CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-128

PHARMACOLOGY REVIEW

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

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PRODUCT:	**SELZENTRY**
INTENDED CLINICAL POPULATION:	**Treatment of HIV-1 infection**
SPONSOR:	**Pfizer**
DOCUMENTS REVIEWED:	**Electronically**
REVIEW DIVISION:	**Division of Antiviral Products (HFD-530)**
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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: There are no nonclinical pharmacology and toxicology issues which would preclude the approval of this NDA.

B. Recommendation for nonclinical studies: To support clinical use, the nonclinical toxicity profile of maraviroc was characterized in an extensive battery of in vitro and in vivo studies including carcinogenicity studies in Sprague-Dawley rats and rasH2 transgenic mice. The pivotal toxicology studies supporting the safety of maraviroc were appropriately designed and conducted in compliance with Good Laboratory Practice (GLP) regulations. In conclusion, the results of extensive nonclinical toxicology and pharmacokinetic evaluation programs support the proposed use of maraviroc in humans.

C. Recommendations on labeling: The issue of labeling will be carried out separately.

II. Summary of nonclinical findings

A: Brief overview of nonclinical findings: Maraviroc is a CCR5 antagonist indicated for treatment experienced patients infected with CCR5-tropic HIV-1, in combination with other retroviral agents. The recommended doses of maraviroc are 300 mg BID (AUC = 3.6 μg*hr/ml) and 150 mg BID (AUC was not available for this dose).

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 (200 mg/kg/day), rat (100 mg/kg/day), dog (5 mg/kg/day) and cynomolgus monkey (120 mg/kg/day). The exposure in animals in toxicology studies at the reference dose was compared to the human clinical dose (4.3 mg/kg) in terms of dose, Cmax and AUC24h for both total and unbound maraviroc. Since there was a 2-fold variation in the unbound fraction of maraviroc across species (0.25 in human to 0.52 in cynomolgus monkey), unbound exposure was considered to be a more relevant comparison by the sponsor. At the reference dose, all toxicology species were 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. Thus the choice of mouse, rat, dog and cynomolgus monkey were appropriate for the evaluation of the toxicology of maraviroc and were relevant to human safety.

Maraviroc was not mutagenic or genotoxic in a battery of in vitro and in vivo assays including a bacterial reverse mutation (Ames), chromosome aberrations in human lymphocytes and rat bone marrow micro nucleus. Maraviroc was evaluated for carcinogenic potential by oral gavage administration to rasH2 transgenic mice for 6 months and rats for up to 96 weeks (females) and 104 weeks (males). Daily doses of 200, 800 and 1500 mg/kg/day were administered to transgenic mice and doses of 50, 100, 500 and 900 mg/kg/day were administered to rats. In the rats or mice, maraviroc did not

cause a statistically significant increase in the incidence of any tumor. There were no indications of carcinogenic potential for humans.

B. Pharmacological activity: 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. Site directed mutagenesis and computer modeling studies locate the likely binding site of maraviroc to a pocket within the transmembrane region of CCR5. 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 and inhibits gp120/CCR5-mediated membrane fusion with an IC_{50} of 0.22 nM.

C. Nonclinical issues relevant to clinical use: The Exec CAC concluded that maraviroc did not produce tumors in Sprague-Dawley rats and rasH2 transgenic mice and these results suggested that maraviroc was not a potential cancer hazard to patients. Maraviroc can be classified as Pregnancy Category B. Maraviroc should be used during pregnancy only if clearly needed.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

UK-427,857 (Maraviroc) 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, and maraviroc is the first CCR5 inhibitor to be considered for approval.

Maraviroc has been formulated as film-coated tablets (150 mg BID and 300 mg BID) for oral administration. The maximum well tolerated therapeutic dose was established at 300 mg BID. 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 was assessed in the toxicology species using doses and plasma exposures (Cmax and AUC24h) higher than those administered to human patients.

In the rat, bioavailability of maraviroc was relatively low at 5%. Absorption in this species was found to be incomplete (20-30%) from hepatic portal vein concentration data. Bioavailability was 40-42% in the dog and absorption was found to be high based on anticipated first-pass extraction with respect to dog liver blood flow. Unchanged drug was observed in the feces of all species (ranging from 19% in the female dog to 77% in

the male rat) and may have resulted from biliary excretion and/or incomplete absorption.

Maraviroc was not mutagenic or genotoxic in a battery of in vitro and in vivo assays including a bacterial reverse mutation assay (Ames), chromosome aberrations in human lymphocytes and rat bone marrow micronucleus. Maraviroc did not impair mating or fertility of male or female rats and did not affect sperm of treated male rats up to 1000 mg/kg/day.

The full toxicology profile of maraviroc was assessed by using high daily doses (up to 2000 mg/kg/day in mice, 1500 mg/kg/day in rats, 250 mg/kg/day in dogs and 800 mg/kg/day in monkeys). The dose range studied provided plasma exposures higher than those found at the maximum therapeutic dose in humans, 300 mg BID.

In mice, mortality at 1000 mg/kg/day was associated with local gastrointestinal pathology rather than systemic toxicity, and gave a NOAEL of 200 mg/kg/day. In rats, the dose of 900 mg/kg/day 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/day and bile duct hyperplasia from 300 mg/kg/day were seen in the 6-month study and established the NOAEL in rats at 100 mg/kg/day.

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

In monkeys, the daily dose of 800 mg/kg/day was not well-tolerated, being associated with cardiovascular changes (reduced blood pressure and heart rate) and treatment reactions (reduced activity, prostration, and loss of balance). The dose of 400 mg/kg/day produced similar, though less severe findings, as well as an increase in QTc interval, in the 9 month study. The NOAEL, established from the 9-month study, was 120 mg/kg/day in monkeys. There was no evidence of cardiac arrhythmias in dogs or monkeys over the dose range studied. In conclusion, maraviroc was well tolerated in toxicology species and was not considered to represent a risk for human patients at the therapeutic dose.

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Information to sponsor: No

Sponsor and/or agent: Pfizer Global Research & Development
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New London, CT 06320

Manufacturer for drug substance: Pfizer Global Research & Development

Sandwich, Kent, CT13 9NJ, UK

Reviewer name: Pritam S. Verma, Ph.D.

Division name: Division of Antiviral Products

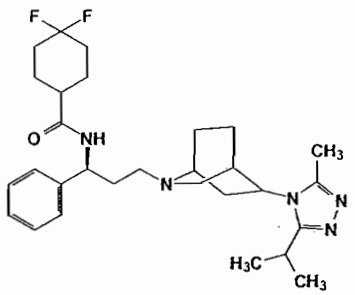
HFD #: 530

Review completion date: 04/11/07

Drug:

Trade name: SELZENTRY^R
Generic name: Maraviroc
Code name: UK-427,857
Chemical name: 4,4-difluoro-N-{1S)-3-[exo-3-(3-isopropyl-5-methyl-4H-1,2,4-triazol 4-yl)-8-azabicyclo[3.2.1]oct-8-yl]-1-phenylpropyl} cyclohexanecarboxamide
CAS registry number: 376348-65-1
Molecular formula: $C_{29}H_{41}F_2N_5O$
Molecular weight: 513.7
Melting Point: 193.5 degrees C
Solubility: >25.6 mg/ml in 0.1 M HCl
Description: White solid of uniform appearance

Structure:



Relevant IND: 65,229

Drug class: CCR5 Antagonist

Intended clinical population: HIV-1 infected adults

Clinical formulation: Tablet dosage form

Route of administration: oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-128 are owned by Pfizer Company or are data for which Pfizer Company has obtained a written right of reference. Any information or data necessary for approval of NDA 22-128 that Pfizer Company does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Pfizer Company does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-128.

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Studies reviewed within this submission:

Table 1
Nonclinical toxicology studies

Study type/duration	Route of administration	Test system
Single dose	Oral	Rat and mouse
	IV	Rat and mouse
Repeat dose		
1. 2 week	Oral	Mouse and dog
2. One-month	Oral	Mouse, rat, dog and monkey
3. 3-month	Oral	Mouse
4. 6-month	Oral	Rat and dog
5. 9-month	Oral	Monkey
Genetic toxicology		
6. Ames assay	In vitro	Salmonella and E. Coli
7. Chromosomal Aberration assay	In vitro	Human lymphocytes
8. Micronucleus assay	Oral	Mouse
Reproductive/developmental		
9. Fertility/embryonic development	Oral	Rat
10. Embryo-fetal development	Oral	Rat and rabbit
11. Pre- and post-natal development	Oral	Rat
Local Tolerance		
1. Dermal toxicity	Skin application	Rat
2. Skin Irritation	Skin application	Rabbit
3. Eye Irritation	Eye instillation	Rabbit
4. Murine local lymph node	Skin sensitization	Mice
Carcinogenicity		
1. 4-week toxicology	Oral	Transgenic mouse
2. 6-month assay	Oral	Transgenic mouse
3. 2-yr bioassay	Oral	Rat
Special toxicology		
1. 4- week immunotox	Oral	Monkey
2. 4-week thyroid & liver changes	Oral	Rat
3. 7-day iv irritation	IV	Rat
Safety pharmacology		
1. MIP-1 β binding	In vitro	HEK-293 cell
2. macaque CCR-5 cells	In vitro	FLIPR assay
3. M3 muscarinic receptor	In vitro	Calcium flux assays
4. Affinity for ion channel	In vitro	Isolated tissues
5. Cardiovascular system	In vivo, oral	Dogs
6. QT Intervals	In vitro	hERG
7. CNS and Peripheral nervous system	In vivo, oral	Rats
8. Blood gases	In vivo, oral	Rats
9. Renal/Urinary	In vivo, oral	Rats

Table 2
Nonclinical pharmacokinetic studies

Type of study	Test system	Method of administration
2.6.5.3 Pharmacokinetic studies after a single dose		
Plasma concentration and bioavailability	Sprague-Dawley rats	Single, po, iv
Plasma concentration and bioavailability	Beagle dogs	Single, po, iv
Plasma concentration	Wild type mice	Single, po
2.6.5.4 Toxicokinetics	Mice	2-week, po
Toxicokinetics	Mice	4-week, po
Toxicokinetics	Rats	4-week, po
Toxicokinetics	Dogs	2-week, po
Toxicokinetics	Dogs	4-weeks, po
Toxicokinetics	Dogs	6-month, po
Toxicokinetics	Monkeys	4-week, po
2.6.4.4 Distribution		
CNS Penetration	Rats	Single, iv
Protein Binding	Plasma from various sources including humans	In vitro
Tissue distribution	Rats	Single, iv
In vitro binding to albumin	humans	In vitro
Plasma concentration ratio in rat dog and human blood	Blood	In vitro
2.6.4.5 Metabolism		
Pharmacokinetics in isolated liver	Rats	In vitro, single
Hepatic microsomal fractions	Human liver	In vitro
Metabolism and excretion	Mice, rats and dogs	In vivo, po, single
Metabolism cytochrome P450	Human liver	In vitro
Inhibition cytochrome P450	Human liver	In vitro
Inhibition recombinant cytochrome P450		In vitro
Profiling of metabolites	Human, dog, monkey hepatocytes	In vitro
2.6.4.6 Excretion		
Mechanism of clearance	rats	In vivo, po
Excretion studies	Male mice	In vivo, po
Excretion studies	dogs	In vivo, po
Biliary excretion	Male rats	Single, iv

Studies <u>not</u> reviewed within this submission: exploratory studies in mice and rats were not reviewed.

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2.6.2 PHARMACOLOGY

Mechanism of action

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

Cytotoxicity

Fresh uninfected human peripheral blood lymphocytes that express the complement of chemokine receptors including CCR5 AND CXCR4 were incubated with UK-427,857 at concentrations up to 10 μM. UK-427,857 did not show any toxicity in these studies. In addition PM-1 cells (an immortalized CD4+ T-cell line naturally expressing CCR5 and CXCR4) showed no evidence of cytotoxicity at 10 μM.

2.6.2.1 Brief summary

Effect on cardiovascular system: Oral (unbound Cmax at highest dose investigated = 168 nM) and intravenous (unbound Cmax at highest dose investigated = 1823 nM) 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 hemodynamic 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. In a series of 2-minute postural challenges, maraviroc (at mean unbound plasma concentration of 1227 nM) produced a statistically significant larger 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.

Effect on QT interval: Maraviroc, at 1 μM, did not inhibit binding of [3H] dofetilide to the hERG channel and was devoid of effects on the canine Purkinje fiber action potential morphology. However, at 3 - 10 μM maraviroc, there was a concentration-dependent inhibition of [3H] dofetilide binding (43% inhibition at 10 μM) and of the hERG potassium current (19% inhibition at 10 μM) and prolongation of the Purkinje fiber action potential duration (up to 31% prolongation at 90% repolarization). 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 repolarization 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 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 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.

Maraviroc caused no statistically significant changes to the QT interval following oral administration to conscious freely moving dogs at 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. 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, that of human.

Effect on central and peripheral nervous system: Groups of conscious male rats were given single doses of UK-427,857 at dose levels of 0 (vehicle control), 1000 (low) and 2000 mg/kg (high). At the high dose, decreased breathing, piloerection, salivation and irregular and labored breathing were observed. Posture, skin color, grip, strength and core temperature were normal throughout the experiment. At the low dose, mild effects on appearance and behavior were observed when compared to the controls. The Cmax was 2.8 μg/ml (5.5 μM) at the maximum tolerated dose (low).

Effect 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.

Effect 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.

2.6.2.4 Safety pharmacology

Summary reports were provided:

In vitro Pharmacology

1. Inhibition of [^{125}I]-MIP-1 β binding by UK-427,857 to recombinant macaque CCR-5 expressed in HEK-293 cell membrane preparation (UK427857/CG/011/020)

The aim of this study was to determine the radioligand binding affinity of UK-427,857 for the macaque recombinant CCR-5, stably expressed in a HEK-293 cell line. The test compound, UK-427,857 and the standard, UK-396,794-03 inhibited the binding of [^{125}I]-MIP-1β macaque CCR5 with mean IC_{50} values of 17.5 nM and 27.8 nM, respectively (n=5). The affinity of UK-427,857 for the macaque CCR5 is similar to the affinity for the human CCR-5.

2. Characterization of UK-427,857 binding in recombinant macaque CCR-5 cells using a FLIPR assay (UK427857/CG/009/02)

The functional activity of UK-427,857 at the macaque CCR5 receptor was determined using a recombinant cell-line in a fluorescence assay run on the Fluorometric Imaging Plate Reader (FLIPR).

The assay quantitatively measures cytosolic calcium flux induced by exogenously added human Macrophage Inflammatory Protein 1b (MIP-1b) to demonstrate antagonism of the macaque CCR5 receptor by UK-427,857.

A total of 4 separate experiments were completed, and the percentage inhibition of the maximum (*i.e.* no added compound) MIP-1b-induced FLIPR fluorescence signal with a range of concentrations of UK-427,857 (0.78-800 nM, 0.40-411 ng/ml) for each experiment was calculated.

The 4 IC50 values for the percentage inhibition of the assay by UK-427,857 were: 30 nM (15.4 ng/ml), 13 nM (6.7 ng/ml), 14 nM (7.2 ng/ml) and 17 nM (8.7 ng/ml) - *i.e.* a range of 13-30 nM (6.7 - 15.4 ng/ml). This range compares closely with the range obtained in previous studies with UK-427,857 on a recombinant human CCR5 receptor cell-line (7 - 30 nM (4 - 15 ng/ml).

3. Potency of UK-427,857 versus CCR2 receptor and M3 muscarinic receptor in functional fluorescent imaging calcium flux assays (UK-427,857/DI/008/1)

The selectivity of UK-427,857 for functional antagonism of CCR5 over the CCR2 and M3 receptors has been determined. UK-427,857 is neither an agonist nor antagonist to either the endogenously expressed CCR2b receptor on a human acute monocytic leukemia cell line (THP-1) or the endogenously expressed M3 muscarinic receptor on CCR5 stable transformed HEK-293 cells up to doses of 10 μM. The complete lack of inhibition of muscarinic signaling by carbachol on HEK-293/CCR5 cells confirms that CCR5 ligand-induced calcium flux inhibition by UK-427,857 at 1μM (n=3) is specific to

CCR5, and does not involve other signal transduction steps that may be common to G protein-coupled receptors. UK-427,857 is therefore unlikely to have a biological effect on other chemokine receptors needed for immune cell function or other 7TM G-coupled receptors.

4. Affinity for other receptors, enzymes and ion channel and effects on isolated tissues (DI/073/06)

The affinity of UK-427,857 for a range of physiologic receptors, ion channels and enzymes is shown in Table 3. UK-427,857 at 1 µM (0.513 µg/ml) did not inhibit binding of dofetilide to the hERG channel and was devoid of effects on the canine cardiac action potential morphology in vitro. At 3 and 10 µM, there was concentration dependent inhibition of dofetilide binding, inhibition of the hERG potassium current and prolongation of the action potential duration at both 50% and 90% of repolarization. These results showed that UK-427,857 was active at the human cardiac hERG channel and suggested that UK-427,857 had the potential to block the IKr current and affected cardiac repolarization in vivo at free plasma concentrations greater than 3µM or 1.54µg/ml (approximately 40 times predicted free Cmax at the 100 mg dose of UK-427,857).

Table 3
Effect of UK-427,857 on ion channels and isolated tissues

Experiment	Species (n)	Concentration	Standard	Result/observation
hERG (IKr)	Human recombinant (6)	0.03 n M to 10 µM (0.0154 to 5136.8 ng/ml)	Dofetilide	100 n M: no effect 10µM: 24% inhibition 3.16 µM: 15% inhibition
hERG potassium channels (patch clamp assay)	Human cloned ion channel (4)	200 n M (102.74 ng/ml) 3 & 10 µM (1541 & 5136.8 ng/ml)	Dofetilide	200 n M: no effect 3µM: 5.74% inhibition 10µM: 19% inhibition
Dog isolated Purkinje fibers: action potential	Dog (5)	0.2, 0.5, 1, 3 & 10 µM (0.102, 0.256, 0.513, 1.54 & 5.1 µg/ml)	d-sotolol	0.2,0.5&1.0µM: no effect 3µM: 8.8% prolongation of APD50, 9.5% prolongation of APD90 10µM: 25.9% prolongation of APD50 30.6% prolongation of APD90
Hemodynamic and ECG parameters	Dog (4)	0.05, 0.15, 0.5 & 1.5 mg/kg	None	No effect Cmax 114.36 ng/ml (free) Total=238.25 ng/ml
Respiratory system	Rat (4) iv	1 mg/kg	Morphine	No effect. A small but statistically significant ↓ in mean arterial BP and heart rate 10 min after dosing

APD = action potential duration

In vivo Pharmacology

1. Effects on cardiovascular system:

a. Effect of iv administration of UK-427,857 on blood pressure and heart rate during postural change in the conscious dog (CG/007/02)

UK-427,857 (10.5 mg/kg) or vehicle was administered to 4 conscious dogs by a continuous intravenous infusion lasting 180 minutes (except dog number 7FI4 where UK- 427,857 was infused for 120 minutes) to examine the effects on blood pressure and heart rate during postural change.

Basal hemodynamic and electrophysiological parameters were measured with the dogs settled and lying in a canvas support sling, twice pre-dose, and for the minute preceding each postural challenge. Responses to postural change (from the lying to the upright position for 2 minutes) were assessed twice pre-dose, approximately 15 minutes after the start of infusion, and at approximately 20 minute intervals until a total of 9 postural challenges were performed. In dog number 7FI4, only 6 postural challenges were performed during an infusion of UK-427,857 lasting 120 minutes. Arterial blood samples (approximately 4 ml), for assessment of plasma drug levels were taken pre-dose, and at the end of each postural challenge during infusion of UK-427,857 or vehicle. Only blood samples taken during infusion of UK-427,857 were analyzed. Mean plasma concentrations were in the range 1030 nM free to 2410 nM free.

With the dog settled in the lying position, administration of UK-427,857 produced small reductions in systolic, and increases in diastolic blood pressure, relative to vehicle which resulted in a slight, but statistically significant ($p<0.05$) reduction in pulse pressure, compared with vehicle. UK-427,857 administration produced no effects on basal HR compared with vehicle. It is unlikely that the effects of UK-47,857 on basal hemodynamics are of biological importance.

When compared to vehicle, UK-427,857 administration caused no biologically relevant effects on basal ECG parameters, except for a moderate prolongation of the ECG QT interval compared with vehicle which was observed at all concentrations of UK-427,857. This effect was not dose related, with a difference from vehicle in mean QT interval of 13.5 msec at the lowest concentration (1030 nM free) and 12.3 msec at the highest concentration (2410 nM free) of UK-427,857. The greatest increase in QT interval compared with vehicle of 23.1 msec occurred at a free plasma concentration of UK-427,857 of 1310 nM.

Although there were some minor changes, there were no statistically significant differences in average heart rate or mean blood pressure during 2-minute postural challenges between vehicle or UK-427,857 treated dogs. Average pulse pressure during postural challenge gradually increased during vehicle administration but

remained relatively constant during UK-427,857 administration. This profile indicated that over the concentration range achieved in this study, UK-427,857 did not produce obvious postural hypotension in the conscious dog.

UK-427,857 produced effects on the initial response to change to the upright position. From challenge 4 to 9 during administration of UK-427,857, the initial heart rate increase was less than the corresponding increase during vehicle administration. Initial fall in systolic blood pressure was greater at each postural challenge during administration of UK-427,857 than during vehicle administration. This suggested that UK-427,857 produced some impairment of normal reflex control of blood pressure during the change to the upright position. However, once the upright position was established, blood pressure control was maintained at a normal level.

Thus, compared with the effects of vehicle, intravenous infusion of UK-427,857 at mean free plasma concentrations in the range 1030 nM to 2410 nM produced a moderate prolongation of the ECG QT interval, and produced only subtle changes to control of blood pressure during postural change in the conscious dog.

b. Determination of the hemodynamic and ECG effect of UK-427,857 following oral administration in the conscious dog (CG/003/00)

UK-427,857 or vehicle at dose levels of 0 (vehicle), 0.05, 0.15 and 0.5 mg/kg, were administered orally by gavage to 4 conscious, freely moving dogs, on separate occasions, to study the effects upon hemodynamic parameters and the electrocardiogram (ECG) using telemetry techniques, which included a continuous measure of the animals activity.

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

UK-427,857 did not cause any biologically relevant hemodynamic or ECG changes at any dose level when compared to vehicle. In conclusion, UK-427,857, when administered orally to the conscious dog at dose levels of 0.05, 0.15 and 0.5 mg/kg, caused no biologically relevant changes in hemodynamic or ECG parameters.

c. Determination of the hemodynamic and ECG effects of UK-427,857 at 1.5 mg/kg following oral administration in the conscious dog (CG/008/00)

UK-427,857, at a dose level of 1.5 mg/kg, was administered orally by gavage to 4 conscious, freely moving dogs, to study the effects upon hemodynamic parameters and the electrocardiogram (ECG) when compared to vehicle. This was done using telemetry techniques, which included a continuous measure of the animals' activity.

Vehicle treatment in the previous study was not associated with any biologically relevant hemodynamic or ECG effects.

UK-427,857 did not cause any biologically relevant hemodynamic or ECG changes when compared to vehicle. In conclusion, UK-427,857, when administered orally to the conscious dog at a dose level of 1.5 mg/kg, caused no biologically relevant changes in hemodynamic or ECG parameters.

2. Effect on central nervous and peripheral nervous system (CG/009/00)

The study was divided into two experimental phases. The first phase was to determine the effects of UK-427,857 on appearance and behavior in the rat. The second phase was to determine the plasma levels that were achieved using the highest clean dose identified in phase one of the study.

In phase one UK-427,857 was administered orally to male rats, Sprague Dawley [redacted] CD BR, [redacted]) at doses of 1000 and 2000mg/kg to investigate any effects on appearance and behavior.

Both rats treated with vehicle showed mild effects on appearance and behavior in the form of increased respiration immediately after dosing, which persisted for 15 minutes before returning to normal. Activity also increased for 5 minutes after dosing, but then returned to normal until both rats fell asleep. Posture, grip strength and skin color remained normal throughout the experiment, and core temperatures were all within the normal range of 37.0°C to 39.5°C.

Posture, skin color, grip strength and core temperatures remained normal throughout the experiment for animals treated with UK-427,857 at 1000 mg/kg po. Respiration increased immediately after dosing in both rats, and took 15 minutes longer to return to normal than in vehicle-treated animals. However, activity decreased after dosing, and took 15 minutes in one rat, and 30 minutes in the other, to return to normal. At 15 minutes after dosing one rat displayed a wet chin, which was evident for approximately 30 minutes.

Rats treated with UK-427,857 at 2000 mg/kg po., however, displayed adverse effects in the form of increased respiration immediately after dosing, which persisted for 30 minutes before returning to normal. Decreased activity was evident in both rats after dosing, followed by a short spell of increased activity (10 minutes) before a longer period of decreased activity (45 minutes) before returning to normal. Piloerection was evident in both rats after 5 minutes, and persisted for approximately 10 and 30 minutes respectively. Immediately after dosing one rat was visibly salivating, and the other was rubbing his wet chin in the sawdust, this behavior lasting until one hour post dose. One rat developed irregular breathing patterns after 5 minutes which lasted for approximately 10 minutes. After 2 hours this rat started opening his jaws and vocalizing. Breathing became labored and then rapid, and a vet was summoned to inspect the animal over a period of almost an hour, by which time the breathing patterns had returned to normal and both rats fell asleep. Posture, grip strength, skin color and core temperatures remained normal throughout the experiment for both animals.

Body weights in all rats tested either increased or remained the same after the 24 hour

period, except for one rat in the 2000 mg/kg treatment group which lost 2 grams. In phase two of the study rats were dosed with UK-427,857 at 1000 mg/kg po., and blood samples were taken at specific time points during the day. Analysis of the blood samples showed that the maximum free plasma concentration of UK-427,857 was obtained at 5 hours post dose in both rats (4.95μM), although there was little difference between values from 30 minutes to 5 hours post dose This could be a reflection of the high dose of UK-427,857 administered, where absorption is restricted due to a limit on dissolution and/or permeability, hence the profile is prolonged. These free plasma levels were in excess of 2,000 times the CCR5 in-vitro inhibitory activity (IC90 value of 2.2 nM).

Oral administration of UK-427,857 at 1000 mg/kg, therefore, showed mild effects on appearance and behavior when compared to vehicle-treated animals. However, at 2000 mg/kg po. adverse effects were observed in the form of decreased activity, piloerection, salivation, wet chins, irregular and labored breathing, jaw movements and vocalization.

3. Effects of oral administered UK-427,857 on the excretion of fluid and electrolytes in female rats (UK427857/CG/004/01)

The effects of orally administered UK-427,857 (10, 20 and 60 mg/kg) were investigated on the excretion of urine and electrolytes over a five hour period after dosing in conscious normotensive female Sprague Dawley strain rats (12 animals per treatment group), given an oral saline load.

Frusemide (20 mg/kg po.) produced pronounced diuresis and increased ion excretion, thus validating the experimental procedure.

UK-427,857 did not produce any significant differences in urine volume, pH or electrolyte excretion compared to vehicle treated animals. Overall, UK-427,857 had no effect on renal function in the conscious rat at the doses tested.

4. Effects of intravenously administered UK-427,857 on blood gases in the urethane anaesthetized rat (UK427857/CG/001/01)

The effect of a single intravenous dose of UK-427,857 (1.0 mg/kg), on arterial blood pH, pCO2 and pO2 was investigated in urethane anaesthetized male rats. In addition, the effects on mean arterial blood pressure (MABP) and heart rate (HR) were analyzed.

UK-427,857 produced no statistically significant change ($p<0.05$) in arterial blood gases compared with changes in vehicle treated animals in this study.

Following administration of UK-427,857, MABP gradually fell to a maximum of -13.1 ± 4.8 mmHg below pre-dose values at 25 minutes post-dose. HR also fell gradually to a maximum of -16.1 ± 6.3 beats/minute below pre-dose values. The changes in MABP and HR after UK-427,857 administration were statistically significant compared with vehicle treated animals ($p<0.05$) at 10 minutes post-dose.

Morphine (4 mg/kg i.v.) produced statistically significant reductions ($p<0.05$) in arterial blood pH and pO2 and increases in pCO2 compared with vehicle treated animals, thus validating the experimental procedure.

Thus, intravenous administration of UK-427,857 at 1.0 mg/kg to the urethane anaesthetized rat produced no statistical effect on pH, pO2 or pCO2 at any of the time points tested. Significant differences from vehicle treated animals were seen in HR and MABP at 10 minutes post-dose, although the size of these differences was small.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

2.4.3.2. Absorption and bioavailability

Following oral administration maraviroc was absorbed rapidly with a Tmax of 2h or less in animal 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 found to be high based on anticipated first-pass extraction with respect to dog liver blood flow. Unchanged drug was observed in the feces of all species (ranging from 19% in female dog to 77% in male rats) and may have resulted from biliary excretion and/or incomplete absorption. Similarly, after a 300 mg oral dose, unchanged drug in human feces accounted for 25% of the dose. Absolute bioavailability in human after a 100 mg dose was 23%. 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.

Multiple dose pharmacokinetics derived within the toxicology program 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.

Toxicokinetics

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 superproportionally 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.

2.4.3.3. Distribution

Maraviroc showed moderate plasma protein binding in all species with an approximate twofold range in unbound fraction from 0.25 (human) to 0.52 (cynomolgus monkey). 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 α 1-acid glycoprotein.

Maraviroc showed 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. 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. 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.

Following single intravenous administration of tritium-labeled maraviroc to male pigmented rats, distribution of radioactivity was rapid. At early time points, most tissues contained concentrations that were several times higher than the blood. Highest levels were detected in liver, kidney, small intestine, bladder, seminal vesicles and prostate gland. Levels of radioactivity decreased 2- to 13-fold by one hr and were higher than blood in all tissues except brain (4-fold lower). Concentrations continued to decline at later time points and were non-detectable in most tissues by 96 hr.

2.4.3.4. Metabolism

The in vitro metabolism of maraviroc was studied using human liver microsomes and recombinant cytochrome P450 enzymes. 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 contributed significantly to its metabolism. Furthermore, the formation of the circulating N-dealkylated metabolite UK-408,027 was shown to be mediated by CYP3A4. CYP3A4 was therefore responsible for a large proportion of the metabolism of maraviroc and as a consequence its pharmacokinetics could be altered by coadministration of drugs that inhibit this enzyme.

The major pathway identified in vivo in humans was N-dealkylation adjacent to the tropane ring. The metabolism of [14C]-labeled maraviroc was studied in human liver microsomes and hepatocytes. Dual-labeled 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 unlabeled 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 unlabeled 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 was investigated in human liver microsomes, with and without preincubation. Maraviroc did not inhibit any of these enzymes at clinically relevant concentrations (IC_{50} > 30 µM against probe substrates for each enzyme). Maraviroc was 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 was confirmed.

Unchanged drug was the major excreted component in all species, with combined urine and fecal 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. Unchanged maraviroc was 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 (further metabolism of amine group) was present in mouse, dog and cynomolgus monkey at 1-5% of circulating radioactivity.

2.4.3.5. Elimination and excretion

The systemic pharmacokinetics of maraviroc were studied in rat and dog following single intravenous administration. In these species, maraviroc had 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 resulted in a short elimination half-life of 0.9-2 hours. The excretion of [14C]-maraviroc was investigated in mouse, rat and dog. Overall there were no major differences in the qualitative pattern of excretion between species with the major route of elimination being fecal (>72% of the administered dose). Elimination was also rapid and most of the radioactivity was recovered within 48 hours. The predominance of fecal excretion across species probably reflects extensive biliary elimination of maraviroc and its metabolites. In an isolated perfused rat liver preparation, 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, 64% of intravenously dosed radioactivity [3H]-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.

2.6.4.2 Methods of Analysis

The nonclinical pharmacokinetic program for maraviroc comprised detailed single dose studies in mouse, rat and dog to define basic pharmacokinetic parameters. This program 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 program 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 radiolabeled forms of maraviroc – tritium and both single and dual carbon-14 labeled material. Initial metabolism studies performed with [3H]-labeled 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 [14C]-labeled 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-labeled fragment was investigated by specific analysis and also by in vitro studies utilizing a second [14C]-labeled compound (dual label) where radiolabel was retained in all fragment products. In vitro metabolism studies were used to support in vivo studies, to characterize the enzymes involved in maraviroc metabolism and to determine the potential for interactions with co-administered drugs.

2.6.4.3 Absorption

1. Pharmacokinetics of UK-427,857 male Sprague-Dawley rats following a single iv or oral administration (DM1)

Groups of male rats were administered UK-427,857 by bolus iv injection (n=2; 1 mg/kg) and by oral gavage at 3 mg/kg (n=2) and 10 mg/kg (n=2). Blood samples were collected

from each animal at selected time points during the 24-hr postdose period. Plasma UK-427,857 concentrations were determined using a validated LC-MS.-MS method. Results: a summary of calculated pharmacokinetic parameters for UK-427,857 following single iv or oral dose is shown in Table 4. Following oral administration of UK-427,857 (10 mg/kg), AUC was 0.669 µg*hr/ml. The oral bioavailability of UK-427,857 was 5%.

Table 4
Pharmacokinetics of UK-427,857 in male rats following iv or oral single dose administration

Dose (mg/kg)	Route	Cmax (ng/ml)	Tmax (min)	Free Cmax (ng/ml)	$T_{1/2}$ (hr)	CL (ml/min/kg)	VD (L)	F (%)
1	iv	-	-	-	0.9	74	6.5	100
3	PO	-	-	-	-	-	-	-
10	PO	55	2	30	-	-	-	5

"-"= plasma levels could not be detected or parameter could not be measured.

2. Pharmacokinetics of UK-427,857 in beagle dogs following a single iv or oral administration (DM4)

Groups of male and female dogs were administered UK-427,857 by bolus iv injection (n=2/sex; 0.5 mg/kg) and by oral gavage at 1 mg/kg (n=1/sex) and 2 mg/kg (n=1/sex). Blood samples were collected from each animal at selected time points during the 24-hr postdose period. Plasma UK-427,857 concentrations were determined using a validated LC-MS.-MS method. Results: a summary of calculated pharmacokinetic parameters for UK-427,857 following single iv or oral dose is shown in Table 5. Following oral administration of UK-427,857 (1 and 2 mg/kg), AUCs were 0.335 and 0.583 µg*hr/ml, respectively. The oral bioavailability of UK-427,857 was 41%.

Table 5
Pharmacokinetics of UK-427,857 in male and female beagle dogs following iv or oral single dose administration

Dose (mg/kg)	Route	Cmax (ng/ml)	Tmax (min)	Free Cmax (ng/ml)	$T_{1/2}$ (hr)	CL (ml/min/kg)	VD (L/kg)	F (%)
05	iv	-	-	-	2.3	21	4.3	100
1	PO	82	1.5	39	-	-	-	42
2	PO	256	0.75	123	-	-	-	40

"-"= parameter could not be measured.

3. Pharmacokinetics of UK-427,857 in male mdrla/lb knockout and wild type mice following a single oral administration (DM2)

The concentrations and pharmacokinetic parameters of UK-427,857 in male mdrla/lb knockout and wild-type mice following a single oral dose (16 mg/kg) administration were determined. Following oral administration to mdrla/la knockout mice (2 mice/timepoint), the plasma AUC was 1247*hr/ml and elimination half-life was 1 hr. Following oral administration to male wild type mice (2 mice/timepoint), the AUC decreased to 440 ng*h/ml. Elimination half-life was 0.7 hr.

Toxicokinetics

4. Two week oral gavage toxicity and toxicokinetics study of UK-427,857 in CD-1 mice (01-2120-03)

Groups of male and female CD-1 mice (10 animal/sex/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (sterile saline; vehicle control), 20 (low), 200 (mid) 1000 (high) or 2000 mg/kg/day (very high) for 14 consecutive days. Plasma drug concentrations were measured in a separate satellite group of animals (3/sex/dose/timepoint) on days 1 and 13 at 1, 3, 5 and 24 hr postdose and the samples were analyzed by a validated analytical method. Toxicokinetics: data are shown in Table 6. Systemic exposure increased over the dose range and was similar in males and females. The highest mean levels were observed at 1 or 3 hr post dose. Some reduction in exposure occurred on repeated administration primarily in male mice.

Table 6
Toxicokinetics of UK-427,857 on days 1 and 13 following repeated daily oral (gavage) administration to male and female mice.

Dose (mg/kg/day)	Sex	Day 1		Day 13	
		$AUC_{0\text{-}24hr}$ (µg*hr/ml)	Cmax (µg/ml)	$AUC_{0\text{-}24hr}$ (µg*hr/ml)	Cmax (µg/ml)
20	M	1.95	0.18	1	0.25
	F	5.39	1.96	0.92	0.37
200	M	18.1	4.9	12.7	2.38
	F	15.9	1.56	13.8	2.02
1000	M	140	10.7	45.8	6.56
	F	95.8	11.4	59.2	7.38
2000	M	240	21.9	132	11.8
	F	219	14.7	236	12.4

5. One month dose range finding oral gavage toxicity and toxicokinetics study of UK-427,857 in CD-1 mice (02002)

Groups of male and female CD-1 mice [10 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (sterile saline; vehicle control), 200 (low), 500 (mid) or 750 mg/kg/day (high) for 28 consecutive days. Plasma drug concentrations were measured in a separate satellite group of animals (15/sex/dose) on day 26 at 1, 3, 5 and 24 hr postdose and the samples were analyzed by a validated analytical method. Toxicokinetics: data are shown in Table 7. Systemic exposure increased over the dose range and was similar in males and females. The highest mean plasma levels were observed at 3 hr postdose in males and between 1 and 5 hr in females

Table 7
Toxicokinetics of UK-427,857 on day 26 following repeated daily oral (gavage) administration to male and female mice.

Dose (mg/kg/day)	Sex	Day 26	
		$AUC_{0\text{-}24hr}$ (µg*hr/ml)	Cmax (µg/ml)
200	M	5.6	31.6
	F	8.3	35.7
500	M	10	79.4
	F	12.8	107
750	M	13	126
	F	13.9	110

6. One month dose range finding oral gavage toxicity and toxicokinetics study of UK-427,857 in male rats (02072)

Groups of male Sprague-Dawley rats 5 animal/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (sterile saline; vehicle control), 100 (low), 300 (mid) or 1500 mg/kg/day (high) for 28 consecutive days. Plasma drug concentrations were measured in a separate satellite group of animals (5/dose) on days 1 and 23 at 1, 3, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method. Toxicokinetics: data are shown in Table 8. The highest mean plasma levels were observed between 1 and 7 hr post dose.

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Table 8
Toxicokinetics of UK-427,857 on days 1 and 23 following repeated daily oral (gavage) administration to male rats

Dose (mg/kg/day)	Day 1		Day 23	
	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)
100	4.77	0.69	7.4	1.15
300	21.3	2.38	31.2	4.3
1500	84.5	7.26	88.7	7.3

7. Two week oral toxicity and toxicokinetics study of UK-427,857 in beagle dogs (01-2120-05)

Groups of male and female beagle dogs [3 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (sterile saline; vehicle control), 10 (low), 50 (mid) or 250 mg/kg/day (high) for 14 consecutive days. Plasma drug concentrations were measured on days 1 and 13 at 1, 3, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method.
Toxicokinetics: data are shown in Table 9. The highest mean plasma levels were observed at 1 hr post dose. Systemic exposure increased over the dose range and was similar in male and female animals and comparable on days 1 and 13.

Table 9
Toxicokinetics of UK-427,857 on days 1 and 13 following repeated daily oral (gavage) administration to male and female dogs

Dose (mg/kg/day)	Day 1		Day 13	
	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)
10	2.4	1.7	2.2	1.8
50	9.4	4.7	9.3	3.6
250	32.2	5.8	25.7	6.5

8. One month oral dose range finding toxicity study of UK-427,857 in beagle dogs (02003)

Groups of male and female beagle dogs [3 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (sterile saline; vehicle control), 5 (low), 50 (mid) or 150 mg/kg/day (high) for 28 consecutive days. Plasma drug concentrations were measured on days 1 and 19 at 1, 4, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method. Toxicokinetics: data are shown in Table 10. The highest mean plasma levels were observed at 1 hr post dose. Systemic exposures increased over the dose range and were 1.5-2.2 folds higher on day 19 when compared to days 1 values.

Table 10
Toxicokinetics of UK-427,857 on days 1 and 19 following repeated daily oral (gavage) administration to male and female dogs

Dose (mg/kg/day)	Day 1		Day 19	
	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)
5	2.93	1	6.02	0.79
50	26.64	6.99	35.19	8.81
150	61.86	9.32	99.73	9.69

9. Six month oral toxicity and toxicokinetics study of UK-427,857 in beagle dogs (02073)

Groups of male and female beagle dogs (4 animal/sex/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (sterile saline; vehicle control), 5 (low), 15 (mid) or 40 mg/kg/day (high) for 6 months. Plasma drug concentrations were measured on days 1 and 176 at 1, 4, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method. Toxicokinetics: data are shown in Table 11. The highest mean plasma levels were observed at 1 hr post dose. Systemic exposure increased over the dose range and was similar on day 1 and 176.

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Table 11
Toxicokinetics of UK-427,857 on days 1 and 176 following repeated daily oral (gavage) administration to male and female dogs

Dose (mg/kg/day)	Day 1		Day 176	
	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)
5	2.88	0.96	2.42	0.88
15	8.32	2.8	8.53	2.61
40	18.02	4.44	19.82	4

10. One month oral bid dose range finding toxicity study of UK-427,857 in cynomolgus monkeys (911/097)

Groups of male and female cynomolgus monkeys [age: 26-32 months; 2 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 bid at dose levels of 0 (sterile saline; vehicle control), 100 (low), 200 (mid), 400 (high) or 800 mg/kg/day (very high) for 28 consecutive days. Plasma drug concentrations were measured on days 1 and 27 at 1, 4, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method. Toxicokinetics: data are shown in Table 12. The highest mean plasma levels were observed between 1 and 3 hr post dose. Systemic exposure increased over the dose range and was similar on day 1 and 27.

Table 12
Toxicokinetics of UK-427,857 on days 1 and 27 following repeated daily oral (gavage) administration to male and female monkeys

Dose (mg/kg/dose)	Day 1		Day 27	
	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)
50	5.4	1.25	4.2	0.81
100	19.2	2.97	19	3.09
200	66	6.79	68.2	8.34
400	84.7	8.7	-	-

2.6.4.4 Distribution

11. CNS penetration of UK-427,857 in male Sprague-Dawley rats (DM3)

The brain penetration of UK-427,857 was studied in male rats (n=4) following iv infusion administration of 30 μg/min/kg for 75 min. Cerebrospinal fluid (CSF) samples collected at the end of the infusion period provided mean concentrations of UK-427,857 which were equivalent to 5.2% of the concentration present in plasma at the same time.

12. Plasma protein binding and blood partitioning in animals and man (DM12/16/18)

The protein binding of UK-427,857 was studied at concentrations ranging from 1 to 1000 ng/ml in mouse, rat, rabbit, dog and humans by equilibrium dialysis. The protein binding was 66% (mouse), 46% (rat), 50% (rabbit), 52% (dog) and 78% (human). Plasma protein binding values were therefore, moderate in all species studied. UK-427,857 exhibited moderate binding to human plasma proteins albumin (56%) and α1-acid glycoprotein (69%) at physiological concentrations. UK-427,857 showed 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 after addition of 100 ng/ml to blood.

13. The tissue distribution of radioactivity in male rats following a single iv administration of ^{3}H-labelled UK-427,857 (DM6)

The distribution of radioactive in the tissues of male pigmented rats (n=1/time point) was studied at 0.1, 1, 6, 24, 48 and 96 hr following iv administration of ^{3}H-labelled UK-427,857 at a dose level of 3 mg/kg. Radioactivity was measured in tissue homogenates by liquid scintillation counting. Results: following the administration, distribution of radioactivity was rapid; at 5 min postdose, radioactivity was detectable in all tissues analyzed, mostly at concentrations similar to or higher than that of blood. Highest levels were detected in liver, kidney, small intestine, bladder, seminal vesicles and prostate gland. Levels of radioactivity decreased 2- to 13-fold by one hr and were higher than blood in all tissues except brain (4-fold lower). Concentrations continued to decline at later time points and were non-detectable in most tissues by 96 hr.

14. Plasma protein binding of [3H]-UK-427,857 to human albumin and α-1 acidic glycoprotein (DM 18)

A series of experiments have been conducted in order to study the binding of [3H]-UK-427,857 to the plasma constituents albumin and *a-1* -acid glycoprotein (AAG) at physiological concentrations of these proteins. Initial drug concentrations of 1, 30 and 1000 ng/ml were used.

The results show that UK-427,857 has a moderate affinity to the plasma constituents albumin (56% bound) and AAG (69% bound). The mean proportion of drug bound to both albumin and AAG was similar across the concentration range. Under physiological conditions, UK-427,857 is likely to bind to both proteins in similar proportions.

15. [3H]-UK-427,857 in vitro blood: plasma concentration ratio in rat, dog and human blood (DM 16)

The in vitro blood: plasma concentration ratio for [[3H]-UK-427,857 was determined in fresh blood from rat, dog and human at a nominal concentration of 100 ng/ml.

Mean values were 1.1, 0.9 and 0.7 for rat, dog and human respectively.

The results showed that for rat and dog [3H]-UK-427,857 distributed equally between the blood cells and plasma. Human blood indicated that [3H]-UK-427,857 partitioned mainly into plasma.

2.6.4.5 Metabolism

16. Pharmacokinetics of UK-427,857 in the isolated perfused rat liver following a single administration (DM8)

The concentrations and pharmacokinetic parameters of UK-427,857 in an isolated perfused rat liver preparation following a single 1 mg administration to the reservoir were determined.

Hepatic extraction of UK-427,857 by male rat liver was 0.4 and 34% of the dose was recovered as unchanged in the bile.

17. In vitro metabolism of UK-427,857 (DM5/9)

The rates of metabolism of UK-427,857 were determined in hepatic microsomal fractions from 3 individual human livers using standard conditions of 1 μM substrate and 0.5 μM cytochrome P450. All incubations were conducted in triplicate. UK-427,857 was slowly metabolized with half-life values ranging from 80 to 120 min. Qualitatively similar metabolite profiles were obtained from human, dog and monkeys hepatocytes incubated with 1 μM [^{3}H]-UK-427,857. Metabolites were the products of mono-oxidation in various parts of the molecule.

18. In vivo metabolism and excretion in male mice, Sprague-Dawley male and female rats and male and female beagle dogs (DM9/10/13/14/15)

The excretion of ^{3}H-labelled UK-427,857 (100 μCi) was investigated in mice (n=3; 20 mg/kg), rats (n=2/sex; 10 mg/kg) and dogs (n=1/sex; 10 mg/kg) after oral administration. Urine and fecal samples were collected for up to 96 hr. Radioactivity in various biological samples was measured by liquid scintillation counting. Results: are shown in Table 13. The major route of excretion of radioactivity was fecal in all species: mouse (103%), rat (101%) and dog (70%). In the urine, the recovery was: mouse (5%), rat (2%) and dog (12%). There were only minor differences between genders (rat and dog) in route of excretion. The majority of activity was recovered within the first 96 hr in dog and within 24 hr in rats and mice.

In the mouse and rat, the majority of radioactivity in the feces was in the form of unchanged UK-427,857 (81% of dose in mouse and 95% of dose in rats). In the dog, fecal metabolite profiles were qualitatively and quantitatively similar in male and female animals, the major fecal component was unchanged UK-427,857 (29% of dose). Other metabolites were the products of mono-oxidation in various parts of the molecule, individually accounting for up to 11% of the dose. The majority of circulating radioactivity in mouse, rat and dog was unchanged UK-427,857.

Table 13

Excretion of radioactivity after single oral doses of ^{3}H-labelled UK-427,857 (100 μCi) to mice, rats and dogs

Species	Dose	Percentage of dose recovered				
		Urine		Feces		total
		0-24 hr	0-96 hr	0-24 hr	0-96 hr	
Mouse male	20	3.9	5	101.3	103.1	108.5
Rat male	10	1.2	1.5	103.6	106.1	108.3
Rat female	10	1.6	1.9	91.8	96.6	99.1
Dog male	10	10.1	-	62.4	63.2	99.6
Dog female	10	14	-	74.5	77.2	91.6

19. In vitro metabolism of UK-427,857 in human liver microsomes and recombinant cytochrome P450 enzymes (DM 5)

The *in vitro* metabolism of UK-427,857 was studied in hepatic microsomes from human livers with varying CYP 3A4, 2C9 and 2D6 activities and in microsomes prepared from cells expressing individual cytochrome P450 enzymes.

UK-427,857 was slowly metabolized in human liver microsomes with disappearance half-life values of 80 min for HM/29 and >120 min in livers HM/28 and HM/9-2. In microsomes prepared from cells expressing individual cytochrome P450 enzymes, it was only possible to detect metabolism in incubations with CYP3A4 and CYP2D6.

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20. In vitro cytochrome P450 inhibition studies on UK-427,857 in human liver microsomes (DM 22)

The potential for UK-427,857 to inhibit the activity of the five major drugs metabolizing cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) was studied ***in vitro*** in human liver microsomes.

UK-427,857 was demonstrated to be a weak inhibitor of cytochrome P450 activity with estimated IC50 value of > 30 μM against each of the enzymes investigated. UK-427,857 was therefore unlikely to inhibit the metabolism of cytochrome P450 substrates in the clinic.

21. In vitro cytochrome P450 inhibition studies of UK-427,857 in recombinant cytochrome P-450 enzymes (DM 7)

The potential for UK-427,857 to inhibit the activity of five drug metabolizing cytochrome P450 enzymes (CYPlA2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) was studied *in vitro* in recombinant CYPs.

UK-427,857 did not inhibit any of the cytochrome P-450 enzymes investigated except for CYP2D6. However the inhibition by UK-427,857 towards CYP2D6 was weak. A slight increase in potency (< 2-fold) upon pre-incubation of UK-427,857 with CYP2D6 was also observed. In comparison with known mechanism-based inhibitors of CYPs (eg, furafylline) this 2-fold increase in potency of inhibition upon pre-incubation was minimal. In general, UK-427,857 was unlikely to inhibit the metabolism of other substrates for these enzymes.

22. Profiling and characterization of metabolites of [3H]-UK-427,857 in human, dog and monkey hepatocytes (DM 19)

Following incubation of 5 μM [3H]-UK-427,857 with human, dog and monkey hepatocytes, qualitative and quantitative metabolism was studied using 2-hour incubation samples. Samples were analyzed using high-performance liquid chromatography (LC) with on line radioactivity and mass spectrometric detection. Metabolite identities were defined by protonated molecular ion, characteristic product ions and retention time.

Unchanged drug was the major compound detected for each of the three species. Transformation products were mono-hydroxylated derivatives of UK-427,857. Total radioactivity quantified for metabolites represented approximately 6.6% in monkey and less than 1% in dog and human hepatocytes incubate samples.

2.6.4.6 Excretion

23. Mechanism of clearance in Sprague-Dawley rats (DM8/17

The biliary excretion and gut secretion of ^{3}H-labelled UK-427,857 (200 μCi) was

investigated in male bile duct cannulated rats following an iv administration (n=2; 3 mg/kg). Bile samples were collected in aliquots for 6 hr postdosing. Radioactivity was measured by liquid scintillation counting. Results: recovery of radioactivity in bile during the 6 hr following iv administration of the labeled compound was 64%, with a further 15% recovered in the gut, indicating that drug related material was directly secreted into the GI tract. Levels of radioactivity in the urine (13%) and liver (1%) were recovered, yielding a total recovery of 93% of the dose at 6hr postdosing.

At least 4 drug related components were present in bile, the major biliary component (R4) was assigned as UK-427,857. The profile of GI contents showed that a single component was present; this was also assigned as unchanged UK-427,857.

24. Excretion of [3H]-UK-427,857 in male mice following single oral (20 mg/kg) administration (DM9)

The excretion of radioactivity following single oral (20 mg/kg) administration of [3H]-UK-427,857 was studied in male mice.

Following administration, the overall recovery (0-96 hr) of radioactivity was 108.5% for male mice. The major route of excretion of radioactivity was in the feces (103.1% of dose) with the remainder of the dose recovered in the urine (5.0%). The majority of radioactivity was recovered within 24hr of dosing (mean 105.2%). At 4 days after administration, 0.2% of the dose remained in the carcass. Measurement of radioactivity in blood cells and plasma allows calculation of a whole blood: plasma ratio of 1.7 (mean of 1and 4hr).

25. Excretion of [3H]-UK-427,857 in male and female dogs following single oral administration of 10 mg/kg (DM 11)

The excretion of radioactivity following single oral (10 mg/kg) administration of **[3H]**-UK-427,857 was studied in male and female dogs.

Following oral administration of [3H]-UK-427,857, the overall recoveries (0- 192 hr) of radioactivity were 99.6% and 91.6% for male and female dog respectively. The major route of excretion of radioactivity was in the feces (63.2% of dose in male and 77.2% in female) with the remainder of the dose recovered in the urine (10.1 % in male and 14.0% in female). The remainder of the dose for the male dog was recovered in the vomit (26%).

Following oral administration, Cmax, for total radioactivity and unchanged UK-427,857 in plasma was achieved at 0.5hr (male) and l h (female). Unchanged UK-427,857 accounted for 103% (male) and 92% (female) of the circulating drug-related radioactivity.

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26. Biliary excretion of [3H]-UK-427,857 in male bile duct cannulated rats following single iv administration of 3 mg/kg (DM 17)

The biliary excretion and gut secretion of radioactivity was investigated following intravenous administration of [3H]-UK-427,857 to male bile duct cannulated rats at a nominal dose of 3mg/kg.

Recovery of radioactivity in bile during the 6h following intravenous administration of [3H]-UK-427,857 was 64%, with a further 15% recovered in the gut, indicating that drug-related material was directly secreted into the gastro-intestinal tract.

Levels of radioactivity in the urine (13%), and liver (1%) were also measured, yielding a recovery of 93% of the dose at 6hr.

At least four drug related components were present in bile, the major biliary component (R4) was assigned as UK-427,857 on the basis of co-chromatography with authentic standard. The profile of gastro-intestinal contents showed that a single component was present, this was also assigned as unchanged parent drug on the basis of cochromatography.

2.6.4.9 Discussion and Conclusions

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 was shown to be devoid of relevant pharmacological activity. 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 only tested animal species for the evaluation of maraviroc toxicology was appropriate and relevant to human safety. Maraviroc was 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.

Toxicokinetic investigations show that at the NOAELs doses in toxicology studies, animals were exposed to similar or higher concentrations of maraviroc than humans at the maximum expected clinical dose (300 mg BID).

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General Toxicology

Single-Dose Toxicity

1. Acute oral gavage toxicity study of UK-427,857 in CD-1 mice: One group of male and female mice (5 animal/sex/group) received a single oral gavage dose of UK-427,857 in 0.5% methylcellulose/0.1% polysorbate 80 at a dose level 2000 mg/kg. All rats were monitored for mortality, clinical signs at least twice daily for two weeks. Results: all animals survived the treatment. No treatment related clinical signs were observed. There were no treatment related gross findings. Conclusion: the maximum asymptomatic single dose in mice was 2000 mg/kg. The maximum asymptomatic single oral gavage dose of UK-427,857 in the mice was considered to be 2000 mg/kg. Based on the body surface area factor, an equivalent dose in humans would be 162.3 mg/kg or 9.7 g for a 60 kg person.

2. Acute oral gavage toxicity study of UK-427,857 in Sprague-Dawley rats: One group of male and female rats (5 animals/sex/group) received a single oral gavage dose of UK-427,857 in 0.5% methylcellulose/0.1% polysorbate 80 at a dose level of 2000 mg/kg. All rats were monitored for mortality and clinical signs at least twice daily for two weeks. Results: all animals survived the treatment. One animal exhibited salivation beginning at approximately 10 min postdose and was normal approximately 1 hr later. No other treatment related clinical signs were observed. There were no treatment related gross findings. Conclusion: the maximum asymptomatic single dose in rat was 2000 mg/kg. A dose level of 2000 mg/kg may be considered a NOEL in this study. Based on the body surface area factor, an equivalent dose in humans would be 324.6 mg/kg or 19.48 g for a 60 kg person.

3. Acute intravenous toxicity study of UK-427,857 in mice and rats: Two groups of male and female rats [strain: [redacted] CD (SD) IGS BR; age: 7 weeks; 2-3 animal/sex/group] and mice [strain: CD-1/ [redacted] CD-1(ICR) BR; age: 13 weeks; 2-3 animal/sex/group] received a single IV dose of UK-427,857 at dose levels of 20 (low) and 200 mg/kg (high). All animals were monitored for mortality, clinical signs at least twice daily for two weeks. Results: at the high dose, all animals died in both the species within 5 min after drug administration. Clinical signs were recorded only at the high dose (convulsions and dyspnea in all animals). Necropsy findings: marbled liver and red lungs were recorded in male mice (low). The highest non-lethal intravenous dose of UK-427,857 explored in mice and rats was 20 mg/kg. Mortality occurred at the dose of 200 mg/kg in both species.

Repeat-dose toxicity

1. Two-week oral toxicity study of UK-427,857 in CD-1 mice: Groups of male and female CD-1 mice (10 animals/sex/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 20 (low), 200 (mid) 1000 (high) or 2000 mg/kg/day (very high) for 14

consecutive days. Drug related lethality or morbidity occurred in 2/20 animals (high) and 3/20 animals (very high) on day 14. All other animals survived the treatment. Clinical signs that preceded lethality or mobility included decreased activity, hunched posture, pale skin or coldness to touch. Body weight & Food consumption: there were no drug related differences in group mean food consumption: no changes. Clinical chemistry: ALT, AST, triglycerides and cholesterol values were elevated sporadically in the very high dose males and females. Gross Necropsy: no changes were seen. Hematology: while no significant changes were observed in group mean hematology parameters, slight or mild increases in neutrophils, monocytes, basophils and/or fibrinogen were seen in animals (very high). Organ weights: there were no effects on organ weights directly related to drug treatment. Histopathology: findings consisted of slight to mild degeneration of the superficial epithelium of the cecum in 4/10 females (high) and 1/10 males and 3/9 females (very high). Inflammatory cells were predominately neutrophils and infiltrated the mucosa and submucosa. The primary drug related effect was degeneration or the superficial epithelium in the cecum with inflammatory cell infiltration and/or extension to the colon, which was accompanied by increases in leukocytes and fibrinogen in individual animals (high and very high). The mechanism of the epithelial degeneration in the cecum is unclear; however, degenerative changes affecting only the superficial epithelium are likely to be a local effect rather than a systemic toxicity. The NOAEL in the mice was 200 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be 16.22 mg/kg/day (973 mg/day for a 60 kg person. Drug exposure at the 100 mg/kg/day was 2.38 and 2.02 µg/ml (Cmax) and 12.7 and 13.8 µg*hr/ml (AUC) in male and female mice, respectively.

2. Two week oral toxicity study of UK-427,857 in beagle dogs: Groups of male and female beagle dogs [age: 8-9 months; 3 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 10 (low), 50 (mid) or 250 mg/kg/day (high) for 14 consecutive days. Plasma drug concentrations were measured on days 1 and 13 at 1, 3, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method. Results: there were no deaths in the study. Clinical signs: the most frequently observed clinical signs consisted of emesis, skin reddening and mydriasis (dilated pupils) and occurred in all drug-treated groups. The diminished pupillary light response test was consistent with the observation of mydriasis in some these animals. Other ocular signs were observed sporadically and included partial eye closure (mid and high), reddened conjunctiva (low, mid or high), protruding nictitating membrane (mid and high) and lacrimation (high). Salivation occurred in all drug treated animals immediately after dosing. Body weights, Food consumption, body temperature, respiratory rate, heart rate, organ weights, clinical chemistry, hematology and pathology: there were no drug related changes. Drug related cardiovascular effects consisted of decrease in blood pressure (mid and high) and slight increases in QTc interval (mid and high; 11-23 msec above predose values). The incidence of QTc prolongation was 1/6 animals (mid) and 6/6 animals (high). Two of six animals (low) have slight changes in QT intervals. A NOAEL could not be identified in this study. Drug exposure at the low dose (10 mg/kg/day) was 1.8 µg/ml (Cmax) and 2.2 µg*hr/ml (AUC).

3. One month oral gavage range finding toxicity study of UK-427,857 in CD-1 mice: Groups of male and female CD-1 mice (10 animal/sex/group) received oral gavage doses

(10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water; vehicle control), 200 (low), 500 (mid) or 750 mg/kg/day (high) for 28 consecutive days. Results: there were no deaths. A low incidence of partially closed eyes was recorded sporadically in few animals in both sexes (mid or high). There were no drug related effects on body weight and food intake. Treatment produced no clinical pathology or histologic evidence of toxicity. The NOEL in the mice was 200 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be 16.22 mg/kg/day (973 mg/day for a 60 kg person). Drug exposure at the 200 mg/kg/day was 5.6 and 8.3 µg/ml (Cmax) and 31.6 and 35.7 µg*hr/ml (AUC) in male and female mice, respectively

4. One month dose range finding oral gavage toxicity study of UK-427,857 in male rats: Groups of male Sprague-Dawley rats (5 animal/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 100 (low), 300 (mid) or 1500 mg/kg/day (high) for 28 consecutive days. Results: there were no deaths in the study. Clinical signs: all treated animals displayed increased salivation with dose-related frequency. This sign was observed generally before dosing and up to 15 min after dosing. Diarrhea occurred sporadically during the second half of the study at the high dose. Body weight & Food consumption: there were no drug related differences in group mean food consumption: high dose animals had a statistically significantly lower mean body weights gain (11%) than the controls. The treatment induced a toxicologically significant decrease (25%) in mean food consumption during the first week of the study (high). Clinical chemistry: ALT, AST, ALP, GGT and cholesterol values were elevated moderately in the high dose animals. Gross Necropsy: dilatation of the colon and cecum was present in 4/5 and 2/5 animals (high). Multifocal red discoloration that affected all liver lobes was present in 1/5 animals (high). Organ weights: there were statistically significant decreases in absolute heart and spleen weights (high). Absolute thymus weights were slightly decreased at all doses with statistical significance at the mid dose only. Histopathology: the liver, adrenal, pituitary, colon, cecum and joint in legs (mid, 1/5; 2/5 high) had changes that were present in the high dose treated animals only. Necrosis in the liver was mostly located in the centrilobular area with occasional extension in the periportal region in the high dose animals. Minimal to mild vacuolation of the pars distalis was observed in the pituitary gland in the high dose animals. Adverse treatment related findings were restricted to the high dose. Changes in the liver consisted of necrosis in one high dose animal, which was associated with moderate increases in liver enzymes. There was also minimal to mild vacuolation of the par distalis in the pituitary gland. Minimal to mild inflammation of the joints was present in a dose related incidence and severity at mid and high doses. The NOEL in the rat was 100 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be 16.23 mg/kg/day (974 mg/day for a 60 kg person). Drug exposure at the 100 mg/kg/day was 1.15 µg/ml (Cmax) and 7.4 µg*hr/ml (AUC).

5. One month oral dose range finding toxicity study of UK-427,857 in beagle dogs: Groups of male and female beagle dogs (3 animal/sex/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water; vehicle control), 5 (low), 50 (mid) or 150 mg/kg/day (high) for 4 consecutive weeks. There were no deaths in the study. At the mid and high doses were emesis, mydriasis (dilated pupils), partially closed eyes, protruding nictitating membrane, red conjunctiva and increased salivation. Except for emesis, that occurred mostly within 15

min after dosing, these signs were generally observed between 15 min and 5 hr postdosing. Increase in QT intervals was seen at the mid or high. In addition, the high dose produced body weight loss and reduce food intake which represents further indication of toxicity. The NOEL in the dog was 5 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be 2.7 mg/kg/day (162 mg/day for a 60 kg person). Drug exposure at the 5 mg/kg/day was 0.79 µg/ml (Cmax) and 6.02 µg*hr/ml (AUC).

6. One-month oral (bid) dose range finding toxicity study in the cynomolgus monkey: Groups of male and female cynomolgus monkeys received repeated oral gavage doses (bid, 7-8 hr apart) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 100 (low), 200 (mid), 400 (high) or 800 mg/kg/day (highest) daily for a period of 4 weeks. The highest dose level of maraviroc induced severe clinical signs resulting in the euthanasia of the animals. Before being sacrificed, these animals showed signs of abnormal motor activity (including half closed eyes, reduced activity, prostration and loss of balance) associated with vomiting. There were no histopathologic changes that could explain the moribund status of these four animals (highest). No clinical signs were noted in the other groups during the study. The treatment at the high and mid dose induced QT prolongation, lowered diastolic blood pressure and decreased heart rate. A slight QT prolongation was seen at the low dose also. In this study, a NOAEL could not be established. A NOAEL in the monkeys should be below 100 mg/kg/day. Based on the body surface area factor, an equivalent oral dose in humans would be 2 g/day for a 60 kg person. Drug exposure at the 100 mg/kg/day was 2.8 µg/ml (Cmax) and 4.21 µg*hr/ml (AUC).

7.Three month oral gavage range finding toxicity study of UK-427,857 in CD-1 mice:

Groups of male and female CD-1 mice (10 animal/sex/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water; vehicle control), 200 (low), 500 (mid) or 750 mg/kg/day (high) for 28 consecutive days. Results: there were 5 deaths; three of them were attributed to gavage error (one male at mid dose, one male and one female in vehicle controls). The cause of death of other two animals (1 male in high dose and one male at low dose could not be determined. Clinical signs: a low incidence of partially closed eyes was recorded sporadically in few animals in both sexes (mid or high). Body weight and Food consumption: there was a dose related increase in body weight in females (mid up to 10%), but food consumption was not affected. Organ weights: mean absolute and relative kidney weights were slightly increased in females (high up to 16%). Histopathology: no toxicological effect. The NOEL in the mice was 200 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be 16.22 mg/kg/day (973 mg/day for a 60 kg person). Drug exposure at the 200 mg/kg/day was 3.9 and 3.7 µg/ml (Cmax) and 15 and 11 µg*hr/ml (AUC) in male and female mice, respectively.

8. 26 Weeks oral (gavage) toxicology study in rats followed by a 13-week treatment free period: Male and female rats received UK-427,857 via oral gavage at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 30 (low), 100 (mid),

300 (high) or 900 mg/kg/day (highest) for 26 consecutive weeks and to evaluate the regression of any toxic signs during a 13-week reversibility period. Treatment from the low dose (30 mg/kg/day) induced hypersalivation. Bile duct vacuolation was observed at the mid (100 mg/kg/day), high (300 mg/kg/day) and highest (900 mg/kg/day) dose levels. These effects were not considered adverse. Treatment at the high dose(300 mg/kg/day) induced bile duct hyperplasia, follicular cell hypertrophy in the thyroid, decreased T4 levels and increased TSH, decreased bilirubin, increased ALT, increased water consumption and dilatation of the cecum. Treatment at the highest dose (900 mg/kg/day) induced hepatic changes (increased GGT, altered cell foci, liver weight increases, multinucleated hepatocytes), decreased body weights, increased urine volume, cortical vacuolation of the adrenals and stained fur in the urogenital area in females. Treatment related changes at the high and highest doses were reversible except for the decreased body weights and increased liver weights (highest) and the bile duct vacuolation, bile duct hyperplasia and multinucleated hepatocytes in males at the high and highest doses. Under the experimental conditions of this study, a dose level of 100 mg/kg/day may be considered the NOAEL.

9. Six month oral toxicity study of UK-427,857 in beagle dogs: Groups of male and female beagle dogs [age: 8 months; 4 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (sterile saline; vehicle control), 5 (low), 15 (mid) or 40 mg/kg/day (high) for 6 months. Plasma drug concentrations were measured on days 1 and 176 at 1, 4, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method. The treatment resulted in clinical signs including emesis, mydriasis and ocular signs and increase in heart rate and QT intervals (mid or high). The changes were seen at 1 hr postdose, the time at which peak plasma levels occurred with mean values more or equal to 2.48 µg/ml at the mid dose. The effect on QTc was consistent with results from dofetilide binding, hERG channel and dog isolated Purkinje fiber studies suggesting that UK-427,857 has the potential to block the IKr current and affect cardiac repolarization in vivo. The NOAEL in the dog was 5 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be 2.7 mg/kg/day (162 mg/day for a 60 kg person). Drug exposure at the 5 mg/kg/day was 0.88 µg/ml (Cmax) and 2.42 µg*hr/ml (AUC).

10. UK-427,857: 9-month oral (bid) toxicity study in the cynomolgus monkey: Groups of male and female cynomolgus monkeys received repeated oral gavage doses (bid, 7-8 hr apart) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 30 (low), 120 (mid) or 400 mg/kg/day (high) daily for a period of 39 weeks. In the high dose animals, signs of toxicity included moderate clinical signs (subdued behavior/reduced activity, prostration, half-closed eyes, vomiting and liquid feces), slightly low red blood cell parameters, a tendency towards increase in triglyceride and ALT levels, the presence of urinary proteins and cardiovascular changes (decreased in heart rate and blood pressure and increased QT/QTc intervals). Drug plasma levels were similar in males and females and increased superproportionally with dose. A dose level of 120 mg/kg/day may be considered the NOAEL in monkeys. Based on the body surface area factor, an equivalent oral dose in humans would be 2.4 g/day for a 60 kg person. Drug exposure (Cmax and AUC) at the NOAEL was 1.15 µg/ml (Cmax) and 6.6 µg*hr/ml

(AUC).

Genetic Toxicology:

1. UK-427,857: Microbial reverse mutation assay: UK-427,857 was tested for its ability to induce gene mutations in two versions of the Salmonella-E. coli/mammalian-microsome mutagenicity assay, using tester Salmonella strains TA98, TA100, TA1535 and TA1537, and E. coli strains WP2uvrA and pKM101, both in the presence and in the absence of rat liver S9 metabolic activation. The S9 homogenate was prepared from male Sprague-Dawley rats that had been injected (i.p.) with Aroclor™-1250 at a dose level of 500 mg/kg. The concentrations tested, along with vehicle and positive control substances, were 50, 150, 500, 1500, and 5,000 µg/plate. UK-427,857 did not cause a positive increase in the number of revertants per plate in any of the tester stains in either the presence or absence of metabolic activation. Conclusion: in the Ames assay, UK-427,857 exerted no detectable mutagenic activity. Under the conditions of this study, UK-427,857 was negative in the Ames test.

2. UK-427,857: In vitro cytogenetic assay: UK-427,857 (Lot No. R1) was tested for clastogenic activity in vitro in human lymphocyte cultures. Chromosome damage was evaluated by metaphase analysis after 3-hour treatments with and without metabolic activation at concentrations ranging from 538 to 840 µg/ml and 750 to 950 µg/ml, respectively. In addition, chromosome damage was evaluated after 24 hours without metabolic activation at concentrations ranging from 42.3 to 258 µg/ml. In all tests, the highest dose level evaluated produced a 48% to 56% reduction of the mitotic index. There was evidence of test article precipitate in the dosing stocks and culture medium in the 3-hour test with metabolic activation and the 24-hour direct test. There was no significant increase in chromosome damage or polyploidy under any test condition at any concentration evaluated.

3. UK-427,857: Rodent micronucleus assay: Groups of male and female CD-1 mice (strain: [redacted]:CD-1 (ICR)BR; 8 animals/sex/group) were orally gavaged a single dose of UK-427,857 (2 ml/kg) at dose levels of 0 (0.1% Tween 80 in 0.5% methylcellulose, vehicle control), 500 (low), 1000 (mid) or 2000 mg/kg (high) to evaluate the potential to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow. Bone marrow cells were collected 24 or 48 hr after the treatment and were examined for micronucleated polychromatic erythrocytes. Results: no significant increase ($p=0.01$) in micronucleated polychromatic erythrocytes was observed in the treated groups when compared to the controls. The mean highest plasma UK-427,857 concentration occurred 3 or 5 hours after dosing (high) with values of 13.0 and 13.5 µg/ml in males and females, respectively. Mean AUC0-24h values (high) were 184 µg*h/ml in males and 174 µg*h/ml in females. Conclusions: under the conditions of the test and according to the criteria set for evaluating the test results, UK-427,857 was negative in the Micronucleus Assay.

Carcinogenicity:

1. UK-427,857 4-week dose range study in the rasH2 transgenic mice: The key

responses seen at the high dose (reduced spleen and thymus weights, and minimal inflammation of the cecum) were considered to be too small to have an impact on the 6-month carcinogenicity study in the mice. There were no findings in this study that would limit the doses for the 6-moth carcinogenicity study in rasH2 transgenic mice. In this study, a dose level of 1500 mg/kg/day (high) may be considered close to the NOAEL. The high dose produced a plasma exposure many times greater (226 in males and 87 in females) than seen at the maximum therapeutic dose in humans.

2. Six-Month oral carcinogenicity study of UK-427,857 in rasH2 Transgenic mice:
The oncogenicity potential of maraviroc was investigated in male and female transgenic mice at oral gavage dosages of 0 (vehicle control), MNU = 75 mg/kg ip (positive control), 200 (low), 800 (mid) or 1500 mg/kg/day (high) in comparison with the controls for a period of 26 weeks. The protocol was approved by the Exec CAC. The systemic exposures were 33 and 59 times that in humans (300 mg bid, AUCss = 3.6 μg*hr/ml) in male and female mice at the high dose level, respectively. No drug-related malignant neoplasms or non-neoplastic changes were seen in transgenic mice.

3. Two-year oral (gavage) carcinogenicity study UK-427,857 in Sprague-Dawley rats:
The oncogenicity potential of maraviroc was investigated in male and female SD rats at oral gavage dosages of 0 (vehicle control), 50 (low), 100 (mid), 500 (high) or 900 mg/kg/day (highest) for a period of 104 weeks. The protocol was approved by the Exec CAC. The systemic exposures were 13 and 18 times that in humans (300 mg bid, AUCss = 3.6 μg*hr/ml) in male and female mice at the highest dose level, respectively. No statistically significant drug-related malignant neoplasms were seen in the rat. At the high dose, male rats had an increased incidence of cholangiocarcinoma of the liver (2/60) as compared to the vehicle control group. Although, the occurrence of cholangiocarcinoma of the liver is extremely uncommon in treated rats, the increase in this study was not statistically significant.

Reproductive Toxicology:

1. Oral fertility and early embryonic development study of UK-427,857 in rats:

Groups of male and female Sprague-Dawley rats [20 rats/sex/group] received UK-427,857 via esophageal intubation at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80, vehicle control), 100 (low), 300 (mid) or 1000 mg/kg/day (high). In a parallel pharmacokinetics study 3 groups of 3 rats/sex received UK-427,857 at the same dose levels as in the main study for 15 days. There were no deaths. Minimal toxicity was observed at the high dose in males consisting of diarrhea and a slight decrease in mean body weights (up to 6.2%) when compared to the controls. The treatment had no effect on the estrus cycle, on pre-coital time, on copulation and pregnancy rates, on the spermatozoid count in epididymis or on the spermatic motility. In the high dose females, there was a statistically significant increase in the pre-implantation loss (9.53% vs 3.24%) with consequently a smaller number of implants (14 vs 16) and of viable fetuses (14 vs 15) when compared to the controls. Based on the fact that there was no difference in the number of implants and viable fetuses in the treated animals

compared with a decrease in the number of viable fetuses relative to the number of implants in the controls, the above findings were deemed not to be worthy of mention in the label. There was no clinical chemistry or histopathological evidence of toxicity. The treatment resulted in slight toxicity in both sexes at the high dose with diarrhea and a slight decrease in mean body weights in males and an increase in the pre-implantation loss in females. The NOAEL was 300 mg/kg/day (AUC=35.77 μg*hr/ml) for both adult male and female rats. Based on the body surface area factor, an equivalent dose in humans would be 48.7 mg/kg/day (2.9 g/day for a 60 kg person). In the clinic, the test compound is being administered at a dose level of 300 mg/day (AUC=3.609 μg*hr/ml). Thus, at the clinical dose there is an approximately 10-fold safety margin.

2. UK-427,857: Oral study of embryo-fetal development in rats: Groups of pregnant Sprague-Dawley rats 20 rats/group received UK-427,857 during organogenesis (days 6-17 post insemination) via esophageal intubation at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80, vehicle control), 100 (low), 300 (mid) or 1000 mg/kg/day (high). In a parallel pharmacokinetics study 5 inseminated rats received UK-427,857 at the same dose levels from day 6 to 21 post insemination. On day 17 and 18, blood samples were collected and were analyzed by a validated analytical method. Mortality (dams): there were no deaths. Clinical signs (dams): Increased salivation was observed 1/20, 8/20 and 20/20 during the treatment period in the low, mid and high dose, respectively. Body weight (dams): when compared to the controls, mean body weights were slightly but significantly decreased (mid and high) from day 7 until the end of study. At the high dose, the mean body weight gain was also decreased (22.7%) during days 6-17. When compared to the controls, mean corrected maternal weight gain was moderately but significantly decreased at the high dose ($p=0.001$). The NOAEL was 300 mg/kg/day for the pregnant female rat and 1000 mg/kg/day for the fetuses. Based on the body surface area factor, an equivalent dose in humans would be 48.7 mg/kg/day (2.9 g/day for a 60 kg person). Equivalent dose for human fetus would be 162 mg/kg/day. At the NOAELs (300 and 1000 mg/kg/day), exposures (steady state AUC and Cmax) were 45.5 and 4.53 and 102 μg*hr/ml and 7.57 μg/ml, respectively.

3. Oral embryo-fetal development study of UK-427,857 in rabbits: Groups of pregnant New Zealand White rabbits (20 rabbits/group] received UK-427,857 during organogenesis (days 7-19 post insemination) via oral gavage at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80, vehicle control), 30 (low), 75 (mid) or 200 mg/kg/day (high). On day 19, blood samples were collected and were analyzed by a validated analytical method. The treatment resulted in significant mortality at the high dose. At the high dose, 7 fetuses in 6 liters were affected by an external anomaly. One fetus (control) had a short tail. Cleft palate, bent forepaws(s) and short tail have been recorded previously (1992-1995) in control fetuses from oral studies with the same strain of rabbits. Ectrodactyly and cutis aplasia has been observed in control populations of rabbits from the compiled historical databases. These fetal defects are not part of a recognizable syndrome.

There were no other signs of potential adverse effects of the compound on the fetuses: no embryo mortality and no effect on fetal body weight. The NOAEL was 75 mg/kg/day for the pregnant female rabbit. Based on the body surface area factor, an equivalent dose in humans would be 24.19 mg/kg/day (1.4 g/day for a 60 kg person). Equivalent dose for human fetus would be 162 mg/kg/day. At the NOAEL (75 mg/kg/day), exposure (steady state AUC and Cmax) were 27.1 µg*hr/ml and 5.85 µg/ml, respectively.

4. UK-427,857: Oral study of pre- and postnatal development in rats: Groups of presumed pregnant rats (27/group) received UK-427,857 via oral gavage at dose levels of 0 (vehicle controls), 100 (low), 300 (mid) or 1000 mg/kg/day (high) from day 6 of gestation through day 20 of lactation. At the high dose level, maternal toxicity was demonstrated in the F0 dams by reduced body weight and food consumption during gestation. No reproductive effects were noted. There were no drug related findings in the F1 generation offspring at any dose level. UK-427,857 caused drug related weight and food consumption changes in the F0 dams at the high dose level, without affecting the pre- or postnatal development of the F1 generation offspring at any dose level. A dose level of 300 mg/kg/day may be considered the NOEL for F0 dams. Based on the body surface area factor, an equivalent oral dose in humans would be 48.7 mg/kg/day (2.9 g/day for a 60 kg person). A dose level of 300 mg/kg/day may be considered the NOEL for F1 offspring. Based on the body surface area factor, an equivalent oral dose in humans would be 48.7 mg/kg/day (2.9 g/day for a 60 kg person). Drug exposure at the NOEL was 13.13 µg*hr/ml.

Special Toxicology:

1. Seven-day intravenous irritation toxicity study of UK-427,857 in rats: Groups of male and female Sprague-Dawley rats [strain: CD (SD) IGS BR; age: 7 weeks; 5 animal/group] received iv injections of UK-427,857 (10 ml/kg) at dose levels of 0 (sodium acetate buffer; vehicle control), 0.6 (low), 2 (mid) or 10 mg/kg/day (high) for 7 consecutive days. Results: there were no deaths, no treatment related clinical signs, no signs of local irritation and no effects on body weight. There was no histopathologic evidence of irritation at the injection sites. In conclusion: the iv administration of UK-427,857 (up to 10 mg/kg/day for 7 days) to rats was well tolerated and did not produce any signs of irritation at the injection sites.

2. UK-427,857: four week oral (bid) immunotoxicity study in the cynomolgus monkey: Groups of male and female cynomolgus monkeys received repeated oral gavage doses (bid, 7-8 hr apart) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 30 (low), 100 (mid) or 300 mg/kg/day (high) once daily for a period of 4 weeks to determine potential immunotoxic effects. The treatment was not associated with any immunotoxic effects. Moderate clinical signs and mild body weight loss were observed at the high dose level in some animals. Drug plasma levels were similar in males and females and increased superproportionally with dose. There were no adverse treatment related pathological changes at any dose level. A dose level of 100 mg/kg/day may be considered the NOEL in monkeys. Based on the body surface area factor, an

equivalent oral dose in humans would be 2 g/day for a 60 kg person. Drug exposure at the NOEL was 6.695 µg*hr/ml.

3. UK-427,857: Investigation of the mechanism of thyroid hypertrophy and liver changes in rats: UK-427,857 was administered via oral gavage (10 ml/kg/day) at dose levels of 0 (vehicle control; 0.5% w/v methylcellulose + 0.1% w/w Tween 80 in water) or 900 mg/kg/day (high) for 4 consecutive weeks to investigate the mechanism of thyroid hypertrophy and liver changes in rats. The treatment produced an increase in thyroid weights in males and follicular cell hypertrophy in all males and a few females. A mild decrease in T4 plasma levels and a marked increase in TSH associated with a vacuolation of the pars distalis of the pituitary indicated that the thyroid hypertrophy resulted from a pituitary stimulation occurring most probably as a compensatory response to the decreased thyroxin level. A higher UDPGT activity occurred in treated females compared to the controls and the thyroxin clearance was increased in both sexes. In conclusion, the treatment produced thyroid follicular cell hypertrophy. Liver enzymes probably contributed to this change. The mean AUC0-24hr values was 34.9 µg*hr/ml at the dose of 900 mg/kg/day.

2.6.6.2 Single-dose toxicity

1. Study title: Acute oral gavage toxicity study of UK-427,857 in CD-1 mice

Key study findings: One group of male and female mice (5 animal/sex/group) received a single oral gavage dose of UK-427,857 in 0.5% methylcellulose/0.1% polysorbate 80 at a dose level 2000 mg/kg. All rats were monitored for mortality, clinical signs at least twice daily for two weeks. Results: all animals survived the treatment. No treatment related clinical signs were observed. There were no treatment related gross findings. Conclusion: the maximum asymptomatic single dose in mice was 2000 mg/kg.

The maximum asymptomatic single oral gavage dose of UK-427,857 in the mice was considered to be 2000 mg/kg. Based on the body surface area factor, an equivalent dose in humans would be 162.3 mg/kg or 9.7 g for a 60 kg person.

Study no.: 01-2120-04

Volume # and page #: 1 and page # 1-27

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: August 1, 2001

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: R1, [redacted] pure

Methods

Doses: One group of male and female mice (5 animal/sex/group) received a single oral gavage dose of UK-427,857 in 0.5% methylcellulose/0.1% polysorbate 80 at a dose level 2000 mg/kg.

Species/strain: male and female mice [strain: CD-1. [redacted] CD-1(ICR) BR]

Number/sex/group or time point (main study): 5 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage, volume: 10 ml/kg

Age: 13-14 weeks old

Weight: 30.5 to 35.3 g for males and 25.3 to 26.9 g for females.

Mortality and clinical signs: the animals were examined daily for changes in condition and behavior. Observation of moribund or dead animals was made twice daily.

Gross pathology: All mice were anesthetized by carbon dioxide inhalation and exsanguinated on day 15. An external and visual examination was performed on all animals.

Histopathology: not done.

Results

Mortality: All animals survived the treatment and 14-day observation period.

Clinical signs: No treatment-related clinical signs were observed.

Body weights: no changes

Gross pathology: No treatment-related gross findings were observed.

Histopathology: not done.

2. Study title: Acute oral gavage toxicity study of UK-427,857 in Sprague-Dawley rats

Key study findings: One group of male and female rats (5 animal/sex/group) received a single oral gavage dose of UK-427,857 in 0.5% methylcellulose/0.1% polysorbate 80 at a dose level of 2000 mg/kg. All rats were monitored for mortality and clinical signs at least twice daily for two weeks. Results: all animals survived the treatment. One animal exhibited salivation beginning at approximately 10 min postdose and was normal approximately 1 hr later. No other treatment related clinical signs were observed. There

were no treatment related gross findings. Conclusion: the maximum asymptomatic single dose in rat was 2000 mg/kg

A dose level of 2000 mg/kg may be considered a NOEL in this study. Based on the body surface area factor, an equivalent dose in humans would be 324.6 mg/kg or 19.48 g for a 60 kg person.

Study no.: 01-2120-06

Volume # and page #: 1 and page # 1-27

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: August 7, 2001

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: R1, [redacted] pure

Methods

Doses: One group of male and female rats/strain: [redacted] CD (SD) IGS BR; age: 7 weeks; 5 animal/sex/group] received a single oral gavage dose of UK-427,857 in 0.5% methylcellulose/0.1% polysorbate 80 at a dose level of 2000 mg/kg.

Species/strain: male and female rats [strain: [redacted] CD (SD) IGS BR]

Number/sex/group or time point (main study): 5 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage, volume: 10 ml/kg

Age: 7 weeks old

Mortality and clinical signs: the animals were examined daily for changes in condition and behavior. Observation of moribund or dead animals was made twice daily.

Gross pathology: All mice were anesthetized by carbon dioxide inhalation and exsanguinated on day 15. An external and visual examination was performed on all animals.

Histopathology: not done.

Results

Mortality: All animals survived the treatment and 14-day observation period.

Clinical signs: One animal exhibited salivation beginning at approximately 10 min postdose and was normal approximately 1 hr later. No other treatment related clinical signs were observed.

Body weights: no changes

Gross pathology: No treatment-related gross findings were observed.

Histopathology: not done.

3. Study title: Acute intravenous toxicity study of UK-427,857 in CD-1 mice and SD rats

Key study findings: Two groups of male and female rats [strain: [redacted]: CD (SD) IGS BR; age: 7 weeks; 2-3 animal/sex/group] and mice [strain: CD-1[redacted]: CD-1(ICR) BR; age: 13 weeks; 2-3 animal/sex/group] received a single IV dose of UK-427,857 at dose levels of 20 (low) and 200 mg/kg (high). All animals were monitored for mortality, clinical signs at least twice daily for two weeks. Results: at the high dose, all animals died in both the species within 5 min after drug administration. Clinical signs were recorded only at the high dose (convulsions and dyspnea in all animals). Necropsy findings: marbled liver and red lungs were recorded in male mice (low).

Conclusions: The highest non-lethal intravenous dose of UK-427,857 explored in mice and rats was 20 mg/kg. Mortality occurred at the dose of 200 mg/kg in both species.

Study no.: 2148/02149

Volume # and page #: 1 and page # 1-23

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: April 16, 2003

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: R103, [redacted] pure

Methods

Doses: Two groups of male and female rats (2-3 animal/sex/group) and mice (2-3 animal/sex/group) received a single iv dose of UK-427,857 at dose levels of 20 (low) and 200 mg/kg (high). The animals were observed daily for 14 days following treatment for

mortality and clinical signs. Their body weights were recorded on days 1, 7 and 14. All animals were necropsied.

Species/strain: CD1 mice [redacted] CD1 (ICR) IGS BR] and Sprague-Dawley rats [redacted] CD® (SD) IGS BR]

Number/sex/group or time point (main study): There were 3 animals/sex at 20 mg/kg and 2 animals/sex at 200 mg/kg in both species.

Route, formulation, volume, and infusion rate: UK-427,857, dissolved in acidified solution with sodium chloride adjusted to pH 3.5, was administered by intravenous injection into a lateral caudal vein in a volume of 10 ml/kg to mice and rats at 20 mg/kg and 15 ml/kg to mice and rats at 200 mg/kg.

Age: At the start of the study, animals were aged about 7 weeks.

Weight: the mean body weight of male and female mice was about 32 g and 26 g respectively, and for male and female rats was about 238 g and 175 g respectively.

Mortality and clinical signs: the animals were examined daily for changes in condition and behavior. Observation of moribund or dead animals was made twice daily.

Gross pathology: All mice were anesthetized by carbon dioxide inhalation and exsanguinated on day 15. An external and visual examination was performed on all animals.

Histopathology: not done.

Results

Mortality: At the dose level of 20 mg/kg no mortality was recorded in either species. At 200 mg/kg all mice and rats (2/sex) died within 5 minutes after injection.

Clinical signs: At the dose level of 20 mg/kg no clinical signs were recorded in either species. At 200 mg/kg all mice presented convulsions and all rats presented dyspnea.

Body weights: The mean body weights of mice and rats from 20 mg/kg were higher on day 14 than on day 1.

Gross pathology: At 20 mg/kg in mice, marbled liver and red lungs were recorded in two males. No other findings were recorded in mice and rats.

Histopathology: not done.

2.6.6.3 Repeat-dose toxicity

1. Study title: Two week oral gavage toxicity study of UK-427,857 in CD-1 mice

Key study findings: Groups of male and female CD-1 mice (10 animals/sex/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 20 (low), 200 (mid) 1000 (high) or 2000 mg/kg/day (very high) for 14 consecutive days. Drug related lethality or morbidity occurred in 2/20 animals (high) and 3/20 animals (very high) on day 14. All other animals survived the treatment. Clinical signs that preceded lethality or mobility included decreased activity, hunched posture, pale skin or coldness to touch. Body weight & Food consumption: there were no drug related differences in group mean food consumption: no changes. Clinical chemistry: ALT, AST, triglycerides and cholesterol values were elevated sporadically in the very high dose males and females. Gross Necropsy: no changes were seen. Hematology: while no significant changes were observed in group mean hematology parameters, slight or mild increases in neutrophils, monocytes, basophils and/or fibrinogen were seen in animals (very high). Organ weights: there were no effects on organ weights directly related to drug treatment. Histopathology: findings consisted of slight to mild degeneration of the superficial epithelium of the cecum in 4/10 females (high) and 1/10 males and 3/9 females (very high). Inflammatory cells were predominately neutrophils and infiltrated the mucosa and submucosa.

The primary drug related effect was degeneration or the superficial epithelium in the cecum with inflammatory cell infiltration and/or extension to the colon, which was accompanied by increases in leukocytes and fibrinogen in individual animals (high and very high). The mechanism of the epithelial degeneration in the cecum is unclear; however, degenerative changes affecting only the superficial epithelium are likely to be a local effect rather than a systemic toxicity.

The NOAEL in the mice was 200 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be 16.22 mg/kg/day (973 mg/day for a 60 kg person. Drug exposure at the 100 mg/kg/day was 2.38 and 2.02 µg/ml (Cmax) and 12.7 and 13.8 µg*hr/ml (AUC) in male and female mice, respectively.

Study no.: 01-2120-03

Volume # and page #: 1 and 1-243

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: August 1, 2001

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Batch # R1, [redacted]

Methods

Doses: Groups of male and female CD-1 mice (10 animals/sex/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 20 (low), 200 (mid) 1000 (high) or 2000 mg/kg/day (very high) for 14 consecutive days.

Species/strain: male and female CD-1 mice [strain: CD-1/[redacted] CD-1 (ICR) BR

Number/sex/group or time point (main study): 10 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage; 10 ml/kg

Satellite groups used for toxicokinetics: Plasma drug concentrations were measured in a separate satellite group of animals (3/sex/dose/timepoint) on days 1 and 13 at 1, 3, 5 and 24 hr postdose and the samples were analyzed by a validated analytical method.

Age: 6 weeks

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once weekly

Hematology: day 13

Clinical chemistry: day 13

Gross Pathology: all rats found dead were necropsied as soon as possible.

Organ weights: Body weights and the weights of the liver, kidneys (combined), adrenal glands(combined), heart, brain, testes (combined), spleen and thymus were recorded and organ to body weight ratios calculated. Organs were not weighed from animals that died or were euthanized as moribund.

Histopathology: Adequate Battery: yes; Peer review: yes

All mice were fasted overnight, anesthetized by carbon dioxide inhalation and exsanguinated on day 15. Following an external and visual examination, samples of the organs listed below plus a sample of any gross lesion were collected and placed in fixative.

Kidneys prostate, urinary bladder, seminal vesicle, liver (left and right lateral lobes),

ovaries, thymus, uterus, spleen, vagina, mesenteric lymph node, trachea, esophagus lung (both diaphragmatic lobes), stomach, heart, duodenum, peripheral nerve, jejunum, brain (cerebrum, cerebellum and pons), ileum, spinal cord (cervical), cecum, Harderian gland, colon, eyes, pituitary gland, skin, and adnexa (including mammary gland), salivary gland, bone (sternum, including bone marrow), skeletal muscle, gall bladder, pancreas, aorta, adrenal glands, larynx, thyroid gland, stifle joint, parathyroid, cervical lymph node, testes (left and right), tongue, epididymides.

Results

Mortality: drug related lethality or morbidity occurred in 2/20 animals (high) and 3/20 animals (very high) on day 14. All other animals survived the treatment.

Clinical signs: that preceded lethality or mobility included decreased activity, hunched posture, pale skin or coldness to touch.

Body weight and Food consumption: no changes.

Hematology: while no significant changes were observed in group mean hematology parameters, slight or mild increases in neutrophils, monocytes, basophils and/or fibrinogen were seen in animals (very high).

Clinical chemistry: ALT, AST, triglycerides and cholesterol values were elevated sporadically in the very high dose males and females.

Gross Necropsy: no toxicological effect.

Organ weights: no toxicological effect.

Histopathology: findings consisted of slight to mild degeneration of the superficial epithelium of the cecum in 4/10 females (high) and 1/10 males and 3/9 females (very high). Inflammatory cells were predominately neutrophils and infiltrated the mucosa and submucosa.

Toxicokinetics: data are shown in Table 14. Systemic exposure increased over the dose range and was similar in males and females. The highest mean levels were observed at 1 or 3 hr post dose. Some reduction in exposure occurred on repeated administration primarily in male mice.

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Table 14

Toxicokinetics of UK-427,857 on days 1 and 13 following repeated daily oral (gavage) administration to male and female mice.

Dose (mg/kg/day)	Sex	Day 1		Day 13	
		AUC $_{0\text{-}24hr}$ (μg*hr/ml)	Cmax (μg/ml)	$AUC_{0\text{-}24hr}$ (μg*hr/ml)	Cmax (μg/ml)
20	M	1.95	0.18	1	0.25
	F	5.39	1.96	0.92	0.37
200	M	18.1	4.9	12.7	2.38
	F	15.9	1.56	13.8	2.02
1000	M	140	10.7	45.8	6.56
	F	95.8	11.4	59.2	7.38
2000	M	240	21.9	132	11.8
	F	219	14.7	236	12.4

2. Study title: Two week oral toxicity study of UK-427,857 in beagle dogs

Key study findings: Groups of male and female beagle dogs [age: 8-9 months; 3 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 10 (low), 50 (mid) or 250 mg/kg/day (high) for 14 consecutive days. Plasma drug concentrations were measured on days 1 and 13 at 1, 3, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method. Results: there were no deaths in the study. Clinical signs: the most frequently observed clinical signs consisted of emesis, skin reddening and mydriasis (dilated pupils) and occurred in all drug-treated groups. The diminished pupillary light response test was consistent with the observation of mydriasis in some these animals. Other ocular signs were observed sporadically and included partial eye closure (mid and high), reddened conjunctiva (low, mid or high), protruding nictitating membrane (mid and high) and lacrimation (high). Salivation occurred in all drug treated animals immediately after dosing. Body weights, Food consumption, body temperature, respiratory rate, heart rate, organ weights, clinical chemistry, hematology and pathology: there were no drug related changes.

Drug related cardiovascular effects consisted of decrease in blood pressure (mid

and high) and slight increases in QTc interval (mid and high; 11-23 msec above predose values). The incidence of QTc prolongation was 1/6 animals (mid) and 6/6 animals (high). Two of six animals (low) have slight changes in QT intervals.

A NOAEL could not be identified in this study. Drug exposure at the low dose (10 mg/kg/day) was 1.8 µg/ml (Cmax) and 2.2 µg*hr/ml (AUC).

Study no.: 01-2120-05

Volume # and page #: 1 and 1-262

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: August 8, 2001

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: **# R1,** [redacted]

Methods

Doses: 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 10 (low), 50 (mid) or 250 mg/kg/day (high).

Species/strain: beagle dog

Age: 8-9 months

Number/sex/group: 3 animals/sex/group

Route, formulation, volume: oral gavage, 10 ml/kg

Satellite groups used for toxicokinetics: Plasma drug concentrations were measured on days 1 and 13 at 1, 3, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method.

Study design: Groups of male and female beagle dogs [age: 8-9 months; 3 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 10 (low), 50 (mid) or 250 mg/kg/day (high) for 14 consecutive days.

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once daily

Hematology: day 13

Clinical chemistry: day 13

Cardiovascular examinations: days 1, 13

Gross Pathology: full necropsy.

Organ weights: listed in Table 15

Histopathology: Table 3, Adequate Battery: yes; Peer review: yes

Table 15
Dog tissues, weighed, preserved and examined

Organ name	Weighed	Examined microscopically
adrenal glands	X	X
aorta (thoracic)		X
Bone marrow smear (rib)		X
Bone (sternum, femur)		X
bone marrow (sternum, femur)		X
Brain (medulla, pons, cerebrum and cerebellum)	X	X
Epididymides		X
Esophagus		X
Eyes with optic nerve		X
Heart	X	X
Kidneys	X	X
lacrimal glands		X
large intestine (cecum, colon, rectum)		X
Liver	X	X
lungs (with mainstem bronchi)		X
Lymph nodes (mesenteric, mediastinal)		X
mammary gland		X

nerve (sciatic)		X
Ovaries		X
Pancreas		X
pituitary gland		X
prostate gland		X
Salivary glands submandibular)		X
seminal vesicles		X
Skeletal muscle (biceps femoris)		X
Skin		X
Small intestine (duodenum, ileum, jejunum)		X
spinal cord (cervical, thoracic, lumber)		X
Spleen	X	X
Stomach		X
Testes	X	X
Thymus	X	X
Thyroid,/parathyroid glands		X
Trachea		X
Turbinates (skull)		
urinary bladder		X
uterus (body/horns) with cervix		X
Vagina		X
All gross lesions		X

Results

Mortality: no deaths.

Clinical signs: the most frequently observed clinical signs consisted of emesis, skin reddening and mydriasis (dilated pupils) and occurred in all drug-treated groups. The diminished pupillary light response test was consistent with the observation of mydriasis in some these animals. Other ocular signs were observed sporadically and included partial eye closure (mid and high), reddened conjunctiva (low, mid or high), protruding nictitating membrane (mid and high) and lacrimation (high). Salivation occurred in all drug treated animals immediately after dosing.

Body weights and food consumption: no change.

Cardiovascular examinations: Drug related cardiovascular effects consisted of decrease in blood pressure (mid and high) and slight increases in QTc interval (mid and high; 11-23

msec above predose values). The incidence of QTc prolongation was 1/6 animals (mid) and 6/6 animals (high). Two of six animals (low) have slight changes in QT intervals.

Clinical chemistry and hematology: no change.

Urinalysis: no treatment related findings.

Organ weights, Gross and Histopathology: there were no adverse pathological changes.

Toxicokinetics: data are shown in Table 16. The highest mean plasma levels were observed at 1 hr post dose. Systemic exposure increased over the dose range and was similar in male and female animals and comparable on days 1 and 13.

Table 16

Toxicokinetics of UK-427,857 on days 1 and 13 following repeated daily oral (gavage) administration to male and female dogs

Dose (mg/kg/day)	Day 1		Day 13	
	$AUC_{0\text{-}24hr}$ (µg*hr/ml)	Cmax (µg/ml)	$AUC_{0\text{-}24hr}$ (µg*hr/ml)	Cmax (µg/ml)
10	2.4	1.7	2.2	1.8
50	9.4	4.7	9.3	3.6
250	32.2	5.8	25.7	6.5

3. Study title: One month oral gavage range finding toxicity study of UK-427,857 in CD-1 mice

Key study findings: Groups of male and female CD-1 mice [strain: CD-1 [redacted]CD-1(ICR)BR; age: 6 weeks; 10 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water; vehicle control), 200 (low), 500 (mid) or 750 mg/kg/day (high) for 28 consecutive days. Results: there were no deaths. A low incidence of partially closed eyes was recorded sporadically in few animals in both sexes (mid or high). There were no drug related effects on body weight and food intake. Treatment produced no clinical pathology or histologic evidence of toxicity.

The NOEL in the mice was 200 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be 16.22 mg/kg/day (973 mg/day for

a 60 kg person). Drug exposure at the 200 mg/kg/day was 5.6 and 8.3 µg/ml (Cmax) and 31.6 and 35.7 µg*hr/ml (AUC) in male and female mice, respectively.

Study no.: 02002

Volume # and page #: 1 and 1-191

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: July 12, 2002

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Batch # R2, [redacted]

Methods

Doses: Groups of male and female CD-1 mice [strain: CD-1, [redacted]. CD-1(ICR) BR; age: 6 weeks; 10 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water; vehicle control), 200 (low), 500 (mid) or 750 mg/kg/day (high) for 28 consecutive days

Species/strain: male and female CD-1 mice [strain: CD-1 [redacted] CD-1 (ICR) BR

Number/sex/group or time point (main study): 10 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage; 10 ml/kg

Satellite groups used for toxicokinetics: Plasma drug concentrations were measured in a separate satellite group of animals (15/sex/dose) on day 26 at 1, 3, 5 and 24 hr postdose and the samples were analyzed by a validated analytical method.

Age: 6 weeks

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once weekly

Hematology: day 26

Clinical chemistry: day 26

Gross Pathology: all rats found dead were necropsied as soon as possible.

Organ weights: Body weights and the weights of the liver, kidneys (combined), adrenal glands (combined), heart, brain, testes (combined), spleen and thymus were recorded and organ to body weight ratios calculated. Organs were not weighed from animals that died or were euthanized as moribund.

Histopathology: Adequate Battery: yes; Peer review: yes

All mice were fasted overnight, anesthetized by carbon dioxide inhalation and exsanguinated on day 31. Following an external and visual examination, samples of the organs listed below plus a sample of any gross lesion were collected and placed in fixative.

Kidneys prostate, urinary bladder, seminal vesicle, liver (left and right lateral lobes), ovaries, thymus, uterus, spleen, vagina, mesenteric lymph node, trachea, esophagus lung (both diaphragmatic lobes), stomach, heart, duodenum, peripheral nerve, jejunum, brain (cerebrum, cerebellum and pons), ileum, spinal cord (cervical), cecum, Harderian gland, colon, eyes, pituitary gland, skin, and adnexa (including mammary gland), salivary gland, bone (sternum, including bone marrow), skeletal muscle, gall bladder, pancreas, aorta, adrenal glands, larynx, thyroid gland, stifle joint, parathyroid, cervical lymph node, testes (left and right), tongue, epididymides.

Results

Mortality: no deaths

Clinical signs: a low incidence of partially closed eyes was recorded sporadically in few animals in both sexes (mid or high).

Body weight and Food consumption: no changes.

Hematology: no change

Clinical chemistry: no change

Gross Necropsy: no toxicological effect.

Organ weights: no toxicological effect.

Histopathology: no toxicological effect.

Toxicokinetics: data are shown in Table 17. Systemic exposure increased over the dose range and was similar in males and females. The highest mean plasma levels were observed at 3 hr postdose in males and between 1 and 5 hr in females.

Table 17
Toxicokinetics of UK-427,857 on day 26 following repeated daily oral (gavage) administration to male and female mice.

Dose (mg/kg/day)	Sex	Day 26	
		Cmax (µg/ml)	AUC $_{0\text{-}24hr}$ (µg*hr/ml)
200	M	5.6	31.6
	F	8.3	35.7
500	M	10	79.4
	F	12.8	107
750	M	13	126
	F	13.9	110

4. Study title: One month dose range finding oral gavage toxicity study of UK-427,857 in male rats

Key study findings: Groups of male Sprague-Dawley rats (5 animal/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 100 (low), 300 (mid) or 1500 mg/kg/day (high) for 28 consecutive days. Results: there were no deaths in the study. Clinical signs: all treated animals displayed increased salivation with dose-related frequency. This sign was observed generally before dosing and up to 15 min after dosing. Diarrhea occurred sporadically during the second half of the study at the high dose. Body weight & Food consumption: there were no drug related differences in group mean food consumption: high dose animals had a statistically significantly lower mean body weights gain (11%) than the controls. The treatment induced a toxicologically significant decrease (25%) in mean food consumption during the first week of the study (high). Clinical chemistry: ALT, AST, ALP, GGT and cholesterol values were elevated moderately in the high dose animals. Gross Necropsy: dilatation of the colon and cecum was present in 4/5 and 2/5 animals (high). Multifocal red discoloration that affected all liver lobes was present in 1/5 animals (high). Organ weights: there were statistically significant decreases in absolute heart and spleen weights (high). Absolute thymus weights were slightly decreases at all doses with statistical significance at the mid dose only. Histopathology: the liver, adrenal, pituitary, colon and cecum (mid, 1/5; 2/5 high) had changes that were present in the high dose treated

animals only. Necrosis in the liver was mostly located in the centrilobular area with occasional extension in the periportal region in the high dose animals. Minimal to mild vacuolation of the pars distalis was observed in the pituitary gland in the high dose animals. Adverse treatment related findings were restricted to the high dose. Changes in the liver consisted of necrosis in one high dose animal, which was associated with moderate increases in liver enzymes. There was also minimal to mild vacuolation of the par distalis in the pituitary gland. Minimal to mild inflammation of the joints was present in a dose related incidence and severity at mid and high doses.

The NOEL in the rat was 100 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be 16.23 mg/kg/day (974 mg/day for a 60 kg person). Drug exposure at the 100 mg/kg/day was 1.15 µg/ml (Cmax) and 7.4 µg*hr/ml (AUC).

Study no.: 02072

Volume # and page #: 1 and 1-106

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: October, 21, 2002

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: **Batch # R102,** [redacted]

Methods

Doses: Groups of male Sprague-Dawley rats (5 animal/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 100 (low), 300 (mid) or 1500 mg/kg/day (high) for 28 consecutive days.

Species/strain: male rats; strain: CD (SD) IGS BR

Number/sex/group or time point (main study): 5 animals/group

Route, formulation, volume, and infusion rate: oral gavage; 10 ml/kg

Satellite groups used for toxicokinetics: 5 animals/dose group

Age: 7 weeks

Weight: 222-286 g

Sampling times: Plasma drug concentrations were measured in a separate satellite group of animals (5/dose) on days 1 and 23 at 1, 3, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method.

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once weekly

Hematology: day 28

Clinical chemistry: day 28

Gross Pathology: all rats found dead were necropsied as soon as possible.

Organ weights: listed in Table 18

Histopathology: Table 3, Adequate Battery: yes; Peer review: yes

Table 18
Rat tissues, weighed, preserved and examined

Organ name	Weighed	Preserved	Examined microscopically
adrenal glands	X	X	X
aorta (thoracic)		X	X
Bone marrow smear (rib)		X	
Bone (sternum, femur)		X	X
bone marrow (sternum, femur)		X	X
Brain (medulla, pons, cerebrum and cerebellum)	X	X	X
Epididymides		X	X
Esophagus		X	X
Eyes with optic nerve		X	X
Harderian gland		X	X
Heart	X	X	X
Kidneys	X	X	X
lacrimal glands		X	X

large intestine (cecum, colon, rectum)		X	X
Liver	X	X	X
lungs (with mainstem bronchi)		X	X
Lymph nodes (mesenteric, mediastinal)		X	X
mammary gland		X	X
nerve (sciatic)		X	X
Ovaries		X	X
Pancreas		X	X
pituitary gland	X	X	X
prostate gland		X	X
Salivary glands submandibular)		X	X
seminal vesicles		X	X
Skeletal muscle (biceps femoris)		X	X
Skin		X	X
Small intestine (duodenum, ileum, jejunum)		X	X
spinal cord (cervical, thoracic, lumber)		X	X
Spleen	X	X	X
Stomach		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroid,/parathyroid glands		X	X
Trachea		X	X
Turbinates (skull)		X	
urinary bladder		X	X
uterus (body/horns) with cervix		X	X
Vagina		X	X
All gross lesions		X	X

Results

Mortality: no deaths

Clinical signs: all treated animals displayed increased salivation with dose-related frequency. This sign was observed generally before dosing and up to 15 min after dosing. Diarrhea occurred sporadically during the second half of the study at the high dose.

Body weight and Food consumption: there were no drug related differences in group mean food consumption: high dose animals had a statistically significantly lower mean body weights gain (11%) than the controls. The treatment induced a toxicologically significant decrease (25%) in mean food consumption during the first week of the study (high).

Hematology: no significant changes

Clinical chemistry: ALT, AST, ALP, GGT and cholesterol values were elevated moderately in the high dose animals.

Gross Necropsy: dilatation of the colon and cecum was present in 4/5 and 2/5 animals (high). Multifocal red discoloration that affected all liver lobes was present in 1/5 animals (high).

Organ weights: there were statistically significant decreases in absolute heart and spleen weights (high). Absolute thymus weights were slightly decreases at all doses with statistical significance at the mid dose only.

Histopathology: the liver, adrenal, pituitary, colon, cecum and joint (mid, 1/5; 2/5 high) had changes that were present in the high dose treated animals only. Necrosis in the liver was mostly located in the centrilobular area with occasional extension in the periportal region in the high dose animals. Minimal to mild vacuolation of the pars distalis was observed in the pituitary gland in the high dose animals.

Toxicokinetics: data are shown in Table 19. The highest mean plasma levels were observed between 1 and 7 hr post dose.

Table 19
Toxicokinetics of UK-427,857 on days 1 and 23 following repeated daily oral (gavage) administration to male rats

Dose (mg/kg/day)	Day 1		Day 23	
	$AUC_{0\text{-}24hr}$ (µg*hr/ml)	Cmax (µg/ml)	$AUC_{0\text{-}24hr}$ (µg*hr/ml)	Cmax (µg/ml)
100	4.77	0.69	7.4	1.15
300	21.3	2.38	31.2	4.3
1500	84.5	7.26	88.7	7.3

5. Study title: One month oral dose range finding toxicity study of UK-427,857 in beagle dogs

Key study findings: Groups of male and female beagle dogs (3 animal/sex/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water; vehicle control), 5 (low), 50 (mid) or 150 mg/kg/day (high) for 4 consecutive weeks. There were no deaths in the study. At the mid and high doses were emesis, mydriasis (dilated pupils), partially closed eyes, protruding nictitating membrane, red conjunctiva and increased salivation. Except for emesis, that occurred mostly within 15 min after dosing, these signs were generally observed between 15 min and 5 hr postdosing. Increase in QT intervals was seen at the mid or high. In addition, the high dose produced body weight loss and reduce food intake which represents further indication of toxicity.

The NOEL in the dog was 5 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be 2.7 mg/kg/day (162 mg/day for a 60 kg person). Drug exposure at the 5 mg/kg/day was 0.79 µg/ml (Cmax) and 6.02 µg*hr/ml (AUC).

Study no.: 02003

Volume # and page #: 1 and 1-262

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: June 21, 2002

GLP compliance: No

QA reports: No

Drug, lot #, and % purity: # R101, [redacted]

Methods

Doses: 0 (0.5% methylcellulose/0.1%Tween 80 in water; vehicle control), 5 (low), 50 (mid) or 150 mg/kg/day (high).

Species/strain: beagle dog

Age: 15 months

Number/sex/group: 3 animals/sex/group

Route, formulation, volume: oral gavage, 10 ml/kg

Satellite groups used for toxicokinetics: Plasma drug concentrations were measured on

days 1 and 19 at 1, 4, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method.

Study design: Groups of male and female beagle dogs (3 animal/sex/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water; vehicle control), 5 (low), 50 (mid) or 150 mg/kg/day (high) for 4 consecutive weeks. Plasma drug concentrations were measured on days 1 and 19 at 1, 4, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method.

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once daily

Hematology: day 26

Clinical chemistry: day 26

Cardiovascular examinations: days 5, 26

Gross Pathology: full necropsy

Organ weights: listed in Table 20

Histopathology: Table 3, Adequate Battery: yes; Peer review: yes

Table 20
Dog tissues, weighed, preserved and examined

Organ name	Weighed	Examined microscopically
adrenal glands	X	X
aorta (thoracic)		X
Bone marrow smear (rib)		X
Bone (sternum, femur)		X
bone marrow (sternum, femur)		X
Brain (medulla, pons, cerebrum and cerebellum)	X	X
Epididymides		X
Esophagus		X

Eyes with optic nerve		X
Heart	X	X
Kidneys	X	X
lacrimal glands		X
large intestine (cecum, colon, rectum)		X
Liver	X	X
lungs (with mainstem bronchi)		X
Lymph nodes (mesenteric, mediastinal)		X
mammary gland		X
nerve (sciatic)		X
Ovaries		X
Pancreas		X
pituitary gland		X
prostate gland		X
Salivary glands submandibular)		X
seminal vesicles		X
Skeletal muscle (biceps femoris)		X
Skin		X
Small intestine (duodenum, ileum, jejunum)		X
spinal cord (cervical, thoracic, lumber)		X
Spleen	X	X
Stomach		X
Testes	X	X
Thymus	X	X
Thyroid,/parathyroid glands		X
Trachea		X
Turbinates (skull)		
urinary bladder		X
uterus (body/horns) with cervix		X
Vagina		X
All gross lesions		X

Results

Mortality: there were no deaths in the study.

Clinical signs: at the mid and high doses were emesis, mydriasis (dilated pupils), partially closed eyes, protruding nictitating membrane, red conjunctiva and increased salivation. Except for emesis that occurred mostly within 15 min after dosing, these signs were generally observed between 15 min and 5 hr postdosing. There were no other changes observed.

Body weights and food consumption: there were sporadic occurrences of reduced food intake in all groups including controls. One (high) dog had frequent reduction in food intake from day 12 and an absence of food intake on 4 occasions towards the end of the study. This dog (high) lost 26% of its weight at the end of the study, relative to day 1.

Cardiovascular examinations:

QT prolongation: relative to predose values, the mean QT interval was increased after dosing on day 5 (mid and high) and on day 26 (high) as shown in Table 21 below:

Table 21
Changes in mean QT intervals (absolute and % change one hr postdose relative to predose values in dogs (one month toxicology study)

Dose (mg/kg/day)	Day 5		Day 26	
	Absolute (msec)	Change (%)	Absolute (msec)	Change (%)
Control	+8.5	+4	+11	+6
5	+5	+3	+3.5	+2
50	+29.8** 5/6 dogs	+15	+12.4 5/6 dogs	+6
150	+23.8** 6/6 dogs	+11	+20.6 4/6 dogs	+10

** = statistically significant at p=0.01

Clinical chemistry and hematology: At the high dose, some dogs showed a decrease in potassium and increases in sodium, proteins and fibrinogen.

Urinalysis: no treatment related findings.

Organ weights, Gross and Histopathology: there were no adverse pathological changes.

Toxicokinetics: data are shown in Table 22. The highest mean plasma levels were observed at 1 hr post dose. Systemic exposure increased over the dose range and was 1.5-2.2 folds higher on day 19 when compared to days 1 value.

Table 22

Toxicokinetics of UK-427,857 on days 1 and 19 following repeated daily oral (gavage) administration to male and female dogs

Dose (mg/kg/day)	Day 1		Day 19	
	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)
5	2.93	1	6.02	0.79
50	26.64	6.99	35.19	8.81
150	61.86	9.32	99.73	9.69

6. Study title: UK-427,857: One-month oral (bid) dose range finding toxicity study in the cynomolgus monkey

Key study findings: Groups of male and female cynomolgus monkeys received repeated oral gavage doses (bid, 7-8 hr apart) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 100 (low), 200 (mid), 400 (high) or 800 mg/kg/day (highest) daily for a period of 4 weeks. The highest dose level of maraviroc induced severe clinical signs resulting in the euthanasia of the animals. Before being sacrificed, these animals showed signs of abnormal motor activity (including half closed eyes, reduced activity, prostration and loss of balance) associated with vomiting. There were no histopathologic changes that could explain the moribund status of these four animals (highest). No clinical signs were noted in the other groups during the study. The treatment at the high and mid dose induced QT prolongation, lowered diastolic blood pressure and decreased heart rate. A slight QT prolongation was seen at the low dose also. In this study, a NOAEL could not be established.

A NOAEL in the monkeys should be below 100 mg/kg/day. Based on the body surface area factor, an equivalent oral dose in humans would be 2 g/day for a 60 kg person. Drug exposure at the 100 mg/kg/day was 2.8 µg/ml (Cmax) and 4.21 µg*hr/ml (AUC).

Study no.: 911/097

Volume # and page #: 1 and 1-262

Conducting laboratory and location: Pfizer Inc., Amboise cedex, France

Date of study completion: August 1, 2003

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: **# R103,** [redacted]

Methods

Doses: 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 100 (low), 200 (mid), 400 (high) or 800 mg/kg/day (highest) twice daily for one month.

Species/strain: cynomolgus monkeys

Age: 26-32 months

Number/sex/group: 2 animals/sex/group

Route, formulation, volume: oral gavage, 5ml/kg

Satellite groups used for toxicokinetics: blood samples were taken from each animal on days 0 and 27 at predetermined time intervals.

Study design: groups of male and female cynomolgus monkeys (2 animals/sex/group) received repeated oral gavage doses (bid, 7-8 hr apart) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 100 (low), 200 (mid), 400 (high) or 800 mg/kg/day (highest) twice daily for one month.

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once daily

Hematology: day 27

Clinical chemistry: day 27

Cardiovascular examinations: days 1, 26

Gross Pathology: full necropsy

Organ weights: listed in Table 23

Histopathology: Table 3, Adequate Battery: yes; Peer review: yes

Table 23
Monkey tissues, weighed, preserved and examined

Organ name	Weighed	Examined microscopically
adrenal glands	X	X
aorta (thoracic)		X
Bone marrow smear (rib)		X
Bone (sternum, femur)		X
bone marrow (sternum, femur)		X
Brain (medulla, pons, cerebrum and cerebellum)	X	X
Epididymides		X
Esophagus		X
Eyes with optic nerve		X
Heart	X	X
Kidneys	X	X
lacrimal glands		X
large intestine (cecum, colon, rectum)		X
Liver	X	X
lungs (with mainstem bronchi)		X
Lymph nodes (mesenteric, mediastinal)		X
mammary gland		X
nerve (sciatic)		X
Ovaries		X
Pancreas		X
pituitary gland		X
prostate gland		X
Salivary glands submandibular)		X
seminal vesicles		X
Skeletal muscle (biceps femoris)		X
Skin		X
Small intestine (duodenum, ileum, jejunum)		X
spinal cord (cervical, thoracic, lumber)		X

Spleen	X	X
Stomach		X
Testes	X	X
Thymus	X	X
Thyroid,/parathyroid glands		X
Trachea		X
Turbinates (skull)		
urinary bladder		X
uterus (body/horns) with cervix		X
Vagina		X
All gross lesions		X

Results

Mortality: all animals (highest) submitted to necropsy on day 1 due to marked clinical signs. All other animals were euthanized after week 4.

Clinical signs: **Highest dose:** before being sacrificed, these animals showed signs of abnormal motor activity (including half closed eyes, reduce activity, prostration and loss of balance) associated with vomiting. There were no histopathologic changes that could explain the moribund status of these four animals (highest). No clinical signs were noted in the other groups during the study.

Ophthalmology: there were no treatment related ocular findings.

Body weights and food consumption: a tendency toward minimal body weight loss (between 1.7 to 5.6%) and reduce food intake was observed in the high dose animals.

Cardiovascular examinations: the treatment induced a decrease in heart rate (high and highest), together with elongated QT prolongation three hrs after dosing. In addition, QT prolongation with no concurrent decreased in heart rate was observed at the mid dose. At the low dose a slight QT prolongation was also observed.

Hematology: on day 1, monkeys (highest) euthanized moribund had low RBC parameters and WBC counts due to increase in neutrophil counts.

Clinical chemistry: low total protein associated with low globulin levels, slightly elevated total bilirubin, slightly elevated creatinine, increased in AST were seen (highest).

Urinalysis: no treatment related findings.

Organ weights, Gross and Histopathology: there were no adverse pathological changes.

Toxicokinetics: plasma drug concentrations were comparable in males and females and were combined. Highest plasma concentrations generally occurred 1 to 6 hr after the first or second daily dosing. The mean AUC values increased in a dose proportional manner. The mean toxicokinetic parameters are shown in Table 24.

Table 24

Group mean toxicokinetic parameters of UK-427,857 in cynomolgus monkeys

Dose (mg/kg/day)	Mean Cmax (µg/ml)		Mean AUC0-24hr (µg*hr/ml)	
	Day 0	Day 27	Day 0	Day 27
50	1.24	2.8	5.42	4.21
100	2.96	5.0	19.23	19.00
200	6.78	3.8	66.00	68.17
400	8.70	-	84.66	-

7. Study title: Three month oral gavage range finding toxicity study of UK-427,857 in CD-1 mice

Key study findings: Groups of male and female CD-1 mice (10 animal/sex/group) received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water; vehicle control), 200 (low), 500 (mid) or 750 mg/kg/day (high) for 28 consecutive days. Results: there were 5 deaths; three of them were attributed to gavage error (one male at mid dose, one male and one female in vehicle controls). The cause of death of other two animals (1 male in high dose and one male at low dose could not be determined. Clinical signs: a low incidence of partially closed eyes was recorded sporadically in few animals in both sexes (mid or high). Body weight and Food consumption: there was a dose related increase in body weight in females (mid up to 10%), but food consumption was not affected. Organ weights: mean absolute and relative kidney weights were slightly increased in females (high up to 16%). Histopathology: no toxicological effect.

The NOEL in the mice was 200 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be 16.22 mg/kg/day (973 mg/day for a 60 kg person). Drug exposure at the 200 mg/kg/day was 3.9 and 3.7 µg/ml (Cmax) and 15 and 11 µg*hr/ml (AUC) in male and female mice, respectively.

Study no.: 02002

Volume # and page #: 1 and 1-254

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: July 12, 2002

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: **Batch # R103,** [redacted]

Methods

Doses: Groups of male and female CD-1 mice [strain: CD-1[redacted]: CD-1(ICR) BR; age: 6 weeks; 10 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water; vehicle control), 200 (low), 500 (mid) or 750 mg/kg/day (high) for 3 months.

Species/strain: male and female CD-1 mice [strain: CD-1/[redacted]CD-1 (ICR) BR

Number/sex/group or time point (main study): 10 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage; 10 ml/kg

Satellite groups used for toxicokinetics: Plasma drug concentrations were measured in a separate satellite group of animals (13/sex/dose) on day 26 at 1, 3, 5 and 24 hr postdose and the samples were analyzed by a validated analytical method.

Age: 6 weeks

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once weekly

Hematology: day 83

Clinical chemistry: day 83

Gross Pathology: all rats found dead were necropsied as soon as possible.

Organ weights: Body weights and the weights of the liver, kidneys (combined), adrenal glands (combined), heart, brain, testes (combined), spleen and thymus were recorded and organ to body weight ratios calculated. Organs were not weighed from animals that died or were euthanized as moribund.

Histopathology: Adequate Battery: yes; Peer review: yes

All mice were fasted overnight, anesthetized by carbon dioxide inhalation and

exsanguinated on day 91. Following an external and visual examination, samples of the organs listed below plus a sample of any gross lesion were collected and placed in fixative.

Kidneys prostate, urinary bladder, seminal vesicle, liver (left and right lateral lobes), ovaries, thymus, uterus, spleen, vagina, mesenteric lymph node, trachea, esophagus lung (both diaphragmatic lobes), stomach, heart, duodenum, peripheral nerve, jejunum, brain (cerebrum, cerebellum and pons), ileum, spinal cord (cervical), cecum, Harderian gland, colon, eyes, pituitary gland, skin, and adnexa (including mammary gland), salivary gland, bone (sternum, including bone marrow), skeletal muscle, gall bladder, pancreas, aorta, adrenal glands, larynx, thyroid gland, stifle joint, parathyroid, cervical lymph node, testes (left and right), tongue, epididymides.

Results

Mortality: there were 5 deaths; three of them were attributed to gavage error (one male at mid dose, one male and one female in vehicle controls). The cause of death of other two animals (1 male in high dose and one male at low dose could not be determined.

Clinical signs: a low incidence of partially closed eyes was recorded sporadically in few animals in both sexes (mid or high).

Body weight and Food consumption: there was a dose related increase in body weight in females (mid up to 10%), but food consumption was not affected.

Hematology: no change

Clinical chemistry: no change

Gross Necropsy: no toxicological effect.

Organ weights: mean absolute and relative kidney weights were slightly increased in females (high up to 16%).

Histopathology: no toxicological effect.

Toxicokinetics: data are shown in Table 25. Systemic exposure increased over the dose range and was similar in males and females. The highest mean plasma levels were observed at 3 hr postdose in males and between 1 and 5 hr in females

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Table 25
Toxicokinetics of UK-427,857 on day 83 following repeated daily oral (gavage) administration to male and female mice.

Dose (mg/kg/day)	Sex	Day 83	
		Cmax (μg/ml)	AUC $_{0\text{-}24hr}$ (μg*hr/ml)
200	M	3.9	15
	F	3.7	11
500	M	19.3	59
	F	10.1	52
750	M	17.9	251
	F	15.2	164

8. Study title: 1. UK-427,857: 26 Weeks oral (gavage) toxicology study in rats followed by a 13-week treatment free period

Key study findings: male and female rats received UK-427,857 via oral gavage at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 30 (low), 100 (mid), 300 (high) or 900 mg/kg/day (highest) for 26 consecutive weeks and to evaluate the regression of any toxic signs during a 13-week reversibility period. Treatment from the low dose (30 mg/kg/day) induced hypersalivation. Bile duct vacuolation was observed at the mid (100 mg/kg/day), high (300 mg/kg/day) and highest (900 mg/kg/day) dose levels. These effects were not considered adverse. Treatment at the high dose(300 mg/kg/day) induced bile duct hyperplasia, follicular cell hypertrophy in the thyroid, decreased T4 levels and increased TSH, decreased bilirubin, increased ALT, increased water consumption and dilatation of the cecum. Treatment at the highest dose (900 mg/kg/day) induced hepatic changes (increased GGT, altered cell foci, liver weight increases, multinucleated hepatocytes), decreased body weights, increased urine volume, cortical vacuolation of the adrenals and stained fur in the urogenital area in females.

Treatment related changes at the high and highest doses were reversible except for the decreased body weights and increased liver weights (highest) and the bile duct vacuolation, bile duct hyperplasia and multinucleated hepatocytes in males at the high and highest doses. Under the experimental conditions of this study, a dose level of 100 mg/kg/day may be considered the NOAEL.

Tabulated summary of key findings and exposure multiples of human therapeutic doses

are shown in Table 26.

Table 26

Key treatment related findings and exposure multiples achieved at each dose level

Dose levels (mg/kg/day)	Key findings*	Clinical doses	
		100 mg bid	300 mg bid
		**Exposure multiples based on free (total) AUC values	
≥30	↑salivation	6 (3)	1 (0.6)
≥100	NOAEL Bile duct vacuolation in males	44 (22)	8 (4)
≥300	↑water consumption in females, ↓bilirubin in females, ↑ALT in females, ↓T4 levels, ↑TSH in males, cecum dilatation, bile duct vacuolation (not reversible in males), bile duct hyperplasia in males (not reversible), thyroid follicular cell hypertrophy.	147 (73)	26 (13)
900	Hair loss, stained fur (urogenital area) in females, ↓body weight in males, ↑GGT, ↑ALT, ↓bilirubin, ↑urine volumes in males, ↓T4 levels, ↑absolute and relative adrenal weight in males, ↑absolute and relative liver weight in females, alopecia in females, multinucleated hepatocytes (not reversible in males), altered cell foci in females, adrenal cortical vacuolation in males.	298 (147)	52 (26)

* = unless otherwise stated finding affected males and females and were reversible at the 300 and 900 mg/kg/day dose levels.

** = Exposure multiples are rounded values calculated based on the rat AUC (0-24h) and double of the human AUC (0-12h), (i.e., 0.22 µg*hr/ml at 100 mg bid and 1.26 µg*hr/ml at 300 mg bid for free AUCs).

Study no.: 911/092

Volume # and page #: 1-3 and 1-1066

Conducting laboratory and location: [redacted]

Date of study completion: March 29, **2004**

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: **Batch # R103,** [redacted]

Methods

Doses: UK-427,857 was administered via oral gavage (5 ml/kg/day) at dose levels of 0 (vehicle control; 0.5% w/v methylcellulose + 0.1% w/w Tween 80 in water), 30 (low), 100 (mid), 300 (high) or 900 mg/kg/day (highest) for 26 consecutive weeks and to evaluate the regression of any toxic signs during a 13-week reversibility period according to an experimental design shown in Table 27.

Table 27

Experimental design of the oral (gavage) 6-month toxicology study in rats

Dosage group (mg/kg/day	Week 26(1)				Week 40 (2) Main group	
	Main group		Satellite group			
	male	Female	Male	female	Male	female
1. vehicle control	15	15	-	-	10	10
2. low; 30	15	15	3	3	-	-
3. mid; 100	15	15	3	3	-	-
4. high; 300	15	15	3	3	10	10
5. highest; 900	15	15	3	3	10	10

(1) = sacrificed at the end of the treatment period
(2) = sacrificed at the end of the reversibility period (week 40)

Species/strain: male and female rats; strain: Ico: OFA.SD. (IOPS Caw)

Number/sex/group or time point (main study): Table 28

Route, formulation, volume, and infusion rate: oral gavage; 5 to 10 ml/kg

Satellite groups used for toxicokinetics: Table 28

Age: 7 weeks

Weight: 222-286 g for males and 157-200 g for females

Sampling times: Plasma concentrations of UK-427,857 were determined at 1, 3, 7 and 24 hr on day first and 181 of the study.

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once weekly

Ophthalmoscopy: weeks 13 and 26

Hematology: week 5, 14, 26 and 32

Clinical chemistry: weeks 5, 14, 26 and 32

Urinalysis: weeks 10 and 23

Gross Pathology: all rats found dead were necropsied as soon as possible.

Organ weights: listed in Table 29

Histopathology: Table 29, Adequate Battery: yes; Peer review: yes

Table 29
Rat tissues, weighed, preserved and examined

Organ name	Weighed	Preserved	Examined microscopically
adrenal glands	X	X	X
aorta (thoracic)		X	X
Bone marrow smear (rib)		X	
Bone (sternum, femur)		X	X
bone marrow (sternum, femur)		X	X
Brain (medulla, pons, cerebrum and cerebellum)	X	X	X
Epididymides		X	X
Esophagus		X	X
Eyes with optic nerve		X	X
Harderian gland		X	X
Heart	X	X	X
Kidneys	X	X	X
lacrimal glands		X	X
large intestine (cecum, colon, rectum)		X	X
Liver	X	X	X

lungs (with mainstem bronchi)		X	X
Lymph nodes (mesenteric, mediastinal)		X	X
mammary gland		X	X
nerve (sciatic)		X	X
Ovaries		X	X
Pancreas		X	X
pituitary gland	X	X	X
prostate gland		X	X
Salivary glands submandibular)		X	X
seminal vesicles		X	X
Skeletal muscle (biceps femoris)		X	X
Skin		X	X
Small intestine (duodenum, ileum, jejunum)		X	X
spinal cord (cervical, thoracic, lumber)		X	X
Spleen	X	X	X
Stomach		X	X
Testes	X	X	X
Thymus	X	X	X
Thyroid,/parathyroid glands		X	X
Trachea		X	X
Turbinates (skull)		X	
urinary bladder		X	X
uterus (body/horns) with cervix		X	X
Vagina		X	X
All gross lesions		X	X

Results

Mortality: There were 3 unscheduled deaths during the study (one male each (low, mid and highest), none of which were considered to be treatment related.

Clinical signs: were limited to increased salivation at all dose levels, hair loss in males and females (highest) and stained fur in the urogenital area in the females (highest). Hair loss was still present during the 13-week recovery period. No other signs were noted in the recovery period.

Body weight and body weight gain: mean body weight gain was statistically significantly

(p=0.001) reduced (-22% during the first week and –13% overall for the treatment period) for the males (highest) when compared with controls. As a consequence, the mean body weight of males (highest) was 4 to 8% lower than controls during the treatment period and 10% lower at the termination. During the recovery phase, males (highest) maintained a lower body weight than controls. However, this difference (-10%) achieved statistical significance during the first week of reversibility only.

Food consumption: the only drug related finding was a transient decrease in mean food consumption (highest) during the first week of treatment (-19%, p=0.001 for males compared with controls, -8% not statistically significant for females). Therefore, the body weight reduction was directly related to the treatment.

Water consumption: there were statistically significant increases in water consumption in females (high) from day 56 onward (+17% to +27%) and in males and females (highest) during the treatment period (+37% to +57% in males and +50% to +90% in females). A similar trend was noted in males (high) without reaching statistical significance (up to +18% on day 56).

Ophthalmology: there were no treatment-related changes noted.

Hematology: no significant changes

Clinical chemistry: compared with controls, GGT value was increased in both males and females (highest, p=0.01) on week 14 and at the end of the treatment period (up to 6 U/L; p=0.001) for both males and females. Mean ALT value was slightly increased in males and females (highest) at all time points, compared with controls (up to 1.5 times at week 5 and 26, p=0.05). Total bilirubin was consistently lower than controls in females from week 5 onwards (reaching –35% for females (highest) and –22% (high), p=0.001.

Thyroid hormone levels: as shown in Table 30, T4 plasma levels decreased while TSH values increased with dose levels in males (high and highest). In females, T4 was decreased (highest) relative to low dose while no clear effect was present for TSH. Hormone levels returned to control levels following the recovery period.

Table 30
Group mean thyroid hormone levels in rats

Dose (mg/kg/day) & group	Main study (week 26) n=3				Satellite study (week 38) n=10			
	T4 (nm/L)		TSH (ng/ml)		T4 (nm/L)		TSH (ng/ml)	
	male	female	male	Female	Male	female	Male	Female
0, control	-	-	-	-	27.2	19.7	4.8	1.4
30, low	50.9	19.2	4.6	4.5				
100, mid	33.3	16.5	5.5	2.5				
300, high	22.1	16.4	6.6	4.3	29.2	25.6	2.2	1.3
900, highest	14.7	7.1	7.7	3.3	31.3	30.3	3.8	2.8

Urinalysis: no significant changes.

Gross pathology: the incidence of enlarged cecum at the end of the treatment period was higher in both males and females (highest). This trend was already seen at the high dose. No similar change was found in any dose group at the end of the recovery period. Alopecia was more frequent amongst females (11/25, highest) compared with other dose groups or controls. The observation was no longer seen at the end of the recovery period.

Organ weights: drug related mean absolute and relative weights of the liver were higher than control for females (highest) at the end of the 6 month period (+23 and +27%, respectively) and at the end of the recovery period (+11% and +14%, respectively). Mean absolute and relative weights of the adrenal glands were higher than controls for males (highest) at the end of treatment period (+20% and +32%, respectively).

Histopathology:

End of treatment period: several changes were found in the liver. Their incidences are presented in Table 31. The vacuolation noted in the bile ducts was minimal to mild and affected large to medium-sized ducts. The increase in incidence of this change was considered drug-related (mid, high or highest). Bile duct hyperplasia was present in a few animals, including controls. The change was minimal except for one control female, which was graded mild. The hyperplasia was restricted to the portal spaces and consisted of an increased number of differentiated bile ducts. Treatment from the high dose level exacerbated this background age-related change as evidenced by the increased incidence with no concomitant increases in severity. Drug related multinucleated hepatocytes were mostly located around the central veins and contained more than 4 nuclei.

Table 31
Incidence of hepatic changes during the treatment phase in the rat

Findings	Dosage levels (mg/kg/day) n=15 animals/sex/group									
	control (0)		low (30)		mid (100)		high (300)		Highest (900)	
	male	Female	male	Female	male	female	male	Female	male	female
Vacuolation, bile duct	3	0	4	0	6	0	7	4	11	11
Hyperplasia, bile duct	1	1	0	1	2	0	3	2	4	7
Multinucleated hepatocytes	0	1	0	0	0	0	0	1	4	9
Altered cell foci	0	0	0	0	0	0	0	0	0	2

Incidence of follicular cell hypertrophy in the thyroid in male and female rat combined: is shown in Table 32. This change was bilateral and was characterized by follicles with narrow lumens and higher cuboidal epithelium. The follicular cells were more vacuolated than those of the controls.

Table 32
Follicular cell hypertrophy in the thyroid of male and female rats

Findings	Dosage levels (mg/kg/day) n=30 animals/group				
	control (0)	low (30)	mid (100)	high (300)	Highest (900)
Follicular cell hypertrophy	2	-	-	5	14

Incidence and severity of diffuse cortical vacuolation of the adrenals in male rats: are shown in Table 33. There was a minimal increased in incidence and severity of diffuse cortical vacuolation of the adrenal in male rats. This change affected only a few females (4 controls and 2 high dose females only.

Table 33
Incidence and severity of diffuse cortical vacuolation of the adrenal in males

Vacuolation	Dosage levels (mg/kg/day) n=30 animals/group				
	control (0)	low (30)	mid (100)	high (300)	Highest (900)
Minimal	6	4	3	1	2
Mild	2	1	1	6	8
Moderate	1	1	0	1	2
Marked	0	0	0	0	1
Total	9	6	4	8	13

Other drug related changes were present in the cecum and skin as shown in Table 34. The dilatation of the cecum correlated with gross findings. The lining epithelium was normal with no evidence of degeneration. This change was drug-related at high and highest dose. A small number of treated animas had acanthosis and hyperkeratosis. This change was present in animals with a gross diagnosis of alopecia, although in majority of cases, alopecia had no histopathological correlates.

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Table 34
Incidence of changes in cecum and skin in male and female rats combined

Vacuolation	Dosage levels (mg/kg/day) n=30 animals/group				
	Control (0)	low (30)	mid (100)	high (300)	Highest (900)
Cecum:					
Dilatation	0	1	5	5	18
Skin:					
Acanthosis	0	0	3	0	4
Hyperkeratosis	0	0	3	0	3

End of the recovery period:

The liver, thyroid and adrenals were examined.

Liver: the main hepatic findings are presented in Table 35. Proliferation of bile ducts was mostly restricted to the portal space areas. However, aggregates of bile ducts extended occasionally into the adjacent parenchyma. The bile duct hyperplasia was within normal background levels for the treated females whereas the incidence was increased in treated males. The increased incidence of multinucleated hepatocytes in males (highest) was considered treatment related. Minimal necrosis of the liver was present in a higher incidence (highest).

Thyroid: upon examination of the thyroid glands, there was no evidence of follicular cell hypertrophy. This change was reversible during the recovery phase.

Table 35
Incidence of hepatic changes in male and female rats at the end of recovery phase

Findings	Dosage levels (mg/kg/day) n=10 animals/sex/group					
	control (0)		High (300)		Highest (900)	
	male	female	Male	female	Male	female
Vacuolation, bile duct	0	0	3	1	3	0
Hyperplasia, bile duct	0	4	6	4	7	4
Altered cell foci	0	0	0	1	0	0
Multinucleated hepatocytes	0	1	0	1	3	1
Necrosis	0	1	1	0	2	3

Adrenals: diffuse cortical vacuolation of the adrenals in males was noted in several animals with no dose-relationship based on incidence or severity (Table 36). Only 1/10 females (highest) had cortical vacuolation of the adrenal. This finding was considered incidental and reversible upon a recovery period.

Table 36
Incidence and severity of diffuse cortical vacuolation of the adrenal in males

Vacuolation	Dosage levels (mg/kg/day) n=10 animals/group		
	control (0)	high (300)	Highest (900)
Minimal	1	5	0
Mild	1	1	2
Moderate	0	2	2
Marked	1	0	0
Total	3	8	4

Toxicokinetics: mean drug exposure values are shown in Tables 37 and 38. As no consistent differences were noted between sexes, male and female data were combined. Highest UK-427,857 concentrations were recorded at 1, 3 or 7 hr after dosing. Mean AUC values increased with dose though not linearly and were 1.4 to 2.2-fold higher on day 181 than day 1.

Table 37
Mean drug exposure values in rats

Dose (mg/kg/day) & group	Toxicokinetic parameters			
	Cmax (µg/ml)		AUC(0-24h) (µg*hr/ml)	
	Day 1	Day 181	Day 1	Day 181
30, low	0.27	0.60	1.3	2.8
100, mid	1.43	2.98	12.5	20
300, high	3	5.97	38.6	65.9
900, highest	7.04	9.35	97.2	133.6

Table 38
Mean UK-427,857 exposure values (day 181) in rats in 26-week oral toxicology study and HIV positive human subjects after a 10-day dosing of 100 or 300 mg bid

Species	Dose (mg/kg/day)	Free Cmax (µg/ml)	Free AUC(0-24h) (µg*hr/ml)
Rat n=6	30	0.30	1.39
	100	1.46	9.78
	300	2.93	32.3
	900	4.58	65.47
Human	100 mg bid	0.03	0.11
	300 mg bid	0.15	0.63

9. Study title: Six month oral toxicity study of UK-427,857 in beagle dogs

Key study findings: Groups of male and female beagle dogs [age: 8 months; 4 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (sterile saline; vehicle control), 5 (low), 15 (mid) or 40 mg/kg/day (high) for 6 months. Plasma drug concentrations were measured on days 1 and 176 at 1, 4, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method. The treatment resulted in clinical signs including emesis, mydriasis and ocular signs and increase in heart rate and QT intervals (mid or high). The changes were seen at 1 hr postdose, the time at which peak plasma levels occurred with mean values more or equal to 2.48 µg/ml at the mid dose. The effect on QTc was consistent with results from dofetilide binding, hERG channel and dog isolated Purkinje fiber studies suggesting that UK-427,857 has the potential to block the IKr current and affect cardiac repolarization in vivo.

The NOAEL in the dog was 5 mg/kg/day. Based on the body surface area factor, an equivalent dose in humans would be 2.7 mg/kg/day (162 mg/day for a 60 kg person). Drug exposure at the 5 mg/kg/day was 0.88 µg/ml (Cmax) and 2.42 µg*hr/ml (AUC).

Study no.: 02073

Volume # and page #: 1 and 1-694

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: April, 2003

GLP compliance: Yes

QA reports: Yes

Drug, lot #, and % purity: # R101, [redacted]

Methods

Doses: of 0 (sterile saline; vehicle control), 5 (low), 15 (mid) or 40 mg/kg/day (high) for 6 months.

Species/strain: beagle dogs

Age: 8 months

Number/sex/group: 4 animals/sex/group

Route, formulation, volume: oral gavage, 10ml/kg

Satellite groups used for toxicokinetics: Plasma drug concentrations were measured on days 1 and 176 at 1, 4, 7 and 24 hr postdose and the samples were analyzed by a validated analytical method.

Study design: Groups of male and female beagle dogs [age: 8 months; 4 animal/sex/group] received oral gavage doses (10 ml/kg) of UK-427,857 at dose levels of 0 (sterile saline; vehicle control), 5 (low), 15 (mid) or 40 mg/kg/day (high) for 6 months.

Mortality: once daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once weekly

Hematology: weekly

Clinical chemistry: weekly

Cardiovascular examinations: yes

Gross Pathology: full necropsy.

Organ weights: listed in Table 39

Histopathology: Table 39, Adequate Battery: yes; Peer review: yes

Table 39
Dog tissues, weighed, preserved and examined

Organ name	Weighed	Examined microscopically
adrenal glands	X	X
aorta (thoracic)		X
Bone marrow smear (rib)		X
Bone (sternum, femur)		X
bone marrow (sternum, femur)		X
Brain (medulla, pons, cerebrum and cerebellum)	X	X
Epididymides		X
Esophagus		X

Eyes with optic nerve		X
Heart	X	X
Kidneys	X	X
lacrimal glands		X
large intestine (cecum, colon, rectum)		X
Liver	X	X
lungs (with mainstem bronchi)		X
Lymph nodes (mesenteric, mediastinal)		X
mammary gland		X
nerve (sciatic)		X
Ovaries		X
Pancreas		X
pituitary gland		X
prostate gland		X
Salivary glands submandibular)		X
seminal vesicles		X
Skeletal muscle (biceps femoris)		X
Skin		X
Small intestine (duodenum, ileum, jejunum)		X
spinal cord (cervical, thoracic, lumber)		X
Spleen	X	X
Stomach		X
Testes	X	X
Thymus	X	X
Thyroid,/parathyroid glands		X
Trachea		X
Turbinates (skull)		
urinary bladder		X
uterus (body/horns) with cervix		X
Vagina		X
All gross lesions		X

Results

Mortality: there were no deaths in the study.

Clinical signs: at the mid and high doses were emesis, mydriasis (dilated pupils), partially closed eyes, protruding nictitating membrane, red conjunctiva and increased salivation. These clinical signs were generally observed within 1 hr post dosing. There were multiple episodes of emesis/day during the first two weeks of the study (up to 5 and 6). Thereafter, animals vomited generally once or twice/day.

Ophthalmology: there were no treatment related ocular findings.

Body weights and food consumption: no effects

Cardiovascular examinations:

Heart rate: relative to predose values, there were increases in mean heart rate 1 hr postdosing (mid and high) reaching a level of statistical significance primarily at the mid and high doses as shown in Table 40 below:

Table 40
Changes in mean heart rate: absolute changes in beats/min and % changes one hr postdose relative to predose values in dogs (six month toxicology study)

Dose (mg/kg/day)	Day 5	Day 54	Day 110	Day 173
Control	+2.5 (+2%)	-2.9 (-3%)	+5.9 (+6%)	+1.7 (+2%)
5	+2.2 (+2%)	-1.5 (-1%)	+1.6 (+1%)	2.6 (+3%)
15	+8.5 (+8%)	+15.5* (+19%)	+10.1 (+10%)	+3.8 (+4%)
40	+26.7* (+28%)	+13.8** (+14%)	+14.5 (+14%)	+15.8 (+17%)

*= statistically significant at p=0.05
**= statistically significant at p=0.01

Electrocardiogram: relative to predose values, there were increases in mean corrected QT intervals one hr postdosing at the mid and high doses reaching a level of significance on day 54 and 173 as shown in Table 41 below. There were also statistically significant decreases in mean P-wave duration on day 173 (mid and high, p=0.05 and 0.01, respectively) and an increase in mean P-wave amplitude on day 5 (high, p=0.01). The change in PR-interval P-wave amplitude was most likely secondary to the increases in heart rate. There were no drug related changes in the ECG pattern and in QRS duration.

Table 41

Changes in mean QT intervals: (absolute changes in msec and % changes one hr postdose relative to predose values in dogs (six month toxicology study)

Dose (mg/kg/day)	Day 5	Day 54	Day 110	Day 173
Control	+0.4 (+0.2%)	+1.1 (+0.5%)	+3.1 (+1.4%)	-0.2 (-0.1%)
5	+7.9 (+3.7%)	0 (0%)	-4.8 (-2.2%)	0 (0%)
15	+8.1 (+3.8%)	+12.5* (+5.9%)	+6.8 (+3.2%)	+9 (+4.2%)
40	+7.3 (+3.3%)	+10.4* (+4.8%)	+7.8 (+3.5%)	+6.6 (+3%)

*= statistically significant at p=0.05
**= statistically significant at p=0.01

Hematology: no findings.

Clinical chemistry: no changes were seen.

Urinalysis: no treatment related findings.

Toxicokinetics: mean toxicokinetics parameters are shown in Table 42. Male and female dogs had similar plasma AUC values that increased proportionally with dose and remained stable throughout the study. The highest mean plasma levels were observed at 1 hr post dose. Systemic exposure increased over the dose range and was similar on day 1 and 176.

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Table 42

Toxicokinetics of UK-427,857 on days 1 and 176 following repeated daily oral (gavage) administration to male and female dogs

Dose (mg/kg/day)	Day 1		Day 176	
	$AUC_{0\text{-}24hr}$ (µg*hr/ml)	Cmax (µg/ml)	$AUC_{0\text{-}24hr}$ (µg*hr/ml)	Cmax (µg/ml)
5	2.88	0.96	2.42	0.88
15	8.32	2.8	8.53	2.61
40	18.02	4.44	19.82	4

Organ weights, Gross and Histopathology: there were no adverse pathological changes.

10. Study title: UK-427,857: 9-month oral (bid) toxicity study in the cynomolgus monkey

Key study findings: Groups of male and female cynomolgus monkeys received repeated oral gavage doses (bid, 7-8 hr apart) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 30 (low), 120 (mid) or 400 mg/kg/day (high) daily for a period of 39 weeks. In the high dose animals, signs of toxicity included moderate clinical signs (subdued behavior/reduced activity, prostration, half-closed eyes, vomiting and liquid feces), slightly low red blood cell parameters, a tendency towards increase in triglyceride and ALT levels, the presence of urinary proteins and cardiovascular changes (decreased in heart rate and blood pressure and increased QT/QTc intervals). Drug plasma levels were similar in males and females and increased superproportionally with dose. A dose level of 120 mg/kg/day may be considered the NOAEL in monkeys. Based on the body surface area factor, an equivalent oral dose in humans would be 2.4 g/day for a 60 kg person. Drug exposure (Cmax and AUC) at the NOAEL was 1.15 µg/ml (Cmax) and 6.6 µg*hr/ml (AUC).

Study no.: 911/102

Volume # and page #: 1-2 and 1-718

Conducting laboratory and location: Pfizer Inc., Amboise cedex, France

Date of study completion: January 26, 2005

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: **# R103,** [redacted]

Methods

Doses: 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 30 (low), 120 (mid) or 400 mg/kg/day (high) once daily

Species/strain: cynomolgus monkeys

Age: 26-37 months

Number/sex/group: 5 animals/sex/group

Route, formulation, volume: oral gavage, 5ml/kg

Satellite groups used for toxicokinetics: blood samples were taken from each animal on days 0, 133 and 270 at predetermined time intervals.

Study design: groups of male and female cynomolgus monkeys (4 animals/sex/group) received repeated oral gavage doses (bid, 7-8 hr apart) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 30 (low), 100 (mid) or 300 mg/kg/day (high) daily for a period of 39 consecutive weeks.

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once daily

Hematology: weeks 13, 26 and 38

Clinical chemistry: weeks 13, 26 and 38

Cardiovascular examinations: days 1, 88, 176 and 267

Gross Pathology: full necropsy.

Organ weights: listed in Table 43

Histopathology: Table 43, Adequate Battery: yes; Peer review: yes

Table 43
Monkey tissues, weighed, preserved and examined

Organ name	Weighed	Examined microscopically
adrenal glands	X	X
aorta (thoracic)		X
Bone marrow smear (rib)		X
Bone (sternum, femur)		X
bone marrow (sternum, femur)		X
Brain (medulla, pons, cerebrum and cerebellum)	X	X
Epididymides		X
Esophagus		X
Eyes with optic nerve		X
Heart	X	X
Kidneys	X	X
lacrimal glands		X
large intestine (cecum, colon, rectum)		X
Liver	X	X
lungs (with mainstem bronchi)		X
Lymph nodes (mesenteric, mediastinal)		X
mammary gland		X
nerve (sciatic)		X
Ovaries		X
Pancreas		X
pituitary gland		X
prostate gland		X
Salivary glands submandibular)		X
seminal vesicles		X
Skeletal muscle (biceps femoris)		X
Skin		X
Small intestine (duodenum, ileum, jejunum)		X
spinal cord (cervical, thoracic, lumber)		X
Spleen	X	X
Stomach		X
Testes	X	X

Thymus	X	X
Thyroid/parathyroid glands		X
Trachea		X
Turbinates (skull)		
urinary bladder		X
uterus (body/horns) with cervix		X
Vagina		X
All gross lesions		X

Results

Mortality: one male (high) died from an acute gastric dilation on day 196 of the study.

Clinical signs: **High dose:** the signs occurred on many, treatment days and consisted of subdued behavior/reduced activity, prostration and half-closed eyes; liquid feces; and vomiting.

Mid dose: animals appeared subdued or had reduces activity and/or half-closed eyes on only few occasions (1% of the treatment days).

Low dose: the above mentioned signs (mid) occurred rarely. Liquid feces and vomiting were noted on a few occasions in animals (low or mid).

Ophthalmology: there were no treatment related ocular findings.

Body weights and food consumption: two males each (mid and high) had lower body weight gains than the other males. At the end of the study, mean body weight (mid and high) males were 8% and 11% lower, respectively. Some individual animals from all groups had occasional episodes of reduced food intake. All animals, however, ate well during the study and there were no treatment related trends in the food consumption data.

Cardiovascular examinations:

Systolic blood pressure: a decrease in systolic blood pressure was noted in the high dose group, 3 hr after dosing, at week 13 in males and weeks 1, 13, 26 and 39 in females. This decrease attained statistical significance at week 13 with respect to the controls.

No statistically significant effects were noted at the low or mid doses.

Diastolic blood pressure: a decrease in diastolic blood pressure was noted in the high dose group, 3 hr after dosing, at weeks 13 and 39 in males and at all occasions in females. This decrease attained statistical significance at week 39.

No statistically significant effects were noted at the low or mid doses.

Electrocardiography:

Heart rate: a moderate/marked decrease in group mean heart rate was noted in the high dose group, 3 hr after dosing at weeks 13 and 26 in males and at all occasions in females when compared with predose values. This decrease attained statistical significance, when compared with the controls, except on day 1 and week 13.

There were no toxicologically relevant effects on heart rate at the low and mid dose levels.

Cardiac conduction:

PR interval: no treatment related effect was noted on mean values of PR interval at any dose level when compared with the predose and control values.

QRS complex: no treatment related effect was noted on mean values of QRS complex at any dose level when compared with the predose and control values.

QT interval: a prolonged QT interval was noted by comparison with predose values in the high dose, 3 hr after dosing on day 1 and week 13 in males and at all occasions in females. This increase attained statistical significance for males on day 1 and for females at weeks 26 and 39 with respect to the controls.

No treatment related effect was noted on QT interval at the low or mid dose.

Hematology: red blood cell parameters (red blood cell count, hemoglobin level and packed cell volume) were slightly lower in animals (high) compared to the controls. The decrease was of a maximum of 13.6% for RBC, 12.4% for hemoglobin and 10.7% for PCV. White blood cells parameters were not affected by the treatment.

Clinical chemistry: there was a tendency towards increased triglyceride levels in males (high) and one male (mid). This was not seen in the females. At the end of the treatment period, some animals (high) had slightly elevated ALT levels.

Urinalysis: urinary proteins (from traces to moderate levels) were detected in urine of high dose animals.

Organ weights, Gross and Histopathology: there were no adverse pathological changes.

Toxicokinetics: plasma drug concentrations were comparable in males and females and were combined. Highest plasma concentrations generally occurred 1 to 3 hr after the first or second daily dosing. The mean AUC values increased in a dose proportional manner. The mean toxicokinetic parameters are shown in Table 44.

Table 44
Group mean toxicokinetic parameters of UK-427,857 in cynomolgus monkeys

Dose (mg/kg/day)	Mean Cmax (µg/ml)			Mean AUC0-24hr (µg*hr/ml)		
	Day 0	Day 133	Day 270	Day 0	Day 133	Day 270
30	0.19	0.15	0.10	0.50	0.30	0.4
120	1.32	1.62	1.15	7.8	7.5	6.5
400	5.53	11.46	10.43	57.3	86.7	90.6

2.6.6.4 Genetic toxicology

1. UK-427,857: Microbial reverse mutation assay

Key findings: UK-427,857 was tested for its ability to induce gene mutations in two versions of the Salmonella-E. coli/mammalian-microsomes mutagenicity assay, using tester Salmonella strains TA98, TA100, TA1535 and TA1537, and E. coli strains WP2uvrA and pKM101, both in the presence and in the absence of rat liver S9 metabolic activation. The S9 homogenate was prepared from male Sprague-Dawley rats that had been injected (i.p.) with Aroclor™-1250 at a dose level of 500 mg/kg. The concentrations tested, along with vehicle and positive control substances, were 50, 150, 500, 1500, and 5,000 µg/plate. UK-427,857 did not cause a positive increase in the number of revertants per plate in any of the tester stains in either the presence or absence of metabolic activation. Conclusion: in the Ames assay, UK-427,857 exerted no detectable mutagenic activity. Under the conditions of this study, UK-427,857 was negative in the Ames test.

Study no.: 01-2120-07

Volume # and page #: 1 and 1-48

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study Completion: November 7, 2001

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: Batch # R1 and [redacted]

Methods

Strains/species/cell line: Table 45

Table 45
UK-427,857 concentrations tested for the Salmonella and Escherichia coli strains in both the presence and absence of metabolic activation

Test article	Dose level (µg/plate)	Salmonella typhimurium strains					E. coli strains	
		TA 98	TA 100	TA 1535	TA 1537	TA 102	WP2uvaA	pKM 101
DMS (negative control)	100 (µl/plate)	yes	Yes	yes	yes	yes	yes	yes
UK-427,857	50	yes	Yes	yes	yes	yes	yes	yes
	150	yes	Yes	yes	yes	yes	yes	yes
	500	yes	Yes	yes	yes	yes	yes	yes
	1500	yes	Yes	yes	yes	yes	yes	yes
	5000	yes	Yes	yes	yes	yes	yes	yes

Mixed function oxidase: crude rat liver extract (S-9) provided the mixed function oxidase metabolic activation system. The extract was obtained from male Sprague-Dawley rats which were stressed with a single intraperitoneal injection of Aroclor 1250 (500 mg/kg) 5 days prior to sacrifice.

Doses used in definitive study: Table 45

Basis of dose selection: a non-GLP exploratory study.

Negative controls: Table 45.

Positive controls: sodium azide, 9-aminoacridien, 2-nitrofluorene and mitomycin C.

Incubation and sampling times: after solidification of the agar overlay, all plates were incubated aerobically at 37 degree C in darkness for 46-48 hr.

Results

UK-427,857 was assessed for mutagenic potential in the Ames assay at concentrations up to 5000 µg/plate, both with or without S-9 metabolic activation in two independent mutagenicity assays. The metabolic activation system was the S-9 fraction of a rat liver homogenate from animals treated previously with Aroclor 1254 (500 mg/kg). No precipitate was observed in the study.

First mutagenicity assay: In the preliminary plate incorporation assay, five concentrations of UK-427,857 were tested in duplicate with each Salmonella and E. coli strain in both the presence and absence of S9 metabolic activation. Compound levels ranged from 0.010 to 5.0 mg/plate. Insoluble compound was not observed. Dose-related cytotoxicity was not observed at any of the concentrations tested with any of the strains in either the presence or absence of S9 metabolic activation. There was no evidence of significant dose-related increases in the number of revertant colonies compared to the negative

controls with any of the strains tested in either the absence or presence of S9 metabolic activation.

Second mutagenicity assay: Compound levels ranged from 0.050 to 5.0 mg/plate in the definitive assay with each of the strains tested in both the absence and presence of S9 metabolic activation. Insoluble compound was not observed in the overlay agar at any of the concentrations tested after incubation at 37 degree C. Dose-related cytotoxicity was not observed at any of the concentrations tested with any of the strains in either the presence or absence of S9 metabolic activation. There was no evidence of significant dose-related increases in the number of revertant colonies compared to the negative controls with any of the strains tested in either the absence or presence of S9 metabolic activation.

Study validity: valid

Study outcome: UK-427,857 was not mutagenic in the Ames assay. UK-427,857 was not cytotoxic at concentrations of 5000 µg/plate.

2. UK-427,857: In vitro cytogenetic assay

Key findings: UK-427,857 (Lot No. R1) was tested for clastogenic activity in vitro in human lymphocyte cultures. Chromosome damage was evaluated by metaphase analysis after 3-hour treatments with and without metabolic activation at concentrations ranging from 538 to 840 µg/ml and 750 to 950 µg/ml, respectively. In addition, chromosome damage was evaluated after 24 hours without metabolic activation at concentrations ranging from 42.3 to 258 µg/ml. In all tests, the highest dose level evaluated produced a 48% to 56% reduction of the mitotic index. There was evidence of test article precipitate in the dosing stocks and culture medium in the 3-hour test with metabolic activation and the 24-hour direct test. There was no significant increase in chromosome damage or polyploidy under any test condition at any concentration evaluated.

Study no.: 01-2120-09

Volume # and page #: 1 and 1-36

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study initiation: September 19, 1996

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: R1and [redacted]

Methods

Mixed function oxidase: crude rat liver extract (S-9) provided the mixed function oxidase metabolic activation system. The extract was obtained from male Sprague-Dawley rats which were stressed with a single intraperitoneal injection of Aroclor 1250 (500 mg/kg) 5 days prior to sacrifice.

Strains/species/cell line: primary human lymphocytes

Doses used in definitive study: with metabolic activation: 538 to 840 µg/ml and without metabolic activation: 750 to 950 µg/ml

Basis of dose selection: Cytotoxicity information from the preliminary tests is used to select test concentrations for the definitive tests. The maximum concentration is determined on a case-by-case basis taking into account both solubility and relevant cytotoxicity information available on the test article. The highest concentration should produce an approximate 50% suppression of the mitotic index compared to concurrent negative control values. In the absence of insolubility or limiting cytotoxicity 5000 mg/ml is used as the highest concentration. For insoluble compounds that are nontoxic, the lowest precipitating concentration is used as the top concentration. If dose-related cytotoxicity or mutagenicity is noted in the preliminary test, irrespective of solubility, then the top concentration is based on cytotoxicity.

Negative controls: DMSO and solvents

Positive controls: Mitomycin C and Cyclophosphamide

Incubation and sampling times: Human lymphocytes are stimulated by a mitogen and cultured for 46-50 hours. For the 3-hour test with metabolic activation and the initial 24 and 3-hour tests without metabolic activation. Negative solvent controls and positive controls are run concurrently with or without the S9 metabolic activation system. With the appropriate exposure times, two sets of duplicate cultures are tested without the S9 metabolic activation system either continuously with the test or control article in dosing medium (MED+) for 24 hours or for 3 hours followed by an additional 21 hours in MED+ without test or control article. These two tests may be conducted concurrently or independently on different days with different donors. Cultures tested in the presence of the S9 metabolic activation system are treated only for 3 hours in dosing medium (MED-, plus the S9 metabolic activation system), centrifuged and cultured an additional 21 hours in MED+.

Results:

In the preliminary cytotoxicity test, UK-427,857 produced no to minimal mitotic suppression under all exposure conditions over a dose range of 7.80 to 500 mg/ml. There was no evidence of test article insolubility in any of the 100X stock solutions prepared in DMSO or in any of the treated cultures.

The initial 3-hour test without metabolic activation was conducted over a dose range of 417 to 5000 µg/ml. This test was discontinued due to a steep cytotoxic response in which 1270 µg/ml of UK-427,857 was toxic, while the next lower concentration of 1020 µg/ml produced only a 33% reduction in the mitotic index. Subsequently, a second 3-hour test without metabolic activation was conducted over a narrower dose range of 512 to 1400 µg/ml. This test was also discontinued due to a steep cytotoxic response in which 1000 µg/ml of UK-427,857 produced a 66% reduction of the mitotic index with insufficient mitotic cells for analysis, while the next lower concentration, 800 µg/ml, produced only a 12% reduction in the mitotic index. In a third 3-hour test without metabolic activation, UK-427,857 was conducted over a narrower dose range of 700 to 1200 µg/ml. Concentrations > 950 µg/ml were toxic. Three concentrations (850, 750 and 700 µg/ml) producing 18 to 53% mitotic suppression were selected for analysis. However upon initiation of aberration analysis it was observed that there were insufficient metaphase cells for analysis. Therefore, this test was discontinued.

The final 3-hour test without metabolic activation was conducted over a dose range of 600 to 1100 µg/ml (Tables 46-48). There was no evidence of test article insolubility in any of the 100X stock solutions prepared in DMSO or in any of the treated cultures. Cultures treated with concentrations > 1000 µg/ml of UK-427,857 were not evaluated due to insufficient numbers of cells available for analysis. In the three lower concentrations (750, 850 and 950 µg/ml), producing 0 to 53% reduction in the mitotic index, were evaluated for chromosome damage. There was no significant increase in the number of abnormal cells compared to concurrent negative control (DMSO) at any of the concentrations evaluated. There was a slight increase in the frequency of polyploid cells in the UK-427,857 treated cultures (0.5 to 0.7%) compared to the concurrent control (DMSO; 0.2%). However these frequencies are within the historical control range and therefore not considered significant.

In the 3-hour test with metabolic activation, UK-427,857 was tested over a dose range of 538 to 5000 µg/ml. There was evidence of test article insolubility in the dosing stocks > 266 µg/ml and in the cultures treated with final concentrations > 1310 µg/ml. Cultures treated with concentrations > 1050 µg/ml of UK-427,857 were not evaluated due to toxicity. Three lower test concentrations (538, 672 and 840 µg/ml), producing 15 to 48% reduction in the mitotic index, were evaluated for chromosome damage. There was no significant increase in the number of abnormal cells compared to concurrent negative control (DMSO) at any of the concentrations evaluated.

There was a slight increase in the frequency of polyploid cells in the UK-427,857 treated cultures (1.0 to 1.3%) compared to the concurrent control (DMSO; 0.8%). However, the frequency of polyploidy in the treated cultures was very similar to that of the negative control, therefore it was not considered to be significant. The initial 24 hour test without metabolic activation was conducted over a dose range of 430 to 5000 µg/ml. This test was discontinued due to insufficient numbers of mitotic cells available for analysis at the three lowest dose levels available for analysis. Subsequently, a second 24 hour test without metabolic activation was conducted over a lower dose range of 42.3 to 1050

µg/ml. There was evidence of test article insolubility in the dosing stocks at 105 µg/ml and in the cultures treated with a final test article concentration of 1050 µg/ml. Cultures treated with concentrations > 430 µg/ml were not evaluated due to insufficient numbers of cells available for analysis. Three lower test concentrations (42.3, 75.6 and 258 µg/ml), producing 4 to 56% mitotic suppression, were selected to evaluate for chromosome damage. There was no significant increase in the number of abnormal cells compared to concurrent negative control (DMSO) at any of the concentrations evaluated. There was a slight increase in the frequency of polyploid cells (1.1%) at the highest concentration evaluated compared to the concurrent control (DMSO; 0.6%). Based on historical experience, increases in polyploidy at concentrations of test article which induce substantial cytotoxicity (>35%) are most likely the consequence of cytotoxic effects on organelles responsible for cytokinesis. Therefore, this increase in polyploid cells was not considered significant.

Study validity (comment on replicates, counting method, criteria for positive results, etc.): yes

Study outcome: These studies indicate that UK-427,857 does not induce a significant increase in structural chromosome aberrations or polyploidy in human lymphocyte cultures in vitro either with or without metabolic activation when tested up to concentrations that produce approximately 50% suppression of the mitotic index compared to the concurrent negative control.

Table 46
Summary of test of human lymphocyte aberration assay (3-hr treatment without metabolic activation)

Treatment	Mean mitotic suppression (%)	Mean abnormal cells ((%)	p-value	Mean polyploid cells (%)
Negative control: DMSO (1%)	0	0.5	-	0.2
Maraviroc: (µg/ml)				
700	Not evaluated	-	-	-
750	0	1.5	0.312	0.7
850	36	1	0.5	0.5
950	53	2.5	0.108	0.6
1000	Insufficient number of mitotic cells	-	-	-
Positive control:				
Mitomycin C 0.4	28	40	0.001	-

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Table 47
Summary of test of human lymphocyte aberration assay (3-hr treatment without metabolic activation)

Treatment	Mean mitotic suppression (%)	Mean abnormal cells ((%)	p-value	Mean polyploid cells (%)
Negative control: DMSO (1%)	0	2	-	0.8
Maraviroc: (µg/ml)				
538	19	1.5	0.5	1
672	15	0.5	0.5	1.3
840	48	2.5	0.5	1.2
1050	toxicity	-	-	-
Positive control:				
Cyclophosphamide 10	7	15.5	0.000	-

Table 48
Summary of test of human lymphocyte aberration assay (24-hr treatment without metabolic activation)

Treatment	Mean mitotic suppression (%)	Mean abnormal cells ((%)	p-value	Mean polyploid cells (%)
Negative control: DMSO (1%)	0	0.5	-	0.6
Maraviroc: (µg/ml)				
42.3	4	2	0.186	0.3
75.6	47	2	0.186	0.3
258	56	1	0.5	1.1
430	Not evaluated insufficient mitotic cells	-		-
Positive control:				
Mitomycin C: 0.05	22	20	0.001	-

3. UK-427,857: Rodent micronucleus assay

Key findings: Groups of male and female CD-1 mice (strain: [redacted] CD-1 (ICR)BR; 8 animals/sex/group) were orally gavaged a single dose of UK-427,857 (2 ml/kg) at dose levels of 0 (0.1% Tween 80 in 0.5% methylcellulose, vehicle control), 500 (low), 1000 (mid) or 2000 mg/kg (high) to evaluate the potential to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow. Bone marrow cells were collected 24 or 48 hr after the treatment and were examined for micronucleated polychromatic erythrocytes. Results: no significant increase (p=0.01) in micronucleated polychromatic erythrocytes was observed in the treated groups when compared to the controls. The mean highest plasma UK-427,857 concentration occurred 3 or 5 hours after dosing (high) with values of 13.0 and 13.5 µg/ml in males and females, respectively. Mean AUC0-24h values (high) were 184 µg*h/ml in males and 174 µg*h/ml in females.

Conclusions: under the conditions of the test and according to the criteria set for evaluating the test results, UK-427,857 was negative in the Micronucleus Assay.

Study no.: 01-2120-08

Volume # and page #: 1 and 1-32

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study Completion: October 15, 2001

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: R101 and [redacted] pure
Methods

Vehicle: 0.1% Tween 80 in 0.5% methylcellulose

Groups of male and female CD-1 mice (strain: [redacted]: CD-1 (ICR) BR; 8 animals/sex/group) were orally gavaged a single dose of UK-427,857 (2 ml/kg) at dose levels of 0 (0.1% Tween 80 in 0.5% methylcellulose, vehicle control), 500 (low), 1000 (mid) or 2000 mg/kg (high) to evaluate the potential to increase the incidence of micronucleated polychromatic erythrocytes in bone marrow. Mitomycin C, the positive control was given to an additional group of 8 female mice by ip injection at 7 mg/kg for 3 days. Bone marrow cells were collected 24 hr after the treatment and were examined for micronucleated polychromatic erythrocytes (PCEs).

Strains/species/cell line: male and female CD-1 mice/ strain [redacted] CD-1 (ICR) BR

Doses used in definitive study: 500, 1000 and 2000 mg/kg

Basis of dose selection: a non GLP dose range study was performed to determine suitable doses of drug for the definitive study. In this study, doses levels of 0, 500, 1000 and 2000 mg/kg were utilized.

Positive controls: Mitomycin C

Incubation and sampling times: approximately 24 and 48 hr after the last dose administration, femoral bone marrow was sampled for evaluation. Bone marrow toxic effects were evaluated based upon a determination of the percent polychromatic erythrocytes (PCEs or reticulocytes) of the total erythrocyte count.

Results

Clinical Signs and mortality: there were no deaths in this study. No clinical signs were observed.

Micronucleus evaluation: no statistically significant inverse in the number of micronucleated PE above vehicle control was observed in any of the doses in the treated groups. The mean highest plasma UK-427,857 concentration occurred 3 or 5 hours after dosing (high) with values of 13.0 and 13.5 µg/ml in males and females, respectively. Mean AUC0-24h values (high) were 184 µg*h/ml in males and 174 µg*h/ml in females.

Study validity: yes

Study outcome: UK-427,857 was negative for in vivo genotoxic potential in the mice micronucleus assay.

2.6.6.5 Carcinogenicity

1. REVIEW AND EVALUATION OF RAT CARCINOGENICITY STUDY

Name of Drug: Maraviroc (UK-427,857)

Sponsor: Pfizer Global Research & Development
50 Pequot Ave
New London, CT 06320

Number of Studies: one (rat)

Reviewer: Pritam Verma, Ph.D.

Supervisor: James Farrelly, Ph.D.

Project Manager: Kenny Shade, J.D., B.S.N

Specific Submission for Linking Minutes: NDA 22-128.0003

Date for Exec CAC Meeting: February 13, 2007

STUDY IDENTIFICATION

Sponsor's Study Title: UK-427,857: 104-WEEK ORAL (GAVAGE) CARCINOGENICITY STUDY IN THE RAT

Study Number: 2003-0446

- Volume Numbers: Electronic

- Test Facility: [redacted]

  Pfizer Global Research & Development, Ann Arbor, MI 48105
- Study Date initiation: March 23, 2004
- Date of Completion: March 31, 2006
- Date of Submission: December 22, 2006
- GLP Compliance: Yes
- QA Report: Yes
- Study Characteristics
- Reference to Dose-range-finding study: Yes

STUDY PROTOCOL DESIGN AND METHODS

- Study Type: 24-month carcinogenic study in rats; Gavage
- Species/strain of animals used: Rat/ [redacted] CD(SD)IGS BR (Sprague-Dawley) and the animals were purchased [redacted]
- Number of animals per group: 60, details in Table 49.

Table 49
Experimental design of the 104-week oral (gavage) carcinogenicity study of UK-427,857 in the rat

Group Numbers	Dosage of UK-427,857 (mg/kg/day)	Toxicity animals		Toxicokinetics (only day 198)	
		Male	Female	Male	Female
1.	0 (vehicle control, 0.5% methylcellulose/0.1%Tween 80)	60	60	5	5
2.	50 (low)	60	60	5	5
3.	100 (mid)	60	60	5	5
4.	500 (high)	60	60	5	5
5.	900 (highest)	60	60	5	5
6.	0	60	60		

Group #6: Untreated control group. Data will be used for the testing facility historical database and were not used for the evaluation of the carcinogenic potential of UK-427,857.

- Age at start of study: 6-8 weeks

- Animal housing: Individual polycarbonate cages
- Drug Lot/Batch number(s): R105
- Drug Purity / Stability / Homogeneity: [redacted], adequate

DOSES: 0, 50, 100, 500 or 900 mg/kg/day

Basis of Dose Selection: Doses of 50, 100, 500 and 900 mg/kg/day were selected for the present rat carcinogenicity study based on results from a previous 26-week oral toxicity study in rats. The high dose of 900 mg/kg/day represented the maximum tolerated dose, producing an approximate 10% decrease in male body weight when compared with control. Histopathologic changes in the liver (altered cell foci, multinucleated hepatocytes, and bile duct vacuolation and hyperplasia) and thyroid (follicular cell hypertrophy) supported this as a suitable high dose for assessing carcinogenic potential over a 104-week period. The toxicological findings were considered unlikely to affect the survival of the animals at this dose. The low dose of 50 mg/kg/day was expected to produce minor clinical signs, such as salivation, and possibly bile duct vacuolation. Intermediate doses of 100 and 500 mg/kg/day were selected to explore the dose-response relationship over the dose range studied.

Once-daily dosing has been demonstrated to provide adequate systemic exposure in previous oral gavage toxicity studies in rats with UK-427,857. Two years is the accepted length for rat studies examining the carcinogenic potential of test compounds. The oral route was used for dosing as that is the intended clinical route in humans.

CAC Concurrence: Yes (Exec. CAC meeting of 12/2/03; doses of 100, 500 or 900 mg/kg/day were approved.)

Restriction Paradigm for Dietary Restriction Study: N/A
Route of Administration: gavage

- Frequency of Drug Administration: once daily
- Controls Employed: vehicle control (0.5% methylcellulose/0.1% Tween 80)
- Interim Sacrifices: No
- Satellite PK or Special Study Group(s): details in Table 49
- Unscheduled Sacrifices: No
- Deviations from Original Study Protocol: None

STUDY RESULTS AND FREQUENCY OF MONITORING

Observations and times:

Clinical signs: twice pretest; once weekly during the dosing phase

Body weights: weekly

Food consumption: weekly

Ophthalmology: once pretest and on day 366

Hematology & coagulation: days 92, 183 and 365

Clinical chemistry: days 92, 183 and 365

Toxicokinetics: blood collection points: 1, 3, 7 and 24 hr postdose on day 198

Organ weights: not done

Gross Pathology: at termination

Sponsor's statistical analysis: Appropriate statistical analyses were applied to body weight, body weight changes, food consumption, water consumption, clinical chemistry, hematology, and urinalysis from animals from Groups 1 to 5 using the statistical package in the Xybion Path/Tox System. The Xybion Path/Tox System utilized Fisher's Least Significant Difference test (if the variances were equal) or Cochran-Cox modified t-test (if the variances were unequal).

RESULTS

Mortality: Due to the high level of mortality in the vehicle-control females (Group 1), all female groups (excepting Group 6) were terminated following 96 weeks of treatment. Treatment with UK-427,857 had no detrimental effects on survival. There was a statistically positive trend in female survival (increased survival) through the high dose group (p=0.043) with no statistically significant differences in male survival. Survival data are shown in Table 50.

Table 50

Survival of male and female rats following 104 and 96 weeks of treatment with UK-427,857, respectively

	Males					Females				
Doses (mg/kg/day)	0	50	100	500	900	0	50	100	500	900
% Survival	38	33	42	47	32	33	42	39	50	48

Clinical Observations: Treatment-related increases in the occurrence of urogenital staining were observed in males and females at doses of 500 and 900 mg/kg/day. Treatment-related hair loss in the anogenital region and/or the ventral surface of the body occurred in males at doses ≥500 mg/kg/day and in females at doses ≥100 mg/kg/day.

Body Weight: Treatment related statistically significant decreases in mean body weight were observed at most collection intervals in males at doses ≥500 mg/kg/day by Day 64. Treatment related statistically significant decreases in mean body weight in females at a dose of 900 mg/kg/day occurred at each collection interval from Days 232 to 505.

After approximately 1 year of treatment (Day 351), mean body weights in upper mid-dose (500 mg/kg/day) and high-dose (900 mg/kg/day) males were 5% and 12% lower, respectively, in comparison with control. The mean body weight in high-dose females was 7% lower in comparison with control. During the second year of dosing, body weights ranged 3% to 12% lower in upper mid-dose males, 12% to 18% lower in high-dose males, and 5% to 8% lower in high-dose females in comparison with control. Body weight (% differences from vehicle controls) male rats are shown in Table 51.

Table 51
Body weight (% differences from vehicle controls) male rats

Dosage (mg/kg/day)	Study day					
	15	323	449	540	624	722
0 (vehicle control)	-	-	-	-	-	-
50 (low)	0	-1	+1	-1	-2	0
100 (low)	+1	0	0	-1	-1	+1
500 (mid)	-1	-5	-8	-7	-8	-5
900 (high)	-1	-9	-14	-14	-17	-15

Food Consumption: Statistically significant increases in mean food consumption occurred almost weekly in males given UK-427,857 at the highest dose (900 mg/kg/day) and in females at doses ≥500 mg/kg/day from Day 15 through Day 127 when compared with control values and were considered treatment-related. The increases ranged from 1% to 10% in the males and from at least 4% to 15% in the females. Following Day 127, increases in mean food consumption were noted at almost each collection interval in females at the two higher doses, but these increases reached statistical significance less consistently. Feed consumed per day (% differences from vehicle controls) male rats are shown in Table 52.

Table 52
Feed consumed per day (% differences from vehicle controls) male rats

Dosage (mg/kg/day)	Study day					
	15	323	449	540	624	722
0 (vehicle control)	-	-	-	-	-	-
50 (low)	+1	-1	+3	+11	-3	+3
100 (low)	+3	-1	-2	+11	-2	+1
500 (mid)	+3	-2	+1	+11	-3	+9
900 (high)	+7	-1	-3	+9	-9	+6

Water Consumption: Dose-related increases in mean water consumption were observed in males and females at doses of ≥500 mg/kg/day in comparison with control at almost each collection interval throughout the first year of dosing and were considered treatment-related. After the first week of dosing (Day 8), these increases reached statistical significance in comparison with control at almost every collection interval for high-dose (900 mg/kg/day) animals. The increases ranged from 16% to 57% in high-dose males and from 24% to 102% in high-dose females. At the upper mid-dose (500 mg/kg/day) level, the increases reached statistical significance at many of the collection intervals and ranged from 11% to 45% in males (excepting on Day 18 when there was no change and on Day 22 when water consumption was decreased by 1%) and from 4% to 72% in females.

Ophthalmology: no treatment related findings were noted.

Hematology and coagulation: there were no meaningful differences in group mean or individual values related to treatment with UK-427,857.

Clinical Chemistry: there were no meaningful differences in group mean or individual values related to treatment with UK-427,857.

Urinalysis: there were no meaningful differences in group mean or individual values related to treatment with UK-427,857.

Toxicokinetics: data are shown in Table 53.

Table 53
Mean Plasma Toxicokinetic Parameters for UK-427,857 in Rat after Oral Administration for 198 days

Dosage (mg/kg/day)	Sex	Cmax (µg/L)	Tmax (h)	Mean AUC0-24 (µg*h/mL)	Multiples of human AUC achieved
50	Male	0.477	3	3.52	1
50	Female	0.459	3	2.55	<1
50	Overall	0.468	3	3.03	<1
100	Male	0.839	3	9.82	3
100	Female	1.70	3	10.1	3
100	Overall	1.27	3	9.97	3
500	Male	2.74	7	40.7	11
500	Female	4.26	3	38.7	11
500	Overall	3.49	3	39.7	11
900	Male	3.57	3	46.9	13
900	Female	4.12	3	63.1	18
900	Overall	3.84	3	54.7	15

Human Exposure: in the clinic (300 mg bid therapeutic dose) in human, AUCss value is

3.6 µg*hr/ml.

Mean AUC0-24 (exposure) to UK-427,857 increased with dose over the dose range for both male and female rats. Mean Cmax generally increased with dose over the dose range for male and female rats, except between the 500 and 900 mg/kg/day dose groups for females, where the observed mean Cmax values appeared to be similar. The mean combined (male and female) AUC0-24 following oral administration of 50, 100, 500, 900 mg/kg/day were 3.03, 9.97, 39.7 and 54.7 µg*h/mL, respectively.

There were no apparent sex-related differences observed for AUC0-24 for the 50, 100 and 500 mg/kg/day dose groups, however, there may be a sex-related difference for the 900 mg/kg/day dose group. The mean combined (male and female) Cmax following oral administration of 50, 100, 500, 900 mg/kg/day were 0.468, 1.27, 3.49 and 3.84µg/mL, respectively. The Tmax was typically 3 h. There were no sex-related differences observed for Cmax and Tmax over the study dose range.

Postmortem Observations

Necropsy Findings: Gross observations of possible importance were noted only at the terminal necropsy. The liver from high-dose (900 mg/kg/day) Group 5 male No. 515 was diffusely enlarged and dark, with firm nodules on all lobes. The liver from an additional high-dose male, No. 516 had a single small mass at the edge of the lateral lobe. A large irregular pale
yellow cyst was noted on the surface of the papillary lobe of the liver from a Group 4 (500 mg/kg/day) female (No. 424).

Histopathology:

Neoplastic: changes of interest (incidence of benign adenoma) in thyroid gland are shown in (Table 54).

Table 54
Incidence of benign adenoma (thyroid gland) in the 2-year rat carcinogenicity study

Neoplastic change	Vehicle control		50 mg/kg/day (low)		100 mg/kg/day (mid)		500 mg/kg/day (high)		900 Mg/kg/day (highest)	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Total examined	60	60	60	60	60	59	60	60	60	58
Benign adenoma C-Cell	5	4	2	6	10	6	7	4	5	7
Benign adenoma follicular cell	0 (0%)	0 (0%)	2 (3%)	1 (2%)	1 (2%)	0 (0%)	1 (2%)	2 (3%)	5 (8%)	3 (5%)

The source of animals was [redacted]. In the male rat, incidence of benign adenoma follicular cell was 0% (vehicle control), 3% (low), 2% (mid), 2% (high) and 8% (highest), respectively. In the female rat, incidence of benign adenoma follicular

cell was 0% (vehicle control), 2% (low), 0% (mid), 3% (high) and 5% (highest), respectively.

In the thirty recent control studies (SD rats) from [redacted] incidence of thyroid: benign adenoma follicular cell was found to occur within 1.67% to 12%.

Thus, the incidence thyroid: benign adenoma follicular cell seen in the present study in rats was within the incidence values obtained for the neoplasm from the control studies conducted by [redacted].

No tumor incidences reached statistical significance in rats. Cholangiocarcinoma of liver is an extremely rare tumor type in SD rats. Cholangiocarcinomas were found in two male rats treated with 900 mg/kg/day (13 times the maximum recommended human dose based on AUC). Although, the occurrence was not statistically significant in a pairwise comparison with the vehicle control group.

Non-neoplastic changes: incidence of hypertrophy and/or hyperplasia of follicular cells (thyroid) are shown in Table 55.

Table 55
Incidence of hypertrophy and/or hyperplasia of follicular cells (thyroid gland) in the 2-year rat carcinogenicity study

Non-neoplastic change	Vehicle control		50 mg/kg/day (low)		100 mg/kg/day (mid)		500 mg/kg/day (high)		900 Mg/kg/day (highest)	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Total examined	60	60	60	60	60	59	60	60	60	58
Hypertrophy, follicular cells	2	0	2	2	5	0	15	4	20	16
Hyperplasia, follicular cells	0	2	1	0	3	0	3	0	8	3
Total	2	2	3	2	8	0	18	4	28	19
Percent Incidence	3%	3%	5%	3%	13%	0%	30%	7%	47%	33%

In the male rat, total incidence of non-neoplastic changes in thyroid gland was 3% (vehicle control), 5% (low), 13% (mid), 30% (high) and 47% (highest), respectively. In the female rat, total incidence of non-neoplastic changes was 3% (vehicle control), 3% (low), 0% (mid), 7% (high) and 33% (highest), respectively.

At the high dose in male and female rat, systemic exposure was 13 and 18-times the exposure in humans (300 mg bid dose), respectively.

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OVERALL INTERPRETATION AND EVALUATION

Adequacy of the carcinogenicity studies and appropriateness of the test model:

MTD was achieved in this study. The dose levels of 100, 500 and 900 mg/kg/day were approved by the Exec CAC. In selection of dose levels, the sponsor relied upon both the toxicological endpoints and drug exposure in animals (from the 26-week rat toxicology study). The high dose of 900 mg/kg/day was proposed based on the hepatic changes (altered cell foci, bile duct hyperplasia, and multinucleated hepatocytes) and thyroid follicular cell hypertrophy. The decrease in body weight (affecting males only) supported a conclusion that the MTD was 900 mg/kg/day. This dose level was expected to provide exposure (free fraction of drug) multiples of 52 and 298 as compared to clinical exposures at 100 mg bid and 300 mg bid, respectively.

This compound is moderately protein bound and the major route of excretion rats was fecal (79%) as compared to urine (12%), the majority of radioactivity being recovered within 24 hr. The major route of excretion in healthy human male subjects was fecal (76%) as compared to urine (20%). The major component in the plasma from human and mouse was unchanged UK-427,857 (42% human, 74% mouse). The most significant metabolites in human plasma were a mono-oxidized analogue of the N-dealkylation product (metabolite A, 11%) and a product of N-dealkylation (metabolite B, 22%); both were observed in mouse plasma (8% and 5%).

The carcinogenicity study in rat was considered to be acceptable.

Evidence of genotoxicity: Maraviroc was not genotoxic in the reverse mutation bacterial test (Ames test), mouse lymphoma or mouse in vivo micronucleus assays.

SUMMARY AND CONCLUSIONS

The oncogenicity potential of maraviroc was investigated in Sprague-Dawley rats with oral gavage dosages of 50 (low), 100 (mid), 500 (high) or 900 (highest) mg/kg/day in comparison with vehicle controls for a period of 104 weeks in males and 96 weeks in females. (All female groups were terminated early when survival in the female control group dropped to 33% (20 of 60 rats surviving to 96 weeks). The protocol was approved by the Exec CAC. The systemic exposures were 13 and 18 times that in humans (300 mg bid, AUCss = 3.6 μg*hr/ml) in male and female rat at the high dose level, respectively.

No significant increase in neoplasms was noted in male or female animals, although, an increased incidence of follicular cell adenoma of the thyroid was noted in males and females at 900 mg/kg/day. This was accompanied by a dose-related increase in follicular cell hyperplasia and hypertrophy at doses from 100 mg/kg/day in males and from 500 mg/kg/day in females. The tumor incidence was within the historical control range of this strain of rat and no follicular cell carcinomas were found in the thyroid gland.

Cholangiocarcinoma of the liver were found in two males (highest). As for the cholangiocarcinomas, extremely rare in the S-D rat, absence of a statistically significant difference between the incidences observed in the high dose and vehicle control groups, considered the finding less than sufficient to clearly implicate the drug.

APPENDIX LIST

ECAC Minutes: 12/04/2003, attached.

Sponsor's Incidence of Histopathology Findings: attached.

Body weight and food consumption changes vs. dose level: figures attached.

Statistical analysis: attached.

Historical control data set SD rats: attached.

List of Organs and Tissues Examined: attached

2. Carcinogenicity studies in rasH2 transgenic mice

1. FOUR WEEK DOSE RANGE FINDING TOXICITY AND TOXICOKINETICS STUDY OF UK-427,857 IN TgrasH2 MICE

OBJECTIVE: To evaluate the toxicity of UK-427,857 in TgrasH2 mice and to determine doses for 6-month carcinogenicity studies in transgenic mice.

TESTING FACILITY:

AND

SPONSOR: Pfizer, New London, CT

TEST/CONTROL ARTICLE INFORMATION

TEST ARTICLE: UK-427,857

VEHICLE CONTROL: methylcellulose as a 0.5% (w/v) aqueous solution containing 0.1% (w/v) Tween 80

PREPARATION: oral gavage (10 ml/kg) solution, once daily

RATIONALE FOR TEST: the TgrasH2 mouse has been extensively tested using reference compounds and has been shown to detect both genotoxic and non genotoxic carcinogens. This animal model is considered an acceptable alternative to the 2-year mouse bioassay for carcinogen hazard identification of pharmaceuticals. Pharmacokinetic data with UK-427,857 in the TgrasH2 mouse suggest that this model is appropriate.

ARTICLE SELECTION: UK-427,857 is not genotoxic and is being investigated for its carcinogenic potential in humans.

GENOTOXICITY:

UK-427,857 did not display mutagenic activity in vitro tests using Salmonella typhimurium stains TA 1535, TA 1537, TA 98 AND TA 100 and Escherichia coli stain WP2uvrA, pKM101 in either the presence or absence of metabolic activation by the S-9 fraction from the livers of Aroclor 1254-treated rats. Chromosomal damage was not observed in cultures of human lymphocytes when tested up to cytotoxicity concentrations either in the presence or absence of metabolic activation. Chromosome damage was also absent in bone marrow (micronucleus assay) of male and female mice treated orally once a day for 3 days with 500, 1000 or 2000 mg/kg of UK-427,857. Thus UK-427,857 did not display mutagenic activity in bacterial cell in vitro or clastogenic activity in vitro or in vivo.

TEST SYSTEM INFORMATION

TEST SYSTEM: Mouse

STRAIN: Mouse/CB6F1/Jic-Tg(rasH2)

APPROXIMATE AGE: 8-10 weeks

JUSTIFICATION OF SYSTEM: UK-427,857 has a similar metabolic profile in wild type litter mates and of TgrasH2 mice to be used in the 26-week carcinogenicity studies. The TgrasH2 mouse has been extensively tested using reference compounds and has been shown to detect both genotoxic and nongenotoxic carcinogens. This model is considered an acceptable alternative to the 2-year mouse bioassay for carcinogen hazard identification of pharmaceuticals.

METHOD FOR CONTROL OF BIAS: Randomization

Housing : Male and female mice will be housed in the same room in the test facility.

Food: ad libitum

Water: (There are no contaminants in the feed and water that are known to interfere with the purpose and conduct of this study.)

The number of animals, animal procedures and experimental design for this study have been reviewed and were approved by the Institutional Animal Care and Use Committee

STUDY DESIGN

DURATION OF STUDY: 4 Weeks

FREQUENCY OF DOSING: Daily

ROUTE OF ADMINISTRATION: Oral Gavage

REASON FOR CHOICE OF ADMINISTRATION ROUTE: Intended route for clinical use.

NO. OF MICE, GROUP AND DOSE LEVELS (mg/kg): Table 56

DOSE VOLUME (ml/kg): 10 ml/kg

Table 56
Experimental design of the 4-week oral dose range-finding toxicity study in the transgenic mice

Dosage (mg/kg/day)	Toxicity		Toxicokinetics	
	male	female	male	Female
0 (control)	10	10	-	-
500 (low)	10	10	24	24
1000 (mid)	10	10	24	24
1500 (high)	10	10	24	24

BASIS FOR DOSE LEVEL SELECTION: doses were selected based on the results from a 4-week oral toxicity study in wild type mice.

TOXICOLOGY COMPONENT:

BODY WEIGHT AND FOOD CONSUMPTION: although there were no changes in body weights, body weight gain was increased (73%) in males at the high dose and females (19%) at the mid or high dose, and associated with an increase (38%) in food consumption in males at the high dose.

CLINICAL EXAMINATIONS: no noteworthy clinical signs were observed.

TOXICOKINETIC COMPONENT:

1. Toxicokinetics of UK-427,857 in the 4-week oral gavage dose range study in transgenic mice is shown in Table 57. For comparisons, toxicokinetic parameters from a 4-week oral gavage toxicity study in wild type mice are shown in Tables 3.

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Table 57
Mean toxicokinetic parameters of UK-427,857 4-week dose range study in the transgenic mice

Dosage (mg/kg/day)	AUC_{0-24} (µg*hr/ml)		Cmax (µg/ml)	
	Male	Female	Male	female
500 (low)	28.1	51.8	11.1	8.98
1000 (mid)	95.4	95.1	18.4	11.7
1500 (high)	671	259	54.5	22.7

Table 58
Mean toxicokinetic parameters of UK-427,857 in 4-week oral gavage toxicity study in the wild type mice

Dosage (mg/kg/day)	AUC_{0-24} (µg*hr/ml)		Cmax (µg/ml)	
	Male	Female	Male	female
500 (low)	31.1	44.6	5.85	16.6
1000 (mid)	118	415	15.2	33.1
1500 (high)	650	540	52.7	36.3

2. Relevant pharmacokinetic data in TgrasH2 and wild type mice and humans

Mean toxicokinetic parameters of UK-427,857 in transgenic and wild type mice and HIV positive human subjects are shown in Table 59. Exposure was shown to increase with increase in dose for both mouse strains over the dose range studied.

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Table 59

Comparison of mean toxicokinetic parameters of UK-427,857 in transgenic and wild type mice and HIV positive human subjects (data in mice shown as males/females)

Species	Dosage (mg/kg/day)	AUC_{0-24} (μg*hr/ml)		Cmax (μg/ml)
		Total	Free	Total
Tgras H2 mice (4-week oral)	500 (low)	28.1/51.8	11.8/21.8	11.1/8.98
	1000 (mid)	95.4/95.1	40.1/39.9	18.4/11.7
	1500 (high)	671/259	282/109	54.5/22.7
Wild type mice (4-week oral)	500 (low)	31.1/44.6	13.1/18.7	5.85/16.6
	1000 (mid)	118/415	49.6/174	15.2/33.1
	1500 (high)	650/540	273/227	52.7/36.3
Human, n=8, Study # A400-1007	300 mg bid	5.10	1.25	0.618

3. Metabolic profile of UK-427,857 in wild type mice and humans:

The major route of excretion of radioactivity (studies were conducted with carbon-14 labeled UK-427,857) in male wild type mice was fecal (89%) as compared to urine (12%), the majority of radioactivity being recovered within 24 hr. The major route of excretion in healthy human male subjects was fecal (76%) as compared to urine (20%).

HPLC profiling and mass spectrometric analysis of human and wild type mouse excreta and time normalized plasma showed similar metabolism in both species. The major component in the plasma from human and wild type mouse was unchanged UK-427,857 (42% human, 74% wild type mouse). The most significant metabolites in human plasma were a mono-oxidized analogue of the N-dealkylation product (metabolite A, 11%) and a product of N-dealkylation (metabolite B, 22%); both were observed in wild type mouse plasma (8% and 5%).

The major excreted component in humans and mice was unchanged UK-427,857 (33% human, 65% wild type mice). Metabolite A and B and a number of oxidized products were observed as minor metabolites (<10% of total dose) in human and mice.

4. Metabolites of UK-427,857: metabolite concentrations in plasma have not been measured in toxicology studies. However, metabolite levels were estimated using radioactivity profile data. By pooling plasma samples, radiochemical profiling yields quantitative data that are representative of the proportion of the total radioactivity AUC accounted for by each circulating component. The plasma exposure of the principal metabolite UK-408,027 estimated in this way is greater in wild type mice administered 200 mg/kg/day than in humans receiving the maximal clinical dose of 300 mg, bid (Table

60).

Table 60

UK-427,857 and its metabolite (UK-408,027) AUC values in human and wild type mice derived from radiochemical profiling following oral administration of [14C]-UK-427,857

Species & dose	Total AUC (µg eq.*hr/ml)	AUC derived from radioactivity profiling (µg*hr/ml)		% of total AUC	
		Parent	Metabolite (UK-408,027)	parent	Metabolite (UK-408,027)
Wild type mice (200mg/kg/day)	25.3	18.7	0.92	74%	3.6%
Human (300 mg, bid)	4.5	1.9	0.45	42%	10%

CLINICAL PATHOLOGY COMPONENT

Hematology: no changes were noted

Serum Biochemistry: no changes were seen at the low or mid dose. Increased cholesterol levels in both sexes at the high dose level were noted.

PATHOLOGY COMPONENT

GROSS PATHOLOGY: no changes were seen.

ORGAN WEIGHTS: changes are shown in Table 61.

Table 61

Organ weights changes at the end of dosing, for controls, mean are shown. For treated groups, percent differences are shown

Organ	0 (control)		500 (low)		1000 (mid)		1500 (high)	
	male	Female	male	female	male	female	male	female
Thymus: Absolute (g)	0.0575	0.0765	nc	nc	-18%	-12%	-35%	-25%
%body weight	0.2494	0.3961	nc	nc	-20%	-11%	-35%	-23%
% brain weight	12.11	15.56	nc	nc	-17%	-11%	-36%	-21%
Spleen: absolute (g)	nc	0.0918	nc	-8%	nc	-10%	nc	-12%
% body weight	nc	0.4735	nc	-7%	nc	-9%	nc	-10%

nc = no change

HISTOPATHOLOGY: there were no changes at the low or mid dose. At the high dose, in the cecum of some animals, there were minimal inflammatory changes.

1. Summary of key responses and associated systemic exposures in the transgenic mice

The threshold doses producing the key responses in the transgenic mice are shown in Table 62, with the corresponding plasma exposure (AUCs), measured at the end of the study. Also shown are the exposure multiples compared to the free exposure in humans at the maximum therapeutic dose (300 mg, bid).

Table 62

Key threshold responses in the 4-week dose range study in transgenic mice and associated exposures of UK-427,857 at the end of the study and exposure multiples of humans at the maximum therapeutic dose of 300 mg, bid

Dose (mg/kg/day)	Key response(s)	Free AUC0-24 hr (µg*hr/ml)		Exposure multiple	
		Male	female	male	female
500	No treatment related findings	11.8	21.8	9.4	17
1000	Reduced thymus weight, % body weight (12%-18%)	40.1	39.9	32	32
1500	Reduced thymus (23-35%) and spleen weights, % body weight (10% female) Minimal inflammation in cecum	282	109	226	87
Exposure multiples calculated using free AUCss0-24 hr humans at 300 mg bid is 1.25 µg*hr/ml (study #A400-1007)					

CONCLUSIONS:

The key responses seen at the high dose (reduced spleen and thymus weights, and minimal inflammation of the cecum) were considered to be too small to have an impact on the 6-month carcinogenicity study in the mice. There were no findings in this study that would limit the doses for the 6-moth carcinogenicity study in transgenic mice. In this study, a dose level of 1500 mg/kg/day (high) may be considered close to the NOAEL. The high dose produced a plasma exposure many times greater (226 in males and 87 in females) than seen at the maximum therapeutic dose in humans.

2. REVIEW AND EVALUATION OF CARCINOGENICITY STUDIES

Name of Drug: Maraviroc (UK-427,857)

Sponsor: Pfizer Global Research & Development
50 Pequot Ave
New London, CT 06320

Number of Studies: one (rasH2 Transgenic mice)

Reviewer: Pritam Verma, Ph.D.

Supervisor: James Farrelly, Ph.D.

Project Manager: Kenny Shade, J.D., B.S.N

Specific Submission for Linking Minutes: 0201

Date for Exec CAC Meeting: June 4, 2006

STUDY IDENTIFICATION

1. Sponsor's Study Title: 6-Month oral carcinogenicity study of UK-427,857 in rasH2 Transgenic mice

- Study Number: 2004-0091
- Volume Numbers: 1
- Test Facility: [redacted]

  Pfizer Global Research & Development, Ann Arbor, MI 48105
- Study Date initiation: November 18, 2004
- Date of Completion: June 9, 2005
- Date of Submission: March 27, 2006
- GLP Compliance: Yes
- QA Report: Yes
- Study Characteristics
- Reference to Dose-range-finding study: Yes

STUDY PROTOCOL DESIGN AND METHODS

- Study Type: 6-month transgenic mice; Gavage
- Species/strain of animals used: Mouse/CB6F1/Jic-Tg (rasH2) hemizygous (carcinogenicity study) and wild type (toxicokinetic analysis)
- Number of animals per group: 15-25, details in Table 63.

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Table 63
Experimental design of the 26-week oral (gavage) carcinogenicity study in the transgenic and wild type (toxicokinetic analysis) mice

Dosage (mg/kg/day)	Toxicity		Toxicokinetics	
	Male	Female	male	female
0 (vehicle control, 0.5% methylcellulose)	25	25	-	-
MNU = 75 mg/kg, ip, (positive control)	15	15	-	-
200 (low)	25	25	15	15
800 (mid)	25	25	15	15
1500 (high)	25	25	15	15

MNU: N-methyl-N-nitrosourea (single dose)

- Age at start of study: 7-9 weeks
- Animal housing: Individual polycarbonate cages
- Drug Lot/Batch number(s): R112
- Drug Purity / Stability / Homogeneity: [redacted]; adequate

DOSES: 0, 200, 800 or 1500 mg/kg/day

Basis of Dose Selection: Doses proposed by the sponsor for the 6-month carcinogenicity study with maraviroc in transgenic mice were 0, 200, 800 and 1500 mg/kg/day, administered by oral gavage. These doses were chosen based on the results of the 4-week oral gavage dose range-finding study in transgenic mice. The high dose of 1500 mg/kg/day was many times greater (226X in males and 87X in females based on AUC of parent drug) than that seen at the maximum therapeutic dose in humans. The low dose of 200 mg/kg/day is predicted to induce no treatment related findings and to provide a low multiple (4-fold, males) of the plasma exposure at the maximal therapeutic dose.

CAC Concurrence: Yes (Exec. CAC meeting of 8/25/04).

- Restriction Paradigm for Dietary Restriction Study: N/A
- Route of Administration: gavage
- Frequency of Drug Administration: once daily
- Controls Employed: vehicle control (0.5% methylcellulose/0.1% Tween 80) and positive control (N-methyl-N-nitrosourea) or MNU
- Interim Sacrifices: No

- Satellite PK or Special Study Group(s): details in Table 63
- Unscheduled Sacrifices: No
- Deviations from Original Study Protocol: None

STUDY RESULTS AND FREQUENCY OF MONITORING

Observations and times:

Clinical signs: once daily

Body weights: weekly

Food consumption: weekly

Ophthalmology: not done

Hematology & coagulation: at necropsy (excluding positive control)

Clinical chemistry: not performed

Toxicokinetics: day 184

Organ weights: not done

Gross Pathology: at termination

Sponsor's statistical analysis: incidence of neoplasms was analyzed using a one-tailed Cochran-Armitage trend test for evidence of a positive relationship between neoplasm incidence and dose. The ordinal scale was used for the doses.

RESULTS

Clinical Observations: there were no clinical signs noted. Clinical signs for positive control included inactivity, rapid respiration and a variety of skin nodules and/or generalized swelling about the mouth or perivaginal area (tracked as masses). These findings were consistent with the marked carcinogenic response to treatment with maraviroc.

Palpable masses: Table 64 contains an incidence summary of distribution of masses in the main study. A vehicle control male scrotal mass and a mass on the nose (mid) were due to skin findings; the male had a papilloma and epidermal hyperplasia was noted in the female. With MNU, there was a high incidence of palpable masses (positive control females 14/15), most perivaginal. Two of the 15 males had palpable masses on the scrotum.

Table 64

Distribution of masses at necropsy in mice

Dosage (mg/kg/day)	Total number of mice with masses			
	Number of animals	Number of masses in males	Number of animals	Number of masses in females
0 (vehicle control)	25	1	25	0
Positive control	15	2	15	14
200 (low)	25	0	25	0
800 (mid)	25	0	25	1
1500 (high)	25	0	25	0

Mortality: thirty mice were either killed in extremis or died during the study. These early deaths are summarized in Table 65. The vehicle control male had intestinal intussusceptions; the females had malignant neoplasms. The females (mid and high) were considered probably dosing errors. Additionally a toxicokinetic female was found dead on day 88; cause of death probably dosing error. The remaining early deaths (positive control) were consistent with the carcinogenic effects of MNU.

Table 65

Survivability at termination of the Maraviroc oncogenicity study in mice

Dosage (mg/kg/day)	Male			Female		
	N	Mortality	Survival (%)	N	Mortality	Survival (%)
0 (vehicle control)	25	1	96	25	2	92
Positive control	15	12	20	15	12	20
200 (low)	25	0	100	25	0	100
800 (mid)	25	0	100	25	2	92
1500 (high)	25	0	100	25	1	96

Body Weight: there was a slight decrease in mean body weights, relative to vehicle control in the high dose males (Table 66). The high dose male bodyweights were noted to be statistically and biologically significantly decreased from the vehicle control from week 15 through 26. The mean body weight decreases for males (high) ranged from 6% to 9% relative to the vehicle control. In female mice (Table 67), there were sporadic decreases in mean body weights; no consistent pattern for the decrease was seen in the females.

Table 66
Group mean body weight (g) of male mice

Dosage (mg/kg/day)	Mean body weight (g)					
	Week 1	Week 5	Week 10	Week 15	Week 20	Week 26
0 (vehicle control)	23.04	24.94	26.46	28.38	29.62	29.78
Positive control	21.94	24.27	26.94	27.97	29.88	30.67
200 (low)	22.74	24.31	26.73	27.39	29.89	30.07
800 (mid)	22.52	24.43	26.37	27.55	29.36	29.10
1500 (high)	22.78	24.18*	26.08	26.94**	26.94**	27.93**

*= group mean was significantly different for the vehicle control at p=0.05
** = group mean was significantly different for the vehicle control at p=0.01

Table 67
Group mean body weight (g) of female mice

Dosage (mg/kg/day)	Mean body weight (g)					
	Week 1	Week 5	Week 10	Week 15	Week 20	Week 26
0 (vehicle control)	18.26	20.14	21.55	22.24	22.58	22.69
Positive control	17.67	19.75	21.83	22.23	21.56	22.49
200 (low)	18.45	20.03	21.86	21.05**	23.23	22.36
800 (mid)	18.13	20.48	21.85	22.22	23.50*	23.12
1500 (high)	18.30	20.18	21.49	23.02*	22.94	23.29

*= group mean was significantly different for the vehicle control at p=0.05
** = group mean was significantly different for the vehicle control at p=0.01

Food Consumption: Table 68 contains the weekly group mean values (males) for feed consumed per day. Consistent with the body weight changes noted previously, food consumption values for males (high) were noted to be statistically and biologically significantly below (-8% to -14%) vehicle control values. Food consumption values (Table 69) for female mice (high) were increased (7% to 21%) compared to the vehicle control without association to body weight changes. Statistically significant reductions in food consumption values were noted for the low dose males for 15 of the 26 weeks monitored, with most low dose group mean values over this period ranging from -7% to -13% below respective control values. These decreases were not associated with any body weight changes, and similar findings were not observed for the mid dose males.

Surviving positive control mice were noted to have increased food consumption levels

over week 18 to 16, with group mean values ranging from 11% to 65% above vehicle control over the period.

Table 68
Group mean food consumption (g) per day by week of male mice

Dosage (mg/kg/day)	Mean food consumption (g)					
	Week 2	Week 6	Week 12	Week 18	Week 22	Week 26
0 (vehicle control)	3.95	4.19	4.30	3.87	4.10	4.10
Positive control	3.17	4.28	4.50	3.87	3.58	4.21
200 (low)	3.60**	4.15	3.91**	3.71	3.55**	3.56**
800 (mid)	3.83	4.34	4.28	3.80	3.5**	3.72**
1500 (high)	3.38**	4.34	4.40	3.89	3.51**	3.51**

*= group mean was significantly different for the vehicle control at p=0.05
** = group mean was significantly different for the vehicle control at p=0.01

Table 69
Group mean food consumption (g) per day by week of female mice

Dosage (mg/kg/day)	Mean food consumption (g)					
	Week 2	Week 6	Week 12	Week 18	Week 22	Week 26
0 (vehicle control)	3.60	4.18	4.23	3.52	3.67	3.36
Positive control	3.77	4.25	4.60	3.92	5.38	4.93
200 (low)	3.55	4.23	4.0	3.56	3.36*	3.26
800 (mid)	3.39	4.17	4.13	3.67	3.45	3.67**
1500 (high)	3.38*	4.36	4.52*	3.95**	3.65	3.69**

*= group mean was significantly different for the vehicle control at p=0.05
** = group mean was significantly different for the vehicle control at p=0.01

Hematology and coagulation: there were statistically significant treatment related decreases in hematology values for red blood cell parameter (red blood cells, hematocrit, and hemoglobin) in high dose animals (2%-4%) relative to vehicle control. There were biologically relevant and statistically significant (1.27 to 1.85-fold) group mean WBC increases noted for the high dose animals relative to the vehicle control. WBC constituent cell increases were most obvious for lymphocyte, segmented neutrophil and eosinophil values.

Gross Pathology: macroscopic findings in the vehicle control and treated animals were similar to those observed in a vehicle control study conducted in rasH2 transgenic mice at

the testing facility. The incidence and distribution of macroscopic finding in the present study did not suggest any treatment related effects.

Most animals (positive control) had macroscopic finding indicative of neoplastic changes. The most common findings included enlargement, confirmed mass or mass/growth/nodule(s) in the skin (including ear, external vagina and rectum), thymus, lymph nodes and stomach.

Histopathology: the most common neoplasm in this study was hemangiosarcoma (Table 70).

Table 70
Incidence of vascular tumors (hemangioma and hemangiosarcoma) in 6-month carcinogenicity study in transgenic mice

Neoplastic change	Vehicle control		200 mg/kg/day (low)		800 mg/kg/day (mid)		1500 mg/kg/day (high)	
	♂	♀	♂	♀	♂	♀	♂	♀
Hemangioma	0	0	0	0	0	0	0	0
Hemangiosarcoma:								
Colon	1	0	0	0	0	0	0	0
Kidney	1	0	0	0	0	0	0	0
Spleen	0	1	1	0	1	0	0	0
Stifle joint	0	0	0	0	1	0	0	0
Uterus	NA	0	NA	0	NA	0	NA	2
Total number of affected animals	2	1	1	0	2	0	0	2
Percent incidence	8%	4%	4%	0%	8%	0%	0%	8%

In male mice, the incidence of hemangiosarcoma was 8% (vehicle control), 4% (low), 8% (mid) and 0% (high). At the high dose in female mice, systemic exposure was 33-times the exposure in humans (300 mg bid dose). No other tumors were seen in male mice. In female mice, the incidence of hemangiosarcoma was 4% (vehicle control), 0% (low), 0% (mid) and 8% (high). At the high dose in female mice, systemic exposure was 59-times the exposure in humans (300 mg bid dose). No other tumors were seen in male mice.

Additionally, incidence of minimally increased glycogen storage in liver was higher in mid and high dose male and female mice.

There were no significant differences in the nature or incidence of hyperplastic or neoplastic findings in any tissue/organ in maraviroc treated male or female mice compared to the animals in the vehicle control group.

There was a 100% prevalence of tumors in the positive control animals and these findings were similar to those reported by the laboratory with MNU in rasH2 transgenic mice. Fourteen of 15 mice of each sex had at least 1 malignant tumor. Squamous cell carcinoma

and lymphoma were the most common malignant tumors. The remaining two mice without malignant tumors had 1 or more benign tumors. Retinal atrophy (low of photoreceptor cells) was also a common and expected finding in MNU treated mice.

Toxicokinetics: results are presented in Table 71. Plasma concentrations of maraviroc confirmed exposure of male and female transgenic mice to the test article following oral administration of 200, 800 and 1500 mg/kg/day maraviroc for 184 days. Cmax and AUC values of maraviroc generally increased with dose across the dose range in both male and female mice. For males, dose increases of 4x and 7.5x resulted in changes of approximately 0.6x and 1.8x in Cmax and 1.5x and 5.2x in AUC values; for females, increases were 2.2x and 3.3x (Cmax) and, 2.5x and 7.3x (AUC). Exposure (AUC) in females appeared to be higher (2x) than males at the 2 higher doses. Tmax was 1 to 7 hr and typically occurred at later time points as dose increased.

Table 71
Mean toxicokinetic parameters for maraviroc in mice on day 184

Dose (mg/kg/day)	Sex	Cmax (µg/ml)	Tmax (hr)	AUC $_{0-24}$ (µg*hr/ml)	Multiples of human AUC achieved
200 (low)	Male	4.64	1	22.5	6
	Female	4.81	3	29	8
	Pooled	4.51	1	25.8	7
800 (mid)	Male	2.89	1	33.4	9
	Female	10.6	3	71.6	20
	Pooled	6.47	3	52.5	15
1500 (high)	Male	8.54	3	117	33
	Female	16	7	213	59
	Pooled	12	7	165	46

Human Exposure: in the clinic (300 mg bid therapeutic dose) in human, AUCss value is 3.6 µg*hr/ml.

OVERALL INTERPRETATION AND EVALUATION

Adequacy of the carcinogenicity studies and appropriateness of the test model:

MTD was not achieved in this study. The dose levels utilized in the study were approved by the Exec CAC. In selection of dose levels, the sponsor relied upon the toxicokinetic endpoint (AUC) for the determination of the high dose that met the criterion in the ICH Guideline regarding acceptability of 25 times exposure as being adequate. In this study, the human systemic exposures at the therapeutic dose were 33 and 59 times at the high dose in male and female mice, respectively. This compound is moderately protein bound and the major route of excretion male wild type mice was fecal (89%) as compared to urine (12%), the majority of radioactivity being recovered within 24 hr. The major route

of excretion in healthy human male subjects was fecal (76%) as compared to urine (20%). The major component in the plasma from human and wild type mouse was unchanged UK-427,857 (42% human, 74% wild type mouse). The most significant metabolites in human plasma were a mono-oxidized analogue of the N-dealkylation product (metabolite A, 11%) and a product of N-dealkylation (metabolite B, 22%); both were observed in wild type mouse plasma (8% and 5%).

The carcinogenicity study in mice was considered to be acceptable.

Evidence of genotoxicity: Maraviroc was not genotoxic in the reverse mutation bacterial test (Ames test), mouse lymphoma or mouse in vivo micronucleus assays.

SUMMARY AND CONCLUSIONS

The oncogenicity potential of maraviroc was investigated in male and female transgenic mice at oral gavage dosages of 0 (vehicle control), MNU = 75 mg/kg ip (positive control), 200 (low), 800 (mid) or 1500 mg/kg/day (high) in comparison with the controls for a period of 26 weeks. The protocol was approved by the Exec CAC. The systemic exposures were 33 and 59 times that in humans (300 mg bid, AUCss = 3.6 µg*hr/ml) in male and female mice at the high dose level, respectively.

No drug-related malignant neoplasms or non-neoplastic changes were seen in transgenic mice.

APPENDIX LIST

CAC Report: 8/25/2004, attached.

Sponsor's Incidence of Histopathology Findings: attached.

Body weight and food consumption changes vs. dose level: figures attached.

Statistical analysis: attached.

List of Organs and Tissues Examined: complete macroscopic postmortem examinations were performed on all carcinogenicity study animals. A histopathology examination was performed on the following tissues in all animals (Table 72):

Table 72

Mouse tissues preserved and examined from the maraviroc study

Organ name	Preserved	Examined microscopically
Adrenal glands	X	X
Aorta (thoracic)	X	X

Bone marrow smear (rib)	X	x
Bone (sternum, femur)	X	X
bone marrow (sternum, femur)	X	X
brain (medulla, pons, cerebrum and cerebellum)	X	X
Epididymides	X	X
Esophagus	X	X
Eyes with optic nerve	X	X
gall bladder	X	X
Harderian gland	X	X
Heart	X	X
Kidneys	X	X
lacrimal glands	X	X
large intestine (cecum, colon, rectum)	X	X
Liver	X	X
lungs (with mainstem bronchi)	X	X
lymph nodes (mesenteric, mediastinal)	X	X
Mammary gland	X	X
nerve (sciatic)	X	X
Ovaries	X	X
Pancreas	X	X
Pituitary gland	X	X
Prostate gland	X	X
Salivary glands submandibular)	X	X
Seminal vesicles	X	X
Skeletal muscle (biceps femoris)	X	X
Skin	X	X
small intestine (duodenum, ileum, jejunum)	X	X
spinal cord (cervical, thoracic, lumber)	X	X

Spleen	X	X
Stomach	X	X
Testes	X	X
Thymus	X	X
Thyroid,/parathyroid glands	X	X
Trachea	X	X
Turbinates (skull)	X	x
Urinary bladder	X	X
uterus (body/horns) with cervix	X	X
Vagina	X	X
tissue masses and macroscopic findings	X	X

2.6.6.6 Reproductive and Developmental Toxicology

Fertility and early embryonic development

Study title: 1. Oral fertility and early embryonic development study of UK-427,857 in rats

Key study findings: Groups of male and female Sprague-Dawley rats [20 rats/sex/group] received UK-427,857 via esophageal intubation at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80, vehicle control), 100 (low), 300 (mid) or 1000 mg/kg/day (high). In a parallel pharmacokinetics study 3 groups of 3 rats/sex received UK-427,857 at the same dose levels as in the main study for 15 days. There were no deaths. Minimal toxicity was observed at the high dose in males consisting of diarrhea and a slight decrease in mean body weights (up to 6.2%) when compared to the controls. The treatment had no effect on the estrus cycle, on pre-coital time, on copulation and pregnancy rates, on the spermatozoid count in epididymis or on the spermatic motility. In the high dose females (1000 mg/kg/day), there was a statistically significant increase in the pre-implantation loss (9.53% vs 3.24%) with consequently a smaller number of implants (14 vs 16) and of viable fetuses (14 vs 15) when compared to the controls. The results were deemed to be not worthy of placing into the label. The exposure (AUC) at this dose was 20 times that in humans at the highest recommended daily dose. There was no clinical chemistry or histopathological evidence of toxicity. The treatment resulted in slight toxicity in both sexes at the high dose with diarrhea and a slight decrease in mean body weights in males and an increase in the pre-implantation loss in females.

The NOAEL was 300 mg/kg/day (AUC=35.77 µg*hr/ml) for both adult male and female rats. Based on the body surface area factor, an equivalent dose in humans would be 48.7 mg/kg/day (2.9 g/day for a 60 kg person). In the clinic, the test compound is being administered at a dose level of 300 mg/day (AUC=3.609 µg*hr/ml). Thus, at the clinical dose there is an approximately 10-fold safety margin.

Study no.: 02132/133

Volume # and page #: 1 and 1-504

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: July 4, 2003

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: Lot # R102, [redacted]%

Methods

Doses: Groups of male and female Sprague-Dawley rats [redacted] CD (SD) IGS BR; 20 rats/sex/group] received UK-427,857 via esophageal intubation at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80, vehicle control), 100 (low), 300 (mid) or 1000 mg/kg/day (high).

Species/strain: male and female rats/[redacted] CD (SD) IGS BR

Number/sex/group: 20 rats/group

Route, formulation, volume, and infusion rate: oral gavage, 10 ml/kg

Satellite groups used for toxicokinetics: In a parallel pharmacokinetics study 3 groups of 3 rats/sex received UK-427,857 at the same dose levels as in the main study for 15 days. Blood samples were collected on day 15 of treatment at 1, 3, and 7-hr postdosing and on day 16 and were analyzed by a validated analytical method.

Study design: the males were treated for 29 days before mating and throughout the mating period (34-37 days) before their euthanasia. Females were treated 15 days before mating, 1-6 days during the mating period and during early gestation (until day 7 post insemination). The total duration was 63-66 days for the males and 23-28 days for the females.

Results

Mortality: there were no deaths.

Clinical signs: diarrhea was observed in both male and female rats (high).

Body weight: a slight decrease in mean body weights (up to 6.2%) when compared to the controls was seen in males (high).

Food consumption: no change

Toxicokinetics: highest plasma concentrations generally occurred between 1 and 7 hr postdosing with mean values shown in Table 73. As no consistent differences were noted between sexes, male and female data were combined. Plasma drug concentrations increased with dose level in a proportional manner.

Table 73
Toxicokinetics of UK-427,857 on day 15 of study after repeated daily oral administration to rats

Dose (mg/kg/day)	AUC $_{0\text{-}24hr}$ (µg*hr/ml)	Cmax (µg/ml)	Tmax (hr)
100	8.10	1.6	2.7
300	35.77	4.07	3
1000	100.31	6.87	5.3

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): the treatment had no effect on the estrus cycle, on pre-coital time, on copulation and pregnancy rates, on the spermatozoid count in epididymis or on the spermatic motility. In the high dose females, there was a statistically significant increase in the pre-implantation loss (9.53% vs 3.24%) with consequently a smaller number of implants (14 vs 16) and of viable fetuses (14 vs 15) when compared to the controls.

There were no clinical chemistry or histopathological evidence of toxicity.

Embryo fetal development

Study title: 2. **UK-427,857: Oral study of embryo-fetal development in rats**

Key study findings: Groups of pregnant Sprague-Dawley rats 20 rats/group

received UK-427,857 during organogenesis (days 6-17 post insemination) via esophageal intubation at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80, vehicle control), 100 (low), 300 (mid) or 1000 mg/kg/day (high). In a parallel pharmacokinetics study 5 inseminated rats received UK-427,857 at the same dose levels from day 6 to 21 post insemination. On day 17 and 18, blood samples were collected and were analyzed by a validated analytical method. Mortality (dams): there were no deaths. Clinical signs (dams): Increased salivation was observed 1/20, 8/20 and 20/20 during the treatment period in the low, mid and high dose, respectively. Body weight (dams): when compared to the controls, mean body weights were slightly but significantly decreased (mid and high) from day 7 until the end of study. At the high dose, the mean body weight gain was also decreased (22.7%) during days 6-17. When compared to the controls, mean corrected maternal weight gain was moderately but significantly decreased at the high dose ($p=0.001$).

The NOAEL was 300 mg/kg/day for the pregnant female rat and 1000 mg/kg/day for the fetuses. Based on the body surface area factor, an equivalent dose in humans would be 48.7 mg/kg/day (2.9 g/day for a 60 kg person). Equivalent dose for human fetus would be 162 mg/kg/day. At the NOAELs (300 and 1000 mg/kg/day), exposures (steady state AUC and Cmax) were 45.5 and 4.53 and 102 µg*hr/ml and 7.57 µg/ml, respectively .

Study no.: 02025/02028

Volume #, and page #: 1 and 1-544

Conducting laboratory and location: Pfizer Inc., Groton, CT,

Date of study completion: October 10, 2002

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: R101, [redacted]

Methods

Doses: 100 (low), 300 (mid) or 1000 mg/kg/day (high)

Species/strain: Sprague-Dawley rats [redacted]:CD (SD)IGS BR

Number/sex/group: 20 rats/group

Route, formulation, volume, and infusion rate: oral gavage

Satellite groups used for toxicokinetics: In a parallel pharmacokinetics study 5 inseminated rats received UK-427,857 at the same dose levels from day 6 to 21 post

insemination. On day 17 and 18, blood samples were collected and were analyzed by a validated analytical method.

Study design: Groups of pregnant Sprague-Dawley rats 20 rats/group received UK-427,857 during organogenesis (days 6-17 post insemination) via esophageal intubation at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80, vehicle control), 100 (low), 300 (mid) or 1000 mg/kg/day (high).

Parameters and endpoints evaluated: maternal gross necropsy, Cesarean-sectioning and fetal evaluation.

Results

Mortality (dams): there were no deaths.

Clinical signs (dams): Increased salivation was observed 1/20, 8/20 and 20/20 during the treatment period in the low, mid and high dose, respectively.

Body weight (dams): when compared to the controls, mean body weights were slightly but significantly decreased (mid and high) from day 7 until the end of study. At the high dose, the mean body weight gain was also decreased (22.7%) during days 6-17. When compared to the controls, mean corrected maternal weight gain was moderately but significantly decreased at the high dose ($p=0.001$).

Food consumption (dams): when compared to the controls, mean food consumption was statistically significantly decreased (mid, 6.1%) and high (15.6%) during the treatment period.

Toxicokinetics: highest plasma concentrations were generally occurred at 3 or 7 hr postdosing with mean values shown in Table 74.

Table 74

Toxicokinetics of UK-427,857 on day 17 post insemination after repeated daily oral administration to pregnant rats

Dose (mg/kg/day)	AUC_{0-24hr} (µg*hr/ml)	Cmax (µg/ml)	Tmax (hr)
100	15.4	1.85	3.8
300	45.5	4.53	3.8
1000	102.0	7.57	5.4

Postmortem observations: no abnormal findings

Maternal and litter observations at cesarean sectioning: Reproductive parameters, necropsy findings, placental and fetal body weights, external and buccal examination: no abnormal findings.

Visceral and skeletal examination: no abnormal findings.

Fetal observations: no abnormal findings

Study title: 3. Oral embryo-fetal development study of UK-427,857 in rabbits

Key study findings: Groups of pregnant New Zealand White rabbits (20 rabbits/group] received UK-427,857 during organogenesis (days 7-19 post insemination) via oral gavage at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80, vehicle control), 30 (low), 75 (mid) or 200 mg/kg/day (high). On day 19, blood samples were collected and were analyzed by a validated analytical method. The treatment resulted in significant mortality at the high dose (six deaths), so that the high dose outcome should be excluded from analysis. However, for the record, at the high dose, 7 fetuses in 6 liters were affected by an external anomaly. One fetus (control) had a short tail. Cleft palate, bent forepaws(s) and short tail have been recorded previously (1992-1995) in control fetuses from oral studies with the same strain of rabbits. Ectrodactyly and cutis aplasia has been observed in control populations of rabbits from the compiled historical databases. These fetal defects are not part of a recognizable syndrome. There were no other signs of potential adverse effects of the compound on the fetuses: no embryo mortality and no effect on fetal body weight.

The NOAEL was 75 mg/kg/day for the pregnant female rabbit. Based on the body surface area factor, an equivalent dose in humans would be 24.19 mg/kg/day (1.4 g/day for a 60 kg person). Equivalent dose for human fetus would be 162 mg/kg/day. At the NOAEL (75 mg/kg/day), exposure (steady state AUC and Cmax) were 27.1 µg*hr/ml and 5.85 µg/ml, respectively.

Study no.: 02026/02029

Volume # and page #: 1 and 1-544

Conducting laboratory and location: Pfizer Inc., Groton, CT,

Date of study completion: October 16, 2002

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: R101, [redacted]

Methods

Doses: 30 (low), 75 (mid) or 200 mg/kg/day (high)

Species/strain: New Zealand White rabbits

Number/sex/group: 20 rabbits/group

Route, formulation, volume, and infusion rate: oral gavage

Satellite groups used for toxicokinetics: none

Study design: Groups of pregnant New Zealand White rabbits (20 rabbits/group] received UK-427,857 during organogenesis (days 7-19 post insemination) via oral gavage at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80, vehicle control), 30 (low), 75 (mid) or 200 mg/kg/day (high). On day 19, blood samples were collected and were analyzed by a validated analytical method.

Parameters and endpoints evaluated: maternal gross necropsy, Cesarean-sectioning and fetal evaluation.

Results

Mortality (dams): at the high dose level, 6/20 females died between day 8 and 20; no associated clinical signs were observed. Thus in the high dose group, there were 13 females with viable fetuses and one non-pregnant female. In the control, low and mid dose groups, all the females were pregnant with viable fetuses.

Clinical signs (dams): noisy respiration was recorded in mid dose animals.

Body weight (dams): mean body weights and mean body weight gains were similar between control and treated groups.

Food consumption (dams): mean food consumption was decreased (12.7%) at the high dose.

Toxicokinetics: highest plasma concentrations were generally occurred at 1 hr postdosing with mean values shown in Table 75.

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Table 75

Toxicokinetics of UK-427,857 on day 17 post insemination after repeated daily oral administration to pregnant rats

Dose (mg/kg/day)	AUC $_{0\text{-}24hr}$ (µg*hr/ml)	Cmax (µg/ml)	Tmax (hr)
30	35.3	1.87	1
75	27.1	5.85	1
200	127.5	10.11	3

Reproductive parameters, necropsy findings and placental and fetal body weighs: no abnormal findings. Placental examination, external and oral examination: at the high dose, 2 fetuses presented a bent forepaw associated with ectrodactyly in one of them, one fetus presented a cleft palate, three fetuses in 3 litters presented a short tail and one fetus presented cutis aplasia. At the mid dose, there was one fetus with a spina bifida occulta. At the low dose, one fetus had single naris and the fetuses of a litter had a double placenta. In the absence of any dose-relationship, findings at the low and mid doses were considered to be incidental. Visceral examination: there were no effects on fetal development. Skeletal examination: incompletely ossified pubis in 2 fetuses in 2 litters in each of the mid and high dose groups. One fetus in the control group had the same finding. The degree of ossification was not affected by the treatment. Some findings already seen at external examination (ectrodactyly and cleft palate) were confirmed at skeletal examination. The other abnormalities showed no dose-relationship or their incidences were within the historical ranges (sponsor's lab).

Prenatal and postnatal development

Study title: 4. UK-427,857: Oral study of pre- and postnatal development in rats

Key study findings: Groups of presumed pregnant rats (27/group) received UK-427,857 via oral gavage at dose levels of 0 (vehicle controls), 100 (low), 300 (mid) or 1000 mg/kg/day (high) from day 6 of gestation through day 20 of lactation. At the high dose level, maternal toxicity was demonstrated in the F0 dams by reduced body weight and food consumption during gestation. No reproductive effects were noted. There were no drug related findings in the F1 generation offspring at any dose level. UK-427,857 caused drug related weight and food consumption changes in the F0 dams at the high dose level, without affecting the pre- or postnatal development of the F1 generation offspring at any dose level. A dose level of 300 mg/kg/day may be considered the NOEL for F0 dams. Based on the body surface area factor, an equivalent oral dose in humans would be 48.7 mg/kg/day (2.9 g/day for a 60 kg person). A dose level of 300 mg/kg/day may be considered the NOEL for F1 offspring. Based on the body surface area factor, an equivalent oral dose in humans would be 48.7 mg/kg/day (2.9 g/day for a 60 kg person). Drug exposure at the NOEL was

13.13 µg*hr/ml.

Study no.: 02-2120-10

Volume # and page #: 1-3 and 1-1256

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: June 18, 2004

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: **# R103,** [redacted]

Methods

Doses: 0 (0.5% methylcellulose/0.1%Tween 80, vehicle control), 100 (low), 300 (mid) or 1000 mg/kg/day (high) once daily

Species/strain: presumed pregnant rats, [redacted] CD (SD) IGS BR

Age: 70-84 days of age on receipt

Number/sex/group: 24 rats/group

Route, formulation, volume: oral gavage, 10ml/kg

Satellite groups used for toxicokinetics: 3 animals/dose

Study design: presumed pregnant female rats received repeated oral gavage doses of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80, vehicle control), 100 (low), 300 (mid) or 1000 mg/kg/day (high) once daily from day 6 of gestation through day 20 of lactation. Dams were allowed to deliver naturally and the litters were monitored for viability at birth, postnatal survival, appearance and growth. On day 21 of lactation, randomly selected F1 generation pups from each litter were continued on the study and the F0 generation dams and remaining F1 generation pups were euthanized. F1 generation rats that continued on study were evaluated for sexual maturation, sensory perception, motor activity learning, memory and reproductive function. The F1 generation rats were evaluated for reproductive capacity. Within each dose group, rats were assigned to cohabitation, one male per female rat. The cohabitation period consisted of a maximum of 21 days. Female rats were Caesarean-section on gestation day 21 and the F2 generation fetuses were evaluated.

Results

F_0 Generation rats

Mortality, clinical sign and necropsy observation: all rats survived to scheduled necropsy on day 21 of lactation. There were no necropsy findings. There were no drug related clinical signs.

Body weights and body weight changes: no drug related changes occurred in the F0 generation dams (low and mid). At the high dose, drug related reductions in body weight were noted; mean body weights were reduced (0.93 - 0.97x control) from gestation day 6 to lactation day 14. Body weight gain (high) was reduced from lactation days 6-12 (0.78x control) and gestation day 12-20 (0.89x control) resulting in an overall gestation body weight gain of 0.86x control.

Feed consumption: there were no drug related effects on absolute or relative maternal food consumption at the low dose. Drug related reductions in food consumption were noted at the mid and high doses. At the high dose, food consumption was reduced from gestation day 7-19 (0.51-0.95x control); the majority of these reductions were statistically significant. At the mid dose, food consumption was reduced from gestation days 7-10 resulting in a statistically significant reduction (0.93x control).

F0 maternal drug level determination: highest individual drug concentrations occurred between 1 and 7 hr after dosing. The mean AUC0-7hr values are shown in Table 76.

Table 76
Mean AUC values of UK-427,857 over the 0-7 hr period in F0 dams

Dose level (mg/kg/day)	Mean AUC0-7hr (µg*hr/ml)
100	3.37
300	13.13
1000	34.82

Reproductive data of littering F0 females: there were no drug related effects on the length of gestation, number of implantation sites, numbers of live and dead pups, sex ratio, percent of pups born alive or litter sizes at birth.

Natural delivery and litter observations: no effects on natural delivery or litter observations were observed at any dose. All pregnant rats delivered litters.

Clinical and necropsy observation-F_1 generation litter: There were no drug related findings in the F1 generation offspring at any dose level.
F_1 generation rats:

Mortality: there were no drug related deaths. All F1 generation male and female rats survived to scheduled necropsy.

Clinical signs and necropsy observation in rats surviving to scheduled necropsy: no drug related clinical observations occurred in either males or females at any dose level. No drug related gross lesions were identified at scheduled necropsy.

Testes and epididymides weights: absolute and relative weights of the testes and epididymides in the F1 generation male rats were unaffected.

Body weights, body weight changes and feed consumption: were unaffected by administration of UK-427,857 to the F0 generation dams.

Postweaning behavioral evaluations: there were no drug related changes in the F1 generation rats when evaluated during the postweaning period for auditory startle, straight channel swimming, and watermaze learning evaluations.

Motor activity: a slight increase in motor activity was noted for the high dose group male rats at both weaning and as adults. There was no effect on female motor activity and no evidence of increased activity in other behavioral evaluations.

Sexual maturation: there were no changes in the age of preputial separation in F1 generation male rats or vaginal patency in F1 generation females.

Mating and fertility: were unaffected.

Caesarean sectioning and litter observations: UK-427,857 had no effect on caesarean sectioning parameters in the F1 generation dams or the F2 generation litter. Values for corpora lutea, implantations, litter sizes, liver or dead fetuses, resorptions, fetal sex ratios and fetal body weighs were comparable for all groups.

F_2 findings: there were no drug related fetal gross external alterations in the F2 generation fetuses. There was no effect on survival, body weight or clinical signs.

2.6.6.7 Local Tolerance

1. Acute dermal toxicity study of UK-427,857 in Sprague-Dawley rats (709/032474/AC)

Five male and female Sprague-Dawley rats [strain: CD (SD) IGS BR; age: 7 weeks] received a single topical application of at a maximum practical concentration of maraviroc at a dose of 2000 mg/kg, in 1% w/v aqueous methylcellulose for 24 hr. All animals were killed and examined macroscopically on day 15, the end of the observation period.

Results: there were no deaths and no treatment related clinical signs were observed. Very slight dermal irritation was observed in 3/5 males and 3/5 females, but was completely resolved on Day 5. After the 15-day observation period, Peyer patches were observed in the large intestines in two females (marked response) and in one female (slight response) and a pale liver was observed in one female.

In conclusion: the acute lethal dermal dose of UK-427,857 in rats was found to be 2000 mg/kg.

2. Skin irritation study of UK-427,857 in rabbit (710032591)

In a skin irritation study in New Zealand White rabbits (2 males, 1 female), received a single dermal administration of approximately 0.5 g of maraviroc (semi-occlusive application to skin, moistened with 0.5 mL reverse osmosis water). The animals were observed for 4 days.

Results: no dermal irritation was observed.

In conclusion: UK-427,857 in rabbits was found to be nonirritant.

3. Eye irritation study of UK-427,857 in rabbits (711/032861)

In an eye irritation study, three male New Zealand White rabbits were administered a single ocular dose of (mean weight 81 mg) of 0.1 mL maraviroc solution into the left eye and were observed for 15 days. Injection of the conjunctival blood vessels was apparent throughout the first 48 hr after the instillation, persisting in one animal for a further 24 hr and in another to day 8. Very slight discharge and iritis were also evident one hr after the administration.

Instillation of maraviroc produced a slight initial pain response.

Results: The mean scores for the ocular reactions at approximately 24, 28 and 72 hr after the administration were: corneal opacity: 0; iridial lesions: 0; Chemosis: 0; and redness: <1.0.
Conclusion: Maraviroc was not an eye irritant in rabbits.

4. Assessment of skin sensitization potential using the murine local lymph node assay (712032309/LN)

In a skin sensitization (local lymph node assay) study in mice (4 females/dose), maraviroc was administered to the dorsal surface of both ears at daily doses of 0% (control), 10% (low), 25% (mid) and 50% (high) of maraviroc for three days. The proliferative response of cells from the auricular lymph nodes was assessed by measuring incorporation of ^{3}H-methyl Thymidine. Results: the test control ratio obtained were 1.4 (low), 1.9 (mid) and 1 (high), respectively. Thus was no evidence of skin sensitization (delayed contact hypersensitivity) in these animals. The positive control substance, hexyl cinnamic aldehyde, responded as expected, in contemporaneous studies.

Conclusion: maraviroc was not a skin sensitizer in the murine local lymph node assay

2.6.6.8 Special Toxicology studies:

1. Study Title: 7-Day intravenous irritation toxicity study of UK-427,857 in rats

Key Study Findings: Groups of male and female Sprague-Dawley rats [strain: CD (SD) IGS BR; age: 7 weeks; 5 animal/group] received iv injections of UK-427,857 (10 ml/kg) at dose levels of 0 (sodium acetate buffer; vehicle control), 0.6 (low), 2 (mid) or 10 mg/kg/day (high) for 7 consecutive days. Results: there were no deaths, no treatment related clinical signs, no signs of local irritation and no effects on body weight. There was no histopathologic evidence of irritation at the injection sites. In conclusion: the iv administration of UK-427,857 (up to 10 mg/kg/day for 7 days) to rats was well tolerated and did not produce any signs of irritation at the injection sites.

Study no.: 02146

Volume # and page #: 1 and 1-81

Conducting laboratory and location: Pfizer Inc., Groton, CT

Date of study completion: April 16, 2003

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Batch # R103, [redacted]%

Methods

Doses: Groups of male and female Sprague-Dawley rats [strain: CD (SD) IGS BR; age: 7 weeks; 5 animal/group] received iv injections of UK-427,857 (10 ml/kg) at dose levels of 0 (sodium acetate buffer; vehicle control), 0.6 (low), 2 (mid) or 10 mg/kg/day (high) for 7 consecutive days.

Species/strain: male rats; strain: CD (SD) IGS BR

Number/sex/group or time point (main study): 5 animals/group

Route, formulation, volume, and infusion rate: iv; 10 ml/kg

Satellite groups used for toxicokinetics: none

Age: 7 weeks

Weight: 260 g males and 173 g for females

Sampling times: none

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once weekly

Hematology: none

Clinical chemistry: none

Gross Pathology: none

Organ weights: none

Histopathology: none

Results

Mortality: no deaths

Clinical signs: There were no clinical signs, no signs of irritation at the injection sites or evidence of pain during the injection procedure. One male treated at the dose level of 10 mg/kg (M304) showed a purplish, mottled aspect of the tail 5 minutes after injection on day 3, which disappeared 2 hours after treatment. This isolated finding with no histologic correlates and recorded in a single animal was therefore not considered to be of toxicological significance.

Body weight and Food consumption: There was no effect of the treatment on body weight at 0.6 and 2.0 mg/kg. The terminal body weights of males and females given 10.0 mg/kg were slightly lower than that of the control animals (3% and 2%, respectively). However, this difference did not reach statistical significance and was not considered to be an adverse effect.

Microscopic findings: Minimal to moderate perivascular and inflammatory changes were noted at the injection site in all treated and control groups with a similar incidence.

2. Study title: UK-427,857: four week oral (bid) immunotoxicity study in the cynomolgus monkey

Key study findings: Groups of male and female cynomolgus monkeys received repeated oral gavage doses (bid, 7-8 hr apart) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 30 (low), 100 (mid) or 300

mg/kg/day (high) once daily for a period of 4 weeks to determine potential immunotoxic effects. The treatment was not associated with any immunotoxic effects. Moderate clinical signs and mild body weight loss were observed at the high dose level in some animals. Drug plasma levels were similar in males and females and increased superproportionally with dose. There were no adverse treatment related pathological changes at any dose level. A dose level of 100 mg/kg/day may be considered the NOEL in monkeys. Based on the body surface area factor, an equivalent oral dose in humans would be 2 g/day for a 60 kg person. Drug exposure at the NOEL was 6.695 µg*hr/ml.

Study no.: 911/096

Volume # and page #: 2 and 1-589

Conducting laboratory and location: Pfizer Inc., Amboise cedex, France

Date of study completion: August 5, 2004

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity: **# R103,** [redacted]

Methods

Doses: 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 30 (low), 100 (mid) or 300 mg/kg/day (high) once daily

Species/strain: cynomolgus monkeys

Age: 27-37 months

Number/sex/group: 5 animals/sex/group

Route, formulation, volume: oral gavage, 5ml/kg

Satellite groups used for toxicokinetics: blood samples were taken from each animal on days 23 and 24 at predetermined time intervals.

Study design: groups of male and female cynomolgus monkeys received repeated oral gavage doses (bid, 7-8 hr apart) of UK-427,857 at dose levels of 0 (0.5% methylcellulose/0.1%Tween 80 in water, vehicle control), 30 (low), 100 (mid) or 300 mg/kg/day (high) once daily for a period of 4 weeks to determine potential immunotoxic effects.

Mortality: twice daily

Clinical signs: once daily

Body weights: once weekly

Food consumption: once weekly

Hematology: days 14/15 and 28/29

Clinical chemistry: days 14/15 and 28/29

Immunological investigation: lymphocyte subset analysis, natural killer assay, phagocytosis assay, humoral response and saturation of CCR5 receptors on days 14/15 and 28/29.

Gross Pathology: full necropsy.

Organ weights: listed in Table 77

Histopathology: Table 77, Adequate Battery: yes; Peer review: yes

Table 77
Monkey tissues, weighed, preserved and examined

Organ name	Weighed	Preserved	Examined microscopically
adrenal glands	X	X	X
Bone marrow smear (rib)		X	X
Bone (sternum, femur)		X	X
bone marrow (sternum, femur)		X	X
Heart		X	X
lungs		X	X
Lymph nodes (mesenteric, mediastinal)		X	X
Peyer's patches		X	X
Spleen	X	X	X
Thymus	X	X	X
All gross lesions		X	X

Results

Mortality: no animal died during the study.

Clinical signs: occasional clinical signs such as half closed eyes, prostration, reduced activity or inactivity, hunched posture, liquid feces and vomit were seen in the high dose group of animals only following the dosing.

Body weights and food consumption: tendency toward body weight loss (mean of 4.8%, maximum of 10%) was seen in males (high). Incidence of reduced food intake was observed in females at the high dose level.
Hematology: some animals (high) had slightly low RBC parameters after 2 or 4 weeks of treatment.

Clinical chemistry: minor increases in triglycerides and decreases in cholesterol were present in animals at the high dose level.

Immunology: CCR5 occupancy by the test compound was complete at all time points at the high dose level while at the low dose CCR5 was complete at the 1 hr postdose timepoint only. At the high dose level, CCR5 occupancy was approximately 79% at the 7 and 24 hr postdose time points.

The test compound did not effect lymphocytes subset distribution, natural killer cell activity, phagocytosis activity or oxidative burst. All animals were able to mount a humoral primary (IgM) and secondary (IgG) immune response to KLH.

Gross and Histopathology: there were no adverse pathological changes.

Toxicokinetics: plasma drug concentrations were comparable in males and females and increased superproportionally with dose. The mean toxicokinetic parameters are shown in Table 78.

Table 78
Group mean toxicokinetic parameters of UK-427,857 in cynomolgus monkeys

Dose level (mg/kg/day)	Tmax (hr)	Cmax ((µg/ml)	Mean AUC0-24hr (µg*hr/ml)
30	1	0.26	0.948
100	1	1.68	6.695
300	2	4.33	40.09

3. Study title: UK-427,857: Investigation of the mechanism of thyroid hypertrophy and liver changes in rats.

Key study findings: UK-427,857 was administered via oral gavage (10 ml/kg/day) at dose levels of 0 (vehicle control; 0.5% w/v methylcellulose + 0.1% w/w Tween 80 in water) or 900 mg/kg/day (high) for 4 consecutive weeks to investigate the mechanism of thyroid hypertrophy and liver changes in rats. The treatment produced an increase in thyroid weights in males and follicular cell hypertrophy in all males and a few females. A mild decrease in T4 plasma levels and a marked increase in TSH associated with a vacuolation of the pars distalis of the pituitary indicated that the thyroid hypertrophy resulted from a pituitary stimulation occurring most probably as a compensatory response

to the decreased thyroxin level. A higher UDPGT activity occurred in treated females compared to the controls and the thyroxin clearance was increased in both sexes. In conclusion, the treatment produced thyroid follicular cell hypertrophy. Liver enzymes probably contributed to this change. The mean AUC0-24hr values was 34.9 µg*hr/ml at the dose of 900 mg/kg/day.

Study no.: 03165

Volume # and page #: 1 and 1-131

Conducting laboratory and location: Pfizer, PGRD Amboise, France

Date of study completion: August 11, **2004**

GLP compliance: yes

QA report: yes

Drug, lot #, and % purity: Batch # R103, [redacted]

Methods

Doses: UK-427,857 was administered via oral gavage (10 ml/kg/day) at dose levels of 0 (vehicle control; 0.5% w/v methylcellulose + 0.1% w/w Tween 80 in water) or 900 mg/kg/day (high) for 4 consecutive weeks to investigate the mechanism of thyroid hypertrophy and liver changes in rats.

Species/strain: male and female Sprague-Dawley rats; strain: [redacted]:CD(SD)IGS BR

Number/sex/group or time point (main study): 5 animals/sex/group

Route, formulation, volume, and infusion rate: oral gavage; 10 ml/kg

Satellite groups used for toxicokinetics: 5 animals/sex/group

Age: 7 weeks

Weight: 227 g for males and 182.5 g for females

Sampling times: Plasma concentrations of UK-427,857 were determined at 1, 3, 7 and 24 hr on day 4 of the study.

Mortality: once daily

Clinical signs: once daily

Body weights: 6 or 7 days

Food consumption: 6 or 7 days

Plasma chemistry: at the end of the study

Necropsy: liver, pituitary and thyroid

Organ weights: liver, pituitary and thyroid

Histopathology: above listed organs, Adequate Battery: yes; Peer review: yes

Results

Mortality: there were no deaths.

Clinical signs: treatment related diarrhea was observed 24 hr postdose and increased salivation occurred 1 hr postdose.

Body weight and body weight gain: there was a mild, not statistically significant decease in body weight (up to 6%) in the treated group compared to the control.

Food consumption: there was a mild decrease (ranging -2 to -17% in males and females) in food consumption in the treated group compared to the controls.

Plasma chemistry: the treatment resulted in mild to moderate variations in a series of clinical chemistry parameters as shown in Table 79.

Table 79
Changes in plasma chemistry parameters in treated rats (% changes from controls)

Parameters	Males	Females
Cholesterol	+30%*	Nc
Triglycerides	-27%	-33%**
ALP	Nc	+34%*
ALT	+79%*	+31%**

*=statistically significant at p=0.05
**=statistically significant at p=0.01
nc=no change

Thyroid hormone levels: as shown in Table 80. T3, T4 and TSH plasma levels were affected by the treatment. In both sexes, increases in T3 and decreases in T4 remained slight whereas TSH increased markedly.

Table 80
Changes in thyroid hormone levels in treated rats (mean and percentage or fold increase compared to controls)

Sex	Treatment	T3	T4	TSH
Males	Control	0.954	56.1	5.12
	Treated	1.206 +26%	36.32 -35%*	12.80 ×2.5*
Females	Control	0.924	32.14	1.64
	treated	1.256 +36%	27.98 -13%	7.0 ×4.3

*=statistically significant at p=0.05

Clearance of radiolabeled thyroxin: there was no effect of treatment on the elimination half life (beta phase, 6-48 hr) of thyroxin. The overall clearance rate of radiolabeled thyroxin was slightly increased in both sexes but these differences were not statistically significant.

Liver biochemistry: there was a statistically significant increase in the mean total specific content of microsomal cytochrome P450 and UDPGT activity for the treated female group. There were no changes of note in the mean total specific content of microsomal cytochrome P450 or in the mean UDPGT activity for the treated males.

Plasma drug concentration: no consistent differences were noted between male and female values. Highest plasma concentrations occurred 1 to 7 hr after dosing with a mean value of 2.99 µg/ml. The mean AUC0-24hr values was 34.9 µg*hr/ml at the dose of 900 mg/kg/day.

Organ weights: a statistically significant drug related increase in relative (to body) thyroid weights was observed in male rats. A trend towards a drug related increases in absolute thyroid weights was also observed in male rats. Thyroid weights were increased in males rats both in absolute (31%) and relative to body weight values (35%), reaching statistical significance for the later values. There were no noteworthy changes in liver weight.

Necropsy findings: dilatation of the cecum was seen in 3/5 treated male rats

Histopathology:

Thyroid: 5/5 treated males and 2/5 treated females had bilateral, mild, diffuse, follicular cell hypertrophy characterized by a decreased diameter of follicular lumens, large cuboidal to tall columnar follicular cells, decreased eosinophilia of colloid and increased number of follicles. These changes correlated with the increase in thyroid weights of treated animals.

Pituitary: 3/5 treated males had, minimal, diffuse cell vacuolation characterized by

larger cells due to more abundant cytoplasm, which is pale eosinophilic and/or contained large pale eosinophilic glassy vacuoles (hypertrophy). Nuclei were normal. These hypertrophic cells were immunohistochemically slightly diffusely positive for TSH and negative for FSH.

2.6.6.9 Discussion and Conclusions

Single dose studies

Single oral administration of maraviroc to mice and rats produced no effects of treatment at 2000 mg/kg. These studies were conducted in compliance with GLP regulations. These data were broadly consistent with those observed in safety pharmacology studies in rats, in which there were mild effects on appearance and behavior at the oral dose of 1000 mg/kg and adverse effects characterized by decreased activity, respiratory changes and vocalization at 2000 mg/kg. The maximum asymptomatic single oral gavage dose of UK-427,857 in the mice was considered to be 2000 mg/kg. Based on the body surface area factor, an equivalent dose in humans would be 162.3 mg/kg or 9.7 g for a 60 kg person. A dose level of 2000 mg/kg was considered NOEL in the rat. Based on the body surface area factor, an equivalent dose in humans would be 324.6 mg/kg or 19.48 g for a 60 kg person.

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 dyspnea in rats. There were no effects of treatment at the lower dose of 20 mg/kg. Changes in respiratory pattern were also seen in the acute intravenous toxicology study.

Repeat dose studies

Repeat-dose oral toxicity studies were conducted in rats and dogs for up to 6 months, in mice for up to 3 months, and in monkeys for up to 9 months; these species are routinely used in the safety evaluation of new chemical entities. All pivotal studies were conducted in conformance with appropriate ICH guidelines and GLP regulations. Multiples of human exposures achieved in the multiple dose animal toxicology studies are shown in Table 81.

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Table 81
Maraviroc exposures in multiple dose toxicity studies in animals vs humans

Study No.	Study, species & route	Dose (mg/kg/day)	NOEL/NOAEL (mg/kg/day)	AUC (µg*hr/ml) at NOEL/NOAEL	Multiples of human exposures (x)
1.	2-wk mice, po	20			
		200	200	13.25	4
		1000			
		2000			
2.	2-wk dogs, po	10	Not identified	2.2	1
		50			
		250			
3.	1- month mice, po	200	200	33.65	9
		500			
		750			
4.	1- month rats, po	100	100	7.4	2
		300			
		1500			
5.	1-month dogs, po	5	5	6.02	2
		50			
		150			
6.	1-month monkeys, po	100	100	4.21	1
		200			
		400			
		800			
7.	3-month mice, po	200	200	13	4
		500			
		750			
8.	6- month rats, po	30			
		100	100	20	6
		300			
		900			
9.	6-month dogs, po	5	5	2.42	1
		15			
		40			
10.	9-month monkeys, po	30			
		120	120	6.6	2
		400			

The results of repeated dose toxicology studies indicated that the maximum therapeutic dose of 300 mg bid is supported by the NOELs/NOAELs achieved in the studies and maraviroc exposure multiples at the NOELs/NOAELs are higher than that of clinical dose. The following principal target organs/tissues in animal studies were identified:

Blood pressure and Heart rate

Toxicology studies in monkeys indicated reductions in blood pressure at daily doses of 200 and 400 mg/kg/day, accompanied at 400 mg/kg/day by lower heart rates. The doses of 200 mg/kg/day (1-month study) and 400 mg/kg/day (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/day in the 9-month study, with a plasma concentration 5-fold that of the maximum therapeutic dose in man.

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 might be sufficient to cause symptoms of postural dizziness in humans.

QT interval prolongation

In vitro studies showed that maraviroc inhibited dofetilide binding. Maraviroc was active at the human cardiac hERG channel and prolonged the action potential of the dog Purkinje fiber at concentrations ≥3 μM or 1541 ng/mL. These results indicated that the maraviroc has the potential to block the I_{Kr} current and affect cardiac repolarization in vivo at unbound plasma concentrations greater than 3 μM, which was 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/day in dogs and ≥200 mg/kg/day 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/day and 120 mg/kg/day, 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 repolarization, lengthening QT interval and increasing the risk of serious arrhythmias, such as Torsade de pointes. This activity of maraviroc was 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.

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 program. 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.

Liver

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/day and was associated with minimal bile duct hyperplasia from 300 mg/kg/day. 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. These changes are possibly a mild response to the biliary excretion of maraviroc or its metabolite.

Thyroid gland

In rats only, thyroid follicular cell hypertrophy was noted in the 6-month study from 300 mg/kg/day, and was shown to be reversible when treatment was withdrawn. Pituitary vacuolation was observed in the 1-month study at 1500 mg/kg/day. The interdependence of thyroid and liver changes was established in an investigative study in rats. 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. The hepatic changes were consistent with an adaptive response to treatment and were associated with an increase in the activity of hepatic xenobiotic metabolism. Activation of hepatic uridine 5-diphosphate glucuronyl transferase (UDPGT) brought about an increased thyroxine clearance, resulting in a reduction in circulating concentrations of this hormone. Hepatic UDPGT was responsible for conjugation of thyroxine prior to excretion into the bile 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.

Immunotoxicity

Maraviroc was 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/day 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/day 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 (AUC0-24) producing complete and continuous blockade of CCR5 receptors and with an exposure multiple 16-fold greater than observed at 300 mg BID.

Genetic toxicology

The genotoxic potential of maraviroc was evaluated in a battery of well established and validated in vitro and in vivo test systems. The scope of the overall battery of tests and the individual study designs were in conformance with applicable ICH guidelines. 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/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. Summary of results is shown in Table 82.

Table 82
Summary results of genotoxicology studies of maraviroc

Type of study	Species strain	Method of administration	Duration of dosing	Doses	Results
1. Ames assay	S. typhimurium &E. coli	In vitro with/without S-9	46-48 hr	0, 50, 150, 500, 1500, 5000 µg/ml	Negative
2. Cytogenetics study	Human lymphocyte	In vitro with/without S-9	24 hr	538-840 with S9 750-950 w/o S9 µg/ml	Negative
3.Micronucleus study in mice	Male and female	po	48 hr	0, 500, 1000 or 2000 mg/kg	Negative

Carcinogenicity studies

For detail see APPENDIX/ATTACHMENTS: #1 (minutes of the Exec CAC)

Carcinogenicity studies in transgenic mice and SD rats were conducted in conformance with ICH Guidelines and in compliance with GLP regulations. Transgenic mice and SD rats are routinely used in carcinogenicity studies, and were the same strains utilized in pivotal repeat-dose toxicity studies with maraviroc. The carcinogenicity studies were adequately designed, and included verification of exposure at all dosage levels. Dose selection for the carcinogenicity studies was based on ICH guidelines.

In these studies, maraviroc was not found to be a carcinogen in rodents. Multiples of human exposures achieved in the carcinogenic studies are shown in Table 83

Table 83

Maraviroc results and exposures in carcinogenicity studies in rodents vs humans

Study No.	Study, species & route	Dose (mg/kg/day)	AUC (µg*hr/ml)	Multiples of human exposures (x)	Results
1.	26-wk transgenic mice, po	0			Negative
		200	25.8		
		800	52.5		
		1500	165		
2.	104-wk SD rats, po	0			Negative
		50	3.03	1	
		100	9.97	3	
		500	39.7	11	
		900	54.7	15	

Reproductive and developmental toxicology

A complete battery of reproductive toxicity studies was conducted with maraviroc. All pivotal studies were conducted in compliance with GLP regulations, were adequately designed, and met ICH guidelines. Reproduction toxicology studies indicate no effects on fertility at 1000 mg/kg/day. In addition, there was no effect on reproduction parameters or embryo-fetal development at 1000 mg/kg/day in rats and 75 mg/kg/day in rabbits. 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/day. 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/day. Multiples of human exposures achieved in the reproductive toxicology studies are shown in Table 84.

Table 84

Maraviroc exposures in reproductive toxicity studies in rats and rabbits vs humans

Study No.	Study, species & route	Dose (mg/kg/day)	NOEL/NOAEL (mg/kg/day)	AUC (µg*hr/ml) at NOEL/NOAEL	Multiples of human exposures (x)
1.	Seg 1 Rat male and female, po	100			
		300	300	35.77	7
		1000			
2.	Seg 2 Rat, po	100	300 for mother & 1000 for fetus	45.5 mother	9
		300			
		1000		102 fetus	20
3.	Seg 2 Rabbit, po	30	75 for mother and fetus		
		75		27.1	5
		200			
4.	Seg 3 Rat, po	100			
		300	300 for F1	13.13	3
		1000			

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: To support clinical use, the nonclinical toxicity profile of maraviroc was characterized in an extensive battery of in vitro and in vivo studies including carcinogenicity studies in rats and mice. The pivotal toxicology studies supporting the safety of maraviroc were appropriately designed and conducted in compliance with Good Laboratory Practice (GLP) regulations. Pharmacodynamic studies have adequately demonstrated the high potency and selectivity of maraviroc as a CCR5 antagonist agent for the treatment of HIV-1 infection. 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. A toxicology program was completed involving repeat-dose studies, which identified toxicological end-points, together with doses of maraviroc without adverse effects. Maraviroc had no adverse effects on fertility other than a statistically significant increase in pre-implantation loss at the high dose in rats, and has no teratogenic potential. Similarly maraviroc was shown not to be mutagenic or clastogenic in appropriate genetic toxicology assays. Carcinogenicity studies in rats and transgenic mice indicated no carcinogenic potential for humans. In conclusion, the results of extensive nonclinical toxicology and pharmacokinetic evaluation programs support the proposed use of maraviroc in humans.

Unresolved toxicology issues (if any): None

Recommendations: There are no nonclinical pharmacology and toxicology issues which would preclude the approval of this NDA.

Suggested labeling: The issue of labeling will be carried out separately.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

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APPENDIX/ATTACHMENTS:

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#1 Exec CAC minutes pertaining to the rat and mouse carcinogenicity studies

Food and Drug Administration Center for Drug Evaluation and Research Office of New Drugs
FACSIMILE TRANSMITTAL SHEET

DATE: September 1, 2004

To: Leilani V. Kapili

Company: Pfizer

Fax number: 860-732-0870

Phone number: 860-732-9969
From: Adele Seifried

HFD-024

Fax number: 301-480-8329

Phone number: 301-443-5344

Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report – IND 65,229

Total no. of pages including cover: 4

Comments:

Document to be mailed: " YES • NO

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Executive CAC Date of Meeting: August 25, 2004

Committee: Abigail (Abby) Jacobs, Ph.D., HFD-024, Acting Chair
Joseph Contrera, Ph.D., HFD-901, Member
Chuck Resnick, Ph.D., HFD-110, Alternate Member
Jeri El Hage, Ph.D., HFD-510, Alternate Member
Alex Jordan, Ph.D., HFD-530, Acting Team Leader
Pritam (Pete) Verma, Ph.D., HFD-530, Presenting Reviewer

Author of Minutes: Pete Verma

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review [IND 65,229; Submission # 043]

IND: 65,229 Drug Name: UK-427,857 Sponsor: Pfizer

Background:

UK-427,857 is an antagonist of the human chemokine receptor CCR5 and it is intended to help prevent the development and progression of AIDS in individuals positive for HIV-1. In vitro pharmacology studies showed that UK-427,857 is a reversible and selective antagonist of the human chemokine receptor CCR5 and inhibited its binding to endogenous chemokine ligands. Thus, the mode of action of UK-427,857 is to prevent HIV-1 entry into cells through binding to the CCR5 receptor.

Tg (ras)H2 Mouse 6-Month Carcinogenicity Protocol:

Notable design features: Hemizygous mice [CB6F-1/Jic – Tg (rasH2] will be used in the carcinogenicity study. At the start of the study, mice will be 8-9 weeks old and it was proposed that the treated groups will receive 200, 800 and 1500 mg/kg/day of UK-427,857 dissolved in methylcellulose as a 0.5% (w/v) aqueous solution containing 0.1% Tween 80 via esophageal intubation in a volume of 10 ml/kg once daily for 6 months according to an experimental design shown in Table 1. The positive control will be N-methyl-N-nitrosourea.

Table 1

Experimental design of the 6-month oral (gavage) carcinogenicity study in the transgenic and wild type (toxicokinetic analysis) mice The mice will be observed twice daily for mortality and morbidity. It is not clear whether the animals will be housed separately. The assessment of clinical signs, mass tracking, body weights and food consumption will be

performed on weekly basis. Full necropsies will be performed on animals found dead during the study, euthanized as moribund or euthanized at the end of the study. Hematology evaluations will be carried out on 25 animals/sex/dose at the study termination. For toxicokinetics, blood samples will be taken at 1, 3, 7 and 24 hr post dosing at termination. Histopathological examinations will be carried out on an extensive range of tissues in all animals from all groups. The study will be conducted according to the FDA GLP regulations.

Dosage (mg/kg/day)	Toxicity (transgenic mice)		Toxicokinetics (wild type)	
	Male	Female	Male	Female
0 (vehicle control)	25	25	-	-
Positive control	15	15	-	-
200 (low)	25	25	6	6
800 (mid)	25	25	6	6
1500 (high)	25	25	6	6

Transgenic Mouse Dose Selection

Doses proposed by the sponsor for the 6-month carcinogenicity study with UK-427,85 in mice were 0, 200, 800 and 1500 mg/kg/day, administered by oral gavage. These doses were chosen based on the results of the 4-week oral gavage dose range-finding study in transgenic mice.

The high dose of 1500 mg/kg/day was many times greater (226X in males and 87X in females based on AUC of parent drug) than that seen at the maximum therapeutic dose in humans. The low dose of 200 mg/kg/day is predicted to induce no treatment related findings and to provide a low multiple (4-fold, males) of the plasma exposure at the maximal therapeutic dose.

Executive CAC Recommendations and Conclusions:

The committee concurred that the basis of dose selection should be the limit dose. The limit dose applies only in cases where there is no evidence of genotoxicity, the rodent to human AUC exposures ratios are greater than 10-fold, and the maximum therapeutic dose does not exceed 500 mg/day. In the case of UK-427,857, the clinical dose is proposed to be 300 mg BID (600 mg/day). However, the limit dose of 1500 mg/kg/day was deemed sufficiently high given the large systemic rodent to human exposure (AUC) ratios, and granted that 300 mg bid remains as the maximum therapeutic dose. UK-427,857 was negative in a standard battery of genetic toxicology studies.

The Executive CAC concurred with doses of 0, 200, 800 and 1500 mg/kg/day for the 6-month study with UK-427,85 in the rasH2 transgenic mice, administered by oral gavage.

Comments:

Although not stated in the protocol, the sponsor should consider individual housing for the
animals in the study.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\

/Division File, HFD-530
/JFarrelly/Team leader, HFD-530
/PVerma/Reviewer, HFD-530
/MHolloman/CSO/PM, HFD-530
/ASeifried, HFD-024

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/s/

Jeri El Hage
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Food and Drug Administration Center for Drug Evaluation and Research Office of New Drugs

FACSIMILE TRANSMITTAL SHEET

DATE: December 4, 2003

To: Leilani V. Kapili

Company: Pfizer

Fax number: 860-732-0870

Phone number: 860-732-9967
From: Adele Seifried

HFD-024

Fax number: 301-480-8329

Phone number: 301-443-5344

Subject: Response to Carcinogenicity Special Protocol Assessment Request - Final CAC Report – IND 65,229

Total no. of pages including cover: 6

Comments:

Document to be mailed: " YES • NO

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Executive CAC Date of Meeting: December 2, 2003

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-540, Member
Bob Osterberg, Ph.D., HFD-520, Alternate Member
Jim Farrelly, Ph.D., HFD-530, Team Leader and Presenting reviewer

Author of Draft: Jim Farrelly

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

The committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogen bioassay, as this does not affect the sponsor's ability to initiate the bioassay. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following section E of the 'Guidance for Industry, Providing Regulatory Submission in Electronic Format, New Drug Application.'

IND # 65,229
Drug Name: UK-427,857

Sponsor: Pfizer

Background:

UK-427,857 is an antagonist of the human chemokine receptor CCR5 and it is intended to help prevent the development and progression of AIDS in individuals positive for HIV-1. In vitro pharmacology studies showed that UK-427,857 is a reversible and selective antagonist of the human chemokine receptor CCR5 and inhibited its binding to endogenous chemokine ligands. Thus, the mode of action of UK-427,857 is to prevent HIV-1 entry into cells through binding to the CCR5 receptor.

Rat 24-Month Carcinogenicity Study Protocol:

Notable design features: Sprague-Dawley rats [redacted]COBS-VAF-CD(SDBR(USA) will be used in the carcinogenicity study. At the start of the study, rats will be 6 weeks old and it was proposed that the treated groups will receive 50, 200 and 900 mg/kg/day of UK427,857 (dissolved in methylcellulose as a 0.5% (w/v) aqueous solution containing 0.14% (w/v) Tween 80) via esophageal intubation (10 ml/kg) once daily for 24 months according to a experimental design shown in Table 1. It is not clear whether the animals will be housed separately. Morbidity and mortality will be checked twice daily, clinical signs weekly, checking for palpable masses will be carried out monthly from month six, ophthalmology exams will be carried out predose and at one year, body weights will be determined predose, weekly for six months and then monthly, while food consumption will be evaluated weekly for six months and monthly thereafter. At 12-months, blood samples will be taken at 1, 3, 7 and 24 hr post dosing. At termination of the study, clinical chemistry, hematology and urinalysis evaluations will be carried out on 20 animals per group. At necropsy, blood smears will be prepared for all animals and morphology will be examined on five controls and five males and females at the high dose. An appropriate list of tissues are given for histological examination but it is not clear whether all dose groups will be examined. The study will be conducted according to the FDA GLP regulations.

Table 1

Proposed experimental design of the oral (gavage) 24-month carcinogenicity study in rats

Dosage group (mg/kg/day	Number of animals			
	Main group		Toxicokinetic group	
	Male	Female	Male	female
1-2. vehicle control	60	60		
3. low; 50	60	60	6	6
4. mid; 200	60	60	6	6
5. high; 900	60	60	6	6

Rat Carcinogenicity Study Dose Selection:

Doses proposed by the sponsor for the 2-year carcinogenicity study with UK-427,857 in rats were 50, 200, or 900 mg/kg/day, administered by oral gavage. These doses were chosen based on the results of the 39-week oral (gavage) toxicology study that included a 26 week dosing period and a 13-week reversibility period.

The high dose of 900 mg/kg/day was proposed based on hepatic changes (necrosis, multinucleated hepatocytes and bile duct vacuolation and hyperplasia, See Table 2). These treatment-related changes of the 39-week study were not considered sufficient to affect mortality of the animals in a 24-month carcinogenicity study based on limited non-adverse clinical signs and mild increases in liver enzymes.

Table 2

Incidence of hepatic changes in male and female rats at the end of the recovery phase

Findings	Dosage levels (mg/kg/day) n=10 animals/sex/group					
	control (0)		High (300)		Highest (900)	
	male	female	Male	female	male	female
Vacuolation, bile duct	0	0	3	1	3	0
Hyperplasia, bile duct	0	4	6	4	7	4
Altered cell foci	0	0	0	1	0	0
Multinucleated hepatocytes	0	1	0	1	3	1
Necrosis	0	1	1	0	2	3

Executive CAC Recommendations and Conclusions:

The reviewer proposed and the Executive CAC concurred with doses of 100, 500, and 900 mg/kg/day for the two-year study to have a more regular interval between doses (400 mg/kg/dose between the intermediate and the low and high dose) rather than 150 and 700 mg/kg/day between the high - mid and mid - low doses proposed by the sponsor.

The Committee noted that the study could have been proposed to be carried out using multiples of the AUC to set the high dose. UK-427,857 was negative in a standard battery of genetic toxicology studies and the AUC values in rats were substantially higher than those in the clinic at the proposed human doses. In this case, the sponsor must document the similarity of metabolism between the rodent and human, provide documentation on whether AUC comparisons are based on data for the parent, parent with metabolites or metabolites, take interspecies differences in protein binding into account and ensure that human data are derived from studies that use the maximum recommended human daily dose.

Comments:

If the sponsor plans histological evaluation of tissues from only control and high dose treatment groups, they will also need to conduct histopathologic examination of other dose groups under any of the following circumstances:

(a) for any macroscopic findings in the low and mid dose groups for a given tissue, they will need to look at that tissue for all of the dose groups

(b) for an increase in the incidence of tumors (rare or common) in the high dose group for a tissue, even if not statistically significant, they will also need to look at the next lower dose group

(c) for an increase in tumors in an organ for a tumor type that should be analyzed across tissue sites as well as by tissue site (e.g., hemangiosarcoma, lymphoma etc.; see McConnell et al, JNCI 76:283, 1986) they should look at all relevant tissues for that dose level and the next lower dose level,

(d) for an excessive decrease in body weight or survival in the examined dose group, they should examine lower dose groups.

Although not stated in the protocol, the sponsor should consider separate housing for the animals in the study. In addition, it should be pointed out that clinical chemistry, hematology and urinalysis evaluations need not be carried out during a two-year carcinogenicity study.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\ /Division File, HFD 530 /JFarrelly/Team leader, HFD-530 /PVerma/Reviewer, HFD-530 /SBelouin/CSO/PM, HFD-530 /ASeifried, HFD-024

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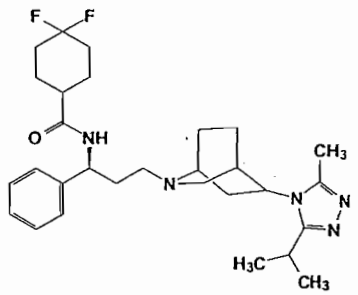
Pritam Verma
6/20/2007 09:16:18 AM
PHARMACOLOGIST

James Farrelly
6/20/2007 10:41:57 AM
PHARMACOLOGIST

REVIEW AND EVALUATION OF RAT CARCINOGENICITY STUDY

NDA: 22-128



Name of Drug: Maraviroc (UK-427,857)

Sponsor: Pfizer Global Research & Development
50 Pequot Ave
New London, CT 06320

Number of Studies: one (rat)

Reviewer: Pritam Verma, Ph.D.

Supervisor: James Farrelly, Ph.D.

Project Manager: Kenny Shade, J.D., B.S.N

Specific Submission for Linking Minutes: NDA 22-128.0003

Date for Exec CAC Meeting: February 20, 2007

STUDY IDENTIFICATION

Sponsor's Study Title: UK-427,857: 104-WEEK ORAL (GAVAGE) CARCINOGENICITY STUDY IN THE RAT

Study Number: 2003-0446

- Volume Numbers: Electronic
- Test Facility: [redacted]

Pfizer Global Research & Development, Ann Arbor, MI 48105

- Study Date initiation: March 23, 2004
- Date of Completion: March 31, 2006
- Date of Submission: December 22, 2006
- GLP Compliance: Yes
- QA Report: Yes
- Study Characteristics
- Reference to Dose-range-finding study: Yes

STUDY PROTOCOL DESIGN AND METHODS

- Study Type: 24-month carcinogenic study in rats; Gavage
- Species/strain of animals used: Rat, CD(SD)IGS BR (Sprague-Dawley) and the animals were purchased f [redacted]

- Number of animals per group: 60, details in Table 1.

Table 1
Experimental design of the 104-week oral (gavage) carcinogenicity study of UK-427,857 in the rat

Group Numbers	Dosage of UK-427,857 (mg/kg/day)	Toxicity animals		Toxicokinetics (only day 198)	
		Male	Female	Male	Female
1.	0 (vehicle control, 0.5% methylcellulose/0.1%Tween 80)	60	60	5	5
2.	50 (low)	60	60	5	5
3.	100 (mid)	60	60	5	5
4.	500 (high)	60	60	5	5
5.	900 (highest)	60	60	5	5
6.	0	60	60		

Group #6: Untreated control group. Data will be used for the testing facility historical database and were not used for the evaluation of the carcinogenic potential of UK-427,857.

- Age at start of study: 6-8 weeks
- Animal housing: Individual polycarbonate cages
- Drug Lot/Batch number(s): R105
- Drug Purity / Stability / Homogeneity [redacted] adequate

DOSES: 0, 50, 100, 500 or 900 mg/kg/day

Basis of Dose Selection: Doses of 50, 100, 500 and 900 mg/kg/day were selected for the present rat carcinogenicity study based on results from a previous 26-week oral toxicity study in rats. The high dose of 900 mg/kg/day represented the maximum tolerated dose, producing an approximate 10% decrease in male body weight when compared with control. Histopathologic changes in the liver (altered cell foci, multinucleated hepatocytes, and bile duct vacuolation and hyperplasia) and thyroid (follicular cell hypertrophy) supported this as a suitable high dose for assessing carcinogenic potential over a 104-week period. The toxicological findings were considered unlikely to affect the survival of the animals at this dose. The low dose of 50 mg/kg/day was expected to produce minor clinical signs, such as salivation, and possibly

bile duct vacuolation. Intermediate doses of 100 and 500 mg/kg/day were selected to explore the dose-response relationship over the dose range studied.

Once-daily dosing has been demonstrated to provide adequate systemic exposure in previous oral gavage toxicity studies in rats with UK-427,857. Two years is the accepted length for rat studies examining the carcinogenic potential of test compounds. The oral route was used for dosing as that is the intended clinical route in humans.

CAC Concurrence: Yes (Exec. CAC meeting of 12/2/03; doses of 100, 500 or 900 mg/kg/day were approved.)

Restriction Paradigm for Dietary Restriction Study: N/A
Route of Administration: gavage

- Frequency of Drug Administration: once daily
- Controls Employed: vehicle control (0.5% methylcellulose/0.1% Tween 80)
- Interim Sacrifices: No
- Satellite PK or Special Study Group(s): details in Table 1
- Unscheduled Sacrifices: No
- Deviations from Original Study Protocol: None

STUDY RESULTS AND FREQUENCY OF MONITORING

Observations and times:

Clinical signs: twice pretest; once weekly during the dosing phase

Body weights: weekly

Food consumption: weekly

Ophthalmology: once pretest and on day 366

Hematology & coagulation: days 92, 183 and 365

Clinical chemistry: days 92, 183 and 365

Toxicokinetics: blood collection points: 1, 3, 7 and 24 hr postdose on day 198

Organ weights: not done

Gross Pathology: at termination

Sponsor's statistical analysis: Appropriate statistical analyses were applied to body weight, body weight changes, food consumption, water consumption, clinical chemistry, hematology, and urinalysis from animals from Groups 1 to 5 using the statistical package

in the Xybion Path/Tox System. The Xybion Path/Tox System utilized Fisher's Least Significant Difference test (if the variances were equal) or Cochran-Cox modified t-test (if the variances were unequal).

RESULTS

Mortality: Due to the high level of mortality in the vehicle-control females (Group 1), all female groups (excepting Group 6) were terminated following 96 weeks of treatment. Treatment with UK-427,857 had no detrimental effects on survival. There was a statistically positive trend in female survival (increased survival) through the high dose group ($p=0.043$) with no statistically significant differences in male survival. Survival data are shown in Table 2.

Table 2
Survival of male and female rats following 104 and 96 weeks of treatment with UK-427,857, respectively

	Males					Females				
Doses (mg/kg/day)	0	50	100	500	900	0	50	100	500	900
% Survival	38	33	42	47	32	33	42	39	50	48

Clinical Observations: Treatment-related increases in the occurrence of urogenital staining were observed in males and females at doses of 500 and 900 mg/kg/day. Treatment-related hair loss in the anogenital region and/or the ventral surface of the body occurred in males at doses ≥500 mg/kg/day and in females at doses ≥100 mg/kg/day.

Body Weight: Treatment related statistically significant decreases in mean body weight were observed at most collection intervals in males at doses ≥500 mg/kg/day by Day 64. Treatment related statistically significant decreases in mean body weight in females at a dose of 900 mg/kg/day occurred at each collection interval from Days 232 to 505.

After approximately 1 year of treatment (Day 351), mean body weights in upper mid-dose (500 mg/kg/day) and high-dose (900 mg/kg/day) males were 5% and 12% lower, respectively, in comparison with control. The mean body weight in high-dose females was 7% lower in comparison with control. During the second year of dosing, body weights ranged 3% to 12% lower in upper mid-dose males, 12% to 18% lower in high-dose males, and 5% to 8% lower in high-dose females in comparison with control. Body weight (% differences from vehicle controls) male rats are shown in Table 3.

Table 3
Body weight (% differences from vehicle controls) male rats

Dosage (mg/kg/day)	Study day					
	15	323	449	540	624	722
0 (vehicle control)	-	-	-	-	-	-
50 (low)	0	-1	+1	-1	-2	0
100 (low)	+1	0	0	-1	-1	+1
500 (mid)	-1	-5	-8	-7	-8	-5
900 (high)	-1	-9	-14	-14	-17	-15

Food Consumption: Statistically significant increases in mean food consumption occurred almost weekly in males given UK-427,857 at the highest dose (900 mg/kg/day) and in females at doses ≥500 mg/kg/day from Day 15 through Day 127 when compared with control values and were considered treatment-related. The increases ranged from 1% to 10% in the males and from at least 4% to 15% in the females. Following Day 127, increases in mean food consumption were noted at almost each collection interval in females at the two higher doses, but these increases reached statistical significance less consistently. Feed consumed per day (% differences from vehicle controls) male rats are shown in Table 4.

Table 4
Feed consumed per day (% differences from vehicle controls) male rats

Dosage (mg/kg/day)	Study day					
	15	323	449	540	624	722
0 (vehicle control)	-	-	-	-	-	-
50 (low)	+1	-1	+3	+11	-3	+3
100 (low)	+3	-1	-2	+11	-2	+1
500 (mid)	+3	-2	+1	+11	-3	+9
900 (high)	+7	-1	-3	+9	-9	+6

Water Consumption: Dose-related increases in mean water consumption were observed in males and females at doses of ≥500 mg/kg/day in comparison with control at almost each collection interval throughout the first year of dosing and were considered treatment-related. After the first week of dosing (Day 8), these increases reached statistical significance in comparison with control at almost every collection interval for high-dose (900 mg/kg/day) animals. The increases ranged from 16% to 57% in high-dose males and from 24% to 102% in high-dose females. At the upper mid-dose (500 mg/kg/day) level, the increases reached statistical significance at many of the collection intervals and ranged from 11% to 45% in males (excepting on Day 18 when there was no change and on Day 22 when water consumption was decreased by 1%) and from 4% to 72% in females.

Ophthalmology: no treatment related findings were noted.

Hematology and coagulation: there were no meaningful differences in group mean or individual values related to treatment with UK-427,857.

Clinical Chemistry: there were no meaningful differences in group mean or individual values related to treatment with UK-427,857.

Urinalysis: there were no meaningful differences in group mean or individual values related to treatment with UK-427,857.

Toxicokinetics: data are shown in Table 5.

Table 5
Mean Plasma Toxicokinetic Parameters for UK-427,857 in Rat after Oral Administration for 198 days

Dosage (mg/kg/day)	Sex	Cmax (µg/L)	Tmax (h)	Mean AUC0-24 (µg*h/mL)	Multiples of human AUC achieved
50	Male	0.477	3	3.52	1
50	Female	0.459	3	2.55	<1
50	Overall	0.468	3	3.03	<1
100	Male	0.839	3	9.82	3
100	Female	1.70	3	10.1	3
100	Overall	1.27	3	9.97	3
500	Male	2.74	7	40.7	11
500	Female	4.26	3	38.7	11
500	Overall	3.49	3	39.7	11
900	Male	3.57	3	46.9	13
900	Female	4.12	3	63.1	18
900	Overall	3.84	3	54.7	15

Human Exposure: in the clinic (300 mg bid therapeutic dose) in human, AUCss value is 3.6 µg*hr/ml.

Mean $AUC_{0\text{-}24}$ (exposure) to UK-427,857 increased with dose over the dose range for both male and female rats. Mean C_{max} generally increased with dose over the dose range for male and female rats, except between the 500 and 900 mg/kg/day dose groups for females, where the observed mean C_{max} values appeared to be similar. The mean combined (male and female) $AUC_{0\text{-}24}$ following oral administration of 50, 100, 500, 900 mg/kg/day were 3.03, 9.97, 39.7 and 54.7 µg*h/mL, respectively.

There were no apparent sex-related differences observed for $AUC_{0\text{-}24}$ for the 50, 100 and 500 mg/kg/day dose groups, however, there may be a sex-related difference for the 900 mg/kg/day

dose group. The mean combined (male and female) C_{max} following oral administration of 50, 100, 500, 900 mg/kg/day were 0.468, 1.27, 3.49 and 3.84µg/mL, respectively. The T_{max} was typically 3 h. There were no sex-related differences observed for C_{max} and T_{max} over the study dose range.

Postmortem Observations

Necropsy Findings: Gross observations of possible importance were noted only at the terminal necropsy. The liver from high-dose (900 mg/kg/day) Group 5 male No. 515 was diffusely enlarged and dark, with firm nodules on all lobes. The liver from an additional high-dose male, No. 516 had a single small mass at the edge of the lateral lobe. A large irregular pale yellow cyst was noted on the surface of the papillary lobe of the liver from a Group 4 (500 mg/kg/day) female (No. 424).

Histopathology:

Neoplastic: changes of interest (incidence of benign adenoma) in thyroid gland are shown in (Table 6).

Table 6
Incidence of benign adenoma (thyroid gland) in the 2-year rat carcinogenicity study

Neoplastic change	Vehicle control		50 mg/kg/day (low)		100 mg/kg/day (mid)		500 mg/kg/day (high)		900 Mg/kg/day (highest)	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Total examined	60	60	60	60	60	59	60	60	60	58
Benign adenoma C-Cell	5	4	2	6	10	6	7	4	5	7
Benign adenoma follicular cell	0 (0%)	0 (0%)	2 (3%)	1 (2%)	1 (2%)	0 (0%)	1 (2%)	2 (3%)	5 (8%)	3 (5%)

[redacted]. In the male rat, incidence of benign adenoma follicular cell was 0% (vehicle control), 3% (low), 2% (mid), 2% (high) and 8% (highest), respectively. In the female rat, incidence of benign adenoma follicular cell was 0% (vehicle control), 2% (low), 0% (mid), 3% (high) and 5% (highest), respectively.

In the thirty recent control studies (SD rats, [redacted], incidence of thyroid: benign adenoma follicular cell was found to occur within 1.67% to 12%.

Thus, the incidence thyroid: benign adenoma follicular cell seen in the present study in rats was within the incidence values obtained for the neoplasm from the control studies conducted by [redacted]

No tumor incidences reached statistical significance in rats. Cholangiocarcinoma of liver is an extremely rare tumor type in SD rats. Cholangiocarcinomas were found in two male rats treated with 900 mg/kg/day (13 times the maximum recommended human dose based on AUC). Although, the occurrence was not statistically significant in a pairwise comparison with the vehicle control group.

Non-neoplastic changes: incidence of hypertrophy and/or hyperplasia of follicular cells (thyroid) are shown in Table 7.

Table 7
Incidence of hypertrophy and/or hyperplasia of follicular cells (thyroid gland) in the 2-year rat carcinogenicity study

Non-neoplastic change	Vehicle control		50 mg/kg/day (low)		100 mg/kg/day (mid)		500 mg/kg/day (high)		900 Mg/kg/day (highest)	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Total examined	60	60	60	60	60	59	60	60	60	58
Hypertrophy, follicular cells	2	0	2	2	5	0	15	4	20	16
Hyperplasia, follicular cells	0	2	1	0	3	0	3	0	8	3
Total	2	2	3	2	8	0	18	4	28	19
Percent Incidence	3%	3%	5%	3%	13%	0%	30%	7%	47%	33%

In the male rat, total incidence of non-neoplastic changes in thyroid gland was 3% (vehicle control), 5% (low), 13% (mid), 30% (high) and 47% (highest), respectively. In the female rat, total incidence of non-neoplastic changes was 3% (vehicle control), 3% (low), 0% (mid), 7% (high) and 33% (highest), respectively.

At the high dose in male and female rat, systemic exposure was 13 and 18-times the exposure in humans (300 mg bid dose), respectively.

OVERALL INTERPRETATION AND EVALUATION

Adequacy of the carcinogenicity studies and appropriateness of the test model:

MTD was achieved in this study. The dose levels of 100, 500 and 900 mg/kg/day were approved by the Exec CAC. In selection of dose levels, the sponsor relied upon both the toxicological endpoints and drug exposure in animals (from the 26-week rat toxicology study). The high dose of 900 mg/kg/day was proposed based on the hepatic changes (altered cell foci, bile duct hyperplasia, multinucleated hepatocytes) and thyroid follicular cell hypertrophy. The decrease in body weight (affecting males only) supported a conclusion that the MTD was 900 mg/kg/day. This dose level was expected to provide exposure (free fraction of drug) multiples of 52 and 298 as compared to clinical exposures at 100 mg bid and 300 mg bid, respectively.

This compound is moderately protein bound and the major route of excretion rats was fecal (79%) as compared to urine (12%), the majority of radioactivity being recovered within 24 hr. The major route of excretion in healthy human male subjects was fecal (76%) as compared to urine (20%). The major component in the plasma from human and mouse was unchanged UK-427,857 (42% human, 74% mouse). The most significant metabolites in human plasma were a mono-oxidized analogue of the N-dealkylation product (metabolite A, 11%) and a product of N-dealkylation (metabolite B, 22%); both were observed in mouse plasma (8% and 5%).

The carcinogenicity study in rat was considered to be acceptable.

Evidence of genotoxicity: Maraviroc was not genotoxic in the reverse mutation bacterial test (Ames test), mouse lymphoma or mouse in vivo micronucleus assays.

SUMMARY AND CONCLUSIONS

The oncogenicity potential of maraviroc was investigated in Sprague-Dawley rats with oral gavage dosages of 50 (low), 100 (mid), 500 (high) or 900 (highest) mg/kg/day in comparison with vehicle controls for a period of 104 weeks in males and 96 weeks in females. (All female groups were terminated early when survival in the female control group dropped to 33% (20 of 60 rats surviving to 96 weeks). The protocol was approved by the Exec CAC. The systemic exposures were 13 and 18 times that in humans (300 mg bid, AUCss = 3.6 μg*hr/ml) in male and female rat at the high dose level, respectively.

No significant increase in neoplasms was noted in male or female animals, although, an increased incidence of follicular cell adenoma of the thyroid was noted in males and females at 900 mg/kg/day. This was accompanied by a dose-related increase in follicular cell hyperplasia and hypertrophy at doses from 100 mg/kg/day in males and from 500 mg/kg/day in females. The tumor incidence was within the historical control range of this strain of rat and no follicular cell carcinomas were found in the thyroid gland.

Cholangiocarcinoma of the liver were found in two males (highest). As for the cholangiocarcinomas, apparently rare in the S-D rat, absence of a statistically significant difference between the incidences observed in the high dose and vehicle control groups, considered the finding less than sufficient to clearly implicate the drug.

APPENDIX LIST

ECAC Minutes: 12/04/2003, attached.

Sponsor's Incidence of Histopathology Findings: attached.

Body weight and food consumption changes vs. dose level: figures attached.

Statistical analysis: attached.

Historical control data set SD rats [redacted] attached.

List of Organs and Tissues Examined: attached

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/s/

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