						
2003-0392	4-week	PO	500	1000	1500	
2004-0091	6-month	PO	200	800	1500	

2.4.4.5.1. Rats

Maraviroc was administered by oral gavage to rats (60/sex/dose) for 24 months (104 weeks) at daily doses of 0, 50, 100, 500 or 900 mg/kg. The female groups were terminated after 96 weeks of dosing due to mortality in vehicle control females. The high dose of 900 mg/kg was selected as the maximum tolerated dose in rats, based on a reduction in body weight and histopathological changes in the liver in the 6-month study.

Systemic exposure in the carcinogenicity study increased with dose over the dose range for both male and female rats, with no major sex-related differences observed. Treatment with maraviroc for 104 weeks (males) and 96 weeks (females) had no effect on survival. Of the 60 rats/sex/dose, the proportion of surviving animals at the end of the treatment period was 32-47% for males and 33-50% for females.

Dose-related decreases in body weight were observed in males at doses of 500 and 900 mg/kg (up to 12% and 18%, respectively), and in females at 900 mg/kg (up to 8%), compared to controls. This provided evidence of toxicity in male rats at the two higher doses and an appropriate choice of the high dose in this study. Food and water consumption were increased at these dose levels.

An increased incidence of follicular cell adenoma of the thyroid was noted in males and females at 900 mg/kg. This was accompanied by a dose-related increase in follicular cell hyperplasia and hypertrophy at doses from 100 mg/kg in males and from 500 mg/kg in females. Previous studies have demonstrated a relationship between thyroid function as a result of treatment with maraviroc and histopathological findings in the thyroid gland (Study Nos. 911/092 and 03165). These thyroid changes are considered to be indirect effects of hepatic enzyme induction and are not interpreted to be a risk for human patients (Davies, 1993).

Other tumours having a higher incidence in treated than in control groups were liver cholangioma in one 500 mg/kg female, and cholangiocarcinoma, basal skin cell adenoma, Leydig cell adenoma of the testis, and parathyroid adenoma in males at 900 mg/kg (2 males affected for each neoplasm). While cholangiocarcinoma is a rare tumour, the absence of pre-neoplastic changes, the low incidence in this study, together with similar incidence in reported control data and in a control group of a concurrent study at the same laboratory together suggest that they are most likely of spontaneous origin and unrelated to treatment. As the incidence of the other tumours in this study was similar to those reported in the Registry of Industrial Toxicology for Animal data (RITA), they were considered to be spontaneous and incidental.

In conclusion, the treatment produced decreases in mean body weight in males at 500 and 900 mg/kg and in females at 900 mg/kg, and caused thyroid follicular cell adenoma at 900 mg/kg, associated with adaptive liver changes. At plasma AUC exposures 21-fold higher than those found in humans at the maximum therapeutic dose, there was no indication of carcinogenic potential for humans.

2.4.4.5.2. Mice

A 6-month carcinogenicity study in CB6F1/Jic-Tg(rasH2) mice was selected to supplement a 2-year carcinogenicity study in Sprague-Dawley rats. The Tg(rasH2) mouse has been extensively tested using reference compounds and has been shown to detect both genotoxic and nongenotoxic carcinogens (Morton et al, 2002; Takaoka et al, 2003). As indicated in the ICH S1B guidelines, this animal model is considered an acceptable alternative to the 2-year mouse bioassay for carcinogen hazard identification of pharmaceuticals (MacDonald et al, 2004). Pharmacokinetic data with UK-427,857 in the TgrasH2 mouse suggest that this model is appropriate.

Nonpivotal

CB6-Tg(rasH2) hemizygous mice received maraviroc orally at daily doses of 0, 500, 1000, or 1500 mg/kg for 4 weeks to select doses for a 6-month carcinogenicity study. The doses for the nonpivotal study were limited to 1500 mg/kg based on mortality seen at 1000 and 2000 mg/kg in CD1 mice. Additional groups of hemizygous mice and wild type mice were used for pharmacokinetic measurement of maraviroc. Mice had plasma maraviroc concentrations that increased with dose. Exposure to maraviroc was similar in hemizygous

and wild type mice and showed no gender difference, although variability in individual animal plasma concentration data was observed, primarily at 1500 mg/kg.

Maraviroc was well tolerated with no clinical indications of toxicity observed. There were no biologically significant changes in body weight, but mild to moderate increases in total body weight gain over the 4-week treatment period were recorded at doses from 1000 mg/kg, with moderate increases in food consumption in males at 1500 mg/kg. Serum total cholesterol values were slightly increased from 1000 mg/kg. The treatment induced no histopathological evidence of target organ toxicity.

Pivotal

CB6-Tg(rasH2) hemizygous mice received maraviroc orally at daily doses of 0, 200, 800, or 1500 mg/kg for 6-months to assess the carcinogenic potential of the compound. While there were no dose-limiting findings in the non-pivotal study, the dose of 1500 mg/kg was selected for the high dose as the free plasma exposure (AUC24h) in male and female mice respectively, was 39- and 70-fold greater than the exposure in humans (1.275 μg.h/mL) at the maximum therapeutic dose (300 mg BID). ICH guideline S1C(R) indicates that for non genotoxic compounds, as is the case for maraviroc, high doses achieving exposures 25-fold greater than found in humans are generally adequate for carcinogenicity testing. A positive control group received a single intraperitoneal injection of the carcinogen N-methyl-N-nitrosourea (MNU) at a dose of 75 mg/kg. Additional groups of wild type mice were used for pharmacokinetic measurement of maraviroc.

Plasma concentrations of maraviroc increased with dose level and appeared higher in females than in males at the mid and high dose levels. Although parent maraviroc was detected in some control plasma samples, analysis of parent/metabolite levels suggest that this finding is consistent with an ex vivo source of maraviroc contamination in the control plasma samples.

At the end of the study, survival in the control and maraviroc treated groups ranged from 96-100% in males and from 92-100% in females, while survival in the positive control group was 20% in both male and female mice. Maraviroc was well tolerated and there were no treatment-related clinical signs. At 1500 mg/kg, treatment was associated with a slight decrease in mean body weights and food consumption in males, relative to controls, and slightly increased food consumption in females. Also at this dose, there were minor decreases in red blood cell parameters and slight increases in white blood cell counts. In the liver, there was a higher incidence of minimally increased glycogen storage, in most maraviroc-treated groups, relative to controls; this finding was not considered to be toxicologically significant. There were no significant differences in the nature or incidence of hyperplastic or neoplastic microscopic findings in any tissue/organ in maraviroc treated mice compared to control animals. All animals of the MNU-treated positive control group had microscopic findings indicative of neoplastic changes. Squamous cell carcinoma and lymphoma were the most common malignant tumors. Retinal atrophy (loss of photoreceptor cells) was also a common and expected finding in the MNU-treated mice.

In conclusion, the oral exposure to maraviroc at dose levels up to 1500 mg/kg for 6 months did not cause hyperplastic, neoplastic, inflammatory, or degenerative changes in rasH2 transgenic mice. The high dose corresponds to plasma AUC exposures at least 39-times higher than found in humans at the maximum therapeutic dose.

2.4.4.6. Reproduction and Developmental Toxicity

Table 7. Reproduction and Developmental Studies

Study No.	Study Type	Species	Treatment Period (days)	Doses (mg/kg/day)			
02132/02133	Oral fertility and early embryonic development	Rat	M: 29ac – 14 pi F: 15 ac –7 pi		100	300	1000
02005	Oral preliminary foetotoxicity study	Rat	6-17 pi		200	500	1000
02025/02028	Oral embryo-foetal development study	Rat	6-17 pi		100	300	1000
01096	Oral escalating dose study	Rabbit	1 to 5	125	250	500	1000
02004	Oral preliminary foetotoxicity study	Rabbit	7-19 pi		50	150	300
02026/02029	Oral embryo-foetal development study	Rabbit	7-19 pi		30	75	200
02-2120-10	Pre- and post-natal development study	Rat	6 pi – 20 pp		100	300	1000

ac: ante-coitum; pi: post-insemination; pp: post partum

2.4.4.6.1. Fertility

A fertility study was conducted to evaluate the effects of maraviroc on mating performance, the fertility of adult male and female rats and the development of the embryos during the pre and post implantation stages. A high dose of 1000 mg/kg was selected given the liver histopathology (centrilobular necrosis) and decreased body weight and food consumption found at 1500 mg/kg in the 1-month study in rats and the slight maternal toxicity (body weight and food consumption) at 1000 mg/kg in the embryofeotal development study in rats.

Changes in plasma chemistry parameters were expected from repeat-dose studies in rats and included slight decreases in triglycrides (from 100 mg/kg) and bilirubin (from 300 mg/kg), and mild increases in AST and ALT (from 300 mg/kg). There were also mild increases in total and HDL-cholesterol values in males. At 1000 mg/kg, the treatment induced minimal toxicity in males, shown as a decrease in body weight and diarrhea, and in females, as an increase in pre-implantation loss, with a consequent smaller number of implants and viable fetuses, compared to controls. Male and female rats receiving maraviroc showed no adverse effects on mating performance or fertility. There were no treatment changes on the estrus cycle, pre-coital time or on copulation and pregnancy rates. Sperm count in the epididymis or sperm motility was unaffected by the treatment.

The NOAEL for adult male and female rats was 300 mg/kg. There were no effects on fertility up to 1000 mg/kg in either sex.

2.4.4.6.2. Foetotoxicity

In the preliminary study in pregnant rats, maraviroc doses of 500 and 1000 mg/kg were associated with transient decreases in food consumption and in corrected maternal body weight gain, considered to represent slight maternal toxicity. There were no maternal effects at 200 mg/kg and no signs indicative of foetal toxicity up to 1000 mg/kg.

Dose levels of 100, 300 and 1000 mg/kg were chosen for the foetotoxicity study, taking into account the slight maternal toxicity in the preliminary study. The high dose again produced slight maternal toxicity, characterized by a transient decrease in food consumption and a moderate decrease in corrected maternal body weight. There was no effect on reproductive parameters or on embryofoetal development and growth. Consequently the NOAEL was 300 mg/kg for pregnant females and 1000 mg/kg for fetuses. Analysis of drug concentrations in maternal and fetal plasma demonstrates placental transfer of maraviroc in rats.

In a pilot study in non-pregnant rabbits, maraviroc produced deaths and marked clinical signs at doses 500 mg/kg and above. These signs of severe toxicity helped to set doses in the preliminary study in pregnant rabbits at 50, 150 and 300 mg/kg. Treatment at 300 mg/kg provoked the death of two rabbits, a marked reduction in food consumption and sustained body weight loss. At 150 mg/kg, one rabbit died with no premonitory clinical signs or macroscopic findings.

As a result of maternal toxicity in the preliminary study, doses of 30, 75 and 200 mg/kg were chosen for the foetotoxicity study. The treatment induced deaths at 200 mg/kg (6/20 females died in the main study, and 4/5 in the toxicokinetic study). Mean food consumption was slightly reduced at 200 mg/kg. There was no effect of the treatment on reproductive parameters or on the foetuses. The few external anomalies observed in fetuses at 200 mg/kg have been previously observed in untreated rabbits. The NOAEL was 75 mg/kg for pregnant females and 200 mg/kg for the fetuses. Maternal and fetal plasma concentrations of maraviroc increased with dose and demonstrated transplacental passage of the drug.

2.4.4.6.3. Pre and post natal development

In order to investigate effects in rats during the prenatal period, parturition and postnatal development of the offspring, maraviroc was administered at doses levels of 100, 300 and 1000 mg/kg from Day 6-post coitum until the end of lactation. The high dose was established from the slight toxicity to pregnant female rats seen in the preliminary study, in which 500 and 1000 mg/kg were associated with a transient decrease in food consumption and a moderate decrease in corrected maternal body weight gain.

In the pre and postnatal development study, the dose of 1000 mg/kg was associated with maternal toxicity in terms of reduced body weight and food consumption. There were no reproductive effects. The only effect on the F1 pups was a slight increase in motor activity noted in 1000 mg/kg male rats at both weaning and as adults. There was no effect on F1 female motor activity and no evidence of increased activity in other behavioral evaluations.

While of uncertain toxicological relevance, a potential relationship between treatment and the increased motor activity for the 1000 mg/kg male offspring cannot be dismissed. There was no effect on sexual maturation or reproductive competency of the F1 offspring.

Based on reduced body weight and food consumption, the NOAEL for F0 females was 300 mg/kg and for reproduction was 1000 mg/kg. The NOAEL for F1 developmental toxicity was 300 mg/kg, given the increased motor activity in F1 males at 1000 mg/kg.

2.4.4.7. Other Studies

2.4.4.7.1. Immunotoxicity study in monkeys
(Study No. 911/096)

As CCR5 receptors are present on various cells of the immune system, a study was conducted to assess the immunotoxic potential of maraviroc in cynomolgus monkeys. Maraviroc was administered to cynomolgus monkeys for 4 weeks at doses of 0, 30, 100 and 300 mg/kg/day, administered BID. At 300 mg/kg, there were moderate effects of treatment (reduced motor activity and body weight), as outlined earlier (section 2.4.4.3.). These clinical effects were considered responsible for minimal to mild thymic atrophy observed at 300 mg/kg. Occupancy of CCR5 receptors by maraviroc was shown to be complete at all time points at 300 mg/kg and almost complete over 24 hours at 30 mg/kg. Treatment with maraviroc had no effect on lymphocyte subset counts and produced no impairment of Natural Killer activity, the percentage of phagocytic cells (Phagotest) or the oxidative activity (Bursttest) per phagocyte. In addition, there were no treatment-related changes in the primary (IgM) and secondary (IgG) immune response against the antigen KLH, and no direct histopathological changes to the immune system. This study indicates that maraviroc at doses up to, and including, 300 mg BID had no effect on the immune system or function in cynomolgus monkeys with complete CCR5 receptor occupancy over 24 hours.

2.4.4.7.2. The effects of maraviroc on the thyroid of rats
(Study No. 03165)

A study was conducted to assess the relationship between thyroid follicular cell hypertrophy in rats and liver enzyme induction. Rats, treated with maraviroc at a dose of 900 mg/kg for 1 month showed increased thyroxine clearance in animals of both sexes, and a higher hepatic uridine 5-diphosphate glucuronyl transferase (UDPGT) activity and cytochrome P450 content in treated females. The treatment produced an increase in thyroid weights in males and follicular cell hypertrophy in both sexes. A mild decrease in T4 plasma level and a marked increase in thyroid stimulating hormone (TSH) were associated with pituitary vacuolation. These findings suggest that thyroid hypertrophy was secondary to pituitary stimulation, following decreases in plasma thyroxine concentration.

Hepatic UDPGT is responsible for conjugation of thryoxine prior to excretion into the bile (Comer *et al.*, 1985; Lumb & Rust, 1985; Hill *et al.*, 1989). Lower plasma concentrations of thyroxine stimulate the pituitary, through a negative feed-back mechanism, to release TSH, resulting in a hypertrophic response on the thyroid epithelium and eventually to proliferative

changes of thyroid follicular cells (McClain *et al.*, 1988; McClain 1989; Johnson *et al.*, 1993). It is widely accepted that treatment-related increase in follicular cell proliferative changes in the thyroid of rats has no relevance to man (Davies, 1993; Hill *et al.*, 1989).

2.4.4.7.3. Worker Safety Studies on Starting Products and Chemical Intermediates of Maraviroc

(See 2.6.6.7. and 2.6.6.8.3.)

A series of toxicology studies were conducted on UK-427,857 and its intermediates to determine any hazardous properties and hence take appropriate measures to protect the workforce. Additionally, where required, testing to enable compliance with European legislation (NONS) was also undertaken. Further details are provided in the Toxicology Written Summary (2.6.6.7 and 2.6.6.8.3).

Maraviroc produced no evidence of skin sensitization at concentrations of 10, 25 and 50% in a local lymph node assay in mice. In topical studies, maraviroc produced very slight dermal irritation at 2000 mg/kg in rats, which resolved on day 5, but no dermal irritation in rabbits. In an eye irritation study in rabbits, maraviroc produced a very slight discharge and iritis one hour after administration and injection of the conjunctival blood vessels up to 2, 3 and 8 days in the three animals.

Substance 1*, a synthetic intermediate of maraviroc, had no mutagenic activity in bacterial assays, but showed clastogenic activity when tested with and without metabolic activation in mitogen-stimulated human lymphocyte cultures. Further testing in the mouse micronucleus assay, at doses up to 75 mg/kg/day (the maximum tolerated dose), indicated no evidence of an increase in the induction of micronucleated immature erythrocytes or bone marrow toxicity. Therefore, the clastogenic response observed in the in vitro cytogenetics assay was not reproduced in vivo, suggesting that the in vitro response is not relevant in vivo. A local lymph node assay in mice produced no evidence of skin sensitization at concentrations of 10, 25 and 50%. In an oral toxicity study in rats, the acute lethal oral dose to rats was between 200 and 2000 mg/kg. Repeated dosing for 4 weeks produced no evidence of toxicity at the high dose of 300 mg/kg. An acute dermal toxicity study in rats produced very slight dermal irritation at 2000 mg/kg, which resolved on day 3, but no dermal irritation in rabbits. In an eye irritation study in rabbits, Substance 1* produced red conjunctiva, diffuse areas of corneal opacity, iritis, very slight to slight chemosis and very slight to substantial damage during 72 hours after instillation; injection of the conjunctival blood vessels (all animals) and iritis (one animal) persisted until Day 8. Instillation of Substance 1* gave rise to a persistent reaction in one animal (injection of conjunctival vessels) and an irreversible response (pannus) in another.

Substance 2* is a synthetic intermediate of maraviroc: drug metabolism studies indicate that Substance 2* is also a metabolite. Substance 2* has no mutagenic activity and shows no acute oral toxicity or acute dermal toxicity in rats at 2000 mg/kg. A local lymph node assay in mice produced no evidence of skin sensitization at concentrations of 10, 25 and 50%. Repeated dosing for 4 weeks, produced no evidence of toxicity at the high dose of 1000 mg/kg. In an acute dermal toxicity study in rabbits, Substance 2* produced a very

slight erythema which persisted 24 hours. In an eye irritation study in rabbits, there was a minimal conjunctival irritation, which resolved 24 hours after instillation.

Substance 3*, a synthetic intermediate of maraviroc, had no indication of mutagenic activity in bacterial assays. A local lymph node assay in mice produced no evidence of skin sensitization at concentrations of 10, 25 and 50%. In an acute oral toxicity study in the rat, clinical signs included abnormal gait, lethargy, piloerection, hunched posture, reduced body temperature, salivation, flat posture and straub tail, which resolved by Day 4. Repeat-dosing for 4 weeks was well tolerated, with only a minor increase in motor activity (rearing and cage floor activity) at the high dose of 300 mg/kg. Changes in haematology parameters were lower APTT from 150 mg/kg and higher prothrombin time at 300 mg/kg. Blood chemistry changes were reduced albumin, globulin and total protein at 300 mg/kg and increases in cholesterol from 150 mg/kg and ALT at 300 mg/kg. Histopathological findings were noted at 300 mg/kg in the liver (minimal hepatocyte hypertrophy) and the spleen (minimal increase in extramedullary haemopoiesis). In the adrenals, there was diffuse cortical hypertrophy, with a dose-related incidence and degree. There was an increased incidence of luminal dilatation of the jejunum , ileum and rectum of both sexes and the colon of males receiving 300 mg/kg, reflecting distension of the gastrointestinal tract at this dose. In the absence of overt toxicity at 300 mg/kg, this dose was considered to be the NOAEL. In an acute dermal toxicity study in rats at 2000 mg/kg, Substance 3* produced very slight dermal irritation which had resolved on day 5. After the 15-day observation period, Peyer patches were observed (slight to marked response) in the large intestine of some animals. There was no dermal irritation in rabbits. In the eye irritation study in rabbits, Substance 3* gave rise to a slight initial pain response and produced injection of the conjunctival blood vessels (throughout the first 48 hours and in one animal on days 15 and 22), very slight discharge, with or without slight chemosis or iritis (one hour after instillation) and diffuse areas of opacity in one animal for the first two weeks of observation.

Substance 4*, a starting product in the chemical synthesis of maraviroc, had no indication of mutagenic activity in bacterial assays. A local lymph node assay in mice produced no evidence of skin sensitization at concentrations of 10, 25 and 50. Substance 4* showed no acute oral toxicity or acute dermal toxicity in rats at 2000 mg/kg. Repeat-dosing for 4 weeks was well tolerated. Changes in haematology parameters were reduced haemoatocrit and red blood cell count at 300 mg/kg and, in treated female groups, there were higher neutrophil counts and lower APTT, compared to controls. Plasma urea levels were slightly raised in all treated female groups. There were no histopathological findings clearly attributed to treatment. In the absence of overt toxicity, the NOAEL was considered to be 300 mg/kg. In the eye irritation study in rabbits, Substance 4* produced injection of the conjunctival blood vessels for the first 24 hours (and in one case for a further 48 hours), and isolated cases of very slight discharge or iritis one hour after instillation.

Substance 5*, a synthetic intermediate of maraviroc, had no mutagenic activity in bacterial assays. A local lymph node assay in mice produced no evidence of skin sensitization at concentrations of 10, 25 and 50%. In an acute oral toxicity study in the rat, there were severe clinical signs (prostration, tremors, and abnormal gait) and mortality at 2000 mg/kg. At 200 mg/kg, clinical signs were prostration, lethargy, shallow respiration, hunched posture,

partially-closed eyes, reduced body temperature, prostration and flat posture, which resolved by Day 2. Repeated dosing for 4 weeks produced no evidence of toxicity at the high dose of 300 mg/kg. Findings included slight changes in clinical pathology values and an increase in relative liver weight. In an acute dermal toxicity study in rats at 2000 mg/kg, Substance 5* produced very slight to well-defined dermal irritation which was completely resolved on Day 5, but no dermal irritation in rabbits. In an eye irritation study in rabbits, Substance 5* produced injection of the conjunctival blood vessels in all animals throughout the first 72 hours persisting in one case to Day 8 and in another animal to Day 22. Iritis, with or without very slight chemosis and discharge, was seen until Day 2.

* ：新薬承認情報提供時に置き換えた

2.4.5. Integrated Overview and Conclusions

Several specific issues were identified for discussion in the Nonclinical Pharmacology and Toxicology Written Summaries (2.6.2.6 and 2.6.6.9). An integrated overview of these discussions and conclusions is presented here. Exposure multiples have been calculated by comparing unbound plasma concentrations (Cmax or AUC24h) in the toxicology species with those at the maximum therapeutic dose in humans (300 mg BID: Cmax 155 ng/mL; AUC24h 1275 ng.h/mL; Clinical study A4001007). Exposure multiples from each toxicology study are shown in Section 2.6.4.10.2.

2.4.5.1. Nonclinical tolerance of maraviroc in repeated-dose studies

The full toxicology profile of maraviroc has been assessed by using high daily doses (up to 2000 mg/kg in mice, 1500 mg/kg in rats, 250 mg/kg in dogs and 800 mg/kg in monkeys). The dose range studied provided plasma exposures many times higher than found at the maximum therapeutic dose in humans, 300 mg BID (or 8.6 mg/kg/day, assuming a 70 kg subject); the unbound Cmax and AUC values represented exposure multiples of up to 45 and 68 in mice, 30 and 51 in rats, 23 and 28 in dogs and 43 and 37 in monkeys.

In mice, mortality at 1000 mg/kg, associated with local gastrointestinal pathology rather than systemic toxicity, gave a NOAEL of 750 mg/kg (with an exposure multiple of 45 (Cmax) or 68 (AUC).

In rats, the dose of 900 mg/kg, with exposure multiples of 30 (Cmax) or 51 (AUC), was found to be the maximum tolerated dose and was used as the high dose in the 24-month carcinogenicity study. Reductions in body weight at 900 mg/kg and bile duct hyperplasia from 300 mg/kg were seen in the 6-month study and established the NOAEL in rats at 100 mg/kg (with an AUC exposure multiple of 8). However, given the high first pass extraction in this species, this exposure multiple is likely to be an underestimate.

In dogs, there were multiple bouts of emesis from relatively low doses (15 mg/kg), accompanied by body weight loss and frequent reductions or absence of food intake at the dose of 150 mg/kg. This was considered to be the maximum tolerated dose in dogs, with an exposure multiple of 23x (Cmax) or 28x (AUC). In the 1- and 6-month studies, the NOAEL was 5 mg/kg, with an exposure multiple of 2 (Cmax), based on increases in QTc interval at higher doses.

In monkeys, the daily dose of 800 mg/kg was not well-tolerated, being associated with cardiovascular changes (reduced blood pressure and heart rate) and treatment reactions (reduced activity, prostration, loss of balance). The dose of 400 mg/kg produced similar, though less severe, findings, as well as an increase in QTc interval, in the 9 month study at exposure multiples of 14 (Cmax) and 23 (AUC). The NOAEL, established from the 9-month study, was 120 mg/kg in monkeys, with a Cmax exposure multiple of 5. There was no evidence of cardiac arrhythmias in dogs or monkeys over the dose range studied.

The only clinical observations recorded in dogs and monkeys, which may be associated with adverse events with an incidence of ≥2% in human Phase 1 studies are orthostatic hypotension (at 300 mg BID), fatigue, dizziness and dizziness/postural (at 600 mg QD), nausea (at 600 mg BID) and ocular hyperaemia (at 900 mg QD) (Section 2.7.4.2. Summary of Clinical Safety). In the Phase 3 studies, the majority of adverse events reported at incidences of ≥2% in maraviroc treatment groups at 300 mg QD or 300 mg BID (Studies A4001027 and A4001028), were reported at similar incidences to placebo. Of the adverse events listed above, only dizziness appeared in the Phase 3 studies with an incidence greater in the maraviroc 300 mg QD (4.8%) and 300 mg BD (4.9%), than that of placebo (3.8%). (Section 2.7.4.2. Summary of Clinical Safety).

In conclusion, maraviroc is well tolerated in toxicology species and is not considered to represent a risk for human patients at the therapeutic dose.

2.4.5.2. Blood pressure and Heart rate

Although maraviroc had no significant effect on resting blood pressure or heart rate in humans at single doses of up to 1200 mg, postural hypotension was identified as the dose-limiting adverse event during the clinical programme. These events appear to be rare at unit doses of ≤300 mg, where they occur at rates similar to that seen with placebo, and increase in number as the unit dose increases beyond 300 mg.

Studies in dogs indicated no significant changes in blood pressure at plasma concentrations 3-6-fold that of the maximum therapeutic dose and only inconsistent reductions in blood pressure in individual animals at concentrations 5 and 9-fold that at the maximum therapeutic dose. Maraviroc produced a slight impairment of normal reflex control of blood pressure in the dog during the change to the upright position at plasma concentrations 3-6 fold the concentrations at the maximum therapeutic dose. However once the upright position had been established, blood pressure control was maintained at a normal level. While maraviroc produced no obvious postural hypotension in the conscious dog, the effects on the initial reflex response may be sufficient to cause symptoms of postural dizziness in humans.

Toxicology studies in monkeys indicated reductions in blood pressure at daily doses of 200 and 400 mg/kg, accompanied at 400 mg/kg by lower heart rates. The doses of 200 mg/kg (1-month study) and 400 mg/kg (9-month study) were associated with similar unbound plasma concentrations (1815 ng/mL and 1718 ng/mL, respectively) and were approximately 11-fold higher than that at the maximum therapeutic dose. No effects on blood pressure or heart rate

were observed at 120 mg/kg in the 9-month study, with a plasma concentration 5-fold that of the maximum therapeutic dose in man.

A series of in vitro studies in vascular tissue were conducted to investigate potential mechanisms for postural hypotension at higher maraviroc doses in humans. Maraviroc is a weak competitive antagonist at alpha adrenergic receptors expressed in canine saphenous vein. 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 postural hypotension. Alternatively, 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 a CCR5 antagonist (DI/073/06) indicating that there may be a CCR5 component to the observed postural hypotension.

In conclusion, maraviroc produced inconsistent reductions in blood pressure in dogs and no obvious postural hypotension. Studies in monkeys have shown reductions in blood pressure and heart rate at plasma concentrations at least 11-fold that seen at the maximum therapeutic dose, 300 mg BID. Although the mechanism responsible for postural hypotension in humans is unclear, in vitro studies suggest effects of maraviroc on alpha adrenoceptors or vascular CCR5 receptors. The reduction in heart rate seen in monkeys has not been observed throughout the clinical programme and is unlikely to represent a risk for human patients.

2.4.5.3. QT interval prolongation

At therapeutic concentrations, maraviroc had no effect on cardiac repolarisation in either in vitro or in vivo assays. This is consistent with the lack of effect of maraviroc on QT interval in humans (Section 2.5.5.4 Clinical Overview). However at supra-therapeutic concentrations, or in the event of an overdose, maraviroc can block the hERG potassium channel. In vitro studies show that maraviroc inhibits dofetilide binding, is active at the human cardiac hERG channel and prolongs the action potential of the dog Purkinje fibre at concentrations ≥3 μM or 1541 ng/mL. These results indicate that the maraviroc has the potential to block the I_{Kr} current and affect cardiac repolarisation in vivo at unbound plasma concentrations greater than 3 μM, which is approximately 10-fold the Cmax at the maximum therapeutic dose.

These changes were consistent with findings from toxicology studies in which maraviroc increased QTc interval at doses of ≥15 mg/kg in dogs and ≥200 mg/kg in monkeys. The unbound plasma concentrations at these lowest effect doses (899 and 1815 ng/mL) represent exposure multiples of 6- and 12-fold, respectively. In these two species, doses of 5 mg/kg and 120 mg/kg, respectively, had no effect on QTc interval at plasma concentrations 2 and 5-fold the maximum therapeutic concentration. The blockade of cardiac potassium channels can cause prolongation of action potential duration, thereby delaying ventricular repolarisation, lengthening QT interval and increasing the risk of serious arrhythmias, such as Torsade de pointes. This activity of maraviroc is considered to represent a low risk to humans given that the ion channel effects occur at a plasma concentration that was 10-fold the maximum therapeutic concentration (155 ng/mL). Furthermore concentrations in dogs

and monkeys have been explored up to 23- and 43-fold, respectively, those seen at the therapeutic dose, with no evidence of cardiac arrhythmias.

The results from the QT study in humans showed that the mean maximum difference from placebo in QTcI was less than 4 msec for all 3 doses of maraviroc (100, 300, and 900 mg as single doses). None of the subjects had a maximum QTcI interval ≥450 (for males) or 470 msec (for females), or a maximum increase from baseline of ≥60 msec, after receiving maraviroc. In addition, there was no clear relationship between maraviroc plasma concentration and maximum increase in QTcI although PK/PD modeling projects an 0.97 msec increase in QTc for every 1000 ng/mL increase in plasma concentration (Section 2.7.4.2. Summary of Clinical Safety).

In conclusion, in vitro studies and animal data from dogs and monkeys indicated the potential for QT interval prolongation in human patients and provided a cautionary signal to investigators throughout the clinical programme. Cardiovascular testing in these species has confirmed no arrhythmogenic activity at plasma exposures many times greater than those expected in humans at the therapeutic dose. A thorough Phase 1 QT study did not show evidence of clinically significant QT interval prolongation at the doses studied, including a supratherapeutic dose of 900 mg. The range of in vitro, animal and clinical data has served to characterize the action of maraviroc on cardiac repolarisation and to provide reassurance that maraviroc does not increase the arrhythmogenic risk for humans, even when taking concomitant medication that would increase exposure (Section 2.7.4.2. Summary of Clinical Safety). However in the event of a significant overdose of maraviroc, changes in the QT interval may occur and cardiac monitoring should be part of supportive care.

2.4.5.4. Hepatic Findings

Repeat-dose toxicology studies in mice, rats, dogs and monkeys identified the liver as a target organ in rats only. Bile duct vacuolation was present from 100 mg/kg and was associated with minimal bile duct hyperplasia from 300 mg/kg. At higher dose levels, while the incidence of bile duct changes increased, there was no increase in the severity. In male rats, bile duct hyperplasia was still present 3 months after withdrawing the treatment, but was fully reversed in female rats. Bile duct hyperplasia appears morphologically similar to spontaneous changes known to occur in rats (Greaves, 2000). These changes are possibly a mild response to the biliary excretion of maraviroc or its metabolite (section 2.4.3.5).

At 900 mg/kg, additional findings in the liver were altered cell foci and multinucleated hepatocytes. Although altered cell foci occur spontaneously in older rats, they are seen infrequently in rats aged 8 months. Multinucleated hepatocytes were not associated with concurrent evidence of liver damage or hepatocyte proliferation in this study. The presence of multinucleated hepatocytes after withdrawing the treatment for 3-month recovery period is consistent with a long recovery period for this finding (Brughera et al, 1995). Although the pathogenesis of this change remains unknown, its significance in terms of human safety is dubious. At the dose of 1500 mg/kg, there were increases in plasma transaminases, accompanied in one animal by hepatocellular necrosis.

In the 24-month study in rats, bile duct hyperplasia was noted in all groups (including controls) with a similar incidence and severity; although bile duct vacuolation was not observed. As in the 6-month study, there were no indications of atypia, mitosis or pleomorphism of bile duct epithelium. While biliary neoplasms (cholangiocarcinoma and cholangioma) were found only in rats receiving maraviroc, the lack of an indication of pre-neoplastic changes, the low incidence of tumors in this study, along with similar incidences in reported control data and in a control group in a concurrent study at the same laboratory, support the conclusion that these neoplasms are of spontaneous origin and unrelated to treatment. In the 6-month carcinogenic study in transgenic (rasH2) mice, there were no noteworthy findings in the liver.

In humans sporadic liver enzyme elevations have been observed during 4 Phase 1 studies but they did not show any dose relationship and were not related to increases in bilirubin. No elevations in liver enzymes or bilirubin were noted during the healthy volunteer 28-day safety study at 300 mg BID (Section 2.7.4 Summary of Clinical Safety). Similarly, only minor differences were seen between maraviroc and placebo treated patients with respect to grade 3 or 4 ALT/AST and total bilirubin elevations in the Phase 2b/3 programme (Section 2.5.5.2 Clinical Overview). The clinical and laboratory data generated during the clinical development programme does not indicate that maraviroc has an adverse effect on hepatic function.

In conclusion, the liver has been identified as a target organ in rats. There was a weak signal of adverse liver findings at high doses, characterized by slight increases in plasma transaminases and bile duct hyperplasia, at 34-fold and 25-fold, respectively, the AUC exposure at 300 mg BID. No adverse liver effects were observed in rats at 8x the AUC exposure at 300 mg BID. Given the high first pass extraction in this species, the systemic exposure (used to calculate exposure multiples), is likely to under-represent the chemical stress imposed on the liver (Walker, 2004). In mice, dogs and monkeys, no signals of adverse liver findings were seen at 68x, 28x and 37x the AUC exposure at 300 mg BID in humans, respectively. Carcinogenicity studies in rats and transgenic mice at plasma AUC exposures 21- and 39-times higher than those found in humans at the maximum therapeutic dose, indicate no carcinogenic potential for the human liver.

2.4.5.5. Thyroid changes in rats

In rats only, thyroid follicular cell hypertrophy was noted in the 6-month study from 300 mg/kg, and was shown to be reversible when treatment was withdrawn. Pituitary vacuolation was observed in the 1-month study at 1500 mg/kg. The interdependence of thyroid and liver changes was established in an investigative study in rats (See 2.6.6.8.2). The thyroid of rats is particularly sensitive to disturbances in thyroid hormone metabolism as they lack thyroxine-binding globulin, resulting in a shorter half-life of T4 than in humans (McClain, 1989). The hepatic changes are consistent with an adaptive response to treatment and are associated with an increase in the activity of hepatic xenobiotic metabolism. Activation of hepatic UDPGT brought about an increased thyroxine clearance, resulting in a reduction in circulating concentrations of this hormone. Hepatic UDPGT is responsible for conjugation of thyroxine prior to excretion into the bile (Comer et al., 1985; Lumb & Rust,

1985; Hill et al., 1989). Lower plasma concentrations of thyroxine stimulate the pituitary, through a negative feed-back mechanism, to release thyroid stimulating hormone (TSH), resulting in a hypertrophic response on the thyroid epithelium and eventually to proliferative changes of thyroid follicular cells (McClain et al., 1988; McClain 1989; Johnson et al., 1993).

Similarly, in the rat 24-month carcinogenicity study, there was evidence of increased thyroid stimulation characterized by increased incidence of follicular cell hypertrophy and/or hyperplasia at doses ≥100 mg/kg in males and ≥500 mg/kg in females, and an increased incidence of benign thyroid follicular cell adenomas at 900 mg/kg in both sexes. These findings are considered to reflect the hepatic enzyme-inducing properties of maraviroc. Following long-term treatment of rats with compounds known to induce liver enzymes, thyroid stimulation associated with increased TSH plasma levels can be documented and benign follicular cell adenomas routinely follow (Capen et al., 2002). It is widely accepted that treatment-related increases in follicular cell proliferative changes in the thyroid of rats, as seen at the high dose in the present study, have no relevance to humans (Hill, 1989; Davies, 1993).

No effect on T4 or TSH (measured at baseline and 24 weeks) related to use of maraviroc was identified during Phase 2b/3 studies, which provided further evidence for the absence of an effect on the thyroid gland in humans (Section 2.7.4.1 Summary of Clinical Safety).

2.4.5.6. Immunological Considerations

Apart from serving as a co-receptor for viral entry in HIV-1 infection, the roles of CCR5 and CXCR4 in immune homeostasis have not been elucidated. There is a hypothetical risk that blockade of the CCR5 receptor could have implications for immune function, due to decreased or altered lymphocyte/ macrophage function. The lack of CCR5 expression in individuals who are homozygous for the Δ32 mutation has been shown to result in protection against HIV-1 transmission, (Liu et al., 1996; Samson et al., 1996). Although, these early reports indicated no evidence of any adverse effect on health in individuals who lack this receptor, more recent studies suggest an equivocal adverse effect in breast cancer patients (Manes et al, 2003; Degerli et al, 2005). Also, CCR5Δ32 homozygosity has been associated with an increased viral burden and fatal outcome in West Nile virus infection (Glass et al, 2006). However, there is no clinical evidence to date in healthy subjects or in HIV-positive patients, to indicate any increased infection risk in patients treated with maraviroc (Clinical Overview Section 2.5.5.4).

Maraviroc has been investigated in several nonclinical studies. The compound has no activity against a number of in vitro human immune function assays, including activity against a number of related chemokine receptor assays. In repeat-dose toxicology studies in mice, rats, dogs (up to 6 months duration) or monkeys (up to 9 months duration), maraviroc produced no alterations in circulating white blood cell parameters, serum globulins, or noteworthy changes to organ weights or histology of the bone marrow, lymph nodes, spleen or thymus. Similarly there was no increase in the incidence of infections during these studies to suggest impairment of the immune system.

A specific study to investigate the potential of maraviroc to impair the immune system in monkeys showed that treatment for 1-month at daily doses of up to 300 mg/kg induced no changes in lymphocyte subset distribution, NK cell activity, phagocytosis activity or oxidative burst. All animals were able to mount a humoral primary (IgM) and secondary (IgG) immune response against KLH. The daily dose of 300 mg/kg was shown to achieve 100% occupancy of CCR5 receptors over 24 hours. There was no adverse effect of maraviroc on the immune system in monkeys at plasma exposures (AUC24) producing complete and continuous blockade of CCR5 receptors and with an exposure multiple 16-fold greater than observed at 300 mg BID.

There was no suggestion that maraviroc stimulated the immune system. In chronic (6 months or longer) studies in mice, rats, dogs and monkeys there was no evidence of lymphoid hyperplasia in the spleen or lymph nodes, which may be expected upon stimulation of the immune system. Furthermore in the immunotoxicology study in monkeys, there was no potentiation of the antibody response to KLH.

Carcinogenicity studies in rats and transgenic mice at plasma AUC exposures 21- and 39-times, respectively, higher than those found in humans at the maximum therapeutic dose, indicate no carcinogenic potential for humans. Rats and mice have 92% CCR5 sequence identity and 96% sequence conservation (DI/100/06) therefore it can be assumed that maraviroc will bind similarly to the CCR5 receptor in these species. Although maraviroc had no functional antagonist activity against the mouse receptor at 1 μM (DI/092/06) it can be assumed that at 10 μM the degree of binding will be similar to that seen in the rat, i.e. 33% (CG/02/04). In the rat carcinogenicity study at 900 mg/kg, the unbound maraviroc Cmax was 1882 ng/mL (3.7 μM), providing an estimated receptor binding of about 10%. In the Tg mouse carcinogenicity study at 1500 mg/kg, the unbound Cmax in males (the lower of the two genders) was 3587 ng/mL (7 μM), giving approximately 20% binding to the CCR5 receptor.

The results of these nonclinical studies with maraviroc do not suggest a risk to the human immune system.

2.4.5.7. Reproductive toxicology

The assessment of maraviroc in repeat-dose studies in rats, dogs and monkeys using daily dosing for up to 6 or 9 months of treatment demonstrated no histopathological evidence of toxicity on the male or female reproductive systems of these species (Section 2.4.4.3).

Reproduction toxicology studies indicate no effects on fertility at 1000 mg/kg, corresponding to an AUC exposure multiple of 39. In addition, there was no effect on reproduction parameters or embryo-fetal development at 1000 mg/kg in rats (AUC exposure multiple of 39) and 200 mg/kg in rabbits (AUC exposure multiple of 34). In the pre-and postnatal study in rats, there was no effect on the reproductive function of treated females up to the dose of 1000 mg/kg, producing an AUC exposure multiple of 27-fold. This dose produced a slight increase in motor activity in male F1 offspring. Based on this finding, the NOAEL for development toxicity in the offspring of maraviroc-treated female rats was 300 mg/kg,

corresponding to an AUC exposure multiple of 9. A study in lactating female rats has shown that maraviroc is extensively secreted into milk, and this is also likely to occur in humans (Section 2.4.3.5).

Maraviroc binds to the CCR5 receptor in rats with a significantly reduced affinity compared with that in humans. Thus, while maraviroc inhibits binding of [^{125}I]MIP-1β to human CCR5 with a mean IC_{50} of 7.2±0.9 nM, data in the rat and dog indicate that maraviroc inhibits binding by 33.3%±4.4% and 38.7±3.8% at 10 μM (Section 2.4.2.3.3). Similar studies have not been conducted in rabbits. However, the homology of the amino acid sequence of the CCR5 binding site across species strongly suggests that the affinity of maraviroc will lie between that of the rat and the dog.

In the rat embryofoetal toxicity study, the free Cmax concentration at the high dose (1000 mg/kg) was 3707 ng/mL, corresponding to 7.2 μM (Study No. 02025/28). Pregnant rats were therefore exposed to maraviroc concentrations that provide approximately 30% inhibition of MIP-1β binding. This dose produced slight maternal toxicity in terms of reduced maternal body weight. The high dose of the rabbit embryofoetal development study (200 mg/kg) produced a free Cmax of 3436 ng/mL, corresponding to 6.7 μM (Study No. 02026/29). Pregnant rabbits were therefore exposed to maraviroc concentrations that are predicted to achieve approximately 30% inhibition of MIP-1β binding in that species. The high dose produced severe maternal toxicity, including mortality.

The high concentrations used in the rat and rabbit embryofoetal development studies are in the range producing approximately 30% inhibition of the recombinant rat CCR5 receptor. In neither of these studies was there evidence of adverse effects of the treatment on embryo or foetal development. A higher dose (300 mg/kg) was administered to pregnant rabbits in the range-finding study and produced severe maternal toxicity (including mortality) but no evidence of embryofoetal toxicity. However plasma drug exposure levels were not measured in this study.

Two pieces of evidence suggest that antagonism of endogenous CCR5 receptors would not have an adverse effect on the developing embryo or fetus. Firstly, CCR5 receptors have been shown to be absent in a population of humans (Samson et al., 1996; Dean et al.,1996). Secondly, knockout mice have been developed that do not express the CCR5 receptor (Zhou et al., 1998). In both examples, the lack of CCR5 receptors has not been associated with adverse developmental consequences.

In conclusion, the high doses used in rat studies produced plasma concentrations of maraviroc expected to inhibit approximately 30% of endogenous chemokine binding to the CCR5 receptor. The presence of maraviroc in foetal plasma indicated placental transfer in rats and rabbits. Although no comparable affinity studies have been conducted in the rabbit, receptor homology analysis suggests that maraviroc would bind to the CCR5 receptors in the rabbit with an affinity higher than for the rat. The plasma concentrations used in the rabbit embryofoetal toxicology study were expected to bind to the CCR5 receptor. In addition, humans and mice that do not express functional CCR5 receptor on their cell surfaces show no gross developmental abnormalities.

2.4.5.8. Overall Conclusions

The pharmacological, pharmacokinetic and toxicological data presented in this Nonclinical Overview demonstrate a favorable preclinical profile of maraviroc.

Pharmacodynamic studies have adequately demonstrated the high potency and selectivity of maraviroc as a CCR5 antagonist, and the efficacy as an antiretroviral agent for the treatment of HIV-1 infection. Maraviroc acts extracellularly, binding to cell surface CCR5 to inhibit viral entry. The mean IC_{90} (90% inhibitory concentration) for maraviroc against a large panel of CCR5-tropic primary HIV-1 isolates following acute infection of human peripheral blood lymphocytes is 2.03 nM (1.04 ng/mL). When this value is compared to the steady-state pharmacokinetics of maraviroc in HIV-1 infected patients receiving the compound as monotherapy at 300 mg BID, there is a 16-fold window between the protein-adjusted geometric mean inhibition in vitro and the average unbound drug levels achieved at Cmin in vivo. Maraviroc inhibits replication in vitro of viruses from a wide geographical origin and diversity of viral clades and also viruses resistant to existing antiretroviral agents. Receptor binding studies demonstrated that maraviroc had a high and similar affinity for CCR5 receptors in monkeys and humans; monkeys were therefore the most appropriate non-rodent toxicology species.

Pharmacokinetic analysis has established the absorption, metabolism, distribution and elimination profile of maraviroc. The pathways of maraviroc metabolism in human were all represented in toxicology species. The main circulating metabolites in human plasma were the secondary amine UK-408,027 and a hydroxylated metabolite which arose by further metabolism of the amine. UK-408,027 has been shown to be devoid of relevant pharmacological activity, and the same is likely to be true for the hydroxylated metabolite which has a similar structure. All human circulating metabolites were identified in the plasma of at least one of the toxicology species indicating that animals were exposed to these metabolites in repeat-dose safety studies. Consequently, the choice of animal species for the evaluation of maraviroc toxicology was appropriate and relevant to human safety. Maraviroc has been shown to be a substrate for CYP3A4 in vitro. Consequently its pharmacokinetics are likely to be affected by co-administration of inhibitors and inducers of this cytochrome P450 enzyme and a series of clinical interaction studies have been performed to investigate this possibility. Maraviroc is not an inhibitor of the 7 major cytochrome P450 enzymes and is therefore unlikely to affect the metabolism of other co-administered P450 substrates at clinical doses.

A toxicology programme has been completed involving repeat-dose studies, which identified toxicology end-points, together with doses of maraviroc without adverse effects. In mice, given mortality at 1000 mg/kg, the NOAEL was 750 mg/kg, with a plasma AUC exposure 68-fold that seen at the maximum therapeutic dose. Rats were the more sensitive rodent species; adverse liver findings were identified at 300 mg/kg, but not at 100 mg/kg, with a plasma AUC exposure 8-fold that seen at the maximum therapeutic dose.

Dog and monkey studies indicated a signal for QTc interval prolongation of the ECG at doses of 15 and 200 mg/kg, respectively, which was consistent with in vitro indications of

effects on cardiac repolarisation. In these species, no changes in QTc interval were observed at 5 and 120 mg/kg, respectively, with plasma Cmax exposure 2 and 5-fold that seen at the maximum therapeutic dose. There was no evidence of arrhythmogenic activity in dogs and monkeys at plasma Cmax exposures 23 and 43-fold that seen at the maximum therapeutic dose. In addition, monkeys showed decreases in heart rate and blood pressure at Cmax exposures about 11-fold higher than that at the maximum therapeutic dose. No changes were seen at the dose of 120 mg/kg, corresponding to a plasma Cmax exposure 5-fold that seen at the maximum therapeutic dose.

Adverse effects on the liver and changes in QTc interval, heart rate and blood pressure that were identified in toxicology studies have been carefully monitored in clinical Phase 2b/3 studies and were not significant at the dose of 300 mg BID. Postural hypotension was noted with increasing frequency in humans at unit doses of 600 mg or more and is the dose limiting adverse effect in humans.

Maraviroc had no adverse effects on fertility and has no teratogenic potential at AUC plasma exposures 39- and 34-fold, respectively, higher than those found in humans at the maximum therapeutic dose. Similarly maraviroc was shown not to be mutagenic or clastogenic in appropriate genetic toxicology assays. Carcinogenicity studies in rats and Tg mice at plasma AUC exposures 21- and 39-times higher than those found in humans at the maximum therapeutic dose, indicate no carcinogenic potential for humans.

Having reviewed all the preclinical data, maraviroc can, be used safely in humans for oral treatment of HIV-1/AIDS following the manufacturer's instructions as set out in the Labeling documents (Module 1).

2.4.6. List of Literature Citations

Brughera M, Scampini G, John Newman A, et al. Overview of toxicological data on Rifabutin. Exp Toxic Pathol 1995;47:1-9.

Capen C, DeLillis R, Yarrington J. Endocrine system. In Haschek WM, Rousseaux CG, Wallig MA, editors. Handbook of toxicologic pathology. 2nd ed. New York: Academic Press; 2002: p.737-8.

Chen C, Li J, Bot G, et al. Heterodimerization and cross-desensitization between the μ-opioid receptor and the chemokine CCR5 receptor. Eur. J. Pharmacol. 2004;483:175-86.

Comer CP, Chengelis CP, Levin S, et al. Changes in thyroidal function and liver UDPglucurosyltransferase activity in rats following administration of a novel imidazole (SC-37211). Toxicol Appl Pharmacol 1985;80:427-36.

Davies DT. Assessment of rodent thyroid endocrinology: Advantages and Pit-Falls. Comp Haematol Int 1993;3:142-52.

Dean M, Carrington M, Winkler C, et al. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Science 1996;273:1856-62.

Degerli N, Yilmaz E and Bardakci F. The Δ32 allele distribution of the CCR5 gene and its relationship with certain cancers in a Turkish population. Clin Biochem. 2005;38:248-52.

Esté JA. Sch-351125 and Sch-350634 Schering-Plough. Curr. Opin. Invest. Drugs. 2002;3:379-83.

Fermini B and Fossa AA. The impact of drug-induced QT interval prolongation on drug discovery and development. Nature Reviews (Drug Discovery) 2003;2:439-47.

Gillen C, Haurand M, Kobelt DJ, et al. Affinity, potency and efficacy of tramadol and its metabolites at the cloned human μ-opioid receptor. Naunyn-Schmiederberg's Arch. Pharmacol. 2000;362:116-21.

Glass WG, McDermott DH, Lim JK, et al. CCR5 deficiency increases risk of symptomatic West Nile virus infection. J. Exp. Med., 2006;204:35-40.

Greaves P. Histopathology of preclinical Toxicity Studies. Interpretation and relevance in drug safety evaluation. Elsevier, Amsterdam, 2000, p 486.

Greenberg M. Fusion Inhibitors: Activity, Virological Responses, and Mechanisms of Resistance and Associated Costs to Viral Fitness. 2nd European HIV Drug Resistance Workshop 2004; session 3:abstract 8.

Grimm MC, Ben-Baruch A, Taub DD, et al. Opiates Transdeactivate Chemokine Receptors: δ and μ Opiate Receptor-mediated Heterologous Desensitization 1998;188:317-25.

Hanton G, Nahas K, Priou C, et al. The QT interval in the dog ECG: importance in preclinical toxicology and relationship to heart rate. Toxicology Methods 2001;11:21-40.

Hill RN, Erdreich LS, Paynter OE, et al. Thyroid follicular cell carcinogenesis. Fundamental and Applied Toxicology 1989;12:629-97.

Hugues FC, Munyera Y and Le Jeunne C. Drug induced orthostatic hypotension. Rev. Med. Interne. 1992;13:465-70.

Johnson S, McKillop D, Miller J, et al. The effects on rat thyroid function of an hepatic microsomal enzyme inducer. Human Exp Toxicol 1993;12:153-8.

Keating MT and Sanguinetti MC Molecular and Cellular Mechanisms of Cardiac Arrhythmias. Cell 2001;104:569-80.

Kenakin TP. The Classification of Drugs and Drug Receptors in Isolated Tissues. Pharmacol Rev 1984;36(3):165-222.

Leblanc B, Jezequel S, Davies T, et al. Binding of drugs to eye melanin is not predictive of ocular toxicity. Reg. Toxicol Pharmacol 1998; 28:124-32.

Liu R, Paxton WA, Choe S, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. Cell 1996;86:367-77.

Lumb GD, Rust JH. The pathologic response of the liver and thyroid of the rat to potassium prorenoate (SC-23992). Toxicol. Pathol. 1985;13:315-24.

MacDonald J, French JE, Gerson RJ, et al. The utility of genetically modified mouse assays for identifying human carcinogens: a basic understanding and path forward. Toxicological Sciences 2004;77:188-94.

Maeda K, Nakata H, Koh Y, et al. Spirodiketopiperazine-based CCR5 inhibitor which preserves CC-chemokine/CCR5 interactions and exerts potent activity against R5 human immunodeficiency virus type 1 in vitro. J Virol 2004;78(16):8654-62

Mahajan SD, Schwartz SA, Shanahan TC, et al. Morphine Regulates Gene Expression of α and β Chemokines and Their Receptors on Astroglial Cells Via the Opioid μ Receptor. J Immunol 2002;169:3589-99.

Manes S, Mira E, Colomer R, et al. CCR5 expression influences the progression of human breast cancer in a p53-dependent manner. J Exp Med 2003;198:1381-9.

Mathias JC and Kimber JR. Postural Hypotension: Causes, clinical Features, investigation and Management. Ann Rev Med 1999;50:317-36.

McClain RM, Posch RC, Bosakowski T, et al. Studies on the mode of action for thyroid gland tumor promotion in rats by phenobarbital. Toxicol Appl Pharmacol 1988;94:254-65.

McClain RM. The significance of hepatic microsomal enzyme induction and altered thyroid function in rats: implications for thyroid gland neoplasia. Toxicol Pathol 1989;17:294-306.

McCune JM, Namikawa R, Shih CC, Rabin L and Kaneshima H. 1990. Suppression of HIV infection in AZT-treated SCID-hu mice. Science 247:564-566.

Melby T, Sista P, De Masi R, et al. Characterization of envelope glycoprotein gp41 genotype and phenotypic susceptibility to enfuvirtide at baseline and on treatment in the phase III clinical trials TORO-1 and TORO-2. AIDS Res Hum Retroviruses. 2006;22(5):375-85

Meredith, PA. (2001) Is Postural Hypotension a real Problem with Antihypertensive Medication? Cardiology 2001;96 suppl 1:19-24.

Morton D, Alden CL, Roth AJ, et al. The Tg rasH2 mouse in cancer hazard identification. Toxicologic Pathology. 2002;30(1):139-46.

Mosier DE, Gulizia RJ, Baird SM, et al. Human immunodeficiency virus infection of human-PBL-SCID mice. Science 1991;251:791-4.

Nakata H, Maeda K, Miyakawa T, et al. Potent anti-R5 human immunodeficiency virus type 1 effects of a CCR5 antagonist, AK602/ONO4128/GW873140, in a novel human peripheral blood mononuclear cell nonobese diabetic-SCID, interleukin-2 receptor γ-chain-knocked-out AIDS mouse model. J Virol 2005;79:2087-96

Parkin NT, Hellmann NS, Whitcomb JM, et al. Natural variation of drug susceptibility in wild-type human immunodeficiency virus type 1. Antimicrob Agents Chemother 2004; 48: 437-43

Raynor K, Kong H, Mestek A, et al. Characterization of the cloned human mu opioid receptor. J Pharmacol Exp Therapeutics 1995;272:423-8.

Redfern WS, Carlsson L, Davis AS, et al. Relationships between preclinical cardiac electrophysiology, clinical QT interval prolongation and torsade de pointes for a broad range of drugs: evidence for a provisional safety margin in drug development. Cardiovas. Res. 2003;58:32-45.

Samson M, Libert F, Doranz BJ et al. Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. Nature 1996;382:722-5.

Sanguinetti MC, Jiang C, Curran ME, et al. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the I_{Kr} potassium channel. Cell 1995;81:299-307

Schoenberger JA. Drug-induced Orthostatic Hypotension. Drug Safety 1991;6:402-7.

Shu M, Loebach JL, Parker KA, et al. Antagonists of human CCR5 receptor containing 4-(pyrazolyl)piperidine side chains. Part 3: SAR studies on the benzylpyrazole segment. Bioorg. Med. Chem. Lett. 2004;14:947-52.

Strizki JM, Xu S, Wagner NE, et al. SCH-C (SCH 351125), an orally bioavailable, small molecule antagonist of the chemokine receptor CCR5, is a potent inhibitor of HIV-1 infection in vitro and in vivo. Proc. Natl. Acad. Sci. USA, 2001;98(22):12718-23.

Szabo I, Wetzel MA, Zhang N, et al. Selective inactivation of CCR5 and decreased infectivity of R5 HIV-1 strains mediated by opioid-induced heterologous desensitization. J Leukocyte Biol 2003;74:1074-82.

Takaoka M, Sehata S, Maejima T, et al. Interlaboratory comparison of short-term carcinogenicity studies using CB6-F1-rasH2 transgenic mice. Toxicologic Pathology 2003;31(2):191-9.

Valenzano KJ, Miller W, Chen Z, et al. DiPOA ([8-3,3-Diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]dec-3-yl]-acetic Acid), a Novel, Systemically Available, and

Peripherally Restricted Mu Opioid Agonist with Antihyperalgesic Activity: 1. In Vitro Pharmacological Characterization and Pharmacokinetic Properties. 2004;310:783-92.

Veazey RS, Klasse PJ, Ketas TJ, et al. Use of a small molecule CCR5 inhibitor in macaques to treat simian immunodeficiency virus infection or prevent simian-human immunodeficiency virus infection. J Exp Med, 2003;198(10):1551-62.

Walker DK, Abel S, Comby P, et al. Species differences in the disposition of the CCR5 antagonist, UK-427,857, a new potential treatment for HIV. Drug Metab Disp 2005; 33:587-595.

Walker DK. The use of pharmacokinetic and pharmacodynamic data in the assessment of drug safety in early drug development. Br. J. Clin. Pharmacol. 2004; 58:601-608.

Watson C, Jenkinson S, Kazmierski W, Kenakin T. The CCR5 receptor-based mechanism of action of 873140, a potent allosteric noncompetitive HIV entry inhibitor. Mol Pharmacol 2005;67:1268–82. (First published 11 January 2005; 10.1124/mol.104.008565.)

Wolinsky SM, Veazey RS, Kunstman KJ, et al. Effect of a CCR5 inhibitor on viral loads in macaques dual-infected with R5 and X4 primate immunodeficiency viruses. Virology, 2004; 328(1):19-29.

Wood A and Armour D The Discovery of the CCR5 Receptor Antagonist, UK-427,857, A New Agent for the Treatment of HIV Infection and AIDS. Prog Med Chem 2005;43:239-71

Zhou Y, et al. Impaired macrophage function and enhanced T cell-dependent immune response in mice lacking CCR5, the mouse homologue of the major HIV-1 coreceptor. J Imunol 1998;160:4018-25.