レスクリプター錠 200mg
（メシル酸デラビルジン）
に関する資料

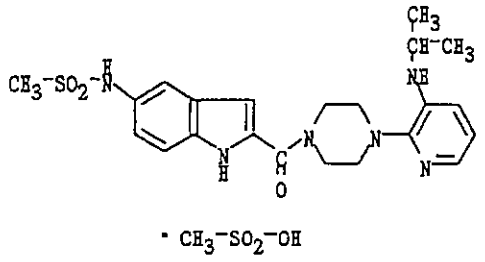
ワーナー・ランバート株式会社

2/1/4～2/1/22

B. PHARMACOLOGIC CLASS, SCIENTIFIC RATIONALE, INTENDED USE, AND CLINICAL BENEFITS

1. Pharmacologic Class

RESCRIPTOR® Tablets contain delavirdine mesylate, a synthetic non-nucleoside reverse transcriptase inhibitor of the human immunodeficiency virus type 1 (HIV-1). The chemical name of delavirdine is piperazine, 1-[3-[(1-methyl-ethyl)amino]-2-pyridinyl]-4-[[5-[(methylsulfonyl)amino]-1H-indol-2-yl]carbonyl]-, monomethanesulfonate. Its molecular formula is $C_{22}H_{28}N_6O_3S \bullet CH_4O_3S$ and its molecular weight is 552.68. The structural formula is:



Delavirdine mesylate is an odorless white-to-tan crystalline powder. The aqueous solubility of delavirdine free base at 23°C is 2942 µg/mL at pH 1.0, 295 µg/mL at pH 2.0, and 0.81 µg/mL at pH 7.4.

Mechanism of Action: Reverse transcriptase (RT), located in the core of the HIV-1 virus and released upon entry into the host cell, uses the viral RNA as a template to form single-stranded DNA. This is a pivotal step in the HIV-1 infection process. Delavirdine is a selective inhibitor of HIV-1 reverse transcriptase. Its selective action is exerted through a binding site on RT distinct from the deoxynucleoside triphosphate (dNTP) substrate binding site. RT inhibition by delavirdine differs from that of nucleoside analog RT inhibitors because it is not competitive with respect to the dNTP substrate. This noncompetitive binding to reverse transcriptase by delavirdine results in reduced enzyme activity and diminished viral replication.

2. Scientific Rationale

Acquired Immune Deficiency Syndrome (AIDS), a condition first described in the early 1980s, is now the leading cause of death in males 25 to 45 years of age in the USA, outstripping the combined mortality of cancer and head injury in that population. By the end of 1995, the Joint United Nations Program on HIV/AIDS (UNAIDS) estimated that some 20.1 million adults are living with HIV/AIDS, including over 11 million males and almost 9 million females. It is also estimated that over 6 million AIDS cases in adults and children have occurred world-wide since the epidemic began. UNAIDS foresees that this cumulative total will triple by the year 2000. Over 5 million adults and children are estimated to have died from AIDS so far

At present there is no cure for AIDS. Pharmacologic agents have been developed from three different classes of compounds (ie, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors and protease inhibitors) for use in treating Human Immunodeficiency Virus (HIV) infections. To date, five nucleoside reverse transcriptase inhibitors, one non-nucleoside reverse transcriptase inhibitor, and three protease inhibitors have received FDA approval for use in treating HIV infections.

2/1/25～2/1/31

3. Intended Use

The intended use of RESCRIPTOR tablets is in combination therapy with approved antiretroviral agents including nucleoside RTIs and protease inhibitors to treat HIV-1 infection in adults and adolescents greater than 13 years of age when antiretroviral therapy is warranted. Patients may be asymptomatic, symptomatic, or have AIDS.

4. Clinical Benefits

It has been demonstrated that when delavirdine mesylate was added to the treatment regimen of patients taking nucleoside analogues, patients experienced increased benefit (ie, increased CD4 counts and reduced viral burden) than nucleoside therapy alone.

2/1/33～2/1/43

C. FOREIGN MARKETING HISTORY

Delavirdine mesylate has no prior marketing history.

ロ．物理的化学的性質並びに規格及び試験方法
ハ．安定性

I．有効成分に関する理化学的知見

1．名　称

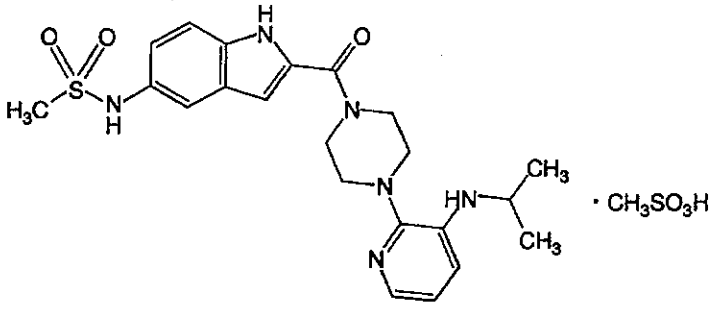
（1）一般名：メシル酸デラビルジン（delavirdine mesilate）

（2）化学名：1-{[3-(1-methylethyl)amino]pyridine-2-yl}-4-({[5-(methylsulfonyl)amino]-1*H*-indol-2-yl}carbonyl)piperazine monomethanesulfonate

（3）分子式：$C_{23}H_{32}N_6O_6S_2$

（4）分子量：552.67

（5）構造式：



2．物理的化学的性質

（1）性　状：本品は白色から黄褐色の粉末である．

（2）溶解性：本品はメタノールにやや溶けやすく，水に溶けにくく，アセトン，テトラヒドロフランに極めて溶けにくく，トルエンにほとんど溶けない．

（3）融解範囲：211～215℃

II．製剤学的事項

1．組成

1錠中にメシル酸デラビルジン200mgを含有する．

2．性　状

本品は白色～灰白色のフィルムコート錠である．

3．安定性

試　　験	保存条件	保存形態	保存期間	結　　果
加速試験	40℃／75％RH	プラスチック瓶（密栓）	6箇月	変化なし
長期保存試験	25℃／60％RH	プラスチック瓶（密栓）	18箇月[1)]	変化なし

1)長期保存試験は現在継続中．

E. NONCLINICAL PHARMACOLOGY AND TOXICOLOGY DATA

1. Introduction

Delavirdine (DLV) mesylate (RESCRIPTOR® Tablets; U-90152 free base [DLV] and E, S and T mesylate salt forms [DLV mesylate]) is a non-nucleoside reverse transcriptase (RT) inhibitor for use in the treatment of HIV-1 infection.

In this integrated summary, the delavirdine mesylate animal pharmacology/virology, toxicology and animal pharmacokinetics data are discussed and the safety of human administration assessed as it relates to the nonclinical data.

2. Pharmacology/Virology

Delavirdine mesylate is a potent, selective non-nucleoside inhibitor of HIV-1 reverse transcriptase (RT), reducing the infectivity of HIV-1 in cell culture. It selectively inhibits HIV-1 RT [2], exhibiting no significant inhibition of cellular DNA polymerase α and β, HIV-2 [3], or other retroviruses [4]. It acts through a binding site on RT distinct from the deoxynucleoside triphosphate (dNTP) substrate binding site. The RT inhibition by delavirdine differs from that of nucleoside analog RT inhibitors because it is not competitive with respect to the dNTP substrate. *In vitro* experiments have shown delavirdine mesylate to be a potent inhibitor of laboratory strains and clinical isolates of HIV-1 in various cell culture systems [2,5-7]. The potent inhibition of replication by delavirdine of both HIV-1 lymphotrophic and monocytotrophic strains was comparable to the antiviral activity of nucleoside or other non-nucleoside RT inhibitors. When delavirdine was used in combination with each of several other antiretroviral agents (zidovudine, didanosine, lamivudine, zalcitabine, alpha-interferon [IFN-α], and an experimental HIV protease inhibitor), a synergistic effect was observed in acute infection experiments using peripheral blood mononuclear cell cultures [8-13]. This strong antiviral efficacy coupled with effective oral delivery of

the drug clinically and an excellent safety profile *in vivo*, make delavirdine mesylate a potentially valuable component of a double or triple drug regimen.

3. Preclinical Safety

a. Overview

Animal safety studies conducted with delavirdine mesylate include single-dose to repeated-dose studies of up to 3 months in mice and cynomolgus monkeys, 6 months in rats and 1 year in dogs. The studies were conducted both as preliminary non-GLP studies (rats, dogs, monkeys, rabbits) and as definitive studies complying with GLPs (mice, rats, dogs, monkeys, rabbits) to support development and registration of delavirdine mesylate. All pivotal repeated-dose toxicity studies were conducted with drug administered as divided doses, two or three (3-month monkey toxicity study only) times daily. In addition, data regarding safety pharmacology, genetic toxicology, effects on reproduction and handler safety were assessed. The main route of administration used was oral (route of human administration) although the intravenous route was used in a limited number of repeated-dose studies; handler safety studies were conducted by topical routes or intratracheally. Oral, intravenous and *in vitro* studies were also conducted to attempt to understand the mechanism of action of delavirdine mesylate in specific toxicity issues.

The rat was selected as the standard species for acute toxicity testing. Repeated-dose studies utilized both rodent and nonrodent species in accordance with current testing theory and guidelines. Systemic exposure to oral delavirdine was assured based on pharmacokinetic data. Doses in all species were high enough to elicit toxicity, were administered by the route of human administration (oral), and included sufficient numbers of animals to judge significance of the findings in each study. Rat and mouse carcinogenicity studies are in progress to address the carcinogenic potential; however, results from a battery of genetic toxicology tests suggest that delavirdine mesylate does not pose a genotoxic hazard in humans.

Nonclinical studies demonstrate that toxic effects of delavirdine mesylate are expressed in the blood vessels, gastrointestinal tract, endocrine system, liver, kidneys, bone marrow, lymphoid tissue, lung, and reproductive system.

The toxicity of delavirdine mesylate in animals is complex and varied with species and pharmacokinetic differences between the species. The absolute oral bioavailability was dose- and species-dependent, being approximately 65%, 100% and 30% in the rat, dog and monkey, respectively. Dose-normalized systemic exposures to DLV-related material, following single oral dose administration of [^{14}C-carboxamide]DLV mesylate, increased in the order rat = rabbit < monkey < human < mouse < dog. Systemic exposure to delavirdine in repeated-dose toxicity studies, on a mg/kg/day basis, increased in the order mouse < rabbit ≤ monkey ≤ rat < dog. Thus, the highest systemic drug levels were experienced in the dog studies. The systemic concentrations of drug to which animals were exposed were generally much higher than those in humans. Keeping these facts in mind aids the review of the various preclinical toxicities described for delavirdine mesylate.

b. Acute Toxicity

Single oral doses of delavirdine mesylate or delavirdine free base at 5000 mg/kg or less in the rat were not lethal and were practically nontoxic providing a wide margin of safety (250 times the recommended human dose) if the total daily dose were inadvertently consumed as a single dose **[14-15]**.

c. Mortalities

Drug-related mortalities (deaths/unscheduled sacrifices) in repeated-dose toxicity studies were associated with high nadir drug serum concentrations. Deaths/sacrifices in mice were attributed to dosing (intubation) problems and not to delavirdine mesylate **[16]**. In rats, drug-related death/unscheduled sacrifice was attributed to multiple organ toxicity at very high dose levels and high mean nadir serum concentrations (≥ 750 mg/kg/day; > 160 µM) **[17,18]**. In dogs **[19-24]** and monkeys **[25]**, the main cause of death/unscheduled sacrifice was gastrointestinal toxicity and associated gastric/duodenal erosions/ulcerations (≥ 50 mg/kg/day [> 180 µM] in dogs, ≥ 240 mg/kg/day [> 190 µM] in monkeys).

d. Delavirdine-Associated Vasculitis/Perivasculitis

Spontaneously occurring vasculitis in beagle dogs has been recognized since 1978 **[26]**. The syndrome of spontaneous vasculitis in beagle dogs has been referred to as polyarteritis **[26,27]**, necrotizing vasculitis **[28-30]**, periarteritis **[31]**, idiopathic febrile necrotizing arteritis **[32]**, beagle pain syndrome **[32]**, panarteritis **[33]** and canine juvenile polyarteritis **[34,35]**. Small- to medium-sized muscular arteries are most often affected and the coronary arteries or their branches are the most commonly affected arteries. Spontaneous vasculitis in beagle dogs can complicate the interpretation of toxicity studies **[30,32,33]**. It has been postulated that necrotizing vasculitis in beagle dogs is a latent condition which can be induced in predisposed dogs by treatment with experimental compounds **[32]**. During the conduct of preclinical toxicity studies with delavirdine mesylate, vasculitis/perivasculitis, a morphologic lesion which is indistinguishable from spontaneous vasculitis, occurred in all oral dog studies when high nadir serum concentrations of delavirdine were achieved.

High doses of delavirdine mesylate (≥ 60 mg/kg/day) given orally to mongrel or beagle dogs for 1 to 3 months resulted in delavirdine-associated vasculitis/perivasculitis in most dogs within 30 days of dose initiation (Table E.1) **[20,21,23]**. A dose of 50 mg/kg/day given orally to beagle dogs for 6 months or 1 year resulted in few dogs with delavirdine-associated vasculitis/perivasculitis and the days of sacrifice for these dogs were variable (study days 29, 101 or 371) **[22,36]**. Mean nadir serum concentrations of delavirdine were generally > 180 µM and in most cases > 270 µM in dogs with vasculitis/perivasculitis. All dogs with delavirdine-associated vasculitis/perivasculitis in the 1-month to 1-year studies had severe clinical signs related to gastrointestinal toxicity; however, there was no association between vasculitis/perivasculitis and gastrointestinal toxicity.

Table E.1. Incidence[a] of Vasculitis/Perivasculitis in Dogs Given Delavirdine Mesylate Orally

Technical Report Number	Study Type	Dose (mg/kg/day)[b]				
		50	76/60	120	150	350
7227-92-034	2-week dose range-finding beagle dog	2/4 (50%)	--	--	4/4 (100%)	4/4 (100%)
7227-92-060	4-week GLP beagle dog	--	--	7/8 (89%)	--	--
7227-95-001	4-week GLP mongrel dog	0/6 (0%)	--	5/6 (83%)	--	--
7227-94-004	3-month GLP beagle dog	--	11/22 (50%)	--	--	--
7226-95-019	6-month GLP beagle dog	1/20 (5%)	--	--	--	--
7227-96-008	1-year GLP beagle dog	2/18 (11%)	--	--	--	--
7227-95-016	4 week experimental beagle dog - immune response	--	--	6/6 (100%)	--	--
7227-95-019	4 week experimental beagle dog - reversibility of vasculitis/perivasculitis	--	--	--	15/16 (94%)	--
7227-96-005	2-week experimental beagle dog - ultrastructural evaluation of vasculitis	--	--	--	3/4 (75%)	--
TOTAL		5/48 (10%)	11/22 (50%)	18/20 (90%)	22/24 (92%)	4/4 (100%)

[a] Number affected/number in group (percent affected)
[b] Delavirdine-associated vasculitis/perivasculitis has not been observed in dogs at oral doses of delavirdine mesylate < 50 mg/kg/day

Coronary arteries of the heart were the most frequently affected arteries. Delavirdine-associated vasculitis/perivasculitis was segmental (focal) and randomly distributed without a defined distribution or site of predilection. All tunics and the entire circumference of the artery wall were affected (transmural and circumferential involvement). Development of the vascular lesions was not synchronous within individual dogs or between dogs. The initial lesion in the development of delavirdine-associated vasculitis/perivasculitis was within the tunica intima. The microscopic characteristics of delavirdine-associated vasculitis/perivasculitis were indistinguishable from spontaneous vasculitis in beagle dogs [35]. Delavirdine-associated vasculitis/perivasculitis was reversible, although the process was not complete within 81 days after the last oral dose of delavirdine mesylate. The primary morphologic change following recovery from delavirdine-associated vasculitis/perivasculitis was a thickening of the tunica intima caused by infiltration/proliferation of smooth muscle cells within the subendothelial space [22].

There was no evidence of an immune-mediated mechanism in the development of delavirdine-associated vasculitis/perivasculitis. The immunologic parameters evaluated included tests/assays for cell-mediated immunity to delavirdine, formation of antibodies to delavirdine, circulating immune complexes, canine immunoglobulin deposits within blood vessels with delavirdine-associated vasculitis/perivasculitis and serum protein electrophoresis **[22,37]**.

Sporadic vascular lesions in species other than the dog occurred in one cynomolgus monkey treated orally with delavirdine mesylate **[25]** and two Dutch-belted rabbits treated orally with delavirdine (free base) **[38]**. The monkey had vasculitis/ perivasculitis in one small muscular (coronary) artery of the heart and the two rabbits had acute inflammation within veins (phlebitis) of the lungs. It is unlikely that the finding of vasculitis/perivasculitis in this monkey was related to treatment with delavirdine mesylate for the following reasons: 1) Only one small muscular artery in one monkey given delavirdine mesylate had vasculitis/perivasculitis; 2) monkeys with signs of toxicity and higher mean nadir serum concentrations of delavirdine (211 µM to 358 µM) did not have vasculitis/perivasculitis (the monkey with vasculitis/ perivasculitis had no signs of toxicity and a mean nadir serum concentration of 138 µM); 3) vasculitis (asymptomatic polyarteritis) of small- and medium-sized muscular arteries (including coronary arteries of the heart) of unknown etiology has been documented in a cynomolgus monkey **[39]**. The morphologic characteristics of asymptomatic polyarteritis as described in the literature were similar to those of the vasculitis/perivasculitis that occurred in the delavirdine mesylate-treated monkey.

However, although unlikely, the possibility that the vasculitis/perivasculitis in the delavirdine mesylate-treated monkey was related to drug administration cannot be ruled out. This was also the opinion of an international panel of multidisciplinary scientists (see Appendix A of Item 5). They concluded that the potential relationship between vasculitis/perivasculitis in this monkey and treatment with delavirdine mesylate could not be ruled out, but that this single case of vasculitis/perivasculitis could not be taken as evidence of drug-induced vasculitis since vasculitis (asymptomatic polyarteritis) has been recorded as a spontaneously occurring lesion in the cynomolgus monkey **[39]**.

Similarly, the potential relationship of acute inflammation within veins (phlebitis) of the lungs of two rabbits to administration of delavirdine is not known. It is unlikely that this finding is related to drug administration for the following reasons: 1) delavirdine-associated vasculitis/perivasculitis in dogs is present in small- to medium-sized muscular arteries of various tissues, whereas only veins of the lungs were affected in the two rabbits; 2) the vascular change in the rabbits (acute phlebitis) was different from those found in dogs (primarily necrotizing vasculitis/perivasculitis); 3) both rabbits died because of delavirdine-induced toxicity; the microscopic findings within veins of the lungs could represent agonal changes; 4) five additional rabbits from the same dose group which died or were sacrificed because of delavirdine-induced toxicity did not have inflammation within veins of the lungs.

Vasculitis/perivasculitis has not been observed in rats treated orally with delavirdine mesylate even though high mean nadir serum concentrations of delavirdine (> 300 µM) have been achieved in individual rats **[17]**.

In conclusion, vasculitis/perivasculitis occurred in all oral dog studies with delavirdine mesylate when high (generally > 180 µM) nadir serum concentrations of delavirdine were achieved. The dog is the only species for which there is a clearly established association between treatment with delavirdine mesylate and the development of vasculitis/perivasculitis. It was the opinion of an international panel of multidisciplinary scientists that delavirdine-associated vasculitis/perivasculitis appeared to be specific to the dog

A complete review of delavirdine-associated vasculitis/perivasculitis is in Item

e. Gastrointestinal Tract Lesions

The gastrointestinal tract was a target organ for the toxicity of orally administered delavirdine mesylate in dogs, monkeys and rats. In most instances, gastrointestinal toxicity was the primary cause of death or unscheduled sacrifice of delavirdine mesylate-treated dogs and monkeys.

Clinical signs of gastrointestinal toxicity included anorexia, emesis, dehydration and weakness in dogs and monkeys. Additional clinical signs included mucoid and blood-containing feces and body weight loss in dogs and distended abdomen in monkeys **[20-23,25,36]**. Anorexia was frequently the first indicator of delavirdine mesylate toxicity in both species. Clinical signs directly related to the gastrointestinal tract, except for decreased food consumption and decreased body weight gain, have not been observed in the rat **[17,18,40]**.

In dogs, signs of gastrointestinal toxicity were related to serum concentrations of delavirdine. When high oral doses (≥ 60 mg/kg/day) of delavirdine mesylate were given to dogs for 1 to 3 months, resulting in high serum concentrations of delavirdine, signs of gastrointestinal toxicity were usually observed within 6 days of dose initiation and most affected individual dogs were sacrificed within 3 weeks of dose initiation **[20,21,23]**. Lower doses of delavirdine mesylate (≤ 50 mg/kg/day) in long-term studies (6 months to 1 year) resulted in periods of time with gastrointestinal toxicity interspersed between periods of time without toxicity in individual dogs. These periods correlated with serum concentrations of delavirdine, which were > 180 µM during the times of adverse clinical signs related to the gastrointestinal tract **[22,36]**.

Altered serum electrolyte values were associated with anorexia, emesis and/or diarrhea in affected dogs and monkeys.

The primary gross and microscopic lesions in dogs were erosions and ulcers within the upper gastrointestinal tract, including the pyloric portion of the stomach and the proximal duodenum. The most severe lesion was frequently within the proximal duodenum, where ulcerations were observed. Additional gross/microscopic findings included hemorrhage and erosions which could be present in any portion of the gastrointestinal tract. Vasculitis/perivasculitis has not been observed at the sites of gastrointestinal hemorrhage, erosions or ulcers in affected dogs.

In monkeys, ulceration of the proximal duodenum was the cause of death or clinical deterioration and unscheduled sacrifice in the 3-month toxicity study **[25]**. Gastric

erosions/ulcers were also present. Nadir serum concentrations of delavirdine at or near the time of death or unscheduled sacrifice were > 190 µM (mean nadir serum concentrations were ≥ 142 µM).

The gastrointestinal tract was also a target tissue in dogs when delavirdine mesylate was given as a continuous intravenous infusion for 1 or 3 months **[24]**. Signs of toxicity included inappetence and diarrhea in most dogs and emesis in some dogs. Gross and microscopic findings included mucosal hemorrhage throughout the gastrointestinal tract and duodenal ulcers.

It is unlikely that direct irritation of orally administered delavirdine mesylate was totally responsible for lesions present in the gastrointestinal tract of dogs and monkeys. This conclusion is based on the observation that intermittent clinical signs of gastrointestinal toxicity in the long-term studies in dogs correlated with high serum concentrations of delavirdine rather than the oral dose of delavirdine mesylate (mg/kg/day) **[22,36]**. In addition, similar gastrointestinal toxicity was observed in dogs given delavirdine mesylate intravenously **[24]**.

Dogs and monkeys have very high concentrations of delavirdine and desalkyl (N-desisopropyl) delavirdine in bile. Mean concentrations of delavirdine in bile were > 1800 µM in dogs (50 mg/kg/day) and monkeys (≥ 240 mg/kg/day). Mean concentrations of desalkyl delavirdine in bile were higher in monkeys than in dogs (> 5700 µM and > 950 µM, respectively) **[41]**. High concentrations of delavirdine and/or delavirdine metabolites in bile deposited in the proximal duodenum and possible reflux of bile into the stomach during emesis may be related to delavirdine mesylate-induced gastrointestinal toxicity in dogs and monkeys.

Most female rats given delavirdine mesylate at a dose of 1500 mg/kg/day for up to 15 days had erosions/ulcers within the stomach without additional lesions in other portions of the gastrointestinal tract **[17]**. Mean nadir serum concentrations of delavirdine were > 175 µM. In studies of 3 months' duration or longer, hyperplasia of the squamous epithelium of the nonglandular portion of the stomach was observed in individual male and female rats given delavirdine mesylate at doses of ≥ 125 mg/kg/day **[18,40]**. These groups had mean nadir serum concentrations of delavirdine that ranged from 18.1±19.00 µM to 174.0±81.0 µM (see Table C-4 in Appendix C of Item 5).

In conclusion, the gastrointestinal tract was a target organ for the toxicity of orally administered delavirdine mesylate in dogs, monkeys and rats and intravenously administered delavirdine mesylate in dogs. In most instances, gastrointestinal toxicity was the primary cause of death or unscheduled sacrifice of delavirdine mesylate-treated dogs and monkeys. The mechanism of delavirdine mesylate-induced gastrointestinal toxicity is not known; however, gastrointestinal toxicity was related to high serum concentrations of delavirdine (> 175 µM) and delavirdine and/or its metabolites in bile may be involved in the dog and monkey.

f. Endocrine Organ Effects

Numerous endocrine-related changes were observed in repeated-dose toxicity studies with delavirdine mesylate, affecting a variety of organs, including the thyroid gland,

the adrenal gland, reproductive organs and the pituitary gland. Several investigational studies were conducted to elucidate the mechanism(s) of these effects. As the hypothalamic-pituitary axis serves as a common denominator in the regulation of these and other endocrine organs, the most logical hypothesis to explain these effects involved disruption of those feedback control mechanisms, with resultant end organ stimulation by -tropic hormones.

1) Thyroid Hypertrophy/Hyperplasia

In studies of 14 days or longer in rats, administration of delavirdine mesylate resulted in increased thyroid gland weights and histopathological evidence of thyroid follicular cell hypertrophy/hyperplasia **[17,40,42]**. A number of studies were conducted in rats to elucidate the potential mechanism for thyroid hypertrophy/hyperplasia noted in the repeat-dose toxicology studies. In studies with levothyroxine supplementation, the delavirdine mesylate-dependent decrease in circulating thyroxine (T_4) levels and elevated thyrotropin (TSH) levels **[43]** were blocked, as were the increase in thyroid gland weight and microscopic evidence of follicular cell hypertrophy **[44]**. The delavirdine mesylate-dependent increases in thyroid weight and follicular cell hypertrophy were also completely blocked in a study using hypophysectomized animals **[45]**. These data clearly indicate that the effects of delavirdine mesylate on the thyroid gland are compensatory in nature, involving normal regulatory mechanisms, in an attempt to maintain a "euthyroid" state. *In vitro* studies indicate that the initial decrease in circulating T_4 concentration is secondary to enhanced clearance as the glucuronide conjugate **[46,47]**, and not an effect of either delavirdine mesylate, or its principal metabolite, desalkyl delavirdine (U-96183), on thyroid hormone biosynthesis **[48]**. In rats, formation of the glucuronide is a primary clearance pathway for thyroid hormone. In contrast, humans are very efficient iodine scavengers and approximately 85% of thyroxine is cleared via deiodination **[49]**, thus compounds which affect thyroid hormone clearance in rats are less likely to do so in humans.

The importance of these data lies in the fact that chronic stimulation of the rat thyroid gland by TSH leads to thyroid adenomas, some of which may progress to exhibit malignant features **[50-52]**. It is reasonable to anticipate that thyroid neoplasms will be observed in rodent carcinogenicity studies, which are currently in progress. As indicated above, differences in thyroid hormone clearance pathways suggest that changes observed in the rat thyroid gland are unlikely to be relevant to humans. Additionally, a substantial body of epidemiological evidence indicates that minimal risk of thyroid carcinoma exists in humans following long-term treatment with agents which induce tumors in rats by this mechanism. Further, in humans there is "strong epidemiological evidence against prolonged stimulation of the thyroid glands of subjects in iodine-deficient areas being associated with increased development of thyroid carcinoma" **[49]**. Thus, the human risk appears minimal even under circumstances in which TSH would be upregulated. An additional indication of the lack of clinical relevance of the thyroid effects comes from the analysis of preliminary (unaudited) data from Protocols M/3331/0040 and M/3331/0046, which indicates that neither T_4 nor TSH were significantly affected by delavirdine mesylate administration (400 mg t.i.d.) for one month (M/3331/0040: report in progress; M/3331/0046: study ongoing).

In summary, given the differences in clearance pathways and negative human epidemiology data regarding other compounds/circumstances which induced identical thyroid changes in rodents, coupled with preliminary confirmation that delavirdine mesylate does not affect the relevant parameters in clinical trials, the effects observed in the rodent thyroid gland are unlikely to translate to a significant human health risk.

2) Adrenal Effects

Increased adrenal gland weights and/or evidence of cortical cell hypertrophy/ hyperplasia was observed following administration of delavirdine mesylate to rats for 14 or more days **[17,40,42]**, to dogs for 4 or more weeks **[20,23]** or to monkeys for 3 months **[25]**. A series of *in vivo* modulation studies was conducted to investigate whether a similar feedback stimulation, by corticotropin (ACTH), could explain the adrenal cortical cell hypertrophy noted in delavirdine mesylate rat and dog toxicity studies. Animals receiving delavirdine mesylate alone exhibited increases in plasma ACTH levels and adrenal gland weight, with histopathological evidence of cortical cell hypertrophy in the zona fasciculata (site of corticosteroid biosynthesis) **[53]**. Supplementation with prednisolone (5, 10, or 20 mg/day), blocked all delavirdine mesylate-dependent adrenal changes, including the increase in adrenal gland weight and adrenal cortical cell hypertrophy **[53]**. A trend toward a decrease in serum corticosterone was observed, but was not statistically significant, presumably due to compensation by the hypertrophied adrenal gland. Similarly, use of hypophysectomized animals also blocked the delavirdine mesylate-induced increase in adrenal gland weight and histopathological evidence of cortical cell hypertrophy **[45]**. Taken together, these studies indicate that delavirdine mesylate-dependent adrenal effects were mediated by compensatory responses of the normal feedback control mechanisms.

In contrast to the case with the thyroid gland changes, discussed above, no literature precedent could be found for initial decreases in circulating glucocorticoid levels occurring secondary to enhanced clearance. However, several chemically diverse compounds that inhibit one or more steps in steroid biosynthetic pathways have been described **[54-56]**. Included among these compounds are ketoconazole, metyrapone and aminobenzotriazole. The effects of delavirdine mesylate, or its principal metabolite, desalkyl delavirdine (U-96183), on two mitochondrial P450 enzymes, P450 cholesterol side chain cleavage and steroid 11β-hydroxylase, as well as the microsomal P450, steroid 21-hydroxylase, were assessed. Delavirdine mesylate inhibited steroid 21-hydroxylase, with an IC_{50} of approximately 50 μM **[57]**. Such inhibition of 21-hydroxylation can completely account for the adrenal enlargement observed in repeated-dose preclinical toxicity studies, by inhibiting steroidogenesis, which effects a compensatory increase in corticotropin secretion, thus stimulating adrenal growth.

Similar compensatory changes have been noted for other agents which inhibit steroid biosynthetic pathways. As an example, ketoconazole, a clinically used antifungal agent, inhibits adrenal steroid 11β-hydroxylase, P450 cholesterol side chain cleavage, and with lesser potency, steroid 21-hydroxylase activities **[58-61]** resulting in decreased corticosteroid levels. A compensatory increase in corticotropin secretion induces adrenal hypertrophy **[62,63]**. In chronic studies this inhibition is overcome by the hypertrophic adrenal gland, reestablishing an euadrenal state **[62]**. From a risk

assessment perspective, ketoconazole is negative in rodent carcinogenicity assays [64], further suggesting that the compensatory responses to such an effect are self-limiting.

The likelihood of significant risk to humans is considered small since 1) the observed adrenal effects involve compensatory stimulation wherein normal regulatory control mechanisms remain intact; 2) numerous clinically used compounds induce adrenal changes by this mechanism; and 3) the apparent self-limiting nature of these changes.

3) Gonadal Effects

Increased testicular weight in rats [18,40], Leydig cell hyperplasia in dogs [23], decreased seminal vesicle weight in rats [18] and increased ovarian weight in female rats [18], were noted in repeated-dose toxicity studies. While no specific studies were conducted to address these issues, it is logical to speculate that similar mechanisms (inhibition of steroid biosynthesis) may explain the gonadal effects of delavirdine mesylate. To again use ketoconazole as an analogy, inhibition of cytochrome P450 enzymes involved in androgen biosynthesis by this compound [55,65,66], induces a compensatory increase in gonadotropin (LH and FSH) secretion [67-69]. In fact, such inhibition accounts for the clinical utility of this compound versus prostate carcinoma [55,67,70,71]. Chronic stimulation by LH has been invoked as a mechanism for induction of Leydig cell hyperplasia and ultimately Leydig cell tumors in rats [72], while Leydig cell tumors are very rare in man. Increased testicular weight has been reported following FSH administration [73]. Additionally, LH- and/or hCG-dependent increases in testicular weight, secondary to increased interstitial fluid, have been reported [74], although increased testicular weight has not been consistently seen following administration of compounds which induce hypersecretion of LH [72,75]. As an androgen-dependent tissue, seminal vesicle weight would be expected to decline, again an effect that has been reported for ketoconazole [69], when androgen biosynthesis is inhibited. Similar effects on seminal vesicle weights have been observed with the antiandrogen, linuron [72]. Increased ovarian weights may be due to the activity of FSH, which may or may not also be a compensatory response to inhibited steroid biosynthesis, as estrogen exerts negative feedback control on FSH release.

Thus, the mechanism of the effects in reproductive organs remains unconfirmed, and the risk to human health cannot be definitively estimated. However, it should be noted that these effects were generally confined to animals receiving doses well in excess of those used clinically, and were reversible on cessation of drug administration.

4) Pituitary Effects

In studies of 1 month or longer in rats, delavirdine mesylate induced hypertrophy of the basophilic cells of the pituitary gland [40,42], which, at least in longer-term studies, was accompanied by a significant increase in pituitary gland weights. Similar increases in pituitary gland weights were observed in female dogs and female cynomolgous monkeys in 3-month studies [24,25]. Such changes are entirely consistent with upregulation of -tropic hormone secretion as basophilic cells of the anterior pituitary are comprised of the three cell types one would reasonably expect to be upregulated given the observations already discussed, ie, Thyrotrophs, Corticotrophs and Gonadotrophs [76].

Thus, the effects of delavirdine mesylate on the pituitary gland in pre-clinical studies appear to be compensatory responses to effects on other endocrine organs (discussed above) as opposed to direct effects of the drug. As such, the probability of significant human health risk is considered low.

g. Pseudopregnancy in Mice

Oral delavirdine mesylate administration in mice produces clinical signs (vaginal discharge) and morphologic changes in the reproductive tissues and mammary glands typical of pseudopregnancy at doses of 250 and 500 mg/kg/day when administered twice daily for 3 months [16]. These changes did not revert to normal by the end of the 1-month reversibility period. Similar changes were not evident in rat [18] or monkey studies [25, 77]. The one incidence of pseudopregnancy in dogs occurred on the 1-year toxicity study and was considered to be an incidental finding [36]. The mechanism for pseudopregnancy induction is the unique capacity of the mouse to generate a metabolite, N-isopropylpyridinepiperazine designated U-88703, which has a high affinity for the dopamine-D2 receptor [78], is a pure receptor antagonist [79], reaches high levels in the brain [80,81], and stimulates prolactin secretion [82]. The species-specific nature of the metabolism of delavirdine indicates that similar hormonal effects would not be expected in other species including humans. Thus, the occurrence of pseudopregnancy related to delavirdine mesylate administration was considered a species-specific effect in the mouse and not of relevance to human administration.

The marked hormonal changes induced in mice by delavirdine mesylate treatment, in particular the increase in prolactin secretion, can be expected to have a significant effect on the results of chronic toxicity studies conducted in mice. Compounds which stimulate increased prolactin secretion such as reserpine and neuroleptic compounds have been shown to increase the incidence of hormonally dependant tumors such as mammary gland neoplasms in mice. A large published data base indicates that humans are not at risk of a similar carcinogenic response due to this hormonal mechanism.

h. Deposition of Delavirdine Crystals in Tissues

Crystals of delavirdine (anhydrous free base) with or without associated inflammation (foreign body granulomas) have been observed in tissues of rats and dogs after prolonged exposure to high serum concentrations of delavirdine.

Individual rats given delavirdine mesylate orally at doses of ≥ 300 mg/kg/day for 3 months [18] or ≥ 175 mg/kg/day for 6 months [40] had deposition of crystals and associated inflammation in multiple tissues including lip, tongue, salivary glands, esophagus, gastrointestinal tract, lymphoid tissues, heart, bone marrow and lungs. Similar deposition of crystals in tissues was not observed in 2-week or 1-month oral studies. The crystals in tissues were determined to be anhydrous delavirdine free base [18]. Deposition of delavirdine crystals in tissues was most likely related to maximum serum concentrations of delavirdine. The group mean steady state maximum serum concentrations of delavirdine for male and female rats given delavirdine mesylate at a dose of 175 mg/kg/day for 6 months were 130±30 µM and 180±40 µM, respectively. The mean steady state nadir serum concentration of delavirdine in individual rats

with delavirdine crystals in tissues was 141 µM with a range of 56 µM to 277 µM. The concentration of soluble delavirdine in pooled rat serum *in vitro* (equilibration for 72 hours at 37°C) was 72±1 µM **[83]**. The insoluble form of delavirdine in pooled rat serum *in vitro* after equilibration for 72 hours was anhydrous delavirdine free base, the same form found in rat tissues. Resolution of the deposition of delavirdine crystals and associated inflammation in tissues was not complete within a 2-month reversibility phase.

Dogs given escalating doses of delavirdine mesylate via continuous intravenous infusion for 3 months had multiple rhomboid/round/oval clear spaces and associated granulomatous inflammation in lymphoid tissues (including lymphoid tissues of ileum), liver (Kupffer cells), bone marrow, heart and tongue **[24]**. Although not identified by analytical methods, these clear spaces were interpreted to be sites of delavirdine crystal deposition. The mean serum concentrations of delavirdine in these dogs increased as the dose of delavirdine mesylate increased until mean serum concentrations of delavirdine plateaued at approximately 270 µM to 300 µM. The finding of delavirdine crystals in tissues and the plateauing of serum concentrations of delavirdine suggests that the carrying capacity of the blood had been exceeded. The concentration of soluble delavirdine in pooled dog serum *in vitro* (equilibration for 72 hours at 37°C) was 48±0 µM **[83]**. Deposition of delavirdine crystals in tissues was not observed in dogs in oral toxicity studies of up to 1 year's duration when individual dogs had mean nadir steady state concentrations of > 175 µM **[36]** or in dogs in a 1-month continuous intravenous infusion study when individual dogs had mean serum concentrations of > 300 µM **[24]**.

The concentration of soluble delavirdine in pooled human serum *in vitro* (equilibration for 72 hours at 37°C) was higher (116±6 µM) than the concentration in pooled rat or dog serum **[83]**. Because of the higher solubility of delavirdine in pooled human serum and the low serum concentrations of delavirdine achieved with therapeutic use of delavirdine mesylate in humans, it is unlikely that deposition of delavirdine crystals in tissues will occur in humans.

In conclusion, deposition of delavirdine crystals with or without associated inflammation has been observed in tissues of rats given delavirdine mesylate orally for ≥ 3 months and in dogs given delavirdine mesylate as a continuous intravenous infusion for 3 months. It is likely that tissue deposition of delavirdine crystals was related to prolonged exposure to high serum concentrations of delavirdine and the relative insolubility of delavirdine in the serum of these species. Based on the relatively higher *in vitro* solubility of delavirdine in pooled human serum and the low serum concentrations of delavirdine achieved with therapeutic doses of delavirdine mesylate in humans, it is unlikely that deposition of delavirdine crystals in tissues will occur in humans. It was also the opinion of an international panel of multidisciplinary scientists that deposition of delavirdine crystals in tissues was associated with high serum concentrations of delavirdine and that crystal deposition is unlikely to be a consequence of the therapeutic use of delavirdine mesylate in humans

i. Effects on the Liver

The liver was a target organ for the toxicity of orally administered delavirdine mesylate primarily in rodents and dogs. Hepatocellular hypertrophy and liver weight increases were present in rats and mice. Both rats and dogs had biochemical and microscopic changes that indicated an effect on biliary function suggestive of bile stasis. Hepatic toxicity was increased in pregnant rats compared to nonpregnant rats.

Rats administered delavirdine mesylate in repeated-dose oral toxicity studies of 2 weeks' to 6 months' duration had treatment-related increases in liver weight and periacinar hepatocellular hypertrophy **[17,18,40,42]**. The hepatic changes in rats on the 3-month and 6-month studies were present at doses of ≥ 58 mg/kg/day. The rats in these groups had mean nadir serum concentrations of delavirdine that ranged from 5.12±4.7 µM to 174.0±81.0 µM (see Table C-4 in Appendix C of Item 5). Liver weight increases that were present in male and female rats on the 3-month and 6-month studies at doses of ≥ 125 mg/kg/day were not reversible in the female rats on either study after a 2-month reversibility phase. The delavirdine mean nadir serum concentrations for female rats in these groups ranged from 37.6±19.0 µM to 162.0±88.0 µM. Likewise, the hepatocellular hypertrophy that was present in male and female rats at doses of ≥ 175 mg/kg/day in these two studies was not reversible in female rats on the 6-month study. The delavirdine mean nadir serum concentrations for these groups of female rats ranged from 85.9±36.0 µM to 162.0±88.0 µM.

The presence of increased liver weights and periacinar hepatocellular hypertrophy in rats did not correlate with increased serum alanine aminotransferase or aspartate aminotransferase, except in the 3-month study at doses of ≥ 300 mg/kg/day.

Hepatic toxicity was increased in pregnant rats at late gestation/parturition compared to nonpregnant rats in the repeated-dose toxicity studies. Moderate-to-severe acute coagulative necrosis of the periacinar hepatocytes was present in pregnant females administered 200 mg/kg/day, a dose level associated with delavirdine maximum and minimum serum concentrations of 180±29 µM and 71±20 µM, respectively **[84]**. Hepatic toxicity was also seen in pregnant rabbits at doses of ≥ 200 mg/kg/day **[38]**.

Less remarkable liver changes were observed in repeated-dose oral toxicity studies in mice (3 months) **[16]**, dogs (3 months to 1 year) **[22,23,36]** and monkeys (3 months) **[25]**. In mice, liver weights were increased (≥ 125 mg/kg/day; mean nadir serum concentrations < 1 µM) and hepatocellular hypertrophy was present (500 mg/kg/day) but both changes were reversible during a 1-month reversibility phase. Individual dogs and monkeys with marked clinical responses to delavirdine mesylate tended to have increased liver weight values and elevated serum liver enzymes. However, the few statistically significant liver weight increases for dogs and monkeys that were present after the completion of the dosing phase were not correlated with microscopic change.

Delavirdine mesylate-treated rats and dogs had biochemical and microscopic changes that indicated an effect on biliary function suggestive of bile stasis. Both species had increased serum alkaline phosphatase (rat ≥ 125 mg/kg/day; dog 50 mg/kg/day) and rats had increased gamma glutamyl transpeptidase (≥ 300 mg/kg/day). The serum enzyme changes were reversible. Bile duct hyperplasia was observed in rats at doses

of ≥ 100 mg/kg/day for ≥ 3 months. The change was reversible, except in rats given delavirdine mesylate at doses of ≥ 175 mg/kg/day for 6 months with a 2-month reversibility phase. Retention of bile pigment in the liver was present and not reversible in 1 month in dogs at doses of ≥ 30 mg/kg/day for 6 months and 1 year. Bilirubin excretion into the urine was increased in dogs on the 1-year study at doses of ≥ 30 mg/kg/day.

Several other liver-related serum biochemical changes were observed. Serum cholesterol levels were reversibly increased in all species examined and at low nadir serum concentrations of delavirdine (mean nadir serum concentrations of ≥ 31 µM in monkeys, ≥ 2.5 µM in dogs and < 1 µM in rats and mice). Plasma fibrinogen concentrations were reversibly increased in all dog studies at doses of ≥ 50 mg/kg/day. Serum bilirubin levels, both direct and indirect, were reversibly increased in all species except the mouse. In addition to altered hepatic function, the increased indirect bilirubin may also have resulted from red blood cell destruction, since in rats and dogs, the elevated indirect bilirubin levels correlated with reduced red blood cell counts. Serum albumin was decreased in rats, dogs and monkeys, especially at the high delavirdine mesylate doses. In dogs, the decreased serum albumin may have been related to anorexia, but may, in part, also have been the result of hepatic functional changes. In monkeys, orally administered delavirdine mesylate resulted in decreased sulfobromophthalein clearance [85].

In conclusion, delavirdine mesylate administration to rats resulted in increased liver weights, periacinar hepatocellular hypertrophy and bile duct hyperplasia with no apparent microscopic changes in hepatocellular integrity after chronic administration. Chronic delavirdine mesylate administration to dogs resulted in evidence of bile stasis and changes in several liver-related biochemical parameters. Serum cholesterol was reversibly increased in all species and at most dose levels. Hepatic toxicity was increased in pregnant rats compared to nonpregnant rats.

j. Effects on the Kidneys

The kidney was a target organ for the toxicity of orally administered delavirdine mesylate in rats, the only species in which consistent treatment-related effects on kidneys or kidney function occurred. Treatment-related renal toxicity was also observed in pregnant rabbits.

Increased kidney weights (≥ 58 mg/kg/day), hyperplasia of the transitional epithelial cells of the renal pelvis (≥ 50 mg/kg/day) and/or an increased incidence of progressive nephropathy (≥ 58 mg/kg/day) were present in rats given delavirdine mesylate for 1, 3 or 6 months. These groups had mean nadir serum concentrations of delavirdine that ranged from 0.913±1.50 µM to 174.0±81.00 µM (see Table C-4 in Appendix C of Item 5). After a 2-month reversibility phase, kidney weights were still increased in female rats of the ≥ 125 mg/kg/day groups. The mean nadir serum concentrations of delavirdine for these groups were ≥ 37.6 µM. Serum electrolyte changes included decreased chloride (≥ 50 mg/kg/day), sodium (≥ 750 mg/kg/day) and calcium (≥ 175 mg/kg/day) and increased inorganic phosphorus (≥ 50 mg/kg/day). Most mean electrolyte values were within normal ranges but were statistically significantly different from control values. Increased water consumption (polydipsia), urine volume

(polyuria) and excretion of chloride and potassium in the urine and decreased urine specific gravity were present at doses of ≥ 175 mg/kg/day **[18,40,42]**.

The microscopic findings in affected kidneys were not considered to be of sufficient severity to be responsible for the increased kidney weights or changes related to kidney function (polydipsia/polyuria, altered serum electrolytes and increased excretion of electrolytes in urine). Increased kidney mass (weight) is typically due to enlargement (hypertrophy) of cells of the nephron (primarily cells of the proximal convoluted tubules) rather than cell proliferation **[86]**. The increased kidney weights in delavirdine mesylate-treated rats were likely the result of compensatory cellular hypertrophy in response to altered renal function. These functional changes were most likely related to altered endocrine control mechanisms and not a direct effect of delavirdine on the kidneys. Findings that could have been related to altered endocrine control of renal function included: 1) polydipsia and polyuria; 2) decreased serum calcium with concurrent increased inorganic phosphorus (parathyroid hormone); and 3) decreased serum chloride and increased excretion of potassium and chloride in urine (aldosterone) **[87]**.

Renal toxicity was present in pregnant rats and rabbits in the reproductive toxicology studies. Rats given delavirdine mesylate orally at a dose of 200 mg/kg/day during late gestation and parturition had marked maternal toxicity including premature deliveries and dystocia; all affected females died or were sacrificed *in extremis*. Microscopic findings in affected females were consistent with ischemic injury to the kidneys (thrombosis within glomerular capillaries and acute proximal tubular necrosis). Treatment with delavirdine mesylate at a dose of 200 mg/kg/day during late gestation/parturition apparently altered the normal physiologic changes occurring at that time, resulting in premature deliveries, dystocia and marked toxicity. Mean maximum and minimum serum concentrations of delavirdine associated with renal toxicity at late gestation/parturition were 180±29 and 71±20 µM, respectively **[84]**.

In pregnant rabbits given delavirdine (free base) orally on gestation days 6 through 20, marked maternal toxicity occurred at doses of 200 and 400 mg/kg/day. Rabbits which died or were sacrificed *in extremis* had tubular nephritis characterized by degeneration/necrosis of individual tubular cells with simultaneous tubular cell hyperplasia **[38]**. Mean minimum serum concentrations of delavirdine for females of the 200 and 400 mg/kg/day groups on dose day 15 were 84±110 and 100±80 µM, respectively.

There were no consistent treatment-related effects on kidneys or kidney function in dogs or monkeys given delavirdine mesylate orally. Serum chemistry changes related to kidney function, increased kidney weights or microscopic findings in kidneys were occasionally but inconsistently present in individual dogs or monkeys **[20-23,25,36]**.

In conclusion, the kidney was a target organ for the toxicity of orally administered delavirdine mesylate in rats as indicated by increased kidney weights; microscopic findings; altered serum electrolytes; and increased water consumption, urine volume and urinary electrolyte excretion. Pregnant rats and rabbits with delavirdine-induced toxicity had microscopic findings of renal tubular necrosis. There were no consistent treatment-related changes indicative of renal toxicity in dogs or monkeys.

k. Effects on Peripheral Blood and Bone Marrow Hematology Parameters and Lymphoid Tissues

Effects of oral delavirdine mesylate on peripheral blood and bone marrow hematology parameters and lymphoid tissues occurred in dogs, monkeys and rats. The type and severity of the effects depended upon the species, serum concentration of delavirdine attained in each species and individual animal susceptibility to delavirdine mesylate-induced toxicity.

The most frequent peripheral blood hematology findings in dogs sacrificed (unscheduled) within 3 weeks of dose initiation because of severe toxicity included increased red blood cell (RBC) parameters (RBC numbers, hematocrit values, hemoglobin concentrations, mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume) and increased white blood cell (WBC) numbers. Dehydration was the most likely cause of the increased RBC parameters and the changes in WBCs were consistent with a stress (steroid) response (increased WBCs, neutrophils and monocytes and decreased lymphocytes and eosinophils) **[19-21,23]**. In contrast, peripheral blood hematology findings in other dogs sacrificed (unscheduled) within 4 weeks of dose initiation because of severe toxicity included decreased WBCs and/or platelets **[20,22,23]**. Irrespective of increased or decreased hematology parameters in the peripheral blood of these dogs, the microscopic findings within the bone marrow were the same and included decreased cellularity, hemorrhage, necrosis and/or increased myeloid to erythroid ratio with increased immature cells of the myeloid series. Delavirdine mesylate at doses of ≥ 50 mg/kg/day and mean nadir serum concentrations of delavirdine of ≥ 250 µM were associated with the aforementioned findings.

Peripheral blood and bone marrow hematology findings in other dogs given delavirdine mesylate at doses of ≥ 30 mg/kg/day for 3 months to 1 year were variable **[22,23,36]**. Transient peripheral blood hematology changes included decreased RBC parameters, decreased WBC numbers (neutropenia) and/or decreased platelet numbers in individual dogs. These peripheral blood hematology changes were present when there were signs of gastrointestinal toxicity and they returned to normal values while the dogs were still receiving delavirdine mesylate. Decreased RBC parameters were likely due to hemorrhage within the gastrointestinal tract. Nadir serum concentrations of delavirdine generally approached or exceeded 200 µM during times of abnormal peripheral blood hematology findings. Minimal-to-mild myelofibrosis was present in the bone marrow of several dogs at the scheduled sacrifices of the 1-year study, suggesting that recovery from delavirdine mesylate-induced effects was accompanied by fibrosis in the bone marrow.

A finding with a low incidence in studies of 3 months or longer at doses of ≥ 30 mg/kg/day was pancytopenia (decreased RBCs, WBCs and platelets) observed in two dogs of the 3-month and two dogs of the 1-year toxicity studies **[23,36]**. The primary microscopic finding within the bone marrow was increased cellularity. Dose administration of delavirdine mesylate for the two dogs of the 1-year study was stopped when neutropenia became severe. Recovery from the anemia, neutropenia and thrombocytopenia occurred within 1 to 2 weeks after stopping dose administration. Upon rechallenge with delavirdine mesylate, these dogs again became neutropenic within 2 to 3 weeks. The mechanism of the pancytopenia is not known;

however, preliminary data suggest that the mechanism is not a humoral immune-mediated process. Mean nadir serum concentrations of delavirdine were ≥ 37 µM (range of 37 to 145 µM) for these four affected dogs.

In monkeys sacrificed (unscheduled) during a 3-month toxicity study because of severe toxicity, peripheral blood hematology changes included decreased RBC parameters and/or decreased numbers of WBCs. Microscopic bone marrow hematology findings in monkeys which died or were sacrificed because of severe toxicity included decreased cellularity and increased myeloid to erythroid ratio with increased immature cells of the myeloid series. These changes were present in individual monkeys given high doses of delavirdine mesylate (≥ 240 mg/kg/day) which had high mean nadir serum concentrations of delavirdine (> 140 µM) **[25]**.

Decreased RBC parameters were occasionally but inconsistently present in male and female monkeys given delavirdine mesylate at doses of ≥ 240 mg/kg/day for 1 or 3 months **[25,77]**.

The primary peripheral blood hematology finding in male and female rats given delavirdine mesylate at doses of ≥ 100 mg/kg/day was macrocytic regenerative anemia **[18,40]**. The mean nadir serum concentrations of delavirdine in these groups of rats ranged from 18.1±19.00 µM to 174.0±81.0 µM (see Table C-4 in Appendix C of Item 5). The maximum decrease of RBC numbers compared to respective control values was 23% in rats given delavirdine mesylate at a dose of 750 mg/kg/day for 3 months. Erythroid regeneration was characterized by increased numbers of reticulocytes, increased cellularity of the erythroid series in the bone marrow and increased extramedullary hematopoiesis (spleen weights were increased in association with the increased extramedullary hematopoiesis). The regenerative anemia was most likely the result of peripheral RBC destruction or loss, rather than bone marrow suppression. After a 2-month reversibility phase, RBC parameters were still decreased in female rats at doses of ≥ 125 mg/kg/day although there was a clear trend toward reversal of the effects at all dose levels. Mean nadir serum concentration of delavirdine for female rats of the 125 mg/kg/day dose group was 37.6±19 µM.

Delavirdine mesylate-induced hematology findings in mice given delavirdine mesylate orally for 3 months have not been observed; however, mean serum concentrations of delavirdine in mice (mean maximum concentration ≤ 115 µM and mean nadir concentration < 12 µM) were generally lower than other species **[16]**.

Male and female rats given delavirdine mesylate at doses of ≥ 300 mg/kg/day for 3 months or ≥ 58 mg/kg/day for 6 months had increased peripheral blood lymphocyte numbers. Mean nadir serum concentrations of delavirdine in male and female rats given delavirdine mesylate at a dose of 58 mg/kg/day were 5.12±4.7 and 14.4±8.5 µM, respectively **[18,40]**.

In dogs and monkeys, atrophy of lymphoid tissues (including lymph nodes, lymphoid tissues of the intestinal tract, thymus and spleen) was present when high doses of delavirdine were given (≥ 50 mg/kg/day in dogs and ≥ 240 mg/kg/day in monkeys). Most of the affected animals were sacrificed because of overt signs of delavirdine mesylate-induced toxicity.

In conclusion, delavirdine mesylate treatment was associated with changes in peripheral blood and bone marrow in individual monkeys and dogs. Peripheral blood and bone marrow hematology findings in dogs were variable, with increases or decreases in the same parameter present in individual animals of the same dose group. The hematology changes in dogs occurred most often when there were other signs of toxicity. Decreases in peripheral blood and bone marrow hematology parameters were present in monkeys at times of severe toxicity. Regenerative anemia was the primary finding in delavirdine mesylate-treated rats. No delavirdine mesylate-induced hematology findings have been observed in mice. In dogs and monkeys, atrophy of lymphoid tissues was present at times of severe toxicity.

l. Effects on the Lungs

Delavirdine mesylate-associated changes in the lung included histiocytosis in rats and inflammation in dogs. Histiocytosis was present at doses of ≥ 58 mg/kg/day for 6 months and was not reversible following a 2-month reversibility phase at doses of ≥ 175 mg/kg/day **[40]**. The mean nadir serum concentrations of delavirdine for rats administered 175 mg/kg/day were 67.9±28.0 µM and 85.9±36.0 µM, in males and females, respectively (see Table C-4 in Appendix C of Item 5). Inflammation in the lungs was present in individual dogs which had severe signs of toxicity associated with oral delavirdine mesylate treatment. Descriptive terms used for the lung lesions included acute inflammation (infiltrates of neutrophils and alveolar macrophages), pyogranulomatous inflammation and bronchopneumonia. Special stains did not reveal an infectious agent and the lesions were considered to be a toxic manifestation of treatment with delavirdine mesylate. Inflammation in the lungs was present in individual dogs given delavirdine mesylate at doses of 150 mg/kg/day for 2 weeks **[19]**, 120 mg/kg/day for 1 month **[20,21]**, 76/60 mg/kg/day for 3 months **[23]** and 50 mg/kg/day for 6 months **[22]** and 1 year **[36]**. Dogs with delavirdine-associated inflammation in the lungs were sacrificed between days 8 and 101 and had mean nadir serum concentrations of > 180 µM.

Treatment-related changes in the lung were not observed in monkeys given delavirdine mesylate orally at doses of ≥ 240 mg/kg/day for 3 months **[25]**.

In conclusion, delavirdine mesylate-associated changes in the lung included histiocytosis in rats and inflammation in dogs. Histiocytosis was reversible in rats at doses ≤ 100 mg/kg/day. Inflammation was present only in individual dogs that also had severe signs of toxicity.

m. Effects on Reproduction

Reproductive toxicology studies have been completed in Sprague-Dawley rats and Dutch-belted rabbits. In rats, oral administration of delavirdine mesylate resulted in marked toxicity in F_0 pregnant females during late gestation/parturition and a low incidence of septal defects (interventricular septal defects) in F_1 fetuses and pups.

When delavirdine mesylate was given orally to male and female rats at doses of 0, 20, 100 and 200 mg/kg/day from prior to cohabitation through gestation day 13, there were no effects on male or female fertility (estrous cycling, mating, preimplantation embryo development and implantation) **[84]**. The no-observed-adverse-effect level

(NOAEL) was 20 mg/kg/day. However, when delavirdine mesylate was given orally to female rats at a dose of 200 mg/kg/day from prior to cohabitation through lactation, there was marked maternal toxicity during late gestation/parturition including premature deliveries and dystocia; all affected females died or were sacrificed *in extremis*. Microscopic findings in affected females were consistent with ischemic injury to the liver and kidneys. Similar findings were not present in pregnant rats given delavirdine mesylate orally at a dose of 100 mg/kg/day **[84]** or in pregnant rats given delavirdine mesylate at a dose of 200 mg/kg/day when exposure was limited to gestation days 6 through 15 **[88]**. Treatment with delavirdine mesylate during late gestation/parturition apparently altered the normal physiologic changes occurring at that time, resulting in premature deliveries, dystocia and marked toxicity. Mean maximum and minimum serum concentrations of delavirdine associated with marked toxicity at late gestation/parturition at a dose of 200 mg/kg/day were 180±29 and 71±20 µM, respectively.

The incidence of septal defects was increased in F_1 fetuses and pups from F_0 female rats given delavirdine mesylate at doses of 50 to 200 mg/kg/day. The primary malformation was interventricular septal defect. In the rat fetus, the interventricular septum normally closes by gestation day 17. Interventricular septal defects are more common in the membranous septum than in the muscular septum and most defects are located immediately below the aortic valve **[89]**. The normal incidence of membranous interventricular septal defects in fetuses (gestation days 20 or 21) of control Sprague-Dawley rats is 0.15% **[90]**.

Table E.2 gives the incidence of heart malformations in fetuses (gestation day 20) and pups (postpartum days 0 through 21) from F_0 female rats given delavirdine mesylate. A dose of 200 mg/kg/day given to F_0 female rats caused a 4.3% overall incidence of septal defects in F_1 offspring (range of 2.0% to 6.7%). The overall incidence of heart malformations at doses of 50 and 100 mg/kg/day was 0.2% and 0.3%, respectively, which is approximately equal to the published incidence (0.15%) of interventricular septal defects in fetuses of control Sprague-Dawley rats. However, because similar septal defects were not observed in any fetuses or pups of the control or low-dose (20 or 25 mg/kg/day) groups and because there was an increased incidence of similar septal defects in fetuses and pups of the 200 mg/kg/day dose groups, the septal defects in 1 pup of the 50 mg/kg/day dose group and in 1 fetus and 1 pup of the 100 mg/kg/day dose group may have been treatment-related. Mean nadir (prior to a.m. dose) serum concentrations of delavirdine in F_0 female rats at dose levels associated with septal defects in F_1 offspring were 71±20 µM on dose day 14 **[84]** and 110±40 µM on dose day 10 **[88]** for the 200 mg/kg/day groups, 12±6 µM **[91]** and 24±11 µM **[88]** on dose day 10 for the 100 mg/kg/day groups and 4.0±2.7 µM **[91]** on dose day 10 for the 50 mg/kg/day group.

Table E.2. Incidence[a] of Heart Malformations in F_1 Offspring from F_0 Female Sprague-Dawley Rats Given Delavirdine Mesylate Orally

Study		Dose				
		0 mg/kg/day	20 or 25 mg/kg/day	50 mg/kg/day	100 mg/kg/day	200 mg/kg/day
Segment I[b] (TR 7227-94-024)	F_1 Pups	0/139 (0%)	0/139 (0%)	–	0/145 (0%)	10/149 (6.7%)[c]
Segment II[d] (TR 7227-95-030)	F_1 Fetuses	0/142 (0%)	–	0/138 (0%)	1/143 (0.7%)[e]	5/122 (4.1%)[e]
	F_1 Pups	0/154 (0%)	–	0/179 (0%)	0/166 (0%)	3/153 (2.0%)[f]
Segment III[g] (TR 7227-96-004)	F_1 Pups	0/151 (0%)	0/155 (0%)	1/153 (0.7%)[h]	1/151 (0.7%)[i]	–
Total		0/586 (0%)	0/294 (0%)	1/470 (0.2%)	2/605 (0.3%)	18/424 (4.3%)

[a] Number affected/number examined (percent affected)
[b] Segment I (study of fertility and general reproductive performance): F_0 female rats were dosed for 14 days prior to cohabitation and during cohabitation, gestation and lactation through postpartum day 20
[c] Includes 9 F_1 pups with interventricular septal defects on postpartum days 0, 1, 2, 3 or 4 and 1 F_1 pup with an interventricular septal defect on postpartum day 21
[d] Segment II (study of effects on embryo-fetal development [teratology] and postnatal development): F_0 female rats were dosed on gestation days 6 through 15
[e] Includes interventricular septal defects in F_1 fetuses on gestation day 20
[f] Includes (one each) an interventricular septal defect, imperforate interventricular septal defect and atrial septal defect with associated malformations in F_1 pups on postpartum day 21
[g] Segment III (study of effects on prenatal and postnatal development, including maternal function): F_0 female rats were dosed from gestation day 6 through remaining gestation and during lactation through postpartum day 20
[h] Includes one F_1 pup with interventricular septal defect and associated heart malformations on postpartum day 21
[i] Includes one F_1 pup with heart malformation other than septal defect on postpartum day 21

Additional findings in the rat reproductive toxicology studies included reduced pup survival on postpartum day 0 at a dose of 100 mg/kg/day **[84]** and embryotoxicity, developmental delay and reduced pup survival (postpartum day 0) at a dose of 200 mg/kg/day **[88]**. Learning and memory, survival and reproductive parameters of the F_1 generation were not affected when F_0 female rats were given delavirdine mesylate at oral doses of 0, 25, 50 and 100 mg/kg/day from gestation day 6 through lactation **[91]**. The NOAEL for the rat reproductive toxicology studies was 25 mg/kg/day.

In an embryo-fetal development (teratology) study, pregnant rabbits were given delavirdine (free base) orally at doses of 0, 100, 200 and 400 mg/kg/day on gestation days 6 through 20 **[38]**. Marked maternal and embryo toxicity occurred at doses of 200 and 400 mg/kg/day. Treatment-related malformations were not apparent at any dose; however, only a limited number of fetuses were available for examination in the 200 and 400 mg/kg/day groups because of the maternal and embryo toxicity. Mean minimum serum concentrations of delavirdine for females of the 200 and

400 mg/kg/day groups on dose day 15 were 84±110 and 100±80 µM, respectively. The NOAEL for maternal and embryo-fetal developmental toxicity in the pregnant rabbit was 100 mg/kg/day.

In conclusion, delavirdine mesylate was teratogenic (septal defects) in rats at a dose of 200 mg/kg/day. Delavirdine mesylate was possibly teratogenic at doses of 50 and 100 mg/kg/day based on one case of septal defect at each of these doses. No evidence of teratogenic effects was apparent in rabbits. There were no effects on reproduction or development through gestation day 13 at any dose in rats; however, marked maternal toxicity was present when delavirdine mesylate was given at a dose of 200 mg/kg/day to female rats during late gestation and parturition. Based on these findings, the potential benefit of therapy in pregnant women must be weighed against potential risk to the mother and fetus.

n. Mutagenic Potential

A battery of genetic toxicology tests was conducted with delavirdine mesylate including three Ames assays **[92-94]**, a mammalian cell mutation assay **[95]**, an *in vitro* cytogenetics (chromosome aberration) assay in human peripheral lymphocytes **[96]**, three unscheduled DNA synthesis (UDS) assays **[97-99]**, and a micronucleus test in mouse bone marrow **[100]**. The results were negative for mutagenic potential in all tests except the initial Ames assay **[92]**, in which Lot#(A)26162-MAL-29 tested positive in one strain (TA-98) of *Salmonella typhimurium* at concentrations of 500 µg/plate and higher only in the presence of metabolic (S9) activation. The results were negative in the four other *Salmonella* strains. The positive results were probably due to a process-related impurity. This could not be confirmed since insufficient material from the original lot remained for chemical or biological testing. A second Ames assay with delavirdine mesylate was conducted with a different lot of drug (Lot#(A)26162-MAL-45) **[94]**. The results were negative in the absence and presence of metabolic (S9) activation. A third Ames assay was then conducted on another lot (Lot#(B2)1500-0081-JLH-114) and the results were again negative in the absence and presence of metabolic (S9) activation **[93]**. Based on these subsequent assays, the results in the initial assay were considered to be due to a process-related impurity. Thus, delavirdine mesylate is considered to be nonmutagenic in the Ames assay.

The results with delavirdine mesylate do not support a designation of this drug as a mutagen. However, it is pertinent to point out that several well-known and widely used drugs have been shown to exhibit mutagenic properties. For example, many marketed quinolone antibiotics are bacterial mutagens. Ciprofloxacin is positive in the UDS assay and the mouse lymphoma cell forward gene mutation assay **[101]**. Azidothymidine (AZT) is positive in the Ames assay in one *Salmonella typhimurium* strain, TA-102. Other drugs, notably metronidazole, have shown mutagenic activity in a number of *in vitro* genetic toxicology tests; another of these drugs, metronitizide, is also known as a carcinogen in rodents **[102]**. Furthermore, the immunosuppressive drug cyclophosphamide is used in non-life-threatening diseases such as rheumatoid arthritis and kidney disease **[103]** despite the fact that it is a potent positive control in many genetic toxicology tests.

In conclusion, the results of collective genetic toxicology tests conducted with delavirdine mesylate suggest that delavirdine mesylate does not pose a genotoxic hazard in humans.

o. Safety Pharmacology

Slight and transient increases in heart rate and the rate of left ventricular pressure/ time (dP/dt)/P were associated with single delavirdine mesylate intravenous doses of 3 and 10 mg/kg in dogs **[104]**.

Electrocardiograms obtained during the 4-week oral toxicity study in beagle dogs indicated that delavirdine mesylate doses of ≤ 38 mg/kg/day did not alter the electrocardiographic measurements **[20]**. This finding is in agreement with the lack of electrocardiogram changes in the 14-day oral dog study at 350 mg/kg/day **[19]**. The only significant finding in the 3-month oral dog study was a slight increase in the mean QTc interval noted at the high dose of 76/60 mg/kg/day on day 8 only (not on day 29, 57 or 85) **[23]**. This finding was not considered to impact human safety.

A single 500 mg/kg delavirdine mesylate oral dose administered to rats resulted in decreased rectal temperatures and decreased activity **[105]** and is not considered to impact human safety.

In conclusion, there were no findings in the safety pharmacology studies which are considered to have an impact on the safety of delavirdine mesylate therapy in humans.

p. Local Tolerance

Ocular and dermal irritation studies were conducted in rabbits with both delavirdine free base **[106,107]** and delavirdine mesylate **[108,109]**. The free base was minimally irritating to the eye and skin, but delavirdine mesylate would be classified as a primary eye irritant at a single dose of 100 mg/eye or at 20 mg/eye/day for 5 days and as a primary dermal irritant at a single dose of 500 mg/site or at 100 mg/site/day for 5 days in rabbits. Since delavirdine mesylate is administered as a tablet, contamination and irritation of eye and skin are considered very remote consequences of drug therapy and not a health concern. Occupational exposure to delavirdine mesylate powder is a concern but it is unlikely that a sufficient amount of drug would contaminate the eye or irritate the skin of a worker. Delavirdine mesylate was practically nontoxic when administered intratracheally to rats at a single dose of 25 mg/kg **[110]** and would not pose a health concern even if an entire day's dose (20 mg/kg) were inhaled.

In conclusion, oral administration of delavirdine mesylate tablets in humans does not pose an eye, dermal or intratracheal health concern even if exposed to an entire day's dose.

4. Animal Pharmacokinetics (Absorption, Distribution, Metabolism and Excretion [ADME])

Absorption, distribution, metabolism and excretion (ADME) studies conducted in mouse, rat, dog, monkey, rabbit and human demonstrated that the pharmacokinetics

of delavirdine (DLV, U-90152) were nonlinear in all species **[16,25,36,38,40,80,111-113]**. Most, if not all, of the nonlinearity appeared to be related to the fact that the biotransformation of delavirdine to its N-desisopropyl metabolite (desalkyl delavirdine, desalkyl DLV, MET-5, U-96183) is, at least in part, saturable or inhibitable **[16,25,36,38,40,80,113]**. Desalkyl delavirdine was the principal metabolite in systemic circulation in all species **[80,114-123]**. Clearances were species-dependent and were low-to-moderate in most species following administration of single intravenous or oral doses above 15 mg/kg **[111,112,124]**. Mean systemic clearances increased in the order dog < rat < monkey, with the clearance an order of magnitude higher in the rat than the dog. Absolute oral bioavailabilities were species- and dose-dependent and increased in the order monkey < rat < dog, being approximately 30%, 65% and 100% in each of these species, respectively, when equivalent intravenous and oral doses of drug were administered **[111,112,124]**. Dose-normalized systemic exposures to delavirdine-related material, following single oral dose administration of [^{14}C-carboxamide]delavirdine mesylate, increased in the order rat ≈ rabbit < monkey < human < mouse < dog **[80,122,123,125-127]**. The systemic exposure to delavirdine relative to delavirdine-related material increased in the order mouse < rabbit < monkey < human < rat < dog, whereas systemic exposure to desalkyl delavirdine relative to delavirdine-related material increased in the converse order dog < rat ≤ human < monkey < rabbit < mouse. Thus, the dog had the highest exposure to delavirdine-related material and delavirdine and the lowest exposure to desalkyl delavirdine due to the high absorption efficiency and low presystemic metabolism of delavirdine in the dog.

Comparison of mean steady state delavirdine pharmacokinetic data from multiple oral dose toxicity studies indicated that systemic exposure to delavirdine, on a mg/kg/day dosage basis, increased in the same rank order as was observed in the single-dose studies of [^{14}C-carboxamide]delavirdine mesylate (mouse < rabbit ≤ monkey ≤ rat < dog), while steady state concentrations of desalkyl delavirdine increased in the converse order, except for the mouse, dog < mouse < rat < rabbit ≤ monkey **[16,18,23,25,38]**. Maximum delavirdine concentrations in excess of 100 μM, 200 μM, 300 μM, 500 μM and 500 μM were observed in the mouse, rabbit, monkey, rat and dog, respectively, in toxicity studies **[16,17,20,38]**. On a mg/kg dosage basis, exposure to delavirdine in the dog was nearly an order of magnitude higher than in the rat and the exposure in the rat was nearly an order of magnitude higher than in the mouse. The systemic concentrations of delavirdine to which animals were exposed were generally much higher than those in humans. Steady state desalkyl delavirdine concentrations, relative to simultaneous delavirdine concentrations of approximately 20 μM, were approximately 20%, 8%, 3%, 100% and 30% in the mouse, rat, dog, monkey and rabbit, respectively. The lowest systemic concentrations of desalkyl delavirdine were observed in the dog and the highest concentrations were observed in the monkey. Inter-subject and inter-day variabilities in systemic concentrations of delavirdine were high in all species. Significant gender differences in systemic exposure to delavirdine ($p < 0.05$) were observed in the mouse for all doses and in the rat for doses less than 300 mg/kg/day. Accumulation of delavirdine occurred in all species except the mouse following multiple-dose administration, with systemic exposure to delavirdine in rats, dogs and monkeys being highly persistent. Comparison of mean steady state maximum (C_{max}) and average (C_{ave}) systemic concentrations and oral clearances suggested that more than proportional increases in delavirdine occurred in all species, at least at lower drug doses **[16,25,36,38,40,113]**.

At high doses in the rat, C_{max} and C_{ave} appeared to reach a plateau, which suggested that the absorption of delavirdine was saturated in rats for drug doses of 300 mg/kg/day or higher [17,18]. An alternative explanation for the observed plateau in delavirdine C_{max} and C_{ave} concentrations is that the *in vivo* solubility of delavirdine may have been exceeded, since crystals of delavirdine were observed in the tissues of rats from the 3-month [18] and 6-month [40] toxicity studies and in the tissues of dogs from the 3-month escalating intravenous dose toxicity study [24]. The mean steady state C_{max} of delavirdine in rats at a dose of 750 mg/kg/day (370 µM) and the maximum delavirdine concentrations observed in the intravenous dose dog study (approximately 300 µM) are consistent with the aqueous solubility of the free base trihydrate form of the drug [128] when protein binding is taken into account [129], and with the measured *in vitro* solubility of the trihydrate form of the drug at 37°C in rat and dog serum, respectively [83]. Absorption of delavirdine mesylate appeared to be saturated in the bred rabbit at doses of 200 mg/kg/day and 400 mg/kg/day [38]. In multiple oral dose studies with [^{14}C-carboxamide]delavirdine mesylate [80,122,125,130], dose-normalized systemic exposures to delavirdine-related material, delavirdine, and desalkyl delavirdine were consistent with those following single oral dose administration of [^{14}C-carboxamide]delavirdine mesylate and with dose-normalized systemic exposures to delavirdine and desalkyl delavirdine observed in multiple oral dose toxicity studies with delavirdine mesylate.

Tissue distribution studies in the rat showed that delavirdine and its metabolites distributed rapidly and extensively to tissues and organs [131-133], including feto-placental tissue [131], melanin-containing tissues [131,134,135], and milk [136]. Concentrations of delavirdine-related material were highest in the liver, kidney and adrenal glands and lowest in the brain. Accumulation in thyroid and adrenal glands was observed following multiple dosing [133]. Distribution of delavirdine in brain was limited to approximately 1% to 6% of simultaneous plasma or serum concentrations in mice [116], rats [119,137] and dogs [137,138]. Concentrations of delavirdine in cerebrospinal fluid (CSF) were < 0.5% of simultaneous plasma or serum concentrations in dogs and humans [138,139]. *In vivo* [80,122,123,125-127,130,132,133,140] and *in vitro* [141] red blood cell/plasma partitioning studies in mice, rats, dogs, monkeys, rabbits, and humans indicated that delavirdine-related material and delavirdine did not preferentially partition into red blood cells (red blood cell/plasma partition coefficients (K_p) ranged from 0.00 to 0.726 within 8 h following drug administration). Delavirdine was highly protein bound (> 96%) [129], and had a moderate volume of distribution (≥ 5 times plasma volume). The binding capacity of human serum for delavirdine was 2-fold greater than in rat, beagle dog and monkey serum, and about equivalent with mongrel dog serum [129]. Because of the greater protein binding capacity of human serum for delavirdine as compared to rat and dog serum, the tissue deposition of delavirdine that was observed in rats and dogs at very high systemic drug concentrations should not be a problem in humans under prescribed clinical use conditions.

Radiolabeled studies indicated that delavirdine was well absorbed (> 80%) after a 10 mg/kg single oral dose. In single and multiple oral dose studies, the majority of delavirdine-related radioactivity was excreted in feces. The percentage of the administered dose excreted in feces ranged from a low of 44.3% in humans to a high of 88.4% in rats [80,114,123,125-127,130,142-144]. Urinary excretion of delavirdine-related material ranged from 6.9% in dogs to 50.8% in humans, and

increased in the order dog ≤ rat < rabbit < mouse < human. Excretion of delavirdine-related material was dose-dependent in all species; at higher doses and following multiple-doses an increased percentage of the administered radioactivity was recovered in feces, except for mouse and human. Gender-related differences in excretion were not observed in any species.

Biotransformation of delavirdine was extensive (Figure E.1) **[80,114-123,140,144-146]**. Delavirdine was the major component in systemic circulation in the rat, dog, and human, while desalkyl delavirdine was the major component in systemic circulation in the mouse and monkey at the doses of drug administered. In the rabbit, desalkyl delavirdine was the major circulating component at 10 mg/kg, while delavirdine was the major component in circulation at 200 mg/kg. N-Isopropylpyridinepiperazine (U-88703) was observed as a minor metabolite in circulation in mouse only. In the species examined, except mouse, most of the dose excreted in urine consisted of desalkyl delavirdine. In the mouse, indole carboxylic acid (U-96364) and U-88703–a metabolite that has been implicated in delavirdine-mediated pseudopregnancy in mice **[78,82,147,148]**–were observed as major urinary metabolites (see Figure E.1). The species-specific metabolism of delavirdine to U-88703 in the mouse indicated that pseudopregnancy would not be expected in other species. The major components in rat bile were 6′-pyridinol delavirdine (MET-7) and its glucuronide (MET-6) and sulfate (MET-8) conjugates. The major constituents in feces of mouse, dog, and rabbit were pyridine-cleaved delavirdine (U-102466) and its conjugate (MET-1). In rat, the major fecal components were pyridine-cleaved delavirdine and its conjugate, 6′-pyridinol delavirdine, and delavirdine. In human feces, pyridine-cleaved delavirdine and desalkyl delavirdine were the primary constituents. Parent drug was observed in feces of all species. Bacterial metabolism of delavirdine and chemical degradation may account for the presence of 6′-pyridinol delavirdine and pyridine-cleaved delavirdine and its conjugate in feces. Taking into account both urine and feces (and bile in the case of the rat), desalkyl delavirdine was the major metabolite in human only, while pyridine-cleaved delavirdine and its conjugate were the major metabolites in mouse, rat, dog and rabbit. All of the metabolites found in human were observed in the preclinical species. The metabolic pathway of delavirdine (Figure E.1) involved N-desalkylation, pyridine ring hydroxylation, amide bond cleavage, and pyridine ring cleavage. Amide bond hydrolysis of delavirdine was observed as a significant pathway in mouse only.

Desalkyl delavirdine was the primary metabolite in mouse, rat, dog, monkey, rabbit and human liver microsomes **[149]**. A metabolite tentatively identified as 6′-pyridinol delavirdine was observed as a minor microsomal metabolite. The *in vitro* metabolism of delavirdine was consistent with the *in vivo* metabolism, except that mouse liver microsomes did not generate the indole carboxylic acid and U-88703 metabolites. *In vitro* intrinsic clearance (V_{max}/K_M) was the lowest in the dog and highest in the monkey and increased in the order dog < rat < human < monkey. The apparent K_M for *in vitro* N-desalkylation was 4.4 μM in monkey, 6.8 μM in human, 9.9 μM in rat and 12.6 μM in dog. Delavirdine was primarily metabolized by CYP3A, which catalyzed both N-desalkylation and 6′-hydroxylation, and secondarily by CYP2D6 which catalyzed N-desalkylation only. There was no apparent enzyme induction in the rat, dog or monkey, however, delavirdine appeared to partially inactivate or inhibit CYP3A in the rat, dog, monkey and human **[149,150]**. The inhibition of CYP3A observed *in vitro* suggests that CYP2D6 may play a more significant role in the *in vivo*

metabolism of delavirdine after multiple-dose administration compared with single-dose administration. Accordingly, the observed nonlinearity in delavirdine pharmacokinetics could be explained as a consequence of both metabolic saturation and partial loss of metabolic capacity. These findings indicate the potential for clinical interactions with co-administered drugs which are metabolized by or influence the activity of CYP3A or CYP2D6.

5. Conclusions

Delavirdine mesylate nonclinical toxicity studies conducted by systemic (mainly oral) routes included acute toxicity, local tolerance, safety pharmacology and reproductive toxicity studies and repeated-dose toxicity studies of up to 3 months in mice and cynomolgus monkeys, 6 months in rats and 1 year in dogs. *In vitro* and *in vivo* genetic toxicology tests were completed and carcinogenicity studies in rats and mice are in progress. The results of these studies indicate that delavirdine mesylate affects multiple organ systems of animals and that toxicity more closely correlated with serum drug concentrations rather than dose administered. Target organs included arteries, gastrointestinal tract, endocrine system, liver, kidneys, peripheral blood and bone marrow, lymphoid tissue, lung and reproductive system. The toxicity of delavirdine mesylate in animals varied by species (eg, vasculitis/perivasculitis in dogs, pseudopregnancy in mice).

Single oral doses of delavirdine mesylate or delavirdine free base given to rats at doses of 5000 mg/kg or less were not lethal and were practically nontoxic providing a wide margin of safety (250 times the recommended human dose) if the total daily dose were inadvertently consumed as a single dose.

Drug-related mortalities (deaths/unscheduled sacrifices) in repeated-dose toxicity studies were associated with high nadir serum concentrations of delavirdine. In rats, drug-related death/unscheduled sacrifice was attributed to multiple organ toxicity at high mean nadir serum concentrations of delavirdine of > 160 µM. In dogs and monkeys, the main cause of death/unscheduled sacrifice was gastrointestinal toxicity (erosions/ulcers). Nadir serum concentrations of delavirdine at the time of gastrointestinal toxicity were > 180 µM in dogs and monkeys.

Vasculitis/perivasculitis in arteries occurred in all oral toxicity dog studies completed with delavirdine mesylate when high mean nadir serum concentrations of delavirdine (generally > 180 µM) were achieved. The dog is the only species for which there is a clearly established association between treatment with delavirdine mesylate and the development of vasculitis/perivasculitis. It was the opinion of an international panel of multidisciplinary scientists that delavirdine-associated vasculitis/perivasculitis appeared to be specific to the dog.

Numerous endocrine-related changes were observed in the repeated-dose toxicity studies with delavirdine mesylate, affecting a variety of organs, including the thyroid gland, adrenal gland, pituitary gland and reproductive organs. Results of mechanistic studies in rats included enhanced clearance (thyroxine) and inhibition of biosynthesis (corticosterone) of hormones. Organ hypertrophy was considered to be compensatory in nature, involving normal regulatory mechanisms, in an attempt to maintain normal hormone levels. Effects on the pituitary gland were consistent with compensatory responses to the effects on other endocrine organs. Pseudopregnancy in mice was associated with the ability of mice to form a metabolite of delavirdine which has a high affinity for the dopamine-D2 receptor, resulting in increased blood prolactin levels. Additional investigational studies on the effects of delavirdine mesylate on the reproductive system were not completed; however, inhibition of hormone biosynthesis may be involved. Based on different clearance pathways, the human safety profile of other drugs which cause similar effects in rodents and/or the lack of effects of

delavirdine mesylate in clinical trials, it is unlikely that similar endocrine-related changes will occur in humans.

Deposition of delavirdine crystals with or without associated inflammation has been observed in tissues of rats (oral) and dogs (intravenous) and is likely related to prolonged exposure (≥ 3 months) to high maximum serum concentrations (rats: ≥ 130 µM; dogs: ≥ 270 µM) of delavirdine and the relative insolubility of delavirdine in the serum of these species. Based on the relatively higher *in vitro* solubility of delavirdine in human serum and the low serum concentrations of delavirdine achieved with therapeutic doses of delavirdine mesylate in humans, it is unlikely that deposition of delavirdine crystals in tissues will occur in humans. This was also the conclusion of an international panel of multidisciplinary scientists.

Delavirdine mesylate was teratogenic (septal defects) in rats at a dose of 200 mg/kg/day. Delavirdine mesylate was possibly teratogenic at doses of 50 and 100 mg/kg/day based on one case of septal defect at each of these doses. The mean nadir serum concentration of delavirdine for rats of the 50 mg/kg/day dose group was 4 µM. No evidence of teratogenic effects was apparent in rabbits. Pregnant rats and pregnant rabbits given delavirdine at doses ≥ 200 mg/kg/day had unique microscopic findings in the liver and the kidney that indicate a heightened susceptibility in pregnant animals to delavirdine-induced renal and hepatic toxicities. Based on these findings, the potential benefit of therapy in pregnant women must be weighed against potential risk to the mother and fetus.

The liver, kidney, lung and peripheral blood were target organs in the rat at low serum concentrations of delavirdine (mean nadir serum concentrations of delavirdine ≥ 5.12 µM). Some kidney, liver and peripheral blood changes were not reversible after a 2-month recovery period in rats which had a mean nadir serum concentration of 37.6 µM. Similarly, increased serum cholesterol values in rats, mice and dogs and liver changes in mice were observed at low serum concentrations of delavirdine. The relevance of these findings to human safety is not known.

The results of a genetic toxicology profile conducted with delavirdine mesylate suggest that delavirdine mesylate does not pose a genotoxic hazard in humans. There were no findings in the safety pharmacology studies which are considered to have an impact on the safety of delavirdine mesylate therapy in humans.

Local tolerance studies indicate that delavirdine mesylate is a primary eye and dermal irritant. However, since delavirdine mesylate is administered orally as a tablet, eye contamination and skin irritation are considered remote consequences of drug therapy and not a health concern. Delavirdine mesylate was practically nontoxic when administered intratracheally to rats at a dose of 25 mg/kg.

In summary, there is a safety margin for the two most significant toxicities noted in animals - vasculitis/perivasculitis in dogs and gastrointestinal erosions/ulcers in dogs and monkeys. This is based on a mean minimum serum concentration of 12 µM in humans versus nadir serum concentrations of > 180 µM in dogs and monkeys when these toxicities were present.

The no-observed-adverse-effect levels (based on mean nadir serum concentrations of delavirdine) for several toxicities in the nonclinical toxicology studies were less than serum concentrations of delavirdine achieved in humans. The relevance of these toxicities observed in animals to humans is not known and the potential for similar toxicities to occur in humans must be determined by the analysis of safety data from the human clinical trials. The principal adverse effect in the human clinical trials with delavirdine mesylate is a skin rash which has not been observed in animals.

The absence of adverse effects in humans similar to the toxicities observed in the nonclinical toxicology studies, plus a significant safety margin for the two most significant toxicities noted in animals, would support the safety of delavirdine mesylate administration in humans at the recommended dose of 1200 mg/day (20 mg/kg/day for a 60 kg person; 800 mg/m^2/day).

6. References/Study Reports/Publications

1. Brown JL. Incomplete labeling of pharmaceuticals: a list of "inactive" ingredients. New Eng J Med 1982;309(7):439-41.
2. Dueweke TJ, Poppe SM, Romero DL, Swaney SM, So AG, Downey KM, et al. U-90152, A potent inhibitor of human immunodeficiency virus type 1 replication. Antimicrob Agents Chemother 1993;37(5):1127-31. . . . . .
3. Descamps D, Collin G, Loussert-Ajaka I, Saragosti S, Simon F, Brun-Vezinet F. HIV-1 group O sensitivity to antiretroviral drugs. AIDS 1995;9(8):977-8. . . . . .
4. Romero DL, Busso M, Tan CK, Reusser F, Palmer JR, Poppe SM, et al. Nonnucleoside reverse transcriptase inhibitors that potently and specifically block human immunodeficiency virus type 1 replication. Proc Natl Acad Sci 1991;88:8806-10. . . . . .
5. Chen ISY, Tarpley WG, Bruce BJ. Antiviral effects of U-90152 in HIV-infected macrophages. Upjohn Technical Report 7252-92-037, 10 November 1992. . . . . .
6. Mayers DL, Bruce BJ. Susceptibility of HIV-1 clinical isolates to U-90152. Upjohn Technical Report 7252-92-038 10 November 1992. . . . . .
7. Batts DH. Open-label, escalating multiple-dose study of the safety, tolerance and pharmacokinetics of oral U-90152S/T (delavirdine mesylate, Rescriptortm tablets) in HIV-1–infected males and females with CD4 counts of 200 to 500/mm^3, who are maintained on a stable dose of AZT (M/3331/0003, BC 1003). Upjohn Technical Report 7217-93-015, 10 June 1996. . . . . .
8. Chong KT, Pagano, PJ. Delavirdine in multiple drug combination against acute and viral-spread infection of human immunodeficiency virus type 1 in vitro. Upjohn Technical Report 7252-95-048, 1 June 1995. . . . . .
9. Chong KT, Pagano PJ. Synergistic effect of delavirdine (U-90152) in combination with lamivudine (3TC) or didanosine (DDI) against HIV-1 replication in vitro. Upjohn Technical Report 7252-95-061, 29 December 1995. . . . . .
10. Chong KT, Pagano PJ. In vitro inhibition of the replication of human immunodeficiency virus type 1 by combination of delavirdine mesylate (DLV or BHAP U-90152) with protease inhibitor U-75875 or recombinant alpha-interferon. Upjohn Technical Report 7252-94-024, 13 May 1994. . . . . .

11. Pagano PJ, Chong KT. Comparison of antiviral effects of convergent three-drug combination with delavirdine mesylate (DLV), AZT, and ddI versus AZT monotherapy on HIV-1 spread infection in human peripheral blood mononuclear cells in vitro. Upjohn Technical Report 7252-94-025, 16 May 1994. .....

12. Chong, KT, Pagano PJ, Hinshaw RR. Bisheteroarylpiperazine reverese transcriptase inhibitor in combination with 3'-Azido-3'-Deoxythymidine or 2',3'-Dideoxycytidine synergistically inhibits human immunodeficiency virus type 1 replication in vitro. Antimicrob Agents Chemother 1994;38(2):288-93. .....

13. Chong KT, Pagano PJ. Synergistic inhibition of human immunodeficiency virus type 1 replication in vitro by two- and three-drug combination of delavirdine, lamivudine and zidovudine. Upjohn Technical Report 7252-96-001, 20 February 1996. .....

14. Rop DA, Leong BKJ, Sabaitis CP. U-90152S: Industrial toxicology evaluation - acute oral toxicity study in albino rats. Upjohn Technical Report 7224-92-072, 15 September 1992. .....

15. Rop DA, Sabaitis CP, Leong BKJ. U-90152: Industrial toxicology evaluation - acute oral toxicity study in albino rats. Upjohn Technical Report 7224-93-041, 17 November 1993. .....

16. White KA, Hall AD, Yancey MF. U-90152T: Three-month oral toxicity study and a one-month reversibility phase in CD-1 mice. Pharmacia & Upjohn Technical Report 7226-96-003, 12 March 1996. .....

17. White KA, Jensen RK, Adams WJ. U-90152S: Two week oral toxicity study in female Sprague-Dawley rats. Upjohn Technical Report 7226-93-011, 21 October 1993. .....

18. White KA, Lund JE, Adams WJ. U-90152S: Three-month oral toxicity study and a two-month reversibility phase in the Sprague-Dawley rat. Pharmacia & Upjohn Technical Report 7226-96-002, 12 March 1996. .....

19. Jensen RK, Stewart NL, March SM, Motte YH, DuCharme DW, Adams WJ. U-90152E: Fourteen-day oral dose toxicity study in male and female beagle dogs. Upjohn Technical Report 7227-92-034, 16 July 1992. .....

20. Jensen RK, Stewart NL, March SM, DuCharme DW, Adams WJ. U-90152E: Four-week oral dose toxicity study and a four-week reversibility phase in male and female beagle dogs. Upjohn Technical Report 7227-92-060, 13 October 1992. .....

21. Jensen RK, Motte YH, Stewart NL, Rodriquez CA. U-90152S: A one-month oral dose toxicity study in male and female mongrel dogs. Upjohn Technical Report 7227-95-001, 15 November 1995. .....

22. Hanson LA, Kolaja GJ, Adams WJ. U-90152S: A six-month oral toxicity study with a one-month reversibility phase in male and female beagle dogs. Pharmacia & Upjohn Technical Report 7226-95-019, 15 January 1996. .....

23. Jensen RK, Stewart NL, March SM, Motte YH, Adams WJ. U-90152S: A three-month oral dose toxicity study with a one-month reversibility phase in male and female beagle dogs. Upjohn Technical Report 7227-94-004, 18 August 1994. .....

24. Jensen RK, Motte YH, Stone JJ, Lin JM. U-90152T: Preliminary one-month and three-month dose escalating continuous dose toxicity studies in male and female beagle dogs. Pharmacia & Upjohn Technical Report 7227-96-006, 8 February 1996. .....

25. Jensen RK, Motte YH, Prough MJ, Adams WJ. U-90152T: Three-month, three-times-a-day oral dose toxicity and toxicokinetic study with a one-month recovery phase in male and female cynomolgus monkeys. Pharmacia & Upjohn Technical Report 7227-96-009, 19 February 1996. .....

26. Harcourt RA. Polyarteritis in a colony of beagles. Vet Rec 1978;102:519-22. .....

27. Albassam MA, Houston BJ, Greaves P, Barsoum N. Polyarteritis in a beagle. J Am Vet Med Assoc 1989;194:1595-97. .....

28. Brooks PN. Necrotizing vasculitis in a group of beagles. Lab Anim 1984;18:285-90. .....

29. Scott-Moncrieff JCR, Snyder PW, Glickman LT, Davis EL, Felsburg PJ. Systemic necrotizing vasculitis in nine young beagles. J Am Vet Med Assoc 1992;201:1553-8. .....

30. Stejskal V, Havu N, Malforms T. Necrotizing vasculitis as an immunological complication in toxicity study. Arch Toxicol 1982;5;283-6. .....

31. Spencer A, Greaves P. Periarteritis in a beagle colony. J Comp Pathol 1987;97:121-8. .....

32. Hayes TJ, Roberts GKS, Halliwell WH. An idiopathic febrile necrotizing arteritis syndrome in the dog: beagle pain syndrome. Toxicol Pathol 1989;17:129-37. .....

33. Ruben Z, Deslex P, Nash G, Redmond NI, Poncet M, Dodd DC. Spontaneous disseminated panarteritis in laboratory beagle dogs in a toxicity study: a possible genetic predilection. Toxicol Pathol 1989:17:145-52. .....

34. Felsburg PJ, HogenEsch H, Somberg RL, Snyder PW, Glickman LT. Immunologic abnormalities in canine juvenile polyarteritis syndrome: a naturally occurring animal model of Kawasaki disease. Clin Immunol Immunopathol 1992;65:110-118. . . . . .

35. Snyder PW, Kazacos EA, Scott-Moncrieff JC, HogenEsch H, Carlton WW, Glickman LT, Felsburg PJ. Pathologic features of naturally occurring juvenile polyarteritis in beagle dogs. Vet Pathol 1995;32:337-345. . . . . .

36. Hanson LA, Hall AD, Adams WJ, Brown PK. U-90152T: A one-year oral toxicity study with a one-month reversibility phase in male and female beagle dogs. Pharmacia & Upjohn Technical Report 7227-96-008, 26 March 1996. . . . . .

37. Jensen RK, Stone JJ, Motte YH, Schroder MA, Padbury GE. U-90152S: Determination of immune response to U-90152 in male and female beagle dogs given U-90152S orally for up to 28 days. Upjohn Technical Report 7227-95-016, 9 October 1995. . . . . .

38. Terry RD, Jensen RK, Zhong WZ, Adams WJ. U-90152: A Segment II teratology study (oral) in Dutch-Belted rabbits. Pharmacia & Upjohn Technical Report 7227-96-007, 9 February 1996. . . . . .

39. Albassam MA, Lillie LE, Smith GS. Asymptomatic polyarteritis in a cynomolgus monkey. Lab Anim Sci 1993;43(6):628-9. . . . . .

40. Brown PK, Hall AD, Adams WJ. U-90152S: 6-month oral toxicity study and a 2-month reversibility phase in the Sprague-Dawley rat. Pharmacia & Upjohn Technical Report 7226-96-001, 7 February 1996. . . . . .

41. Adams WJ, Friis JM, Hosley JD, Sood VK, Chang M. Bile concentrations of delavirdine and its N-desalkyl metabolite (U-96183) in the beagle dog (P&T Study No. 93-081 and P&T Study No. 93-309) and cynomolgus monkey (P&T Study No. 94-034). Pharmacia & Upjohn memo to RKJensen and LAHanson. 3 April 1996. . . . . .

42. Ratke CC, Stout CL, Adams WJ. U-90152E: Four-week oral dose toxicity study with a four-week reversibility phase and toxicokinetic study in male and female Sprague-Dawley rats. Upjohn Technical Report 7227-92-064, 28 October 1992. . . .

43. Petry TW, Blakeman DP, Lund JE, Jolly RA. U-90152S: Two week oral toxicity study, with levothyroxine supplementation in Sprague-Dawley rats. Upjohn Technical Report 7228-94-101, 27 October 1994. . . . . .

44. Petry TW, Blakeman DP, Lund JE, Jolly RA. U-90152T: Two week oral toxicity study, with high dose levothyroxine supplementation, in Sprague-Dawley rats. Upjohn Technical Report 7228-95-026, 21 March 1995. . . . . .

45. Petry TW, Blakeman DP, Lund JE, Jolly RA. U-90152T: Two week oral toxicity study in hypophysectomized Sprague-Dawley rats. Upjohn Technical Report 7228-95-055, 27 April 1995. . . . . .

46. Petry TW, Blakeman DP, Jolly RA. U-90152S: Evaluation of U-90152S as an inducer of liver microsomal thyroxine UDP-glucuronosyltransferase activity in rats. Upjohn Technical Report 7228-94-138, 5 December 1994. . . . . .

47. Petry TW, Blakeman DP, Jolly RA. U-90152T: Evaluation of U-90152T +/- levothyroxine supplementation as an inducer of hepatic microsomal thyroxine UDP-glucuronosyltransferase activity in rats. Upjohn Technical Report 7228-95-078, 1 August 1995. . . . . .

48. Blakeman DP, Jolly RA, Petry TW. U-96183: Evaluation of U-96183 as a reducing co-substrate and mechanism-based peroxidase inhibitor. Upjohn Technical Report 7228-95-050, 18 April 1995. . . . . .

49. Curran PG, DeGroot LJ. The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. Endocr Rev 1991;12(2):135-50. . . . . .

50. Japundzic MM. The goitrogenic effect of phenobarbital-Na on the rat thyroid. Acta Anat 1969;74:88-96. . . . . .

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

52. Morris HP. The experimental development and metabolism of thyroid gland tumors. Adv Cancer Res 1955;3:51-115. . . .

53. Petry TW, Blakeman DP, Lund JE, Jolly RA. U-90152T: 14-Day oral toxicity study with high-dose prednisolone supplementation in Sprague-Dawley rats. Upjohn Technical Report 7228-95-129, 8 January 1996. . . . . .

54. Gower DB. Modifiers of steroid-hormone metabolism: a review of their chemistry, biochemistry and clinical applications. J Steroid Biochem 1974;48:501-23. . . . . .

55. Feldman D. Ketoconazole and other imidazole derivatives as inhibitors of steroidogenesis. Endocr Rev 1986;7:409-20. . . .

56. Murray M, Reidy GH. Selectivity in the inhibition of mammalian cytochromes P-450 by chemical agents. Pharmacol Rev 1990;42:85-101. . . . . .

57. Petry TW, Blakeman DP, Jolly RA. U-90152T: Effects of U-90152T and U-96183 on rat adrenocortical steroid biosynthetic enzyme activities in vitro. Pharmacia & Upjohn Technical Report 7228-96-002, 17 January 1996. .....

58. Loose DS, Kan PB, Hirst MA, Arcus RA, Feldman D. Ketoconazole blocks adrenal steroidogenesis by inhibiting cytochrome P-450-dependent enzymes. J Clin Invest 1983;71:1495-9. .....

59. Eckhoff C, Oelkers W, Bahr V. Effects of two oral antimycotics, ketoconazole and fluconazole, upon steroidogenesis in rat adrenal cells in vitro. J Steroid Biochem 1988;31:819-23. .....

60. Higashi Y, Omura M, Suzuki K, Inano H, Oshima H. Ketoconazole as a possible universal inhibitor of cytochrome P-450 dependent enzymes: its mode of inhibition. Endocrinol Japan 1987;34:105-15. .....

61. Ayub M, Levell MJ. Inhibition of adrenal steroidogenic enzymes in vitro by imidazole drugs including ketoconazole. J Steroid Biochem 1989;32:515-24. .....

62. Burrin JM, Yeo TH, Ashby MJ, Bloom SR. Effect of ketoconazole on adrenocorticotrophic hormone secretion in vitro and in vivo. J Endocr 1986; 108:37-41. .....

63. Lamberts SW, Uitterlinden P, deJong FH. Rat prostatic weight regression in reaction to ketoconazole, cyproterone acetate and RU23908 as adjuncts to a depot formulation of gonadotropin-releasing hormone analogue. Cancer Res 1988;48:6063-8. .....

64. Davies TS, Monro A. Marketed human pharmaceuticals reported to be tumorigenic in rodents. J Amer Coll Toxicol 1995;14:90-107.. .....

65. Malozowski S, Young I, Garcia H, Simoni C, Loriaux DL, Cassorla F. Effects of ketoconazole on rat testicular steroidogenic enzymatic activities. Steroids 1985;46:659-63.

66. DeCoster R, Cone MC, VanCamp C, VanCamp K, Beerens D, Cools W. Comparative effects of ketoconazole on rat, dog and human steroidogenesis. J Enzyme Inhibition 1989;2:261-8. .....

67. Tapazoglou E, Subramanian MG, Al-Sarraf M, Kresge C, Decker DA. High-dose ketoconazole therapy in patients with metastatic prostate cancer. Amer J Clin Oncol 1986;9:369-75. .....

68. Glass AR. Ketoconazole-induced stimulation of gonadotropin output in men: basis for a potential test of gonadotropin reserve. J Clin Endocrinol Metab 1986;63:1121-5. .....

69. Bhasin S, Sikka S, Fielder T, Sod-Moriah U, Levine HB, Swerdeloff RS, et al. Hormonal effects of ketoconazole in vivo in the male rat: mechanism of action. Endocrinology 1986;118:1229-32. .....

70. Trachtenberg J, Pont A. Ketoconazole therapy for advanced prostate cancer. Lancet 1984;2:433-5. .....

71. Trachtenberg J. Ketoconazole therapy for advanced prostate cancer. J Urol 1984;132:61-3. .....

72. Cook JC, Mullin LS, Frame S, Biegel LB. Investigation of a mechanism for Leydig cell tumorigenesis by linuron in rats. Toxicol Appl Pharmacol 1993;119:195-204. .....

73. Closset J, Hennen G. Biopotency of highly purified porcine FSH and human LH on gonadal function. J Endocrinol 1989;120:89-96. .....

74. Sharpe RM. Gonadotrophin-induced accumulation of 'interstitial' fluid in the rat testis. J Reprod Fert 1979;55:365-71. .....

75. Viguier-Martinez MC, Hochereau-de-Reviers MT, Barenton B, Perreau C. Endocronological and histological changes induced by flutamide treatment on the hypothalamo-hypophyseal-testicular axis of the adult male rat and their incidences on fertility. Acta Endocrinol (Copenh) 1983;104:246-52. .....

76. Fawcett DW. A textbook of histology. 12th ed. New York: Chapman and Hall, 1994, 473-89. .....

77. Jensen RK, Cole SL, Zaya RM, Kakuk TJ, Adams WJ, Voorman RL. U-90152E: Four-week oral dose toxicity study in female cynomolgus monkeys. Upjohn Technical Report 7227-92-067, 28 October 1992. .....

78. Smith MW, Dinh DM, Lawson CF, Schlachter SK, Branstetter DG. Monoaminergic receptor binding of metabolites of U-90152T (delavirdine mesylate). Upjohn Technical Report 7295-95-021, 9 May 1995. .....

79. VonVoigtlander PF, Huff RM, Chio CL. Results with U-88703. Pharmacia & Upjohn memo to DBranstetter. 15 March 1996. .....

80. Chang M, Sood VK, Wilson GJ, Hauer MJ, Sanders PE, Verburg MT, Koets MD, Cox SR, Turner SW, Kloosterman DA, Batts DH. Metabolism and excretion of delavirdine (U-90152) in healthy male volunteers following single and multiple oral dose administration of [^{14}C]delavirdine mesylate (M/3331/0036, BC 1042). Pharmacia & Upjohn Technical Report 7215-95-047, 29 February 1996. .....

81. Chang M, Sood VK, Hauer MJ, Fagerness PE, Norris LR, Jones BW, Rousch KE. Metabolism and tissue distribution of delavirdine (U-90152) in female Sprague-Dawley rats after single oral administration of [2-^{14}C-pyridine]delavirdine mesylate at 250 mg/kg (DMR Protocol No. 95-299). Pharmacia & Upjohn Technical Report 7256-95-070, 29 January 1996. . . . . .

82. Branstetter DG, Zhang W, Marks TA. U-88703: Pulsatile prolactin secretion following single intravenous administration of U-88703 in the female Sprague-Dawley rat. Pharmacia & Upjohn Technical Report 7228-95-137, 18 December 1995. . . . . .

83. Bothwell BE, Meulman PA, Bombardt PA. Crystal forms and solubility of delavirdine in human, monkey, dog, rat and rabbit serum following in vitro equilibration of U-90152T with serum at 37°C. Pharmacia & Upjohn Technical Report 7256-95-068, 13 February 1996. . . . . .

84. Black DL. U-90152S: A Segment I fertility and general reproductive performance study (oral) in Sprague-Dawley (Crl:CD[BR]) rats. Upjohn Technical Report 7227-94-024, 17 November 1994. . . . . .

85. Jensen RK, Prough MJ. U-90152S: Multiple day oral dose toxicity study with an emphasis on liver function in cynomolgus monkeys. Upjohn Technical Report 7227-95-005, 13 March 1995. . . . . .

86. Alden CL, Frith CH. Urinary system. In: Haschek WM, Rousseaux CG, editors. Handbook of toxicologic pathology. San Diego: Academic Press, Inc., 1991: 315-87. . . . . .

87. Riley JH, Cornelius LM. Electrolyte, blood gasses, and acid base balance. In: Loeb WF, Quimby FW, editors. The clinical chemistry of laboratory animals. Oxford: Pergamon Press Inc., 1989: 345-413. . . . . .

88. Morris, DF, Adams WJ, Jensen RK. U-90152S: A Segment II teratology study (oral) in Crl:CD[BR] Sprague-Dawley rats, modified to include postnatal assessments until weaning. Upjohn Technical Report 7227-95-030, 15 November 1995. . . . . .

89. Warkany J. Ventricular septal defects. In: Warkany J, editor. Congenital malformations. Chicago: Year Book Medical Publishers, Inc., 1971: 489-94. . . . . .

90. Middle Atlantic Reproduction and Teratology Association and Midwest Teratology Association. Historical control data (1992-1994) for developmental and reproductive toxicity studies using Crl:CD®BR rats. Published and distributed by Charles River Laboratories; December, 1995. . . . . .

91. Tasker EJ, Jensen RK. U-90152S: A Segment III peri-postnatal study (oral) in rats. Pharmacia & Upjohn Technical Report 7227-96-004, 1 February 1996. .....

92. Mayo JK, Aaron CS. U-90152S: Evaluation of U-90152S in the *Salmonella*/microsome test (Ames Assay). Upjohn Technical Report 7228-92-039, 4 August 1992. .....

93. Mayo JK, Aaron CS. U-90152S: Evaluation of U-90152S in the *Salmonella* mutagenicity test (Ames Assay). Upjohn Technical Report 7228-93-050, 20 January 1994. .....

94. Wang Y, Aaron CS. U-90152S: Evaluation of U-90152S in the *Salmonella typhimurium* mutation assay (Ames Assay) with or without exogenous metabolic activation. Upjohn Technical Report 7228-94-024, 30 March 1994. .....

95. Wang Y, Aaron CS. U-90152S: Evaluation of U-90152S in the AS52/XPRT mammalian cell mutation assay with and without metabolic activation. Upjohn Technical Report 7228-94-006, 28 January 1994. .....

96. Wang Y, Aaron CS. U-90152S: Evaluation of U-90152S in the in vitro chromosome aberration assay in cultured human peripheral lymphocytes. Upjohn Technical Report 7228-94-008, 1 February 1994. .....

97. Harbach PR, Filipunas AL, Wang Y, Aaron CS. U-90152A: Evaluation of U-90152A in the in vitro unscheduled DNA synthesis (UDS) assay in rat primary hepatocytes. Upjohn Technical Report 7228-92-027, 29 July 1992. .....

98. Harbach PR, Filipunas AL, Wang Y, Aaron CS. U-90152S: Evaluation of U-90152S in the in vitro unscheduled DNA synthesis (UDS) assay in rat primary hepatocytes. Upjohn Technical Report 7228-93-012, 20 April 1993. .....

99. Harbach PR, Filipunas AL, Wang Y, Aaron CS. U-90152S: Evaluation of U-90152S (B process) in the in vitro unscheduled DNA synthesis (UDS) assay in rat primary hepatocytes. Upjohn Technical Report 7228-94-003, 25 March 1994. .....

100. Wang Y, Aaron CS. U-90152S: Evaluation of U-90152S in the micronucleus test in mouse bone marrow. Upjohn Technical Report 7228-94-007, 28 January 1994. .....

101. Physicians' Desk Reference. 46th rev. ed. Oradell, NJ: Medical Economics, 1992: 1592. .....

102. Physicians' Desk Reference. 46th rev. ed. Oradell, NJ: Medical Economics, 1992: 2131. .....

103. Physicians' Desk Reference. 46th rev. ed. Oradell, NJ: Medical Economics, 1992: 726. .....

104. Robinson FG, DuCharme DW. U-90152E: Acute cardiovascular and hemodynamic effects of intravenously administered U-90152E in anesthetized dogs. Upjohn Technical Report 7224-92-062, 13 August 1992. .....

105. Bastianse RG, Kirton KT. U-90152S: A functional observational battery to assess neurotoxic-pharmacological potential in Sprague-Dawley rats in a single dose toxicity study. Upjohn Technical Report 7224-92-067, 8 September 1992. .....

106. Rop DA, Sabaitis CP, Leong BKJ. U-90152: Industrial toxicology evaluation - acute eye irritation study in albino rabbits. Upjohn Technical Report 7224-93-042, 17 November 1993. .....

107. Sabaitis CP, Rop DA, Leong BKJ. U-90152: Industrial toxicology evaluation - acute dermal irritation study in albino rabbits. Upjohn Technical Report 7224-93-043, 17 November 1993. .....

108. Leong BKJ, Rop DA, Sabaitis CP. U-90152S: Industrial toxicology evaluation - acute eye irritation study in albino rabbits. Upjohn Technical Report 7224-92-073, 15 September 1992 .....

109. Rop DA, Leong BKJ, Sabaitis CP. U-90152S: Industrial toxicology evaluation - acute dermal irritation study in albino rabbits. Upjohn Technical Report 7224-92-074, 15 September 1992. .....

110. Sabaitis CP, Rop DA, Leong BKJ. U-90152S: Industrial toxicology evaluation - acute intratracheal inhalation toxicity study in albino rats. Upjohn Technical Report 7224-93-058, 16 December 1993. .....

111. Adams WJ, Hosley JD, Jones BW, Thayer PA, Sedlock ML, Anstadt RA, Morozowich W, Smith DP, Douglas SL. Pharmacokinetics, absolute oral bioavailability and dose proportionality of the bisheteroarylpiperazine HIV-1 reverse transcriptase inhibitors U-90152A and U-94262 following administration of single intravenous and oral doses in male Sprague-Dawley rats (Cancer and Infectious Diseases Protocol No. 91-327). Upjohn Technical Report 7256-92-061, 24 November 1992. .....

112. Adams WJ, Hosley JD, Friis JM, Jones BW, Thayer PA. Pharmacokinetics and systemic oral bioavailability of reference solution and solid dosage formulations of delavirdine in the male beagle dog (DMR Protocol 91-479). Pharmacia & Upjohn Technical Report 7256-95-065, 4 January 1996. .....

113. Jensen RK, Prough MJ, Adams WJ, Schlukebir DS, Easter BD. U-90152S: Intravenous toxicity and pharmacokinetic study in male and female cynomolgus monkeys. Upjohn Technical Report 7227-93-029, 21 September 1993. .....

114. Chang M, Sood VK, Kloosterman DA, Sanders PE, Wilson GJ, Norris LR, Rousch KE, VandeGiessen TL. Studies on the metabolism of delavirdine in CD-1 mice: Isolation and identification of metabolites, metabolite profiles, and excretion following 10 mg/kg single-oral dose and 200 mg/kg/day multiple-oral dose administration of [^{14}C] and [$^{13}C_3$, ^{14}C]delavirdine mesylate (DMR Protocols No. 93-356 and 93-417). Upjohn Technical Report 7256-94-089, 28 October 1994. .....

115. Chang M, Wilson GJ, Sood VK, Sanders PE. Plasma levels of delavirdine and its N-desisopropyl metabolite, metabolite characterization, and metabolite profiles of plasma and urine following a single-oral dose administration of [^{14}C]delavirdine mesylate to male and female CD-1 mice at 10 or 250 mg/kg (DMR Protocol No. 93-314). Upjohn Technical Report 7256-94-110, 20 February 1995. .....

116. Sood VK, Norris LR, Ouding RJ, Rousch KE, Zhang W, Branstetter DG, Chang M. Tissue distribution of [2-^{14}C-pyridine]delavirdine mesylate (U-90152T) and metabolites in female CD-1 mice after single oral administration at 250 mg/kg (P & T Protocol No. 95-131). Upjohn Technical Report 7256-95-049, 20 November 1995. .

117. Chang M, Wilson GJ, Sood VK, Sanders PE. Plasma levels of delavirdine and its N-desisopropyl metabolite, metabolite characterization, and metabolite profiles of plasma and urine following single- and multiple-oral dose administration of [^{14}C]delavirdine mesylate to male and female Sprague-Dawley rats at 10 or 125 mg/kg (DMR Protocol No. 93-439). Upjohn Technical Report 7256-94-096, 7 December 1994. .....

118. Chang M, Sood VK, Sanders PE, Jones BW, Norris LR, Rousch KE. Metabolism of delavirdine in male Sprague-Dawley rats after single intravenous dose administration of [^{14}C]delavirdine mesylate at 10 mg/kg: Pyridine ring hydroxylation is not mediated by gut metabolism (DMR Protocol No. 94-300). Upjohn Technical Report 7256-94-071, 22 February 1995. .....

119. Chang M, Sood VK, Hauer MJ, Fagerness PE, Norris LR, Jones BW, Rousch KE. Metabolism and tissue distribution of delavirdine (U-90152) in female Sprague-Dawley rats after single oral administration of [2-^{14}C-pyridine]delavirdine mesylate at 250 mg/kg (DMR Protocol No. 95-299). Pharmacia & Upjohn Technical Report 7256-95-070, 29 January 1996. .....

120. Chang M, Sood VK, Wilson GJ, Kloosterman DA, Sanders PE. Metabolism of delavirdine in the beagle dog: Isolation and identification of metabolites and metabolite profiles of plasma, urine, and feces following single-oral dose administration of [^{14}C]delavirdine mesylate at 4 or 40 mg/kg (DMR Protocol No. 92-451). Upjohn Technical Report 7256-94-093, 21 November 1994. . . . . .

121. Chang M, Wilson GJ, Sood VK. Plasma levels of delavirdine (U-90152) and its N-desisopropyl metabolite and metabolite profiles of plasma and urine following single and multiple oral administration of [^{14}C]delavirdine mesylate to male and female beagle dogs at 4 or 20 mg/kg (DMR Protocol No. 93-361). Upjohn Technical Report 7256-95-062, 9 November 1995. . . . . .

122. Chang M, Sood VK, Kloosterman DA, Hauer MJ, Sanders PE, Vrbanac JJ. Metabolism of delavirdine in male and female cynomolgus monkeys after single and multiple oral dose administration of [^{14}C]delavirdine mesylate: Identification of novel N-acetylglucosamine and pyridinol sulfate conjugates (DMR Protocol No. 94-161). Upjohn Technical Report 7256-95-007, 10 July 1995. . . . . .

123. Chang M, Sood VK, Wilson GJ, Hauer MJ, Sanders PE, Norris LR, VandeGiessen TL, Thayer PA, Brewer JE, Koets MD, Bombardt PA. Metabolism and excretion of delavirdine in female dutch-belted rabbits following single and multiple oral dose administration of [^{14}C]delavirdine mesylate at 10 or 200 mg/kg (DMR Protocol No. 93-447). Pharmacia & Upjohn Technical Report 7256-95-032, 16 January 1996. . . . . .

124. Adams WJ, Friis JM, Hosley JD. Pharmacokinetics and systemic oral bioavailability of delavirdine mesylate (U-90152S) in female and male Cynomolgus monkeys (DMR Protocol 92-393). Pharmacia & Upjohn Technical Report 7256-95-064, 6 March 1996. . . . . .

125. Chang M, Sood VK, Wilson GJ, VandeGiessen TL, Norris LR, Jones BW, Ouding RJ, Koets MD. Disposition and excretion of drug-related materials in male and female Sprague-Dawley rats after multiple-oral dose administration of [^{14}C]delavirdine mesylate at 10 or 125 mg/kg (DMR Protocol No. 93-439). Upjohn Technical Report 7256-94-084, 9 June 1995. . . . . .

126. Chang M, Sood VK, Wilson GJ, VandeGiessen TL, Norris LR, Ouding RJ, Rousch KE. Disposition and excretion of drug-related materials in male and female CD-1 mice following single-oral dose administration of [^{14}C]delavirdine mesylate at 10 or 250 mg/kg (DMR Protocol No. 93-314). Upjohn Technical Report 7256-94-083, 6 January 1995. . . . .

127. Chang M, Wilson GJ, Rousch KE, Norris LR, Jones BW. Disposition and excretion of [^{14}C]U-90152S in male beagle dogs following single oral dose administration at 4 mg/kg or 40 mg/kg (Protocol 92-451). Upjohn Technical Report 7256-93-145, 3 January 1994. .....

128. Douglas SL. U-90152 free base hydrate: Preparation, micronization, water solubility and aqueous suspension preparation. Upjohn interoffice memo to list, 2 May 1994. .

129. Bombardt PA, Brewer JE. Studies of serum protein binding of delavirdine (U-90152) in humans, Cynomolgus monkeys, dogs, and Sprague-Dawley rats. Pharmacia & Upjohn Technical Report 7256-95-067, 5 February 1996. .....

130. Aboagye E, Peterson MK, Goldensoph C, Chang M. Disposition and excretion of drug-related materials after multiple-oral dose administration of [^{14}C]U-90152S to male and female beagle dogs at 4 mg/kg or 20 mg/kg (DMR Protocol No. 93-361). Upjohn Technical Report 7256-95-051, 28 November 1995. .....

131. Weber GL, Judy RW, Schuette MR, Loux SJ. Tissue distribution of [^{14}C]U-90152S and metabolites in the rat by quantitative whole-body autoradiography after single oral administration at 10 mg/kg (DMR Protocol 93-119). Upjohn Technical Report 7256-94-050, 12 June 1995. .....

132. Hattori K, Hayashi M, Igarashi M. U-90152T: Tissue distribution of radioactivity in male and female Sprague-Dawley rats by tissue excision after single oral administration of [^{14}C]delavirdine mesylate at 10 mg/kg (Protocol DMACR/94/012). Pharmacia & Upjohn Technical Report 1470-95-043, 13 December 1995. .....

133. Hattori K, Hayashi M, Igarashi M. Tissue distribution of radioactivity in male and female Sprague-Dawley rats by tissue excision after multiple oral administration of [^{14}C]delavirdine mesylate at 20 mg/kg/day (twice daily) (Protocol DMACR/94/006). Pharmacia & Upjohn Technical Report 1470-95-044, 14 December 1995. .....

134. Voorman RL, Judy RW, Schuette MR. Preliminary study on the tissue distribution of [^{14}C]U-90152S and metabolites in male Long Evans rats by whole body autoradiography (Protocol 92-364). Upjohn interoffice memo to list, 30 October 1992. .....

135. Wilson GJ, Sood VK, Norris LR, Chang M. Tissue distribution of [^{14}C]delavirdine mesylate (U-90152S) and metabolites in pigmented male Long-Evans rats by tissue excision after single oral dose administration at 10 mg/kg (DMR Protocol No. 93-441). Upjohn Technical Report 7256-95-022, 30 March 1995. .....

136. Hattori K, Hayashi M, Igarashi M. Lacteal secretion of [^{14}C]U-90152T in female Sprague-Dawley rats after single oral dose administration at 10 mg/kg and 125 mg/kg (Protocol DMACR/94/005). Pharmacia & Upjohn Technical Report 1470-95-037, 12 December 1995. . . . . .

137. Voorman, RL. Evaluation of brain parenchymal uptake of U-90152 in rats and dogs. Upjohn interoffice memo to list, 4 August 1992. . . . . .

138. Yancey MF, Shobe EM, Cathcart KS, Jones BW, Rousch KE, Thayer PA. Determination of U-90152 (delavirdine) and its N-desisopropyl metabolite, U-96183, in dog serum, brain tissue, cerebral spinal fluid and mesenteric lymph nodes after 7 days of dosing at 20 and 50 mg/kg/day (DMR Protocol 93-459). Upjohn Technical Report 7256-95-069, 20 November 1995. . . . . .

139. Staton BA, Bombardt PA. U-90152 levels in HIV+ human cerebrospinal fluid: Quantitative determination by HPLC and fluorescence detection. Upjohn Technical Report 7256-93-132, 23 November 1993. . . . . .

140. Chang M, Sood VK, Wilson GJ. Species comparison of the disposition, metabolism, and excretion of delavirdine (U-90152) in rat, dog, monkey, rabbit, mouse, and man. Pharmacia & Upjohn Technical Report 7256-95-078, 31 January 1996. . . . . .

141. Sood VK, Chang M. In vitro partitioning of [^{14}C]delavirdine mesylate (U-90152T) and [^{14}C]atevirdine mesylate (U-87201E) to red blood cells of rat, dog, mouse, rabbit, monkey, and human. Pharmacia & Upjohn Technical Report 7256-95-045, 30 January 1996. . . . . .

142. Chang M, Jones BW, Rousch KE, Norris LR. Elimination of drug-related materials in bile-duct-cannulated male Sprague-Dawley rats following a single-oral dose of [^{14}C]U-90152S at 10 mg/kg or 100 mg/kg (Protocol 92-489). Upjohn Technical Report 7256-93-154, 20 January 1994. . .

143. Voorman RL, Maio SM, Sood VK, Koets MD. Absorption and excretion of [^{14}C]U-90152S following a single oral dose (10 mg/kg) to male and female Sprague-Dawley rats (DMR Protocol 92-310): An addendum of reanalyzed data (amends TR 7256-92-069). Upjohn Technical Report 7256-95-001, 26 September 1995. . . . . .

144. Chang M, Sood VK, Sanders PE, Norris LR, Ouding RJ, Rousch KE. Metabolism and excretion of delavirdine in male CD-1 mice following single-oral dose administration of [2-^{14}C-pyridine]delavirdine mesylate at 250 mg/kg (DMR Protocol No. 94-301). Upjohn Technical Report 7256-95-003, 13 March 1995. . . . . .

145. Chang M, Wilson GJ. Metabolite profiles of bile, urine, and feces in male Sprague-Dawley rats following single-oral dose administration of [^{14}C]delavirdine mesylate at 10 mg/kg or 100 mg/kg (Protocol 92-489). Upjohn Technical Report 7256-94-059, 29 April 1994. . . . . .

146. Chang M, Sood VK, Jones BW, Norris LR. Elimination of drug-related materials in bile-duct-cannulated male Sprague-Dawley rat following multiple-oral dose administration of [^{14}C]U-90152S at 100 mg/kg/day (Protocol 92-524). Upjohn Technical Report 7256-94-013, 11 March 1994. . . . . .

147. Branstetter DG, Zhang W. U-88703: Fifteen-day oral toxicity study in the female CD-1 mouse. Pharmacia & Upjohn Technical Report 7228-96-008, 16 January 1996. . . .

148. Branstetter DG, Zhang W. U-88703: Fifteen-day oral toxicity study in the female Sprague-Dawley rat. Pharmacia & Upjohn Technical Report 7228-96-004, 18 January 1996 .

149. Voorman RL, Maio SM, Hauer MJ, Sanders PE, Payne NA, Ackland MJ. Metabolism of delavirdine (U-90152), an HIV-1 reverse transcriptase inhibitor, by microsomal cytochrome P450 in humans, rats and other species: Probable involvement of CYP3A subfamily. Pharmacia & Upjohn Technical Report 7256-95-071, 20 February 1996. . . . . .

150. Voorman RL, Maio SM, Payne NA, Wang X. Microsomal metabolism of delavirdine (U-90152): Evidence for mechanism-based inactivation of human cytochrome P450 3A by delavirdine. Pharmacia & Upjohn Technical Report 7256-95-072, 6 February 1996. . . . . .

F. HUMAN PHARMACOKINETICS AND BIOAVAILABILITY DATA

1. Overall Summary and Conclusions

a. Absorption and Dose-Proportionality

Delavirdine is rapidly absorbed following oral administration of delavirdine mesylate tablets to either healthy volunteers or HIV-1–infected patients, with peak plasma concentrations occurring at about 1 hour **[1-15]**. Although the absolute bioavailability of delavirdine is not known, the extent of drug absorption is at least 50% based on urinary recovery of a radiolabeled dose of delavirdine mesylate solution **[7]**. Bioavailability is not reduced when a slurry of delavirdine is prepared by allowing delavirdine mesylate tablets to disintegrate in water before administration **[6]**. When delavirdine mesylate tablets are taken with food under multiple-dose conditions, the extent of drug absorption is not affected **[15]**. Thus, delavirdine mesylate may be taken either on an empty stomach or with meals.

Single-dose pharmacokinetic assessments showed that delavirdine has a relatively high oral clearance (40-140 L/h) which tended to decrease with increasing dose, a relatively short apparent terminal half-life (about 1.4 hours), and relatively large intersubject variability in pharmacokinetic parameter values **[1-4]**. Following total daily doses of delavirdine mesylate ranging from 60 to 1200 mg, the steady-state pharmacokinetics of delavirdine are dose-dependent, as evidenced by a 10-fold decrease in delavirdine oral clearance values, a prolongation of the apparent elimination half-life, and a reduction in the steady-state plasma concentration ratio of the primary inactive metabolite to parent delavirdine **[2-5]**. The dose-dependency is apparently related to capacity-limited formation of the primary metabolite **[2-5]**. The pharmacokinetics of delavirdine are also time-dependent as evidenced by a substantial decrease in apparent oral clearance within a fixed dose level after multiple dosing compared with single (or first) dosing **[2-4]**. The time-dependency may be related to a partial loss of metabolic capacity resulting from delavirdine inhibiting its own metabolism **[3,4,5,16,17]**. Mean steady-state delavirdine concentrations (C_{ss}) are highly variable between individuals; in contrast, intrasubject variability (as determined for C_{ss}) is relatively low, with CVs of approximately 25% **[4,18,19,20]**. Comparison of delavirdine pharmacokinetic parameters across studies suggests that delavirdine pharmacokinetics are similar in healthy volunteers, HIV-1–infected patients receiving delavirdine mesylate monotherapy, and HIV-1–infected patients receiving delavirdine mesylate in combination with zidovudine **[2,3,4]**.

b. Distribution

Estimating the volume of distribution (V_z/F) of delavirdine is confounded by whether the assessment is made based on single- or steady-state doses. Mean V_z/F is approximately 170 L following single doses ranging from 20 to 400 mg **[1,2,3,4]**. Estimates of V_z/F are dose-dependent following multiple-dose administration, varying from approximately 105 L (doses of 20 to 50 mg q8h) to 34 L (doses of 100 to 400 mg q8h) **[2,3,4]**.

Delavirdine is extensively bound (approximately 98%) to plasma proteins, primarily albumin **[3,5,21]**. Protein binding is independent of delavirdine concentration over a

broad concentration range (0.5 to 196 μM), and bound delavirdine is not displaced by its primary circulating metabolite, desalkyl delavirdine [21]. The amount of delavirdine-related radioactivity associated with erythrocytes averaged 14% ± 2.3% in samples collected from a study of [^{14}C]delavirdine mesylate in healthy volunteers [22]. In a study of 5 HIV-1–infected patients whose total daily dose of delavirdine ranged from 600 to 1200 mg, cerebrospinal fluid concentrations of delavirdine averaged 0.4% ± 0.07% of the corresponding plasma delavirdine concentrations, a value lower than corresponding concentrations of unbound plasma delavirdine [19,23]. Steady-state delavirdine concentrations in saliva and semen are about 6% and 2%, respectively, of the corresponding plasma delavirdine concentrations collected at the end of a dosing interval [2,7,24].

c. Metabolism and Elimination

Delavirdine is extensively converted to several inactive metabolites. The major metabolic pathways for delavirdine are N-desalkylation and pyridine hydroxylation [7]. Delavirdine is primarily metabolized by cytochrome P450 3A (CYP3A), but in vitro data suggest that delavirdine may also be metabolized by cytochrome P450 2D6 (CYP2D6) [16]. In a study of [^{14}C]delavirdine in 6 healthy volunteers who received multiple doses of delavirdine tablets 300 mg q8h, about 44% of the radiolabeled dose was recovered in feces, with the pyridine-cleaved metabolite and the N-desalkyl metabolite accounting for approximately 29% and 28%, respectively, of fecal recovery [7]. About 51% of the dose was excreted in urine; approximately 85% of the urinary recovery was the N-desalkyl metabolite and about 5% was unchanged drug [7]. Renal clearance is therefore a minor route of elimination for delavirdine [2,7].

In vitro and in vivo studies have shown that delavirdine reduces CYP3A activity and inhibits its own metabolism [3,4,5,16,17]. This inhibitory effect on CYP3A activity is reversible within one week after discontinuation of drug [5]. Inhibition of CYP3A suggests that CYP2D6 may play a more significant role in the metabolism of delavirdine after multiple compared with single dosing [7]. The nonlinearity observed in delavirdine pharmacokinetics in vivo could be a consequence of both metabolic saturation and partial loss of metabolic capacity [5]. These findings indicate the potential for clinical interactions with coadministered drugs which are metabolized by, or influence the activity of, CYP3A and/or CYP2D6 [5,16,17].

d. Gender

Based on analyses of data pooled from pharmacokinetic studies and trough concentration data from long-term clinical studies with 897 subjects (121 females and 776 males), plasma delavirdine concentrations tend to be higher in females than in males [20]. Given the high intersubject variability characteristic of delavirdine plasma concentrations, gender differences are not considered clinically important.

e. Race/Ethnicity

The population pharmacokinetic data base for delavirdine contains data from 636 White, 159 Black, and 90 Hispanic patients [20]. No significant differences in the pharmacokinetics of delavirdine were observed among these racial or ethnic groups.

f. Drug-Drug Interactions

The nonlinearity observed in delavirdine pharmacokinetics in vivo could be a consequence of both metabolic saturation and partial loss of metabolic capacity due to inhibition of CYP3A [3,4,5,16,17]. These findings indicate the potential for clinical interactions with coadministered drugs which are metabolized by, or influence the activity of, CYP3A and/or CYP2D6 [5,16,17]. When coadministered with either fluconazole, clarithromycin, or ketoconazole, each of which is a known or suspected inhibitor of CYP3A, no clinically important effect on the steady-state pharmacokinetics of delavirdine is observed [10,11,20]. In contrast, the coadministration of rifampin, rifabutin, phenytoin, phenobarbital, and/or carbamazepine, all known inducers of CYP3A, results in clinically important reductions in plasma delavirdine concentrations [12,13,20]. These results indicate that delavirdine alone substantially reduces CYP3A activity such that concurrent administration of other CYP3A inhibitors does not further alter the pharmacokinetics of delavirdine. However, concurrent administration of known CYP3A inducers appears to overcome delavirdine-associated CYP3A inhibition such that delavirdine clearance is substantially increased and plasma delavirdine concentrations consequently reduced. The hypothesis that CYP2D6 may play a significant role in the metabolism of delavirdine after multiple dosing is supported by the finding that concurrent dosing of fluoxetine, an inhibitor of CYP2D6, increases trough delavirdine concentrations [20]. This increase, like that observed for concurrent dosing of delavirdine mesylate and CYP3A inhibitors, is not considered clinically important.

A second group of pharmacokinetic drug interaction studies were conducted based on the potential for drugs which influence gastric pH to alter the bioavailability of delavirdine. Simultaneous administration of 300 mg delavirdine mesylate tablets with alumina and magnesia oral suspension resulted in a 48% reduction in mean delavirdine AUC relative to delavirdine taken alone [8]. Antacids should not be administered within at least one hour of delavirdine mesylate dosing. Consistent with this finding, didanosine, which is orally formulated as a buffered solution to neutralize gastric pH, slightly reduced the rate and extent of delavirdine absorption when coadministered with delavirdine mesylate [15]. This effect was not considered clinically important. These results, in concert with the antacid study results, suggest that the bioavailability of delavirdine may be reduced by conditions which result in elevated gastric pH.

As expected, no significant pharmacokinetic drug interactions were found following concurrent dosing of delavirdine mesylate and either zidovudine, sulfamethoxazole, or trimethoprim/sulfamethoxazole, compounds which are not known to be extensively metabolized by cytochrome P450 [3,20].

2. References/Study Reports/Publications

1. Ward P, Cox SR, Donaldson KM, Fenton CM, Blood P, Harry JD. Escalating single dose safety, tolerance and pharmacokinetic study of U-90152S in healthy male volunteers (M/3331/0001). Upjohn Technical Report 1424-96-006, 02 April 1996. .....

2. Batts DH, Cox SR, Peel BG, Hanover CK, Francom SF, Wathen LK, et al. Randomized, double-blind, placebo-controlled, escalating multiple-dose study of the safety, tolerance, and pharmacokinetics of oral U-90152S in normal, healthy male volunteers (Protocol M/3331/0002 [BC994]). Upjohn Technical Report 7217-94-009, 11 April 1996. .....

3. Batts DH, Cox SR, Wathen LK, Peel BG, Hanover CK, Busboom SD, et al. Open-label, escalating multiple-dose study of the safety, tolerance, and pharmacokinetics of oral U-90152S in HIV-1 infected males and females with CD4 counts of 200 to 500/mm^3, who are maintained on a stable dose of AZT (M/3331/0003 [BC1003]). Upjohn Technical Report 7217-93-015, 10 June 1996. .....

4. Ward P, Cox SR, Fenton CM, Harry JD. Escalating multiple-dose, safety, tolerance, and pharmacokinetic study of delavirdine mesylate (U-90152S) in nucleoside-naive, HIV-positive patients (Protocol M/3331/0006). Upjohn Technical Report 1424-96-008, 13 May 1996. .....

5. Cox SR, Chambers JH. Preliminary Results, Protocol M/3331/0026, A steady-state pharmacokinetic evaluation of delavirdine mesylate in HIV positive patients. Upjohn memo to list, 15 March 1996. .....

6. Turner SW, Cox SR, Batts DH, Carel BJ. Relative bioavailability of delavirdine mesylate tablets, tablet suspension, and solution in healthy volunteers (Protocol M/3331/0035). Upjohn Technical Report 7215-94-027, 12 September 1994. .....

7. Chang M, Sood VK, Wilson GJ, Hauer MJ, Sanders PE, Verburg MT, et al. Metabolism and excretion of delavirdine (U-90152) in healthy male volunteers following single and multiple oral dose administration of [^{14}C]delavirdine mesylate (M/3331/0036, BC 1042). Upjohn Technical Report 7215-95-047, 02 February 1996. .....

8. Della-Coletta AA, Fleishaker JC, Cox SR, Carberry PA, Freimuth WW. The effects of food and antacid on the bioavailability of delavirdine after administration of delavirdine mesylate tablets to healthy volunteers (M/3331/0015). Upjohn Technical Report 7215-95-045, 17 November 1995. .....

9. Della-Colletta AA, Fleishaker JC, Cox SR, Carberry PA, Freimuth WW. An Evaluation of the Influence of Food on Steady-State Delavirdine Mesylate Pharmacokinetics in HIV-Infected Patients (M/3331/0019). Upjohn Technical Report 7215-95-051, 16 January 1996. .....

10. Borin MT, Cox SR, Herman BD, Freimuth WW, Carel BJ, Carberry PA, et al. An evaluation of the effect of fluconazole on the steady-state pharmacokinetics of delavirdine mesylate (U-90152S) in HIV-positive patients (Protocol M/3331/0016). Upjohn Technical Report 7215-95-039, 30 November 1995. .

11. Borin MT, Chambers JC, Cox SR, Freimuth WW, Carel BJ, Carberry PA, et al. An evaluation of the effect of clarithromycin on the steady-state pharmacokinetics of delavirdine mesylate (U-90152T) in HIV positive patients (Protocol M/3331/0029). Upjohn Technical Report 7215-95-054, 06 February 1996. .....

12. Borin MT, Chambers JH, Freimuth WW, Carel BJ, Carberry PA, Driver MR. An evaluation of the effect of rifabutin on the steady-state pharmacokinetics of delavirdine mesylate (U-90152S) in HIV-positive patients (Protocol M/3331/0025). Upjohn Technical Report 7215-95-021, 06 October 1995. ...

13. Borin MT, Chambers JH, Freimuth WW, Carel BJ, Carberry PA. An evaluation of the effect of rifampin on the steady-state pharmacokinetics of delavirdine mesylate (U-90152S) in HIV-positive patients (Protocol M/3331/0028). Upjohn Technical Report 7215-95-043, 15 December 1995. .....

14. Driver MR, Cox SR, Batts DH, Carel BJ, Freimuth WW. Bioequivalency evaluation of U-90152S and U-90152T tablets given as a single 300-mg dose in healthy volunteers (Protocol M/3331/0033). Upjohn Technical Report 7215-94-010, 29 April 1994. .....

15. Della-Coletta AA, Fleishaker JC, Cox SR, Carberry PA, Freimuth WW. An Evaluation of the Multiple-Dose Pharmacokinetic Interaction Between Didanosine and Delavirdine Mesylate in HIV Positive Patients (M/3331/0030). Upjohn Technical Report 7215-96-001, 16 January 1996. .....

16. Voorman RL, Maio SM, Hauer MJ, Sanders PE, Payne NA, Ackland MJ. Metabolism of delavirdine (U-90152), an HIV-1 reverse transcriptase inhibitor, by microsomal cytochrome P450 in humans, rats and other species: Probable involvement of CYP3A subfamily. Upjohn Technical Report 7256-95-071, 20 February 1996. .....

17. Voorman RL, Maio SM, Payne NA, Wang X. Microsomal metabolism of delavirdine (U-90152): Evidence for mechanism-based inactivation of human cytochrome P450 3A by delavirdine. Upjohn Technical Report 7256-95-072, 06 February 1996. .....

18. Ward P, Cox SR, DeGeest G, Wathen LK, Nickens DJ, Knuth DW. A study of the tolerance and efficacy of combinations of U-90152S and nucleoside therapy in the treatment of HIV infected patients (24-week report; Protocol M/3331/0013A). Upjohn Technical Report 1340-96-006, 19 June 1996. .....

19. Freimuth WW, Greenwald C, Daenzer CL, Cox SR, Wathen LK. Interim report: A randomized, dose-escalating multi-dose phase I/II trial of oral U-90152S compared to conventional combination nucleoside therapy in HIV-1 infected patients (Protocol M/3331/0007). Upjohn Technical Report 9156-96-008, 1 July 1996. .....

20. Knuth DW, Cox SR. A blinded pharmacokinetic report to the delavirdine drug safety monitoring board summarizing the therapeutic drug monitoring and pharmacokinetic evaluations through 31 December 1995. Upjohn Technical Report 7215-96-023, 29 March 1996. .....

21. Bombardt PA, Brewer JE. Studies of serum protein binding of delavirdine (U-90152) in humans, Cynomolgus monkeys, dogs, and Sprague-Dawley rats. Upjohn Technical Report 7256-95-067, 05 February 1996, amended 22 March 1996. .

22. Sood VK, Chang M. In vitro partitioning of [^{14}C]delavirdine mesylate (U-90152T) and [^{14}C]atevirdine mesylate (U-87201E) to red blood cells of rat, dog, mouse, rabbit, monkey, and human. Upjohn Technical Report 7256-95-045, 30 January 1996. .....

23. Staton BA, Bombardt PA. U-90152 levels in HIV+ human cerebrospinal fluid: Quantitative determination by HPLC and fluorescence detection. Upjohn Technical Report 7256-93-132, 23 November 1993. .....

24. Staton BA, Bombardt PA. U-90152 levels in HIV$^+$ human saliva: Quantitative determination by HPLC and fluorescence detection. Upjohn Technical Report 7256-94-060, 02 June 1994. .....

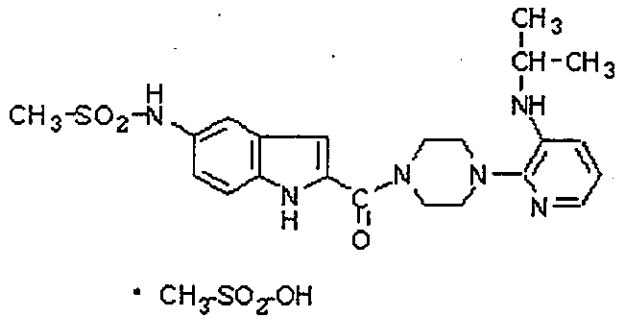
G. MICROBIOLOGY SUMMARY

1. Introduction

Delavirdine mesylate (DLV, U-90152) is a potent inhibitor of HIV-1 replication. The Mechanism of Action section contains a description of how delavirdine selectively inhibits HIV-1 reverse transcriptase (RT), exhibiting no significant inhibition of cellular DNA polymerase α and δ, HIV-2, or other retroviruses. Its selective action is exerted through a binding site on RT distinct from the dNTP substrate binding site. In the Pharmacokinetics section, the absorption, distribution, metabolism and excretion (ADME) studies conducted in mouse, rat, dog, monkey, rabbit and human demonstrate that the pharmacokinetics of delavirdine are nonlinear in all species. In vitro experiments show delavirdine mesylate to be a potent inhibitor of laboratory strains and clinical isolates of HIV-1 in several cell culture systems and the data are summarized in the Antimicrobial Activity section of this summary (page 110). Tissue culture and recombinant RT experiments investigating in vitro resistance are provided in the In Vitro Resistance section (page 117) and demonstrate that the predominant HIV-1 amino acid substitution attributable to delavirdine in vitro is P236L. Delavirdine has significant inhibitory activity against nucleoside resistant forms of RT and most non-nucleoside resistant forms of RT. Efficacy and resistance assays and their predictive values are discussed in the Clinical Laboratory Test Methods section (page 126). Viral isolates obtained in the clinical investigations of delavirdine were evaluated for growth, drug susceptibility and RT genotypes and that data are discussed in the In Vitro Studies Conducted During the Clinical Trials section (page 134).

2. Mechanism of Action

Delavirdine mesylate is a selective inhibitor of HIV-1 reverse transcriptase (RT). It has a molecular formula of $C_{22}H_{28}N_6O_3S \cdot CH_4O_3S$ and a relative molecular weight of 552.68.



Delavirdine mesylate (DLV) lacks structural similarity to known antiviral drugs, several of which are analogous to the nucleoside building blocks of nucleic acids. The HIV RT inhibitors zidovudine (ZDV), didanosine (ddI), and zalcitabine (ddC) are nucleoside analogs.

HIV-1 replication is dependent on the activities of reverse transcriptase. Host cell infection by HIV-1 is initiated by attachment of the viral envelope glycoprotein, gp120, to its major cellular receptor CD4 found on T-lymphocytes, monocytes and macrophages. Entry into the cell of the viral nucleocapsids of bound HIV virions follows gp41-mediated virus-cell membrane fusion. Upon entry into the cytoplasm, replication begins with reverse transcription of the nucleocapsid-associated viral genomic RNA (first strand cDNA synthesis) by the virion-associated heterodimeric RT. Complete double-stranded cDNA synthesis of the viral RNA in the preintegration complex is accomplished by the RNA-and DNA-dependent polymerase and RNase-H activities of RT. The complex is transported to the nucleus for integration of the viral DNA (or provirus) into the host genome by the viral integrase enzyme. Proviral DNA is transcribed by cellular RNA polymerase II to produce viral mRNAs for synthesis of viral proteins as well as to produce viral genomic RNAs for packaging into progeny virions. Since the multifunctional HIV-1 RT plays an essential role in the early events of the viral replication cycle, it is an important target for anti-retroviral intervention.

Early HIV-1 antiviral research led to the discovery that nucleoside RT inhibitors (NRTIs) such as zidovudine (ZDV), didanosine (ddI), zalcitabine (ddC) and stavudine (D4T) could inhibit viral DNA synthesis by RT competing with intracellular deoxynucleoside triphosphates (dNTPs). NRTIs are similar to the dNTP substrate of polymerases and thus can occupy the substrate binding site and prevent the nucleotide addition required for polymerization. NRTI action also includes termination of the nascent DNA chain, where the drug becomes incorporated but prevents further elongation due to lack of a 3'-OH. NRTI similarity to dNTPs prevents significant selectivity for viral polymerases over host polymerases, and similarity among NRTIs precludes true synergy at the enzyme level (where two drug molecules would simultaneously bind to RT).

Unlike the NRTIs, the non-nucleoside RT inhibitors (NNRTIs) such as delavirdine show very high selectivity for HIV-1 RT over host polymerases. Delavirdine inhibits recombinant HIV-1 RT by 50% at a concentration of 0.26 μM (IC_{50}), but fails to achieve comparable inhibition of DNA polymerases α and δ at 440 μM (Table G.1) [1] and in our preliminary experiments the γ polymerase was similarly unaffected [YC Cheng, personal communication]. Like most NNRTIs, delavirdine is also specific for HIV-1, having no detectable inhibitory effect on HIV-2 RT or virus [2]. These observations suggest that the NRTIs and the NNRTIs do not share a common mechanism of action involving competition for the dNTP substrate. Distinct modes of interaction with RT would allow for the two types of drugs to inhibit RT with synergy, which has been observed in vitro [3]. As discussed below, NNRTIs such as delavirdine are now known to bind RT at a site distinct from the dNTP binding site and thus the site of NRTI interaction. Recent evaluations have shown that delavirdine blocked RT mediated generation of DNA products in the absence of chain termination [4]. In addition, these data suggested that delavirdine and nevirapine, two different NNRTIs, interact with different chemical groups that constitute the RT hydrophobic pocket.

The kinetics of RT inhibition by delavirdine also support the model of delavirdine-RT interaction presented below. Applying a modified Briggs-Haldane analysis to an ordered RT polymerization model, inhibition of both the RNA- and DNA-directed DNA polymerase activity of RT by delavirdine is not competitive with respect to either the dNTP or template: primer substrates [5]. Delavirdine shows a higher binding affinity

for the enzyme-substrate complex than for the free enzyme, and thus does not directly impair the functions of the substrate binding sites. Delavirdine's preference for the enzyme-substrate complexes requires that its inhibition be described as "mixed" rather than "non-competitive," where binding to all forms of the enzyme is equivalent. This kinetic analysis suggests that delavirdine impairs an event occurring after the formation of the enzyme-substrate complex, perhaps inhibiting phosphodiester bond formation or translocation of the template:primer following bond formation.

From the crystalline structure of the HIV-1 RT it has been shown that the polymerase (pol) domain of the 66-kilodalton subunit has a large cleft analogous to that of the Klenow fragment of Escherichia coli DNA polymerase I [6]. This pocket does not exist in the p51 subunit [6], explaining the previous observations that p66 is the main target of this class of drugs [7]. Like the other NNRTIs, delavirdine mesylate binds to this cleft. The drug-binding pocket is formed on one side by the central three strands [8-10] of a five-stranded β-sheet in the palm domain, and on the other side by residues Phe-227 to Pro-236 from strands 12-14, a β-meander at the base of the thumb, and by Tyr-319 from the C terminus of β-strand 15. A solvent-accessible entrance to the cavity is formed by residues from the termini of strands 9 and 10 (Val179 and Ser191), the loop between β-strands 5 and 6 (Leu-100 to Lys-103) and Glu-138 located on the loop between β-strands 7 and 8 of the fingers domain of p51 [11]. Fan and coworkers [12] have constructed an HIV-2 RT substituted with the corresponding amino acids 176-190 from HIV-1 and showed that delavirdine sensitivity was indistinguishable from the wild type HIV-1 RT. Based on the fact that wild type HIV-2 is insensitive to delavirdine, it can be concluded that the 176-190 domain in the HIV-1 RT is involved in delavirdine binding.

While attempts to determine the crystalline structure of HIV-1 RT complexed with delavirdine have been unsuccessful, a model of delavirdine in complex with HIV-RT has been developed, based upon: (a) a structural model of the nevirapine/RT complex [13] and (b) a consensus binding site model. The structural model was developed by a combination of manual model building and constrained energy minimization techniques, relying on published pictures of the nevirapine/RT crystal structure as a guide [6,11,14]. The consensus binding site model was developed using similarity matching from electrostatic potential and/or molecular steric volume fields of several structurally diverse NNRTI molecules [15]. Data for multiple conformations and orientations of nevirapine, TIBO and L-697661 were matched to data for multiple conformations of an earlier generation BHAP, U-88204 [13]. The resulting pseudo-binding model was then used to model complexes of TIBO, L-697661, and BHAPs with RT [13]. The position of nevirapine was used as a template for positioning the pseudo-binding model into the RT structure at the binding site. Other inhibitors were then evaluated as to how well they fit into the binding site cavity. Further refinement of the model was made following publication of the coordinates for the crystal structure of an α-APA analog/RT complex. [16,17].

Delavirdine was positioned into the binding site cavity of the published RT crystalline structure by similarity matching the molecular steric volume and electrostatic potential of the α-APA molecule as described above. The binding site lies near, but is distinct from the polymerase active site of RT at positions 183 to 186. The delavirdine mesylate molecule fits into the pocket in a manner very similar to that determined by the earlier model. The isopropyl-amine moiety at one end of delavirdine mesylate

extended deep into the pocket and was located in a hydrophobic cleft which included the Pro236 residue. The delavirdine pyridine ring adjacent to the amine was also positioned in a hydrophobic region which seemed to be stabilized by a ring-ring stacking interaction with Tyr181. The indole ring system at the other end of the delavirdine molecule extended backward into the channel between Lys101, Val179, and Glu138 which leads from the binding pocket to the solvent exposed exterior of the protein. The binding seemed to be entirely hydrophobic in nature, since no hydrogen bonding involving delavirdine was determined in this binding model.

These modelling experiments, together with the observed kinetics of inhibition, suggest two possible mechanisms of RT inhibition by NNRTIs. Both are distinct from the NRTI mechanisms of inhibition and could potentially work in concert if NNRTIs and NRTIs were used in combination.

1) Binding of delavirdine may interfere with the movement of the thumb domain and thereby suppress translocation of the primer:template following nucleotide incorporation.

2) Delavirdine may alter the conformation of the β-strands. (Recombinant RT experiments have suggested that β strands 9 and 10 [residues 176-190] and/or β13-β14 [residue 236] play a role in the delavirdine binding domain) and thus the orientation of nearby conserved carboxylate side chains involved in catalysis.

In summary, delavirdine mesylate is a potent inhibitor of HIV-1 replication with the following properties:

- It selectively inhibits HIV-1 RT, exhibiting no significant inhibition of cellular DNA polymerase α and δ, or HIV-2;
- Its selective action is exerted through a binding site on RT distinct from the dNTP substrate binding site;
- RT inhibition by delavirdine differs from that of nucleoside analog RT inhibitors because it is not competitive with respect to the dNTP substrate.

Table G.1. *In vitro* Inhibition of HIV-1 RT and Normal Cellular Polymerases

Compound	IC_{50} (μM) for *:		
	HIV-1 RT	Polymerase α	Polymerase δ
Delavirdine Mesylate	0.26	440	>550
AZT triphosphate	0.15	60	140
2', 3'-Dideoxythymidine triphosphate	0.025	100	100

* Determined from the slopes of median effect plots of combined data from a minimum of two independent determinations. The correlation coefficients for the plots were ≥0.98.

3. Antimicrobial Activity of Delavirdine Mesylate

In vitro experiments have shown delavirdine mesylate to be a potent inhibitor of laboratory strains and clinical isolates of HIV-1 in various cell culture systems (summarized in Table G.2). The drug-sensitive phenotypes of laboratory strains of HIV-1 allow direct comparison of the antiviral activity of delavirdine to that of approved drugs like 3'-azido-3'-deoxythymidine (ZDV) [18]. Delavirdine inhibited replication of HIV-1_{IIIB} in MT-2 cells by 50% (IC_{50}) at 0.01 µM where a ZDV IC_{50} of 0.07 µM is required. In peripheral blood mononuclear cells (PBMC) infected with the HIV-1 laboratory strain D34, delavirdine had an IC_{50} of 0.1 nM while the ZDV IC_{50} was 1 nM. The in vitro antiviral activities of delavirdine, ZDV, and 2',3'-dideoxycytidine (ddC) had also been compared in acute infection experiments using HIV-1_{IIIB} in H9 cell culture and using the monocytotropic variant HIV-1_{JRCSF} replicating in PBMC (Table G.3). In both culture systems, delavirdine was at least as potent as ZDV or ddC and displayed IC_{50}s of approximately 6 nM. Chen [19] tested the delavirdine antiviral activity in primary human macrophage cultures infected with HIV-1_{JRFL}, another monocytotrophic strain of HIV-1. From day 7 through day 21 in culture, both 0.5 and 0.1 µM delavirdine inhibited viral replication by >90%. In addition, direct comparison of the antiviral activities of delavirdine and the non-nucleoside reverse transcriptase inhibitors (NNRTI) L-697661 and α-APA (R88703) against HIV-1_{IIIB} infection in MT4 cells revealed that the delavirdine IC_{90} (0.05 µM) was comparable to the other two NNRTIs tested (0.11 µM and 0.15 µM, respectively) [20].

The antiviral activity of delavirdine was also evaluated using panels of clinical HIV-1 isolates using the AIDS Clinical Trials Group (ACTG) standardized PBMC drug susceptibility culture assay [21]. Table G.4 shows the IC_{50}s of delavirdine and three nucleoside RT inhibitors (ZDV, didanosine (ddI), and ddC) against 25 patient isolates of HIV-1. Delavirdine was a potent inhibitor (mean IC_{50} = 0.068 ± 0.138 µM) of all the HIV-1 isolates tested [22]. A mean ZDV IC_{50} for the tested panel would reflect several isolates that are phenotypically resistant to ZDV, but even consideration of the most ZDV-susceptible isolates showed delavirdine to have potency comparable to ZDV. In these studies, 100 µM delavirdine caused less than 8% decrease in PBMC viability. Against an additional 24 clinical isolates (Table G.5) obtained from ZDV experienced patients (median ZDV experience = 17 months), delavirdine was a highly effective inhibitor of viral replication (mean IC_{50} = 0.017). ZDV was less effective against these cultured isolates due to extensive patient pre-exposure to ZDV [23].

In contrast to the potent inhibition of HIV-1 replication in human lymphocytes, no inhibition of HIV-2_{ROD} virus was observed at delavirdine concentrations up to 20 µM (Table G.2) [24]. For comparison, the IC_{50} for ZDV in this assay was <0.01 µM. This observation was consistent with the inability of all NNRTIs to inhibit recombinant HIV-2 RT activity in vitro. BHAPs closely related to delavirdine also lack antiviral activity against simian immunodeficiency virus (SIVmac252, B670), Rauscher murine leukemia virus, and the feline immunodeficiency virus in cell culture [2].

In addition to the T-lymphoid and PBMC culture systems described, a human brain cell culture model of HIV-1 infection was adapted for the purpose of testing the antiviral potential of delavirdine mesylate. In the microglial cells acutely infected with HIV-1 SF162, a macrophagetropic cerebrospinal fluid isolate [25], delavirdine

mesylate inhibited virus (97% p24 reduction) at a concentration of 1.0 μM [26]. When these experiments were extended to 21 days in culture, delavirdine mesylate demonstrated significant inhibition of viral replication.

The antiviral activity of delavirdine combined with other drugs has been assessed in a variety of experiments. The potential benefit of using delavirdine in combination with ZDV was assessed in a cell culture experiment that employed drug concentrations which allowed cell-to-cell spread of HIV-1_{IIIB} in MT-4 cells over periods up to several weeks. Infected MT-4 cells were co-cultivated with uninfected MT-4 cells at a ratio of 1:1000 in the presence of a range of concentrations of delavirdine, ZDV, or the two combined. In this system, the time until rapid viral spread through the culture (exponential increase in viral p24 antigen) was inversely related to the drug concentration present [1]. ZDV at 3 μM inhibited rapid viral replication for about 7 days, delavirdine at 1 μM delayed such replication about 30 days, and 3 μM delavirdine completely prevented detectable spread of HIV-1. When used in combination, lower concentrations of ZDV and delavirdine achieved comparable results: each at 0.5 μM combined blocked HIV-1 replication. When the drugs were removed from this culture at day 24, no virus emerged upon further drug-free growth of the culture. Prevention of viral spread coupled with death and/or dilution of the initially infected cells apparently removed all detectable virus from the cultures. These experiments suggested that low concentrations of delavirdine and ZDV used in combination achieved antiviral effects that would require relatively high concentrations of each drug used individually. When delavirdine was used in combination with each of several other antiretroviral agents (zidovudine, didanosine, lamivudine, zalcitabine, alpha-interferon [IF-α] and an experimental HIV-1 protease inhibitor), a synergistic effect was observed in acute infection experiments using peripheral blood mononuclear cell cultures [27-29].

To more rigorously assess the potential benefit of combining delavirdine with known anti-HIV drugs, inhibition of HIV-1 replication by delavirdine combined with ZDV, ddC, ddI, or 3TC at multiple molar ratios was evaluated [3,30]. Concentrations of each drug (1.0, 3.0, 10.0 and 30.0 nM) in combination with the others were assayed in a checkerboard manner (Table G.6). The combination indices (CI) were calculated using the multiple drug effect equation [31]. The CI values at various fractional inhibitions (50, 90, and 95%) were used to determine whether the combinations were synergistic (CI at 50% <1), additive (CI at 50% = 1), or antagonistic (CI at 50% >1). For each combination, and the majority of ratios, their respective CI values were less than 1, which suggested that these regimens inhibit in vitro replication in a synergistic manner. It should be noted that when delavirdine mesylate was administered orally to HIV-1–infected patients at 400 mg three times a day in well controlled pharmacokinetic trials, the observed mean peak plasma concentration of DLV was 41 ± 18 μM and mean trough concentrations of ZDV were 23 ± 12 μM. With oral doses of 10 mg of ZDV per kg, plasma concentrations were maintained above 1 μM in HIV-1–infected patients [32]. Thus, the concentrations of the drugs that were employed in these in vitro synergy experiments were much lower than the plasma concentrations achievable in patients.

The triple combinations of delavirdine, ZDV, and 3TC or delavirdine, ZDV, and ddI can also be used advantageously to inhibit HIV-1 in PBMC cultures (Table G.7). Greater suppression of viral growth was achieved with the three-drug "cocktail" than

with two-drug combinations at drug concentrations equal to those tested in the triple drug regimen [30]. The in vitro combinatorial effects of delavirdine, ZDV, and ddI on viral spread in PBMC was investigated in experiments where viral growth was monitored. HIV-1_{JRCSF} infected PBMC were grown with IC_{50-70} concentrations of the three drugs for 7 days. Drug-free medium was used to feed the cells on day 7 and thereafter. Measurement of p24 antigen in the media on days 2, 6, 8 and 12 post viral challenge demonstrated that the convergent combination provided more complete virus suppression than ZDV monotherapy [33]. The enhanced efficacy of these regimens supports the hypothesis that reverse transcriptase convergent therapy may be highly beneficial in HIV-1–infected patients. In an extensive in vitro comparison of selected triple-drug combinations for suppression of HIV-1 replication, it was clearly shown that for drugs that are not antagonistic, combination regimens with three drugs can have potent antiviral effects [34].

Taking into consideration the recent revelations on the dynamics of HIV-1 replication in humans [35-37], severe restriction of viral replication in vivo should lessen the destruction of target immune cells resulting in sustained immune function. Limiting widespread viral propagation may also minimize neoplastic complications and opportunistic infections, extend the life span of an infected person, and potentially reduce viral sexual and in utero transmission. Data from the studies briefly described above confirm that delavirdine mesylate is capable of completely inhibiting HIV-1 replication under in vitro conditions. Multiple combinations of delavirdine with ZDV, ddI, ddC, 3TC, alpha-interferon (IFN-α), or an HIV protease inhibitor (U-75875) demonstrated that these combination regimens are more potent than use of these compounds as single agents. Delavirdine in concert with any of these agents as double or triple therapy diminished or prevented HIV-1 replication in vitro.

Table G.2. Comparison of Delavirdine Antiviral Activity in Multiple HIV Isolates

Drug Regimen	HIV Isolate	Cell Type	IC_{50} (μM)	IC_{90} (μM)	Investigator	Geographic Location
Delavirdine	HIV-1_{IIIB}	H-9	0.006	.05	Chong	Kalamazoo, MI
		H-9	.01	.05	Pagano	Kalamazoo, MI
		MT4	ND	.05	Olmsted	Kalamazoo, MI
	HIV-1_{MF}	MT4	ND	0.1	Dueweke	Kalamazoo, MI
	HIV-1_{JRCSF}	PBMC	ND	0.1	Dueweke	Kalamazoo, MI
		PBMC	.017	.08	Chong	Kalamazoo, MI
		PBMC	.008	.04	Pagano	Kalamazoo, MI
		PBMC	.005	.04	Chong	Kalamazoo, MI
	Clinical	PBMC	0.07	ND	Mayers	Bethesda, MD
		PBMC	0.017	ND	Batts	Kalamazoo, MI
	HIV-1_{F162}	Microglial	0.01-0.03	ND	Peterson	Minneapolis, MN
	HIV-2_{ROD}	PBMC	>20	ND	Descamps	Paris, France
	HIV-$1_{group\ 0}$	PBMC	0.06->20	ND	Descamps	Paris, France
	HIV-1_{JRFL}	PBMC	0.02	ND	Chen	Los Angeles, CA

ND = not determined

Table G.3. Comparison of Antiviral Activity of Delavirdine Mesylate in Cell Culture With Those of ZDV and ddC*

HIV-1 Isolate	Cell Type	DLV		ZDV†		ddC	
		IC_{50}	IC_{90}	IC_{50}	IC_{90}	IC_{50}	IC_{90}
IIIB	H9	6.0 ± 2.0 (4-8)	45.0 ± 16.0 (26-65)	80.4 ± 60.0 (38-123)	1575.2 ± 1440 (437-2713)	31.8 ± 14.0 (13-47)	206.2 ± 148.0 (81-435)
JRCSF	PBMC	5.3 ± 4.0 (1-8)	39.3 ± 16.0 (18-47)	5.9 ± 4.0 (3-10)	19.0 ± 5.0 (16-25)	25.0 ± 9.0 (17-34)	152.7 ± 83.0 (102-248)

* Data represent mean IC_{50} and IC_{90} values in nanomoles ± standard error of the mean of five separate experiments (each using three replicate wells) for HIV-1_{IIIB}/H9 determinations and three experiments for HIV-1_{JRCSF}/PBMC determinations. Values in parentheses are the ranges of values.
† ZDV values vary widely due to H9 phosphorylation aberrancies.

Table G.4. Antiviral Activity of Delavirdine and Nucleoside Analog RT Inhibitors Against Clinical HIV-1 Isolates

Isolate	IC_{50} (μM) of*:			
	ZDV	ddI	2',3'-Dideoxycytidine	Delavirdine†
1	4.2	3.9	0.02	<0.005
2	0.005	0.3	0.02	0.005
3	0.003	2.6	0.003	0.006
4	0.008	0.2	0.01	0.007
5	0.2	1.9	0.02	0.008
6	0.2	0.6	0.03	0.01
7	4.3	1.4	0.03	0.01
8	7.4	2.2	0.02	0.01
9	0.05	9.8	0.08	0.02
10	6.6	2.8	0.03	0.02
11	0.5	2.1	0.02	0.02
12	0.02	4.3	0.02	0.03
13	<5.0	15.5	0.05	0.03
14	3.5	16.7	0.15	0.04
15	1.0	2.5	0.01	0.04
16	0.1	6.0	0.02	0.05
17	0.02	3.1	0.04	0.06
18	2.9	3.1	0.03	0.08
19	2.1	6.5	0.05	0.10
20	0.1	2.7	0.06	0.11
21	0.05	9.5	0.12	0.18
22	0.6	15.4	0.07	0.16
23	3.1	5.8	0.03	0.69
24	0.02	0.4	<0.01	<0.005
25	0.005	0.05	<0.01	<0.005

* Determined with the median effect equation and triplicate wells at each of six drug concentrations.

† DLV at 100 μM caused less than 8% reduction in cell viability.

Table G.5. Sensitivity of Clinical Isolates to Delavirdine Mesylate and ZDV

Patient	Delavirdine Mesylate IC_{50} (μM)	ZDV IC_{50} (μM)
1	0.04	0.45
2	0.001	0.02
3	0.03	0.13
4	0.02	5.0
5	0.02	5.0
6	0.001	0.05
7	0.005	0.02
8	0.007	0.91
9	0.02	0.01
10	0.006	0.69
11	0.009	0.20
12	0.002	0.24
13	0.004	0.11
14	0.004	0.02
15	0.004	0.04
16	0.02	3.0
17	0.02	0.05
18	0.007	0.22
19	0.005	0.01
20	0.006	0.02
21	0.03	0.02
22	0.002	0.64
23	0.0005	0.01
24	0.002	5.0

Table G.6. Combination Index (CI) Values for Two-Drug Combination Regimens of Delavirdine (DLV) with Lamivudine (3TC), Zidovudine (ZDV), Didanosine (ddI) and Zalcitabine (ddC) Against HIV-1 Replication in H9 Cells or PBMC

Exp #	Cell Type & Virus	Combination	Ratio†	CI* at % inhibition	
				50	95
1	H9; HIV-1_{IIIB}	DLV + ddC	1:1 1:3 1:10	0.68 0.52 0.34	0.57 0.67 1.32
2	H9; HIV-1_{IIIB}	DLV + ddC	1:1 1:3 1:10	0.33 0.24 0.30	0.45 0.63 0.46
3	PBMC; HIV-1_{JRCSF}	DLV + ddC	1:1 1:3 1:10	0.66 0.57 0.14	0.45 0.37 1.26
4	PBMC; HIV-1_{JRCSF}	DLV + ZDV	1:1 1:3 3:1	0.42 0.20 0.21	0.39 0.68 0.78
5	PBMC; HIV-1_{JRCSF}	DLV + ZDV	1:1 1:3 3:1	0.29 0.56 0.19	0.64 0.82 0.72
6	PBMC; HIV-1_{JRCSF}	DLV + 3TC	1:1 1:3 1:10	0.97 0.61 0.58	0.61 0.47 0.48
7	PBMC; HIV-1_{JRCSF}	DLV + 3TC	1:1 1:3 1:10	0.75 0.68 0.68	0.53 0.50 0.57
8	PBMC; HIV-1_{JRCSF}	DLV + 3TC	1:1 1:3 1:10	0.88 0.66 0.61	0.68 0.41 0.55
9	PBMC; HIV-1_{JRCSF}	DLV + ddI	1:10 1:30 1:100	0.92 0.96 0.65	0.30 0.30 0.24
10	PBMC; HIV-1_{JRCSF}	DLV + ddI	1:10 1:30 1:100	0.68 0.64 0.83	0.53 0.43 0.91

* CI values of <1, 1, and >1 indicate synergism, additive effects, and antagonism, respectively.
† Molar ratio of compounds in nM.

Table G.7. Combination Index (CI) Values for Three-Drug Combination Regimens of Delavirdine (DLV) with Lamivudine (3TC), Zidovudine (ZDV), Didanosine (ddI), and Zalcitabine (ddC) Against HIV-1 Replication in H9 Cells or PBMC

Exp #	Cell Type & Virus	Combination	Ratio†	CI* at % inhibition	
				50	95
1	PBMC; HIV-1_{JRCSF}	DLV + 3TC	1:1	.57	.66
		ZDV + DLV	1:3	.95	.71
		ZDV + 3TC	1:10	.90	.60
		ZDV + DLV + 3TC	1:3:10	.88	.85
2	PBMC; HIV-1_{JRCSF}	DLV + 3TC	1:1	.64	.70
		ZDV+DLV	1:3	1.02	.64
		ZDV+3TC	1:10	.97	.62
		ZDV+DLV+3TC	1:3:10	.98	.82
3	PBMC; HIV-1_{JRCSF}	DLV + ddI	1:150	1.24	.68
		ZDV + DLV	1:2	.94	.65
		ZDV + ddI	1:300	1.01	.69
		ZDV + DLV + ddI	1:2:300	.59	.58

* CI values of <1, 1, and >1 indicate synergism, additive effects, and antagonism, respectively.
† Molar ratio of compounds in nM

4. Assessment of Resistance

The rapidity with which drug resistant variants of HIV-1 are selected in vivo by nucleoside and non-nucleoside inhibitors of reverse transcriptase (RT), as well as viral protease inhibitors, can be explained by recent studies of the dynamics of HIV-1 replication in infected individuals which revealed an extremely high rate of virus replication and turnover **[35,36]**. High levels of replication combined with the infidelity of the RT DNA polymerase inevitably lead to the generation of the viral quasispecies from which drug resistant variants, harboring resistance conferring mutations, are selected during drug therapy. The selection and characterization of resistant HIV-1 variants in vitro has lead to a better understanding of the potential for resistance development in vivo to a particular drug and the genotypes (ie, mutations) responsible for the sensitivity/resistant phenotypes observed. The following studies describe the phenotypic and genotypic analyses of HIV-1 in vitro resistance to delavirdine.

a. Selection of Delavirdine Resistant Variants of HIV-1 in Cell Culture: Drug Sensitivity Phenotype and RT Genotype Analyses

To assess the in vitro development of resistance to delavirdine, two drug sensitive strains of HIV-1 that display different cell tropism phenotypes were serially passed in cell cultures in the presence of delavirdine or atevirdine, a closely related BHAP homolog (U-87201E)**[38]**. HIV-$1_{JR\text{-}CSF}$ is a monocytotrophic isolate whose in vitro replication is restricted to PBMCs **[39]**. HIV-1_{MF} is highly cytopathic for both peripheral blood mononuclear cells (PBMC) and T-lymphoid CD4+ cell lines (eg, MT4 cells) **[40]**. Serial propagation of HIV-$1_{JR\text{-}CSF}$ in PBMCs and of HIV-1_{MF} in MT-4 cells in the presence of increasing concentrations of delavirdine and atevirdine, respectively, led to the selection and isolation of drug resistant variants from both parent viruses

[38]. Drug sensitivity analyses revealed IC_{90} estimates of >10 μM and >3 μM for delavirdine against the drug resistant variants of HIV-1_{JR-CSF} and HIV-1_{MF}, respectively, compared to IC_{90} estimates of ~ 0.1 μM and 0.06 μM against their respective parent viruses. The genotypic basis for the delavirdine resistant phenotypes displayed by these two variants was investigated by sequence analysis of their RT genes. Relative to the parental drug sensitive HIV-1_{JR-CSF}, delavirdine resistant HIV-1_{JR-CSF} contained mutations in the RT gene that encoded two amino acid substitutions, a leucine to phenylalanine at amino acid 228 (L228F) and a proline to leucine at amino acid 236 (P236L). Similar analysis of the RT gene of the atevirdine resistant HIV-1_{MF}, which displayed a delavirdine resistant phenotype, revealed RT substitutions at codon 236 (P236L) and 273 (G273R). These studies clearly demonstrated that high level resistance to delavirdine can be selected in vitro and the resistance phenotype was conferred by a proline to leucine substitution at RT codon position 236 [38].

In a subsequent study, a panel of NNRTI resistant variants was obtained that included two delavirdine resistant viruses derived from the drug sensitive strains, HIV-1_{MF} and HIV-1_{IIIB} [20]. Virus stocks were harvested when growth was sustained in 10 μM of the NNRTI used for the resistance selection. Genotypic analysis of the delavirdine resistant HIV-1_{MF} variant, HIV-1_{MF}-BHAP^r, revealed the presence of the P236L RT substitution as described in the previous study [38]. The P236L substitution conferred high level resistance to delavirdine (IC_{90} >10 μM), however and as shown previously [38], this substitution did not confer cross-resistance to two other NNRTIs tested. HIV-1_{MF}-BHAP^r remained susceptible to the NNRTIs, L-697661 (pyridinone), and α-APA (R88703) (Table G.8)[20]. The second delavirdine resistant variant from this study, HIV-1_{IIIB}-BHAP^r, harbored substitutions at RT codons 100 (Leu→Ile) and 230 (Met→Leu). These substitutions conferred high level resistance to delavirdine (IC_{90} >10 uM) but in contrast to the P236L RT genotype of HIV-1_{MF}-BHAP^r, these mutations conferred significant cross-resistance to the antiviral effects of L-697661 and α-APA (IC_{90}s: 4.0 and 6.5 μM, respectively; Table G.8).

b. Delavirdine Activity Profile versus RTI Resistant HIV-1 Variants

Early studies on the characterization of NNRTI resistant HIV-1 variants derived by single drug treatments in cell culture demonstrated that a tyrosine to cysteine substitution at RT codon 181 (Y181C) conferred broad cross resistance to most NNRTIs [41,42]. From the study shown in Table G.8 [20], the variants selected to grow in 10 μM pyridinone or α-APA harbored the Y181C RT mutation and displayed significant resistance to the antiviral effects of delavirdine (IC_{90}s: 5.2 μM and 1.0 μM, respectively). A separate study has characterized the NNRTI drug sensitivity/resistance spectrum of HIV-1_{IIIB} resistant variants that emerged from thiocarboxanilide derivative selection [43]. Five of seven variants harbored single mutations at RT codons 100 and 103, whereas two variants carried double substitutions at either codons 101 and 141 or 101 and 190. All the NNRTIs tested against these mutant viruses, including delavirdine, were less effective against these virus strains than against wild-type virus (Table G.9). The delavirdine IC_{50} estimates against this panel of mutant viruses were all below 3 μM which is a drug level achievable in humans administered a 400 mg tid dose regimen.

Laboratory strains of HIV-1 resistant to (-)2',3'-dideoxy-3'-thiacytidine (3TC) containing the M184V RT substitution or ddC (which contain the K65R substitution) and two clinical strains resistant to 3TC (M184R) were assessed for cross-resistance to delavirdine in cell culture (Table G.10) [44]. The IC_{50} estimates demonstrated that delavirdine was equally effective in inhibiting the resistant variants carrying the M184V or K65R RT substitutions compared to drug sensitive wild-type control HIV-1s (HxB2 or HIV-1_{IIIB}). Thus, neither of the two RT substitutions that confer resistance to the nucleoside RT inhibitors 3TC or ddC reduced the susceptibility of these variants to delavirdine.

Of equal importance is the assessment of the effects of ZDV resistance conferring RT mutations on delavirdine antiviral activity. Thirteen HIV-1 pre-treatment M/3331/0003 clinical isolates, each containing one or more ZDV resistance conferring RT mutations, were tested in PBMC cultures with delavirdine. These isolates had amino acid substitutions at RT codons 41, 67, 70, 215 and 219 (Table G.11). The 13 ZDV resistant clinical isolates tested were fully susceptible to the inhibitory effects of delavirdine (IC_{50} range: 0.001 µM to 0.039 µM). These data suggest that the presence of single or multiple ZDV resistant mutations does not significantly reduce susceptibility to delavirdine.

Various combinations of 5 RT substitutions (M41L, D67N, K70R, T215Y, and K219Q) have been shown to be responsible for conferring ZDV resistance [45]. It has been shown that the NNRTI resistance conferring substitution Y181C diminishes the ZDV resistance conferred by a number of combinations of these known ZDV resistance conferring substitutions [46]. To examine what effects the delavirdine resistance conferring substitution P236L had on the accumulation of ZDV resistance conferring substitutions, a highly delavirdine resistant variant, HIV-1_{MFE30} [38], was passaged in the presence of 10 µM delavirdine alone or with delavirdine and increasing concentrations of ZDV (0.02 to 5 µM). Despite the continued presence of 10 µM delavirdine, in virus passaged with delavirdine and ZDV, the P236L substitution reverted to wild type. Before any known ZDV resistance conferring substitutions appeared, the P236L substitution had completely reverted to wild type, and two other substitutions accumulated, namely K103N and M230L. Only at this point did the ZDV resistance conferring substitutions D67N, K70R, and T215Y begin to appear [47]. In comparison, virus similarly passaged in the presence of delavirdine alone showed no change from the P236L genotype. These data suggest that there may be some replicative disadvantage to virus containing the P236L and ZDV resistance conferring substitutions, but that this disadvantage can be overcome by using different substitutions to confer resistance to delavirdine.

c. Delavirdine Susceptibility of Mutant Recombinant Reverse Transcriptases

As HIV-1 variants resistant to delavirdine or other RT inhibitors were characterized, the observed mutations in the RT genes were re-created in plasmids to encode recombinant RT enzymes with specific amino acid substitutions. Assay of such recombinant RTs in vitro allowed for rapid comparison of different RT mutants and the effects of different non-nucleoside drugs on these enzymes. This approach identified the P236L RT substitution as the predominant cause of resistance in the BHAP resistant HIV-1_{MF} and/or HIV-1_{JR-CSF} described above. Also, by evaluating the effects of different RT inhibitors on recombinant P236L RT, it became apparent that

other non-nucleosides such as L-697661 and TIBO R82913 have lower IC_{50}s versus this mutant than against the wild type enzyme. With the NNRTIs, drug susceptibility of mutant HIV in cell culture often reflects the susceptibility of the corresponding mutant RTs in vitro, and HIV-1_{MF} with the P236L RT substitution was more sensitive to L-697661 than the parental HIV-1_{MF} [38]. Inhibition of recombinant RT with the P236L substitution as well as two other non-nucleoside-associated resistance mutations (K103N and Y181C) by a few NNRTIs is shown in Table G.12. The RT substitutions Y181C and K103N were identified from HIV grown in the presence of L-697661 and nevirapine [41,42] and shown to cause cross resistance to other NNRTIs. Y181C and K103N RTs were less susceptible to delavirdine, which suggests that delavirdine has a mode of action similar to other NNRTIs. However, the disparate effect of the P236L substitution on the potency of delavirdine versus that of other NNRTIs reveals a significant difference in their exact binding interactions with RT.

To further characterize the NNRTI binding pocket of the HIV-1 RT, RTs with various substitutions at amino acid 236 were expressed in *E. coli*: P236L, P236T, P236H, P236R, and P236A [48]. Relative to the wild type, the IC_{50} was highest for the P236L mutant and lowest for the P236A mutant (P236L>P236T>P236H>P236R>P236A). Relative to wild type RT, all these P236 mutants had enhanced susceptibility to L-697661, with the effect of P236L being greatest. The resistance trends did not correlate well with the polarity or size of the amino acids present at position 236 of HIV-1 RT, suggesting that binding of delavirdine to RT may not involve a specific interaction between the drug and the side chain of the 236 residue of HIV-1 RT. Fan and coworkers concluded that substitution of alternative amino acids for proline 236 alters the geometry of the inhibitor-binding pocket [48]. The altered pocket geometry may cause decreased affinity for delavirdine (resistance) while increasing affinity for L-697661.

Amino acid substitutions at other positions thought to lie near the NNRTI binding pocket of HIV-1 RT also affect the NNRTI susceptibility of RT. The Y181C substitution and its effects are described above. NNRTI resistant viral variants with substitutions such as Y181I, Y188L, or V106I have been identified from viral passage in cell culture or from HIV-1–infected patients [41,49-52]. These substitutions in recombinant RT confer less resistance to delavirdine than to the pyridinone L-697661 [12] (Table G.13).

S-2720 and other members of the quinoline/quinoxaline class of HIV-1 non-nucleoside reverse transcriptase inhibitors have been shown to select for a glycine to glutamate substitution at residue 190 (G190E) of the RT in cell culture. Mutant HIV-1 reverse transcriptase enzymes (G190 E, D, H, R, P, F, Q, L, V, S, A) were derived from the HIV-1_{MN} RT sequence using site-directed mutagenesis and assayed for resistance to various NNRTIs [53]. Delavirdine was clearly the most active non-nucleoside RTI tested against these mutants (Table G.14).

There has been no evidence that substitutions which confer resistance to the nucleoside RTIs affect susceptibility of RT to inhibition by delavirdine. As described above however, the NNRTI resistance conferring substitutions can have profound effects on pre-existing ZDV resistance. When delavirdine resistant HIV-1 containing the P236L substitution was passaged in delavirdine and increasing concentrations of ZDV, two substitutions, M230L and K103N, were selected for as the P236L

substitution reverted to wild type. To determine the effects of the M230L and K103N substitutions on delavirdine resistance, recombinant RTs containing various combinations of these substitutions along with ZDV resistance conferring substitutions were expressed in E. coli and tested for resistance to delavirdine. As shown in Table G.15, both the M230L and K103N substitutions individually confer only low level resistance to delavirdine. The IC_{50}s of delavirdine against the M230L or K103N substituted RTs (4.2 and 3.4 μM respectively) were not as high as that obtained with RT containing the P236L substitution and considerably lower than the 10 μM in which the virus was propagated. When the M230L and K103N substitutions are contained within the same RT however, the RT is highly resistant to delavirdine. Two substitutions were apparently necessary to maintain delavirdine resistance as the RT remodelled to confer resistance to ZDV **[47]**.

Tissue culture and recombinant RT experiments have demonstrated that the predominant HIV-1 amino acid substitution attributable to delavirdine in vitro was P236L. Rather than conferring cross resistance to other RT inhibitors, this substitution sensitized RT 7-10 fold to TIBO R82913 and L-697661 without influencing sensitivity to nucleoside analogue RT inhibitors **[38]**. Although the RT amino acid substitutions K103N and Y181C, which confer cross-resistance to several non-nucleoside inhibitors, also decrease the potency of delavirdine, this drug retains significant activity against these mutant RTs in vitro **[38]**. Mutations which confer resistance to one drug can result in increased sensitivity to a second drug **[46,54]**. The Y181C mutation in a ZDV resistant background has been shown to significantly suppress resistance to ZDV **[46]**. Similarly, the codon 184 mutation conferring resistance to 3TC suppresses resistance to ZDV **[55]**. Emergence of the Y181C or another substitution during delavirdine therapy could cause resensitization of ZDV resistance. Delavirdine has significant inhibitory activity against nucleoside resistant forms of RT and most non-nucleoside resistant forms of RT. Although the elucidation of patterns of HIV-1 drug resistance for the different classes (NRTI, NNRTI or protease inhibitor) is far from complete, it is evident that within each category of inhibitor, resistance to one compound does not necessarily lead to resistance to the other compounds of that same class **[56]**.

Table G.8. Antiviral Activity of Delavirdine and Other Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI) Against *in vitro* Selected NNRTI Resistant HIV-1 Variants

RTI	IC_{90} (μM)[a]				
	HIV-1_{IIIB} (wild type)[c]	HIV-1_{MF}-BHAP^{r} [b] (Leu-236)	HIV-1_{IIIB}-BHAP^{r} (Ile-100; Leu-230)	HIV-1_{IIIB}-Pyr^{r} (Cys-181)	HIV-1_{IIIB}-α-APA^{r} (Cys-181)
Delavirdine (BHAP)	0.05	>10	>10	5.2	1.0
L-697661 (Pyr)	0.11	0.43	4.0	>10	7.7
α-APA (R88703)	0.15	0.08	6.5	>10	>10

[a] IC_{90}, concentration of drug that inhibited p24 production by 90% in infected MT4 cells.
[b] Delavirdine (U-90152T) was used for the selections of the BHAP resistant HIV-1_{MF} and HIV-1_{IIIB} variants.
[c] RT genotype.

Table G.9. Spectrum of Sensitivity/Resistance of HIV-1 Thiocarboxanilide Resistant Mutants to Delavirdine Inhibition

HIV-1 Mutant Strains	IC_{50} (μM)[a]
HIV-1 IIIB (Wild Type)	0.03[b]
103N	0.55
100I	1.50
103N	0.11
103T	1.00
101I + 141E	0.22
103N	1.10
101E + 190E	2.8[b]

[a] IC_{50}, concentration of drug that inhibited viral growth 50%.
[b] Values for BHAP U-88204 (DLV data are comparable)

Table G.10. Delavirdine Inhibition of Laboratory and Clinical HIV-1 Variants Resistant to 3TC or ddC.

Virus	IC_{50} μM[a]			
	Delavirdine	Nevirapine	ZDV	d4T
HxB2	0.006	0.3	0.002	0.3
HIV-1IIIB	0.004	0.2	0.002	0.4
HXB2-M184V	0.008	0.3	0.001	0.3
M184V- clin*A	0.005	0.5	0.003	0.5
M184V- clin*B	0.004	0.4	0.001	0.4
HxB2-K65R	0.002	0.6	0.001	0.6

[a] IC_{50}, concentration of drug that inhibited viral growth 50%.
*clin = clinical isolate.

Table G.11. Inhibition of ZDV Resistant Clinical Isolates by Delavirdine

Clinical Isolate	ZDV Mutation(s) in RT	ZDV IC_{50} (μM)[a]	Delavirdine IC_{50} (μM)[a]
1	K70R	0.296	0.039
2	M41L, T215Y	0.019	0.001
3	M41L	0.131	0.028
4	M41L, T215Y	5.000	0.020
5	K70R, T215Y	4.040	0.022
6	D67N, K70R, K219Q	0.512	0.011
7	K70R	0.022	0.005
9	M41L, T215Y	0.709	0.007
16	D67N, K70R	0.454	0.002
17	K70R	0.114	0.004
23	D67N, K70R, K219Q	3.044	0.016
30	K70R	0.102	0.027
34	M41L, T215Y	4.403	0.002

[a] IC_{50}, concentration of drug that inhibited viral growth 50%.

Table G.12. *In vitro* Inhibition of Recombinant HIV_{IIIB} RT Mutants

RT	IC_{50} μM			
	Delavirdine	Nevirapine	L-697661	R82913
Wild Type	0.26 ± 0.04	3.1 ± 0.3	0.80 ± 0.08	3.8 ± 0.6
Y181C	8.32 ± 0.70	>60*	>60*	38 ± 7
K103N	7.7 ± 0.6	>60*	15 ± 4.1	>60*
P236L	18.0 ± 2.1	0.32 ± 0.02	0.11 ± 0.01	0.32 ± 0.05
Y181C/P236L	>60*	6 ± 1	10.0 ± 1.6	8.7 ± 1.1

RNA-dependent DNA polymerase activity of the mutant RTs was assayed and IC_{50} values were determined by nonlinear least-squares fit of data from triplicate points at 12 drug concentrations. Computed best-fit value for IC_{50} ± asymptotic standard deviation is shown.
* Highest drug concentration tested

Table G.13. Inhibition of RNA-Dependent DNA Polymerase Activity of Mutant Enzymes of HIV-1 RT by Delavirdine and L-697661

HIV-1 RT	Delavirdine IC_{50} (μM)*	L-697661 IC_{50} (μM)*
Wild Type	0.29 ± 0.01	0.23 ± 0.01
Y181I	3.60 ± 0.15	>100
Y188L	0.71 ± 0.02	35.2 ± 1.50
V106I	0.30 ± 0.01	0.21 ± 0.01

* The RT activity assays were carried out in the presence of poly(rA):oligo(dT) as the template primer. IC_{50} values greater than 100 μM cannot be determined accurately due to solubility limits. The data represent mean ± S.D. (n = 3).

Table G.14. Analysis of Various Mutants with Amino Acid Substitutions at Position 190 of the HIV-1 RT

Enzyme	Amino Acid at Position 190	Activity*	IC_{50} (μM)† DLV	IC_{50} (μM) Nevirapine	IC_{50} (μM) L697661
RT-1 MN	Gly	1.0	1.4	7.5	1.5
RT-1 E	Glu	0.04	200(143)	>500 (>67)	>500 (>333)
RT-1 D	Asp	0.03	>500 (>357)	>500 (>67)	>500 (>333)
RT-1 H	His	0.04	29 (21)	>500 (>67)	>500 (>333)
RT-1 R	Arg	0.08	>500 (>357)	>500 (>67)	>500 (>333)
RT-1 P	Pro	0.36	0.33 (0.24)	>500 (>67)	68 (45)
RT-1 F	Phe	0.03	40 (29)	>500 (>67)	270 (180)
RT-1 Q	Gln	1.0	43 (31)	>500 (>67)	>500 (>333)
RT-1 L	Leu	0.1	0.2 (0.14)	>500 (>67)	11 (7)
RT-1 V	Val	0.39	0.1 (0.07)	>500 (>67)	6.1 (4)
RT-1 S	Ser	2.9	0.5 (0.36)	>500 (>67)	3.2 (2)
RT-1 A	Ala	1.1	0.5 (0.36)	>500 (>67)	5.4 (4)

* Activity of mutant enzymes are compared to the HIV-1 MN wild type enzyme.
† Fold increase in inhibitory concentrations compared with the HIV-1 MN wild type enzyme are given in parentheses.

Table G.15. Delavirdine Inhibition of Recombinant Mutant Reverse Transcriptases

HIV-1 Mutant Enzymes	IC_{50} (μM)*
Wild Type	0.22± 0.02
D67N/K70R	0.13 ± 0.03
K103N	3.41 ± 0.64
M230L	4.21 ± 0.67
K103N/M230L	106 ± 16
K70R/K103N/M230L	128 ± 33
K70R/K103N	4.44 ± 0.21
D67N/K70R/M230L	1.31 ± 0.01
P236L	18.0± 2.1

*IC_{50}s were determined by nonlinear best fit to :$v_{inhib}/v_{uninhib} = 1/(1+[I]/IC_{50})$ using Sigma Plot (Jandel Scientific, San Rapheal, CA). Mean values and deviations were calculated from two or more assays.

5. Clinical Laboratory Test Methods

a. Quantitation of HIV-1 RNA in Plasma

The RNA from HIV-1 virions in plasma was extracted with a guanidine thiocyanate solution and reverse transcribed into DNA. The resulting cDNA was then amplified using the polymerase chain reaction (PCR) process. PCR amplification was achieved by repetitive cycles of DNA denaturation, annealing HIV-1 gag primers, and primer extension, resulting in exponential generation of specific HIV-1 genetic material. The amplification product was captured using an avidin coated microtiter plate and hybridized with a horseradish peroxidase labeled HIV-1 probe. Comparison of the sample optical densities to the optical densities of a standard curve material derived from recombinant RNA corresponding to nucleotides 679-2095 of the gag region provided quantitation of the specimens. A complete description of the procedures involved in this assay is detailed in Item 7, Appendix A.

This quantitative human immunodeficiency virus type 1 (HIV-1) RNA polymerase chain reaction assay has been validated analytically and clinically in over 13,000 samples [57]. The assay was highly reproducible with intra- and inter- assay precision of 16 and 19%, respectively. In 1542 of 1548 subjects with CD4+ counts of 0-500 cells per mm^3, viral RNA levels were quantifiable and ranged from about 3000-52,200,000 copies per milliliter. Median plasma HIV-1 RNA values were inversely proportional to CD4+ counts from 0-400 cells per mm^3. When patients were off antiretroviral therapies for 2-4 weeks prior to the initial baseline RNA PCR evaluation, the mean variance between the two baseline values obtained 7 days apart was 23% (0.1 log). Ninety-five percent of these patients with 0-500 CD4+ cell counts had a sufficient plasma viral load to quantitate at least a 10 fold (1 log) diminution in viral load caused by antiviral therapy. In contrast, only 20% and 45% of these subjects had sufficient p24 and immune complex dissociated (ICD) p24 levels to detect a 50% diminution in viral antigen virus. The high precision and reproducibility of this quantitative RNA PCR assay provided an enhanced means of evaluating therapeutic drug regimens for HIV-1.

HIV-1 RNA burden in plasma samples was measured using this quantitative polymerase chain reaction (PCR) assay from over 2000 patients participating in two ongoing blinded trials who received DLV in combination with either ZDV or ddI, or ZDV or ddI alone. In these trials, about 11.2% of patients experienced clinical progression, (ie, AIDS defining illness or death). The prognostic value of baseline RNA levels and changes from baseline in RNA PCR levels (viral burden), CD4+ T-lymphocyte count, and ICD p24 antigen following antiretroviral therapy were compared using the Cox proportional hazards regression model. Protocol M/3331/0017 enrolled ZDV-experienced patients with CD4+ cell counts <300 cells/μL who received ddI with or without DLV (400 mg tid). Protocol M/3331/0021 enrolled patients (60% antiretroviral therapy naive) with CD4+ cell counts between 200 and 500 cells/μL who received ZDV alone or in combination with one of three doses (200, 300, or 400 mg tid) of DLV. From these analyses, baseline HIV-1 RNA levels in plasma, decrease in HIV-1 RNA levels from baseline, and baseline CD4+ cell count in subjects receiving antiretroviral therapy (six different regimens were tested) were predictive of clinical progression [58]. Patients having a 0.5 $\log_{10}$ (68%) decrease in HIV-1 RNA level while receiving antiretroviral therapy reduced the risk of clinical progression by about 56%. A therapeutic reduction of 1 $\log_{10}$ (90%) lowered the risk of experiencing a clinical outcome by about 80% within the timeframe assessed. After as little as 8 weeks of therapy, the predictability of the clinical outcome based on the RNA response was statistically detectable. Risk of HIV-1 disease progression was small (≈1%) for subjects with a baseline HIV-1 RNA level <100,000 copies/mL. Initial ICD p24 antigen levels and decrease in ICD p24 during antiretroviral therapy were not statistically significant predictors of clinical progression. In addition, increase in CD4+ cell count from baseline was less predictive of clinical outcome than viral burden [58].

b. Quantitation of HIV-1 DNA in Lymphocytes

Peripheral blood mononuclear cells (PBMCs) were separated from whole blood and lysed. HIV-1 proviral DNA, incorporated in host cell DNA, was amplified by polymerase chain reaction using primers specific to the gag region of HIV-1. PCR amplification was achieved by repetitive cycles of DNA denaturation, annealing HIV-1 gag primers, and primer extension, resulting in exponential generation of specific HIV-1 target sequences. The amplification product was quantified using an avidin coated capture plate and a commercially generated horseradish peroxidase labeled probe. Comparison of the sample optical densities to the optical densities of a standard curve material acquired commercially (plasmid DNA that contained the entire, though rearranged genome of the HIVZ6 [59]) provided quantitation of the specimens. Item 7, Appendix B contains the complete standard operating procedure for this assay.

The levels of HIV-1 DNA (provirus load) in infected patients has been studied due to the association of CD4+ cell decline and disease progression. HIV-1 DNA copy frequencies have been reported to be about 1:100 CD4+ cells in patients with acquired immunodeficiency syndrome (AIDS) [60]. In asymptomatic patients frequencies vary between 1:10000 and 1:1000 CD4+ cells. A longitudinal study has shown that an increasing provirus load in peripheral blood CD4+ cells is directly associated with a progressive decline in CD4+ cells and deteriorating clinical course [60]. Patients with high viral DNA burden have been found to have a high percentage of CD8+CD11+

suppressor cells, which can down-regulate immune responses, and low or undetectable cytotoxic T-lymphocyte activity [61]. Luque and coworkers [62] found an intrinsic rate of HIV-1 proviral load increase in untreated patients. These studies showed a reduction in this rate in zidovudine treated patients. In addition, they found patients with the RT codon 215 mutation did not respond to ZDV therapy. Others have found the number of HIV-1 proviruses in the lymphocytes of non-progressor subjects varied from 5 to 160 copies/μg DNA, values ten times lower than those detected in fast progressors and AIDS patients [63]. These studies illustrate that there is a correlation of HIV-1 viral DNA load with increased disease progression. Some patients responding to antiretroviral therapy have diminished proviral load, and when mutations diminish drug effectiveness, HIV-1 DNA can also be effected.

c. Analysis of CD4+ and CD8+ Cell Count

Whole blood samples were obtained by venipuncture and collected in ACD anticoagulant vacutainer tubes. Fluorescent-labeled monoclonal antibodies to CD4, CD8 and CD3 cell surface antigens were incubated with whole blood. Red blood cells were removed using a lysis technique. Samples were washed with phosphate buffered saline and preserved with paraformaldehyde. Enumeration of the CD4+ and CD8+ lymphocytes was accomplished by using a flow cytometer to detect dual stained CD4+/CD3+ and CD8+/CD3+ cells. Cells were separated on the basis of forward and 90° light scatter and on the color of the particular fluorescent-labeled monoclonal antibodies which attached to the specific lymphocyte subtypes. The CD4+ and CD8+ cell counts were determined by multiplying the percentage CD4+/CD3+ and percentage CD8+/CD3+ cells by the absolute lymphocyte count obtained for that sample. Item 7, Appendix C contains the standard operating procedures used for these evaluations.

The prognostic value of many markers for HIV-1 disease progression has been investigated [64-66] but the CD4+ lymphocyte count is the best characterized and is currently used as a guide for antiretroviral therapy. Longitudinal studies have shown a low and constant rate of decline of CD4+ cells in groups of asymptomatic HIV-1 infected persons and a constant but more rapid loss of CD4+ cells in groups of patients who finally progress to AIDS [67-69]. Phillips and coworkers therefore concluded that differences in CD4+ cell numbers at the time of infection, may account for the variation observed in duration of the asymptomatic period [67]. In other studies over a 5-year follow-up, nonprogressors to AIDS showed continuous and steady decline of the mean number of CD4+ cells, from a mean of 0.7 $X10^9$/L at 9 months to 0.4 x 10^9/L at 54 months [70]. In progressors, until 18 months before onset of AIDS, the decline of CD4+ lymphocytes was slow and steady, after which, when mean CD4+ cell numbers were 0.4 x 10^9/L, the decline was much faster. At the time of diagnosis of AIDS mean CD4+ cell numbers were 0.14 x 10^9/L. The regression coefficients of trend lines showed that nonprogressors lose their CD4+ at a rate between 3.3 x 10^9/L/month and 6.9 x 10^9/L/month. CD4+ cell decline in the progressors in the period >18 months before diagnosis of AIDS was comparable to decline in nonprogressors. However, in the last 18 months preceding diagnosis, the decline was three to five times faster (15.7 x 10^6/L/month). In more recent studies, a dynamic interaction between the replication of HIV-1 and destruction of CD4+ lymphocytes has been identified [35,36]. When HIV-1 replication declines steeply at the initiation of therapy, CD4+ lymphocyte count tends to increase. Although the CD4+ cell count has been a useful marker in

determining stages of HIV-1 disease, it has not been particularly useful as a marker of the clinical response to antiretroviral therapy. In data presented by Volberding and coworkers, the baseline CD4+ cell count was highly correlated with the progression to AIDS (P<.001). However, only a small proportion of the effect of zidovudine on this progression was statistically explained by the effect of the drug on CD4+ count. In another study, the duration of the increase in the CD4+ lymphocyte count in response to antiretroviral therapy appeared to be more important in predicting disease progression than the magnitude of the initial response **[71,72]**. In a preliminary analysis of data from Protocols M/3331/0017 and M/3331/0021, baseline CD4+ cell count, but not change in CD4+ cell count from baseline was found to be a statistically significant predictor of clinical progression **[58]**. Persons with similar CD4+ cell counts may differ in their future rate of CD4+ cell decline and risk of clinical progression, limiting the usefulness of CD4+ cell counts as sole criterion for therapeutic intervention during the asymptomatic period of the disease.

d. Coulter Immune Complex Dissociation (ICD) Preparation of Samples for p24 Analysis

P24 antigen/antibody immune complex formation prevents the direct detection of p24 antigen by enzyme immunoassay in patients with high levels of circulating p24 antibodies. This procedure was used to dissociate HIV-1 antigen/antibody complexes in patient plasma or serum samples prior to performing the Coulter HIV-1 p24 antigen assay. Dissociating the antigen from the antibody allowed a more accurate measurement of circulating p24 antigen. Serum or plasma samples were treated with a glycine-containing immune complex dissociating reagent. The acidified samples were neutralized with a Tris buffer before aliquots were transferred to the HIV-1 p24 antigen reaction plate. An immune complex reagent provided by Coulter serves as a positive control. Normal human serum served as a negative control. Assay plates were incubated overnight at 37°C before proceeding with the first wash step in the procedure described for the Coulter HIV-1 p24 antigen assay. The complete standard operating procedure for this sample pretreatment is found in Item 7, Appendix D.

e. Coulter HIV-1 p24 Antigen Assay

The Coulter HIV-1 p24 antigen enzyme immunoassay quantitatively measured the amount of p24 HIV core antigen present in plasma, serum, or tissue culture medium. The assay was performed with a commercial kit and was conducted in microtiter plate wells that were coated with murine monoclonal antibody against HIV-1 p24 protein. Samples were placed in the wells and incubated at 37°C for an hour. If p24 protein was present, it bound to the antibody in the well. Duplicate positive controls, consisting of tissue culture medium spiked with HIV-1 p24 antigen, were run in parallel. Negative controls, consisting of tissue culture medium containing the same ingredients as the test samples, were run in triplicate. After washing wells thoroughly, biotinylated antibodies to p24 were added to the microtiter wells and again incubated. Wells were washed thoroughly before a solution containing streptavidin-horseradish peroxidase was added and plates were incubated. Finally, plates were again washed, and tetramethylbenzidine (TMB) substrate solution was added. The peroxidase reacted with hydrogen peroxide in the presence of TMB, resulting in color development. The reaction was stopped after 30 minutes. The color

intensity was directly proportional to the amount of HIV-1 antigen present and the quantity of antigen present in each sample was determined by comparing the optical density of the standard curve wells to that of the test sample. A complete description of the standard operating procedure for this assay is presented in Item 7, Appendix E.

HIV-1 p24 antigen is an indirect marker of viral replication and has been shown to correlate with disease progression [73-77]. In addition, the detection of p24 antigen can be used as a criterion for entry into clinical trials and subsequently for monitoring antiviral therapy [49,78,79]. Various studies have shown that treating serum samples with acid [80,81] or base [82] can dissociate p24 antigen complexed with anti-p24 antibodies and thus increase the rate of positivity for p24 antigen. In addition, immune complex dissociation can be helpful for the evaluation of antiretroviral therapy in HIV-1–infected adults [83] and may contribute to the diagnosis of infection in infants [84]. Recently, Henrard and coworkers demonstrated that the main benefit of ICD treatment was to detect p24 antigenemia approximately 1 year before regular p24 assay detection [85]. In our studies, subjects with CD4+ cell counts 250-500 had detectable ICD p24 in only 49% of samples and in patients with CD4+ cell counts 0-500 approximately half of the subjects had a high enough ICD p24 level to quantitate a 50% decline [57]. These data suggest that ICD p24 quantitation may be of limited utility in patients above 300 CD4+ cell count.

f. PBMC Endpoint Dilution Assay

This PBMC co-culture assay was used for the detection of replicating HIV-1 from patient PBMCs as measured by the appearance of p24 antigen in culture supernatant fluids and is a method for estimating the patients' circulating cell-associated viral burden. Patient lymphocytes were isolated and quantified. Four replicates of dilutions containing $10^{5.5}$, 10^5, $10^{4.5}$, 10^4 patient cells were co-cultured with $2.0x10^5$ PHA stimulated PBMCs from HIV seronegative donors. Cells were co-cultured for a total of 28 days. On days 3/4, 7, 14, and 21, the culture supernatants were removed and replaced with fresh medium containing $2x10^5$ PHA stimulated donor PBMC. On days 10, 17, and 24, supernatants were removed and replaced with an equal volume of fresh culture medium. The Coulter HIV-1 p24 antigen enzyme immunoassay was performed on day 7 and 28 supernatants. Values were calculated using the Spearman - Karber formula: $M = xk + d[0.5 - (1/n) \times (\text{sum of } r)]$, where $M = TCID_{50}$ expressed as Log_{10}, xk = dose of highest dilution, r = number of negative wells, d = spacing between dilutions, and n = wells per dilution. The antilog of M was the relative dilution of starting cells that resulted in a 50% infection rate. The 50% tissue culture infectious dose ($TCID_{50}$) was calculated by dividing the original number of patient cells by the antilog of M. Item 7, Appendix F contains the complete standard operating procedure for this method.

Quantitative measures of virus load by culture methods have shown a consistent correlation between increasing amounts of circulating virus, virus-infected cells, decreasing CD4+ cells and disease progression. Initially this relationship was defined in reports from Ho [78] and Coombs [73], who observed a correlation between dilution assays of culturable virus from the plasma or PBMCs, stage of disease, and CD4+ cell numbers. Evaluation of viral load by quantitative microculture, in which serial dilutions of patients' PBMCs were added to donor lymphocytes and assayed for p24 antigen production, have been incorporated into several ACTG trials of antiretroviral

drugs and biologics [86,87]. Quantitative culture titers have been shown in a number of natural history and cross-sectional studies to reflect advancing stages of disease and decreasing CD4+ cell count. The primary drawback for the use of quantitative culture is the wide variability of the assay. The titer of infectious virus in the quantitative culture reflects the variability in donor cells, culture conditions, and efficiency of in vitro infection, each of which can lead to differences in titer of more than 10 fold between replicate samples for the same individual over a 1 to 2 week interval, even when drug treatment is absent or held constant [88]. One advantage of the quantitative culture technique is the initiation of the growth of a virus isolate for the generation of a virus stock for drug susceptibility and genotypic testing.

g. MT-2 Syncytium-Inducing Assay

This assay evaluated the syncytia-inducing phenotypes of patient HIV-1 isolates. MT-2 cells were co-cultured in duplicate with the infected patient's PBMCs for a total of 14 days. Three types of positive controls were run in parallel:

1) MT-2 cells infected with HIV-1_{RF},
2) PHA stimulated PBMCs infected with HIV-1_{RF}, and
3) PHA stimulated uninfected donor PBMCs co-cultured with patient PBMCs.

Uninfected MT-2 and PHA stimulated PBMC cultures served as negative controls. The MT-2 cultures were examined microscopically for syncytium formation on days 3, 7, and 14. On days 3, 7, and 10, half of the volume was removed from MT-2 cultures and replaced with an equal volume of fresh medium; at the same time, supernatants from the PBMC cultures were removed and replaced with an equal volume of fresh medium. A culture was considered syncytium-inducing (SI) positive if 5 or more syncytia (per well) were observed. If no syncytia were seen by day 14, a p24 enzyme immunoassay was run on the PBMC co-culture controls to insure that infectious virus was present in the patient sample. Cultures with measurable p24 levels but no syncytia were considered non-syncytium-inducing. When no syncytia were observed and no p24 was detected the result was reported as no viable virus detected (NVVD). The complete standard operating procedure for this assay is contained in Item 7, Appendix G.

Biological differences between different HIV-1 isolates have been proposed as one important factor in pathogenesis. Several studies have demonstrated that in vitro differences in virus replication rate, syncytium-inducing capacity, and ability to infect tumor cell lines correlate with the severity of the immunodeficiency [89-91]. A classification system has been developed based on the capacity of primary isolates to induce syncytia in culture. HIV-1 isolates have been classified as syncytium-inducing (SI) or non-syncytium inducing (NSI). Subjects harboring SI viruses have a more rapid course of disease progression than those harboring NSI viruses, and this biological phenotype has been shown to be an independent predictor of progression to AIDS [92]. The presence of SI variants correlates with a rapid CD4+ lymphocyte decline [92,93]. In untreated asymptomatic individuals, conversion from NSI to SI viruses has been reported to be followed usually by a more rapid decline in the number of CD4+ lymphocytes [92,93]. The MT-2 syncytium assay has been employed in clinical trials to improve the early recognition of progressive disease.

h. HIV-1 Isolation and Expansion in Tube Co-Culture

This procedure was used to obtain virus stocks for use in phenotyping and genotyping protocols. Cultures containing 3 x 10^6 or 3 x 10^5 patient PBMCs were co-cultured with 2 x 10^6 PHA stimulated PBMC from HIV-1 seronegative donors. On day 3 or 4, 7, 14, 21, 28, and 35 culture supernatants were removed and replaced with an equal volume of fresh medium containing 2 x 10^6 PHA-stimulated donor PBMCs. Culture supernatants from day 7 and 7 day intervals thereafter were assayed for p24 antigen. When a significant amount of p24 antigen was detected, cultures were expanded for an additional 14 days. During the expansion process, culture supernatant was removed and replaced with an equal volume of PHA-stimulated PBMC in fresh culture medium on days 4, 7, and 11. Day 4 and 7 supernatants were assayed for p24 antigen. Cultures were harvested on day 14 by separating cells from supernate by centrifugation. Supernatants were titrated and frozen for later use as viral stocks. Item 7, Appendix H references the complete standard operating procedure for this method.

i. HIV-1 Drug Sensitivity/Susceptibility Assay (Phenotyping)

This tissue culture assay was used to measure the extent that drug inhibits HIV-1 replication in PBMCs. PHA stimulated PBMCs were infected at a multiplicity of infection (MOI) of 1, and incubated for an hour. Non-absorbed virus was removed and infected cells were then mixed with culture medium containing drug. Six concentrations of drug were tested in triplicate. Control wells were prepared containing infected cells with no drug added. On day 4 post infection, approximately half of the culture volume was removed and replaced with fresh drug-containing medium. Supernatants from day 7 cultures were assayed for HIV-1 p24 antigen to determine the extent of viral replication. Drug 50% inhibitory concentration (IC_{50}) values were computer generated using software based on the median effect principle developed by Chou and Chou. The equation (F_a) = $1/[1+(IC_{50}/\text{drug concentration})^m]$ is used and a curve is fit to data points F_a and drug concentration using nonlinear regression modelling, where F_a is the fraction affected (= % reduction from untreated control x 0.01) and m is the slope of the curve. The complete standard operating procedure for this assay is found in Item 7, Appendix I.

A number of studies have shown that drug resistance is temporally associated with, and may be a factor in, the reversal of benefit obtained from antiretroviral drug therapy **[94-97]**. In the first study reporting resistance to ZDV **[98]**, HIV-1 patient isolates from PBMC were analyzed and several conclusions emerged from that work: HIV-1 isolated from subjects not treated with ZDV had a narrow range of ZDV susceptibility; the 50% inhibitory concentration ranged from 0.001 to 0.04 μM. Almost all isolates from patients with AIDS or advanced AIDS-related complex showed reductions in their drug susceptibilities after 6 months of therapy. Sequential isolates from individuals being treated with ZDV displayed progressive, stepwise increments in resistance. Increases of >100 fold in the IC_{50} of ZDV were observed in several isolates. Several groups of investigators have reported 4 to 10 fold reductions in the susceptibilities of sequential isolates from patients receiving other nucleoside reverse transcriptase inhibitors **[54,99,100]**. More recently, phase I and II clinical trials with the non-nucleoside RT inhibitors L-697,661 and nevirapine have shown rapid selection for resistant virus in cell culture **[49,50]**. The significance of different levels of drug

susceptibility which appear over different periods of time may be quite different clinically. Recent work with assays to detect mutations in clinical specimens suggest that an HIV-1–infected person may have a mixture of viruses with different resistance phenotypes circulating simultaneously **[42,101,102]**. It is therefore not clear-cut whether any immediate consequence will occur from diminished viral isolate sensitivity nor what proportion of the virus population or quasispecies must have this phenotype to initiate any clinical repercussion.

j. HIV-1 RNA Extraction, Reverse Transcription, PCR Amplification, and Sequencing (Genotyping)

This procedure was used to determine the nucleotide and respective deduced amino acid sequences of the HIV-1 RT genes to identify the genetic changes in patient isolates before and after drug therapy. The supernatants from cultures (virus expansion) were used as the source of patient virus for genotypic and phenotypic analyses. Virus was first separated from the supernatant by centrifugation and the viral RNA was extracted according to the protocol in Item 7, Appendix J. Preparations were first treated with a guanidinium/phenol solution to solubilize the viral protein coat and release RNA to the aqueous phase. Yeast carrier tRNA was added to the aqueous phase, and RNAs were precipitated with isopropanol. After centrifugation the RNA pellet was dissolved in buffer containing carrier yeast tRNA. Viral RNA was reverse transcribed into cDNA using MMLV RT in the presence of NE1' primer as described in Appendix K of Item 7. The NE1' primer contained a sequence located within the pol gene of the HXB_{II} viral genome between nucleotides 3299 and 3315. Reactions were terminated after 30 minutes by heat inactivation. Viral cDNA was then amplified by polymerase chain reaction in the presence of primer A. The primer A contains a sequence located within the pol gene of the HXB_{II} viral genome between nucleotides 2529 and 2563. The reaction was terminated after 40 cycles and the amplified DNA product was purified using Amicon microconcentrators according to Appendix L of Item 7. DNA concentration was determined by agarose gel electrophoresis, staining with ethidium bromide, photographing the resulting band of DNA, and comparing band intensity with a commercially available photo table of a low DNA mass ladder. Sequencing reactions using dye-terminator chemistry were set up according to Appendix M of Item 7. Approximately 15 ng of DNA was prepared in the presence of primer using a commercially available dye terminator kit. Reactions were terminated after 25 cycles. Preparations were purified either by running them through a Centri-Sep spin column or by ethanol precipitation. Samples were sequenced using an automated sequencer according to Appendix N of Item 7. Samples were run on a 4% acrylamide gel in an ABI377 automatic sequencer for 3 to 10 hours. Sequence data was collected and analyzed using three software programs. ABI Sequence Analysis software processed the gel and generated sequence data. The Factura program removed low quality sequence data generated by the Sequence Analysis program. AutoAssembler assembled sequences into a contiguous set representing the region of interest.

By far the majority of knowledge about the clinical significance of HIV-1 viral genotypic mutations has been derived from patients treated with ZDV. The RT mutations most commonly associated with ZDV resistance were the Thr→ Tyr or Phe substitution at codon 215. In many cases a Met→Leu substitution at codon 41 was found as well. Introduction of these two mutations into RT of HIV-1 molecular clones

resulted in a 60 fold less susceptible virus. When HIV-1 isolates were obtained from patients on ACTG 116B/117 trials, the presence of mutations at both codons 215 and 41 conferred an increased risk for progression and an increased risk for death [103]. It was also shown that the benefit of switching to didanosine compared with continued zidovudine therapy was independent of the presence of these mutations. Thus, genotypic analysis provides basic information on the mutations that arise during therapy and may be helpful in determining advantageous subsequent therapy. However, only when more knowledge is gained about the interaction of mutations will we know how to use genotypic information to manage patient therapy.

6. In Vitro Studies Conducted During the Clinical Trials

Clinical trials have shown delavirdine to have strong antiretroviral activity against HIV-1. Delavirdine also achieves high plasma concentrations and has an excellent safety profile in vivo. These characteristics make delavirdine a potentially valuable component of double or triple drug regimens. To help understand which drugs would be best combined with delavirdine, virus isolates were regularly obtained from patients in selected trials. These isolates were characterized to determine the prevalence of syncytium-inducing phenotypes, drug sensitivity phenotypes, and reverse transcriptase genotypes. The HIV-1 burden in peripheral blood mononuclear cells (PBMC) was also monitored.

When early studies showed that HIV-1 variants resistant (ie, reduced drug susceptibility) to non-nucleoside reverse transcriptase inhibitors (NNRTIs) selected with one drug displayed considerable cross-resistance to other NNRTIs [41,42,104,105] a view emerged that development of reduced drug susceptibility to one inhibitor would preclude the utility of other NNRTIs for subsequent therapy. As an example, the Tyr181→Cys substitution in reverse transcriptase (RT) confers broad cross-resistance to most NNRTIs under development [41,42]. However, succeeding studies on the Tyr181→Cys and other RT resistance conferring NNRTI substitutions [106], have shown that these substitutions do not have equivalent effects on all NNRTIs [38,107].

Various mutations have been identified in HIV-1 passaged in vitro at high concentrations of delavirdine or delavirdine plus ZDV. The delavirdine resistant isolates were found to have amino acid substitutions in RT: Leu228→Phe, Pro236→Leu, Gly273→Arg, Leu100→Ile, Met230→Leu [20,38,47]. The Pro236→Leu (P236L) substitution was found to confer a high-level of delavirdine resistance in cell culture [38]. However, instead of conferring cross-resistance to other NNRTIs, the P236L substitution sensitized RT 7 to 10 fold to TIBO R82913 and L-697661 without influencing sensitivity to nucleoside analogue reverse transcriptase inhibitors [1]. To assess the in vivo development of reduced susceptibility to delavirdine and which RT amino acid substitutions were predominant, viral isolates from patients enrolled in protocols to evaluate safety, tolerance, and pharmacokinetics during monotherapy or combination therapy were studied.

a. Selection of HIV-1 Variants During Delavirdine Therapy In Vivo: Drug Sensitivity Phenotype and Reverse Transcriptase Genotypic Analyses

A randomized, dose-ranging, open label trial to study the safety and tolerance of delavirdine monotherapy and the reductions in viral burden associated with three levels of drug was conducted at 9 primary sites (ACTG 260). Preliminary delavirdine sensitivity data has been presented from 16 (6 pretrial antiretroviral naive, 10 ZDV-experienced) patients in ACTG 260 who were randomized to receive doses of delavirdine that would give targeted serum trough levels of either 3-10, 11-30, or 31-50 µM **[108]**. Delavirdine IC_{50} values were determined for viral isolates at weeks 0 and 8. The median delavirdine IC_{50} value was 0.021 µM (range 0.01-0.13 µM) prior to therapy and 10.28 µM (range 0.018-27.53 µM) at week 8. Against variants isolated at week 8 from 15/16 patients (94%), the delavirdine IC_{50} was greater than 1.0 µM. The delavirdine IC_{50} against the remaining patient's week 8 isolate continued unchanged from baseline. Trough delavirdine blood levels achieved in these 16 patients exceeded the delavirdine IC_{50} value for their cultured virus in the majority (9/16) of patients. Isolates from 8 (4 pretrial antiretroviral naive and 4 ZDV experienced) patients were analyzed for RT mutations by direct sequencing of PCR amplified proviral DNA from PBMCs at termination of culture. The RT genes (encoding RT amino acids 30-250) of paired isolates (weeks 0 and 8) were sequenced and by week 8 virus samples were shown to contain RT substitutions previously associated with NNRTI resistance **[49,50,109]**. The spectrum of substitutions identified included Y181C (1 patient), K103N (1 patient), K103N + Y181C (4 patients), K103T (1 patient), and P236L (1 patient). The clinical significance of the decreased susceptibility (median IC_{50} = 10.28 µM) associated with the above mutations is not precisely known. However, it is possible that sustained high plasma concentrations of drug in which trough levels remain greater than the IC_{50} of circulating virus can achieve enduring antiretroviral activity.

Viral phenotypic and genotypic changes in HIV-1 isolates were studied from patients in a well controlled pharmacokinetic study involving delavirdine combination therapy. In an open-label, escalating dose investigation of delavirdine mesylate in combination with a stable dose of ZDV (M/3331/0003), 34 highly ZDV-experienced HIV-1–infected subjects were studied **[23]**. Patients were initially treated with five different delavirdine regimens (100 mg qid, 150 mg qid, 200 mg qid, 300 mg tid and 400 mg tid) in combination with ZDV (200 mg tid). The delavirdine dose was adjusted to ensure that each participant maintained trough plasma concentrations greater than 1 µM. The whole blood samples collected and processed for plasma and PBMCs at predose and weeks 6, 12, 16, 24, 32, 36 and 44 were virologically evaluated. Isolates were tested for ZDV and delavirdine susceptibility, PBMCs were monitored for quantity of infectious virus, and isolates were sequenced to determine reverse transcriptase genotypes.

Virus was recovered from the predose samples of 24 of the 34 patients treated with delavirdine and ZDV as shown in Table G.16. A high level of susceptibility to delavirdine was observed for the predose isolates (average IC_{50} = 0.0175 µM). Isolates with reduced susceptibility to delavirdine emerged in all dose groups. Isolates with the highest delavirdine IC_{50}s (>25 µM) were obtained from 6 patients from the 100, 150, 200 qid and 300 tid dose groups. During the first 8 months of therapy, the mean delavirdine IC_{50} of these isolates from the 200 mg qid and 400 mg tid treated patients

remained below the average delavirdine plasma concentrations (Table G.17). The average steady state delavirdine plasma trough concentration achieved in the 400 mg tid treatment group was 23.0 ± 11.9 μM. Eighty-eight percent (23/26) of the patients with recoverable virus during the treatment phase of the protocol had variants that remained susceptible in vitro to delavirdine concentrations below 23.0 μM. All isolates obtained from participants on the 400 mg tid arm of the study had delavirdine IC_{50} less than 23.0 μM for at least 32 weeks.

The genetic basis for the reduced susceptibility of virus isolates obtained from M/3331/0003 patients was defined by genotyping the RT (encoding RT amino acids 20-250) of previously phenotyped viral stocks. The accumulated substitutions are shown in Table G.18. During the first 24 weeks of therapy, 79% (15/19) of the patients on delavirdine + ZDV developed a Lys103→Asn substitution. The clinical isolates with the Lys103→Asn substitutions exhibited a 75 fold or greater decrease in delavirdine susceptibility. Only two of the nineteen patients developed a Pro236→Leu RT substitution, which resulted in an average 168 fold reduction in virus susceptibility to delavirdine. After 32 weeks of therapy, one of the subjects with a Pro236→Leu amino acid change detected by week 24, had developed the Lys103→Asn and Tyr181→Cys and the codon 236 mutation was no longer evident. No isolates with the Leu228→Phe, Leu100→Ile, or Met230→Leu RT substitutions were detected in these patients.

During the M/3331/0003 study, virus isolates were tested for drug sensitivity to ZDV (Table G.19). There was an average 86 fold increase in the ZDV sensitivity of all patient isolates after 24 weeks on the delavirdine and ZDV combination therapy. Three patients experienced as much as a 130 fold increase in ZDV sensitivity (Subject 5, 21, 23). This resensitization to a more ZDV susceptible viral phenotype was seen in 22/24 patients; the remaining two patients were already highly ZDV sensitive (IC_{50}s of 0.019 and 0.021μM). The average increase in ZDV sensitivity in patients with ZDV-resistant (>0.2 μM IC_{50}) predose samples was 118 fold (range 1.1-761). Six patients developed additional ZDV associated mutations (Asp67→Asn, Lys70→Arg, Thr215→Tyr or Phe, and Lys219→Gln) during the combination therapy (Subject 2, 5, 6, 16, 17, 18; Table G.18). Four of these six subjects had at least two ZDV associated mutations prior to starting the delavirdine + ZDV combination regimen (Subject 2, 5, 6, 16; Table G.20). The increase in sensitivity to ZDV in spite of the increase in abundance of ZDV associated substitutions suggests that delavirdine substitutions may have an influence on the diminished sensitivity usually associated with the ZDV mutations.

In a pharmacokinetic study (M3331/0030) of delavirdine plus didanosine (ddI), delavirdine susceptibilities and reverse transcriptase genotypes of patient isolates were evaluated [**110**]. Nine NNRTI-naive patients with extensive nucleoside reverse transcriptase inhibitor (NRTI) experience (median duration of prior ZDV and ddI were 26 and 15 months, respectively) were evaluated. Seven patients received delavirdine plus ddI and 2 received the triple drug regimen, delavirdine + ddI + ZDV. HIV-1 isolates from 89% (8/9) patients had substitutions associated with nucleoside resistance at entry (Table G.21). After 10 weeks of delavirdine and ddI therapy, 5 of 7 patients had developed the Y181C substitution. All but one of these Y181C changes were in combination with K103N (Table G.21). Isolates from one patient remained genotypically unchanged during therapy and one developed only the K103N substitution. In this limited study the Y181C was readily detected, in contrast to the M/3331/0003 data with delavirdine and ZDV combination therapy. Neither patient on

the triple drug regimen (delavirdine, ddI, ZDV) developed isolates with the Y181C mutation. Both participants treated with this triple combination developed the K103N substitution, one in combination with P236L.

As illustrated by the three clinical protocols described above, the K103N substitution has been the predominant RT genotypic change observed within the RT coding region in isolates from patients on monotherapy and combination therapies with delavirdine. Variants with the Y181C were observed in isolates from patients treated with delavirdine alone or delavirdine with ddI. A regimen of delavirdine combined with ZDV resulted in isolates with predominantly K103N substitutions; only a single subject receiving this therapy developed an isolate with the Y181C substitution. These data support previous studies **[46,111]** suggesting that Y181C may be incompatible with ZDV resistance mutations. Finally, unlike in vitro passage of the virus with delavirdine **[38]**, patient samples have had relatively few proline to leucine substitutions at RT amino acid 236.

b. Characterization of Virus Isolated from Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells (PBMC) were isolated from participants on the M/3331/0003 protocol treated with delavirdine (100 mg qid, 150 mg qid, 200 mg qid, 300 mg tid and 400 mg tid) in combination with ZDV (200 mg tid). These PBMC were co-cultured with phytohemagglutinin (PHA) stimulated lymphocytes from normal donors and monitored for HIV-1 proliferation by measurement of p24 levels in the culture supernatant at days 7 and 28 of culture. The estimated levels of infectious units per million PBMC did not increase over the first 24 weeks of therapy (Table G.22) and the peak diminutions occurred after 12 weeks of therapy (data not shown). Four M/3331/0003 patients had a greater than 1 log decrease in PBMC viral burden **[23]**.

HIV-1 isolates differ in their capacity to induce syncytia in vitro. On the basis of both this capacity and other in vitro biological properties such as replication rate and cellular tropism, syncytium-inducing (SI) and non-SI (NSI) HIV-1 isolates have been distinguished **[90]**. The appearance of SI variants is associated with a rapid decline in CD4+ cells leading to the onset of Acquired Immunodeficiency Syndrome (AIDS) **[89,92,93]** and has been used as a marker of disease progression in HIV-1 infection **[112]**. Syncytium and non-syncytium inducing virus variants were also isolated from the PBMC of the patients on the delavirdine/ZDV combination therapy trial described above. In BC 1003, the sixteen patients with culturable virus prior to therapy were phenotyped and all (16/16) retained their predose syncytium-inducing or non-syncytium-inducing status during therapy (Table G.23). None of the 10 predose NSI patients converted to the SI phenotype. The SI phenotype was strongly correlated with ZDV resistant variants in this study (Tables G.20 and G.23) in which all six patients with SI phenotype were ZDV resistant ($IC_{50} \geq 0.2\mu M$). Karlsson and coworkers **[113]** have noted that SI positive isolates were more likely than NSI isolates to show reduced sensitivity to zidovudine and didanosine. The patients who developed reduced delavirdine susceptibility while on study did not develop the SI phenotype during therapy.

In conclusion, in vitro analysis of patient isolates on clinical trials has revealed that delavirdine and ZDV therapy was predominantly associated with the K103N RT

substitution. Both the K103N and Y181C substitutions were frequently detected after delavirdine monotherapy or delavirdine in combination with ddI. The high plasma concentrations of delavirdine achieved in the 400 mg tid delavirdine plus ZDV regimen of M/3331/0003, suggest that even with the K103N substitution, sufficient viral replication may be inhibited to allow some continual benefit from delavirdine combination therapy. Moreover, treatment of patients with delavirdine and ZDV restored partial sensitivity to previously highly ZDV resistant isolate phenotypes. Finally, the lack of increased peripheral blood mononuclear cell viral burden and lack of progression to SI phenotype demonstrated by the M/3331/0003 trial, is consistent with apparent benefit of delavirdine therapy.

Table G.16. Delavirdine Sensitivity[a] of Clinical Isolates from Patients on Delavirdine and ZDV (200 mg tid) Therapy (IC_{50} expressed in μM)

Subject	Delavirdine Dose (mg)	Week							
		0	6	12	16	24	32	36	44
1	100 qid	0.029	2.508	0.266	1.282	0.311	6.286	31.22	†
2	100 qid	0.000	†	0.018	†				
3	100 qid	0.028	†	†	†	7.624	>50.0		
4	100 qid	0.020	†	†	0.948	3.162			
5	100 qid	0.017	0.458	1.679	1.772	0.980	†		
6	100 qid	0.006	4.497	1.264	29.70		>50.0		
7	150 qid	0.005	0.371	1.191	0.715	3.162	†		
8	150 qid	†	0.077	†	†	†	†	†	†
9	150 qid	0.005	0.445	†	†	1.785	33.90		†11.8
10	150 qid	0.021	†	0.006	†	†	†		
11	150 qid	†	0.001	†	†	†	0.097	†	4.926
12	150 qid	†	†	†	†	†	†	†	†
13	200 qid	0.006	†	†	†	†	†	†	†
14	200 qid	†	†	†	†	†	†	†	†
15	200 qid	0.103	6.639	†					
16	200 qid	0.050	1.136	1.288	7.402	6.155	5.422	†	25.89
17	200 qid	0.004	†	†	†	0.426	†	†	†
18	200 qid	0.004	†	†	†	7.847			
19	300 tid	†	†	†	†	2.963	†	†	†
20	300 tid	†	†	†	†	†	†	†	†
21	300 tid	0.004	0.015	0.308					
22	300 tid	†	1.201	>50.0		29.70		†	20.34
23	300 tid	0.016	†	†	†	4.951			
24	300 tid	0.021	†	†	0.357	†	†	†	†
25	400 tid	0.006	†	†	†	†	†	†	†
26	400 tid	0.003	†	†	1.212				
27	400 tid	†	†	†					
28	400 tid	†	†	†	†				
29	400 tid	0.006	†	†					
30	400 tid	0.022	0.017	5.652	6.943	6.744	10.12		†
31	400 tid	0.001	0.550	8.582	†	7.937	†	†	
32	400 tid	0.005	†	†	1.288	†			
33	400 tid	†	0.882	†	†	†	†	†	†
34	400 tid	0.002	0.206	4.706	8.736	19.31		†	

† remaining on therapy, but no culturable virus

[a] Assay described in 7.J-Appendix I.

Table G.17. Mean Delavirdine Sensitivity of Viral Isolates During Delavirdine and ZDV Therapy (IC_{50} expressed in μM) for Each Treatment Group

Group	DLV Treatment in Combination with ZDV	Average Delavirdine Trough Plasma Concentration	Average Predose*	WK 6	WK 12	WK 16	WK 24	WK 32	WK 36	WK 44
1	100 qid	1.8	0.02	2.49	0.81	8.43	12.42	28.14	31.22	
2	150 qid	3.53	0.01	0.22	0.60	0.72	2.47	17.00		8.37
3	200 qid	5.43	0.03	3.89	1.29	7.40	4.81	5.42		25.89
4	300 tid	9.09	0.01	0.61	25.15	15.03	3.96	20.34		
5	400 tid	23.0	0.01	0.41	6.31	4.54	11.33	10.12		

* Average of available predose values.

Table G.18. Reverse Transcriptase Amino Acid Substitutions Accumulated in Patient Isolates During Delavirdine and ZDV Therapy (M/3331/0003)

	CODON																																				
Subject	20	35	64	67	70	101	103	104	105	122	123	135	142	160	162	166	172	174	177	178	179	181	196	200	207	211	215	219	236	241	245	248	249
	K	I	K	D	K	K	K	K	K	K	D	I	I	F	S	K	R	Q	D	I	V	Y	G	A	R	R	T	K	P	V	V	E	K
1							N																										
2				N	R																							Q					
3		V/T					K/N					T										C							P/L				
4							N																										
5				N			N							S/F														Q					
6							N				E		V														F						
7							N																							K			
9							N																		Q/E		Y/S						
10					R				N																K/E								
15																													L				
16			R/K	N/D			N	N																T				Q		A/V			
17				N			N																										
18					R		N									R												Q		A			
21							K/N								S/C						D/V/E										M/V			
23							N																E										
24	T/K						N																										
30							N	N									K									R/K							
32		V					N			E	N																						
34							N				N		VT						N	M											T	Q	Q

The RT coding region was sequenced from about codons 20-250. If a substitution was not shown, it was not detected in any isolate.

Table G.19. ZDV Sensitivity[a] of Clinical Isolates from Patients on Delavirdine and ZDV Therapy

Sub.	Delavirdine Dose (mg)	IC_{50} expressed in µM at Week							
		0	6	12	16	24	32	36	44
1	100 qid	0.296	0.225	0.076	0.083	0.045	0.007	0.008	†
2	100 qid	0.019	†	0.370	†				
3	100 qid	0.131	†	†	†	0.001	0.011		
4	100 qid	5.000	†	†	0.199	0.214			
5	100 qid	4.040	0.784	0.307	0.212	0.025	†		
6	100 qid	0.512	5.000	1.271	1.760	0.467			
7	150 qid	0.022	0.052	0.004	0.076	0.014	†		
8	150 qid	†	0.007	†	†	†	†	†	†
9	150 qid	0.709	1.319	†	†	0.017	0.191	†	0.674
10	150 qid	0.011	†	0.003	†	†	†		
11	150 qid	†	0.007	†	†	†	0.001	†	0.002
12	150 qid	†	†	†	†	†	†	†	†
13	200 qid	0.685	†	†	†	†	†	†	†
14	200 qid	†	†	†	†	†	†	†	†
15	200 qid	1.055	0.106	†					
16	200 qid	0.454	0.022	0.128	0.091	0.017	0.079	†	0.119
17	200 qid	0.114	†	†	†	0.015	†	†	†
18	200 qid	0.021	†	†	†	0.065			
19	300 tid	†	†	†	†	0.084	†	†	†
20	300 tid	†	†	†	†	†	†	†	†
21	300 tid	0.044	0.006	0.000					
22	300 tid	†	0.030	0.004	0.135	†	0.045	†	†
23	300 tid	3.044	†	†	†	0.004			
24	300 tid	0.052	†	†	0.001	†	†	†	†
25	400 tid	0.141	†	†	†	†	†	†	†
26	400 tid	0.008	†	†	0.002				
27	400 tid	†	†	†					
28	400 tid	†	†	†	†				
29	400 tid	0.016	†	†					
30	400 tid	0.102	0.025	0.005	0.173	0.005	0.004	†	
31	400 tid	0.335	0.035	0.238	†	0.005	†	†	
32	400 tid	0.008	†	†	0.004	†			
33	400 tid	†	0.001	†	†	†	†	†	†
34	400 tid	4.403	0.291	0.992	0.088	0.953	†		

† remaining on therapy, but no culturable virus. [a] Assay described in 7.J-Appendix I.

Table G.20. Inhibition of ZDV Resistant Clinical Isolates by Delavirdine

Clinical Isolate	ZDV Mutation(s) in RT	ZDV IC_{50} (μM)[a]	Delavirdine IC_{50} (μM)[a]
1	K70R	0.296	0.039
2	M41L, T215Y	0.019	0.001
3	M41L	0.131	0.028
4	M41L, T215Y	5.000	0.020
5	K70R, T215Y	4.040	0.022
6	D67N, K70R, K219Q	0.512	0.011
7	K70R	0.022	0.005
9	M41L, T215Y	0.709	0.007
16	D67N, K70R	0.454	0.002
17	K70R	0.114	0.004
23	D67N, K70R, K219Q	3.044	0.016
30	K70R	0.102	0.027
34	M41L, T215Y	4.403	0.002

[a] IC_{50}, concentration of drug that inhibited viral growth by 50%.

Table G.21. HIV-1 DLV Susceptibilities and RT Mutations in Patients Treated with Delavirdine and ddI or Delavirdine, ddI and ZDV

Patient	Treatment	Week #	DLV IC_{50} (μM)	Nucleoside Resistance Mutations	NNRTI Resistance Mutations
1	DLV + ddI	00	0.14	none	none
1	DLV + ddI	04	22.48	L74V	K103N, Y181C
2	DLV + ddI	00	0.09	T215Y	none
2	DLV + ddI	03	9.12	T215Y	K103N, Y181C
3	DLV + ddI	00	0.01	M41L, T215Y	none
3	DLV + ddI	04	1.34	M41L, L74V, T215Y	K103N, Y181C
3	DLV + ddI	10	5.91	M41L, L74V, T215Y	K103N, Y181C
4	DLV + ddI	00	0.01	L74V, M184V	none
4	DLV + ddI	04	19.61	L74V, M184V	K103N
4	DLV + ddI	10	32.34	L74V, M184V	K103N, Y181C
5	DLV + ddI	00	<0.01	M41L, T215Y	none
5	DLV + ddI	04	0.05	M41L, T215Y	K103N
5	DLV + ddI	10	0.10	M41L, T215Y	K103N
6	DLV + ddI	00	0.01	M41L, L74V, T215Y	none
6	DLV + ddI	04	1.46	M41L, L74V, T215Y	K103N
6	DLV + ddI	10	9.97	M41L, L74V, T215Y	K103N, Y181C
7	DLV + ddI	00	0.01	K70R	none
7	DLV + ddI	04	0.05	K70R	none
7	DLV + ddI	10	<0.01	K70R	none
8	DLV + ddI + ZDV	00	0.01	M41L, D67N, K70R, T215Y	none
8	DLV + ddI + ZDV	04	0.14	M41L, D67N, K70R, T215Y	K103N, P236L
8	DLV + ddI + ZDV	10	1.63	M41L, D67N, K70R, T215Y	K103N, P236L
9	DLV + ddI + ZDV	00	0.07	A62V, V75I, F116Y	none
9	DLV + ddI + ZDV	04	3.24	A62V, V75I	K103N
9	DLV + ddI + ZDV	10	11.21	A62V, V75I, F116Y	K103N

Table G.22. Estimated Infectious Units Per Million (IUPM) Lymphocytes* Prior To and During Delavirdine Plus ZDV Therapy

Subject	IUPM After 7 Days in Culture		IUPM After 28 Days in Culture	
	Predose	16-24 Weeks of DLV + ZDV	Predose	16-24 Weeks of DLV + ZDV
1	39	29	92	52
2	5	1	12	1
3	<1	2	<1	92
4	12	39	92	69
5	9	12	16	29
6	4	2	9	2
7	2	<1	>123	>123
8	<1	<1	2	<1
9	4	2	92	4
10	<1	2	2	2
11	<1	<1	<1	7
12	<1	<1	2	3
13	<1	<1	16	<1
14	<1	<1	2	<1
16	7	5	>123	9
17	<1	2	4	2
18	2	<1	2	2
19	2	<1	4	>123
20	<1	<1	4	<1
22	<1	3	2	5
23	2	2	12	22
24	<1	2	<1	16
25	3	<1	>123	>123
26	1	3	2	3
28	<1	<1	<1	<1
30	12	<1	52	29
31	<1	<1	12	3
32	3	39	22	39
33	<1	<1	<1	<1
34	4	3	29	4

* Assay methodology described in 7.J-Appendix F.

Table G.23. Syncytium-Inducing (SI) and Non-Syncytium-Inducing (NSI) Phenotypes* of HIV-1 Isolates from Patients Treated with Delavirdine and ZDV Combination Therapy

Subject	Pre-Therapy	During Therapy
1	SI	SI
2	NSI	NSI
4	SI	SI
5	SI	SI
6	SI	SI
7	NSI	NSI
9	NSI	NSI
10	NSI	NSI
15	SI	SI
17	NSI	NSI
18	NSI	NSI
21	NSI	NSI
23	NSI	NSI
24	NSI	NSI
30	NSI	NSI
34	SI	SI

NSI = Non-Syncytium Inducing Virus
SI = Syncytium Inducing Virus
* Assay methodology described in 7.J-Appendix G.

7. Conclusions

Delavirdine mesylate (U-90152S/T, DLV) is a potent, selective non-nucleoside inhibitor of HIV-1 reverse transcriptase (RT) and infectious HIV-1 in cell culture. It selectively inhibits HIV-1 RT [1], exhibiting no significant inhibition of cellular DNA polymerase α and δ, HIV-2 [24], or other retroviruses [2]. It acts through a binding site on RT distinct from the dNTP substrate binding site. The RT inhibition by delavirdine differs from that of nucleoside analog RT inhibitors because it is not competitive with respect to the dNTP substrate. In vitro experiments have shown delavirdine mesylate to be a potent inhibitor of laboratory strains and clinical isolates of HIV-1 in various cell culture systems [1,19,22,23]. The potent inhibition of replication by delavirdine of both HIV-1 lymphotrophic and monocytotrophic strains was comparable to the antiviral activity of nucleoside or other non-nucleoside RT inhibitors. When delavirdine was used in combination with each of several other antiretroviral agents (zidovudine, didanosine, lamivudine, zalcitabine, alpha-interferon [IF-α] and an experimental HIV-1 protease inhibitor), a synergistic effect was observed in acute infection experiments using peripheral blood mononuclear cell cultures [3,27-30,33]. Strong antiviral activity coupled with effective oral delivery and an excellent safety profile, will make delavirdine a valuable component of a double or triple drug regimen.

Tissue culture and recombinant RT experiments have demonstrated that the predominant HIV-1 amino acid RT substitution attributable to delavirdine resistance in vitro was P236L. Rather than conferring cross resistance to other RT inhibitors, this substitution sensitized RT 7-10 fold to TIBO R82913 and L-697661 without influencing sensitivity to nucleoside analogue RT inhibitors [1]. Although the RT amino acid substitutions K103N and Y181C, which confer cross-resistance to several non-nucleoside inhibitors, also decrease the potency of delavirdine, this drug retains significant activity against these mutant RTs in vitro [1]. Mutations which induce resistance to one drug can induce sensitivity to a second drug [46,54]. The Y181C mutation in an ZDV resistant background has been shown to significantly suppress resistance to ZDV [46]. Similarly, the codon 184 mutation conferring resistance to 3TC suppresses resistance to ZDV [55]. Emergence of the Y181C or another substitution during delavirdine therapy mediated the observed resensitization of ZDV resistance. Finally, delavirdine had significant inhibitory activity against nucleoside resistant forms of RT and most non-nucleoside resistant forms of RT.

In vitro analysis of patient isolates on clinical trials revealed that with delavirdine therapy the predominant RT substitution was K103N. A spectrum of substitutions was identified including Y181C, K103N, K103N + Y181C, K103T, and P236L. However, treatment with delavirdine and ZDV prevented the emergence of the Y181C for at least 6 months and this substitution occurred in only a single patient. The high plasma concentrations achieved with the 400 mg tid delavirdine plus ZDV regimen suggest that even with the K103N substitution, sufficient viral replication may be inhibited to allow some continued benefit from delavirdine therapy. The lack of increased peripheral blood mononuclear cell viral burden, and lack of progression to syncytium-inducing phenotype demonstrated by the patients on the M/3331/0003 trial, is consistent with the apparent benefit of delavirdine therapy. Although the patterns of HIV-1 drug resistance for the different classes (NRTI, NNRTI or protease inhibitor) are far from complete, it is evident that within each category of inhibitor, resistance to one compound does not necessarily lead to complete resistance to the other compounds

of that same class [56]. In addition, complex interactions between one substitution and others have been shown to ameliorate susceptibility losses seen with single substitutions, suggesting that multiple drugs in combination may be able to maintain clinical benefit despite the emergence of mutations specific to any of the individual agents.

8. References/Study Reports/Publications

1. Dueweke TJ, Poppe SM, Romero DL, Swaney SM, So AG, Downey KM, et al. U-90152, A potent inhibitor of human immunodeficiency virus type 1 replication. Antimicrob Agents Chemother 1993;37(5):1127-31. . . . . .

2. Romero DL, Busso M, Tan CK, Reusser F, Palmer JR, Poppe SM, et al. Nonnucleoside reverse transcriptase inhibitors that potently and specifically block human immunodeficiency virus type 1 replication. Proc Natl Acad Sci 1991;88:8806-10. . . . . .

3. Chong, KT, Pagano PJ, Hinshaw RR. Bisheteroarylpiperazine reverese transcriptase inhibitor in combination with 3'-Azido-3'-Deoxythymidine or 2',3'-Dideoxycytidine synergistically inhibits human immunodeficiency virus type 1 replication in vitro. Antimicrob Agents Chemother 1994;38(2):288-93. . . . . .

4. Gu Z, Quan Y, Li Z, Arts EJ, Wainberg MA. Effects of non-nucleoside inhibitors of human immunodeficiency virus type 1 in cell-free recombinant reverse transcriptase assays. J Biol Chem 1995;270(52):31046-31051. . . . . .

5. Althaus IW. Kinetic studies with the non-nucleoside HIV-1 reverse transcriptase inhibitor U-90152E. Upjohn Technical Report 7295-93-014, June 21, 1993. . . . . .

6. Kohlstaedt LA, Wang J, Friedman JM, Rice PA, Steitz TA. Crystal structure at 3.5 Å resolution of HIV-1 reverse transcriptase complexed with an inhibitor. Science 1992;256:1783-90. . . . . .

7. Wu JC, Warren TC, Adams J, Proudfoot J, Skiles J, Raghavan P, et al. A novel dipyridodiazepinone inhibitor of HIV-1 reverse transcriptase acts through a nonsubstrate binding site. Biochemistry 1991;30:2022-2026. . . . . .

8. Pauwels R, Andries K, Desmyter J, Schols D, Kukla MJ, Breslin HJ, et al. Potent and selective inhibition of HIV-1 replication in vitro by a novel series of TIBO derivatives. Nature 1990;343:470-4. . . . . .

9. Miyasaka T, Tanaka H, Baba M, Hayakawa H, Walker RT, Balzarini J, et al. A novel lead for specific anti-HIV-1 agents: 1-[(2-Hydroxy-ethoxy)methyl]-6(phenylthio)thymine. J Med Chem 1989;32(12):2507-2509. . . . . .

10. Kopp EB, Miglietta JJ, Shrutkowski AG, Shih CK, Grob PM, Skoog MT. Steady state kinetics and inhibition of HIV-1 reverse transcriptase by a non-nucleoside dipyridodiazepinone, BI-RG-587, using a heteropolymeric template. Nuc Acids Res 1991;19(11):3035-9. . . . . .

11. Smerdon SJ, Jager J, Wang J, Kohlstaedt LA, Chirino AJ, Friedman JM, et al. Structure of the binding site for nonnucleoside inhibitors of the reverse transcriptase of human immunodeficiency virus type 1. Proc Natl Acad Sci USA 1994;91:3911-15. .....

12. Fan N, Rank KB, Evans DB, Thomas RC, Tarpley WG, Sharma SK. Simultaneous mutations at Tyr-181 and Tyr-188 in HIV-1 reverse transcriptase prevents inhibition of RNA-dependent DNA polymerase activity by the bisheteroarylpiperazine (BHAP) U-90152s. FEBS Letters 1995;370:59-62. .....

13. Howe WJ, Nugent RA, Rohrer DC. Analysis of binding site models of HIV-1 reverse transcriptase/inhibitor complexes. Upjohn Technical Report 7247-95-002, June 5, 1995. .....

14. Tong L, Cardozo M, Jones PJ, Adams J. Preliminary structural analysis of the mutations selected by non-nucleoside inhibitors of HIV-1 reverse transcriptase. Bioorganic & Medicinal Chemistry Letters 1993;3(4):721-6. .

15. Rohrer DC. An internally consistent 3D molecular similarity model for non-nucleotide HIV-1 reverse transcriptase inhibitors. Upjohn Technical Report 7247-94-001, July 25, 1994. .....

16. Ding J, Das K, Tantillo C, Zhang W, Clark AD, Jessen S, et al. Structure of HIV-1 reverse transcriptase in a complex with the non-nucleoside inhibitor α-APA R 95845 at 2.8 Å resolution. Structure 1995;3(4):365-79. .....

17. Ren J, Esnouf R, Garman E, Somers D, Ross C, Kirby I, et al. High resolution structures of HIV-1 RT from four RT-inhibitor complexes. Nature Structural Biology 1995;2(4):293-302. .....

18. Romero DL, Morge RA, Genin MJ, Biles C, Busso M, Resnick L, et al. Bis(heteroaryl)piperazine (BHAP) reverse transcriptase inhibitors: structure-activity relationships of novel substituted indole analogues and the identification of 1-[(5-Methanesulfonamido-1H-indol-2-yl)-carbonyl]-4-[3-[(1-methylethyl) amino]-pyridinyl]piperazine Monomethanesulfonate (U-90152S), a second-generation clinical candidate. J Med Chem 1993;36(10):1505-8. .....

19. Chen ISY, Tarpley WG, Bruce BJ. Antiviral effects of U-90152 in HIV-infected macrophages. Upjohn Technical Report 7252-92-037, November 10, 1992. .....

20. Olmsted RA, Slade DE, Kopta LA, Poppe SM, Poel TJ, Newport SW, et al. (Alkylamino) piperidine BHAP analogs are potent, broad-spectrum nonnucleoside reverse transcriptase inhibitors of drug-resistant isolates of HIV-1 and select for drug-resistant variants of HIV-1_{IIIB} with reduced replication phenotypes. J Virol 1996; in press. ....

21. Japour AJ, Mayers DL, Johnson VA, Kuritzkes DR, Beckett LA, Arduino JM, et al. Standardized peripheral blood mononuclear cell culture assay for determination of drug susceptibilities of clinical human immunodeficiency virus type 1 isolates. Antimicrob Agents Chemother 1993;37(5):1095-1101. ....

22. Mayers DL, Bruce BJ. Susceptibility of HIV-1 clinical isolates to U-90152. Upjohn Technical Report 7252-92-038, November 10, 1992. ....

23. Batts DH. Open-label, escalating multiple-dose study of the safety, tolerance and pharmacokinetics of oral U-90152S/T (delavirdine mesylate, Rescriptor™ tablets) in HIV-1 infected males and females with CD4 counts of 200 to 500/mm^3, who are maintained on a stable dose of AZT (M/3331/0003, BC 1003). Upjohn Technical Report 7217-93-015, June 10, 1996.

24. Descamps D, Collin G, Loussert-Ajaka I, Saragosti S, Simon F, Brun-Vezinet F. HIV-1 group O sensitivity to antiretroviral drugs. AIDS 1995;9(8):977-8. ....

25. Cheng-Mayer C, Levy JA. Distinct biological and serological properties of human immunodeficiency viruses from the brain. Ann Neurol 1988;23(S):58-61. ....

26. Peterson PK, Gekker G, Hu S, Chao CC. Anti-human immunodeficiency virus type 1 activities of U-90152 and U-75875 in human brain cell cultures. Antimicrob Agents Chemother 1994;38(10):2465-8. ....

27. Chong KT, Pagano, PJ. Delavirdine in multiple drug combination against acute and viral-spread infection of human immunodeficiency virus type 1 in vitro. Upjohn Technical Report 7252-95-048, June 1, 1995. ....

28. Chong KT, Pagano PJ. Synergistic effect of delavirdine (U-90152) in combination with lamivudine (3TC) or didanosine (DDI) against HIV-1 replication in vitro. Upjohn Technical Report 7252-95-061, December 29, 1995. ....

29. Chong KT, Pagano PJ. In vitro inhibition of the replication of human immunodeficiency virus type 1 by combination of delavirdine mesylate (DLV or BHAP U-90152) with protease inhibitor U-75875 or recombinant alpha-interferon. Upjohn Technical Report 7252-94-024, May 13, 1994. ....

30. Chong KT, Pagano PJ. Synergistic inhibition of human immunodeficiency virus type 1 replication in vitro by two- and three-drug combination of delavirdine, lamivudine and zidovudine. Upjohn Technical Report 7252-96-001, February 20, 1996. . . . . .

31. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul 1984;22:27-55. . . . . .

32. Klecker RW, Collins JM, Yarchoan R, Thomas R, Jenkins JF, Broder S, Myers CE. Plasma and cerebrospinal fluid pharmacokinetics of 3'-azido-3'-deoxythymidine: a novel pyrimidine analog with potential application for the treatment of patients with AIDS and related diseases. Clin Pharmacol Ther 1987;41(4):407-12. . . . . .

33. Pagano PJ, Chong KT. Comparison of antiviral effects of convergent three-drug combination with delavirdine mesylate (DLV), AZT, and ddI versus AZT monotherapy on HIV-1 spread infection in human peripheral blood mononuclear cells in vitro. Upjohn Technical Report 7252-94-025, May 16, 1994. . . . . .

34. St. Clair MH, Pennington KN, Rooney J, Barry DW. In vitro comparison of selected triple-drug combinations for suppression of HIV-1 replication: the inter-company collaboration protocol. J Acquir Immune Def Syndr Hum Retrovirol 1995;10(S2):s83-s91. . . . . .

35. Wei X, Ghosh ME, Taylor ME, Johnson VA, Emini EA, Deutsch JD, et al. Viral dynamics in human immunodeficiency virus type 1 infection. Nature 1995;373:117-22. . . . . .

36. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 1995;373:123-6. . . .

37. Coffin JM. HIV population dynamics in vivo: Implications for genetic variation, pathogenesis, and therapy. Science 1995;267:483-9. . . . . .

38. Dueweke TJ, Pushkarskaya T, Poppe SM, Swaney SM, Zhao JQ, Chen ISY, et al. A mutation in reverse transcriptase of bis(heteroaryl)piperazine-resistant human immunodeficiency virus type 1 that confers increased sensitivity to other nonnucleoside inhibitors. Proc Natl Acad Sci USA 1993;90:4713-7. . . . . .

39. Koyanagi Y, Miles S, Mitsuyasu RT, Merrill JE, Vinters HV, Chen ISY. Dual infection of the central nervous system by AIDS viruses with distinct cellular tropisms. Science 1987;236:819-22. . . . . .

40. Stevenson M, Haggerty S, Lamonica C, Mann AM, Meier C, Wasiak A. Cloning and characterization of human immunodeficiency virus type 1 variants diminished in the ability to induce syncytium-independent cytolysis. J Virol 1990;64(8):3792-803. . . . . .

41. Nunberg JH, Schleif WA, Boots EJ, O'Brien JA, Quintero JC, Hoffman JM, et al. Viral resistance to human immunodeficiency virus type 1-specific pyridinone reverse transcriptase inhibitors. J Virol 1991;65(9):4887-92. . . . . .

42. Richman D, Shih C-K, Lowy I, Rose J, Prodanovich P, Goff S, et al. Human immunodeficiency virus type 1 mutants resistant to nonnucleoside inhibitors of reverse transcriptase arise in tissue culture. Proc Natl Acad Sci USA 1991;88:11241-5. . . . . .

43. Balzarini J, Pérez-Pérez M-J, Vélazquez S, San-Félix A, Camarasa M-J, De Clercq E, Karlsson A. Suppression of the breakthrough of human immunodeficiency virus type 1 (HIV-1) in cell culture by thiocarboxanilide derivatives when used individually or in combination with other HIV-1-specific inhibitors (i.e., TSAO derivatives). Proc Natl Acad Sci USA 1995;92:5470-4. . . . . .

44. Wainberg MA, Drosopoulos WC, Salomon H, Hsu M, Borkow G, Parniak MA, et al. Enhanced fidelity of 3TC-selected mutant HIV-1 reverse transcriptase. Science 1996;271:1282-1285. . . . . .

45. Kellam P, Boucher CAB, Larder BA. Fifth mutation in human immunodeficiency virus type 1 reverse transcriptase contributes to the development of high-level resistance to zidovudine. Proc Natl Acad Sci USA 1992;89:1934-8. . . . . .

46. Larder BA. 3'-azido-3'-deoxythymidine resistance suppressed by a mutation conferring human immunodeficiency virus type 1 resistance to nonnucleoside reverse transcriptase inhibitors. Antimicrob Agents Chemother 1992;36(12):2664-2669. . . . . .

47. Slade DE, Poppe SM, Swaney SM, Wisniewsky SM, Sharova N, Stevenson M, et al. The molecular basis of HIV-1 resistance to a BHAP RT inhibitor changes during acquisition of co-resistance to BHAP and AZT. Upjohn Company Technical Report 7252-94-047, 20 October 1994. . .

48. Fan N, Evans DB, Rank KB, Thomas RC, Tarpley WG, Sharma SK. Mechanism of resistance to U-90152S and sensitization to L-697,661 by a proline to leucine change at residue 236 of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase. FEBS Letters 1995;359:233-8. . . . . .

49. Saag MS, Emini EA, Laskin OL, Douglas J, Lapidus WI, Schleif WA, Whitley RJ et al. A short-term clinical evaluation of L-697,661, a non-nucleoside inhibitor of HIV-1 reverse transcriptase. N Engl J Med 1993;329(15):1065-72. .

50. Richman DD, Havlir D, Corbeil J, Looney D, Ignacio C, Spector SA, et al. Nevirapine resistance mutations of human immunodeficiency virus type 1 selected during therapy. J Virol 1994;68(3):1660-6. . . . . .

51. Vandamme A-M, Debyser Z, Pauwels R, DeVreese K, Goubau P, et al. Characterization of HIV-1 strains isolated from patients treated with TIBO R82913. AIDS Res Human Retro 1994;10(1):39-46. . . . . .

52. DeClercq E. HIV Resistance to reverse transcriptase inhibitors. Biochem Pharma 1994; 47(2):155-69. . . . . .

53. Kleim J-P, Bender R, Kirsch R, Meichsner C, Paessens A, Rieb G. Mutational analysis of residue 190 of human immunodeficiency virus type 1 reverse transcriptase. Virol 1994;200:696-701. . . . . .

54. St. Clair MH, Martin JL, Tudor-Williams G, Bach MC, Vavro CL, King DM, et al. Resistance to ddI and sensitivity to AZT induced by a mutation in HIV-1 reverse transcriptase. Science 1991;253:1557-9. . . . . .

55. Larder BA, Kemp SD, Harrigan PR. Potential mechanism for sustained antiretroviral efficacy of AZT-3TC combination therapy. Science 1995;269:696-9. . . . . .

56. Balzarini J, Karlsson A, Perez-Perez M-J, Vrang L, Walberg J, Zhang H,et al. HIV-1-specific reverse transcriptase inhibitors show differential activity against HIV-1 mutant strains containing different amino acid substitutions in the reverse transcriptase. Virology 1993;192:246-53. . . . . .

57. Wathen LK, Crampton DJ, Patel RK, Nuorala KW, Poppe SM, Dueweke TJ, et al. Validation of a quantitative RNA PCR assay for HIV-1 in human plasma. Upjohn Technical Report 7262-95-004. October 12, 1995. . . . . .

58. Wathen LK, Freimuth WW, Nickens DJ, Greenwald CA, Chuang-Stein CJ, Crampton DJ, et al. The level of HIV-1 viral burden at baseline or its reduction following antiretroviral therapy is highly correlated with risk of HIV-1 disease progression. Upjohn Published Document 7262-96-003. JAMA 1996; submitted. . . . . .

59. Hart C, Chang S-Y, Kwok S, Sninsky J, Ou C-Y, Schochetman G. A replication-deficient HIV-1 DNA used for quantitation of the polymerase chain reaction (PCR). Nucleic Acids Research 1990;18:4029-30. . . . . .

60. Schnittman SM, Greenhouse JJ, Psallidopoulos MC, Baseler M, Salzman NP, Fauci AS, et al. Increasing viral burden in CD4+ T cells from patients with human immunodeficiency virus (HIV) infection reflects rapidly progressive immunosuppression and clinical disease. Ann Int Med 1990;113:438-43.

61. Kundu SK, Merigan TC. Relationship of HIV-1 provirus load, CD8+ CD11+ T cells and HIV-1 envelope-specific cytotoxic T lymphocytes in HIV-infected asymptomatic patients. Immunology 1994;83:81-5.

62. Luque F, Caruz A, Pineda JA, Torres Y, Larder B, Leal M. Provirus load changes in untreated and zidovudine-treated human immunodeficiency virus type 1-infected patients. J Inf Dis 1994;169:267-73.

63. Garbuglia AR, Salvi R, DiCaro A, Montella F, DiSora F, Recchia O, et al. Peripheral lymphocytes of clinically non-progressor patients harbor inactive and uninducible HIV proviruses. J Med Vir 1995;46:116-21.

64. Fahey JL, Taylor JMG, Detels R, Hoffman B, Melmed R, Nishanian P, et al. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. N Engl J Med 1990;322:166-72.

65. Valentine FT, Jacobson MA. Immunological and virological surrogate markers in the evaluation of therapies for HIV infection. AIDS 1990;4(S1)S201-6.

66. Lagakos SW. Surrogate markers in AIDS clinical trials: conceptual basis, validation, and uncertainties. Clin Infect Dis 1993;16(S1):S22-5.

67. Phillips AN, Lee CA, Elford J, Janossy G, Timms A, Bofill M, et al. Serial CD4 lymphocyte counts and development of AIDS. Lancet 1991;337:389-92.

68. Eyster ME, Gail MH, Ballard JO, Al-Mondhiry H, Goedert JJ. Natural history of human immunodeficiency virus infections in hemophiliacs: effects of T-cell subsets, platelet counts, and age. Ann Intern Med 1987;107:1-6.

69. Lange JMA, DeWolf F, Goudsmit J. Markers for progression in HIV infection. AIDS 1989;3(S1):S153-60.

70. Schellekens PTA, Tersmette M, Roos MTL, Keet RP, deWolf F, Coutinho RA, et al. Biphasic rate of CD4+ cell count decline during progression to AIDS correlates with HIV-1 phenotype. AIDS 1992;6:665-9.

71. Graham NMH, Piantadosi S, Park LP, Phair JP, Rinaldo CR, Fahey JL. CD4+ lymphocyte response to zidovudine as a predictor of AIDS-free time and survival time. J Acquir Immune Defic Syndr 1993;6:1258-66.

72. Choi S, Lagakos SW, Schooley RT, Volberding PA. CD4+ lymphocytes are an incomplete surrogate marker for clinical progression in persons with asymptomatic HIV infection taking zidovudine. Ann Intern Med 1993;118:674-80. . . . . .

73. Coombs RW, Collier AC, Allain J-P, Nikora B, Leuther M, Gjerset G, et al. Plasma viremia in human immunodeficiency virus infection. N Engl J Med 1989;321:1626-31. . . . . .

74. DeWolf F, Goudsmit J, Paul DA, Lange JMA, Hooijkaas C, Schellekens P, et al. Risk of AIDS related complex and AIDS in homosexual men with persistent HIV antigenaemia. Br. Med J. 1987;295:569-72. . . . . .

75. MacDonell KB, Chmiel JS, Poggensee L, Wu S, Phair JP. Predicting progression to AIDS: combined usefulness of CD4 lymphocyte counts and p24 antigenemia. Am J Med 1990;89:706-12. . . . . .

76. Moss AR, Bacchetti P, Osmond D, Krampf W, Chaisson RE, Stites D, et al. Seropositivity for HIV and the development of AIDS or AIDS related condition: three year followup of the San Francisco General Hospital cohort. Br Med J 1988;296:745-50. . . . . .

77. Paul DA, Falk LA, Kessler HA, Chase RM, Blaauw B, Chudwin DS, et al. Correlation of serum HIV antigen and antibody with clinical status in HIV-infected patients. J Med Virol 1987;22:357-63. . . . . .

78. Ho DD, Moudgil T, Alam M. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. N Engl J Med 1989;321:1621-5. . . . . .

79. Rinaldo C, Kingsley L, Neumann J, Reed D, Gupta P, Lyter D, et al. Association of human immunodeficiency virus (HIV) p24 antigenemia with decrease in CD4+ lymphocytes and onset of acquired immunodeficiency syndrome during the early phase of HIV infection. J Clin Microbiol 1989;27:880-4. . . . . .

80. Kageyama S, Yamada O, Mohammad S-S, et al. An improved method for the detection of HIV antigen in the blood of carriers. J Virol Methods 1988;22:125-31. . . . . .

81. Kestens L, Hoofd G, Gigase PL, Deleys R, van der Groen G. HIV antigen detection in circulating immune complexes. J Virol Methods 1991;31:67-76. . . . . .

82. Phillips SK, Parekh B, George JR. Comparison of methods for HIV p24 antigen tests and methods for dissociation of immune complexes, abstr. 174. In First National Conference on Human Retroviruses, Washington, D.C. 1993. . . . . .

83. Bollinger RC, Kline RL, Franics HL, Moss MW, Bartlett JG, Quinn TC. Acid dissociation increases the sensitivity of p24 antigen detection for the evaluation of antiviral therapy and disease progression in asymptomatic human immunodeficiency virus-infected persons. J Infect Dis 1992;165:913-6.

84. Palomba E, Gay V, de Martino M, Fundaro C, Perugini L, Tovo P-A. Early diagnosis of human immunodeficiency virus infection in infants by detection of free and complexed p24 antigen. J Infect Dis 1992;165:394-5.

85. Henrard DR, Wu S, Phillips J, Wiesner D, Phair J. Detection of p24 antigen with and without immune complex dissociation for longitudinal monitoring of human immunodeficiency virus type 1 infection. J Clin Microb 1995;33(1):72-5.

86. Hollinger FB, Bremer JW, Myers LE, Gold JWM, McQuay L. Standardization of sensitive human immunodeficiency virus coculture procedures and establishment of a multicenter quality assurance program for the AIDS clinical trials group. J Clin Microb 1992;30:1787-94.

87. Hammer S, Crumpacker C, D'Aquila R, Jackson B, Lathey J, Livnat D, et al. Use of virologic assays for detection of human immunodeficiency virus in clinical trials: recommendations of the AIDS clinical trials group virology committee. J Clin Microb 1993;31:2557-64.

88. Fiscus SA, DeGruttola V, Gupta P, Katzenstein DA, Meyer WA III, LaFaro ML, et al. Human immunodeficiency virus type 1 quantitative cell microculture as a measure of antiviral efficacy in a multicenter clinical trial. J Infect Dis 1995;171:305-11.

89. Asjo B, Morfeldt-Manson L, Albert J, Biberfeld G, Karlsson A, Lidman K, et al. Replicative capacity of human immunodeficiency virus from patients with varying severity of HIV infection. Lancet 1986,ii:660-2.

90. Tersmette M, DeGoede REY, Al BJM, Winkel IN, Gruters RA, Cuypers HT, et al. Differential syncytium-inducing capacity of human immunodeficiency virus isolates: frequent detection of syncytium-inducing isolates in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. J Virol 1988;62(6):2026-32.

91. Von Briesen H, Becker WB, Henco K, Helm EB, Gelderblom HR, Brede HD, et al. Isolation frequency and growth properties of HIV-variants: multiple simultaneous variants in a patient demonstrated by molecular cloning. J Med Virol 1987;23:51-66.

92. Koot M, Keet IPM, Vos AHV, deGoede REY, Roos MTL, Coutinho RA, et al. Prognostic value of HIV-1 syncytium-inducing phenotype for rate of CD4+ cell depletion and progression to AIDS. Ann Intern Med 1993;118:681-8. . . . .

93. Tersmette M, Lange JMA, DeGoede REY, DeWolf F, Eeftink-Schattenkerk JKM, Schellekens PTA, et al. Association between biological properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. Lancet 1989;i:983-5. . . . . .

94. Richman DD. Resistance of clinical isolates of human immunodeficiency virus to antiretroviral agents. Antimicrob Agents Chemother 1993;37(6):1207-13. . . . . .

95. Erice A, Balfour HH. Resistance of human immunodeficiency virus type 1 to antiretroviral agents: a review. Clin Infect Dis 1994;18:149-56. . . . . .

96. Tudor-Williams G, St Clair MH, McKinney RE, Maha M, Walter E, Santacroce S, et al. HIV-1 sensitivity to zidovudine and clinical outcome in children. Lancet 1992;339:15-19. . . . . .

97. Kozal MJ, Shafer RW, Winters MA, Katzenstein DA, Merigan TC. A mutation in human immunodeficiency virus reverse transcriptase and decline in CD4 lymphocyte numbers in long-term zidovudine recipients. J Infect Dis 1993;167:526-32. . . . . .

98. Larder BA, Darby G, Richman DD. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. Science 1989;243:1731-4. . . . . .

99. McLeod GX, McGrath JM, Ladd EA, Hammer SM. Didanosine and zidovudine resistance patterns in clinical isolates of human immunodeficiency virus type 1 as determined by a replication endpoint concentration assay. Antimicrob Agents Chemother 1992;36:920-5. . . . . .

100. Reichman RC, Tejani N, Lambert JL, Strussenberg J, Bonnez W, Blumberg B, et al. Didanosine (ddI) and zidovudine (ZDV) susceptibilities of human immunodeficiency virus (HIV) isolates from long-term recipients of ddI. Antiv Res 1993;20:267-77. . . . . .

101. Boucher CAB, Tersmette M, Lange JMA, Kellam P, deGoede REY, Mulder JW, et al. Zidovudine sensitivity of human immunodeficiency viruses from high-risk, symptom-free individuals during therapy. Lancet 1990;336:585-90. . . . . .

102. Lopez-Galindez C, Rojas JM, Najera R, Richman DD, Perucho M. Characterization of genetic variation and 3'-azido-3'-deoxythymidine-resistance mutations of human immunodeficiency virus by the RNase A mismatch cleavage method. Proc Natl Acad Sci USA 1991;88:4280-4. . . . . .

103. Japour AJ, Welles S, D'Aquila RT, Johnson VA, Richman DD, Coombs RW, et al. Prevalence and clinical significance of zidovudine resistance mutations in human immunodeficiency virus isolated from patients after long-term zidovudine treatment. J Infect Dis 1995;171:1172-9. .

104. Mellors JW, Dutschman GE, Im G-J, Tramontano E, Winkler SR, Cheng Y-C. In vitro selection and molecular characterization of human immunodeficiency virus-1 resistant to non-nucleoside inhibitors of reverse transcriptase. Mol. Pharmacol. 1992;41:446-51. . . . . .

105. Sardana VV, Emini EA, Gotlib L, Graham DJ, Lineberger DW, Long WJ, et al. Functional analysis of HIV-1 reverse transcriptase amino acids involved in resistance to multiple nonnucleoside inhibitors. J Bio Chem 1992;267(25):17526-17530. . . . . .

106. Mellors JW, Larder BA, Schinazi RF. Mutations in HIV-1 reverse transcriptase and protease associated with drug resistance. International Antiviral News. 1995;3:8-13. . . . .

107. Balzarini J, Karlsson A, Meichsner C, Paessens A, Riess G, DeClercq E, et al. Resistance pattern of human immunodeficiency virus type 1 reverse transcriptase to quinoxaline S-2720. J. Virol. 1994;68:7986-92. . . . . .

108. Demeter LM, Shafer RW, Para M, Morse G, Freimuth W, Merigan T. Delaviridine (DLV) susceptibility of HIV-1 isolates obtained from patients (pts) receiving DLV monotherapy (ACTG 260). Fourth International HIV Drug Resistance Workshop. Abstract no. 23. Sardinia, Italy, July 6-9, 1995. . . . . .

109. Staszewski S, Massari FE, Kober A, Gohler R, Durr S, Anderson KW, et al. Combination therapy with zidovudine prevents selection of human immunodeficiency virus type 1 variants expressing high-level resistance to L-697661, a nonnucleoside reverse transcriptase inhibitor. J Infect Dis 1995;171:1159-65. . . . . .

110. Demeter LM, Meehan P, Morse G, Gerondelis P, Dexter A, Berrios, et al. HIV-1 drug susceptibilities and reverse transcriptase mutations in patients receiving combination therapy with didanosine and delavirdine. (Submitted). . . . . .

111. Byrnes VW, Emini EA, Schleif WA, Condra JH, Schneider CL, Long WJ, et al. Susceptibilities of human immunodeficiency virus type 1 enzyme and viral variants expressing multiple resistance-engendering amino acid substitutions to reverse transcriptase inhibitors. Antimicrob Agents Chemother 1994;38:1404-7. . . . . .

112. Koot M, Vos AHV, Keet RPM, DeGoede REY, Dercksen MW, Terpstra FG, et al. HIV-1 biological phenotype in long-term infected individuals evaluated with an MT-2 cocultivation assay. AIDS 1992;6:49-54. . . . . .

113. Karlsson A, Parsmyr K, Aperia K, Sandstrom E, Fenyo EM, Albert J. MT-2 cell tropism of human immunodeficiency virus type 1 isolates as a marker for response to treatment and development of drug resistance. J Infect Dis 1994;170:1367-75. . . . . .

H. CLINICAL DATA SUMMARY

1. Background/Overview of Clinical Studies

The delavirdine mesylate clinical program is being conducted in patients with HIV-1 infection who are at least 13 years of age and who have a CD4 cell count of no more than 500 cells/mm^3. Data from two ongoing, double-blind, randomized, comparative phase II/III studies (Protocols M/3331/0017 and M/3331/0021) provide the basis for the evaluation of the effectiveness (antiviral effect) of delavirdine as an antiretroviral agent in the treatment of HIV-1 infection. These two studies constitute the adequate and well-controlled pivotal efficacy/safety studies for the treatment of HIV-1 infection and AIDS. Because these studies are currently ongoing, data for clinical endpoints (including death) remain blinded. Data from five phase I or II studies in patients with HIV-1 infection provide supportive evidence for the efficacy of delavirdine (M/3331/0003, M/3331/0007, M/3331/0013A, M/3331/0018, M/3331/0023). Several additional studies provide data related to pharmacokinetics, drug interactions, and safety; these studies are protocols M/3331/0006, M/3331/0016, M/3331/0019, M/3331/0025, M/3331/0026, M/3331/0028, M/3331/0029, and M/3331/0030. Protocol M/3331/0030 also included collection of some additional limited efficacy and resistance data; however, these data are presented as a part of the integrated summary of efficacy

The safety data base for delavirdine mesylate includes information from over 2600 subjects who have been treated in completed or ongoing studies as of August 31, 1995. The study blind has only been broken by group for safety data not classified as clinical endpoints (including death) from the two ongoing pivotal studies (Protocols M/3331/0017 and M/3331/0021); data from these two studies are summarized in this submission by individual study. Safety results are described in the Safety Summary

2. Efficacy Summary

a. Background

The clinical program is being conducted in patients with HIV-1 infection who are at least 13 years of age and who have a CD4 cell count of no more than 500 cells/mm^3. Data from 1614 patients in two ongoing, double-blind, randomized, comparative phase II/III studies (Protocols M/3331/0017 [1] and M/3331/0021 [2]) provide the basis for the evaluation of the effectiveness (antiviral effect) of delavirdine as an antiretroviral agent in the treatment of HIV-1 infection and are summarized in Table H.1. These two studies constitute the adequate and well-controlled pivotal efficacy/safety studies for the treatment of HIV-1 infection and AIDS. Because these studies are currently ongoing, data for clinical endpoints (including death) remain blinded. Data from five Phase I or II studies in patients with HIV-1 infection provide supportive evidence for the efficacy of delavirdine (M/3331/0003, M/3331/0007, M/3331/0013A, M/3331/0018, and M/3331/0023)[3-7]. These studies are summarized in Table H.2. Protocol M/3331/0030 also includes a collection of some additional limited efficacy data; these data are presented as a part of the integrated summary of efficacy [8].

Table H.1. Pivotal Clinical Studies with Delavirdine Mesylate (U-90152S/T)
(Ongoing Studies Included in NDA 20-705)

Protocol Number	Study Design/Description	DLV Dosage	Other Therapy	No. of Pts.[a]	Study Dates[b]	Mean Age, Range (yr)	M/F W/B/O
M/3331/0017	A double-blind, randomized, comparative study of delavirdine mesylate (U-90152S) in combination with didanosine (ddI) versus ddI alone in HIV-1–infected individuals with CD4 counts of ≤ 300/mm^3 (2 years)	0 mg tid 400 mg tid	ddI 200 or 125 mg bid	896	Apr 94–Aug 95	37.5 (20-78)	775/121 617/171/109
M/3331/0021	A double-blind, randomized, dose-response study of three fixed doses of delavirdine mesylate (U-90152S) in combination with zidovudine (ZDV) versus ZDV alone in HIV-1–infected individuals with CD4 counts of 200-500/mm^3 (2 years)	0 mg tid 200 mg tid 300 mg tid 400 mg tid	ZDV 200 mg tid (ddI 200 or 125 mg bid)[d]	718	Mar 94–Aug 95	34.8 (15-70)	579/139[c] 498/132/88[c]

Abbreviations: bid = twice daily; ddC = zalcitabine; ddI = didanosine; DLV = delavirdine; ZDV = zidovudine; M/F = males/females; Pts = patients; q6h = every 6 hours; q8h = every 8 hours; qid = four times daily; tid = three times daily; W/B/O = white/black/other; Yr = years

[a] For ongoing studies, numbers reflect those patients in the efficacy populations in interim Technical Reports. When not all patients were evaluable, numbers are presented as randomized/evaluable.

[b] For ongoing studies, end dates reflect cut-off dates for data included in interim Technical Reports.

[c] Demographic data not available for some patients in this ongoing study.

[d] Added in cases of immunologic deterioration, disease progression, ZDV intolerance, etc.

Table H.2. Supportive Clinical Studies with Delavirdine Mesylate (U-90152S/T): Completed and Ongoing Studies Included in NDA 20-705

Protocol No.	Study Design/Description	DLV Dosage	Other Therapy	No. of Pts.[a]	Study Dates[b]	Mean Age, Range (yr)	M/F W/B/O
Supporting Clinical and Pharmacokinetic Studies in HIV-1 Positive Patients							
M/3331/0003	Open-label, escalating multiple-dose study of the safety, tolerance and pharmacokinetics of oral U-90152S in HIV-1 infected males and females with CD4 counts of 200 to 500/mm^3 who are maintained on a stable dose of ZDV (BC1003)	100 mg qid	ZDV 200 mg tid	6	Mar 93–Feb 94	33.9 (20-56)	24/10 28/2/4
		150 mg qid		6			
		200 md qid		6			
		300 mg tid		6			
		400 mg tid		10			
M/3331/0013A	A study of the tolerance and efficacy of combinations of U-90152S and nucleoside therapy in the treatment of HIV-infected patients (Part A)	0 mg tid	ZDV 200 mg tid or ZDV 200 mg + ddC 0.75 mg tid	26	Oct 93–Jan 95	37.7 (21–65)	87/16 96/5/2
		200 mg tid		26			
		400 mg tid		25			
		Escalating[c]		26			
M/3331/0023	Optional, open-label, extended use delavirdine mesylate treatment in triple combination for HIV-1+ patients who participated in other delavirdine mesylate protocols	≤ 400 mg tid	ZDV 500-600 mg qd + ddI 125 or 200 mg bid	128	Mar 94-Dec 95	37 (19-64)	255/30 215/36/34
			ZDV 500-600 qd + ddC 0.75 mg tid	157			
M/3331/0018 (ACTG 260)	Randomized, phase I/II, dose-ranging, open-label trial of the anti-HIV activity of delavirdine mesylate (DLV, U-90152S)	400 mg tid (increased to target trough concentration)	ZDV 200 mg tid or ddI 200 mg bid	115	Oct 94–Jul 95	37 (19-61)	97/18 80/23/12
M/3331/0007A	A randomized, dose-escalating multiple-dose Phase I/II trial of oral U-90152S compared to conventional combination nucleoside therapy in HIV-1 infected patients (Part A)	0 mg qid	ZDV 200 mg tid + ddI 100 mg bid	15	Apr 93-Aug 95	37 (20-59)	45/2 44/1/2
		100 mg qid		5			
		150 mg qid		5			
		200 mg qid		5			
		250 mg qid		5			
		300 mg qid		12			
M/3331/0007B	A randomized, dose-escalating multiple-dose Phase I/II trial of oral U-90152S compared to conventional combination nucleoside therapy in HIV-1–infected patients (Part B)	400 mg tid	None	19	Aug 93–Aug 95	37 (27-49)	34/4 35/1/2
			ZDV 200 mg tid	19			

Abbreviations: bid = twice daily; ddC = zalcitabine; ddI = didanosine; DLV = delavirdine; ZDV = zidovudine; M/F = males/females; Pts = patients; q6h = every 6 hours; q8h = every 8 hours; qid = four times daily; tid = three times daily; W/B/O = white/black/other; Yr = years

[a] For ongoing studies, numbers reflect those patients in the efficacy populations in interim Technical Reports. When not all patients were evaluable, numbers are presented as randomized/evaluable.

[b] For ongoing studies, end dates reflect cut-off dates for data included in interim Technical Reports.

[c] Escalating dose for protocol 0013A = 7 days each of 50 mg tid, 100 mg tid, 200 mg tid, then 400 mg tid for duration of study. Amendment #2 (8/4/93; serial no. 027, submitted 9/24/93 to IND 41,207) eliminated 100 mg tid dose.

b. Pivotal Studies (Protocols M/3331/0021 and M/3331/0017)

1) *Overview of Methodology for the Pivotal Studies*

Protocol M/3331/0021 was a randomized, double-blind, parallel group, comparative study of three dosages (200 mg, 300 mg, or 400 mg three times daily) of delavirdine plus zidovudine (DLV+ZDV) versus zidovudine (ZDV) therapy alone. In addition, didanosine therapy could be added for patients in any treatment group under specified conditions (CD4 cell decline or disease progression). Protocol M/3331/0017 was a randomized, double-blind, parallel group, comparative study of a single dose of delavirdine plus didanosine versus didanosine therapy alone. Both studies were multiple center trials conducted simultaneously at approximately 100 study centers throughout the United States and Canada for a duration of 2 years. Both studies included a 6-month administrative data analysis.

2) *Efficacy Evaluations*

The Upjohn Company is seeking accelerated review and approval for this submission under 21 CFR 314.5. In seeking accelerated approval, The Upjohn Company is not including a clinical endpoint analysis between therapeutic groups but rather data and analyses based on surrogate endpoints. The pivotal studies (M/3331/0017 and M/3331/0021) remain blinded with respect to clinical endpoints.

Parameters to be measured for surrogate marker response in the pivotal studies included CD4 lymphocyte counts, tests for viral burden (plasma RNA and PBMC DNA PCR) and serum p24 and ICDp24 antigen levels. Other parameters considered as efficacy measures (clinical changes, progression in minor characteristics of HIV disease, survival, and occurrence of disease progression) will be addressed in the full NDA submission.

- *Primary Endpoints for the Interim Analyses*

Methodology for the following surrogate marker assays is described in Item Microbiology, Section . Clinical Laboratory Test Methods of this submission and its appendices.

- CD4 lymphocytes were to be measured in a quality controlled, central flow cytometry laboratory, and the absolute number of cells calculated using a WBC and a machine differential count. Sequential changes in the percent and absolute number of CD4 cells were evaluated for individual patients. An increase in CD4 cell count of 25 cells/mm^3 or ≥25% increase sustained at two consecutive measurements at least 4 weeks apart were considered a positive response. Trends in these sequential values for each patient were also to be assessed

- RNA and DNA PCR (PBMC) and PBMC co-culture viral titer were to be performed. RNA PCR was to be measured for all patients but lymphocyte DNA PCR and PBMC co-culture were to be determined in a subset of patients. Later, DNA PCR was expended to include all patients. A 0.5 log or 1 $\log_{10}$ or greater reduction in the number of infectious units per volume of plasma or

cells was considered a positive response for these surrogate markers. PBMC co-culture data were not available for inclusion in the interim reports for the pivotal studies

- Plasma p24 and ICD p24 antigen measurements were to be performed in a central, quality-controlled laboratory. Sequential changes in ICD p24 and p24 antigen levels for individual patients were evaluated. A 50% reduction in p24 and ICD p24 antigen from the mean of two before-treatment measurements sustained at least four weeks were considered a significant response

- *Secondary Endpoints for the Interim Analyses*

HIV-1 syncytia-inducing phenotype, changes in genotype mutation, and phenotype sensitivity to delavirdine (IC_{50}) will be evaluated for a subset of patients, but are not available for the interim reports for the pivotal studies.

The **EFFICACY POPULATION** consists of all patients who were enrolled on or prior to 31 May 1995. Data pertaining to samples collected on or prior to 31 December 1995 and available for analysis on 29 February 1996 in this patient population formed the basis of efficacy summary. Two sets of efficacy evaluations were conducted in this population. The first was *on-treatment* and the second was *intent-to-treat analysis*. The *on-treatment analysis* included only data acquired during the pivotal studies and within 3 days beyond the patients' last dose in the pivotal studies. For the *intent-to-treat analysis*, all available surrogate marker data for subjects, regardless of their timing relative to the blinded medications in Protocol M/3331/0017 or Protocol M/3331/0021, were included in the analysis. This included data after the patients permanently stopped the blinded medications in their respective studies and data acquired for those patients who participated in the salvage study Protocol M/3331/0023.

3) *Results*

In Protocol M/3331/0021, investigators enrolled 718 patients through 31 May 1995. A total of 718 patients are included in the efficacy analysis; 175 patients in the ZDV-treated group and 173 patients in the DLV+ZDV 200 mg tid treatment group, 189 patients in the DLV+ZDV 300 mg tid treatment group, and 181 patients in the DLV+ZDV 400 mg tid treatment group. Data on surrogate markers and trough delavirdine concentrations for the efficacy population from visits that occurred through 31 December 1995 are provided for samples analyzed by 29 February 1996. The number of patients enrolled per investigator ranged from to .

There was a higher proportion of patients who enrolled in the study with no prior zidovudine experience (62%) than those with six months or less experience (38%). There was no apparent difference across treatment groups and between DLV+ZDV dose groups in this proportion with respect to the prior zidovudine experience categorization.

Of the 718 patients in the efficacy population of Protocol M/3331/0021, efficacy data are included for 137 who had completed the week-68 follow-up visit, 35 in the

zidovudine group and 102 in the DLV+ZDV groups. Data from as far out as the week-84 follow-up visit are included for 12 patients (2 patients in the ZDV-treated group and 10 patients in the DLV+ZDV groups).

Protocol M/3331/0017 enrolled HIV-1–infected patients with an entry CD4 count of 0 to 300 cells/mm^3 who were zidovudine experienced and had ≤ 4 months of prior didanosine therapy were randomized to receive one of two therapies: didanosine monotherapy (200 mg two times daily for patients >60 kg and 125 mg two times daily for patients <60 kg), or 400 mg three times daily of delavirdine combined with didanosine (200 mg two times daily for patients >60 kg and 125 mg two times daily for patients <60 kg). The effect of these therapies on a variety of surrogate markers (CD4, CD4%, CD8, CD8%, CD4/CD8 ratio, HIV-1 RNA, HIV-1 DNA, ICD p24 and p24) was studied over the duration of the trial. Subpopulations defined by didanosine experience (none [naive] or ≤4 months of prior didanosine therapy), CD4 cell stratum (≤49 cells/mm^3, 50 to 149 cells/mm^3, and 150 to 300 cells/mm^3), delavirdine trough concentrations achieved, race, and sex were analyzed.

As results from patients with CD4 ≥50 cells/mm^3 are the primary analyses, their results will be emphasized in this discussion. In general, the surrogate marker results from all patients are comparable to those with CD4 ≥50 cells/mm^3. Results for the all patient population are provided in the Integrated Summary of Efficacy

In Protocol M/3331/0017, 96 investigators enrolled 896 patients through 31 May 1995 for the efficacy population (441 in the DLV+ddI group and 455 in the ddI-treated group). Data on surrogate markers and trough delavirdine concentrations for the efficacy population from visits that occurred through 31 December 1995 are provided for samples analyzed by 29 February 1996. The number of patients per investigator ranged from . to patients. Of these, 1 patient had more than 4 months of previous didanosine exposure (4.5 months), while 838 had not been treated previously with didanosine. Of the 896 patients in the efficacy population, data are included for 107 who had completed the week-68 visit, 54 in the ddI group and 53 in the DLV+ddI group. Data up to the week-84 visit are included for 4 patients, 1 patient in the ddI-treated group and 3 patients in the DLV+ddI group.

Table H.3 summarizes the duration of prior exposure to zidovudine and didanosine for patients in Protocols M/3331/0021 and M/3331/0017 and the frequency of detectable p24 and ICD p24 antigens by treatment group at baseline. There were no statistically significant differences across treatment groups in prior antiretroviral therapy or in the frequency of detectable antigen in either protocol at baseline.

Table H.3. Summary of Prior Antiretroviral Therapy; Detectable Antigen at Baseline

	Protocol M/3331/0021				Protocol M/3331/0017	
		DLV Treatment Group[A] (mg tid)			Efficacy Population	
	ZDV (N = 175)	200 (N = 173)	300 (N = 181)	400 (N = 181)	ddI	DLV+ddI
Previous antiviral therapy (months)						
Zidovudine mean range	n = 175 0.95 (0-6.0)	n = 172 0.91 (0-6.0)	n = 189 1.04 (0-6.0	n = 181 0.94 (0-6.0)	 17.1 0.1-108	 17.9 0-104
Didanosine mean range	ND	ND	ND	ND	 0.35 0-5.0	 0.45 0-4.0
Detectable HIV p24 antigen (number of pts & %)						
ICD p24[B]	58 (33.1)	58 (33.5)	60 (31.7)	75 (41.4)	127 (28)	116 (27)
p24[C]	21 (12.0)	18 (10.4)	21 (11.1)	27 (14.9)	243 (53)	225 (51)

A All patients also received 200 mg of ZDV three times daily.

B ICD p24 = immune-complex dissociated p24 antigen, detectable at baseline at levels of ≥ 32 pg/mL.

C p24 antigen was detectable at baseline at levels of ≥ 16 pg/mL.

Abbreviations: ND = not done

Source: Tables: Refs 1&2: B15-1A, B17-1A [Ref 2], B15-1, B17-1 [Ref 1].

In both protocols, CD4 counts, measures of viral p24 antigen, and tests of viral burden were done at baseline. Table H.4 shows the mean values for the CD4 cell counts, percent CD4 cells, p24 and ICD p24 antigen, and RNA and DNA PCR at baseline for the efficacy population in Protocols M/3331/0021 and M/3331/0017. No statistically significant differences were observed between treatment groups or among DLV+ZDV dose groups for any of these measures.

In Protocol M/3331/0017, the treatment groups were comparable at baseline with regard to demographics, immunologic and virologic characteristics, and medical history.

Table H.4. Baseline Immunological Status[A]

	Protocol M/3331/0021				Protocol M/3331/0017	
		DLV+ZDV			Efficacy Population	
	ZDV N = 175	200 mg tid N = 173	300 mg tid N = 189	400 mg tid N = 181	ddI	DLV+ddI
CD4 Lymphocytes						
CD4 Count (cells/mm^3) Mean Range	n = 175 337.7 96-696	n = 173 329.6 124-613	n = 189 333.7 (121-686)	n = 181 326.3 (75-615)	n = 455 136 0-495	n = 437 139 3-541
CD4 cells (%) Mean Range	n = 175 21.9	n = 173 21.9	n = 189 22.0	n = 181 21.4	n = 455 11 0-36	n = 437 11 1-36
HIV p24 Antigen[B]						
p24 (pg/mL)[C] Mean Range	n = 21 116.3	n = 18 41.9	n = 21 61.2	n = 27 132.9	n = 127 196 16-4478	n = 116 167 17-3693
ICD p24 (pg/mL)[D] Mean Range	n = 58 369.9	n = 58 324.9	n = 60 353.4	n = 75 526.6	n = 243 661 32-23435	n = 225 700 33-22510
Viral Burden						
RNA PCR (log cps/mL) Mean Range	n = 175 5.3	n = 172 5.3	n = 189 5.2	n = 181 5.2	n = 455 5.8 3.9-7.3	n = 438 5.8 3.3-7.3
DNA PCR[E] (log cps/3M) Mean Range	n = 147 3.5	n = 143 3.5	n = 160 3.4	n = 157 3.4	n = 198 3.7 2.1-4.8	n = 188 3.7 2.2-4.7

[A] Baseline characteristics were not reported for 1 patient.
[B] For HIV p24 antigen assays, only those patients with detectable levels of p24 ≥ 16 pg/mL or ICD p24 ≥ 32 pg/mL were included in the summary statistics.
[C] p24 antigen was detectable at baseline at levels of ≥ 16 pg/mL.
[D] ICD p24 = immune complex dissociated HIV p24 antigen, detectable at baseline at levels of ≥ 32 pg/mL.
[E] DNA PCR was measured in copies per 3 million cells.
Abbreviations: DLV = delavirdine mesylate, tid = three times daily, ZDV = zidovudine.
Source: Tables: Refs 1&2: BS16-1A, B16-1A [Ref 2]; BS16-1, B16-1 [Ref 1].

Throughout the results section of this report, many subgroups are analyzed in the safety and efficacy populations, and at times the baseline values are statistically significantly different; however, the magnitude of these differences are usually less than 5% to 10% between treatment groups. Efficacy results of surrogate markers are analyzed by change from baseline, which reduces the effect of baseline differences. Baseline values are calculated on the patients with available data at each time point during the trial before the change from baseline values are calculated.

Results from the Pivotal Studies: Protocol M/3331/0021

In Protocol M/3331/0021, the 300-mg and 400-mg DLV+ZDV groups show statistically significant greater increases in CD4/CD4% counts and in greater reductions in viral burden as measured by HIV-1 RNA, HIV-1 DNA, ICD p24, and p24 levels for up to 60 weeks when compared with zidovudine monotherapy. For all surrogate markers studied, there is evidence of both a sustained dose-response relationship and a concentration-response relationship. The results, in general, are comparable between the 300-mg and 400-mg delavirdine dose groups which usually have statistically significantly better surrogate marker responses than 200-mg delavirdine dose group, which has a limited duration of response. Analysis of results by the delavirdine trough concentrations achieved suggests that patients with concentrations of more than 1.5 μM have greater and more sustained surrogate marker responses. In addition, patients with trough levels greater than 7.5 μM often have incrementally greater responses than patients with lower delavirdine concentrations. The majority of patients in the 300-mg and 400-mg dose groups or in the "1.5 to 7.5 μM" and "more than 7.5 μM" concentration groups have sustained reduction of HIV-1 RNA of 0.5 $\log_{10}$ copies/mL (68%) or more for 52 weeks. A ≥0.5 $\log_{10}$ reduction in HIV-1 RNA was found to be associated with a two- to three-fold reduction in the risk for disease progression in combination data from protocols M/3331/0021 and M/3331/0017.

Delavirdine administered at 300-mg three times daily or 400-mg three times daily combined with zidovudine has an excellent safety profile and, compared to zidovudine monotherapy, provides significantly better surrogate marker responses of at least one year duration. The greater efficacy of 300-mg and 400-mg DLV+ZDV combination therapies over zidovudine monotherapy with little added risk suggests that this combination therapy will be beneficial for long-term treatment of HIV-1 patients.

Results from the Pivotal Studies: Protocol M/3331/0017

In Protocol M/3331/0017, patients on 400 mg tid of delavirdine combined with didanosine (weight adjusted dose bid) show statistically significant greater increases in CD4/CD4% counts and in greater reductions in viral burden as measured by HIV-1 RNA, HIV-1 DNA, ICDp24 and p24 antigen levels for up to 24 weeks when compared with didanosine monotherapy. There was evidence of a concentration-response relationship for CD4, HIV-1 RNA, and ICDp24 antigen surrogate markers. The majority of patients in the DLV+ddI group have sustained mean DAVG reduction of HIV-1 RNA ≥0.5 $\log_{10}$ copies/mL after week 4 to 60 weeks. This magnitude of viral burden reduction had been associated with a two-to-three fold reduction in the risk of disease progression.

In Protocol M/3331/0030, 5 of 7 highly nucleoside-experienced patients developed the Y181C substitution in combination with K103N after 10 weeks of delavirdine and didanosine therapy. These mutations were associated with decreased susceptibility to delavirdine. If there is a more rapid emergence of Y181C and K103N to DLV+ddI therapy in highly nucleoside RTI-experienced patients in Protocol M/3331/0017, it may contribute to the limited period of maximum reduction in HIV-1 RNA observed in the DLV+ddI group. Two of these patients who had less than 3 months of didanosine experience had little or no change to delavirdine susceptibility. Unlike combination therapy with delavirdine and zidovudine, the delavirdine and didanosine combination

did not prevent emergence of the Y181C substitution. The more rapid emergence of Y181C and K103N in the DLV+ddI group may contribute to increase in viral burden in the DLV+ddI group after week 4.

Delavirdine administered at 400 mg three times a day combined with didanosine provides significantly better surrogate marker response of up to 24 weeks compared to didanosine monotherapy. A more durable surrogate marker response was observed in some subgroups of didanosine-naive patients with higher CD4 counts. The greater efficacy of 400-mg DLV+ddI combination therapy over didanosine monotherapy, with little added risk, suggest that this combination therapy will be beneficial for the treatment of HIV-1 patients.

c. Association of Surrogate Marker Response with Clinical Progression

The correlation of surrogate markers (CD4, HIV-1 RNA, and ICDp24) with HIV-1 disease progression (defined as new or recurring AIDS defining illness or death) was studied in the pivotal delavirdine protocols M/3331/0017 and M/3331/0021. Analysis was performed maintaining the blind of individual patients and their assigned therapy. The effect of the antiretroviral treatment on clinical outcome was not examined and remains blinded.

Using the Cox proportional hazards ratio model, baseline HIV-1 RNA levels in plasma ($p<0.0001$), decrease in HIV-1 RNA levels from baseline ($p<0.0001$), and baseline CD4+ cell count ($p<0.0001$) in subjects receiving antiretroviral therapy were found to be predictive of clinical progression. A 0.5 $\log_{10}$ (68%) decrease in HIV-1 RNA level, while the patient was receiving antiretroviral therapy, was associated with a 54% reduction in the risk of clinical progression. A 1.0 $\log_{10}$ (90%) decrease was related to a 78% reduction in risk of clinical progression. After 8 weeks of therapy, the predictability of the clinical outcome based on the HIV-1 RNA response was evident. The tendency for enduring viral suppression to be associated with decreased incidence of clinical outcomes was maintained at least through 60 weeks of antiretroviral therapy. Risk of HIV-1 disease progression was 0.8% (4/511) for subjects with a baseline HIV-1 RNA level less than 5 $\log_{10}$ copies/mL. Change in CD4+ cell count, initial ICD p24 antigen levels, and decrease in ICD p24 levels during antiretroviral therapy were less strongly associated with risk of clinical progression.

In HIV-1–infected subjects, measurement of plasma HIV-1 RNA at baseline, change in HIV-1 RNA from baseline, and CD4+ cell count at baseline were more closely associated with HIV-1 disease progression than CD4+ cell count change or ICD p24 measurements. In addition, moderate decreases of 0.5 $\log_{10}$ (68%) in viral burden in subjects receiving antiretroviral therapy resulted in clinically relevant reduced risk (two- to three-fold) of disease progression. There appeared to be a level of plasma HIV-1 RNA (5 $\log_{10}$ copies/mL) below which clinical progression was infrequent during an average 9.2 months of antiretroviral therapy.

d. Supportive Studies

Changes in genotype mutation, phenotype sensitivity to delavirdine (IC_{50}), and HIV-1 syncytia-inducing phenotype, were evaluated in the supportive studies (Protocols M/3331/0003, M/3331/0007, M/3331/0013A, M/3331/0018, and M/3331/0023).

In these delavirdine supportive clinical studies, evidence of marked or significant initial increases in CD4 and reduction in viral burden has been demonstrated in delavirdine monotherapy or combination therapy with one or two nucleoside RTIs in a diverse population of HIV-1–infected patients. There were trends of better surrogate marker responses in patients treated with ≥ 900 mg daily doses of delavirdine in combination therapy. Surrogate marker responses of CD4 and viral burden are of greater magnitude and duration with combination therapy, in which delavirdine triple-therapy was better than delavirdine double-therapy, which was better than delavirdine monotherapy. This result appears to be associated with synergy of delavirdine with nucleoside RTIs as evidenced by greater and more durable surrogate marker responses compared to single or dual nucleoside RTI therapy in comparable patient populations. In addition, combination therapy of DLV+ZDV appears to delay emergence of genotypic changes and decreased susceptibility to delavirdine compared to delavirdine monotherapy. This effect is more pronounced in zidovudine-naive or zidovudine-sensitive patients receiving DLV+ZDV combination therapy than zidovudine-experienced or zidovudine-resistant patients. Patients that are naive to antiretroviral therapy or who are sensitive to zidovudine at initiation of delavirdine therapy tend to have greater and more durable responses than in zidovudine-experienced or zidovudine-resistant patients. In addition, a majority of patients examined have increased sensitivity to zidovudine during DLV+ZDV combination therapy. The demonstrated viral synergy and delay of emergence of resistance to delavirdine and increased sensitivity to zidovudine during DLV+ZDV therapy likely contribute to the increased effectiveness of this combination therapy compared to zidovudine or delavirdine monotherapy.

e. Genotypic and Phenotypic Analysis of Antiviral Resistance

In vitro analysis of patient isolates on the supportive clinical trials described above revealed that, with delavirdine therapy, the predominant RT substitution was K103N. This substitution can cause reduced viral susceptibility to delavirdine but isolates with this substitution alone have been shown to remain partially sensitive to the drug. The high plasma concentrations achieved with the 400-mg tid delavirdine dose suggest that, even with the K103N substitution, sufficient viral replication may be inhibited to allow some continued benefit from delavirdine therapy. In addition to the predominant K103N, a spectrum of other substitutions was identified including K103T, Y181C, P236L, K103N + Y181C, and K103N + P236L. Delavirdine monotherapy and combination delavirdine plus didanosine therapy resulted in patient isolates with more dual substitutions (K103N + Y181C and K103N + P236L) than delavirdine plus zidovudine therapy. Treatment with delavirdine and zidovudine in the Protocols M/3331/0003 and M/3331/0013A prevented the emergence of the Y181C substitution for at least 6 months in 98% (55/56) of the patients. In Protocol M/3331/0003, there was an average 86-fold increase in the zidovudine sensitivity of all patient isolates after 24 weeks on DLV+ZDV combination therapy. This resensitization was seen in 22 of 24 patients. In addition, in Protocol M/3331/0013A, 33% (5/15) patients had varying degrees of increasing sensitivity to zidovudine over 12 weeks of DLV+ZDV combination therapy. The increase in sensitivity to zidovudine experienced by formerly ZDV-resistant patients suggests that delavirdine substitutions may have a positive influence on the phenotypic effects usually associated with the zidovudine mutations.

In conclusion, the majority of patients on delavirdine trials have had viral isolates with IC50 values below the median trough drug concentrations achieved with the 400-mg tid dose regimen for at least 24 weeks. Although the relative abundance of a few substitutions has increased in the patient isolates, zidovudine susceptibility and CD4 cell count have also increased in patients on this dosing regimen. Moreover, significant and sustained diminution in plasma viral burden has also been demonstrated with the DLV+ZDV combination therapy. Finally, due to the complex interactions between one substitution and others, multiple drugs in combination may be able to maintain clinical benefit despite the emergence of mutations specific to any of the individual agents.

3. Safety Summary

a. Introduction

1) *Scope of the Clinical Program*

Over 2700 subjects participated in the 21 completed/ongoing studies that comprise this NDA. Table H.5 is a summary of the number of patients who have participated in each of the different types of studies according to the study medication administered.

Table H.5. Summary of Patient Participation

Population	Number of Studies	Number of Patients/Subjects Dosed			
		Placebo	Active Control	Delavirdine	Total
Normal Volunteers	6	55[A]	–	150[A]	158[A]
Upjohn Company Supporting Studies in HIV+ Individuals	12	–	41	517	558
ACTG Supporting Study in HIV+ Individuals	1	–	29	106[B]	115
Combination Therapy in HIV+ Individuals	2	–	735	1148	1883
Total	21	55	805	1921	2714

[A] 48 placebo-treated patients crossed over to also receive DLV
[B] 20 active control-treated patients received DLV when enrolled in Protocol M/3331/0023

2) *Organization of the Safety Data*

The 21 studies that comprise this NDA have been grouped primarily by subject type and study design. Discussions of safety for each group of studies include serious medical events, discontinuations, and deaths at a minimum. The first group discussed are the studies conducted in normal volunteers. The next group of studies are the supportive studies in HIV-1+ individuals. The study designs in this group are diverse and include 12 studies conducted by The Upjohn Company and one conducted by the

ACTG. Data from the 12 conducted by The Upjohn Company are summarized together based on the daily dose administered to each patient (≤900 mg, >900 mg, or comparator). The ACTG study is discussed alone. The final group discussed consists of the two large efficacy, safety, and pharmacokinetic studies (Protocols M/3331/0017 and M/3331/0021). Each of these is treated separately because different drugs were co-administered with delavirdine, and in different patient populations at different stages of disease progression. Topics of special discussion include correlation of medical event data with plasma concentration of delavirdine, development of rash, and effect on liver function.

b. Summary of Safety in Healthy Volunteers

In healthy volunteers with delavirdine doses of ≤900 mg/day, there have been no deaths, serious medical events, or significant laboratory abnormalities. A 1.3% incidence of a mild skin rash was reported.

c. Summary of Safety in Supportive Studies in HIV-1+ Patients

Examination of serious medical events and laboratory shift frequencies shows that delavirdine was well tolerated by the patients in these 12 diverse supportive clinical studies. Serious medical events reported by 1% or more of the patients treated with delavirdine were limited to fever, Kaposi's sarcoma, depressive symptoms, and pneumonia. Of these events, the investigators considered only fever to be related to study drug treatment. Nine deaths were reported; only one of these occurred during treatment with delavirdine or within 30 days following the completion of delavirdine therapy. None of the deaths was considered to be related to delavirdine treatment. There was no apparent relationship between sex and the types and frequencies of medical events. Data on race were limited; however, it is possible that the frequency of events was greater in white patients than in black patients.

d. Summary of Safety in Supportive Monotherapy Study in HIV-1+ Patients

Delavirdine was well tolerated in Protocol M/3331/0018 (ACTG 260), with the exception of skin rash, a known side effect of delavirdine treatment. Drug-related skin rash occurred in 30 of the 86 delavirdine-treated patients (34.9%). There were no serious safety concerns. Of the patients who developed skin rash, approximately 90% could be dosed through the rash or successfully rechallenged.

e. Summary of Safety in the Pivotal Studies

1) *Protocol M/3331/0017*

Delavirdine, in combination with didanosine, was well-tolerated in this study. The overall frequency of medical events was similar between treatment groups; however, a significantly higher proportion of the DLV+ddI-treated patients experienced drug-related medical events than ddI-treated patients; the drug-related medical events reported more frequently by the DLV+ddI-treated patients included (in decreasing order of frequency) rash, maculopapular rash, pruritus, insomnia, change in dreams, tingling, and arthralgia. The frequencies of serious medical events and Grade 3 or 4 medical events were similar between the two treatment groups. Total serious medical

events were fewer (159) in the DLV+ddI treatment group compared with the group treated with ddI (184) alone. During the reporting period for this interim report, 42 deaths occurred, none of which was attributed to study medication. Thirty-six of the deaths occurred more than 30 days after discontinuation of study medication.

Patients who received DLV+ddI were significantly more likely to discontinue treatment because of a medical event than those who received ddI; rash and maculopapular rash were the causes of discontinuation that occurred significantly more often in patients treated with DLV+ddI (p-value ≤ 0.05).

There was no clinically meaningful difference in the types or frequencies of medical events reported by race or sex. However, the frequency of skin rash was lower in black patients who received DLV+ddI, which may have been due to the difficulty in visualizing the rash on dark skin. Women reported a higher frequency of drug-related and Grade 3 or 4 medical events than men in both treatment groups, but there was no significant difference in serious or drug-related Grade 3 or 4 medical events by sex or treatment group.

Of the drug-related medical events that were reported by 2 or more patients and which showed a statistical difference between treatment groups, abnormal stools, leg cramps, insomnia, change in dreams, rash, and pruritus were reported somewhat more frequently in patients with delavirdine plasma concentrations >7.5 μM than in patients with delavirdine plasma concentrations >0 but ≤7.5 μM.

For all treatment-emergent skin rash and drug-related skin rash, there was a significant increase in the occurrence of skin rash in the DLV+ddI treatment group compared with patients treated with ddI alone. The incidence of all treatment-emergent skin rash (rash, maculopapular rash, and urticaria) was 25% in ddI-treated patients and 42% in DLV+ddI-treated patients; the incidence of drug-related skin rash was 13% and 29%, respectively. The skin rash was usually a diffuse, maculopapular rash that occurred in the first four weeks of therapy (median time ~ 14 days) unless study medication was interrupted. This time-of-onset of skin rash is approximately 3 to 4 days longer than that observed in other delavirdine trials and is likely due to the two-week dose-escalation of delavirdine that preceded initiation of the full 1200-mg daily doses that were administered in this protocol.

The frequency of all treatment-emergent skin rash and drug-related skin rash increased in both treatment groups in proportion to decreases in CD4 cell counts. In the ddI treatment group, the incidence of all treatment-emergent skin rash was 32% in patients with baseline CD4 cell counts of <50 cells/mm^3, 28% in patients with CD4 cells counts of 50-149 cells/mm^3, and 19% in patients with CD4 cell counts of 150-300 cells/mm^3 compared with 57%, 45%, and 31%, respectively, in patients treated with DLV+ddI. The increase in the incidence of skin rash in patients with lower CD4 cell counts in both treatment groups may be related to increased immune dysfunction and/or increased frequency of administration of noninvestigational concomitant medications. The incidence of all types of skin rash, including drug hypersensitivity reactions, increases with HIV-1 disease progression and the concurrent decline in CD4 cell count **[9-16]**.

The overall frequency of skin rash was similar in the DLV+ddI-treated patients whose delavirdine plasma concentrations were >7.5 μM (40.4%) and in patients whose delavirdine plasma concentrations were >0 but ≤7.5 μM (40.6%); but somewhat higher for drug-related skin rash (incidence was 29% and 23%, respectively). However, the inverse relationship between CD4 cell count and rash frequency appears to hold for all treatment groups.

There were no significant differences between the ddI and DLV+ddI treatment groups in the incidence of Grade 3/4 skin rash, all serious treatment-emergent skin rash, or serious drug-related skin rash. Only 9 (1.8%) of 508 patients in the ddI treatment group and 17 (3.4%) of 499 patients in the DLV+ddI treatment group reported Grade 3/4 rash. Overall, only 3 (0.6%) of 499 patients in the DLV+ddI treatment group reported a serious skin rash, with complete resolution of all symptoms within 1 to 2 weeks. For serious drug-related skin rash, 1 (1.5%) of 65 patients in the ddI treatment group and 3 (2.1%) of 145 patients in the DLV+ddI treatment group reported a serious rash.

Of 208 patients in the DLV+ddI treatment group with skin rash, 26 (12.5%) discontinued treatment. Most patients (>85%) were dosed through a rash episode or were successfully rechallenged after the skin rash disappeared following interruption of therapy.

There were no clinically meaningful differences between treatment groups in vital signs measured during the study.

Laboratory safety measurements obtained in the study showed no clinically important difference between treatment groups. Serious Grade 3 or 4 laboratory abnormalities were also similar between treatment groups, and where information was available, were no more frequent than those reported in the Videx® labeling. It is possible that there may be a greater frequency of Grade 3 or 4 increases in prothrombin time in the DLV+ddI group than in the ddI group; however, the total number of such increases was small.

2) *Protocol M/3331/0021*

Delavirdine was well tolerated in this study. The overall frequency of medical events was similar for patients who received ZDV alone and for patients who received DLV+ZDV at doses of 200, 300, or 400 mg tid. No statistically significant differences were observed between treatment groups in the proportion of patients who experienced drug-related medical events, medical events of Grade 3 or 4, or serious medical events. Likewise, no statistically significant differences were observed between treatment groups in the proportion of patients who discontinued due to medical events.

Of the medical events the investigators considered to be drug related, only rash was reported significantly more often in patients treated with delavirdine. Nausea was the reason for study discontinuation significantly more often among patients who received DLV+ZDV at 300 or 400 mg than for patients who received ZDV alone. Elevated ALT/SGPT was the reason for discontinuation significantly more frequently in patients who received ZDV alone.

The overall incidence of treatment-emergent skin rash was 19% in the ZDV treatment group and 33% in the combined DLV+ZDV treatment groups. For drug-related skin rash, the incidence was 10% for the ZDV treatment group and 24% in the combined DLV+ZDV groups. The frequency of the treatment-emergent skin rash in all treatment groups was greater for patients with baseline CD4 cell counts less than 300 cells/mm^3 than those with baseline CD4 cell counts greater than 300 cells/mm^3 (35% versus 26%, treatment-emergent, and 26% versus 18%, drug-related, respectively). The increased incidence of skin rash in patients with lower CD4 cell counts may be related to increased immune dysfunction and/or increased frequency of concomitant medications.

In treatment-emergent and drug-related skin rashes, there was a significant difference in incidence of skin rash between the ZDV treatment group and any of the DLV+ZDV treatment groups. There was a trend of increased incidence of skin rash with the 400-mg DLV+ZDV group versus the lower dose groups, but this was not significantly different, except in subgroup patients with CD4 cell counts less than 300 cells/mm^3 at baseline. There was no significant difference in incidence of grade 3/4 or serious treatment-emergent or drug-related skin rashes between the ZDV treatment group and any of the DLV+ZDV treatment groups. There was only 1 serious drug-related skin rash (0.6%) reported for all the patients (160) in any of the DLV+ZDV treatment groups reporting a drug-related skin rash. The skin rash is usually a diffuse, erythematous maculopapular rash that occurs in the first three weeks of therapy with little frequency later, unless drug interruption occurs. Most patients (>85%) can be dosed through or successfully rechallenged after rash disappears as only 11 (6.9%) of 160 patients in DLV+ZDV treatment groups discontinued due to skin rash.

There were no clinically meaningful differences between treatment groups in vital signs measured throughout the study.

Significant decreases occurred in red blood cells, hemoglobin, and hematocrit during the study. These changes were probably due primarily to ZDV, but a small additional effect due to delavirdine could not be excluded; there was no increase in the frequency of Grade 3 or 4 anemia in patients who received DLV+ZDV as compared with those who received ZDV alone. No evidence of an effect of delavirdine on any liver function tests was observed. There appeared to be a very small effect of delavirdine on serum bilirubin and creatinine, but the mean changes in these measurements that occurred during treatment were very small.

Overall, the three delavirdine plus zidovudine combination therapies were very well-tolerated in over 600 HIV-1–infected patients for up to 68 weeks. A diffuse maculopapular drug-related skin rash usually of mild-to-moderate intensity occurred in about 24% of patients on the three delavirdine combination therapies. Although an increased incidence of skin rash was noted at the 400-mg delavirdine dose level, there was no increase observed in incidence of grade 3/4 or serious drug-related skin rashes compared with those seen in the ZDV monotherapy group. No other medical events or grade 3/4 lab abnormalities occurred with significantly greater frequency in the delavirdine dose groups compared with the ZDV monotherapy group. In addition, no other significant correlation was noted of a higher delavirdine dose or concentration with greater incidence of a medical event or a grade 3/4 lab abnormality. Indeed, there were fewer total serious medical events observed in the delavirdine combination

therapy and a lower incidence of some medical events. Delavirdine administered at 300-mg three times daily or 400-mg three times daily combined with ZDV has an excellent safety profile.

f. Summary of Data on Patients with High Delavirdine Concentrations

Twenty-seven patients from Protocols M/3331/0017, M/3331/0021, M/3331/0013A, and M/3331/0003 had blood levels ≥60 μM or ≥75 μM, as identified by routine population pharmacokinetics. Ninety-six percent (26/27) of the patients reported treatment-emergent medical events. In blinded Protocols M/3331/0017 and M/3331/0021, 78% of subjects with delavirdine ≥60 μM reported drug-related medical events during their participation in the study. Two of the three patients in Protocol M/3331/0013A had asymptomatic grade 3-4 increases in AST and ALT, but were associated with infectious epididymitis and bacterial pneumonia and other non-investigational agents known to be hepatotoxic. The causality of these increased LFTs was unknown. There were two patients with plasma delavirdine concentrations ≥60 μM in Protocols M/3331/0017 and M/3331/0021 with drug-related ALT ≥ grade 3, blocking their participation in the trial. These data suggest that reversible elevations in hepatic transaminases may be associated with trough levels of delavirdine ≥60 μM and other ongoing medical events such as infections. Only one serious drug-related medical event, a Grade 3 skin rash, was reported among all patients described with delavirdine ≥60 μM. The Drug Safety Monitoring Board for Protocols M/3331/0017 and M/3331/0021 reviewed 8 patients with elevated delavirdine concentrations and concluded no serious events and no clear pattern of minor medical events correlated with delavirdine concentration ≥75 μM. The overall incidence and descriptions of medical events seen in patients with delavirdine concentrations ≥60 μM appears comparable to that observed in all patients participating in delavirdine clinical trials.

g. Drug-Drug Interactions

When coadministered with either fluconazole, clarithromycin, or ketoconazole, each of which is a known or suspected inhibitor of CYP3A, no clinically important effect on the steady-state pharmacokinetics of delavirdine is observed **[21-23]**. In contrast, the coadministration of rifampin, rifabutin, phenytoin, phenobarbital, and/or carbamazepine, all known inducers of CYP3A, results in clinically important reductions in plasma delavirdine concentrations **[23-25]**. These results indicate that delavirdine alone substantially reduces CYP3A activity such that concurrent administration of other CYP3A inhibitors does not further alter the pharmacokinetics of delavirdine. However, concurrent administration of known CYP3A inducers appears to overcome delavirdine-associated CYP3A inhibition such that delavirdine clearance is substantially increased and plasma delavirdine concentrations consequently reduced. The interaction of delavirdine with known inducers and inhibitors of CYP3A was studied in Protocols M/3331/0017 and M/3331/0021. The limited drug interaction data from these two studies suggests that various inhibitors of cytochrome P450 do not have a clinically important effect on the pharmacokinetics of delavirdine. These findings are in general agreement with the results of delavirdine interaction studies with fluconazole (Protocol M/3331/0016) and clarithromycin (Protocol M/3331/0029). The extremely limited data from this study regarding the interaction of delavirdine with phenytoin, carbamazepine, or phenobarbital are also consistent with the results of previous drug interaction studies with rifabutin and rifampin, inducers of drug

metabolism (Protocols M/3331/0025 and M/3331/0028), and suggest that the concomitant use of delavirdine mesylate with inducers of CYP3A should be discouraged.

h. Drug-Demographic Interactions

There were no clinically significant differences between races or sex in serious medical event rates or Grade 3 or 4 laboratory abnormalities.

i. Drug-Disease Interactions

1) *Hepatic Transaminase Elevation and Pretreatment Hepatitis B and C*

Patients with prior history of hepatitis B and/or C have an increased incidence of developing Grade 3/4 increase in AST or ALT during participation in either Protocol M/3331/0017 or M/3331/0021. Patients with prior history of hepatitis B and/or C accounted for 44% and 67% of all grade 3/4 elevations in AST or ALT in Protocols M/3331/0017 and M/3331/0021, respectively, though only accounting for about 20% of the population in each protocol. In Protocol M/3331/0017, the incidence of treatment-emergent and drug-related grade 3/4 increase in AST or ALT are comparable between treatment groups (5.3%/4.3% vs 4.0%/3.6%) but somewhat higher in the DLV+ddI group in a proportion similar to the increased number of hepatic B or C patients in the DLV+ddI group (22%) compared to ddI alone (18%). In Protocol M/3331/0021, the incidence of Grade 3/4 AST or ALT in all three DLV+ZDV doses is consistently 2 to 3-fold lower than the ZDV group for all patients and those with prior history of viral hepatitis. In Protocol M/3331/0021, there is a trend of lower incidence of grade 3/4 AST or ALT in patients with prior viral hepatitis administered higher doses of delavirdine, in particular the 400-mg delavirdine dose. In other supporting delavirdine clinical trials with HIV-1+ patients, the overall incidence of Grade 3/4 ALT of 4.3% and 3.9% in patients with >900 mg or ≤900 mg daily dose, respectively, is comparable to what is seen in Protocols M/3331/0017 and M/3331/0021 with ZDV or ddI monotherapy (4% to 5%).

In Protocol M/3331/0018 (ACTG 260), 2 of 86 patients (2.3%) on delavirdine monotherapy, at doses often higher (up to 850 mg tid) than in the previously described trials, had Grade 3 ALT elevations that were transient and reversible. Both patients were able to continue in the trial without recurrence of elevated liver function tests. Overall, it appears that delavirdine in combination with ZDV, ddI, ZDV+ddI, or ZDV+ddC does not increase the risk of developing increased hepatic transaminase and may be associated with a decreased incidence in patients with prior hepatitis B or C taking ZDV plus delavirdine 400 mg tid.

2) *Rash*

In studies with delavirdine monotherapy or combination therapy with nucleoside RTIs, skin rashes were the primary adverse event noted. There is sufficient evidence, that in addition to the expected rashes in the HIV-1 population, a unique rash with some characteristic clinical signs is found with exposure to delavirdine. The rash has not been fully characterized with regards to its pathophysiology, pharmacogenesis, pathology, and epidemiology. However, clinical experience and data collected during

the above-noted studies suggest that the following description delineates the broad outlines of the rash.

The characteristic skin eruption seen with delavirdine therapy is a diffuse, erythematous maculopapular rash that occurs in the first three weeks of therapy. The median time to onset following initiation of therapy is 11 days. The rash is seen in 30% to 40% of exposed subjects and appears to be both dose-dependent and related to the CD4 count. The intensity of the eruption and its extent of distribution varies from patient to patient. In patients with darker skin the rash is not as noticeable as in their lighter-skinned counterparts. At times, the rash occurs after restarting the drug following a temporary interruption of treatment with delavirdine. The rash's morphological description is an erythematous macule or maculo-papule of 2 to 5 mm in diameter. Confluency of macules leading to a more diffuse erythematous picture occurs, more often on the head than elsewhere. The distribution of the rash is mainly cephalic with decreasing intensity of the lesions on the neck and shoulders, and from there, progressively less on the rest of the trunk and limbs. The suffused red ears and nose can be a particularly noticeable aspect of the dermatological picture. Swelling of the face may occur, primarily involving the lips. Fewer lesions are seen on the palms and soles. In the more severe cases mucosal involvement with injection of lips, oral mucosa, and conjunctiva may be seen. The degree of pruritus varies from patient to patient. Most patients (more than 85%) can be dosed through or successfully rechallenged after rash disappears. In a few cases when the 'typical' rash was biopsied, the pathology shows mild infiltration of predominately lymphocytes with a few neutrophils and eosinophils around the superficial vessels with no obvious interface dermatitis or epidermal changes. Occurrence of a delavirdine related rash after one month of therapy is uncommon unless prolonged interruption of delavirdine dosing occurs.

Skin rash is a common side effect noted in clinical trials with HIV+ patients that increases in frequency with disease progression and decreasing CD4 count **[9-20]**. The exact mechanism of the delavirdine rash is unclear. One can draw some insight from the experience with trimethoprim/sulfamethoxazole and other drug hypersensitivity reactions seen in HIV-1 infected patients **[13-16,18-20]**.
Trimethoprim/sulfamethoxazole, the preferred therapy for PCP, is strongly associated with skin disorders; over 30% of exposed patients develop a skin eruption **[19,26]**. It has been postulated that the mechanism of such reactions is the build- up of hydroxylamine metabolites of sulfamethoxazole because of relative glutathione deficiency in HIV-infected individuals **[27,28]**. Given that the mesylate salt contains a sulfone moiety, this mechanism could be plausible for delavirdine also.
Sulfamethoxazole-substituted human serum proteins occur after sulfonamide undergoes oxidative metabolism to yield reactive metabolites that haptenate serum proteins **[20]**. Relationship between haptanization of serum proteins and hypersensitivity reactions needs further study. It has also been postulated that immunodysregulation by HIV-1 infection enhances CD8 T-cell sensitivity to drugs allowing hypersensitivity to develop **[16]**. Drug specific IgE has been postulated to mediate some hypersensitivity reactions in HIV-1 patients **[16]**. In healthy normal volunteers treated with daily doses of up to 900 mg of delavirdine, mild skin rash was rare, occurring in only 1.3% (2/150) subjects; however, recent experience with a 1200-mg daily dose indicates an increased incidence of 36.6% (11/30 subjects) of Grade 1 or 2 skin rash, often pruritic in nature. Skin rash has has not been observed in

animal studies with delavirdine. Whatever the underlying explanation for the mechanism of the delavirdine rash is, the rash does appear to be specific in its characteristic time to onset, its emphasis for cephalic distribution, and its variable degree of intensity in patients on the same dosage.

It is now known that even if the rash re-appears on rechallenge with delavirdine, it is possible to treat through the skin rash in the majority of affected subjects. Therefore the management of this side effect is to continue to treat the subject without dose adjustment. Under these circumstances, over 90% of exposed subjects are able to continue on therapy without interruption **[29]**. In studies of another NNRTI, nevirapine, it was reported that the incidence of skin reactions was lower in subjects exposed to lower doses for 2 weeks following initiation of therapy **[30]**.

Drug-related skin rash of a serious nature was infrequent, occurring in 4/1148 (0.34%) patients in the pivotal, well-controlled double-blind trials in HIV-1 patients treated with delavirdine combination therapy with CD4 cell counts of 0-500 cells/mm^3. Whether these events were directly related to the delavirdine only or to another concomitant drug or disease can be difficult to ascertain from the data in the studies compiled in this report. The equal frequency of the serious and ACTG Grades 3 and 4 reports in the delavirdine and comparator groups suggest that the more problematic cutaneous disorders are not predominant finding in the use of delavirdine. There is a trend of increased incidence of skin rash with higher doses (≥400 mg tid) and the higher concentrations associated with these doses. However, there is no significant increased incidence of ACTG Grade 3 or 4 skin rash or serious medical events associated with higher doses.

In summary, the skin eruption associated with delavirdine therapy appears to be non-serious and readily recognizable, and usually occurs in the first 3-4 weeks of therapy. Its characteristic time to onset and predominately cephalic distribution makes for an easy diagnosis and its benign course allows for decisive ongoing therapy.

j. Overall Summary of Delavirdine Safety Data

Delavirdine, in monotherapy or in combination therapy with nucleoside reverse transcriptase inhibitors, administered orally at daily doses of up to 400 mg tid in more than 150 healthy volunteers and at doses of up to 850 mg tid in 1771 HIV-1–infected patients for up to 3 years, has been very well tolerated.

In healthy volunteers with delavirdine doses of ≤900 mg/day, there have been no serious medical events or significant laboratory abnormalities. A 1.3% incidence of a mild skin rash was reported.

Safety information from over 500 HIV-1–infected patients in supportive delavirdine clinical studies with CD4 counts ranging from 2 to 1509 cells/mm^3 and different stages of disease progression had a 3% incidence of drug-related serious medical events during their participation in the delavirdine studies. Fever and/or chills, reported in fewer than 1% of the patients, was the most common drug-related serious medical event. Grade 3 or 4 elevations in lipase (6%), AST/ALT (4%), and amylase (2%) were the most common laboratory abnormalities noted in these studies.

In an open-label concentration-controlled delavirdine monotherapy study with doses of 200 to 850 mg tid (Protocol M/3331/0018, ACTG 260) the most common drug-related serious medical events (ACTG-defined) were skin rashes (maculopapular, urticarial, and erythematous). Drug-related rashes (Grades 1, 2, 3, or 4) were reported in 35% (30/86) of the delavirdine patients.

The pivotal studies (Protocols M/3331/0017 and M/3331/0021) involved 1148 HIV-1 patients with CD4 counts between 0 to 500 cells/mm^3 at enrollment. In these studies, the combination of delavirdine (400 mg tid+ddI), or delavirdine (200, 300, or 400 mg tid) + ZDV was well tolerated. In Protocol M/3331/0017, the overall frequency of medical events was similar between treatment groups; however, a significantly higher proportion of the DLV+ddI-treated patients experienced drug-related medical events than ddI-treated patients. The frequencies of serious medical events and Grade 3 or 4 medical events were similar between the two treatment groups. Total serious medical events were fewer (159) in the DLV+ddI treatment group compared with the group treated with ddI (184) alone. During the reporting period for this interim report, 42 deaths occurred, none of which was attributed to study medication. Thirty-six of the deaths occurred more than 30 days after discontinuation of study medication. In Protocol M/3331/0021, the overall frequency of medical events was similar for patients who received ZDV alone and for patients who received DLV+ZDV at doses of 200, 300, or 400 mg tid. No statistically significant differences were observed between treatment groups in the proportion of patients who experienced drug-related medical events, medical events of Grade 3 or 4, or serious medical events. Likewise, no statistically significant differences were observed between treatment groups in the proportion of patients who discontinued due to medical events. There were fewer total serious medical events in the delavirdine dose groups (18 at 200 mg, 19 at 300 mg, and 14 at 400 mg) than there were in the ZDV (30) group.

The statistically significant differences between treatment groups for treatment-emergent, drug-related, and serious medical events, as well as discontinuations due to medical events, both by and within body systems, are summarized in Table H.6 for Protocol M/3331/0017 and in Tables H.7 through H.10 for Protocol M/3331/0021.

There were no significant differences between treatment groups in the frequency of serious medical events within body systems for either protocol, except an increased frequency of dehydration in the ZDV group in Protocol M/3331/0021.

In Protocol M/3331/0017, the DLV+ddI group had a significantly increased frequency of the following drug-related medical events (in decreasing order of frequency): rash, maculopapular rash, pruritus, insomnia, change in dreams, tingling, and arthralgia. In Protocol M/3331/0021, the only significantly increased incidence of drug-related medical events for DLV+ZDV patients was rash, occurring in each of the three delavirdine treatment groups. Therefore, skin rash was the only treatment-emergent drug-related medical event that had a significantly increased incidence in the delavirdine combination therapy group in both protocols. For the treatment groups not receiving delavirdine, there were significantly increased incidence of drug-related dry skin rash (ddI group, Protocol M/3331/0017); and constipation, amnesia, and pharyngitis (ZDV group, Protocol M/3331/0021).

Statistically significant increases in discontinuation frequencies were seen in delavirdine patients for rash (Protocol M/3331/0017) and nausea (Protocol M/3331/0021), and in ZDV patients for elevated SGPT (Protocol M/3331/0021).

Across all delavirdine clinical trials it appears that skin rash is the primary consistent medical event associated with delavirdine monotherapy or combination therapy. (In some trials increased frequencies of nausea and pruritus associated with skin rash have accompanied delavirdine dosing.) There were no significant Grade 3 or 4 laboratory abnormalities consistently associated with delavirdine therapy. Delavirdine doses of up to 850 mg tid and trough concentrations ≥60 μM in 27 patients for up to 1 to 2 months have been well tolerated without serious medical events. There were no clinically significant differences between races or sex in serious medical event rates or Grade 3 or 4 laboratory abnormalities. Systemic vasculitis, observed in dogs, has not been reported in any delavirdine-treated patients or observed upon autopsy.

Drug-related skin rash of a serious nature was infrequent, occurring in 4/1148 (0.34%) patients in the pivotal, well-controlled double-blind trials in HIV-1 patients treated with delavirdine combination therapy with CD4 cell counts of 0-500 cells/mm^3. Whether these events were directly related to the delavirdine only or to another concomitant drug or disease can be difficult to ascertain from the data in the studies compiled in this report. The equal frequency of the serious and ACTG Grades 3 and 4 reports in the delavirdine and comparator groups suggest that the more problematic cutaneous disorders are not predominant finding in the use of delavirdine. There is a trend of increased incidence of skin rash with higher doses (≥400 mg tid) and the higher concentrations associated with these doses. However, there is no significant increased incidence of ACTG Grade 3 or 4 skin rash or serious medical events associated with higher doses.

Delavirdine is primarily metabolized by cytochrome P450 3A. Drug interaction data from Protocols M/3331/0017 and M/3331/0021 suggest that various inhibitors of cytochrome P450 3A do not have clinically important effects on the pharmacokinetics of delavirdine. However, limited data on inducers of cytochrome P450 3A suggest that these drugs reduce delavirdine blood concentrations to varying degrees.

In summary, delavirdine mesylate has an excellent safety profile in both healthy normal volunteers and HIV-1-infected patients of various stages of disease progression and CD4 counts. Delavirdine is well tolerated, with a mild-to-moderate skin rash being the primary medical event associated with its use in monotherapy or combination therapy. The rash develops in 30% to 40% of patients in the first month of therapy and can be dosed through in over 85% of cases.

Table H.6. Statistically Significant Treatment-Emergent Medical Events - Protocol M/3331/0017

Statistically Significant Treatment-Emergent Frequencies of MEs - by Body System	
Musculo-Skeletal	0.0082
Respiratory	0.0184
Skin	<0.0001
Statistically Significant Treatment-Emergent Frequencies of MEs - within Body System	
Nervous System	
change in dreams	0.0017
tremor	0.0391
disorientation	0.0276
Skin	
rash	<0.0001
pruritus	0.0003
maculo-papular rash	0.0358
seborrhea	0.0399 (ddI)
dermatitis	0.0401 (ddI)
contact dermatitis	0.0260
hair loss	0.0340 (ddI)
Special Senses	
ear pain	0.0125
Urogenital	
infection urinary tract	0.0042
Statistically Significant Drug-Related Frequencies of MEs - by Body System	
Nervous	0.0496
Skin	<0.0001
Statistically Significant Drug-Related Frequencies of MEs - within Body System	
Digestive	
nausea	[0.0525]
Muscolo-Skeletal	
arthralgia (single and multiple joint)	0.0278
Nervous	
insomnia	0.0355
tingling	0.0390
change in dreams	0.0031
Skin	
rash	<0.0001
maculo-papular rash	0.0010
pruritus	0.0009
dry skin NAS	0.0274 (ddI)
Statistically Significant Discontinuation Frequencies due to MEs - by Body System	
Skin	0.0012
Statistically Significant Discontinuation Frequencies due to MEs	
Skin	
rash	0.0073
maculo-papular rash	0.0273
Statistically Significant Frequencies of Serious MEs - by Body System	
Musculo-Skeletal	0.0400

Source: Ref 1

Table H.7. Statistically Significant (p≤0.05) Treatment-Emergent Medical Events -- Protocol M/3331/0021

	ZDV N = 200		Delavirdine Treatment Group[a] 200 mg tid N = 199		300 mg tid N = 212		400 mg tid N = 206		p-Value
	n	p-Value[B]	n	p-Value	n	p-Value	n	p-Value	
By BODY SYSTEM									
Skin	87	NS[D]	103	NS	117	0.0177	119	0.0041	0.0258
Within BODY SYSTEM									
Body as a Whole									
Environmental allergy	9	NS	3	NS	3	NS	12	NS	0.0247
Cardiovascular									
Palpitation	1	NS	1	NS	0	NS	5	NS	0.0347
Digestive									
Disorder rectal (ZDV)[C]	9	NS	5	NS	2	0.0258	1	0.0085	0.0184
Disorder tongue (ZDV)	7	NS	1	0.0319	1	0.0268	1	0.0236	0.0093
Gingivitis	0	NS	5	0.0230	3	NS	0	NS	0.0271
Hepatomegaly (ZDV)	5	NS	0	0.0251	0	0.0209	1	NS	0.0081
Nervous									
Anxiety (ZDV)	22	NS	13	NS	8	0.0049	14	NS	0.0384
Somnolence	6	NS	0	0.0141	1	0.0480	6	NS	0.0216
Respiratory									
Wheezing	0	NS	1	NS	5	0.0295	0	NS	0.0141
Skin									
Rash	31	NS	47	0.0417	49	[0.0516]	65	0.0002	0.0026
Special Senses									
Taste perversion	0	NS	5	0.0239	7	0.0098	2	NS	0.0469
Urogenital									
Urinary frequency	3	NS	0	NS	7	NS	2	NS	0.0410

[a] All patients also received concurrent ZDV (200 mg tid).

[b] p-Value: DLV dose group compared with ZDV-treated group using Cochran Mantel Haenszel Test for general association with prior ZDV experience as strata.

[c] These medical events occurred with higher frequencies in the ZDV-treated groups.

[D] NS = not significant; p>0.05

Abbreviations: DLV = delavirdine mesylate, tid = three times daily, ZDV = zidovudine.

Source: Ref 2: Tables ME2-1A, ME3-1A, ME2-1B, ME3-1B.

Table H.8. Statistically Significant (p≤0.05) Drug-Related Treatment-Emergent Medical Events -- Protocol M/3331/0021

	ZDV N = 200		Delavirdine Treatment Group[a] 200 mg tid N = 199		300 mg tid N = 212		400 mg tid N = 206		
	n	p-Value[B]	n	p-Value	n	p-Value	n	p-Value	p-Value
By BODY SYSTEM									
Skin	35	NS[D]	54	0.0209	60	0.0093	72	0.0001	0.0013
Within BODY SYSTEM									
Digestive									
Constipation (ZDV)[C]	8	NS	3	NS	2	0.0440	0	0.0041	0.0102
Nervous									
Amnesia (ZDV)	5	NS	0	0.0251	1	NS	1	NS	0.0312
Respiratory									
Pharyngitis (ZDV)	6	NS	1	[0.0584]	0	0.0112	1	[0.0513]	0.0094
Skin									
Rash	13	NS	32	0.0025	33	0.0035	50	<0.0001	<0.0001

[a] All patients also received concurrent ZDV (200 mg tid).
[b] p-Value: DLV dose group compared with ZDV-treated group using Cochran Mantel Haenszel Test for general association with prior ZDV experience as strata.
[c] These medical events occurred with higher frequencies in the ZDV-treated groups.
[D] NS = not significant; p>0.05

Abbreviations: DLV = delavirdine mesylate, tid = three times daily, ZDV = zidovudine.
Source: Ref 2:Tables ME6-1A, ME7-1A, ME7-1B.

Table H.9. Statistically Significant (p≤0.05) Serious Medical Events -- Protocol M/3331/0021

	ZDV N = 200		Delavirdine Treatment Group[a] 200 mg tid N = 199		300 mg tid N = 212		400 mg tid N = 206		p-Value[B]
	n	p-Value	n	p-Value	n	p-Value	n	p-Value	
By BODY SYSTEM									
Metabolic and Nutritional (ZDV)[C]	4	NS[D]	0	0.0451	1	NS	0	0.0402	0.0294
Within BODY SYSTEM									
Metabolic and Nutritional									
Dehydration (ZDV)	3	NS	0	NS	0	NS	0	NS	0.0245

[a] All patients also received concurrent ZDV (200 mg tid).
[b] p-Value: DLV dose group compared with ZDV-treated group using Cochran Mantel Haenszel Test for general association with prior ZDV experience as strata.
[c] These medical events occurred with higher frequencies in the ZDV-treated groups.
[D] NS = not significant; p>0.05
Abbreviations: DLV = delavirdine mesylate, NS = not significant (p>0.05),tid = three times daily, ZDV = zidovudine.
Source: Ref 2:Tables ME9-1A, ME10-1A, ME10-1B.

Table H.10. Statistically Significant (p≤0.05) Drop-out due to Treatment-emergent Medical Events -- Protocol M/3331/0021

	ZDV N = 200		Delavirdine Treatment Group[a] 200 mg tid N = 199		300 mg tid N = 212		400 mg tid N = 206		p-Value
	n	p-Value[B]	n	p-Value	n	p-Value	n	p-Value	
By BODY SYSTEM									
Digestive	2	NS[d]	7	NS	14	0.0032	12	0.0076	0.0235
Within BODY SYSTEM									
Digestive									
Nausea	2	NS	5	NS	11	0.0148	11	0.0124	0.0465
Metabolic and Nutritional									
SGPT Elevated (ZDV)[C]	4	NS	0	0.0451	1	NS	0	0.0402	0.0294

[a] All patients also received concurrent ZDV (200 mg tid).
[b] p-Value: DLV dose group compared with ZDV-treated group using Cochran Mantel Haenszel Test for general association with prior ZDV experience as strata.
[c] These medical events occurred with higher frequencies in the ZDV-treated groups.
[d] NS = not significant; p>0.05
Abbreviations: DLV = delavirdine mesylate, tid = three times daily, ZDV = zidovudine.
Source: Ref 2: Tables ME16-1A, ME17-1A, ME16-1B.

4. References/Study Reports/Publications

1. Freimuth WW, Carberry PA, Wang Y, Edge BA, Daenzer CL, Mickelson MM, et al. Interim analysis: a double-blind, randomized study of delavirdine mesylate (U-90152S) in combination with didanosine (ddI) compared with ddI alone in HIV-1 infected individuals with CD4 counts of 300 cells/mm^3 or less (Protocol M/3331/0017). Technical Report 9156-96-003, 25 June 1996 .....

2. Freimuth WW, Wang Y, Carberry PA, Daenzer CL, Edge BA, Mickelson PM, et al. Interim report: a double-blind, randomized, dose-response study of three fixed doses of delavirdine mesylate (U-90152S) in combination with zidovudine (ZDV) versus ZDV alone in HIV-1 infected individuals with CD4 counts of 200-500/mm^3. (Protocol M/3331/0021). Technical Report 9156-96-004, 3 June 1996

3. Batts DH, Cox SR, Wathen LK, Peel BG, Hanover CK, Busboom SD, et al. Open-label, escalating multiple-dose study of the safety, tolerance, and pharmacokinetics of oral U-90152S in HIV-1 infected males and females with CD4 counts of 200 to 500/mm^3, who are maintained on a stable dose of AZT (M/3331/0003 [BC1003]). Upjohn Technical Report 7217-93-015, 10 June 1996 .....

4. Freimuth WW, Greenwald C, Daenzer CL, Cox SR, Wathen LK. Interim report: A randomized, dose-escalating multi-dose phase I/II trial of oral U-90152S compared to conventional combination nucleoside therapy in HIV-1 infected patients (Protocol M/3331/0007). Pharmacia & Upjohn Technical Report 9156-96-008, 1 July 1996. .....

5. Ward P, Cox SR, DeGeest G, Wathen LK, Nickens DJ, Knuth. A study of the tolerance and efficacy of combinations of U-90152S and nucleoside therapy in the treatment of HIV infected patients (24-week report; Protocol M/3331/0013A). Upjohn Technical Report 1340-96-006, June 19, 1996 .....

6. Freimuth WW, Peel BG, Nickens DJ. Delavirdine mesylate, phase I/II, randomized, comparator-controlled, dose-ranging, concentration-targeted, open-label trial of safety, tolerance and anti-HIV-1 activity (Protocol M/3331/0018 ACTG 260)—Interim analysis. Pharmacia & Upjohn, Inc. Technical Report 9156-96-010, 14 June 1996 .....

7. Wang Y, Greenwald C, Docsa SR, Freimuth WW. Optional, open-label, extended use delavirdine mesylate treatment In triple combination for HIV-1+ patients who participated in other delavirdine mesylate protocols (Protocol M/3331/0023). Technical Report 9156-96-005, 25 June 1996 .....

8. Demeter LM, Shafer RW, Para M, Morse G, Freimuth WW, Merigan T. Delavirdine (DLV) susceptibility of HIV-1 isolates obtained from patients (pts) receiving DLV monotherapy (ACTG 260). Fourth International Workshop HIV Drug Resistance; Sardinia, Italy. (Abstract 23) 6-9 July 1995 .....

9. Wong D, Shumack S. Managing HIV. Part 5: Treating secondary outcomes. 5.1 HIV and skin disease. MJA 1996;164:352-6 .....

10. Bason MM, Berger TG, Nesbitt LT. Pruritic papular eruption of HIV-disease. Int J Dermatol 1993;32(11):784-9 .....

11. Ray MC, Gately LE. Dermatologic manifestations of HIV infection and AIDS. Infect Dis Clin North Am 1994;8(3):583-605 .....

12. Zalla MJ, Su WPD, Fransway AF. Dermatologic manifestations of human immunodeficiency virus infection. Mayo Clin Proc 1992;67:1089-1108 .....

13. Coopman SA, Johnson RA, Platt R, Stern RS. Cutaneous disease and drug reactions in HIV infection. N Eng J Med 1993;328(23):1670-4 .....

14. Sadick NS, McNutt NS. Cutaneous hypersensitivity reactions in patients with AIDS. Inter J Dermatol 1993;32(9):621-7 .....

15. Harb GE, Jacobson MA. Human immunodeficiency virus (HIV) infection. Does it increase susceptibility to adverse drug reactions? Drug Safety 1993;9(1):1-8 .....

16. Carr A, Garsia R. Part 3: Mechanisms of disease. Managing HIV. 3.5 How HIV leads to hypersensitivity reactions. MJA 1996;164:227-9 .....

17. Roujeau JC, Stern RS. Severe adverse cutaneous reactions to drugs. N Engl J Med 1994;331(19):1272-85 .....

18. Bayard PJ, Berger TG, Jacobson MA. Drug hypersensitivity reactions and human immunodeficiency virus disease. J Acquir Immune Defic Syndr 1992;5:1237-57 .....

19. Federico G, del Forno A, del Borgo C, Ricci F, Tumbarello M, Fantoni M. Adverse reactions to sulphonamides in patients with HIV infection. J Chemother 1995;7(4):190-2 .....

20. Meekins CV, Sullivan TJ, Gruchalla RS. Immunochemical analysis of sulfonamide drug allergy: identification of sulfamethoxazole-substituted human serum proteins. J Allergy Clin Immunol 1994;94(6 part 1);1017-24 .....

21. Borin MT, Cox SR, Herman BD, Freimuth WW, Carel BJ, Carberry PA, et al. An evaluation of the effect of fluconazole on the steady-state pharmacokinetics of delavirdine mesylate (U-90152S) in HIV-positive patients (Protocol M/3331/0016). Upjohn Technical Report 7215-95-039, 30 November 1995 .....

22. Borin MT, Chambers JC, Cox SR, Freimuth WW, Carel BJ, Carberry PA, et al. An evaluation of the effect of clarithromycin on the steady-state pharmacokinetics of delavirdine mesylate (U-90152T) in HIV positive patients (Protocol M/3331/0029). Upjohn Technical Report 7215-95-054, 06 February 1996 .....

23. Knuth DW, Cox SR. A blinded pharmacokinetic report to the delavirdine drug safety monitoring board summarizing the therapeutic drug monitoring and pharmacokinetic evaluations through 31 December 1995. Upjohn Technical Report 7215-96-023, 29 March 1996 .....

24. Borin MT, Chambers JH, Freimuth WW, Carel BJ, Carberry PA, Driver MR. An evaluation of the effect of rifabutin on the steady-state pharmacokinetics of delavirdine mesylate (U-90152S) in HIV-positive patients (Protocol M/3331/0025). Upjohn Technical Report 7215-95-021, 06 October 1995 .....

25. Borin MT, Chambers JH, Freimuth WW, Carel BJ, Carberry PA. An evaluation of the effect of rifampin on the steady-state pharmacokinetics of delavirdine mesylate (U-90152S) in HIV-positive patients (Protocol M/3331/0028). Upjohn Technical Report 7215-95-043, 15 December 1995 .....

26. Toma E, Fournier S. Adverse reactions to co-trimoxazole in HIV infection. Lancet 1991;338:954 .....

27. van der Ven AJDM, Koopmans PP, Vree TB, van der Meer JWM. Adverse reactions to co-trimoxazole in HIV infection. Lancet 1991;338:431-3 .....

28. Staal FJT, Ela SW, Roeberer M, Anderson MT, Herzenberg LA, et al. Glutathione deficiency and human immunodeficiency virus infection. Lancet 1992;339:909-912 .....

29. The Upjohn Company, U-90152S Investigator's Brochure, July 1995

*Submitted to IND 41207, original submission dated 12/4/91; page 1/011. Updates provided in Amendment No. 112, 8/4/95; Amendment No. 114, 8/9/95; Amendment No. 115, 8/14/95.

30. Abrams D, Cotton D, Mayer K, editors. AIDS/HIV treatment directory. New York: American Foundation for AIDS Research (AmFAR), 1994;7(3):50-51 .....

I. BENEFIT/RISK ASSESSMENT

1. Introduction

Acquired Immune Deficiency Syndrome (AIDS), a condition first described in the early 1980s, is now the leading cause of death in males 25 to 45 years of age in the USA, surpassing the combined mortality of cancer and head injury in that population. As of the end of 1995, the Joint United Nations Program on HIV/AIDS (UNAIDS) estimated that some 20.1 million adults are living with HIV/AIDS, including over 11 million males and almost 9 million females. It is also estimated that over 6 million AIDS cases in adults and children have occurred worldwide since the epidemic began. UNAIDS foresees that this cumulative total will triple by the year 2000. Over 5 million adults and children are estimated to have died from AIDS so far [1].

Delavirdine mesylate (U-90152S/T, RESCRIPTOR tablets™, DLV) belongs to a class of compounds known as bisheteroarylpiperazines (BHAPs) that have shown activity in vitro against the reverse transcriptase (RT) of Human Immunodeficiency Virus-Type 1 (HIV-1) in several systems. All compounds in the class are highly specific inhibitors of HIV-1 reverse transcriptase, having no activity against the RT of HIV-2 or the other animal retroviruses **[2,3]**.

The effectiveness and safety of DLV for the treatment of HIV-1 infection were established in 21 completed and ongoing clinical studies in which over 1900 people received treatment with delavirdine mesylate. These studies have identified the following benefits and risks associated with the use of DLV.

2. Benefits

Delavirdine is a member of a new class of antiretroviral agents called non-nucleoside RTIs that have a different mechanism of action that targets the HIV-1 reverse transcriptase enzyme, which is essential for viral replication. Delavirdine has been shown to be active against HIV-1 clinical isolates that are resistant to ZDV, ddI, and ddC. Due to its different mechanism of action and unique binding site in the HIV-1 reverse transcriptase enzyme, it would likely be active against HIV-1 resistant to 3TC and d4T or the three licensed protease inhibitors. The correlation of surrogate markers (CD4, HIV-1 RNA, and ICDp24) with HIV-1 disease progression has been assessed in the two delavirdine pivotal trials involving over 1700 HIV-1–infected patients. Hazard regression analysis has shown that the best predictors of HIV-1 disease progression (new or recurring AIDS defining illness or death) in these populations are baseline CD4 and HIV-1 RNA as well as reduction in HIV-1 RNA following administration of antiretroviral therapy. Sustained average changes (DAVG) in reduction of HIV-1 RNA of 0.5 $\log_{10}$copies/mL with antiviral therapy were associated with a 2- to 3-fold risk of disease progression. CD4 changes during therapy have previously been shown to be predictive of clinical outcome, but in these pivotal studies change in CD4 or ICDp24 antigen levels were not as predictive of clinical outcome as HIV-1 RNA. The clinical significance of other surrogate markers such as cellular HIV-1 DNA levels are yet to be determined. In this application, the following attributes of delavirdine were presented:

Protocol M/3331/0021 comprised 852 HIV-1–infected male and female patients with an entry CD4 count of 200 to 500 cells/mm^3 who had ≤6 months of prior zidovudine therapy and were randomized to receive one of four therapies: zidovudine monotherapy (200 mg three times daily), or 200-mg, 300-mg, or 400-mg three time daily of delavirdine combined with zidovudine (200-mg three times daily). The 300-mg and 400-mg DLV+ZDV groups show statistically significant greater increases in CD4/CD4% counts and in greater reductions in viral burden as measured by HIV-1 RNA, HIV-1 DNA, ICD p24, and p24 levels for up to 60 weeks when compared with ZDV monotherapy. For all surrogate markers studied, there is evidence of both a sustained dose-response relationship and a concentration-response relationship. The results, in general, are comparable between the 300-mg and 400-mg DLV dose groups which usually have statistically significantly better surrogate marker responses than 200-mg DLV dose group, which has a limited duration of response. Analysis of results by the DLV trough concentrations achieved suggests that patients with concentrations of more than 1.5 µM have greater and more sustained surrogate marker responses. In addition, patients with trough levels greater than 7.5 µM often have incrementally greater responses than patients with lower DLV concentrations. The majority of patients in the 300-mg and 400-mg dose groups or in the 1.5 to 7.5 µM and more than 7.5 µM concentration groups have sustained reduction of HIV-1 RNA of 0.5 $\log_{10}$ copies/mL (68%) or more for 52 weeks.

Protocol M/3331/0017 comprised 1031 HIV-1–infected male and female patients with an entry CD4 count of 0 to 300 cells/mm^3 who were zidovudine-experienced and had ≤ 4 months of prior didanosine therapy and were randomized to receive one of two therapies: didanosine monotherapy (200 mg two times daily for patients >60 kg and 125 mg two times daily for patients <60 kg) or 400-mg three times daily of delavirdine combined with didanosine (200 mg twice daily or 125 mg twice daily adjusted by body weight). Patients who received 400 mg tid of delavirdine combined with didanosine (weight adjusted dose bid) show statistically significant greater increases in CD4/CD4% counts and in greater reductions in viral burden as measured by HIV-1 RNA, HIV-1 DNA, ICDp24 and p24 antigen levels for up to 24 weeks when compared with ddI monotherapy. There was evidence of a concentration-response relationship for CD4, HIV-1 RNA, and ICDp24 antigen surrogate markers. The majority of patients in the DLV+ddI group have sustained mean DAVG reduction of HIV-1 RNA ≥0.5 $\log_{10}$ copies/mL after week 4 to 60 weeks.

A mean DAVG reduction of ≥0.5 $\log_{10}$ copies/mL of HIV-1 RNA was found to be associated with a two-to-three fold reduction in the risk of disease progression in combined data from Protocols M/3331/0021 and M/3331/0017.

In delavirdine supportive clinical studies, evidence of marked or significant initial increases in CD4 and reduction in viral burden has been demonstrated in delavirdine monotherapy or combination therapy with one or two nucleoside RTIs in a diverse population of HIV-1–infected patients. There were trends of better surrogate marker responses in patients treated with ≥ 900 mg daily doses of delavirdine in combination therapy. Surrogate marker responses of CD4 and viral burden are of greater magnitude and duration with combination therapy, in which DLV triple-therapy was better than DLV double-therapy, which was better than DLV monotherapy. This result appears to be associated with synergy of DLV with nucleoside RTIs as evidenced by greater and more durable surrogate marker responses compared to single or dual

nucleoside RTI therapy in comparable patient populations. In addition, combination therapy of DLV+ZDV appear to delay emergence of genotypic changes and decreased susceptibility to DLV compared to DLV monotherapy. This effect is more pronounced in ZDV-naive or ZDV-sensitive patients receiving DLV+ZDV combination therapy than ZDV-experienced or ZDV-resistant patients. Patients that are antiretroviral naive or who are sensitive to ZDV at initiation of DLV therapy tend to have greater and more durable responses than in ZDV-experienced or ZDV-resistant patients. In addition, a majority of patients examined have increased sensitivity to ZDV during DLV+ZDV combination therapy. The demonstrated viral synergy and delay of emergence of resistance to DLV and increased sensitivity to ZDV during DLV+ZDV therapy likely contribute to the increased effectiveness of this combination therapy compared to ZDV or DLV monotherapy.

3. Risks

Across all delavirdine clinical trials it appears that skin rash is the primary consistent medical event associated with delavirdine monotherapy or combination therapy. (In some trials increased frequencies of nausea and pruritus associated with skin rash have accompanied delavirdine dosing.) There were no significant Grade 3 or 4 laboratory abnormalities consistently associated with delavirdine therapy. Delavirdine doses of up to 850 mg tid and trough concentrations ≥60 μM in 27 patients for up to 1 to 2 months have been well tolerated without serious drug-related medical events. There were no clinically significant differences between races or sex in serious medical event rates or Grade 3 or 4 laboratory abnormalities.

The characteristic skin eruption seen with delavirdine therapy is a diffuse, erythematous maculopapular rash that occurs usually in the first three weeks of therapy. The median time to onset following initiation of therapy is 11 days. The rash is seen in 30% to 40% of exposed subjects and appears to be both dose-dependent and related to the CD4 count. The intensity of the eruption and its extent of distribution varies from patient to patient. In patients with darker skin the rash is not as noticeable as in their lighter-skinned counterparts. At times, the rash occurs after restarting the drug following a temporary interruption of treatment with delavirdine. The rash's morphological description is an erythematous macule or maculo-papule of 2 to 5 mm in diameter. Confluency of macules leading to a more diffuse erythematous picture occurs, more often on the head than elsewhere. The distribution of the rash is mainly cephalic with decreasing intensity of the lesions on the neck and shoulders, and from there, progressively less on the rest of the trunk and limbs. The suffused red ears and nose can be a particularly noticeable aspect of the dermatological picture. Swelling of the face may occur, primarily involving the lips. Fewer lesions are seen on the palms and soles. In the more severe cases mucosal involvement with injection of lips, oral mucosa, and conjunctiva may be seen. The degree of pruritus varies from patient to patient. Most patients (more than 85%) can be dosed through or successfully rechallenged after rash disappears. In a few cases when the 'typical' rash was biopsied, the pathology shows mild infiltration of predominately lymphocytes with a few neutrophils and eosinophils around the superficial vessels with no obvious interface dermatitis or epidermal changes. Occurrence of a delavirdine related rash after one month of therapy is uncommon unless prolonged interruption of delavirdine dosing occurs.

Drug-related skin rash of a serious nature was infrequent, occurring in 4/1148 (0.34%) patients in the pivotal, well-controlled double-blind trials in HIV-1 patients treated with delavirdine combination therapy with CD4 cell counts of 0-500 cells/mm^3. Whether these events were directly related to the delavirdine only or to another concomitant drug or disease can be difficult to ascertain from the data in the studies compiled in this report. The equal frequency of the serious and ACTG Grades 3 and 4 reports in the delavirdine and comparator groups suggest that the more problematic cutaneous disorders are not predominant finding in the use of delavirdine. There is a trend of increased incidence of skin rash with higher doses (≥400 mg tid) and the higher concentrations associated with these doses. However, there is no significant increased incidence of ACTG Grade 3 or 4 skin rash or serious medical events associated with higher doses.

In Protocol M/3331/0017, the DLV + ddI group had a significantly increased frequency of the following drug-related medical events (in decreasing order of frequency): rash, maculopapular rash, pruritus, insomnia, change in dreams, tingling, and arthralgia. In Protocol M/3331/0021, the only significantly increased incidence of drug-related medical events for DLV + ZDV patients was rash, occurring in each of the three delavirdine treatment groups. Therefore, skin rash was the only treatment-emergent drug-related medical event that had a significantly increased incidence in the delavirdine combination therapy group in both protocols.

Delavirdine is primarily metabolized by cytochrome P450 3A. Drug interaction data from Protocols M/3331/0017 and M/3331/0021 suggest that various inhibitors of cytochrome P450 3A do not have clinically important effects on the pharmacokinetics of delavirdine. However, limited data on inducers of cytochrome P450 3A suggest that these drugs reduce delavirdine blood concentrations to varying degrees. Due to the inhibitory effect of delavirdine on cytochrome P450 3A, its coadministration with drugs primarily metabolized by cytochrome P450 3A may result in increased plasma concentrations of these drugs that could increase or prolong both therapeutic and adverse effects. Therefore, appropriate dose adjustments may be necessary for these drugs.

4. Conclusions

Delavirdine, in monotherapy or in combination therapy with nucleoside reverse transcriptase inhibitors, administered orally at daily doses of up to 400 mg tid in more than 150 healthy volunteers and at doses of up to 850 mg tid in 1771 HIV-1–infected patients for up to 3 years, has been very well tolerated.

In summary, delavirdine mesylate has an excellent safety profile in both healthy normal volunteers and HIV-1-infected patients of various stages of disease progression and CD4 counts. Delavirdine is well tolerated, with a mild-to-moderate skin rash being the primary medical event associated with its use in monotherapy or combination therapy. The rash develops in 30% to 40% of patients in the first month of therapy and can be dosed through in over 85% of cases.

Delavirdine administered at 300-mg three times daily or 400-mg three times daily combined with ZDV has an excellent safety profile and, compared to ZDV monotherapy, provides significantly better surrogate marker responses of at least one

year duration. The greater efficacy of 300-mg and 400-mg DLV+ZDV combination therapies over ZDV monotherapy with little added risk suggests that this combination therapy will be beneficial for long-term treatment of HIV-1 patients.

Delavirdine administered at 400 mg three times a day combined with didanosine provides significantly better surrogate marker response of up to 24 weeks compared to ddI monotherapy. A more durable surrogate marker response was observed in some subgroups of ddI-naive patients with higher CD4 counts. The greater efficacy of 400-mg DLV+ddI combination therapy over ddI monotherapy, with little added risk, suggest that this combination therapy will be beneficial for the treatment of HIV-1 patients.

In supportive studies, delavirdine combination with ZDV+ddI or ZDV+ddC in a diverse HIV-1 population (including patients with CD4 < 50 cells/mm^3 who have failed prior RTI therapy) has been well-tolerated and provided significantly better surrogate marker responses compared to prior RTI combination therapy of monotherapy.

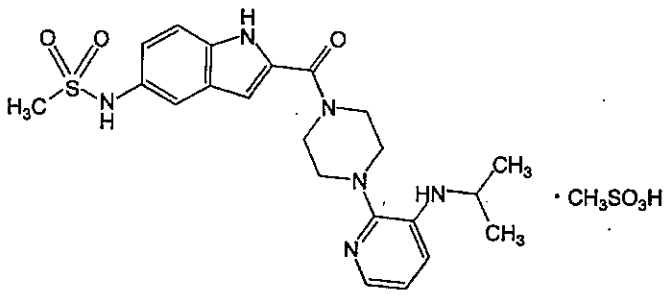
5. References/Study Reports/Publications

1. Mann JS and Tarantola D. Coming to terms with the AIDS pandemic. Issues in Science and Technology 1993; Spring:41-8

2. Romero DL, Busso M, Tan CK, Reusser F, Palmer JR, Poppe SM, et al. Nonnucleoside reverse transcriptase inhibitors that potently and specifically block human immunodeficiency virus type 1 replication. Proc Natl Acad Sci USA 1991;88:8806-10 . .

3. Vasudevachari MB, Battista C, Lane HC, Psallidopoulos MC, Zhao B, Cook J, et al. Prevention of the spread of HIV-1 infection with nonnucleoside reverse transcriptase inhibitors. Virology 1992;190:269-77 . . . . . .

毒薬・劇薬等の指定申請資料のまとめ

化学名・別名	1-{[3-[(1-methylethyl)amino]pyridin-2-yl}-4-({[5-(methylsulfonyl)amino]-1*H*-indol-2-y1}carbonyl) piperazine monomethanesulfonate
構造式	
効能・効果	HIV－１感染症
用法・用量	通常、成人にはメシル酸デラビルジンとして１回 400mg を１日３回経口投与する。なお、投与に際しては必ず他の抗 HIV 薬と併用すること。

劇薬等の指定	劇 薬	原体・塩類・製剤	指 定	原体・塩類・製剤	要指示	原体・塩類・製剤
市販名及び有効成分･分量	製剤：レスクリプター錠２００ｍｇ（１錠中　メシル酸デラビルジン２００ｍｇ含有）					

毒性

急　性

動物種	投与経路	概略の致死量
ラット	経口	＞500mg/kg

亜急性

動物種	投与期間	投与経路	投与量 (mg/kg/日)	無毒性量 (mg/kg/日)	主な所見
ラット	３ヵ月（２ヵ月回復）	ＰＯ	50, 125, 300, 750	♂♀＜50	750mg/kg 群：雌 2 匹, 雄 1 匹死亡（死因不明）大球性貧血, 組織中の本剤結晶に対する肉芽腫, 肥大または過形成性変化（肝臓, 腎臓, 胃, 甲状腺, 副腎, 下垂体）, 新生黄体の増加.
イヌ	３ヵ月（１ヵ月回復）		12, 33, 76/60* *途中減量	♂♀12	76/60mg/kg 群：消化管の糜爛, 潰瘍, 肺炎, 骨髄細胞の減少/壊死, 血管炎/血管周囲炎の増加, 死亡.
サル			240, 300	♂♀＜240	240mg/kg 群：壊死性血管炎/血管周囲炎（300mg/kg群では見られず） 平均最低血中濃度 140μM 以上で消化管糜爛/潰瘍のため死亡または屠殺.

慢　性

動物種	投与期間	投与経路	投与量 (mg/kg)	無毒性量 (mg/kg)	主な所見
ラット	６ヵ月（２ヵ月回復）	ＰＯ	5, 58, 100 170, 300	♂♀ 5	３ヵ月のラット毒性試験で観察されたものに加え可逆性のリンパ球増多症, 局所性の組織球増殖症
イヌ	12 ヵ月（６ヵ月回復）		2, 18, 30, 50	♂♀ 18	30, 50mg/kg 群：嘔吐, 下痢, 粘液様または血様便, 前立腺重量の減少. 50mg/kg 群：血球数減少副腎重量増加, 慢性血管炎/血管周囲炎, 骨髄壊死, 造血機能の上昇, 消化管の糜爛/潰瘍

副作用	Study 0017－Rescriptor+ジダノシン（n=591）

副作用の種類	発現率(%)	臨床検査値異常の種類	発現率(%)
頭痛	5.6	好中球減少（絶対好中球数 ＜ 750/mm3）	5.7
疲労	2.9	貧血（ヘモグロビン ＜ 7.0 g/dL）	0.7
嘔気	4.9	血小板減少（血小板数＜ 50,000 /mm3）	1.5
下痢	4.5	GPT 上昇(＞正常値上限の 5 倍)	6.7
嘔吐	2.4	GOT 上昇(＞正常値上限の 5 倍)	5.6
GPT 上昇	5.2	ビリルビン上昇（＞正常値上限の 2.5 倍）	0.5
GOT 上昇	4.5	アミラーゼ上昇(＞正常値上限の 2.5 倍)	5.2
皮疹	9.8		
斑状丘疹性皮疹	6.6		
そう痒	2.2		

・副作用については、中等度もしくは重度と判定された副作用について、発現率 2%以上のものを記載。
・臨床検査値異常発現率の母数は、各検査が実施された患者数。

Study 0021－Rescriptor+ジドブジン（n=271）

副作用の種類	発現率(%)	臨床検査値異常の種類	発現率(%)
頭痛	5.6	好中球減少（絶対好中球数 ＜ 750/mm3）	3.5
疲労	5.2	貧血（ヘモグロビン ＜ 7.0 g/dL）	1.0
嘔気	10.8	GPT 上昇(＞正常値上限の 5 倍)	3.8
下痢	3.5	GOT 上昇(＞正常値上限の 5 倍)	2.1
嘔吐	2.8	ビリルビン上昇（＞正常値上限の 2.5 倍）	1.0
GPT 上昇	2.4		
GOT 上昇	1.7		
皮疹	12.5		
斑状丘疹性皮疹	4.5		
そう痒	3.1		

・副作用については、中等度もしくは重度と判定された副作用について、発現率 2%以上のものを記載。
・臨床検査値異常発現率の母数は、各検査が実施された患者数。

会　社	ワーナー・ランバート株式会社　　製剤：輸入