เภสัชจลนศาสตร์ของสตาวูดีน ลามิวูดีน และเนวิราพีนในยาสูตรผสม จีพีโอ-เวียร์ เอส30 เปรียบเทียบกับการให้ยาต้นแบบเดี่ยว 3 ชนิดร่วมกัน และการประเมินประสิทธิผล การรักษาและความปลอดภัยของ จีพีโอ-เวียร์ เอส30 ในผู้ป่วยติดเชื้อเอชไอวี

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PHARMACOKINETICS OF STAVUDINE, LAMIVUDINE AND NEVIRAPINE IN A COMBINED FORMULATION GPO-VIR S30 COMPARED TO THREE SINGLE ORIGINAL BRANDS AND ASSESSMENT OF EFFICACY AND SAFETY OF GPO-VIR S30 IN HUMAN IMMUNODEFICIENCY VIRUS (HIV)-INFECTED PATIENTS

Miss Maneerat Luangwattanawilai

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การวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาและเปรียบเทียบเภสัชจลนศาสตร์ของสตาวูดีน ลามิวูดีน และเนวิราพีน ในยาสูตรผสมจีพีโอ-เวียร์ เอส30 กับยาต้นแบบเดี่ยว ในผู้ป่วยติดเชื้อเอชไอวี รวมทั้งติดตามประสิทธิผลการรักษาและความปลอดภัยของยาสูตรผสมจีพีโอ-เวียร์ เอส30 ในผู้ป่วยติดเชื้อเอชไอวี วิธีวิจัยเป็นการวิจัยเชิงทดลอง โดยแบ่งกลุ่มผู้ป่วยเป็น 2 กลุ่มๆ ละ 10 คน กลุ่มที่ 1 รับประทานยาสูตรผสมจีพีโอ-เวียร์ เอส30 กลุ่มที่ 2 รับประทานยาต้นแบบเดี่ยว 3 ชนิด เป็นเวลา 2 สัปดาห์ เก็บตัวอย่างพลาสมา หลังจากนั้นจึงสลับ กลุ่มที่ 1 ให้รับประทานยาต้นแบบเดี่ยว 3 ชนิด และกลุ่มที่ 2 ให้รับประทานยาสูตรผสมจีพีโอ-เวียร์ เป็นเวลา 2 สัปดาห์ เก็บตัวอย่างพลาสมา นำตัวอย่างพลาสมามาตรวจวัดความเข้มข้นของยาแต่ละชนิดด้วยวิธีวิเคราะห์ที่ได้ผ่านการตรวจสอบความถูกต้องแล้ว ติดตามประสิทธิผลการรักษาโดยติดตามการใช้ยาต้านเอชไอวีจีพีโอ-เวียร์ เอส30 เป็นเวลา 12 สัปดาห์ ประสิทธิผลการรักษาพิจารณาจากปริมาณเชื้อเอชไอวีในเลือด จำนวนเม็ดเลือดขาวซีดี 4 ติดตามความปลอดภัยโดยติดตามอาการไม่พึงประสงค์จากการใช้ยา

ผลการวิจัยพบว่าค่าทางเภสัชจลนศาสตร์ของยาสตาวูดีน ลามิวูดีน และเนวิเรพีน จากการรับประทานยาสูตรผสมจีพีโอ-เวียร์ เอส30 ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติ ($P>0.05$) ตามเกณฑ์ของการศึกษาชีวสมมูลพบว่าพื้นที่ใต้กราฟระหว่างความเข้มข้นของยาในพลาสมากับเวลาของยาทั้ง 3 ชนิดอยู่ในช่วงความเท่าเทียมที่กำหนดไว้ คือ 0.8-1.25 ทั้ง 3 ตัวยา ส่วนความเข้มข้นสูงสุดของยาในพลาสมาสำหรับยาเนวิราพีนมีความเท่าเทียมกัน ขณะที่สตาวูดีนและลามิวูดีนจากยาสูตรผสมจีพีโอ-เวียร์ เอส30 ให้ค่าสูงกว่าขอบเขตที่กำหนดไว้สำหรับช่วงบนเล็กน้อย (1.31 และ 1.30 ตามลำดับ) จากผลการศึกษาแสดงว่ายาทั้ง 2 ตำรับมีปริมาณยาที่ถูกดูดซึมเท่าเทียมกันแต่อัตราเร็วในการดูดซึมจากตำรับจีพีโอ-เวียร์ เอส30 อาจจะเร็วกว่าผลิตภัณฑ์ต้นแบบเล็กน้อย อย่างไรก็ตาม เนื่องจากยาทั้ง 3 ตัวที่ศึกษาเป็นยาที่ให้แบบต่อเนื่องระยะยาว ปริมาณยาที่ถูกดูดซึมจะมีความสำคัญต่อผลการรักษาทางคลินิกมากกว่าอัตราเร็วในการดูดซึม ยาทั้ง 3 ตัว จากผลิตภัณฑ์ทั้ง 2 ตำรับ จึงยังถือได้ว่ามีชีวสมมูล ผลการรักษาทางคลินิกของจีพีโอ-เวียร์ เอส30 เป็นเวลา 12 สัปดาห์ พบว่ามีผู้ป่วยที่มีปริมาณเชื้อไวรัสในกระแสเลือดลดลงจนไม่สามารถตรวจวัดได้จำนวน 15 รายจาก 19 ราย ส่วนค่าเฉลี่ยเม็ดเลือดขาวซีดี 4 เพิ่มขึ้นจาก 108 เป็น 206 cells/mm^3 อาการข้างเคียงที่พบได้มากที่สุดคือ ผื่นแพ้ทางผิวหนัง

สตาวูดีน ลามิวูดีน และเนวิราพีนในยาสูตรผสมจีพีโอ-เวียร์ เอส30 ถือได้ว่ามีชีวสมมูลกับผลิตภัณฑ์ต้นแบบ เนื่องจากการทดสอบค่าทางเภสัชจลนศาสตร์ไม่พบว่ามีความแตกต่างอย่างมีนัยสำคัญทางสถิติ และอัตราส่วนพื้นที่ใต้กราฟความเข้มข้นของยาในพลาสมากับเวลาของยาทั้ง 3 ตัว เมื่อทดสอบที่ขอบเขตความเชื่อมั่น 90% พบว่าอยู่ในช่วง 0.8-1.25 ที่กำหนดทั้งหมด การติดตามผลการรักษาและอาการข้างเคียงจากการใช้ยาจีพีโอ-เวียร์ เอส30 เป็นเวลา 12 สัปดาห์พบผลลัพธ์ที่น่าพอใจ

ภาควิชา……เภสัชกรรม……ลายมือชื่อนิสิต……

สาขาวิชา……เภสัชกรรมคลินิก……ลายมือชื่ออาจารย์ที่ปรึกษา……

ปีการศึกษา……2546……ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……

4476598033: MAJOR CLINICAL PHARMACY

KEY WORD: STAVUDINE / LAMIVUDINE / NEVIRAPINE / PHARMACOKINETICS / GPO-VIR

MANEERAT LUANGWATTANAWILAI: PHARMACOKINETICS OF STAVUDINE, LAMIVUDINE AND NEVIRAPINE IN A COMBINED FORMULATION GPO-VIR S30 COMPARED TO THREE SINGLE ORIGINAL BRANDS AND ASSESSMENT OF EFFICACY AND SAFETY OF GPO-VIR S30 IN HUMAN IMMUNODEFICIENCY VIRUS (HIV)-INFECTED PATIENTS. THESIS ADVISOR: ASSOC. PROF. DUANGCHIT PANOMVANA NA AYUDHYA, Ph.D., THESIS CO-ADVISOR: WISIT PRASITHSIRIKUL, M.D., M.Sc., WIYADA AKARAWUT, Ph.D., 117 pp. ISBN 974-17-5506-6

The purposes were to study and compare the pharmacokinetic parameters of stavudine (d4T), lamivudine (3TC) and nevirapine (NVP) after administration as combined drugs formulation, GPO-vir S30, to those after giving three single drug tablets of the original brands in HIV-infected patients and also to determine short-term safety and efficacy of GPO-vir S30 in HIV-infected patients. The opened-label, randomized, 2-ways crossover trial was carried in twenty HIV-infected patients. They were divided into two groups, ten of them received the combined drugs tablet, and another group received the single drug tablets of the original brands for two weeks. Plasma samples were collected. The drug products were then switched, and the second period samples were collected after crossover for another two weeks. Plasma samples were analyzed by the HPLC validated method. Safety and efficacy of GPO-vir S30 were further followed for 12 weeks through viral load (VL) and CD4 counts.

Pharmacokinetics of d4T, 3TC and NVP showed no statistically significant differences between GPO-vir S30 and the original brands products ($P>0.05$). Bioequivalence study based on 90% confidence interval limits indicated that the $AUC_{0\text{-}12hr}$ ratio of d4T, 3TC and NVP were all in the 0.8-1.25 equivalent limits. C_{max} ratio of NVP was also within the equivalent range; however, C_{max} ratio of d4T and 3TC were both met the lower limit but were slightly higher than 1.25 for the upper limit (1.31 and 1.30, respectively). These results indicated that the extent of absorption of d4T and 3TC were equivalent while the rates of absorption of these two drugs from GPO-vir S30 tablet might be slightly faster. Since all these drugs were used in chronic dosing which the extent of absorption were much more related to therapeutic effects than the rate of absorption, the two products could then be interpreted as bioequivalent. Short-term clinical monitoring for therapeutic effect of GPO-vir S30 for 12 more weeks revealed that VL of 15 from the total of 19 patients were decreased to undetectable level, the less 4 patients had VL with in the range of 50-157 copies/mL. Mean CD4 was increased from 108 to 206 cells/mm^3. Majority adverse events were dermatological reaction.

GPO-vir S30 and the original brands products could be interpreted as bioequivalence since both pharmacokinetic parameters of d4T, 3TC and NVP did not showed statistically significant differences and 90% confidence interval of $AUC_{0\text{-}12hr}$ ratio of all three drugs were within the 0.8-1.25 limits. Short-term monitoring for clinical effect of GPO-vir S30 revealed satisfactory results.

Department……Pharmacy……Student's signature……..……....

Field of study……Clinical Pharmacy…….. Advisor's signature……..……..

Academic year……2003…….. Co-advisor's signature……..……..

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ABBREVIATIONS

τ	Dosing interval
μmol	micromolar
3TC	Lamivudine
ABC	Abacavir
AIDS	Acquired immune deficiency syndrome
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
AUC	Area under the concentration-time curve
BUN	Blood urea nitrogen
CAD	Coronary artery disease
C_{avg}	Average concentration
CBC	Complete blood count
CDC	Centers for Disease Control and Prevention
CL	Clearance
CL_{Cr}	Creatinine clearance
C_{max}	Maximum concentration
C_{min}	Minimal concentration
Cr	Creatinine
CV	Coefficient of variation
d4T	Stavudine
ddC	Zalcitabine
ddI	Didanosine
GPO	Government pharmaceutical organization
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
hr	Hour
IS	Internal standard
K_e	Elimination rate constant

ABBREVIATIONS (Cont.)

kg	Kilogram
L	Liter
LFT	Liver function test
LLOQ	Lower limit of quantification
Max	Maximum
mg	Milligram
Min	Minimum
mL	Milliliter
mM	Millimolar
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PCP	*Pneumocystis carinii* pneumonia
PI	Protease inhibitor
QCH	High concentration quality control sample
QCL	Low concentration quality control sample
QCM	Medium concentration quality control sample
QCs	Quality control sample
rpm	Round per minute
SD	Standard deviation
SE	Standard error
sec	Second
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
$t_{1/2}$	Half-life
t_{max}	Time to reach the maximum concentration
UA	Urinalysis
ULN	Upper limit of normal
UNAIDS	Joint united nations programme on HIV/AIDS
v/v	Volume by volume

ABBREVIATIONS (Cont.)

V_d	Volume of distribution
VL	Viral load
WBC	White blood cell
WHO	World Health Organization
yr	year
ZDV	Zidovudine

CHAPTER I

INTRODUCTION

HIV/AIDS (human immunodeficiency virus/ acquired immune deficiency syndrome) is the importance public health problem of the world including Thailand. According to the report from Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health of Thailand since 1984 to 31 Jul 2003, there were 305,848 HIV/AIDS patients. More than 60,000 died.[1] This problem affects economic, social and public health services due to increasing number of patients. Goals of HIV/AIDS therapy are to achieve maximal and durable suppression of viral load, restoration and/or preservation of immunologic function, improvement of quality of life and reduction of HIV-related morbidity and mortality.[2-4]

Combination antiretroviral regimens or highly active antiretroviral therapy (HAART) effectively reduce level of HIV-RNA, improve immune function, delay the progression of HIV disease and improve quality of life.[5, 6] Initial therapy with two nucleoside reverse transcriptase inhibitors (NRTIs) and either one or two protease inhibitors (PIs) or a non-nucleoside reverse transcriptase inhibitor (NNRTI) is now a recommended standard treatment.[2-4] NNRTIs-based regimens are attractive for the treatment of antiretroviral-naïve patients because of convenient dosing (non-food-dependent dosing regimen and lower pill burden), good tolerance, preservation of PIs for salvage intervention and minimization of long-term toxicity classically ascribed to PIs (e.g. lipodystrophy and lipid abnormalities).[7-11] Recent studies have shown that stavudine/lamivudine/nevirapine (d4T/3TC/NVP) containing regimen is highly effective in reducing viral load. Shalit et al.[12] have investigated the long-term safety and efficacy of this regimen and found that 92% (24/26) had viral loads of less than 50 copies/mL within a mean of 19 months (range 7-38 months) follow-up. French et al.[13] have compared the efficacy of three backbone NRTIs, i.e.; zidovudine/lamivudine (AZT/3TC), stavudine/lamivudine (d4T/3TC) and stavudine/didanosine (d4T/ddI) when combined with nevirapine (NVP). They had found that all three-drug combinations were equally

effective in suppressing viral load and increasing CD4 T-cell counts within 52 weeks follow-up. Yozviak et al.[14] had reviewed the charts of 73 patients receiving d4T/3TC/NVP and reported that 78.1% (57/73) had viral load of less than 400 copies/mL within 16 weeks follow-up while the mean CD4 cell count increase was 170 cell/mm^3 within 48 weeks. Comparison to PIs-based regimens, NNRTIs-based regimens can provide more convenient administration, no food restrictions and easily incorporated into their daily routine.[15] While, PIs-based regimen are known to have long-term side effects as lipodystrophy and lipid abnormalities that may expose patients to an increased risk of coronary artery disease (CAD).[16]

D4T/3TC/NVP is one of the recommended regimens in standard treatment guideline of Thailand.[17] Government Pharmaceutical Organization (GPO), Thailand, has produced a fixed-dose combined tablet of d4T/3TC/NVP, namely, GPO-vir S30 and GPO-vir S40. These combined tablets require only one pill twice daily, in contrast to taking three pills of original drugs twice daily. This simplier administration can improve patient adherence. However, pharmacokinetic data of this combined tablet needs to be examined. The purposes of this study were to compare the pharmacokinetics of d4T, 3TC and NVP from a combined formulation, GPO-vir S30 with three separate original brands in HIV-infected patients and to determine short-term safety and efficacy of GPO-vir S30 for 12 weeks.

Objectives

The objectives of the study are

1. To study the pharmacokinetic parameters of d4T, 3TC and NVP in combined drugs formulation, GPO-vir, S30 and three single original brands (three reference single drugs) in HIV-infected patients. These parameters are elimination rate constant (K_e), apparent volume of distribution (apparent V_d), half-life ($t_{1/2}$), apparent clearance (apparent CL), area under the concentration-time curve (AUC), maximum concentration (C_{max}) and time to reach the maximum concentration (t_{max}).
2. To compare the pharmacokinetic parameters of d4T, 3TC and NVP in combined drugs formulation, GPO-vir S30, with three single original brands (three reference single drugs) in HIV-infected patients.
3. To determine short-term safety and efficacy of GPO-vir S30 in HIV-infected patients for 12 weeks.

Significance of the study

1. This study will provide information on the pharmacokinetic parameters of d4T, 3TC and NVP in combined drugs formulation; GPO-vir S30, compared with three single original brands (three reference single drugs) in HIV-infected patients.
2. This study will provide information on short-term safety and efficacy of GPO-vir S30 in HIV-infected patients.
3. Patient will receive quality and efficacious drug products with appropriate price.

CHAPTER II

LITERATURE REVIEW

1. HIV and AIDS[18]

The acquired immunodeficiency syndrome (AIDS) was first recognized in 1981. A retrovirus, human immunodeficiency virus type 1 (HIV-1) is the major cause of AIDS. A second retrovirus, HIV-2, has also been recognized to cause AIDS, although it is less prevalent than HIV-1. The lymphocyte and other cells bearing the CD4 surface receptors are the target of viral infection. During early infection, HIV antibody is gradually produced until it can be detected after 3-4 weeks by recently developed screening test. When HIV antibody can be detected, that person will be called "HIV positive". The immune system is suppressed, gradually destroyed and finally turn to AIDS. This advance stage of HIV infection is commonly progressed with many of opportunistic infections.

1.1 Epidemiology[1, 19]

The UNAIDS/WHO estimates those about 42 million people living with HIV/AIDS at the end 2002. Newly infected with HIV about 5 million and more than 3 million died due to AIDS during 2002. In Thailand, the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health reported that since 1984 to 31 Jul 2003, there were 305,848 HIV/AIDS patients. Classified as 223,476 AIDS patients and 82,372 symptomatic HIV infected patients. More than 60,000 patients have already died.

1.2 Clinical Manifestations[18, 20]

Clinical manifestations of HIV infection and AIDS can be divided into 3 stages as followed:

1.2.1 The acute HIV syndrome

The acute clinical syndrome occurs approximately 3 to 6 weeks after primary infection. Varying degrees of clinical severity have been reported. The typical clinical findings are listed in Table 1; they occur along with a burst of plasma viremia. The syndrome is typical of an acute viral characterized by nonspecific flu-like symptoms and usually persist for 1 to several weeks and gradually subside as an immune response to HIV develops and the levels of plasma viremia decrease. Opportunistic infections have been reported during this stage of infection, reflecting the immunodeficiency that results from reduced number of CD4 cells and likely also from the dysfunction of CD4 cells associated with the extremely high levels of plasma viremia. The number of total lymphocytes and T cell (CD4 and CD8) are initially reduced. An inversion of the CD4/CD8 ratio occurs later because of a rise in the number of the CD8 cells. The total circulating CD8 cell count may remain elevated or return to normal; however, CD4 cell levels usually decrease, although there may be a slight rebound towards normal. Lymphadenopathy occurs in approximately 70% of individuals with primary HIV infection. Most patients recover spontaneously from this syndrome and many are left with only a mildly depressed CD4 cell count before beginning progressive decline.

1.2.2 The asymptomatic stage (clinical latency)

Although the length of time from initial infection to the development of clinical disease varies greatly, the median time for untreated patients is approximately 10 yr. The rate of disease progression is directly correlated with HIV RNA or viral load level. During the asymptomatic period of HIV infection, the average rate of CD4 cell decline is approximately 50 cells/mm^3 per yr. When the CD4 cell count falls <200 cells/mm^3, the resulting state of immunodeficiency is severe enough to place the patient at high risk for opportunistic infection and neoplasms, and hence for clinically apparent disease.

Table 1 Clinical findings in the acute HIV syndrome

General	*Neurologic*
Fever	Meningitis
Pharyngitis	Encephalitis
Lymphadenopathy	Peripheral neuropathy
Headache/retroorbital pain	Myelopathy
Arthralgias/myalgias	*Dermatologic*
Lethargy/malaise	Erythematous maculopapular rash
Anorexia/weight loss	Mucocutaneous ulceration
Nausea/vomiting/diarrhea	

1.2.3 Symptomatic disease

Symptoms of HIV disease can appear at any time during the course of HIV infection. The more severe and life-threatening complications of HIV infection occur in patient with CD4 cell counts <200 cells/mm^3. A diagnosis of AIDS is made in anyone with HIV infection and a CD4 cell counts <200 cells/mm^3 and in anyone who develops one of the HIV-associated diseases in category C (Table 2) Most of AIDS patients are death as a direct result of opportunistic infections.

The Centers for Disease Control and Prevention (CDC) in the United State have established the criteria for categorizes HIV infection and AIDS patients on the basic of clinical conditions associated with HIV infection and CD4 cell counts. This classification system defined for adolescents (greater than or equal to 13 yr of age) and adults which classified into 3 clinical categories A, B and C (Table 2). Each category is divided into 3 levels based on CD4 cell counts as followed:

Category A: Consists of one or more of the conditions list below in an adolescent or adult (> 13 yr) with documented HIV infection. Conditions listed in categories B and C not have occurred.

Asymptomatic HIV infection

Persistent generalized lymphadenopathy

Acute (primary) HIV infection with accompanying illness or history of acute HIV infection

Table 2 1993 Revised Classification System for HIV Infection and Expanded AIDS Surveillance Case Definition for Adolescents and Adults[20]

CD4 T cell Categories (cells/mm^3)	Clinical categories		
	A Asymptomatic, acute (primary) HIV or PGL	B Symptomatic, not A or C conditions	C AIDS-indicator conditions
> 500	A1	B1	C1
200-499	A2	B2	C2
< 200	A3	B3	C3

Category B: Consist of symptomatic condition in an HIV-infected adolescent or adult that are not included among conditions listed in clinical category C and that meet at least one of the following criteria:

(1) The conditions are attributed to HIV infection or are indicative of a defect in cell-mediated immunity; or

(2) The conditions are considered by physicians to have a clinical course or to require management that is complicated by HIV infection. Examples include, but are not limited to, the following:

Bacillary angiomatosis

Candidiasis, oropharyngeal (thrush)

Candidiasis, vulvovaginal; persistent, frequent, or poorly responsive to therapy

Cervical dysplagia (moderate or severe)/cervical carcinoma in situ

Constitutional symptoms, such as fever (38.5°C) or diarrhea > 1 month

Hairy leukoplakia, oral

Herpes zoster (shingles), involving at least two distinct episodes or more than one dermatome

Idiopathic thrombocytopenic purpura

Listeriosis

Pelvic inflammatory disease, particularly if complicated by tuboovarian abscess

Category C: This category includes the symptomatic conditions found in AIDS patients. Examples of conditions in this category include:

Candidiasis of bronchi, trachea, or lung

Candidiasis, esophageal

Cervical cancer, invasive

Coccidioidomycosis, disseminated or exterapulmonary

Cryptococcosis, extrapulmonary

Cryptosporidiosis, chronic intestinal (> 1 month's duration)

Cytomegalovirus disease (other than liver, spleen, or nodes)

Cytomegalovirus retinitis (with loss of vision)

Encephalopathy, HIV-related

Herpes simplex: chronic ulcer(s) (> 1 month's duration); or bronchitis, pneumonia, or esophagitis

Histoplasmosis, disseminated or extrapulmonary

Isosporiasis, chronic intestinal (> 1 month's duration)

Kaposi's sarcoma

Lymphoma, Burkitt's (or equivalent term)

Lymphoma, primary, of brain

Mycobacterium avium complex or *M.kansasii*, disseminated or extrapulmonary

Mycobacterium tuberculosis, any site (pulmonary or extrapulmonary)

Mycobacterium, other species or unidentified species, disseminated or extrapulmonary

Pneumocystic carinii pneumonia

Pneumonia, recurrent

Progressive multifocal leukoencephalopathy

Salmonella septicemia, recurrent

Toxoplasmosis of brain

Wasting syndrome due to HIV

All persons within Category C as well as persons in subset 3 (Table 2) with a CD4 T-lymphocyte count less than 200 cells/mm^3 or less than 14% meet the surveillance criteria for a definition of AIDS.

1.3 Pathogenesis[18]

A number of steps in replicate of HIV are recognized that may be targets of drug therapy. A rational approach to antiretroviral therapy requires a basic understanding of the HIV replication cycle.

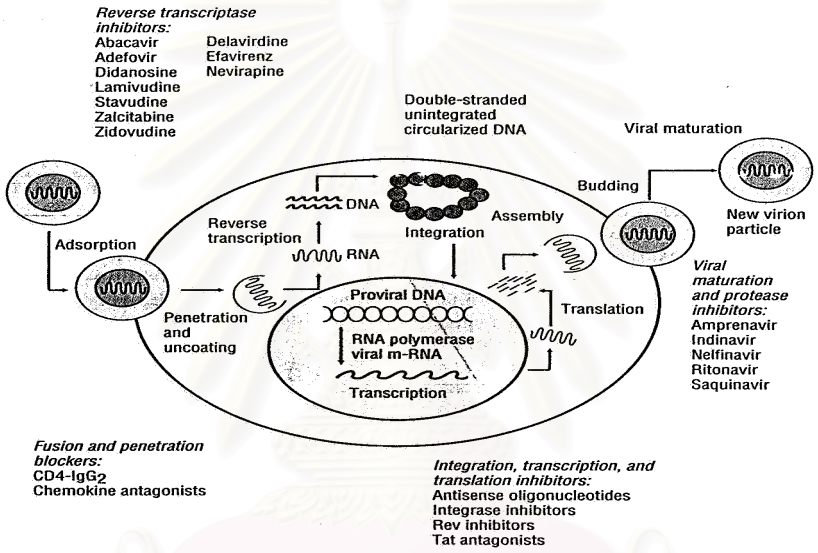


Figure 1 Live cycle of HIV with potential targets where replication may be interrupted and site of action of antiretroviral agents

The life cycle of HIV is complicated but useful to understand since the strategies employed in the treatment of HIV target various points in this cycle. Once HIV enters a human body, the outer glycoprotein (gp160) expressed on the virus allows HIV to bind to CD4 receptor, proteins present on cell surface of T-helper (Th) lymphocytes, monocytes, macrophages and dendritic cells. The glycoprotein consists of two subunits, gp120 and gp41. The gp120 subunit has high affinity for the CD4 receptor and responsible for the initial binding of the virus to the CD4 cell. Once initial binding occurs, the intimate association of HIV with the cell is further enhanced by chemokine coreceptors. Genetic defects in the expression of chemokine receptors appear to

protect some individuals from developing AIDS despite their being exposed to the virus. Attachment of HIV to the cell promotes fusion and internalization (adsorption) of the virus, a process mediated by the gp41 subunit.

After adsorption, the virus is uncoated in preparation for replication. The genetic material of the virus is positive-sense, single-strand RNA (ssRNA); the virus must transcribe this RNA into DNA to optimally replicate in human cells. HIV is equipped with a unique enzyme, RNA-dependent DNA polymerase (reverse transcriptase). Following reverse transcription, the final dsDNA product migrates into chromosome by integrase, another enzyme unique to HIV.

The integration of HIV into the host chromosome is troublesome for several reasons. First, HIV can establish a chronic and persistent infection, particularly in certain cells of immune system, such as memory T lymphocyte. Second, integration is random, thus making it difficult to target and extract integrated HIV. Last, random integration of HIV may cause cellular abnormalities leading to cancer. When HIV replication is induced, the host RNA polymerase transcribes the integrated proviral DNA into messenger RNA (mRNA) with subsequent translation of the mRNA into viral proteins.

Assembly of new virion particles occurs in a stepwise manner beginning with the coalescence of HIV proteins beneath the host cell lipid bilayer. Viral subunits are formed then buds through the plasma membrane, acquiring the lipid bilayer of the host along with some host proteins. After the virus buds, the maturation process begins. Within the virion an enzyme, HIV protease, begins cleaving a large precursor polypeptide (*gag* and *gag-pol*) into functional proteins that are necessary to produce a complete virus and infect other CD4 cells. The process is summarized in Figure 1.

2. Antiretroviral therapy

2.1 Therapy goals[3, 4]

Eradication of HIV infection cannot be achieved with available antiretroviral regimens, chiefly because the pool of latently infected CD4 T cells is established during the earliest stages of acute HIV infection and persists with a long-life, even with prolonged suppression of plasma viremia to < 50 copies/mL. The primary goals of antiretroviral therapy are maximal and durable suppression of viral load, restoration and preservation of immunologic function, improvement of quality of life and reduction of HIV-related morbidity and mortality.

Plasma viremia is a strong prognostic indicator in HIV infection. Furthermore, reductions in plasma viremia achieved with antiretroviral therapy account for substantial clinical benefits. Therefore, suppression of plasma viremia as much as possible for as long as possible is a critical goal of antiretroviral therapy, but this goal must be balanced against the need to preserve effective treatment options.

2.2 When to start treatment[2-4]

With currently available antiretroviral agents, eradication of HIV infection is not likely to be possible. The aim of treatment is thus to prolong life and improve quality of life by maintaining suppression of virus replication for as long as possible. The recommendations are summarized in Table 3.

Patients with established HIV infection are discussed in two arbitrarily defined clinical categories:

1. Asymptomatic infection or
2. Symptomatic disease (i.e., wasting, thrush or unexplained fever for > 2 weeks) including AIDS, as classified by CDC in 1993.

Table 3 Indications for the initiation of antiretroviral therapy in the HIV-infected patient

	BHIVA [a] (2003)			DHHS[b] (2003)			IAS [c] (2000)			Thai MOPH[d] (2002)		
Clinical category	CD4 cell (cells/mm^3)	Viral load (copies/mL)	Recommen-dation	CD4 cell (cells/mm^3)	Viral load (copies/mL)	Recommen-dation	CD4 cell (cells/mm^3)	Viral load (copies/mL)	Recommen-dation	CD4 cell (cells/mm^3)	Viral load (copies/mL)	Recommen-dation
Sympto-matic	Any value	Any value	Treat	Any value	Any value	Treat	Any value	Any value	Treat	Any value	Any value	Treat
Asympto-matic	<200	Any value	Treat	<200	Any value	Treat	<350			<200	Any value	Treat
	200-350	Any value	Consider	200-350	Any value	Should be offered	350-500	>5,000 <5,000	Treat Consider	200-350	Any value	f/u q 3 mo
	>350	Any value	Defer[e]	>350	>55,000 <55,000	Defer, [f] Defer, [g]	>500	>30,000 5000-30,000 <5,000	Treat Consider Defer[e]	>350	Any value	f/u q 6 mo

[a]BHIVA : British HIV Association Guidelines; Jul, 2003[4]

[b]DHHS : Department of Health And Human Services Guidelines; Jul, 2003[3]

[c]IAS : International AIDS Society, USA Panel Guidelines; Jan 2000 (JAMA, 2000)[2]

[d]Thai MOPH : Ministry of Public Health, Thailand; 2002[17]

[e]Defer : Monitor CD4 cell count and viral load every 3 months

[f] : Recognizing that the 3-yr risk of developing AIDS in untreated patient >30 %

[g] : Recognizing that the 3-yr risk of developing AIDS in untreated patient <15 %

All patients in the second category or CD4 count consistently < 200 cells/mm^3, or who have diagnosed with AIDS or severe/recurrent HIV related illness or tumor at any CD4 count should be offered antiretroviral therapy. Initiating antiretroviral therapy among patients in first category with CD4 > 200 cells/mm^3 must balance the readiness of the patient for treatment, consideration of the prognosis for disease-free survival as determined by baseline CD4 count and viral load levels, and assessment of the risks and potential benefits. Before therapy for any patient is initiated, however, the following evaluation should be performed:

- Complete history and physical
- Complete blood count, chemistry profile, including serum transaminase and lipid profile
- CD4 T-lymphocyte count
- Plasma HIV RNA measurement

2.3 Initial regimen[2-4]

There are no definitive data regarding superiority of one acceptably potent initial regimen over another and recommendations for specific combinations of individual drugs cannot is made. Choice of a regimen should be individualized based on the strength of supporting data and on regimen potency, tolerability, adverse effect profile, likely drug-drug interaction, convenience and adherence likelihood, potential for alternative treatment options if initial regimen fails and possible resistance testing results. Each possible regimen for initial therapy has advantages and disadvantages (Table 4).

2.3.1 NNRTI-based regimens[3, 4]

Efavirenz (EFV) and nevirapine (NVP) are both recommended for initial therapy. These combinations have now been evaluated in a controlled, comparative trial using surrogate endpoints. The 2NN study yielded data comparing the two drugs in a randomized trial, showing that EFV and NVP were comparable in efficacy but there appears to be more safety concerns (particularly, higher incidence and more serious skin rash and hepatotoxicity) about using NVP over EFV.

Table 4 Advantages and disadvantages of antiretroviral regimens[3, 4]

Regimens	Advantages	Disadvantages	Drug interaction complication	Impact on future options
1. PI-based HAART regimen (NNRTI-sparing)	♦ Clinical, virologic and immunologic efficacy well-documented ♦ Resistance requires multiple mutations ♦ Avoid NNRTI-associated side effect ♦ Targets HIV at 2 steps of viral replication (RTI & PI)	♦ Some regimens are difficult to use and adhere to ♦ Long-term side effects often include lipodystrophy, hyperlipidemia and insulin resistance	♦ Mild to severe inhibition of cytochrome P450 pathway; ritonavir is most potent inhibitor (but this effect can be exploited to boost levels of other PIs)	♦ Preserves NNRTIs for use in treatment failure ♦ Resistance primes for cross-resistance with other PIs
2. NNRTI-based HAART regimen (PI-sparing)	♦ Virologic and immunologic efficacy well-documented ♦ Spares PI-related side effects ♦ Easier to use and adhere to, compare with PIs	♦ Resistance conferred by a single or limited number of mutations	♦ Fewer drug interactions compared with PIs	♦ Preserves PIs for use in treatment failure ♦ Resistance usually leads to cross-resistance across entire NNRTI class
3. Triple NRTIs (NNRTI- and PI-sparing)	♦ Generally easier to use and adhere to, compare with PIs ♦ Sparing PI and NNRTI side effects ♦ Cross-resistance to all drugs in the NRTI class is unlikely with initial regimen failure	♦ Virologic efficacy inferior to PI- and NNRTI- based regimen	♦ No cytochrome P450 interaction	♦ Preserves both PI and NNRTI classes for use in treatment failure

2.3.2 PI-based regimens[3, 4]

These regimens have shown clinical and surrogate marker efficacy in clinical practice. Many clinicians use PIs in combination with RTV to provide a pharmacokinetic boosting effect when starting antiretroviral in naïve patients on PI-based regimen. The advantage of this strategy is that to enhance the pharmacokinetic profiles, either by raising the trough levels of PIs or extending their half-life. This may improve potency and may be associated with a reduced risk of resistance development. In addition, a boosted PI may improve convenience by reducing dosage frequency and pill burden; this may facilitate better adherence. However, there is also some suggestions that toxicities, such as nephrotoxicity, dry skin and nail dystrophy with IDV/RTV, gastrointestinal toxicity with other boosted PIs and lipid abnormalities, may be more frequent with boosted PIs compared with using a single PI.

2.3.3 Triple NRTIs regimen[3, 4, 21, 22]

There is now surrogate marker endpoint data suggesting that ZDV/3TC/ABC is less potent than combining two NRTIs with either a NNRTI or a PI. The major advantages of triple NRTIs regimen are the simpler regimen, good tolerability, a relative lack of drug-drug interactions, a low incidence of side effects and a low pill burden. The exception to this is ABC hypersensitivity, a multiple system disorder often presenting with fever and rash, which occurs in up to 3% of patients. Re-challenging affected individuals has resulted in severe toxicity and death on occasion. Triple NRTIs regimen consisting of ZDV/3TC/ABC or d4T/3TC/ABC may be used as an alternative to a NNRTI-based or a PI-based regimen in treatment-naïve patients where the other options may be less desirable due to concerns over toxicities, drug interaction or regimen complexity. In addition, this regimen would not recommend initiation in patients with baseline viral load > 100,000 copies/mL.

3. Stavudine

3.1 Chemical structure and properties[23]

The chemical name for stavudine (d4T) is 2',3'-didehydro-3'deoxythymidine. D4T has the following structure formula:

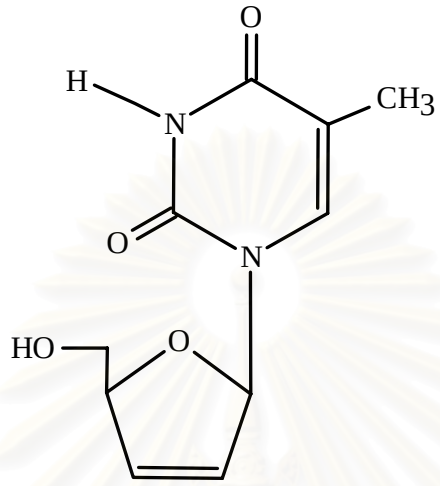


Figure 2 Chemical structure of stavudine

D4T is a white to off-white crystalline solid with the molecular formula $C_{10}H_{12}N_2O_4$ and a molecular weight of 224.2. The solubility of d4T at 23°C is approximately 83 mg/mL in water and 30 mg/mL in propylene glycol. The n-octanol/water partition coefficient at 23°C is 0.144.

3.2 Pharmacokinetic properties

The pharmacokinetic properties of d4T have been investigated following oral administration to pediatric and adult patients with HIV infection. Also, the pharmacokinetic of d4T in volunteers with hepatic and renal impairment have been investigated, but data from elderly patients have yet to be reported.

3.2.1 Absorption and distribution

After oral administration of d4T to patients with HIV infection, the bioavailability was 86.4 ± 18.2%. Peak plasma concentration (C_{max}) and area under the concentration –time curve (AUC) of d4T increased proportionally with dose, and time to

C_{max} (t_{max}) was 0.50-0.75 hr post dose. A apparent volume of distribution is 48.2±8.0 L was observed after administration of 40 mg single dose.[23-25]

Table 5 Pharmacokinetic parameters of stavudine on adults HIV-infected patients[23,24]

Parameter	Value, mean ± SD
F, %	86.4 ± 18.2
AUC, mg/h·L (40 mg single dose)	1.86 ± 0.17
C_{max}, mg/L (40 mg single dose)	0.84 ± 0.20
t_{max}, hr	0.50 - 0.75
$t_{1/2}$, hr	1.55 ± 0.24
V_d/F, L	48.2 ± 8.0
CL/F, L/hr	21.6 ± 1.9

The absorption rate of d4T was affected by food intake, but the overall bioavailability was not, in 17 asymptomatic HIV patients with CD4 cell counts of >200 cells/mm^3. Compared with fasting, postprandial and pre-prandial administration, the C_{max} of d4T following postprandial administration was approximately 50% lower ($p \leq 0.0001$) and t_{max} was delayed by 1 hr ($p \leq 0.0001$). However, values for AUC were similar for all administration schedules indicating that d4T may be taken with or without meals.[26]

At the steady-state, d4T pharmacokinetic were comparable with those observed after a single dose, with no evidence of accumulation.[27] Pharmacokinetic modeling techniques indicate that adjustments in d4T dosage are not required for patients weighing between 40 and 100 kg. It is currently recommended that dosage should be adjusted according to whether patients weigh < 60 kg or ≥ 60 kg.[28]

3.2.2 Metabolism and elimination[25, 27-29]

In vitro study indicates that d4T undergoes intracellular phosphorylation by the enzymes thymidine kinase, thymidylate kinase and pyrimidine nucleoside diphosphate kinase to stavudine-5'-mono-,di- and triphosphate, respectively.

The phosphorylation pathways of d4T and ZDV are probably the same. But d4T has a lower affinity to thymidine kinase, while stavudine monophosphate has higher affinity to thymidylate kinase; thus, the rate-determining step for activation of d4T is the initial conversion to the monophosphate form by thymidine kinase. This results in high intracellular ratios of triphosphorylated drug relative to the other intermediate or unchanged form. In contrast, ZDV is rapidly converted to zidovudine monophosphate; further conversion to the triphosphorylated form is slow, resulting in high intracellular accumulation of zidovudine monophosphate. This process which may be linked to the toxicity of ZDV. In contrast, none of the phosphates of d4T accumulate and the concentration of stavudine-5'-triphosphate is linearly related to that of its parent compound.

In vitro studies, it has been hypothesis that zidovudine-5'-monophosphate may inhibit the production of stavudine-5'-monophosphate (but not vice versa) and therefore the combination of d4T and ZDV may antagonistic. This suggests that a clinical combination of these agents would offer no advantage over ZDV alone.

The elimination half-life ($t_{1/2}\beta$) of d4T was 1.55 $\pm$ 0.24 hr. However, *in vitro* intracellular half-life of the active metabolite, stavudine-5'-triphosphate has been estimated to be 3-3.5 hr in human peripheral blood mononuclear cells.

3.2.3 Effect of hepatic and renal impairment[24, 29]

Dosage adjustments are necessary in the patients with renal impairment (Table 6) as d4T clearance decreased and AUC and $t_{1/2}\beta$ increased as creatinine clearance declined. In patients with hepatic impairment, no initial dosage adjustment is necessary.

Table 6 Stavudine dosage recommendations for patients with normal renal function and renal impairment based on CL_{Cr} [24, 29]

Creatinine clearance	Dosage (mg) and frequency	
	Body weight ≥60kg	Body weight < 60kg
Normal or >3.0 L/h (>50 mL/min)	40 every 12 hr	30 every 12 hr
1.6-3.0 L/hr (26-50 mL/min)	20 every 12 hr	15 every 12 hr
0.6-1.5 L/hr (10-25 mL/min)	20 every 24 hr	15 every 24 hr
Hemodialysis[#]	20 every 24 hr	15 every 24 hr

[#] The recommended dose is to be administered after completion of hemodialysis and at the same time on non-dialysis days

3.2.4 Adolescents, children and infants over 3 months[25, 29, 30]

The pharmacokinetic properties of d4T in children are similar to those in adults except those children need approximately double the dose per unit body weight to achieve similar values for C_{max}. This may be party explained by slightly lower bioavailabity of d4T in children (61 to 78%).

3.3 Pharmacodynamic properties

3.3.1 Mechanism of action[25, 30, 31]

D4T enters cell membranes via non-facilitated diffusion and subsequently phosphorylated by cellular enzymes to monophosphate, diphosphate and triphosphate form. The 5'-triphosphate derivative inhibits HIV reverse transcriptase and hence the incorporation of thymidine-5'-triphosphate into viral DNA. Incorporation of stavudine-5'-triphosphate into DNA results in chain termination because the drug lacks the 3'-hydroxy group found in thymidine which is required to form a 3'-5'-phosphodiester linkage with the next base added. This mechanism of action is similar to that of other nucleoside reverse transcriptase inhibitors (e.g. ZDV and 3TC).

3.3.2 Antiviral activity[25, 30]

In vitro studies have demonstrated the activity of d4T against the HIV and Moloney murine leukemia virus. D4T generally produced 50% viral inhibition (IC_{50}) at

concentrations of around 0.05 to 0.5 μmol/L, with 5- to 10-fold lower potency than ZDV. However, d4T was around 100-fold less potent than ZDV against HIV in the Tall 1 human cell line. The antiviral activity of d4T was proportional to its concentration over the range of 0.0001 to 10.0 μmol/L in human peripheral blood mononuclear cells infected with HIV-1_{LAV}. A similar concentration-dependent effect has been found in human T cell lines. HIV-1 IIIB and HIV-2 LAV-2 were similarly inhibited by d4T and ZDV *in vitro*.

The *in vitro* antiviral activity of nucleoside analogues may not be good measure of *in vivo* efficacy. All *in vivo* studies have evaluated extracellular drug concentrations to investigate anti-HIV activity. However, the 5'-triphosphate of this drug is the active metabolite and it has been shown that the intracellular concentration of reverse trancriptase inhibitors provides a better measure of *in vivo* antiviral activity. Estimations of *in vitro* anti-HIV activity only provide an approximate indication of the potential clinical efficacy of a drug.

3.4 Drug interaction[29, 30]

Since d4T is actively secreted by the renal tubules, interactions with other actively secreted medicinal products are possible, e.g. trimethoprim. No clinical pharmacokinetic interaction has, however, been seen with 3TC.

ZDV may inhibit the intracellular phosphorylation of d4T. ZDV is therefore not recommended to use in combination with d4T. The activation of d4T is inhibited by doxorubicin but not by other medicines used in HIV infection which are similarly phosphorylated, e.g. ddI, ddC, 3TC, ganciclovir, and foscanet.

When administered of d4T and ddI simultaneously, no significant changes in pharmacokinetic parameters were observed for either d4T or ddI. Combination with ddI and d4T in the clinical practice, pancreatitis, peripheral neuropathy and liver function abnormalities occur more frequently. Patients treated with this combination should be closely monitored for signs of liver toxicity.

3.5 Dosage and administration[25, 29]

D4T dosage should be adjusted according to bodyweight and creatinine (Table 6). Patients with normal kidney function should receive 40 mg twice daily if they weigh ≥ 60 kg; this is decreased to 30 mg twice daily for patients < 60 kg. In pediatric patients who weigh ≥ 30 kg should receive adult dosing and decreased to 1 mg/kg twice daily if they weigh < 30 kg. If d4T is taken twice daily it should be at 12-hr intervals. D4T may be taken with or without food. Dosage recommendations for adolescents, children and infants over 3 months are shown in Table 7.

Table 7 Stavudine dosage recommendations for adolescents, children and infants over 3 months

Bodyweight	Dosage (mg) and frequency
< 30kg	1 mg/kg every 12 hr
≥ 30kg	Adult dosing

Patients should be monitored for development of clinically relevant hepatic abnormalities or peripheral neuropathy (usually characterized by persistent numbness, tingling or pain in the feet and/or hands) during d4T treatment. If either condition develops d4T should be discontinued until resolution occurs. D4T-related peripheral neuropathy should resolve if therapy is withdrawn promptly although some patients may experience a temporary worsening of symptoms following discontinuation. If symptoms resolve satisfactorily, resumption of treatment with d4T at half of previous dosage may be considered. Patients with a previous occurrence of, or pre-existing, peripheral neuropathy, either disease– or drug-related, may be more likely to have exacerbation or reoccurrence of peripheral neuropathy during d4T treatment than patients without a history of peripheral neuropathy.

3.6 Tolerability[25, 29, 30]

Many of the serious undesirable effects reported in clinical trials with d4T are consistent with the course of HIV infection or the side effects of concomitant

therapies. Accordingly, it may be difficult to distinguish adverse effects caused by anti-HIV drug treatment from effects of HIV infection and associated disorders. The major clinical toxicity in monotherapy trials is dose-related peripheral neuropathy requiring done modification. This is characterized by painful tingling sensations in the toes and feet, areflexia, mild muscle weakness and distal sensory loss. However, peripheral neuropathy can also be caused by HIV infection. Additionally, d4T and other dideoxynucleosides (such as ddI and ddC) can exacerbate existing peripheral neuropathy

Peripheral neuropathy was graded according to severity:

- Minor discomfort not interfering with daily life (grade 1)
- Analgesics required for pain relief (grade 2)
- Interference with daily life and narcotics required for pain relief (grade 3)
- Obvious physical signs such as foot drop (grade 4)

If patients had grade 1 or 2, d4T was discontinued until resolution, at which time the drug was restarted at half the previous dosage. If grade 3 or 4 peripheral neuropathy occurred, permanent discontinuation of d4T should be recommended.

Incidence of peripheral neuropathy has been reported as 17 and 23 %, respectively, of patients receiving d4T 20 and 40 mg twice daily for 24 weeks. Peripheral neuropathy led to discontinuation (grade 3 and 4, or repeated grade 2) in 11 and 13% of patients.

Pancreatitis, occasionally fatal, has been reported in up to 2-3% of patients in monotherapy clinical studies and occurs more frequently when combined with ddI. Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with d4T as with other nucleoside analogues alone or in combination with other antiretrovirals. Associated symptoms include malaise, nausea, vomiting, abdominal pain, and tachypnea. The mechanism of lactic acidosis is mitochondrial toxicity. Modest elevations in serum transaminases were commonly observed in

controlled trials. Redistribution/accumulation of body fat including central obesity, dorsocervical fat enlargement (buffalo hump), peripheral wasting, facial wasting, breast enlargement, and "cushingoid appearance" have been observed in patients receiving d4T-containing antiretroviral therapy.

Animal data showed embryo-fetal toxicity at very high exposure levels. D4T was genotoxic *in vitro* tests in human lymphocytes possessing triphosphorylating activity, in mouse fibroblasts and *in vivo* test for chromosomal aberrations. Similar effects have been observed with other nucleoside analogues.[29]

D4T was carcinogenic in mice (liver tumors) and rats (liver tumors: cholangiocellular, hepatocellular, mixed hepatocholangiocellular and/or vascular; and urinary bladder carcinomas) at very high exposure levels. No carcinogenicity was noted at dose of 400 mg/kg/day in mice and 600 mg/kg/day in rats, corresponding to exposures about 39 and 168 times the expected human exposure, respectively, suggestion an insignificant carcinogenic potential of d4T in clinical therapy.[29]

4. Lamivudine

4.1 Chemical structure and properties[32]

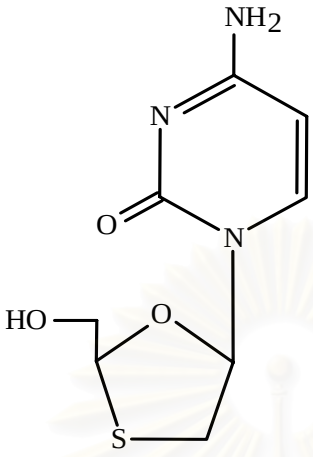


Figure 3 Chemical structure of lamivudine

Lamivudine (also known as 3TC), the negative (*cis*) enantiomer of 2'-doexy-3'-thiacytidine (Figure 3), is a synthetic cytidine 2'-3'-dideoxynucleoside analogue with unnatural 2R, 5S absolute configuration. Like ZDV, ddI, ddC and d4T, 3TC belongs to the nucleoside analogue class of antiretroviral drugs.

4.2 Pharmacokinetic properties

4.2.1 Absorption[33, 34]

Orally administered 3TC is rapidly absorbed and has good systemic bioavailability. The bioavailability of the drug in adults and adolescents was 80 to 88 % and in children were approximately 66 to 68 %. The time to reach C_{max} (t_{max}) is approximately 0.5 to 3 hr after 300 mg single dose. Food delays the peak serum concentration (C_{max}) and t_{max} however, there is no significant difference in area under the serum concentration-time curve (AUC) (indicating systemic drug exposure). Therefore, 3TC may be administered either with food or on an empty stomach, depending on the preference of the patient.

4.2.2 Distribution[33-36]

3TC is widely distributed, crosses the blood-brain barrier and distributed into the cerebrospinal fluid (CSF) to a limited extent. The mean apparent volume of

distribution (V_d/F) of 3TC was 117-139 L after 150 mg twice daily. Distribution of lamvudine at steady- state was dose-independent and did not correlate with body-weight. 3TC penetrates the CSF in adults with HIV infection, but the CSF: serum ratio is lower than has been reported for the other nucleoside analogues. In 6 HIV-infected adults, the mean CSF: serum ratio was 0.06, whereas for ZDV, ddI, ddC and d4T have been reported as 0.4, 0.2, 0.2 and 0.2 respectively.

Table 8 Pharmacokinetic parameters of lamivudine

Parameter	Value, range
F, %	80 - 88
AUC, mg/h·L (2 mg/kg single dose)	4.58
C_{max}, mg/L (2 mg/kg single dose)	1.73
t_{max}, hr	0.5 – 3.0
$t_{1/2}$, hr	6.8 – 7.7
V_d/F, L (150 mg twice daily)	117 – 139
CL/F, L/hr (150 mg twice daily)	23.8 – 26.4

The extent of 3TC plasma protein binding is low (<36%). No data are yet available on the penetration of 3TC into breast milk. Preliminary data suggest that 3TC diffuse freely across the placenta from the maternal circulation to the fetal circulation. In 13 woman and their neonates, 3TC concentrations were similar in the maternal circulation, umbilical cord blood and neonate circulation.

4.2.3 Metabolism and elimination[33, 34, 36]

3TC is metabolized intracellularly to its active 5''-triphosphate metabolite, which inhibits HIV reverse transcriptase activity. The majority of 3TC is eliminated unchanged in the urine (68 to 71%); approximately 5.2% of the trans-sulfoxide metabolite are excreted in the urine within 12 hr. The renal clearance of 3TC is greater than the glomerular filtration rate; indicating that 3TC is predominately eliminated by active renal tubular secretion.

The terminal elimination half-life ($t_{1/2}\beta$) of 3TC was 6.8-7.7 hr in adults after 300 mg single dose. Half-life of the active intracellularly metabolite, lamivudine triphosphate is 11 to 15 hr.

4.2.4 Effect of renal impairment[33, 34, 36, 37]

As 3TC is eliminated primarily as unchanged drug via the kidneys, its pharmacokinetics are significantly affected in patients with renal impairment. In HIV-infected patients with moderate or severe renal impairment, 3TC C_{max}, AUC and $t_{1/2}\beta$ values increased with decreasing renal function. Thus, 3TC dosage modification is required in patents with a creatinine clearance of < 50 mL/min (3 L/hr) as shown in Table 9.

Table 9 Lamivudine dosing recommendation for patients with renal dysfunction

Creatinine clearance (mL/min)	First dose (mg)	Maintenance dose (mg)	Interval
≥50	150	150	every 12 hr
30-49	150	150	every 24 hr
15-29	150	100	every 24 hr
5-14	150	50	every 24 hr
<5	50	25	every 24 hr

Based on dosage recommended for combination therapy

4.3 Pharmacodynamic properties

4.3.1 Mechanism of action[34, 36]

As with other nucleoside analogues, the mechanism of antiviral activity of 3TC is related to its conversion intracellular to the 5'-triphosphate derivative, initially by deoxycytidine kinase and then by other human cellular kinase enzymes. 3TC triphosphate inhibit viral reverse transcriptase by competing with 2'-deoxycytidine-5'-triphosphate for incorporation into HIV DNA. Because lamivudine triphosphate lacks the 3'-hydroxy group required for nucleic acid replication, viral DNA chain elongation is terminated and HIV replication prevented.

In vitro studies with combination of nucleoside analogues showed that 3TC did not affect the phosphorylation of ZDV in peripheral blood mononuclear cells (PBMCs). In contrast, ddC phosphorylation was significantly impaired by 3TC in PBMCs and in MOLT-4 and U937 cell lines. This finding may be clinically important and should be considered when selecting drugs for use in combination.

4.4 Drug interaction[36, 38]

Co-trimoxazole (trimethoprim-sulfamethoxazole; 160/800 mg) once daily increases 3TC exposure (area under the concentration-time curve; AUC). The effect of higher doses of co-trimoxazole on 3TC pharmacokinetics has not been investigated. At doses prescribed to prevent *Pneumocystis carinii* pneumonia (PCP), it is unlikely that co-trimoxazole will cause a significant increase in 3TC concentrations. The effects on 3TC pharmacokinetics at higher doses of co-trimoxazole used to treat PCP need to be investigated. In 14 patients with asymptomatic HIV infection who received a single 300 mg oral dose of 3TC after 4 days' treatment with co-trimoxazole 800/160 mg/day (administered as PCP prophylaxis). There was a 44% increase in 3TC AUC (compared with administration of 3TC alone) from 10.1 to 14.5 mg/L·hr. This was accompanied by a 35% decrease in 3TC CL_R (from 16.6, with 3TC alone, to 10.8 L/hr), which probably because of competitive inhibition of renal tubular secretion of 3TC by trimethoprim. Nevertheless, the increase of systemic exposure to 3TC was not associated with an increase in concentration dependent toxicity of the drugs. Whether the interaction between 3TC and co-trimoxazole is of greater clinical importance in individuals receiving the higher dosages of co-trimoxazole used to treat PCP requires further investigation. The pharmacokinetics co-trimoxazole 800/160mg daily were unaffected by administration of a single 300 mg dose of 3TC.

In a randomized, single center, open-label, crossover study, no significant differences were seen in area under the concentration-time curve (AUC) or total clearance for 3TC or ZDV when the two drugs were administered together to 12 asymptomatic HIV-positive adult patients. Dosage modification is not necessary when these 2 drugs are administered in combination. Drug interaction studies have also shown no clinically significant interaction between 3TC and d4T.

3TC and ddC may inhibit the intracellular phosphorylation of one another, increasing 3TC and ddC exposure. The concurrent administration of ddC and 3TC is not recommended.

4.5 Dosage and administration[33, 34, 36]

The recommended dosage of 3TC for adults and adolescents (aged over 16 years) is 150 mg twice daily in combination with other antiretrovirals. 3TC should be administered in combination with other antiretrovirals and is not recommended as monotherapy.

The recommended dosage of 3TC for infants and children (aged 3 months to 16 yr) is 4 mg/kg twice daily (maximum 150 mg twice daily). As in adults and adolescents, it is recommended that 3TC is administered in combination with other antiretrovirals and the drug should not be given as monotherapy. The dosage of 3TC should be reduced to 2 mg/kg twice daily in neonates (younger than 3 months).

As the systemic clearance of 3TC decreases with declining renal function, the dosage of 3TC should be adjusted according to creatinine clearance values as in Table 9.

4.6 Tolerability[33, 34, 36]

Lactic acidosis and severe hepatomegaly with steatosis, including fatal cases, have been reported with the use of nucleoside analogs, including 3TC, alone or in combination. A majority of these cases have been in women. 3TC is given in combination with other antiretroviral agents. Some side effects, such as pancreatitis, peripheral neuropathy and hematologic abnormalities may be seen with other antiretroviral agents, such as ZDV, and/or severe HIV disease; therefore, differentiation between the side effects of 3TC and other medications or the complications of HIV disease may be difficult. 3TC appears to be better tolerated than ZDV, with gastrointestinal adverse events and neutropenia observed less frequently. Diarrhea,

malaise, fatigue and headache were the most common adverse events reported during treatment with 3TC monotherapy in dosage ranging from 0.5 to 2 mg/kg/day.

Other adverse events reported in > 15% of 3TC treatment included nausea, vomiting and abdominal discomfort. Peripheral neuropathy, arthalgia, myalgia, skin rash and pruritus were reported less frequently. Pancreatitis (nausea; vomiting; severe abdominal or stomach pain) has occurred more frequent in children.

5. Nevirapine

5.1 Chemical structure and properties[39, 40]

Nevirapine (NVP) is a NNTRI with activity against HIV-1. NVP is structurally a member of dipyridodiazepinone chemical class of compounds. The chemical name of NVP is 11-cyclopropyl-5,11-dihydro-4-methyl-6H-dipyrido (3,2-b:2',3'-)(1,4) diazepin-6-one. NVP is a white to off-white crystalline powder with the molecular weight of 266.3 and the molecular formula $C_{15}H_{14}N_4O$. It is a low-molecular-weight compound which is lipophilic (partition coefficient = 83) and a weak base (pKa = 2.8). It is highly soluble at pH < 3, but Its aqueous solubility decreases to approximately 0.1 mg/mL at neutral pH. NVP has the following structural formula:

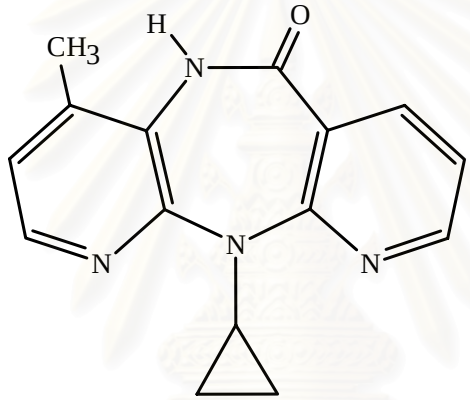


Figure 4 Chemical structure of nevirapine

5.2 Pharmacokinetic properties

5.2.1 Absorption[39-42]

NVP is readily absorbed (> 90%) after oral administration in healthy volunteers and in adults with HIV-1 infection. Peak plasma NVP concentrations of 2±0.4 mg/L were attained by 4 hr following a 200-mg single dose. Following multiple doses, NVP peak concentrations appear to increase linearly in the dose range of 200 to 400 mg/day. Steady-state trough NVP concentrations of 4.2 ± 1.4 mg/L were attained at 200 mg twice daily. When NVP (200 mg) was administered to 24 healthy adults (12 female, 12 male), with either a high fat breakfast or an antacid, the extent of NVP absorption (AUC) was comparable to that observed under fasting conditions. NVP may be administered with or without food, antacid or ddI.

5.2.2 Distribution[39, 42-44]

NVP is highly lipophilic and is essentially non-ionized at physiologic pH. The apparent volume of distribution (V_{dSS}) of NVP was 1.21±0.09 L/kg, suggesting that NVP is widely distributed in humans. NVP readily crosses the placenta and is distributed into breast milk. NVP is about 60% bound to plasma proteins in the plasma concentration range of 1-10 mg/L. NVP concentrations in human cerebrospinal fluid were 45% (±5%) of the concentrations in plasma; this ratio is approximately equal to the fraction not bound to plasma protein. NVP concentrations in seminal plasma are dependent on the time after ingestion. NVP achieve therapeutic concentrations in both the testes and prostate and the seminal vesicles throughout the dosing interval.

Table 10 Pharmacokinetic parameters of nevirapine after 200 mg twice daily

Parameter	Value, range or mean ± SD
F, %	> 90
AUC, mg/h·L	61.98 ± 13.56
C_{max}, mg/L	7.0 ± 1.2
t_{max}, hr	4.0
$t_{1/2}$, hr	25 – 30
V_d/F, L/kg	1.21 ± 0.09
CL/F, L/hr	3.36 ± 0.68

5.2.3 Metabolism an elimination[39, 42, 45]

In vivo studies in humans and *in vitro* studies with human liver microsomes have shown that NVP is extensively biotransformed via cytochrome P450 (oxidative) metabolism to several hydroxylated metabolites. *In vitro* studies with human liver microsomes suggest that oxidative metabolism of NVP is mediated primarily by cytochrome P450 isozymes from the CYP3A family, although other isozymes may have a secondary role. Cytochrome P450 metabolism, glucuronide conjugation, and urinary excretion of glucuronidated metabolites represent the primary route of NVP

biotransformation and elimination in humans. Renal excretion plays a minor role in elimination of the parent compound.

NVP has been shown to be an inducer of hepatic cytochrome P450 metabolic enzymes. The pharmacokinetics of autoinduction are characterized by an approximately 1.5 to 2 fold increase in the apparent oral clearance of NVP as treatment continues from a single dose to two-to-four weeks of dosing with 200-400 mg/day. Auto-induction also results in a corresponding decrease in the terminal phase half-life of NVP in plasma from approximately 45 hr (single dose) to approximately 25-30 hr following multiple dosing with 200-400 mg/day.

5.2.4 Pharmacokinetic in special populations[39, 42]

Renal/Hepatic Dysfunction: The pharmacokinetics of NVP has not been evaluated in patients with either renal or hepatic dysfunction.

Race: An evaluation of NVP plasma concentrations (pooled data from several clinical trials) from HIV-1-infected patients (27 Black, 24 Hispanic, 189 Caucasian). No marked difference in NVP steady-state trough concentrations (median $C_{min}ss$ = 4.7 mg/L Black, 3.8 mg/L Hispanic, 4.3 mg/L Caucasian) with long-term NVP treatment at 400 mg/day. However, the pharmacokinetics of NVP has not been evaluated specifically for the effects of ethnicity.

Geriatric Patients: NVP pharmacokinetics in HIV-1 infected adults do not appear to change with age (range 18 to 68 yr); however, NVP has not been extensively evaluated in patients older than 55 yr of age.

Pediatric Patients: After a single dose administration (7.5 mg, 30 mg or 120 mg per m^2; n=3 per dose), the mean NVP apparent clearance adjusted for body weight was greater in children compared to adults. In a multiple dose study, NVP suspension or tablets (240 or 400 mg/m^2/day) were administered as monotherapy or in combination with ZDV or ZDV+ddI to 37 HIV-1-infected pediatric patients with median

age of 11 months (range: 2 months-15 yr). The majority of these patients received 120 mg/m^2/day of NVP for approximately 4 weeks followed by 120 mg/m^2/b.i.d. (patients >9 yr of age) or 200 mg/m^2/b.i.d. (patients =9 yr of age). NVP apparent clearance adjusted for body weight reached maximum values by age 1 to 2 yr and then decreased with increasing age. NVP apparent clearance adjusted for body weight was at least two-fold greater in children younger than 8 yr compared to adults. The pediatric dosing regimens were selected in order to achieve steady-state plasma concentrations in pediatric patients that approximate those in adults.

5.3 Pharmacodynamic properties

5.3.1 Mechanism of action[39, 42]

NVP is a non-nucleoside reverse transcriptase inhibitor (NNRTI) of HIV-1. NVP binds directly to reverse transcriptase (RT) and blocks the RNA-dependent and DNA-dependent DNA polymerase activities by causing a disruption of the enzyme's catalytic site. The activity of NVP does not compete with template or nucleoside triphosphates. HIV-2 RT and eukaryotic DNA polymerases (such as human DNA polymerases α, β, γ or δ) are not inhibited by NVP.

5.3.2 *In vitro* HIV susceptibility[39, 42]

The *in vitro* antiviral activity of NVP was measured in peripheral blood mononuclear cells, monocyte derived macrophages, and lymphoblastoid cell lines. IC_{50} values (50% inhibitory concentration) ranged from 10-100 nM against laboratory and clinical isolates of HIV-1. In cell culture, NVP demonstrated additive to synergistic activity against HIV-1 in drug combination regimens with ZDV, ddI, d4T, 3TC, saquinavir, and indinavir. The relationship between *in vitro* susceptibility of HIV-1 to NVP and the inhibition of HIV-1 replication in humans has not been established.

5.4 Drug interaction

Nucleoside analogues: No dosage adjustments are required when NVP is taken in combination with ZDV, ddI, or ddC. Results from studies in HIV-1 infected patients who were administered NVP with different combinations of ddI or ddC, on a

background of ZDV therapy, indicated that no clinically significant pharmacokinetic interactions occurred when the nucleoside analogues were administered in combination with NVP.[39]

Protease Inhibitors: amprenavir, indinavir, nelfinavir, ritonavir and saquinavir When NVP is concurrently administered with a protease inhibitor, as the plasma concentrations of the protease inhibitors may be reduced to subtherapeutic concentrations due to increased hepatic metabolism by NVP. NVP decreases the AUC and C_{max} of indinavir, saquinavir and ritonavir; NVP and nelfinavir do not appear to interact significantly. In contrast, protease inhibitors do not appear to affect pharmacokinetics of NVP.[39, 42, 46]

Ketoconazole: NVP and ketoconazole should not be administered concomitantly. Ketoconazole AUC and C_{max} decreased by a median 63% (95%CI -95, +33) and 40% (95%CI -52, +11), respectively. Comparison of the pharmacokinetics from this study to historical data suggested that coadministration with ketoconazole may result in a 15-30% increase in NVP plasma concentrations. The clinical significance of this observation is not known.[39, 42]

Rifabutin: Rifabutin accelerates the metabolism of non-nucleoside reverse transcrip-tase inhibitors (NNRTIs), such as NVP through induction of P450 cytochrome isoenzymes, resulting in subtherapeutic levels of NVP. In addition NVP retards the metabolism of rifabutin, resulting increased serum levels of rifabutin; a dosage adjustment may be required when rifabutin is administered with NVP.[42]

Rifampin: Rifampin accelerates the metabolism of NNRTIs, such as NVP through induction of hepatic P450 cytochrome isoenzymes, resulting in subtherapeutic levels of NVP. In addition NVP retards the metabolism of rifampin, resulting in increased serum levels of rifampin and the likelihood of increased toxicity; data are insufficient to assess whether dose adjustments are necessary when rifampin is

coadministered with NVP. Concurrent use of NVP with rifampin should be considered only if clearly indicated and with careful monitoring.[42, 47]

Cimetidine and macrolides antibiotic: Monitoring of NVP plasma concentrations in patients who received long-term NVP treatment indicate that steady-state NVP trough plasma concentrations were elevated in patients who received cimetidine (+21%, n=11) and macrolides (+12%, n=24), known inhibitors of CYP3A.[42]

Contraceptives and estrogen-containing: There are no clinical data on the effects of NVP on the pharmacokinetics of oral contraceptives. NVP may decrease plasma concentrations of oral contraceptives (also other hormonal contraceptives); therefore, these drugs should not be administered concomitantly with NVP.[42]

Methadone: Based on the known metabolism of methadone, NVP may decrease plasma concentrations of methadone by increasing its hepatic metabolism. Narcotic withdrawal syndrome has been reported in patients treated with NVP and methadone concomitantly. Methadone-maintained patients beginning NVP therapy should be monitored for evidence of withdrawal and methadone dose should be adjusted accordingly.[42, 48]

St. John's wort: Concurrent use of St. John's wort (*Hypericum Perforatum)* or St. John's wort containing products with NVP is expected to substantially decrease NVP concentrations and may result in suboptimal levels of NVP. Possible lead to loss of virologic response and possible resistance to NVP; concurrent use is not recommended.[42]

5.5 Dosage and administration[39, 42]

Adults:

The recommended dose for NVP is one 200 mg tablet daily for the first 14 days (this lead-in period should be used because it has been found to lessen the

frequency of rash), followed by one 200 mg tablet twice daily, in combination with antiretroviral agents.

Pediatric patients:

The recommended oral dose of NVP for pediatric patients 2 months up to 8 yr of age is 4 mg/kg once daily for the first 14 days followed by 7 mg/kg twice daily thereafter. For patients 8 yr and older the recommended dose is 4 mg/kg once daily for two weeks followed by 4 mg/kg twice daily thereafter. The total daily dose should not exceed 400 mg for any patient.

5.6 Tolerability[3, 39, 42]

Clinical practice has shown that the most serious adverse reactions associated with NVP are clinical hepatitis/hepatic failure, Stevens-Johnson syndrome, toxic epidermal necrolysis, and hypersensitivity reactions. Clinical hepatitis/hepatic failure may be isolated or associated with signs of hypersensitivity which may include severe rash or rash accompanied by fever, general malaise, fatigue, muscle or joint aches, blisters, oral lesions, conjunctivitis, facial edema, and/or hepatitis, eosinophilia, granulocytopenia, lymphadenopathy, and renal dysfunction. Severe and life-threatening hepatotoxicity, and fatal fulminant hepatitis have been reported in patients treated with NVP. Hepatic adverse events have been reported to occur more frequently during the first 12-16 weeks of treatment, but such events may occur at any time during treatment.

In clinical trials, the risk of clinical hepatic events with NVP at 1 yr was approximately 2- fold that of placebo. Approximately one-half of these events occurred within the first 12-16 weeks of treatment. Increased SGOT or SGPT levels and/or seropositivity for hepatitis B and/or C, and CD4+ cell count >350 cells/mm^3, were associated with a greater risk of hepatic adverse events for both NVP and control groups. The risk of hepatic events at 1 yr of NVP treatment was less than 2% among patients who were hepatitis B and/or C negative. It appears that women may be at higher risk for NVP-associated hepatic events.

The most common clinical toxicity of NVP is rash. Severe or life-threatening rash occurred in approximately 2% of NVP-treated patients, most frequently within the first 6 weeks of therapy. Rashes are usually mild to moderate, maculopapular erythematous cutaneous eruptions, with or without pruritus, located on the trunk, face and extremities. Women tend to be at higher risk for development of NVP associated rash.

Liver function test abnormalities (SGOT, SGPT) were observed more frequently in patients receiving NVP than in controls. Asymptomatic elevations in GGT occur frequently but are not a contraindication to continue NVP therapy in the absence of elevations in other liver function tests. Other laboratory abnormalities (bilirubin, anemia, neutropenia, thrombocytopenia) were observed with similar frequencies in clinical trials. Because clinical hepatitis has been reported in NVP-treated patients, intensive clinical and laboratory monitoring, including liver function tests is essential at baseline and during the first 12-16 weeks of treatment. Monitoring should continue at frequent intervals thereafter, depending on the patient's clinical status.

CHAPTER III

PATIENTS AND METHODS

The study was conducted from February 2003 to December 2003 at Bamrasnaradura Infectious Disease Institute, Nonthaburi, Thailand.

1. Patients

Twenty HIV-infected patients were recruited for this study based on the following criteria:

1.1 Inclusion criteria

The patients who had all of these characteristics were enrolled in this study.

1. An aged 18-45 yr.
2. Antiretroviral-naïve patients.
3. Body weights less than 60 kg and body mass index (BMI) between 18-24 kg/m^2.
4. CD4 count between 50-200 $cells/mm^3$.
5. Hemoglobin not less than 9 g/dL.
6. Platelet not less than 75,000 $cells/mm^3$.
7. SGOT/SGPT/ALP not more than 3 times of upper limit o normal (ULN).
8. Serum creatinine not more than 1.5 times of ULN.
9. All patients consented to enroll in the study.

1.2 Exclusion Criteria

The patients who had either one of these characteristics were excluded from this study.

1. The patients had gastrointestinal disease that effect to drug absorption.
2. The patients currently smoking or smoking cessation less than 1 month prior enroll to this study.
3. The patients had drug addiction, chronic alcoholism or alcoholic cessation less than 2 weeks.
4. The patients had immunologic stimulant within 1 month.

5. The patients had active opportunistic infection.
6. The patients were pregnancy or lactation.
7. The patients had known hypersensitivity to d4t, 3TC or NVP.
8. The patients had history of pancreatitis, significantly impaired liver and/or renal function.
9. The patients had currently peripheral neurophathy.
10. The patients received myelosuppressive, neurotoxic, pancreatoxic, hepatotoxic or cytotoxic within 1 month.
11. The patients were diagnosed from physicians to be inappropriate to enroll in this study.

1.3 Sample Size Determination

Sample size was calculated from the equation as followed:[49]

$$n \geq \left(\frac{\sigma}{\Delta}\right)^2 (Z_\alpha + Z_\beta)^2$$

When n = sample size

$Z_{\alpha, 0.05}$ (two-tailed) = 1.96

$Z_{\beta, 0.20}$ (one-tailed) = 0.84

σ = Standard deviation of AUC

Δ = Difference between 2 groups, 20 % of AUC

Since GPO-vir S30 is the combined of three drugs, so sample size determination are calculated from each drug data as followed:

1) As Murphy et al.[46] had studied the interaction of NVP and indinavir. They had reported mean AUC of NVP was 61.98±13.56 h·mg/L and mean C_{max} was 7±1.2 mg/L.

Calculation from AUC; $n \geq \left(\frac{13.56}{0.2 \times 61.98}\right)^2 (1.96 + 0.84)^2$

$$\geq \quad 9.38 \quad \cong \quad 10$$

Calculation from C_{max}; $n \geq \left(\frac{1.2}{0.2x7} \right)^2 (1.96 + 0.84)^2$

$$\geq \quad 5.76 \quad \cong \quad 6$$

2) Horton et al.[50] had reported mean $AUC_{0-\infty}$ of 3TC was 11.187±1.796 h·mg/L and C_{max} was 3.2835±0.6308 mg/L.

Calculation from AUC; $n \geq \left(\frac{1.796}{0.2x11.187} \right)^2 (1.96 + 0.84)^2$

$$\geq \quad 5.05 \quad \cong \quad 6$$

Calculation from C_{max}; $n \geq \left(\frac{0.6308}{0.2x3.2835} \right)^2 (1.96 + 0.84)^2$

$$\geq \quad 7.23 \quad \cong \quad 8$$

3) In the study of Grasela et al.[24] found that mean $AUC_{0-\infty}$ of d4T was 18.64±1.73 h·mg/L and mean C_{max} was 0.836±0.196 mg/L.

Calculation from AUC; $n \geq \left(\frac{1.73}{0.2x18.64} \right)^2 (1.96 + 0.84)^2$

$$n \geq \quad 1.69 \quad \cong \quad 2$$

Calculation from C_{max}; $n \geq \left(\frac{0.196}{0.2x0.836} \right)^2 (1.96 + 0.84)^2$

$$n \geq \quad 10.77 \quad \cong \quad 11$$

The number of subjects, at least 11 patients, was needed in this study. However, several others studies had reported larger variation among subject, which

mean that a larger number of subjects are required. This study decided in a number of twenty subjects to include in the study.

2. Study design

This study was designed as an opened-label, randomized, 2-way crossover trial to compare the pharmacokinetic of d4T, 3TC and NVP between combined formulation, GPO-vir S30 and the three single original brands. The protocol was reviewed and approved by the reviewed board at Bamrasnaradura Infectious Disease Institute and the ethic committee of the Ministry of Public Health, Thailand. The subjects of this study were selected from a group of HIV-infected patients at Bamrasnaradura Infectious Disease Institute. Written informed consent had been given.

3. Drug administration and sampling

Twenty HIV-infected patients who met the inclusion criteria were participated in this study. In the first-two weeks, lead-in period of NVP, all patients received d4T (Stavir; GPO, Thailand) 30 mg every 12 hr, 3TC (Lamivir; GPO, Thailand) 150 mg every 12 hr and NVP (Neravir; GPO, Thailand) 200 mg once daily for 2 weeks. This is the lead-in period of NVP, due to NVP autoinducer. Recommended dose was 200 mg once daily for 2 weeks and then 200 mg twice daily.

After lead-in period of NVP, patients were divided into two groups; ten of them received one combined tablet of d4T, 3TC and NVP (GPO-vir S30) every 12 hr. The second group received three single original brands, Zerit 30 mg, Epivir 150 mg and Viramune 200 mg every 12 hr for 2 weeks. Plasma samples of period I were collected in day 14. In the Period II, first group was switched to receive three single original tablets and the second group switched to receive combined tablets. Plasma samples collections of period II were conducted in day 28.

On the day of study, all patients had to fast at least 8 hr before and 2 hr after dose. Drug administration was according to randomization, with 200 mL of water, at approximately 8.00 a.m. Concurrent medication was ingested 2 hr after ingestion of

studied drug during breakfast. Series of plasma samples were collected pre-dose and at 15, 30, 45 min, 1, 1.5, 2, 4, 8 and 12 hr post-dose on day 14. Sample collections in period II were conducted on day 28 in the same manner as those on day 14.

Blood samples were collected in 6-mL heparinized tube and centrifuged at 3000 rpm for 10 min. The plasma portion were separated and stored at –20°C until analysis. Evaluation of patient adherence was performed by drug accountability, interview and patient self-report.

3.1 Products

Test product

Trade name : GPO-vir S30
Manufacturer : Government Pharmaceutical Organization
Dosage form : Tablet
Ingredient : Stavudine (d4T) 30 mg, Lamivudine (3TC) 150 mg and Nevirapine (NVP) 200 mg

Reference product 1

Trade name : Zerit
Manufacturer : Bristol-Myers Squibb
Dosage form : Capsule
Ingredient : Stavudine (d4T) 30 mg

Reference product 2

Trade name : Epivir
Manufacturer : Glaxo Wellcome
Dosage form : Tablet
Ingredient : Lamivudine (3TC) 150 mg

Reference product 3

Trade name : Viramune

Manufacturer : Boehringer Ingelheim

Dosage form : Tablet

Ingredient : Nevirapine (NVP) 200 mg

4. Clinical follow-up

A medical history and physical examination were completed at the screening visit and at weeks 2, 4, 6, 10, 14, 18. Information regarding adverse events and HIV-related illness was collected at each visit. Plasma HIV-RNA was quantitated with the Amplicor HIV-1 Monitor assay from specimen collected twice at baseline and weeks 18. CD4 cell counts, routine hematology, urinalysis and chemistry testing were done at baseline and at weeks 18. LFTs were also done at baseline, weeks 2 and weeks 18.

5. Bioanalysis of d4T, 3TC and NVP

Quantitative analyses of d4T, 3TC and NVP in plasma samples had been developed and validated using HPLC method described by previous study as followed:[51-54]

5.1 Chemicals and reagents

Acetonitrile (Labscan Asia Co., Ltd., Thailand)

Ammonium acetate (Merck. Inc., Germany)

Clozapine (Medipharma (Lot No. 20000905))

Lamivudine (Cadila Healthcare Ltd., India (Lot No. R3-45/00655))

Methanol (Labscan Asia Co., Ltd., Thailand)

Nevirapine (Aurobino Pharma Ltd., India (Lot No. R3-45/00763))

Perchloric acid (Merck. Inc., Germany)

Sodium dihydrogenphosphate dihydrate ($NaH_2PO_4 \cdot 2H2O$) (Merck.Inc., Germany)

Stavudine (Samchully Pharm. Co., Ltd., Korea (Lot No. r3-45/00723))

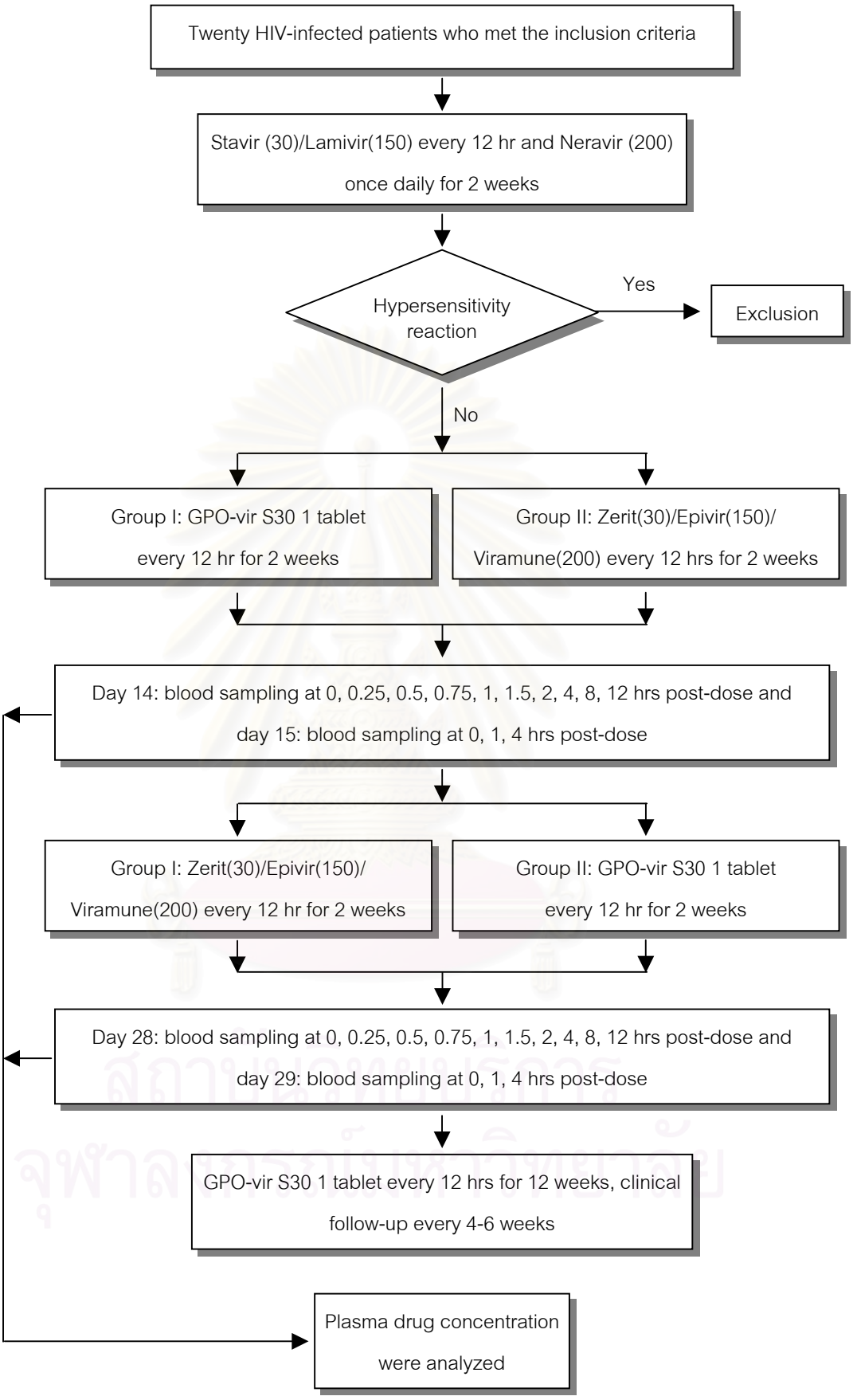


Figure 5 Flow chart of the study

5.2 Determination of d4T and 3TC in plasma

Instruments

High Performance Liquid Chromatography (HPLC) instrument

Shimadzu, Japan

Chromatographic conditions

Column : Hypurity Aquastar C_{18} 5 μm, 4.6x250 mm

Mobile Phase : 10 mM NaH_2PO_4 buffer (pH 6.9): acetonitrile (98:2 v/v)

Internal standard : Bromouracil

Flow Rate : 1 mL/min

Detector : UV 266 nm

Injection Volume : 20 μl

Temperature : Room temperature

5.3 Determination of NVP in plasma

Instruments

High Performance Liquid Chromatography (HPLC) instrument

Hitachi, Japan

Chromatographic conditions

Column : Apollo C_{18} 5 μm, 4.6x250 mm

Mobile Phase : 20 mM NaH_2PO_4 buffer (pH 3.6): acetonitrile (70:30 v/v)

Internal standard : Clozapine

Flow Rate : 1 mL/min

Detector : UV 240 nm

Injection Volume : 100 μl

Temperature : Room temperature

5.4 Validation of HPLC method

5.4.1 Standard preparation

The initial stock solution (S) of d4T, 3TC and NVP (0.5, 0.5 and 1.0 mg/mL, respectively) were prepared in methanol. This solution was appropriately diluted in purified water for the preparation of working solutions (WS).

A stock solution of the internal standard (S_{IS}) for NVP assay; clozapine was prepared in methanol to yield a final concentration of 2 mg/mL and diluted with 50 % methanol to prepare working solution (W_{IS}) 35 mg/L. The internal standard of d4T and 3TC assay; bromouracil was prepare in purified water at concentration of 50 mg/L and diluted with purified water to prepare working solution 12.5 mg/L. Each solution was stored at -20°C. For preparation of the plasma standard samples an appropriate amount of the working solutions and the internal standard were added to blank plasma to achieve the mentioned range of calibration concentrations.

5.4.2 Preparation of calibration curve

NVP assay: A 500-μl of plasma sample was transferred to 1.5-mL eppendorf tube. Fifty microliter of NVP working solution were added to yield seven concentrations 100, 250, 500, 1000, 5000, 7500 and 10000 ng/mL (Table 11). After vortexing for 10 sec, the sample is then heated at 60°C for 1 hr. Spike with 50 μl of W_{IS} (clozapine). One hundred microliter of 13% perchloric acid was added for protein precipitation and vortex for 20 sec followed by centrifugation at 6000 rpm for 15 min. The supernatant was transferred to a 200-μl autosampler vial insert and 100 μl was injected into the HPLC system.

D4T and 3TC assay: Pipet 1000 μl of plasma into a 1.5-mL eppendorf tube, spike with 100 μl of WS for calibration curve to yield seven level concentrations as shown in Table 11, and vortex for 10 sec. The sample is then heated at 60°C for 1 hr followed by centrifugation at 6000 rpm for 15 min.

Table 11 Concentrations of spiked plasma for calibration curve

Calibration No.	Conc. of NVP in spiked plasma (ng/mL)	Conc. of d4T in spiked plasma (ng/mL)	Conc. of 3TC in spiked plasma (ng/mL)
1	100	50	50
2	250	100	100
3	500	250	500
4	1000	500	1000
5	5000	1000	2000
6	7500	1500	3500
7	10000	2000	5000

Extraction cartridges (Water Oasis HLB 1 cc 30 mg) were placed on a vacuum elution manifold and rinse with 1 mL of methanol follow by 1 mL of purified water. Nine hundred and fifty microliter of the spiked plasma sample was then load onto the solid phase extraction (SPE) cartridges and vacuum applied. The cartridges were then wash with 3 mL of purified water follow by vacuum suction for 1 min. Water-acetonitrile (1 mL, 80:20, v/v) was used to elute the analytes. The eluted solution was mixed well, spiked with 100 μl of internal standard and evaporated to dryness under gentle stream of nitrogen gas at 40°C. The residue were reconstituted with 120 μl of 2% acetonitrile. Transfer the solution to 150-μl autosampler vial insert and 20 μl was injected into the HPLC system.

5.4.3 Preparation of quality control samples

Working solution for quality control samples were prepared by dilute appropriately standard stock solution of NVP, d4T and 3TC in purified water. On each validation day, QC samples were prepared at each level as shown in Table 12 by the same procedure as the preparation of calibration curve described above.

Table 12 Concentrations of spiked plasma for quality control samples

QCs No.	Conc. of NVP in spiked plasma (ng/mL)	Conc. of d4T in spiked plasma (ng/mL)	Conc. of 3TC in spiked plasma (ng/mL)
LLOQ	100	50	50
QCL	200	100	100
QCM	2000	750	1500
QCH	9000	1750	4000

5.5 Calibration curve and linearity

Quantitative analysis of d4T, 3TC and NVP was performed using the internal standard method. The calibration curves were obtained by weighted (1/concentration squared) least squares linear regression of the peak area ratio of d4T, 3TC and NVP to IS versus concentrations of d4T, 3TC and NVP, respectively. The calibration was established over the range of 50-2000 ng/mL for d4T, 50-5000 ng/mL for 3TC and 100-10000 ng/mL for NVP.

5.6 Specificity and selectivity

In order to evaluate levels of endogenous compounds with potential for interference with the analytical method, analysis of six different blank samples was performed.

5.7 Limit of quantification

For the concentration to be accepted as the lowest limit of quantification (LLOQ) the percent deviation from the nominal concentration (relative deviation; RD, measure of accuracy) and the coefficient of variation (CV, measure of precision) has to be less than 20%.

5.8 Accuracy , precision , linearity and recovery

Intra-day accuracy and precision of the method were determined by measuring five replicate QC samples (QCs) at four different concentrations (LLOQ: lower limit of quantification, QCL: low concentration QCs, QCM: medium concentration QCs, QCH: high concentration QCs) of NVP (100, 200, 2000, 9000 ng/mL), d4T, (50, 100, 750, 1750 ng/mL) and 3TC (50, 100, 1500, 4000 ng/mL), respectively. To obtain the inter-day accuracy and precision, each concentration was analyzed at three different days. Accuracy was calculated as the relative deviation of the nominal concentration. The mean value should be within 15 % of the actual value except at LLOQ, where it should not deviate by more than 20 %. Precision was expressed in terms of coefficient of variation (CV) for each test concentration. The precision determined at each concentration level should not exceed 15 % of the CV except for the LLOQ, where it should not exceed 20 % of the CV. Daily standard curves were evaluated by analysis of seven concentrations of spiked plasma samples for each drug.

The recovery of each drug in the extraction procedure was determined comparing the detected concentrations of each drug at concentrations of QCL, QCM and QCH in three replicate extracted spiked plasma samples to those of non-processed standard solutions including internal standard. Recovery should be in range of 80-120%.

5.9 Analysis of patient samples

Plasma samples derived from 20 HIV-infected patients during antiretroviral therapy with combination of d4T, 3TC and NVP. A total of n=400 plasma samples from all patients were obtained by a standardized procedure. Plasma was separated by centrifugation at 3000 g for 10 min and viro-inactivated at 60°C for 1 hr before stored at -20°C until further analysis.

5.10 Stability of spiked plasmas and extracts

Triplicate of two concentrations (QCL and QCH) of d4T, 3TC and NVP in plasma were prepared and stored under various conditions. The stability was evaluated as percentage of d4T, 3TC and NVP remaining at each condition relative to the concentration after fresh preparation. The stability was performed under the conditions as followed:

5.10.1 Stability of plasma samples kept frozen at -20°C

5.10.2 Stability of plasma samples after three freeze–thaw cycles

5.10.3 Stability of plasma samples left at room temperature for 6 hr

5.10.4 Stability of final extract samples at room temperature (in autosampler), 4°C for 12 hr and –20°C for 48 hr.

5.11 In-study validation

In the analysis of plasma samples in each analytical run should compose of drug-free plasma, spiked calibration curve (at least 5 concentration levels), one of QC sample (OQL, OQM and QCH). Plasma samples from each patient, period I and II were analyzed in the same analytical run.

6. Statistical analysis

The data of general characteristics and information obtained from the subjects including laboratory data were recorded and analyzed using descriptive statistics.

The data composed of 10 concentrations at various times after drug administered, 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12 hr. The pharmacokinetic parameters of d4T, 3TC and NVP were derived as followed; elimination rate constant (K_e), apparent volume of distribution (apparent V_d), half-life ($t_{1/2}$), apparent clearance (apparent CL), area under the concentration-time curve ($AUC_{0\text{-}12hr}$), maximum concentration (C_{max}) and time to reach the maximum concentration (t_{max}) using pharmacokinetic program.

Comparisons of each parameter of d4T, 3TC and NVP between GPO-vir S30 and the reference products were performed by analysis of variance (ANOVA). The statistical significance was determine at $p<0.05$. The equivalence of two products was assessed. The 90% confidence interval of $AUC_{0\text{-}12hr}$ and C_{max} in log-transformed data was constructed for the ratio of two products. The local products, GPO-vir S30 would be concluded to be equivalent when the 90% confidence interval for the ratio of two products is in the range of 0.8 to 1.25.

Safety and efficacy of GPO-vir S30 was followed up after 18 weeks. Plasma HIV-RNA and CD4 cell count indicate the efficacy of therapy. Goals of therapy were HIV-RNA less than 50 copies/mL and increasing of CD4 cell count. Routine hematology, urinalysis and chemistry testing were performed to monitor safety of therapy.

CHAPTER IV

RESULTS AND DISCUSSION

1. Study population

During February 2003 to December 2003, twenty patients who met the inclusion criteria were enrolled in this study at Bamrasnaradura Infectious Disease Institute. All patients signed their consent to participate in the study. All twenty patients received the antiretroviral d4T/3TC/NVP regimen. After lead-in period of NVP, they were divided into two groups; each group composed of ten patients. The first group received one combined tablet of d4T, 3TC and NVP (GPO-vir S30) every 12 hr. The second group received three single original brand tablets, Zerit 30 mg, Epivir 150 mg and Viramune 200 mg every 12 hr. After two weeks, the first period blood samples were collected. The drug products were then switched, and the second period samples were collected after crossover for another two weeks. After blood sampling for pharmacokinetic study was finished, safety and efficacy of GPO-vir S30 were further followed for 12 weeks.

Demographic data

Of the 20 patients enrolled in the study, 14 patients (70%) were female, the range of age 22-44 yr and the mean age was 32.25 $\pm$ 6.70 yr (mean $\pm$ SD). Most of the patients (85%) had acquired HIV infection through heterosexual contact. Mean hematocrit level was 36.25 $\pm$ 5.05 percentage, which was within the normal range value. White blood cell count showed the mean which was 4.39 $\pm$ 1.49 ($x10^3$ cell/mm^3), the low white blood cell count levels were commonly found in HIV-infected patients. Both blood urea nitrogen (BUN) and creatinine were within the normal range with the mean values equaled to 11.00 $\pm$ 2.76 mg/dL and 0.80 $\pm$ 0.17 mg/dL, respectively. Laboratory data of all patients, i.e., ALP, SGOT, SGPT and serum lactates were also within the normal ranges. Mean baseline CD4 cell count was 107 $\pm$ 43 cells/mm^3 and mean viral load was 268,258 copies/mL (5.24 $\log_{10}$). Demographic data were shown in Table 13.

Table 13 Demographic data of patients enrolled into the study

Characteristic	Frequency, mean±SD (range)	
Gender, n (%)		
Male	6 (30)[#]	
Female	14 (70)[#]	
Risk factors, n (%)		
Heterosexual	17 (85)[#]	
Homosexual	2 (10)[#]	
Injection drug use	1 (5)[#]	
Age, yr	32.25 ± 6.70	(22-44)
BMI, kg/m^2	20.26 ± 2.00	(18.05-23.83)
Infected time, yr	4.20 ± 2.65	(1-8)
Hematocrit, %	36.25 ± 5.05	(31-49)
Hemoglobin, g/dl	11.96 ± 1.86	(9.09-16.50)
Platelet, $x10^3$ cells/mm^3	272.85 ± 54.23	(173-388)
WBC, $x10^3$ cells/mm^3	4.39 ± 1.49	(1.68-6.97)
BUN, mg/dL	11.00 ± 2.76	(7.0-16.0)
Creatinine, mg/dL	0.80 ± 0.17	(0.60-1.23)
ALP, U/L	97.30 ± 29.63	(59-162)
SGOT, U/L	29.75 ± 9.46	(16-57)
SGPT, U/L	21.20 ± 11.46	(9-55)
Lactate, mmol/L	0.96 ± 0.45	(0.3-2.4)
CD4 cell count, cells/mm^3	106.70 ± 43.13	(51-198)
%CD4	7.70 ± 2.96	(2-13)
Viral load, copies/mL	268,258 ± 219,113	(7,470- >750,000)
Viral load, log_{10}copies/mL	5.24 ± 0.49	(3.87- > 5.87)

[#] : Data are number (%) of patients

2. Bioanalysis of d4T, 3TC and NVP

2.1 Calibration curve and linearity

The linearity was determined from seven level of calibration curve between concentrations of 50 to 2000, 50 to 5000 and 100 to 10000 ng/mL for d4T, 3TC and NVP, respectively. The representative calibration curve of each drug is shown in Figure 6-8. The weighted (1/concentration squared) least squares linear regression equation was

D4T; $y = 0.00087x - 0.01491$, $R^2 = 0.9980$

3TC; $y = 0.00088x + 0.00334$, $R^2 = 0.9998$

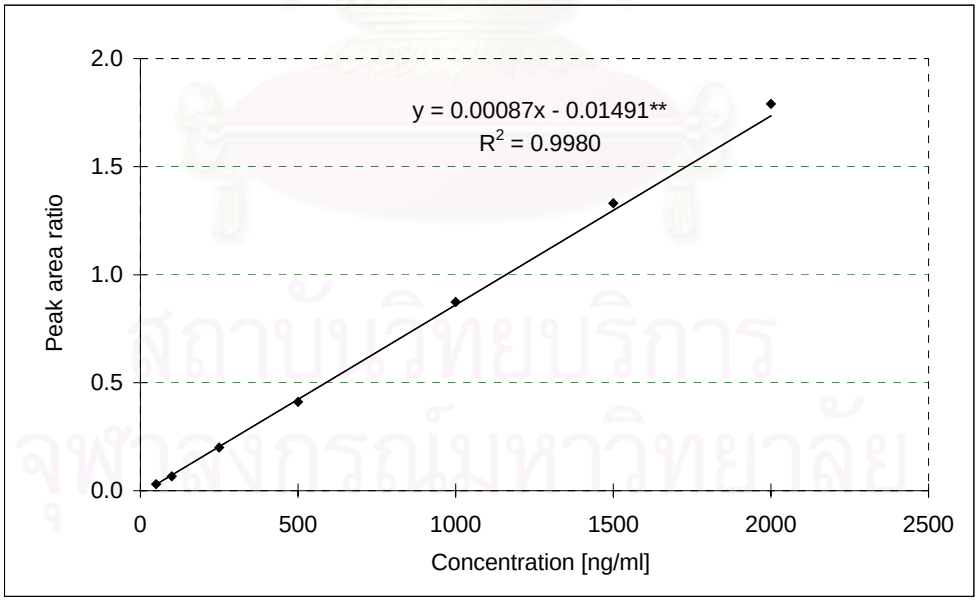
NVP; $y = 0.00037x - 0.00653$, $R^2 = 0.9973$

Where

x = Plasma drug concentration

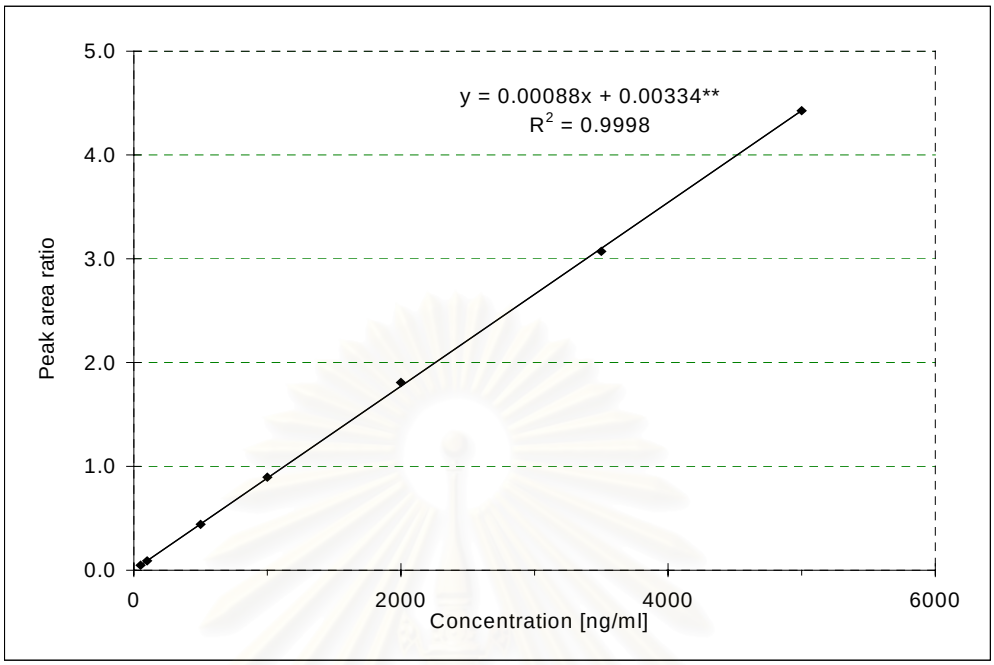
y = Peak area ratio of drug to internal standard (IS)

R^2 = Coefficient of determination



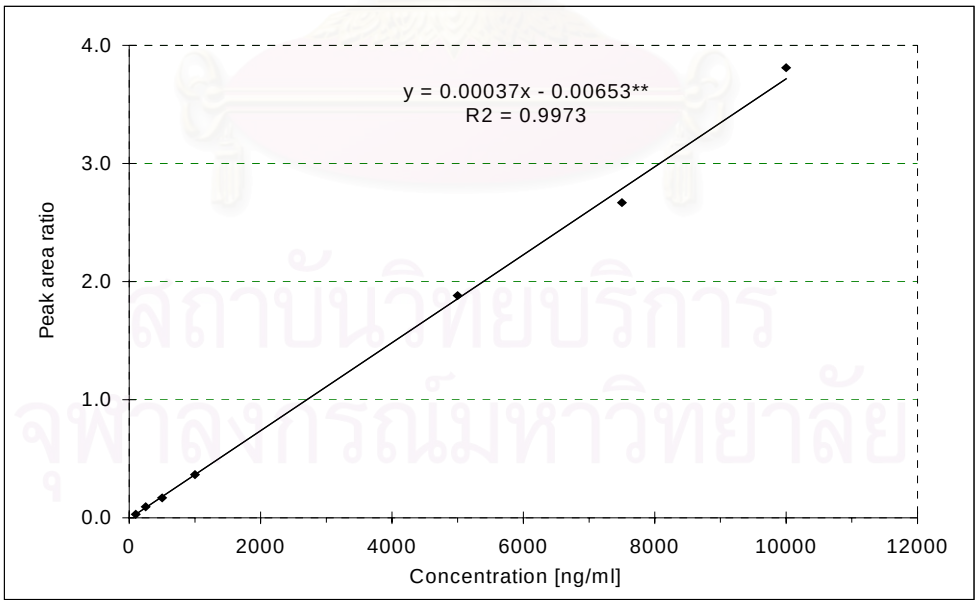
** *weighted (1/concentration squared) least squares linear regression equation*

Figure 6 Calibration curve of d4T



** *weighted (1/concentration squared) least squares linear regression equation*

Figure 7 Calibration curve of 3TC



** *weighted (1/concentration squared) least squares linear regression equation*

Figure 8 Calibration curve of NVP

2.2 Specificity and selectivity

Chromatograms of drug-free plasma, spiked plasma are demonstrated in Figure 9-12. No interference from endogenous substances in plasma was observed.

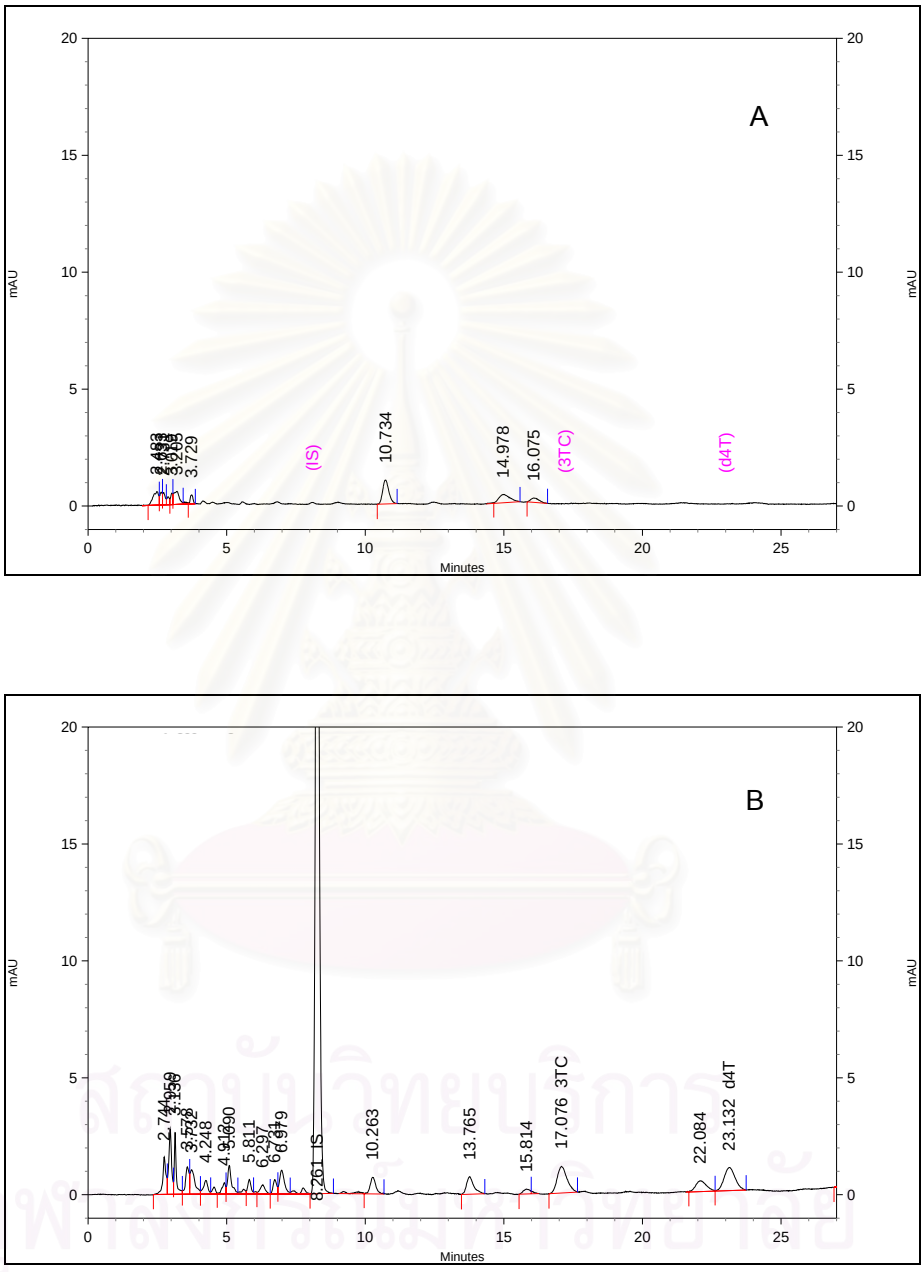
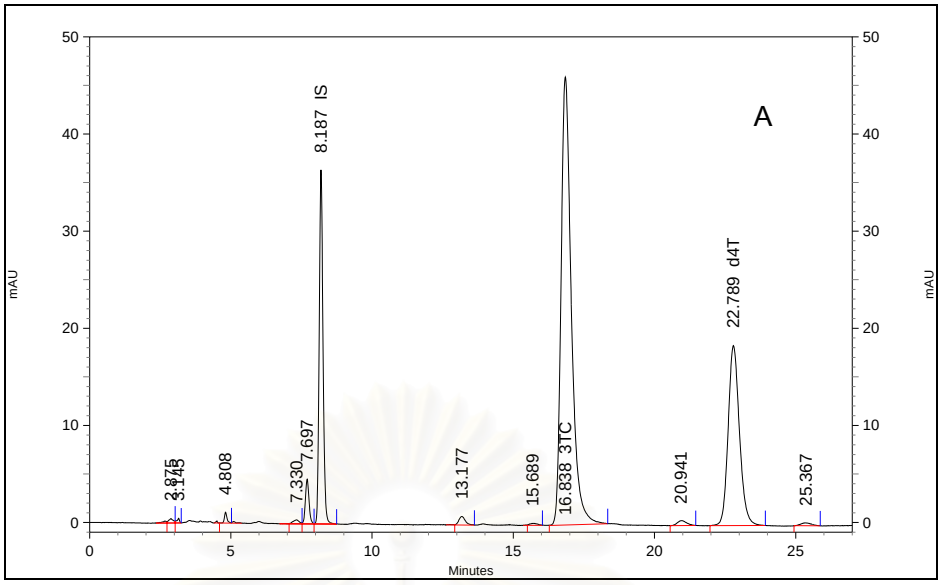


Figure 9 Chromatogram of

A: Drug-free plasma for d4T and 3TC assay

B: Spiked plasma of IS (bromouracil), d4T and 3TC at 1250, 100 and 100 ng/mL, respectively



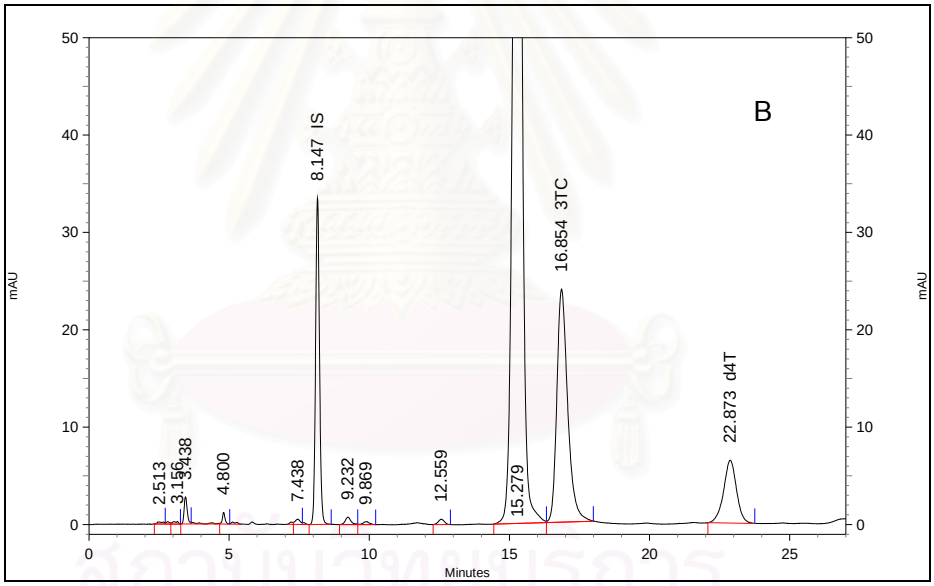


Figure 10 Chromatogram of

A: Spiked plasma of IS (bromouracil), 3TC and d4T at 1250, 4000 and 1750 ng/mL, respectively

B: Patient samples containing IS (bromouracil), 3TC and d4T at 1250, 2295.65 and 717.61 ng/mL, respectively

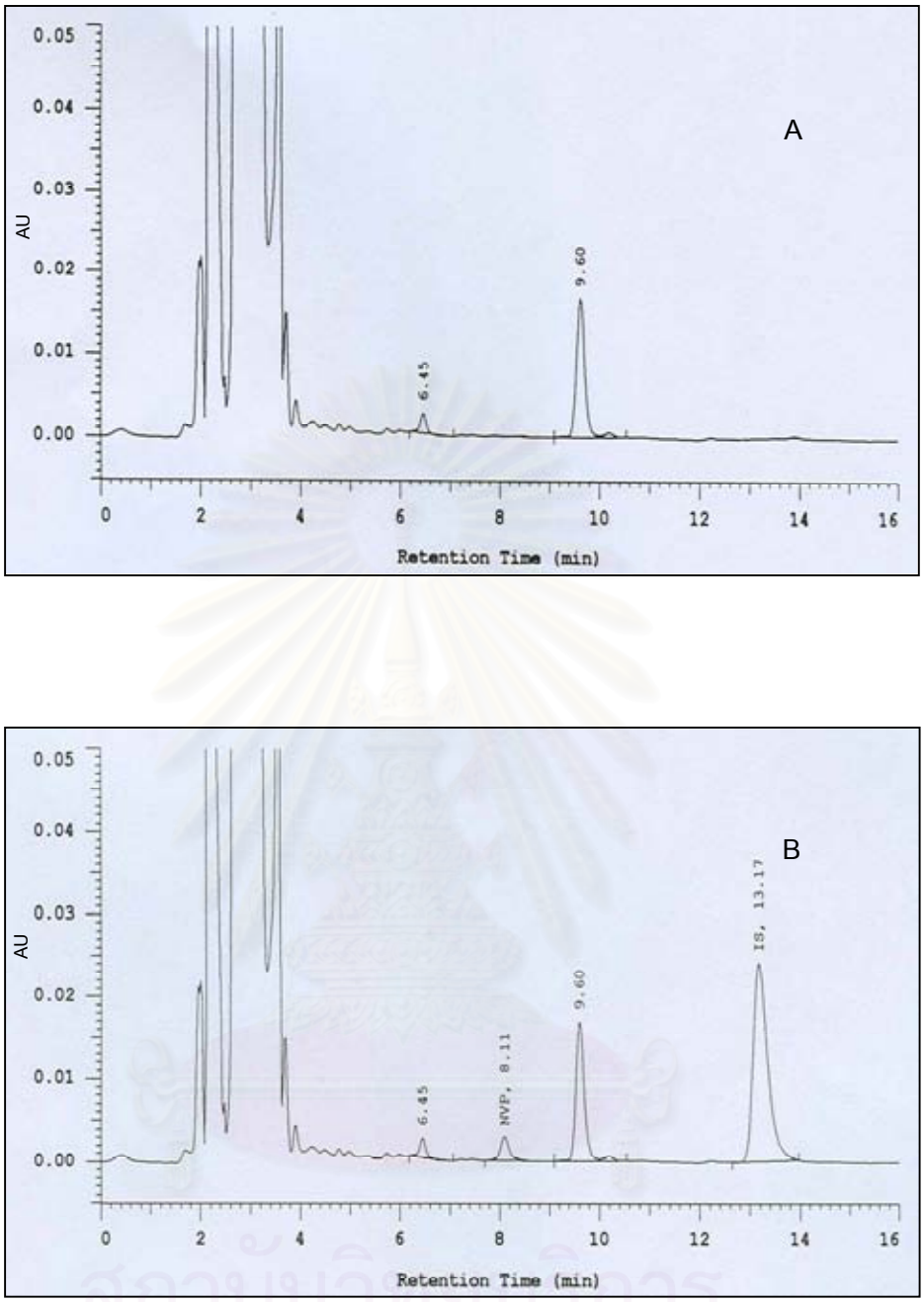


Figure 11 Chromatogram of

A: Drug-free plasma for NVP assay

B: Spiked plasma of IS (clozapine) and NVP at 3500 and 200 ng/ml, respectively

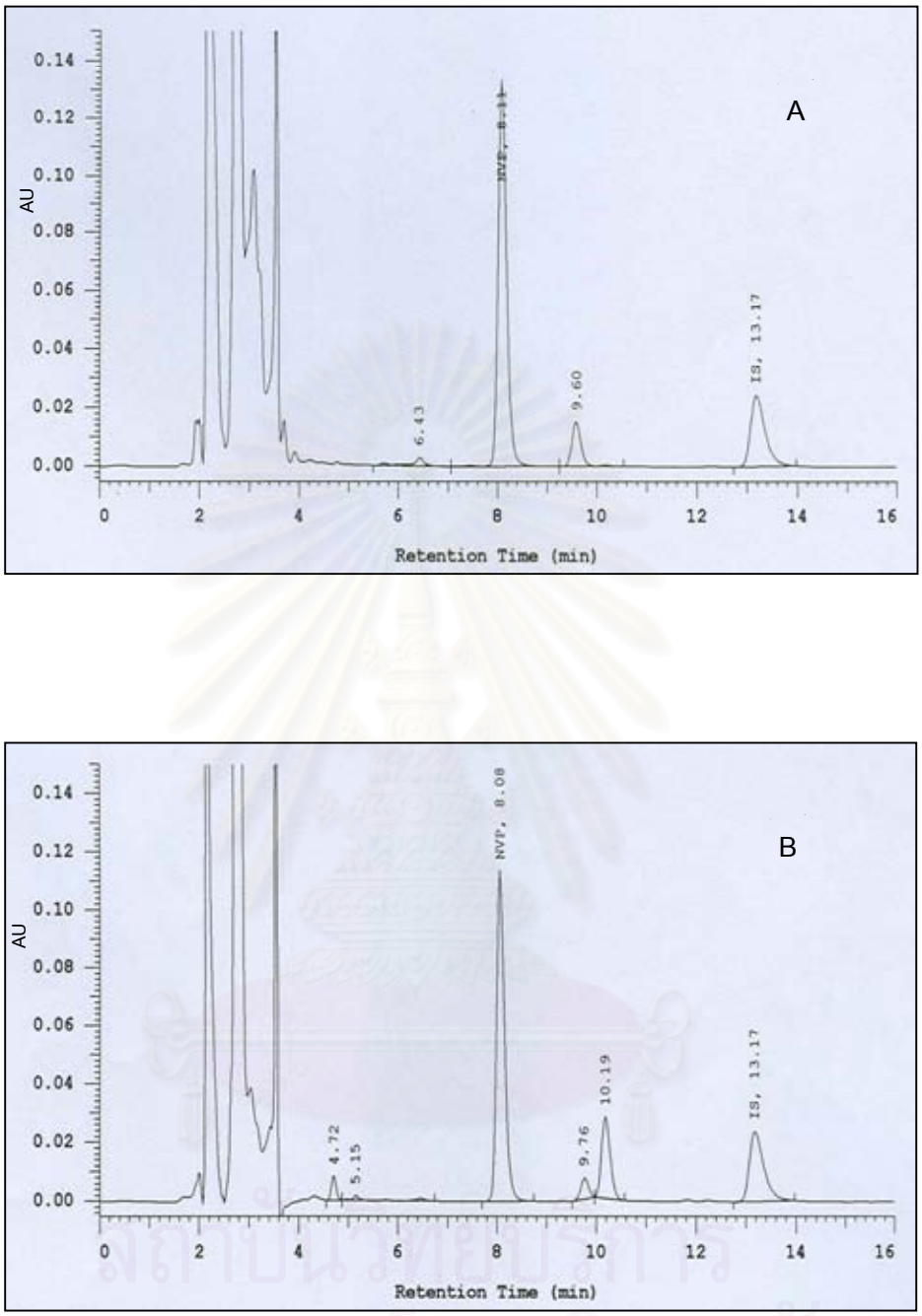


Figure 12 Chromatogram of

A: Spiked plasma of IS (clozapine) and NVP at 3500 and 9000 ng/ml, respectively

B: Patient samples containing IS (clozapine) and NVP at 3500 and 7511.99 ng/mL, respectively

2.3 Limit of quantification

The lowest concentration on the calibration curve which was linearity correlated with peak area of each drug and had acceptable in range ± 20% accuracy (%RD; percent deviation from the nominal concentration = 1.12, 4.50 and 2.72 %) and in range ± 20% precision (%CV; coefficient of variation = 10.17, 12.71, and 9.29 %) for d4T, 3TC and NVP, respectively as shown in table14.

2.4 Accuracy , precision , linearity and recovery

The precision and accuracy of the assay procedure were evaluated from %CV and %RD, respectively. As shown in Table 14, the intra-day and inter-day precision/accuracy were in ranged of acceptable at all concentration levels (acceptable range should be within ±15 %, except at LLOQ should be within ±20 %). Extraction of d4T resulted in lowest percentage recovery which, was approximately 75%.

Table 14 Accuracy and precision of spiked plasma of d4T, 3TC and NVP

Drug	*QCs*	*Acceptable limit (±%)*	*Concentration (ng/mL)*	*Intra-day (n = 5)*		*Inter-day (n = 3)*		*Recovery* (%)*
				Precision (%)	*Accuracy (%)*	*Precision (%)*	*Accuracy (%)*	
d4T	LLOQ	20	50	10.17	1.12	1.2	1.97	-
	QCL	15	100	6.51	-12.24	6.22	-5.65	73.84
	QCM	15	750	1.32	2.54	1.51	0.78	76.22
	QCH	15	1750	0.88	-0.11	1.05	-1.27	74.69
3TC	LLOQ	20	50	12.71	4.50	3.72	8.34	-
	QCL	15	100	4.60	0.96	3.14	-2.11	108.29
	QCM	15	1500	1.75	5.98	3.84	1.49	86.60
	QCH	15	4000	0.54	2.00	2.77	0.76	82.02
NVP	LLOQ	20	100	9.29	2.72	6.82	-1.30	-
	QCL	15	200	8.07	8.76	7.73	0.68	107.47
	QCM	15	2000	3.80	-8.69	5.20	-4.28	92.28
	QCH	15	9000	1.36	-3.46	2.76	-1.28	95.53

* Recovery should in range of 80-120%

2.5 Stability of spiked plasmas and extracts

The stability of plasma samples and extracts left at various conditions was checked as shown in Table 15. The variations of d4T, 3TC and NVP, at QCL and QCH were always higher than 85%, a value comprised within the assay variability, indicating that the plasma samples appear to be stable all through the study.

Table 15 Stability of d4T, 3TC and NVP in plasma samples and final extracts

Conditions	*Stability (%)*					
	d4T		*3TC*		*NVP*	
	QCL	*QCH*	*QCL*	*QCH*	*QCL*	*QCH*
Long-term stability (frozen -20°C)	98.26*	106.61*	109.73*	97.49*	104.58**	96.60**
3 cycles of freeze/thaw	94.01	102.16	105.30	107.58	100.71	108.48
6 hr at room temperature	87.73	97.24	94.49	101.75	96.61	91.20
Final extract						
- In autosampler for 12 hr	90.56	99.32	108.25	102.19	97.81	97.49
- 4°C for 48 hr	89.91	100.58	89.01	97.69	98.75	95.68
- Frozen (-20°C) for 48 hr	87.98	101.08	95.35	105.51	101.35	98.64

* : Long-term stability of d4T and 3TC were tested at 3 months

** : Long-term stability of NVP was tested at 6 months

3. Comparisons of pharmacokinetic parameters (which involve bioavailability) of d4T, 3TC and NVP

3.1 Plasma drug concentration profile

Table 16, 18 and 20 demonstrated steady-state concentrations of each subject at various times after multiple doses, oral administration of combined drugs tablet, GPO-vir S30, for d4T, 3TC and NVP, respectively. Steady-state plasma concentration profiles of reference tablets were shown in Table 17, 19 and 21 for d4T, 3TC and NVP, respectively. Data of 3TC was evaluated for 18 patients only, subject number 5 and 10 were excluded due to interfering peak from concomitant medication in the assay procedures. Mean plasma concentration-time profiles of d4T, 3TC and NVP of both preparations were presented in Figure 13, 14 and 15, respectively.

3.2 Pharmacokinetic parameters involving bioavailability of d4T, 3TC and NVP after administration with a combined drugs formulation, GPO-vir S30, compared to those obtained after three single drug reference tablets: non-compartmental analysis

Pharmacokinetic parameters of d4T, 3TC and NVP were estimated and/or calculated for each individual patient using non-compartmental analysis. These parameters consist of area under the concentration-time curve ($AUC_{0\text{-}12hr}$), maximum concentration (C_{max}) and time to reach the maximum concentration (t_{max}). Table 22, 23 and 24 showed parameters of individual patient of d4T, 3TC and NVP, respectively, after administration with combined tablet, GPO-vir S30, and reference tablets. Log-transformation of ratio of these parameters were also constructed (Table 25-27).

Data revealed that C_{max} and $AUC_{0\text{-}12hr}$ of all components (d4T, 3TC and NVP) in combined drugs formulation, GPO-vir S30, and reference tablets were not statistically significant differences ($P > 0.05$) as presented in Table 28. These results implied that the extent of absorption of d4T, 3TC and NVP were all equivalence between the two preparations. Times to reach maximum concentration (t_{max}) of d4T were 0.79 and 0.78 hr for combined drugs tablet, GPO-vir S30, and reference tablets, respectively which showed no statistically significant difference ($P > 0.05$). Where as t_{max} of 3TC and NVP in combined tablet were slightly faster than reference tablets however these differences

were also not statistically significant ($p > 0.05$). Differences of t_{max} indicated that the rates of absorption of 3TC and NVP in combined drugs tablet might be faster than those of the reference tablets. Equivalence in AUC_{0-12hr} of two preparations revealed that the amount of drug entered general circulation were equal.

Hypothesis tests are inappropriate in that preparations that are very close, but with small variance, may be result in significant different, where as preparations that are widely different, but with large variance may be concluded as equivalent (not significantly different). The use of a confidence interval is more meaningful and has better statistical properties.

Decision regarding the equivalence of alterative products, the confidence limits must lie between 0.8 and 1.25 bases on the ratio of AUC and/or C_{max} alterative to reference products.

In order to test for the bioequivalence between the two preparations, comparisons of each parameter of d4T, 3TC and NVP obtained from GPO-vir S30 to those from the reference products were performed by log-transformation data of the ratio of each parameter of two products as showed in Table 25-27. Equivalence of the two products was assessed by analysis of variance (ANOVA), as presented in Table 28-30. The statistical significance was determine at $p<0.05$. GPO-vir S30 would be concluded to be equivalent when the 90% confidence interval of the ratio of the two products was within the range of 0.8 to 1.25.

Nightly percent confidence interval of ratio of AUC_{0-12hr} of all three drugs, d4T, 3TC and NVP, were 0.87 to 1.08, 0.89 to 1.15 and 1.00 to 1.16, respectively as showed in Table 31. They were all in the range of 0.8 to 1.25. The AUC_{0-12hr} of all components were equivalent between GPO-vir S30 and the reference products. C_{max} of NVP was also in range of 0.8 to 1.25. However, C_{max} of d4T and 3TC were out of range 0.8 to 1.25. Both products met the requirement of higher than 0.8, the lower limit were 0.92 and 0.93 for the C_{max} of d4T and 3TC, respectively. However, the upper limit were slightly higher

than 1.25, they were 1.30 and 1.31 for the C_{max} of d4T and 3TC, respectively. These results might implied that extent of absorption of all three drugs into systemic circulation were equal between combined drugs formulation, GPO-vir S30, and the three single drug reference tablets, however, the rate of absorption of d4T and 3TC from the combined drugs formulation, GPO-vir S30, may be faster than those from the reference tablets.

Bioequivalence interpretation need not be fixed and rigid. US FDA has stated that products may be considered equivalent in the presents of different ratio of absorption, for example, for a drug that is used in chronic dosing, the extent of absorption is probably a much more important than the rate of absorption.

Table 16 Plasma d4T concentrations (ng/mL) at various times after oral administration of combined drugs tablet, GPO-vir S30 (n =20)

Subject No.	*Time (hr)*									
	0	*0.25*	*0.5*	*0.75*	*1*	*1.5*	*2*	*4*	*8*	*12*
1	ND	80.08	688.27	812.99	533.47	329.97	211.61	60.91	ND	ND
2	ND	790.75	989.67	636.04	406.74	306.14	225.36	67.27	ND	ND
3	ND	ND	427.84	1038.67	512.39	387.17	260.03	37.64	ND	ND
4	ND	286.12	942.05	809.66	601.00	374.84	253.24	30.17	ND	ND
5	ND	110.86	188.67	163.67	168.51	223.40	207.75	ND	ND	ND
6	ND	72.41	743.32	841.43	424.37	287.40	206.35	65.54	ND	ND
7	ND	ND	19.70	103.53	320.11	602.86	587.65	177.95	ND	ND
9	ND	117.09	997.23	602.42	483.93	294.02	185.03	50.25	ND	ND
10	ND	51.68	309.83	368.07	323.19	668.69	318.92	55.08	ND	ND
11	ND	55.49	370.95	487.77	541.07	269.75	210.82	51.70	ND	ND
12	ND	201.39	264.68	581.22	718.59	775.83	478.56	NA	ND	ND
13	ND	1122.79	831.40	492.88	372.64	252.01	166.30	57.62	ND	ND
14	ND	601.48	1014.38	830.78	538.83	396.54	254.63	57.17	ND	ND
15	ND	370.01	855.09	694.43	530.01	420.38	359.76	184.06	ND	ND
16	ND	ND	104.97	412.46	620.45	486.05	381.67	135.35	ND	ND
17	ND	819.26	865.62	699.40	598.69	454.11	325.30	93.37	ND	ND
18	ND	204.48	655.51	465.20	386.29	198.08	167.28	56.54	ND	ND
19	ND	53.48	313.45	423.54	274.49	188.74	138.19	57.94	ND	ND
20	ND	561.86	873.90	770.35	650.52	332.43	260.64	76.73	ND	ND
22	ND	961.03	1007.09	694.60	469.51	314.62	183.48	33.22	ND	ND
Mean	-	**380.02**	**623.18**	**596.46**	**473.74**	**378.15**	**269.13**	**74.92**	-	-
SD	-	358.00	337.23	235.16	138.71	155.86	112.69	45.23	-	-
%CV	-	94.21	54.11	39.43	29.28	41.22	41.87	60.37	-	-
Min.	-	51.68	19.70	103.53	168.51	188.74	138.19	30.17	-	-
Max.	-	1122.79	1014.38	1038.67	718.59	775.83	587.65	184.06	-	-
Max.-Min.	-	1071.11	994.68	935.14	550.08	587.09	449.46	153.89	-	-

ND: Not detectable NA: Not available

Table 17 Plasma d4T concentrations (ng/mL) at various times after oral administration of single drug reference tablets (n =20)

Subject No.	*Time (hr)*									
	0	*0.25*	*0.5*	*0.75*	*1*	*1.5*	*2*	*4*	*8*	*12*
1	ND	ND	ND	211.61	899.47	479.11	247.57	80.40	ND	ND
2	ND	54.96	317.99	744.46	594.42	417.86	355.36	96.05	ND	ND
3	ND	ND	50.62	77.10	219.42	389.28	340.74	81.39	ND	ND
4	ND	138.09	260.37	536.35	858.57	553.89	322.98	42.87	ND	ND
5	ND	794.30	851.50	470.02	308.61	197.91	111.53	4.84	ND	ND
6	ND	ND	675.21	888.39	622.81	335.88	233.06	58.58	ND	ND
7	ND	60.00	613.32	653.97	591.75	428.56	321.09	85.72	ND	ND
9	ND	361.92	500.39	710.02	645.75	374.27	236.89	61.20	ND	ND
10	ND	81.95	193.65	317.08	530.27	669.06	285.36	52.97	ND	ND
11	ND	ND	53.68	552.96	516.88	440.26	320.39	51.63	ND	ND
12	ND	146.33	235.83	434.01	654.21	432.90	437.32	93.10	ND	ND
13	ND	170.21	889.79	592.26	436.83	276.29	196.96	40.81	ND	ND
14	ND	ND	93.68	436.42	511.15	506.17	314.15	72.23	ND	ND
15	ND	218.22	390.90	717.61	766.80	531.66	467.08	288.05	ND	ND
16	ND	600.56	578.21	500.37	369.70	351.38	317.20	115.47	ND	ND
17	ND	1256.30	916.53	622.82	535.06	368.94	273.30	19.34	ND	ND
18	ND	567.27	721.00	532.68	428.58	301.90	199.90	83.70	ND	ND
19	ND	429.37	392.04	314.25	254.08	181.86	113.05	59.25	ND	ND
20	ND	53.74	155.16	282.88	554.08	484.30	416.12	109.25	ND	ND
22	ND	22.25	556.85	544.98	439.18	302.94	180.16	33.43	ND	ND
Mean	-	**330.36**	**444.56**	**507.01**	**536.88**	**401.22**	**284.51**	**76.51**	-	-
SD	-	348.84	284.33	197.62	180.74	119.83	97.25	57.53	-	-
%CV	-	105.59	63.96	38.98	33.66	29.87	34.18	75.19	-	-
Min.	-	22.25	50.62	77.10	219.42	181.86	111.53	4.84	-	-
Max.	-	1256.30	916.53	888.39	858.57	669.06	467.08	288.05	-	-
Max.-Min.	-	1234.05	865.91	811.29	639.15	487.20	355.55	283.21	-	-

ND: Not detectable

Table 18 Plasma 3TC concentrations (ng/mL) at various times after oral administration of combined drugs tablet, GPO-vir S30 (n =18)

Subject	*Time (hr)*									
No.	*0*	*0.25*	*0.5*	*0.75*	*1*	*1.5*	*2*	*4*	*8*	*12*
1	104.79	140.13	991.43	1687.52	2247.02	1926.78	1365.05	1010.83	209.89	33.96
2	122.62	1049.12	2327.16	2194.72	1825.19	1454.70	1101.15	1140.52	257.36	68.55
3	151.09	161.32	575.93	2642.37	2217.25	1656.92	1302.34	701.81	149.19	76.55
4	263.39	240.93	1416.59	2152.47	2298.05	1952.25	1488.42	824.50	251.84	119.07
6	110.88	166.23	1072.94	2053.28	2715.09	2804.45	1661.99	615.29	104.93	89.32
7	227.32	187.67	206.69	300.82	496.12	1507.40	1683.80	1590.84	396.35	125.41
9	145.19	179.79	1298.00	1444.58	1233.55	1021.00	797.35	553.73	242.14	70.52
11	111.85	178.43	452.57	1596.97	2291.99	2083.34	1651.81	718.68	165.35	69.86
12	302.40	300.48	523.46	1285.59	2092.72	2798.48	2420.14	274.31	NA	107.84
13	158.12	1192.58	1823.42	1777.35	1659.45	1637.78	1355.32	666.97	261.63	83.82
14	148.61	501.03	1744.28	2842.82	3114.95	2441.31	1571.55	705.39	249.87	110.69
15	168.94	583.16	1626.11	2554.16	2924.63	2281.00	1880.49	862.59	315.33	144.09
16	160.39	259.21	299.73	925.20	1636.28	2242.89	1726.41	774.07	448.94	678.79
17	271.71	1298.77	2197.89	2392.28	2182.38	1891.52	1952.36	1545.58	386.63	174.69
18	71.32	222.38	1951.15	2206.00	2265.04	1646.71	1197.91	818.70	169.66	45.07
19	78.45	121.13	286.98	784.91	1212.37	1079.48	854.67	562.79	145.06	104.79
20	153.23	540.55	1250.53	1417.02	1712.69	1230.55	1168.26	1303.76	336.01	110.33
22	239.99	1410.88	3368.39	3865.30	3033.59	2477.95	1761.76	751.67	427.24	230.90
Mean	166.13	485.21	1300.74	1895.74	2064.35	1896.36	1496.71	856.78	265.73	135.79
SD	67.72	440.47	853.07	838.81	678.60	541.81	402.40	344.34	104.95	143.28
%CV	40.76	90.78	65.58	44.25	32.87	28.57	26.89	40.19	39.50	105.51
Min.	71.32	121.13	206.69	300.82	496.12	1021.00	797.35	274.31	104.93	33.96
Max.	302.40	1410.88	3368.39	3865.30	3114.95	2804.45	2420.14	1590.84	448.94	678.79
Max.-Min.	231.09	1289.75	3161.70	3564.48	2618.83	1783.45	1622.79	1316.53	344.01	644.83

NA: Not available

Table 19 Plasma 3TC concentrations (ng/mL) at various times after oral administration of single drug reference tablets (n =18)

Subject No.	*Time (hr)*									
	0	*0.25*	*0.5*	*0.75*	*1*	*1.5*	*2*	*4*	*8*	*12*
1	46.58	252.86	157.74	383.42	1965.15	2795.45	1707.09	835.05	156.83	25.19
2	122.27	841.24	1811.70	2873.74	3241.07	2260.02	1728.88	1018.11	416.48	118.16
3	125.49	150.51	354.85	684.51	1116.51	1701.54	1408.30	1012.57	274.69	118.98
4	210.16	600.55	1705.30	2113.84	2610.45	3785.90	2605.93	1251.65	210.09	93.03
6	175.47	213.23	800.83	1749.58	1873.66	1722.91	1327.07	1048.18	353.11	155.16
7	273.53	270.24	986.59	1473.02	1710.02	1697.34	2630.77	1139.31	276.59	10.71
9	373.66	898.83	2342.51	1726.55	2281.13	2509.63	1988.36	1443.54	491.34	219.07
11	143.54	1235.61	1341.25	1470.99	1517.59	1404.79	1415.29	549.88	172.34	74.27
12	65.07	569.12	1493.15	1529.43	1713.56	1782.10	1734.79	608.96	85.39	82.96
13	54.45	908.35	1838.52	1592.41	1389.92	1098.54	1106.50	531.94	164.54	90.40
14	223.17	389.16	1226.81	1837.10	2117.57	1913.22	1628.97	843.80	221.68	116.28
15	187.32	1076.35	1835.37	2295.65	2261.84	1775.58	1590.20	985.19	413.42	219.80
16	159.29	1318.08	1966.02	2085.18	1707.57	1457.80	1528.53	714.75	227.43	75.74
17	297.31	1662.25	2285.02	2489.76	2156.47	1750.19	1570.44	1179.68	412.89	191.49
18	109.20	738.94	2019.89	1955.02	1675.27	1532.89	1152.12	700.26	187.11	66.55
19	55.42	374.26	1152.46	1222.70	1189.16	1133.66	933.94	492.14	154.39	82.68
20	196.68	216.59	350.22	557.55	899.72	1071.18	1046.68	1347.67	272.64	153.03
22	193.44	362.35	1242.52	1437.83	1205.09	885.75	785.81	402.95	286.11	109.92
Mean	167.34	671.03	1383.93	1637.68	1812.88	1793.25	1549.43	894.76	265.39	111.30
SD	89.38	442.37	660.29	650.97	580.48	700.39	499.09	311.36	112.15	58.50
%CV	53.41	65.92	47.71	39.75	32.02	39.06	32.21	34.80	42.26	52.56
Min.	46.58	150.51	157.74	383.42	899.72	885.75	785.81	402.95	85.39	10.71
Max.	373.66	1662.25	2342.51	2873.74	3241.07	3785.90	2630.77	1443.54	491.34	219.80
Max.-Min.	327.08	1511.74	2184.76	2490.32	2341.35	2900.15	1844.95	1040.59	405.95	209.08

Table 20 Plasma NVP concentrations (ng/mL) at various times after oral administration of combined drugs tablet, GPO-vir S30 (n = 20)

Subject No.	*Time (hr)*									
	0	*0.25*	*0.5*	*0.75*	*1*	*1.5*	*2*	*4*	*8*	*12*
1	12540.55	12632.61	13374.69	13787.18	15526.53	17212.31	18058.00	13629.60	12206.92	11465.68
2	6293.19	6495.34	6665.02	6982.25	6848.67	6563.23	6337.25	6009.97	5897.63	5523.62
3	5555.61	5732.82	7146.43	7033.82	5928.16	7040.09	6392.51	5543.85	5137.00	4079.88
4	10234.59	10702.41	10762.77	12194.44	13881.83	13307.20	13223.03	10867.91	10363.50	9239.85
5	7720.41	7890.19	8283.61	9749.14	8336.91	8146.22	8764.92	9597.90	7925.36	6036.30
6	9697.43	7812.18	11571.71	11569.96	13031.40	11213.98	11686.01	9624.90	11074.82	9857.87
7	13346.51	13465.90	13327.28	14034.89	14537.89	14468.79	15948.61	13294.98	13752.83	10722.61
9	11338.11	11079.74	11786.78	11791.58	12529.86	13926.77	12995.73	11026.87	10000.81	9385.23
10	9236.69	9184.53	9435.56	8948.04	9165.00	8467.39	9233.13	9025.94	8963.64	8772.74
11	6847.88	7156.33	7198.69	7554.71	8236.59	8561.23	9514.42	7272.80	6750.64	5450.49
12	6414.55	6133.11	5797.07	6470.34	6955.88	7711.70	7698.69	5361.17	5041.68	3962.36
13	10661.77	12177.49	13266.88	13638.03	14589.32	14920.75	13724.35	10171.46	10012.25	9117.05
14	10386.38	10328.28	11041.26	12241.19	11095.41	13943.27	11650.83	9771.42	9709.76	9449.94
15	7648.24	8295.74	9151.62	9765.28	9406.77	9475.89	9958.84	7541.72	6809.19	6309.88
16	6701.73	6829.71	6813.19	6902.08	7369.09	8925.97	8099.51	6444.78	6309.44	5197.13
17	7277.20	7741.24	7952.08	8540.73	8517.42	9871.20	10918.72	7605.79	7511.99	6524.92
18	7747.20	8050.46	8322.46	9212.72	8816.40	8578.47	8834.21	7708.72	6862.88	6212.80
19	6585.74	7257.62	7232.98	7102.19	6876.56	7662.34	7193.54	8464.63	7155.10	5748.11
20	5783.29	6275.19	6938.76	7517.46	7826.12	7736.97	7637.74	7169.02	5610.04	5673.86
22	7861.20	9827.57	9480.77	10232.71	9935.76	11650.67	12081.73	8180.83	8580.17	8009.74
Mean	**8493.91**	**8753.42**	**9277.48**	**9763.44**	**9970.58**	**10469.22**	**10497.59**	**8715.71**	**8283.78**	**7337.00**
SD	2233.67	2248.21	2379.52	2477.71	2930.41	3046.46	3097.16	2261.24	2362.54	2193.29
%CV	26.30	25.68	25.65	25.38	29.39	29.10	29.50	25.94	28.52	29.89
Min.	5555.61	5732.82	5797.07	6470.34	5928.16	6563.23	6337.25	5361.17	5041.68	3962.36
Max.	13346.51	13465.90	13374.69	14034.89	15526.53	17212.31	18058.00	13629.60	13752.83	11465.68
Max.-Min.	7790.90	7733.08	7577.62	7564.55	9598.37	10649.08	11720.75	8268.43	8711.15	7503.32

Table 21 Plasma NVP concentrations (ng/mL) at various times after oral administration of single drug reference tablets (n =20)

Subject No.	*Time (hr)*									
	0	*0.25*	*0.5*	*0.75*	*1*	*1.5*	*2*	*4*	*8*	*12*
1	11411.88	10357.91	11134.75	10720.77	11720.76	14664.46	13863.88	11650.00	12343.25	10893.43
2	7327.96	8161.29	7972.91	8495.98	8415.78	7772.92	8658.33	7677.22	6914.49	7095.70
3	4313.03	4400.95	4464.34	4715.65	5503.76	6302.66	6545.56	6253.42	4768.43	3849.44
4	11189.34	11237.88	10570.41	11257.60	11459.17	11732.35	11168.60	9980.96	9584.75	8475.20
5	7440.97	9288.49	9222.99	8481.20	9299.06	8452.85	8293.38	7141.37	6236.74	5370.68
6	8950.16	8594.20	9921.64	11585.12	10691.54	12489.79	11746.49	10548.67	8660.46	7394.35
7	7135.46	7260.30	7653.96	7997.01	8215.11	8757.70	8867.62	7195.78	6650.19	5791.16
9	11706.55	12679.08	15720.06	13991.97	14821.19	13968.63	14791.55	10527.01	9875.83	9371.93
10	8112.66	7870.34	8916.14	8495.90	7631.62	9152.42	8491.22	7507.10	8479.64	7910.57
11	6886.59	7752.04	7651.11	8709.08	8574.04	8571.63	8853.61	6965.09	6634.85	5938.15
12	4315.75	4515.80	4877.28	5390.05	6202.90	6692.62	7470.43	5136.74	4059.86	3417.90
13	11410.18	11989.21	11873.75	11947.99	12139.39	11938.66	13179.06	9049.44	9383.76	8463.95
14	8508.79	9151.52	9100.06	8752.85	11095.41	11251.55	10526.53	8808.86	9280.32	7418.50
15	7653.38	7831.21	8350.61	8558.31	8968.92	8528.24	7792.32	7497.21	6155.99	5456.96
16	7277.20	7741.24	7952.08	8540.73	8517.42	9871.20	10918.72	7605.79	7511.99	6524.92
17	6701.73	6829.71	6813.19	6902.08	7369.09	8925.97	8099.51	6444.78	6309.44	5197.13
18	8091.34	8853.73	10102.00	10131.70	10233.68	10944.01	10165.22	8975.39	8337.21	7137.48
19	4077.22	4128.55	5077.23	4973.79	4970.25	5775.58	5249.73	4652.97	4113.18	3697.68
20	6744.35	7024.40	7174.27	6788.22	8079.96	7687.00	8360.11	6976.96	6735.69	5192.88
22	11791.31	11714.68	12237.19	13459.38	11903.12	12642.11	12327.65	11091.27	11136.55	9725.97
Mean	8052.29	8369.13	8839.30	8994.77	9290.61	9806.12	9768.48	8084.30	7658.63	6716.20
SD	2426.76	2429.45	2712.67	2603.07	2469.10	2533.45	2508.54	1938.20	2222.83	2053.77
%CV	30.14	29.03	30.69	28.94	26.58	25.84	25.68	23.97	29.02	30.58
Min.	4077.22	4128.55	4464.34	4715.65	4970.25	5775.58	5249.73	4652.97	4059.86	3417.90
Max.	11791.31	12679.08	15720.06	13991.97	14821.19	14664.46	14791.55	11650.00	12343.25	10893.43
Max.-Min.	7714.09	8550.53	11255.72	9276.32	9850.94	8888.88	9541.82	6997.03	8283.39	7475.53

Table 22 Pharmacokinetic parameters of d4T of individual patient after administration a combined drugs formulation, GPO-vir S30, compared to those after three single drug reference tablets (n = 20)

Subject No.	C_{max} *(ng/mL)*		$AUC_{0\text{-}12hr}$ *(hr*ng/mL)*		t_{max} *(hr)*	
	GPO	*REF*	*GPO*	*REF*	*GPO*	*REF*
1	812.99	899.47	1085.79	1072.52	0.75	1.00
2	989.67	744.46	1258.68	1251.44	0.50	0.75
3	1038.67	389.28	1168.52	822.50	0.75	1.50
4	942.05	858.57	1268.97	1279.21	0.50	1.00
5	223.40	851.50	342.63	887.89	1.50	0.50
6	841.43	888.39	1040.61	1226.70	0.75	0.75
7	602.86	653.97	1367.25	1255.09	1.50	0.75
9	997.23	710.02	1039.21	1179.69	0.50	0.75
10	668.69	669.06	1091.67	1091.22	1.50	1.50
11	541.07	552.96	881.55	1024.45	1.00	0.75
12	775.83	654.21	1038.85	1305.57	1.50	1.00
13	1122.79	889.79	1143.01	1002.03	0.25	0.50
14	1014.38	511.15	1387.45	1053.92	0.50	1.00
15	855.09	766.80	1522.59	1756.96	0.50	1.00
16	620.45	600.56	1230.61	1246.08	1.00	0.25
17	865.62	1256.30	1547.63	1445.00	0.50	0.25
18	655.51	721.00	840.84	1100.48	0.50	0.50
19	423.54	429.37	625.60	670.69	0.75	0.25
20	873.90	554.08	1364.22	1202.28	0.50	1.00
22	1007.09	556.85	1261.63	855.81	0.50	0.50
Mean	**793.61**	**707.89**	**1125.36**	**1136.48**	**0.79**	**0.78**
SD	230.56	199.81	291.84	238.83	0.41	0.36
%CV	29.05	28.23	25.93	21.02	51.78	46.69
Min.	223.40	389.28	342.63	670.69	0.25	0.25
Max.	1122.79	1256.30	1547.63	1756.96	1.50	1.50
Max.-Min.	899.39	867.02	1205.00	1086.28	1.25	1.25

GPO: GPO-vir S30, REF: Reference tablet

Table 23 Pharmacokinetic parameters of 3TC of individual patient after administration a combined drugs formulation, GPO-vir S30, compared to those after three single drug reference tablets (n = 18)

Subject No.	C_{max} *(ng/mL)*		$AUC_{0\text{-}12hr}$ *(hr*ng/mL)*		t_{max} *(hr)*	
	GPO	*REF*	*GPO*	*REF*	*GPO*	*REF*
1	2247.02	2795.45	8170.17	7655.70	1.00	1.50
2	2327.16	3241.07	8784.41	10860.04	0.50	1.00
3	2642.37	1701.54	7006.94	7717.42	0.75	1.50
4	2298.05	3785.90	8402.84	12041.84	1.00	1.50
6	2804.45	1873.66	7779.07	8803.06	1.50	1.00
7	1683.80	2630.77	9855.45	10040.75	2.00	2.00
9	1444.58	2509.63	5489.30	12618.49	0.75	1.50
11	2291.99	1517.59	7494.06	6558.05	1.00	1.00
12	2798.48	1782.10	7577.28	6942.54	1.50	1.50
13	1823.42	1838.52	7568.51	5980.02	0.50	0.50
14	3114.95	2117.57	8980.84	8328.76	1.00	1.00
15	2924.63	2295.65	9937.09	10097.84	1.00	0.75
16	2242.89	2085.18	9759.70	7847.58	1.50	0.75
17	2392.28	2489.76	12243.44	10866.82	0.75	0.75
18	2265.04	2019.89	7498.81	7009.25	1.00	0.50
19	1212.37	1222.70	4848.97	5133.81	1.00	0.75
20	1712.69	1347.67	9015.02	7926.64	1.00	4.00
22	3865.30	1437.83	11195.68	5235.03	0.75	0.75
Mean	**2338.42**	**2149.58**	**8422.64**	**8425.76**	**1.03**	**1.24**
SD	640.82	674.02	1818.76	2229.05	0.38	0.81
%CV	27.40	31.36	21.59	26.46	37.21	65.25
Min.	1212.37	1222.70	4848.97	5133.81	0.50	0.50
Max.	3865.30	3785.90	12243.44	12618.49	2.00	4.00
Max.-Min.	2652.93	2563.20	7394.47	7484.68	1.50	3.50

Table 24 Pharmacokinetic parameters of NVP of individual patient after administration a combined drugs formulation, GPO-vir S30, compared to those after three single drug reference tablets (n = 20)

Subject No.	C_{max} (ng/mL)		$AUC_{0\text{-}12hr}$ (hr*ng/mL)		t_{max} (hr)	
	GPO	REF	GPO	REF	GPO	REF
1	18058.00	14664.46	161165.13	144257.46	2.00	1.50
2	6982.25	8658.33	72261.40	89819.85	0.75	2.00
3	7146.43	6545.56	64745.77	62864.41	0.50	2.00
4	13881.83	11732.35	130619.73	119021.53	1.00	1.50
5	9749.14	9299.06	98169.13	82871.02	0.75	1.00
6	13031.40	12489.79	126941.64	114657.90	1.00	1.50
7	15948.61	8867.62	160838.68	84934.21	2.00	2.00
9	13926.77	15720.06	129842.84	132921.01	1.50	0.50
10	9435.56	9152.42	107736.46	97647.77	0.50	1.50
11	9514.42	8853.61	85317.72	84767.94	2.00	2.00
12	7711.70	7470.43	65664.57	57731.15	1.50	2.00
13	14920.75	13179.06	129987.61	118985.70	1.50	2.00
14	13943.27	11251.55	122450.11	109144.28	1.50	1.50
15	9958.84	8968.92	90954.90	82539.08	2.00	1.00
16	8925.97	10918.72	78291.10	94661.37	1.50	2.00
17	10918.72	8925.97	94661.37	78291.10	2.00	1.50
18	9212.72	10944.01	89006.24	104849.41	0.75	1.50
19	8464.63	5775.58	87133.73	53175.33	4.00	1.50
20	7826.12	8360.11	77551.16	81672.79	1.00	2.00
22	12081.73	13459.38	107903.97	134292.96	2.00	0.75
Mean	**11081.94**	**10261.85**	**104062.16**	**96455.31**	**1.49**	**1.56**
SD	3191.15	2659.34	28995.62	25517.65	0.80	0.46
%CV	28.80	25.91	27.86	26.46	53.84	29.31
Min.	6982.25	5775.58	64745.77	53175.33	0.50	0.50
Max.	18058.00	15720.06	161165.13	144257.46	4.00	2.00
Max.-Min.	11075.75	9944.48	96419.36	91082.13	3.50	1.50

GPO: GPO-vir S30 REF: Reference tablet

Table 25 Ln(C_{max}) and Ln(AUC_{0-12hr}) ratio of d4T of individual patient between administration a combined drugs formulation, GPO-vir S30, and three single drug reference tablets (n = 20)

Subject No.	*Ln (C_{max})*		*Ln ($C_{max(GPO)}$/ $C_{max(REF)}$)*	*Ln (AUC_{0-12hr})*		*Ln ($AUC_{0-12hr(GPO)}$/ $AUC_{0-12hr(REF)}$)*
	GPO	*REF*		*GPO*	*REF*	
1	6.7007	6.8018	-0.1011	6.9901	6.9778	0.0123
2	6.8974	6.6127	0.2847	7.1378	7.1321	0.0058
3	6.9457	5.9643	0.9814	7.0635	6.7123	0.3511
4	6.8481	6.7553	0.0928	7.1460	7.1540	-0.0080
5	5.4090	6.7470	-1.3380	5.8366	6.7888	-0.9522
6	6.7351	6.7894	-0.0543	6.9476	7.1121	-0.1645
7	6.4017	6.4831	-0.0814	7.2206	7.1350	0.0856
9	6.9050	6.5653	0.3397	6.9462	7.0730	-0.1268
10	6.5053	6.5059	-0.0006	6.9955	6.9951	0.0004
11	6.2935	6.3153	-0.0217	6.7817	6.9319	-0.1502
12	6.6539	6.4834	0.1705	6.9459	7.1744	-0.2285
13	7.0236	6.7910	0.2326	7.0414	6.9098	0.1316
14	6.9220	6.2367	0.6854	7.2352	6.9603	0.2750
15	6.7512	6.6422	0.1090	7.3282	7.4713	-0.1432
16	6.4304	6.3979	0.0326	7.1153	7.1278	-0.0125
17	6.7634	7.1359	-0.3725	7.3445	7.2759	0.0686
18	6.4854	6.5806	-0.0952	6.7344	7.0035	-0.2691
19	6.0486	6.0623	-0.0137	6.4387	6.5083	-0.0696
20	6.7730	6.3173	0.4557	7.2183	7.0920	0.1264
22	6.9148	6.3223	0.5925	7.1402	6.7521	0.3881
Mean	***6.6204***	***6.5255***	***0.0949***	***6.9804***	***7.0144***	***-0.0340***

Table 26 Ln(C_{max}) and Ln(AUC_{0-12hr}) ratio of 3TC of individual patient between administration a combined drugs formulation, GPO-vir S30, and three single drug reference tablets (n = 18)

Subject No.	*Ln (C_{max})*		*Ln ($C_{max(GPO)}$/ $C_{max(REF)}$)*	*Ln (AUC_{0-12hr})*		*Ln ($AUC_{0-12hr(GPO)}$/ $AUC_{0-12hr(REF)}$)*
	GPO	*REF*		*GPO*	*REF*	
1	7.7174	7.9357	-0.2184	9.0082	8.9432	0.0650
2	7.7524	8.0837	-0.3313	9.0807	9.2928	-0.2121
3	7.8794	7.4393	0.4401	8.8547	8.9512	-0.0966
4	7.7398	8.2390	-0.4992	9.0363	9.3961	-0.3598
6	7.9390	7.5356	0.4033	8.9592	9.0829	-0.1237
7	7.4288	7.8750	-0.4462	9.1958	9.2144	-0.0186
9	7.2756	7.8279	-0.5523	8.6106	9.4429	-0.8324
11	7.7372	7.3249	0.4123	8.9219	8.7884	0.1334
12	7.9368	7.4855	0.4513	8.9329	8.8454	0.0875
13	7.5085	7.5167	-0.0082	8.9318	8.6962	0.2356
14	8.0440	7.6580	0.3859	9.1028	9.0275	0.0754
15	7.9809	7.7388	0.2422	9.2040	9.2201	-0.0160
16	7.7155	7.6426	0.0729	9.1860	8.9680	0.2181
17	7.7800	7.8199	-0.0399	9.4127	9.2935	0.1193
18	7.7253	7.6108	0.1145	8.9225	8.8550	0.0675
19	7.1003	7.1088	-0.0085	8.4865	8.5436	-0.0571
20	7.4458	7.2061	0.2397	9.1066	8.9780	0.1287
22	8.2598	7.2709	0.9889	9.3233	8.5631	0.7602
Mean	***7.7204***	***7.6289***	***0.0915***	***9.0154***	***9.0057***	***0.0097***

Table 27 Ln(C_{max}) and Ln(AUC_{0-12hr}) ratio of NVP of individual patient between administration a combined drugs formulation, GPO-vir S30, and three single drug reference tablets (n = 20)

Subject No.	*Ln (C_{max})*		*Ln ($C_{max(GPO)}$/ $C_{max(REF)}$)*	*Ln (AUC_{0-12hr})*		*Ln ($AUC_{0-12hr(GPO)}$/ $AUC_{0-12hr(REF)}$)*
	GPO	*REF*		*GPO*	*REF*	
1	9.8013	9.5932	0.2082	11.9902	11.8794	0.1108
2	8.8511	9.0663	-0.2152	11.1880	11.4056	-0.2175
3	8.8744	8.7865	0.0878	11.0782	11.0487	0.0295
4	9.5383	9.3701	0.1682	11.7800	11.6871	0.0930
5	9.1849	9.1377	0.0473	11.4944	11.3250	0.1694
6	9.4751	9.4327	0.0425	11.7515	11.6497	0.1018
7	9.6771	9.0902	0.5870	11.9882	11.3496	0.6385
9	9.5416	9.6627	-0.1211	11.7741	11.7975	-0.0234
10	9.1522	9.1218	0.0305	11.5874	11.4891	0.0983
11	9.1606	9.0886	0.0720	11.3541	11.3477	0.0065
12	8.9505	8.9187	0.0318	11.0923	10.9636	0.1288
13	9.6105	9.4864	0.1241	11.7752	11.6868	0.0884
14	9.5428	9.3283	0.2145	11.7155	11.6004	0.1150
15	9.2062	9.1015	0.1047	11.4181	11.3210	0.0971
16	9.0967	9.2982	-0.2015	11.2682	11.4581	-0.1899
17	9.2982	9.0967	0.2015	11.4581	11.2682	0.1899
18	9.1283	9.3005	-0.1722	11.3965	11.5603	-0.1638
19	9.0437	8.6614	0.3823	11.3752	10.8813	0.4938
20	8.9652	9.0312	-0.0660	11.2587	11.3105	-0.0518
22	9.3994	9.5074	-0.1080	11.5890	11.8078	-0.2188
Mean	***9.2749***	***9.2040***	***0.0709***	***11.5166***	***11.4419***	***0.0748***

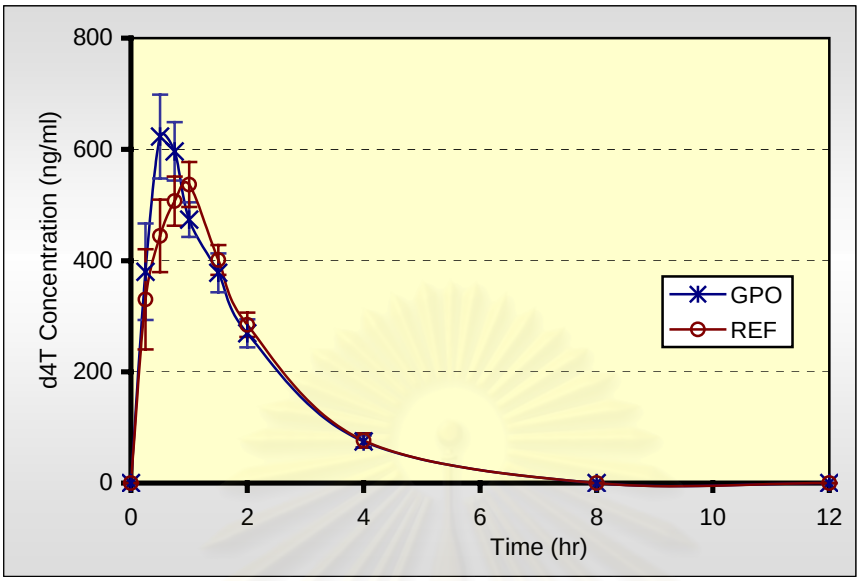


Figure 13 Compared average plasma concentration – time profiles of d4T between reference tablet and GPO-vir S30 (mean ± SE)

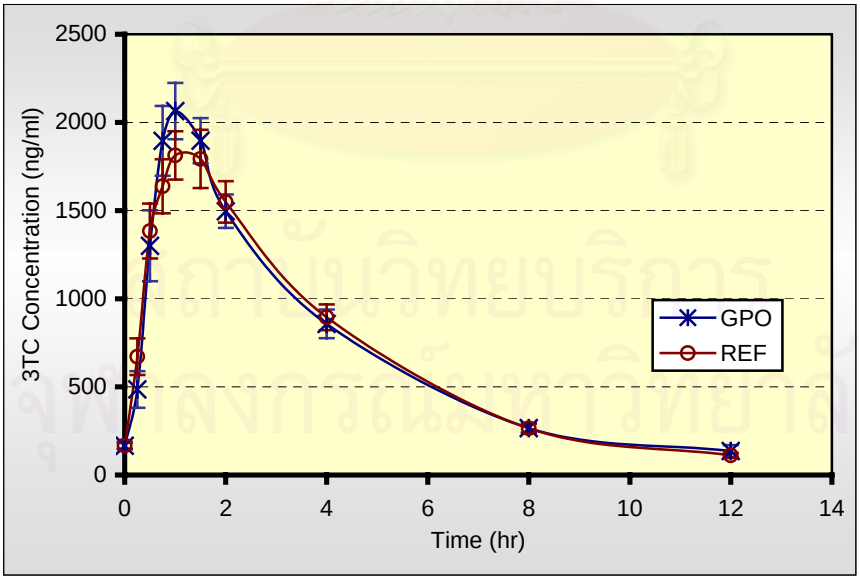


Figure 14 Compared average plasma concentration – time profiles of 3TC between reference tablet and GPO-vir S30 (mean ± SE)

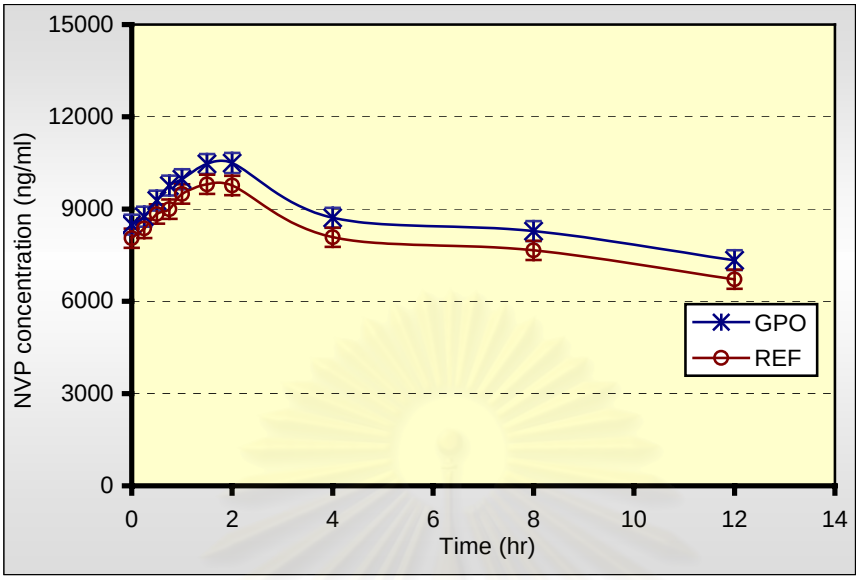


Figure 15 Compared average plasma concentration – time profiles of NVP between reference tablet and GPO-vir S30 (mean ± SE)

Table 28 ANOVA table of Ln (C_{max}) and Ln ($AUC_{0\text{-}12hr}$) of d4T (n = 20)

Source of Variation	*DF*	*Sum of Squares*	*Mean Square*	*F*	*Sig.*
Ln (C_{max})					
Treatment	1	0.09009	0.09009	0.8589	0.3663
Sequence	1	0.05014	0.05014	0.4780	0.4981
Period	1	0.14760	0.14760	1.4072	0.2509
Subject (Sequence)	18	2.14689	0.11927	1.1371	0.3941
Error	18	1.88799	0.10489		
Total	39	4.32271			
Ln ($AUC_{0\text{-}12hr}$)					
Treatment	1	0.01155	0.01155	0.2861	0.5993
Sequence	1	0.13731	0.13731	3.4009	0.0817
Period	1	0.02050	0.02050	0.5078	0.4852
Subject (Sequence)	18	2.23208	0.12400	3.0712	0.0110
Error	18	0.72677	0.04038		
Total	39	3.12821			

Table 29 ANOVA table of Ln (C_{max}) and Ln ($AUC_{0\text{-}12hr}$) of 3TC (n = 18)

Source of Variation	*DF*	*Sum of Squares*	*Mean Square*	*F*	*Sig.*
Ln (C_{max})					
Treatment	1	0.07536	0.07536	0.9532	0.3434
Sequence	1	0.14639	0.14639	1.8516	0.1925
Period	1	0.11168	0.11168	1.4126	0.2520
Subject (Sequence)	16	1.42432	0.08902	1.1260	0.4077
Error	16	1.26496	0.07906		
Total	35	3.02271			
Ln ($AUC_{0\text{-}12hr}$)					
Treatment	1	0.00084	0.00084	0.0171	0.8975
Sequence	1	0.02452	0.02452	0.4975	0.4908
Period	1	0.03417	0.03417	0.6932	0.4173
Subject (Sequence)	16	1.24175	0.07761	1.5745	0.1867
Error	16	0.78867	0.04929		
Total	35	2.08995			

Table 30 ANOVA table of Ln (C_{max}) and Ln ($AUC_{0\text{-}12hr}$) of NVP (n = 20)

Source of Variation	*DF*	*Sum of Squares*	*Mean Square*	*F*	*Sig.*
Ln (C_{max})					
Treatment	1	0.05028	0.05028	3.3098	0.0855
Sequence	1	0.00987	0.00987	0.6497	0.4308
Period	1	0.09693	0.09693	6.3803	0.0211
Subject (Sequence)	18	2.43402	0.13522	8.9006	0.0000
Error	18	0.27347	0.01519		
Total	39	2.86457			
Ln ($AUC_{0\text{-}12hr}$)					
Treatment	1	0.05592	0.05592	3.1800	0.0914
Sequence	1	0.00007	0.00007	0.0038	0.9513
Period	1	0.11082	0.11082	6.3017	0.0218
Subject (Sequence)	18	2.45817	0.13657	7.7657	0.0000
Error	18	0.31654	0.01759		
Total	39	2.94152			

Table 31 Comparisons of pharmacokinetic parameters of d4T, 3TC and NVP between combined formulation, GPO-vir S30 and three single reference tablets

Parameters		*Mean*	*90% CI of ratio*	*Sig.*	*Power*
d4T (n = 20)					
Ln (C_{max})	GPO	6.62	0.92 to 1.31	0.3663	0.6695
	REF	6.53			
Ratio (% of REF)	109.96				
Ln (AUC_{0-12hr})	GPO	6.98	0.87 to 1.08	0.5993	0.9538
	REF	7.01			
Ratio (% of REF)	96.66				
t_{max} (hr)	GPO	0.79			
	REF	0.78			
Ratio (% of REF)	101.61				
3TC (n = 18)					
Ln (C_{max})	GPO	7.72	0.93 to 1.30	0.3434	0.7330
	REF	7.63			
Ratio (% of REF)	109.58				
Ln (AUC_{0-12hr})	GPO	9.02	0.89 to 1.15	0.8975	0.8887
	REF	9.01			
Ratio (% of REF)	100.97				
t_{max} (hr)	GPO	1.03			
	REF	1.24			
Ratio (% of REF)	83.15				
NVP (n = 20)					
Ln (C_{max})	GPO	9.27	1.02 to 1.15	0.0855	0.9996
	REF	9.20			
Ratio (% of REF)	107.35				
Ln (AUC_{0-12hr})	GPO	11.52	1.00 to 1.16	0.0914	0.9989
	REF	11.44			
Ratio (% of REF)	107.76				
t_{max} (hr)	GPO	1.49			
	REF	1.56			
Ratio (% of REF)	95.20				

GPO: GPO-vir S30 REF: Reference tablet

4. General pharmacokinetics of d4T, 3TC and NVP

4.1 Stavudine

Mean values of general pharmacokinetic parameters of d4T after 30 mg twice daily dosage were shown in Table 32 for both preparations (combined drugs tablet, GPO-vir S30 and three single drug reference tablets). Figure 13 depicts mean plasma concentration – time profiles of d4T in 20 patients. Absorption was rapid, with maximum concentrations measured within 1.5 hr after drug administration in all patients.

Multiple dose pharmacokinetic parameters, K_e, $t_{1/2}$, CL/F and Vd/F obtained from this study were comparable with those observed after a single dose, with no evidence of accumulation.[22] Peak plasma concentration which was above concentration associated with antiretroviral activity *in vitro*[27]. However, the concentrations since 8 hr after drug administration until trough concentration at 12 hr were often undetectable. (below the sensitivity of HPLC assay).

Antiretroviral activity of stavudine requires activation of the intracellular to the triphosphorylated forms. The rate-determining step for activation of stavudine is the initial conversion to the monophosphate form by thymidine kinase. These results in high intracellular ratios of triphosphorylated form related to the other intermediates or unchange form. The decline in triphosphorylated forms in the intracellular is slow, with a longer half-life than extracellular. However, plasma pharmacokinetic profiles still importance. Therefore, the preparations that provide adequate and reproducible systemic exposure to drug are importance to concern. In this study, average AUC of two preparations was equivalence implied that the systemic exposure to d4T after either preparation was equal. Even C_{max} ratio was slightly higher than the 20% bioequivalence limit, but the $AUC_{0\text{-}12hr}$ were equivalent implied that the total amount of drug absorbed were equivalent.

4.2 Lamivudine

Of the 20 patients enrolled in the study, a total of 18 patients were included in the 3TC pharmacokinetic analysis; two patients were excluded because there were interfering peak (from concurrent medication) in the assay procedures. The pharmacokinetics of 3TC after multiple dose of 150 mg twice daily was evaluated by non-compartmental analysis. General pharmacokinetic parameters at steady-state of 3TC of individual patient were shown in Table 33, for both preparations (combined tablet, GPO-vir S30 and reference tablet). Figure 14 presents mean plasma concentration – time profile of 3TC in 18 patients.

Observed 3TC apparent volume of distribution (Vd/F) and clearance (CLss/F) were similar to the published data of 3TC. Moore et al. had reported CL/F of 3TC when administration alone and concomitant with co-trimoxazole to be 29.6 L/hr (27.6-31.8) and 20.8 L/hr (19.4-22.3), respectively.[35] All patients in this study received concomitant medication, co-trimoxazole for prophylaxis of *Pneumocystis carinii* pneumonia. The competitive inhibition of renal tubular secretion of 3TC by trimethoprim resulted in decreasing of CL and in turn increasing of AUC.

Antiviral activity of 3TC requires activation in the intracellular to the triphosphorylated form. Studies have observed that plasma 3TC concentrations correlate poorly with intracellular triphosphate concentrations. The average target concentration (C_{avg}) of 3TC at steady-state was 440 ng/mL.[56-57] Mean C_{avg} of 3TC was above target concentration. Because of high inter-patient variability the range of C_{avg} was 404.08 – 1051.54 ng/mL (Table 35), there were a few patients with C_{avg} slightly lower than the target concentration.

4.3 Nevirapine

Of the 20 patients enrolled in the study, all of them were included in the NVP pharmacokinetic analysis. The pharmacokinetics of NVP after multiple dose of 200 mg twice daily was calculated by non-compartmental analysis. Hepatic metabolism of NVP is mainly through the activity of CYP2B6 and CYP3A4. NVP also induces its own metabolism by activation both CYP2B6 and CYP3A4.[58] The pharmacokinetic study should be performed at the steady-state. This study was conducted after 4 weeks of initial treatment. Pharmacokinetic parameters at steady-state of NVP were shown in Table 28 and 34, for both preparations (combined tablet, GPO-vir S30 and reference tablet). Figure 15 presented mean plasma concentration – time profile of NVP in 20 patients.

The results of this study demonstrated that total exposure to NVP is not significantly different between a combined tablet, GPO-vir S30 and reference tablet. NVP peak plasma levels (C_{max}) and $AUC_{0\text{-}12hr}$ were higher than values those reported in the literature. Murphy et al. reported mean C_{max} and $AUC_{0\text{-}12hr}$ at steady-state of NVP as 7.0 $\pm$ 1.2 $x10^3$ng/mL and 61.98 $\pm$ 13.56 $x10^3$hr*ng/mL, respectively.[46] However, apparent volume of distribution (Vd/F) was higher and clearance (CLss/F) was lower to those published data of NVP.[42, 46] No relationship between C_{max}, AUC and toxicity were discerned in this study. The secondary C_{max}, which was observed 8 hr after ingestion of NVP, was in agreement with previous findings. The presence of secondary C_{max} suggests enterohepatic cycling, which had been shown to occur in rats.[46, 59]

Veldkamp et al. showed that plasma NVP concentration correlated with virological response.[60] The IC_{50} concentration of NVP was 3400 ng/mL.[3] The present study showed that C_{min} of NVP was above IC_{50} concentration, as showed in Table 35.

Table 32 Pharmacokinetic parameters: K_e, $t_{1/2}$, CL/F and Vd/F of d4T of individual patient after administration a combined drugs formulation, GPO-vir S30, and after administration three single drug reference tablets (n = 20)

Subject No.	*K_e (hr^{-1})*			*$t_{1/2}$ (hr)*			*CL/F (L/hr)*			*Vd/F (L)*		
	GPO	*REF*	*Average*	*GPO*	*REF*	*Average*	*GPO*	*REF*	*Average*	*GPO*	*REF*	*Average*
1	0.6607	0.6706	0.6657	1.05	1.03	1.04	25.47	25.16	25.31	38.55	37.51	38.03
2	0.6057	0.6150	0.6104	1.14	1.13	1.14	21.90	21.31	21.61	36.16	34.66	35.41
3	0.9420	0.6517	0.7969	0.74	1.06	0.90	24.82	31.67	28.25	26.35	48.59	37.47
4	1.0037	1.0196	1.0117	0.69	0.68	0.69	23.09	22.71	22.90	23.01	22.27	22.64
5		1.5085	1.5085		0.46	0.46		33.67	33.67		22.32	22.32
6	0.5862	0.6962	0.6412	1.18	1.00	1.09	26.03	22.89	24.46	44.41	32.87	38.64
7	0.5193	0.6447	0.5820	1.33	1.08	1.20	17.54	21.61	19.58	33.79	33.52	33.65
9	0.6910	0.7107	0.7009	1.00	0.98	0.99	26.98	23.70	25.34	39.05	33.35	36.20
10	0.9642	0.9652	0.9647	0.72	0.72	0.72	26.11	26.18	26.15	27.08	27.12	27.10
11	0.6728	0.8731	0.7730	1.03	0.79	0.91	31.30	27.69	29.49	46.53	31.71	39.12
12		0.6453	0.6453		1.07	1.07		20.69	20.69		32.07	32.07
13	0.5730	0.7825	0.6778	1.21	0.89	1.05	24.12	28.46	26.29	42.10	36.37	39.24
14	0.7559	0.7663	0.7611	0.92	0.90	0.91	20.50	26.13	23.32	27.13	34.10	30.61
15	0.3317	0.2442	0.2880	2.09	2.84	2.46	14.44	10.22	12.33	43.53	41.84	42.69
16	0.5092	0.4623	0.4858	1.36	1.50	1.43	20.05	20.06	20.05	39.37	43.38	41.38
17	0.6302	1.2208	0.9255	1.10	0.57	0.83	17.69	20.54	19.11	28.07	16.82	22.45
18	0.5132	0.5586	0.5359	1.35	1.24	1.30	31.55	23.99	27.77	61.47	42.96	52.21
19	0.4616	0.5484	0.5050	1.50	1.26	1.38	39.94	38.52	39.23	86.53	70.25	78.39
20	0.5936	0.6165	0.6051	1.17	1.12	1.15	20.09	21.75	20.92	33.84	35.28	34.56
22	0.8864	0.8624	0.8744	0.78	0.80	0.79	23.09	33.54	28.31	26.05	38.88	32.47
Mean	0.6611	0.7531	0.7096	1.13	1.06	1.09	24.15	25.02	24.61	39.06	35.79	37.34
SD	0.1854	0.2752	0.2384	0.34	0.49	0.42	5.97	6.18	6.02	15.20	11.15	13.14
%CV	28.04	36.54	33.61	29.78	46.12	38.36	24.73	24.72	24.46	38.92	31.16	35.20
Min.	0.3317	0.2442	0.2442	0.69	0.46	0.46	14.44	10.22	10.22	23.01	16.82	16.82
Max.	1.0037	1.5085	1.5085	2.09	2.84	2.84	39.94	38.52	39.94	86.53	70.25	86.53
Max.-Min.	0.6720	1.2643	1.2643	1.40	2.38	2.38	25.50	28.31	29.72	63.52	53.43	69.70

Table 33 Pharmacokinetic parameters: K_e, $t_{1/2}$, CL/F and Vd/F of 3TC of individual patient after administration a combined drugs formulation, GPO-vir S30, and after administration three single drug reference tablets (n = 18)

Subject No.	*K_e (hr^{-1})*			*$t_{1/2}$ (hr)*			*CL/F (L/hr)*			*Vd/F (L)*		
	GPO	*REF*	*Average*	*GPO*	*REF*	*Average*	*GPO*	*REF*	*Average*	*GPO*	*REF*	*Average*
1	0.4242	0.4376	0.4309	1.63	1.58	1.61	18.36	19.59	18.98	43.28	44.77	44.03
2	0.3515	0.2693	0.3104	1.97	2.57	2.27	17.08	13.81	15.44	48.59	51.28	49.93
3	0.3203	0.2584	0.2894	2.16	2.68	2.42	21.41	19.44	20.42	66.84	75.23	71.04
4	0.2728	0.3589	0.3159	2.54	1.93	2.24	17.85	12.46	15.15	65.44	34.71	50.08
6	0.3356	0.2277	0.2817	2.07	3.04	2.56	19.28	17.04	18.16	57.46	74.84	66.15
7	0.3176	0.5349	0.4263	2.18	1.30	1.74	15.22	14.94	15.08	47.93	27.93	37.93
8	0.2464	0.2314	0.2389	2.81	3.00	2.90	27.33	11.89	19.61	110.90	51.37	81.13
11	0.3314	0.2502	0.2908	2.09	2.77	2.43	20.02	22.87	21.44	60.40	91.40	75.90
12	0.2915	0.3269	0.3092	2.38	2.12	2.25	19.80	21.61	20.70	67.91	66.09	67.00
13	0.2719	0.2619	0.2669	2.55	2.65	2.60	19.82	25.08	22.45	72.88	95.79	84.34
14	0.2315	0.2778	0.2547	2.99	2.50	2.74	16.70	18.01	17.36	72.15	64.83	68.49
15	0.2237	0.2019	0.2128	3.10	3.43	3.27	15.10	14.85	14.97	67.48	73.56	70.52
16	0.1175	0.2806	0.1991	5.90	2.47	4.19	15.37	19.11	17.24	130.86	68.12	99.49
17	0.2372	0.2182	0.2277	2.92	3.18	3.05	12.25	13.80	13.03	51.66	63.25	57.45
18	0.3624	0.3011	0.3318	1.91	2.30	2.11	20.00	21.40	20.70	55.19	71.07	63.13
19	0.2371	0.2549	0.2460	2.92	2.72	2.82	30.93	29.22	30.08	130.44	114.61	122.53
20	0.3087	0.2719	0.2903	2.25	2.55	2.40	16.64	18.92	17.78	53.90	69.59	61.74
22	0.1475	0.1872	0.1674	4.70	3.70	4.20	13.40	28.65	21.03	90.81	153.07	121.94
Mean	**0.2794**	**0.2862**	**0.2828**	**2.73**	**2.58**	**2.65**	**18.70**	**19.04**	**18.87**	**71.90**	**71.75**	**71.82**
SD	0.0755	0.0854	0.0795	1.04	0.60	0.84	4.57	5.14	4.80	26.76	29.17	27.59
%CV	27.0124	29.8609	28.1245	38.31	23.33	31.78	24.45	26.98	25.41	37.22	40.65	38.41
Min.	0.1175	0.1872	0.1175	1.63	1.30	1.30	12.25	11.89	11.89	43.28	27.93	27.93
Max.	0.4242	0.5349	0.5349	5.90	3.70	5.90	30.93	29.22	30.93	130.86	153.07	153.07
Max.-Min.	0.3067	0.3477	0.4174	4.27	2.41	4.61	18.68	17.33	19.05	87.57	125.14	125.14

Table 34 Pharmacokinetic parameters: K_e, $t_{1/2}$, CL/F and Vd/F of NVP of individual patient after administration a combined drugs formulation, GPO-vir S30, and after administration three single drug reference tablets (n = 18)

Subject No.	K_e (hr^{-1})			$t_{1/2}$ (hr)			CL/F (L/hr)			Vd/F (L)		
	GPO	REF	Average	GPO	REF	Average	GPO	REF	Average	GPO	REF	Average
1	0.0216	0.0228	0.0222	32.07	30.34	31.21	1.24	1.39	1.31	57.42	60.69	59.06
2	0.0124	0.0192	0.0158	55.94	36.15	46.04	2.77	2.23	2.50	223.37	116.12	169.75
3	0.0414	0.0607	0.0511	16.75	11.43	14.09	3.09	3.18	3.14	74.64	52.46	63.55
4	0.0203	0.0279	0.0241	34.17	24.84	29.50	1.53	1.68	1.61	75.48	60.22	67.85
5	0.0580	0.0356	0.0468	11.96	19.46	15.71	2.04	2.41	2.23	35.14	67.76	51.45
6	0.0160	0.0463	0.0312	43.24	14.96	29.10	1.58	1.74	1.66	98.27	37.64	67.96
7	0.0331	0.0271	0.0301	20.96	25.53	23.25	1.24	2.35	1.80	37.61	86.75	62.18
9	0.0201	0.0145	0.0173	34.40	47.71	41.06	1.54	1.50	1.52	76.44	103.57	90.01
11	0.0361	0.0199	0.0280	19.23	34.76	26.99	2.34	2.36	2.35	65.02	118.33	91.67
12	0.0613	0.0509	0.0561	11.31	13.61	12.46	3.05	3.46	3.26	49.72	68.03	58.87
13	0.0137	0.0340	0.0239	50.67	20.40	35.53	1.54	1.68	1.61	112.47	49.46	80.97
15	0.0223	0.0376	0.0300	31.09	18.45	24.77	2.20	2.42	2.31	98.64	64.50	81.57
16	0.0450	0.0192	0.0321	15.41	36.18	25.79	2.55	2.11	2.33	56.78	110.27	83.53
17	0.0192	0.0450	0.0321	36.18	15.41	25.79	2.11	2.55	2.33	110.27	56.78	83.53
18	0.0270	0.0327	0.0299	25.70	21.18	23.44	2.25	1.91	2.08	83.32	58.28	70.80
19	0.0484	0.0287	0.0386	14.33	24.13	19.23	2.30	3.76	3.03	47.45	130.94	89.19
20	0.0338	0.0422	0.0380	20.49	16.42	18.45	2.58	2.45	2.51	76.23	58.00	67.12
22	0.0305	0.0220	0.0263	22.73	31.52	27.13	1.85	1.49	1.67	60.78	67.73	64.25
Mean	0.0311	0.0326	0.0318	27.59	24.58	26.09	2.10	2.26	2.18	79.95	75.97	77.96
SD	0.0148	0.0126	0.0136	13.11	9.76	11.49	0.58	0.68	0.62	42.51	27.68	35.41
%CV	47.50	38.82	42.63	47.50	39.70	44.04	27.45	29.88	28.63	53.18	36.43	45.42
Min.	0.0124	0.0145	0.0124	11.31	11.43	11.31	1.24	1.39	1.24	35.14	37.64	35.14
Max.	0.0613	0.0607	0.0613	55.94	47.71	55.94	3.09	3.76	3.76	223.37	130.94	223.37
Max.-Min.	0.0489	0.0462	0.0489	44.63	36.28	44.63	1.85	2.37	2.52	188.23	93.29	188.23

Table 35 C_{avg} of d4T, 3TC and NVP and C_{min} of 3TC and NVP of individual patient after administration a combined drugs formulation, GPO-vir S30, compared to those after three single drug reference tablets

Subject No.	d4T (n = 18)			3TC (n = 18)						NVP (n = 20)					
	C_{avg} (ng/ml			C_{min} (ng/ml)			C_{avg} (ng/ml			C_{min} (ng/ml)			C_{avg} (ng/ml		
	GPO	REF	Total	GPO	REF	Total	GPO	REF	Total	GPO	REF	Total	GPO	REF	Total
1	90.48	89.38	89.93	33.96	25.19	29.58	680.85	637.97	659.41	11465.68	10357.91	10911.80	13430.43	12021.45	12725.94
2	104.89	104.29	104.59	68.55	118.16	93.36	732.03	905.00	818.52	5523.62	6914.49	6219.06	6021.78	7484.99	6753.39
3	97.38	68.54	82.96	76.55	118.98	97.77	583.91	643.12	613.51	4079.88	3849.44	3964.66	5395.48	5238.70	5317.09
4	105.75	106.60	106.17	119.07	93.03	106.05	700.24	1003.49	851.86	9239.85	8475.20	8857.53	10884.98	9918.46	10401.72
5	28.55	73.99	51.27	NA	NA	122.24	NA	NA	690.92	6036.30	5370.68	5703.49	8180.76	6905.92	7543.34
6	86.72	102.23	94.47	89.32	155.16	68.06	648.26	733.59	829.01	7812.18	7394.35	7603.27	10578.47	9554.82	10066.65
7	113.94	104.59	109.26	125.41	10.71	144.80	821.29	836.73	754.49	10722.61	5791.16	8256.89	13403.22	7077.85	10240.54
9	86.60	98.31	92.45	70.52	219.07	72.07	457.44	1051.54	585.50	9385.23	9371.93	9378.58	10820.24	11076.75	10948.49
10	90.97	90.94	90.95	NA	NA	86.46	NA	NA	604.99	8467.39	7507.10	7987.25	8978.04	8137.31	8557.68
11	73.46	85.37	79.42	69.86	74.27	69.14	624.51	546.50	564.52	5450.49	5938.15	5694.32	7109.81	7064.00	7086.90
12	86.57	108.80	97.68	107.84	65.07	113.49	631.44	578.55	721.23	3962.36	3417.90	3690.13	5472.05	4810.93	5141.49
13	95.25	83.50	89.38	83.82	54.45	165.71	630.71	498.34	834.79	9117.05	8463.95	8790.50	10832.30	9915.47	10373.89
14	115.62	87.83	101.72	110.69	116.28	118.07	748.40	694.06	733.64	9449.94	7418.50	8434.22	10204.18	9095.36	9649.77
15	126.88	146.41	136.65	144.09	187.32	183.09	828.09	841.49	962.93	6309.88	5456.96	5883.42	7579.58	6878.26	7228.92
16	102.55	103.84	103.20	160.39	75.74	55.81	813.31	653.97	604.50	5197.13	6524.92	5861.03	6524.26	7888.45	7206.35
17	128.97	120.42	124.69	174.69	191.49	66.94	1020.29	905.57	415.95	6524.92	5197.13	5861.03	7888.45	6524.26	7206.35
18	70.07	91.71	80.89	45.07	66.55	131.68	624.90	584.10	705.90	6212.80	7137.48	6675.14	7417.19	8737.45	8077.32
19	52.13	55.89	54.01	78.45	55.42	170.41	404.08	427.82	684.61	5748.11	3697.68	4722.90	7261.14	4431.28	5846.21
20	113.68	100.19	106.94	110.33	153.03	105.26	751.25	660.55	702.02	5610.04	5192.88	5401.46	6462.60	6806.07	6634.33
22	105.14	71.32	88.23	230.90	109.92	53.05	932.97	436.25	167.08	7861.20	9725.97	8793.59	8992.00	11191.08	10091.54
Mean	93.78	94.71	94.24	105.53	104.99	50.40	701.89	702.15	23.80	7208.83	6660.19	6934.51	8671.85	8037.94	8354.89
SD	24.32	19.90	21.94	48.86	58.36	10.71	151.56	185.75	404.08	2164.40	1986.61	2069.33	2416.30	2126.47	2269.45
%CV	25.93	21.02	23.28	46.30	55.59	230.90	21.59	26.46	1051.54	30.02	29.83	29.84	27.86	26.46	27.16
Min.	28.55	55.89	28.55	33.96	10.71	220.19	404.08	427.82	659.41	3962.36	3417.90	3417.90	5395.48	4431.28	4431.28
Max.	128.97	146.41	146.41	230.90	219.07	29.58	1020.29	1051.54	818.52	11465.68	10357.91	11465.68	13430.43	12021.45	13430.43
Max.-Min.	100.42	90.52	117.86	196.94	208.36	93.36	616.21	623.72	613.51	7503.32	6940.01	8047.78	8034.95	7590.18	8999.15

C_{min} of d4T was below the sensitivity of HPLC assay, NA: Not available

5. Short-term clinical efficacy and safety of GPO-vir S30 in HIV-infected patients

The study had included overall 33 patients. Thirteen patients were excluded before completely blood sampling for pharmacokinetic study. Among them, nine patients were excluded due to adverse drug reaction, two patients developed tuberculosis, one was poor adherence while another one developed HIV-related disease as shown in Figure 16. Twenty patients had completed the blood sampling process for pharmacokinetic study. After blood samples were completely collected for comparative bioavailability study, clinical efficacy and safety of GPO-vir S30 were followed-up for 12 weeks. Nineteen patients had completed this step; one patient could not tolerate skin rash adverse effect and was excluded during week 6^{th}. Information of adverse events and HIV-related illness were collected at each visit (4 – 6 week interval). During follow-up period, there were two patients who developed new opportunistic infection. One patient had oral candidiasis in week 10^{th}, the other developed genital herpes simplex virus in week 2^{nd}. No other serious opportunistic infection was observed.

Viral load decreased and CD4 cell counts increased after treatment for 18 weeks. The virological and immunological profiles were shown in Table 36 and 37, respectively. The reduction in plasma HIV-RNA from 5.24 $\log_{10}$ copies/mL (range 3.87 to > 5.87 $\log_{10}$ copies/mL) to 1.73 $\log_{10}$ copies/mL (range <1.69 to 2.20 $\log_{10}$ copies/mL) was observed. A viral load of less than 50 copies/mL was found in 78.9 % of patient (15 from 19). Among the patients whose baseline viral load were $\geq$ 100,000 copies/mL (n = 15), there were only three patients who still had detectable viral load (66, 81 and 157 copies/ml) after 18 weeks of treatment. High baseline viral load in these patients (432,000, 385,000 and >750,000 copies/ml, respectively) could be the affected factor for detectable viral load after 18 weeks. Among the 4 patients with baseline viral load < 100,000 copies/mL (n = 4), 3 patients had undetectable viral load (< 50 copies/ml), while another one had viral load equal to 50 copies/ml at the end of 18 weeks of treatment.

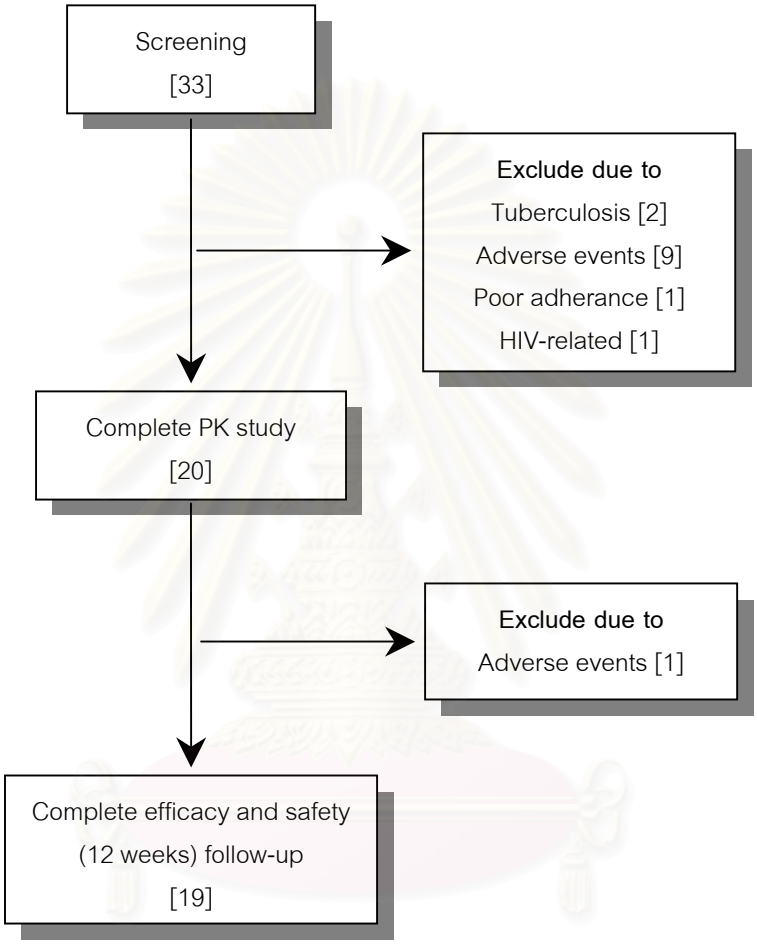


Figure 16 Number of patients included in different process of study

The immunological response to GPO-vir S30 mirrored the virological response. A mean increase in CD4 count to 206.72 cells/mm^3 (range 107 to 366 cells/mm^3) was seen at the end of 18 weeks follow-up, as presented in Table 37. The virological and immunological profiles from this study were only the initial evaluation for efficacy of antiretroviral treatment. Viral load also predicts therapeutic outcome. For every decrease in viral load of 1 log scale after 6 months of therapy, there is corresponding 43-72% decrease in the risk of disease progression and death.[61] In this study, decreasing of viral load of all patients was more than 1 log scale at the end of 18 weeks follow-up indicate that good therapeutic effectiveness could be predicted. However, the clinical outcome in the long-term should be monitored.

Table 36 Virological profile of patients after 18 weeks follow-up (n = 19)

Initial VL (copies/ml)	*End of study VL (number of patients)*	
	<50 VL copies/ml	*≥ 50 copies/ml*
< 100,000 (n = 4)	3	1
>=100,000 (n = 15)	12	3
Total (n=19)	**15**	**4**

Table 37 Immunological profile of patients after 18 weeks follow-up (n = 18)

	Mean (range), (cells/mm^3),
Initial CD4 (n=18)	108.78 (51-198)
End of study CD4 (n=18)	206.72 (107-366)
Initial %CD4 (n=18)	7.89 (2-13)
End of study %CD4 (n=18)	13.83 (6-21)

Safety of the drug treatment was also monitored through laboratory data. Liver transaminase, at baseline, week 2nd and 18th were all in the normal level. Hematology and chemistry testing were all within the normal ranges at the end of week 18th. Comparisons between the means of all laboratory values by paired-sample t test showed no statistically difference between the values at baseline and at the end of week 18th ($P > 0.05$) except for the values of serum lactate as shown in table 39. The serum lactate at week 18th (1.43 mmol/L) increased significantly ($P = 0.0053$) from baseline (0.97 mmol/L), but still in the range of normal level (0.7-2.5 mmol/L). The increasing level of serum lactate indicates that the patients have predisposing factor for lactic acidosis to occur, closely monitoring of this laboratory data for long-term safety should be concerned.

Table 38 Laboratory profiles of patients after 18 weeks follow-up

Laboratory test (Normal range)	*Time of follow-up*	*n*	*Mean*	*SD*	*Min.*	*Max.*	*Sig.*
SGOT (U/L)	Baseline	19	29.68	9.72	16	57	
(10 – 35 U/L)	Week 2	19	27.37	15.49	12	84	0.4298
	Week 18	19	30.05	20.82	15	104	0.9345
SGPT (U/L)	Baseline	19	20.63	11.49	9	55	
(10 – 40 U/L)	Week 2	19	21.11	12.06	8	51	0.8114
	Week 18	19	24.42	14.87	8	75	0.3729
Hematocrit (%)	Baseline	19	36.05	5.10	31	49	
(Male:40-52 %, Female: 34-46 %)	Week 18	19	37.05	4.62	31	46	0.2160
Hemoglobin (g/dl)	Baseline	19	11.90	1.88	9.09	16.50	
(g/dl)	Week 18	19	12.51	1.63	10.10	15.70	0.0531
Platelet ($x10^3$ cell/mm^3)	Baseline	19	275.74	54.11	173	388	
(160-370 $x10^3$ cell/mm^3)	Week 18	19	282.11	44.63	190	352	0.5432
WBC ($x10^3$ cell/mm^3)	Baseline	19	4.37	1.53	1.68	6.97	
(3.8-10.5 $x10^3$ cell/mm^3)	Week 18	19	4.78	1.36	2.70	7.23	0.2058
BUN (mg/dL)	Baseline	19	10.95	2.83	7.0	16.0	
(10 – 20 mg/dL)	Week 18	19	11.74	3.23	5.0	19.0	0.3943
Creatinine (mg/dL)	Baseline	18	0.81	0.18	0.60	1.23	
(0.6 – 1.2 mg/dL)	Week 18	18	0.87	0.28	0.43	1.59	0.2543
ALP (U/L)	Baseline	19	96.74	30.33	59	162	
(32 – 150 U/L)	Week 18	19	110.42	47.95	47	264	0.1018
Lactate (mmol/L)	Baseline	19	0.97	0.46	0.3	2.4	
(0.7 – 2.5 mmol/L)	Week 18	19	1.43	0.47	0.6	2.3	0.0053

Adverse events were reported in Table 39. Overall 13 patients (39 %) of the total 33 patients initially included into the study experienced grade 1 or 2 adverse events. The most common adverse events was dermatological reaction. One patient had elevated SGOT/SGPT levels. Three patients' experienced grade 3 or 4 adverse events, one of them could tolerate after antihistamine and oral corticosteroid, while two could not tolerate and were excluded from the study. These grade 3 or 4 adverse events were dermatological reaction and Steven's Johnson Syndrome. After completion of the blood sampling process for pharmacokinetics study, one patient discontinued therapy after week 6th because of intolerance to skin rash even though it was only in grade 1-2. Adverse events observed in this study were only after short-term follow-up. There are many long-term adverse events which can be occurred due to this antiretroviral regimen, such as hepatitis, lipodystrophy, peripheral neuropathy, lactic acidosis and pancreatitis. Therefore, laboratory testing, physical examination and clinical follow-up should be considered and closely monitors in long-term treatment are highly recommended.

Table 39 Clinical and laboratory adverse events

Adverse events	*Frequency*			
	Included group (n = 20)		*Excluded group (n = 13)*	
	Grade 1 or 2 adverse event	*Grade 3 or 4 adverse event*	*Grade 1 or 2 adverse event*	*Grade 3 or 4 adverse event*
Clinical				
Rash	3	1	7	1
Myalgia/Arthralgia	1	0	1	0
Headache	1	0	1	0
Nausea	1	0	0	0
Fever	1	0	3	0
Face edema	0	0	1	0
weakness	1	0	0	0
Erythema Multiform	0	0	1	0
Steven's Johnson Syndrome	0	0	0	1
Laboratory				
SGOT/SGPT elevated	1	0	0	0
Total	**9**	**1**	**14**	**2**

CHAPTER V

CONCLUSION

1. Study population

Twenty HIV-infected patients were enrolled in the study. All patients received antiretroviral as d4T/3TC/NVP regimen. After lead-in period of NVP, they were divided into two groups; ten of them received one combined tablet of d4T, 3TC and NVP (GPO-vir S30) every 12 hr, while the second group received three single original brand tablets, Zerit 30 mg, Epivir 150 mg and Viramune 200 mg every 12 hr. Blood samples were collected after 2 weeks and the second period blood sampling was performed after crossover for another 2 weeks. Safety and efficacy of GPO-vir S30 were further followed for 3 months.

2. Bioanalysis of d4T, 3TC and NVP

The method for analysis of d4T, 3TC and NVP concentrations in plasma were developed by modified from those methods of Moyer et al. The linearity of each calibration curves were determined from seven concentrations between 50-2000 ng/ml, 50-5000 ng/ml and 100-10000 ng/ml for d4T, 3TC and NVP, respectively. D4T and 3TC were analyzed at the same time using bromouracil as the internal standard. Solid phase extraction was used in the sample preparation. NVP was analyzed in another condition with clozapine used as the internal standard. Protein precipitation by perchloric acid was used in the sample preparation.

The correlation between concentration and peak area ratio was expressed by coefficient of determination (R^2) that was higher than 0.99. Method validation was performed for three days. Accuracy and precision of intra- and inter-day were all within the acceptable ranges. Recovery of extraction was about 75, 92 and 98 % for d4T, 3TC and NVP, respectively. The stability of plasma samples and extracts were tested at various conditions and were always higher than 85%.

3. Pharmacokinetic parameters of d4T, 3TC and NVP obtained from a combined drugs formulation, GPO-vir S30, compared to those from three single drug reference tablets

Pharmacokinetic parameters of d4T, 3TC and NVP calculated from the data of individual patients using non-compartmental analysis after administration with combined drugs tablet, GPO-vir S30, were comparable to those obtained from reference tablets. Analysis of variance using log-transformed data and 90% confidence interval showed that $AUC_{0\text{-}12hr}$ of all drugs were within the 20 percent bioequivalence limit. C_{max} ratio of NVP was also within the bioequivalence limit while C_{max} of d4T and 3TC ratios were slightly higher than 20 percent in the upper limit.

4. Short-term clinical efficacy and safety of GPO-vir S30 in HIV-infected patients

After 3 months of treatment with GPO-vir S30, virological response had been expressed by the reduction of plasma HIV-RNA from 5.24 $\log_{10}$ copies/mL (range 3.87 to > 5.87 $\log_{10}$ copies/mL) to 1.73 $\log_{10}$ copies/mL (range <1.69 to 2.20 $\log_{10}$ copies/mL). A viral load of less than 50 copies/mL were found in 78.9 % of patient (15 from 19). Only four patients still had detectable viral load (50, 66, 81 and 157 copies/ml). High baseline viral load may be the affected factor. The immunological response to GPO-vir S30 was presented by the increment in CD4 count from 108.78 cells/mm^3 (range 51-198 cells/mm^3) to 206.72 cells/mm^3 (range 107 to 366 cells/mm^3) after 18 weeks follow-up.

Adverse events were reported for 7 patients (35 %) of the total 20 patients participated. Majority were experienced with grade 1 or 2 adverse events. The most common adverse events were dermatological reaction.

D4T/3TC/NVP is one of the recommended regimens for HIV/AIDS in standard treatment guideline of Thailand.[17] The combined drugs tablet, GPO-vir S30 and GPO-vir S40, requires only one pill twice daily. The simplier administration can improve patient adherence and the cost was much cheaper. The $AUC_{0\text{-}12hr}$ ratio of all components were within equivalent range indicating that the total amount of drug absorbed were equivalent between the two preparations. The clinical efficacy and safety were satisfied. However, since this study was only performed for a short-term, long-term efficacy and safety should be monitored. These patients may need the antiretroviral for life-long, follow up for long-term adverse events, drug resistance and adherence should be concerned.

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APPENDICES

APPENDICES A

เอกสารแสดงรายละเอียดเกี่ยวกับงานวิจัยสำหรับผู้ป่วย

1. **ชื่อโครงการวิจัย** : เภสัชจลนศาสตร์ของสตาวูดีน ลามิวูดีน และเนวิราพีนในยาสูตรผสม จีพีโอ-เวียร์ เอส30 เปรียบเทียบกับการให้ยาต้นแบบเดี่ยว 3 ชนิดร่วมกัน และการประเมินประสิทธิผลการรักษาและความปลอดภัยของ จีพีโอ-เวียร์ เอส30 ในผู้ป่วยติดเชื้อเอชไอวี
2. **ชื่อผู้วิจัยหลัก** : ภญ. รศ. ดร. ดวงจิต พนมวัน ณ อยุธยา
   ตำแหน่ง : รองศาสตราจารย์ 9
   สถานที่ทำงาน : โครงการจัดตั้งภาควิชาเภสัชกรรมคลินิก คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ถ.พญาไท เขตปทุมวัน กรุงเทพฯ 10330
   โทรศัพท์ : 0-2218-8405
   โทรสาร : 0-2218-8403
3. **เนื้อหาสาระของโครงการวิจัยและความเกี่ยวข้องกับผู้ป่วย**

   3.1 **เหตุผลและความจำเป็นที่ต้องทำการศึกษาวิจัย**

ในปัจจุบันโรคเอดส์ยังไม่มียาที่สามารถรักษาให้หายขาดได้ การรักษาด้วยยาต้านเอชไอวีมีจุดมุ่งหมายเพื่อลดปริมาณเชื้อไวรัสในกระแสเลือดให้มากและยาวนานที่สุด ตลอดจนป้องกันการทำลายและเพื่อให้มีการฟื้นกลับมาของภูมิคุ้มกัน เพื่อเพิ่มคุณภาพชีวิตของผู้ป่วย ลดอัตราการตายและอัตราการป่วยเป็นเอดส์ ปัญหาจากการใช้ยาในระยะยาวก็มีมากเช่นกัน เช่น อาการข้างเคียงจากยา ความร่วมมือในการใช้ยา รวมทั้งปัญหาค่าใช้จ่ายในการรักษา เนื่องจากยาต้านเอชไอวีมีราคาค่อนข้างสูง ตามมาตรฐานการรักษาผู้ป่วยติดเชื้อ/ผู้ป่วยเอดส์นั้นแนะนำให้ใช้ยาต้านเอชไอวีอย่างน้อย 3 ชนิดร่วมกันและจะต้องได้รับยาไปตลอดชีวิต สูตรยาแต่ละสูตรมีข้อดีและข้อเสียแตกต่างกันทั้งทางด้านประสิทธิภาพการรักษา ความสะดวกในการรับประทาน อาการไม่พึงประสงค์จากการใช้ยา และปัญหาเรื่องค่าใช้จ่ายซึ่งเป็นปัญหาหนึ่งที่มีความสำคัญต่อระบบบริการทางสาธารณสุขในการที่จะต้องรับภาระที่จะเกิดขึ้นเนื่องจากจำนวนผู้ป่วยที่เพิ่มมากขึ้น

ในช่วงที่ผ่านมาได้มีการพัฒนาอย่างรวดเร็วเกี่ยวกับยาต้านเอชไอวี ให้มีผลข้างเคียงน้อยลง และสามารถรับประทานได้สะดวกมากขึ้น ประกอบกับองค์การเภสัชกรรมสามารถผลิตยาต้านเอชไอวีหลายชนิดได้ในราคาที่ถูกลง ยาสูตรผสม GPO-vir ประกอบด้วยยา 3 ชนิดได้แก่ d4T, 3TC และ NVP เป็นสูตรหนึ่งที่ผลิตโดยองค์การเภสัชกรรมและอยู่ในระหว่างการศึกษาวิจัยเพื่อหาประสิทธิภาพในการรักษา

การวิจัยนี้เป็นการศึกษาความเท่าเทียมกันของยาต้านเอชไอวีสูตรผสม GPO-vir S30 โดยเปรียบเทียบกับยาต้นแบบ ซึ่งงานวิจัยนี้ได้รับความร่วมมืออย่างดีจากองค์การเภสัชกรรม ในการหาแหล่งทุนสนับสนุนในการวิจัยและจัดหายาสูตรผสม GPO-vir S30 เพื่อการวิจัย สิ่งที่มุ่งหวังในขั้นสุดท้ายคือสามารถนำไปเป็นแนวทางในการผลิตยาในขั้นอุตสาหกรรมเพื่อให้ผู้ป่วยมียาที่มีคุณภาพซึ่งผลิตได้เองในประเทศ ราคาถูกเป็นที่ยอมรับของผู้ป่วย และเป็นที่พอใจของแพทย์ผู้ใช้ยา สามารถลดต้นทุนในการนำเข้ายา โดยลดภาระค่าใช้จ่ายในการรักษาพยาบาลของผู้ป่วยและของประเทศ ซึ่งเหมาะสมกับสถานการณ์ปัจจุบัน และเป็นการพัฒนางานวิจัยที่เกิดขึ้นภายในประเทศให้บังเกิดผลที่สามารถนำมาใช้ได้จริง

3.2 วัตถุประสงค์ของการวิจัย

เปรียบเทียบความเข้มข้นของยาในเลือดของยาสูตรผสม GPO-vir S30 กับการให้ยาต้นแบบเดี่ยว 3 ชนิดร่วมกันในผู้ป่วยติดเชื้อเอชไอวี

3.3 วิธีการศึกษาวิจัยโดยสังเขป

ศึกษาในผู้ป่วยติดเชื้อเอชไอวีจำนวน 20 คน ในการศึกษาผู้ป่วยจะได้รับยาทั้งสองผลิตภัณฑ์ โดยสุ่มให้ได้รับยาผลิตภัณฑ์หนึ่งก่อนและให้รับประทานเป็นเวลา 2 สัปดาห์จึงทำการเก็บตัวอย่างเลือด โดยรับผู้ป่วยเข้านอนในโรงพยาบาลในเช้าวันที่ 14 ของการศึกษาเก็บตัวอย่างเลือดที่เส้นเลือดตำแหน่งท้องแขนหรือหลังมือครั้งละ 6 มิลลิลิตร (ประมาณ 1 ช้อนชาเศษ) ที่เวลาก่อนรับประทานยาคือ 8.00 น. และหลังรับประทานยา 15 นาที, 30 นาที, 45 นาที, 1 ชั่วโมง, 1 ชั่วโมงครึ่ง, 2, 4, 8 และ12 ชั่วโมงตามลำดับโดยให้ผู้ป่วยรับประทานยาพร้อมกับน้ำ 200 mL และให้รับประทานอาหารตามที่โรงพยาบาลจัดให้ในเวลา 10.00 น. จากนั้นจึงให้รับประทานยาผลิตภัณฑ์ที่เหลือเป็นเวลา 2 สัปดาห์และเก็บตัวอย่างเลือดเช่นเดียวกัน โดยรับผู้ป่วยเข้านอนในโรงพยาบาลในวันที่ 28 และเก็บตัวอย่างเลือดเช่นเดียวกับที่ดำเนินการในวันที่ 14 จากนั้นผู้ป่วยทั้ง 2 กลุ่มจะได้รับยา GPO-vir S30 หรือ S40 จนครบ 3 เดือน โดยนัดติดตามผลทุก 1 เดือนและตรวจเลือดเพื่อติดตามความปลอดภัยและประสิทธิผลการรักษาเมื่อครบ 3 เดือน

3.4 ระยะเวลาที่ผู้ป่วยต้องเกี่ยวข้องในการศึกษาวิจัย

ระยะเวลาประมาณ 5 เดือน

3.5 ประโยชน์ที่คาดว่าจะเกิดขึ้น

งานวิจัยนี้เป็นการศึกษาเพื่อเปรียบเทียบค่าทางเภสัชจลนศาสตร์ (pharmacokinetic parameters) ของยาสูตรผสม GPO-vir S30 กับยาต้นแบบ ประโยชน์สูงสุดที่คาดว่าจะได้รับคือสามารถนำไปเป็นแนวทางในการผลิตยาในขั้นอุตสาหกรรมเพื่อให้ผู้ป่วยมียาที่มีคุณภาพ ราคาถูกเนื่องจากสามารถผลิตได้เองในประเทศ ทำให้ลดต้นทุนในการนำเข้ายาและลดภาระค่าใช้จ่ายในการรักษาพยาบาลของผู้ป่วยและของประเทศ

3.6 ความเสี่ยงที่คาดว่าจะเกิดขึ้นกับผู้ป่วย

3.6.1 การเกิดอาการข้างเคียงจากการใช้ยา

อาการไม่พึงประสงค์จากการใช้ยาที่พบบ่อยคือ ผื่นคันตามผิวหนังอาจมีอาการรุนแรงจนต้องหยุดยา ตับอักเสบ ปวดข้อ ปวดกล้ามเนื้อ ต่อมน้ำเหลืองโต เม็ดเลือดขาวต่ำ ปลายประสาทอักเสบ ปวดศีรษะ คลื่นไส้ อาเจียน ตับอ่อนอักเสบ โลหิตจาง

3.6.2 การเจาะเลือด

ผู้เข้าร่วมการวิจัยจะเจ็บปวดจากการเจาะเลือดทั้งหมดดังนี้

- เพื่อหาข้อมูลพื้นฐานของผู้ป่วย เป็นการเก็บตัวอย่างเลือดเพื่อตรวจทางห้องปฏิบัติการเป็นจำนวนประมาณ 25 มิลลิลิตร (5 ช้อนชา)
- เพื่อวิเคราะห์ความเข้มข้นของยาในพลาสมา โดยแบ่งเป็น 2 ช่วง ห่างกัน 2 สัปดาห์ช่วงละ 10 ครั้ง แต่ละช่วงจะเก็บรักษาเส้นเลือดไว้ด้วย heparin lock และปริมาณเลือดที่เก็บครั้งละประมาณ 6 มิลลิลิตร รวมเป็น 120 มิลลิลิตร

การเจาะเลือดเพียงเล็กน้อย โดยทั่วไปจะไม่เกิดอันตรายใดๆ แก่ผู้ป่วยเลย นอกจากอาจมีรอยช้ำบริเวณเจาะเล็กน้อย ซึ่งอาจหายได้เองภายใน 7 วัน

3.7 การป้องกันความเสี่ยง และการแก้ไขกรณีเกิดปัญหา

ในระหว่างการวิจัยแพทย์จะนัดติดตามผลทุก 2 สัปดาห์ในช่วง 6 สัปดาห์ เมื่อเกิดอาการไม่พึงประสงค์จากยาที่รุนแรงจะให้ผู้ป่วยหยุดยาและอาจมีการตรวจติดตามทางห้องปฏิบัติการร่วมด้วย

ในระหว่างการเจาะเลือดนั้นจะมีแพทย์และพยาบาลเป็นผู้ดูแลการเจาะเลือดและปฐมพยาบาลเบื้องต้น โดยสถานที่คือโรงพยาบาลบำราศนราดูร และจัดที่พักให้ผู้ป่วยใช้พักผ่อนได้อย่างสะดวก และปลอดภัย

3.8 การตรวจวินิจฉัยอื่น

ผู้ป่วยจะได้รับประโยชน์จากการตรวจค่าพื้นฐานทางห้องปฏิบัติการเพื่อดูการทำงานของตับ ไต ได้แก่ CBC, UA, BUN, Cr, SGOT, SGPT และ Alk Phos นอกจากนี้ยังได้รับการตรวจปริมาณเชื้อไวรัสในกระแสเลือดและ CD4 cell count ก่อนได้รับยาโดยผู้ป่วยไม่เสียค่าใช้จ่ายใดๆ ทั้งสิ้น

3.9 ขอบเขตการดูแลรักษาความลับของข้อมูลต่าง ๆ ของผู้ป่วย

ข้อมูลของผู้ป่วยจะถูกเก็บเป็นความลับและจะเปิดเผยเฉพาะในรูปแบบที่เป็นสรุปผลการวิจัย

3.10 การดูแลรักษาที่ผู้วิจัยจัดให้

ในวันที่ทำการเก็บตัวอย่างเลือด ผู้ป่วยจะได้รับการดูแล โดยมีแพทย์และพยาบาลเป็นผู้ดูแลการเจาะเลือดและปฐมพยาบาลเบื้องต้น โดยสถานที่คือโรงพยาบาลบำราศนราดูร และจัดที่พักให้ผู้ป่วยใช้พักผ่อนได้อย่างสะดวกสบาย ปลอดภัย

3.11 กรณีเกิดอันตรายหรือผลไม่พึงประสงค์จากการศึกษาวิจัย ผู้ป่วยจะได้รับการดูแลรักษาโดยไม่เสียค่าใช้จ่ายอย่างไรบ้าง

- หากเกิดอาการไม่พึงประสงค์จากการใช้ยา ผู้วิจัยจะรับผิดชอบค่าใช้จ่ายที่เกิดขึ้นทั้งหมด
- ในกรณีที่ผู้ป่วยเกิดการติดเชื้อฉวยโอกาสและจำเป็นต้องได้รับยารักษาโรคติดเชื้อฉวยโอกาส ผู้ป่วยจะถูกคัดออกจากการวิจัยและจะได้รับการรักษาตามมาตรฐานการบำบัดรักษาผู้ติดเชื้อ/ผู้ป่วยเอดส์ของกระทรวงสาธารณสุขต่อไปอีก 1 ปี

3.12 การตอบแทน ชดเชยแก่ผู้ป่วย

- ผู้ป่วยจะได้รับยาสูตรผสม GPO-vir S30 หรือ GPO-vir S40 เป็นเวลานาน 1 ปีโดยไม่เสียค่าใช้จ่ายใดๆ หลังจากนั้นจะได้รับการรักษาตามมาตรฐานการบำบัดรักษาผู้ติดเชื้อ/ผู้ป่วยเอดส์ของกระทรวงสาธารณสุขต่อไปอีก 1 ปี
- ผู้ป่วยจะได้รับการตรวจค่าพื้นฐานทางห้องปฏิบัติการเพื่อดูการทำงานของตับ ไต ได้แก่ CBC, UA, BUN, Cr, SGOT, SGPT และ Alk Phos นอกจากนี้ยังได้รับการตรวจปริมาณเชื้อไวรัสในกระแสเลือดและ CD4 cell count ก่อนได้รับยาโดยผู้ป่วยไม่เสียค่าใช้จ่ายใดๆ ทั้งสิ้น
- ผู้ป่วยจะได้รับค่าตอบแทนเป็นค่าเดินทางครั้งละ 500 บาท ทั้งหมด 3 ครั้ง รวมเป็นเงิน 1,500 บาท
- ถ้าผู้ป่วยเกิดอาการไม่พึงประสงค์จากการใช้ยา ผู้วิจัยจะดูแลผู้ป่วยและให้ความช่วยเหลืออย่างเต็มที่จนกว่าจะหาย และรับผิดชอบค่าใช้จ่ายทั้งหมด
- ในวันที่ทำการเก็บตัวอย่างเลือดจะจัดอาหารให้ผู้ป่วยทั้ง 3 มื้อ

3.13 **ในกรณีเกิดอันตรายถึงขั้นร้ายแรง เช่นพิการ เสียชีวิต ผู้ป่วยหรือทายาทจะได้รับการชดเชยอย่างไร**

หากเกิดอันตรายแก่ผู้ป่วย ผู้วิจัยจะรับผิดชอบค่าใช้จ่ายตามความเหมาะสม

3.14 **ในกรณีที่ผู้ป่วยเกิดอาการข้างเคียงจากการรับประทานยาที่ทำการศึกษา ผู้ป่วยจะได้รับการชดเชยอย่างไร**

ผู้ป่วยสามารถถอนตัวจากโครงการวิจัยได้ทุกเมื่อ โดยไม่กระทบต่อการดูแลรักษาที่ผู้ป่วยพึงได้รับ

3.15 **ชื่อ ที่อยู่ เบอร์โทรศัพท์ของแพทย์ หรือผู้อื่นที่ผู้ป่วยสามารถติดต่อได้สะดวก ทั้งในและนอกเวลาราชการ กรณีมีเหตุจำเป็นหรือฉุกเฉิน**

นพ. วิศิษฏ์ ประสิทธิศิริกุล โรงพยาบาลบำราศนราดูร จ. นนทบุรี (0-1811-5610)

ภญ. มณีรัตน์ เหลืองวัฒนวิไล โครงการจัดตั้งภาควิชาเภสัชกรรมคลินิก

คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย (0-1944-7825)

APPENDICES B

หนังสือแสดงความยินยอม

<u>การวิจัยเรื่อง</u> เภสัชจลนศาสตร์ของสตาวูดีน ลามิวูดีน และเนวิราพีนในยาสูตรผสม จีพีโอ-เวียร์ เอส30 เปรียบเทียบกับการให้ยาต้นแบบเดี่ยว 3 ชนิดร่วมกัน และการประเมินประสิทธิผลการรักษา และความปลอดภัยของ จีพีโอ-เวียร์ เอส30 ในผู้ป่วยติดเชื้อเอชไอวี

วันให้คำยินยอม วันที่…….เดือน…….พ.ศ……..……

ข้าพเจ้า (นาย/นาง/นางสาว)……..……นามสกุล……..……..…
อยู่บ้านเลขที่……..ซอย…….ถนน……..แขวง/ตำบล……..……..…….
เขต/อำเภอ……..…จังหวัด…….……..……รหัสไปรษณีย์……..…...……...

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายและเอกสารแสดงรายละเอียดเกี่ยวกับงานวิจัยจากผู้วิจัย ให้ทราบถึงวัตถุประสงค์ของการวิจัย วิธีวิจัย อันตรายหรืออาการข้างเคียงที่อาจเกิดขึ้นจากการวิจัยหรือจากยาที่ใช้ รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และเข้าใจดีแล้ว

ผู้วิจัยได้ตอบคำถามต่างๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบัง ซ่อนเร้นจนข้าพเจ้าพอใจ

ข้าพเจ้าเข้าร่วมโครงการนี้โดยสมัครใจ และมีสิทธิ์ที่จะบอกเลิกการเข้าร่วมโครงการวิจัยนี้เมื่อใดก็ได้ โดยการบอกเลิกจะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะได้รับต่อไป

ข้าพเจ้าอนุญาตให้ผู้วิจัยเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าในหน่วยงานที่เกี่ยวข้องได้ ตามที่ผู้วิจัยเห็นสมควร ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะเกี่ยวกับตัวข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะรูปแบบที่เป็นสรุปผลการวิจัย

ผู้วิจัยรับรองว่า หากเกิดอันตรายใดๆ จากการวิจัยดังกล่าว ข้าพเจ้าจะได้รับการรักษาพยาบาลโดยไม่คิดมูลค่า และจะได้รับค่าตอบแทนเป็นเงินค่าเดินทางครั้งละ 500 บาท ทั้งหมด 3 ครั้ง รวมเป็นเงิน 1,500 บาท และได้รับยาสูตรผสม GPO-vir S30 หรือ GPO-vir S40 เป็นระยะเวลา 1 ปีโดยไม่เสียค่ายา รายละเอียดเกี่ยวกับการรักษาพยาบาล หรือค่าตอบแทนดังกล่าวข้าพเจ้าสามารถติดต่อได้ที่

ภญ. มณีรัตน์ เหลืองวัฒนวิไล โครงการจัดตั้งภาควิชาเภสัชกรรมคลินิก
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย โทร. 0-1944-7825, 0-2218-8403

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ จึงได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม.....ผู้ยินยอม

ลงนาม.....พยาน

ลงนาม.....พยาน

ข้าพเจ้าไม่สามารถอ่านหนังสือได้ แต่ผู้วิจัยได้อ่านข้อความในใบยินยอมนี้ให้แก่ข้าพเจ้าฟังจนเข้าใจดีแล้ว ข้าพเจ้าจึงลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ลงนาม.....ผู้ยินยอม

ลงนาม.....พยาน

ลงนาม.....พยาน

คำอธิบายของผู้วิจัย

ข้าพเจ้าได้อธิบายรายละเอียดของโครงการวิจัย ตลอดจนประโยชน์ของการทดลองรวมทั้งความเสี่ยงที่อาจจะเกิดขึ้นแก่ผู้ป่วยแล้ว

ลงนาม.....แพทย์ผู้รับผิดชอบการวิจัย

(นพ. วิศิษฎ์ ประสิทธิศิริกุล)

วันที่.....

APPENDICES C

Demographic data of the individual patients

Subject No.	*Sequence*	*Patient's Initial*	*Gender*	*Status*	*Age (yr)*	*Weight (kg)*	*Height (cm)*	*Body Mass Index (kg/m^2)*	*Infected Time (yr)*	*Route of Transmission*
1	1	PP	Male	Single	23	45.7	159	18.08	1	Homosexual
2	2	BT	Female	Widow	35	58.0	156	23.83	2	Heterosexual
3	1	SH	Male	Widow	39	49.0	164	18.22	5	Heterosexual
4	2	SM	Female	Spouse	35	50.0	159	19.78	6	Heterosexual
5	1	TS	Female	Widow	38	52.8	155	21.98	1	Heterosexual
6	2	AJ	Male	Single	24	49.0	163	18.44	1	IVDU
7	1	KS	Female	Spouse	36	50.0	150	22.22	7	Heterosexual
9	1	PB	Female	Spouse	36	50.5	159	19.98	6	Heterosexual
10	2	SG	Female	Spouse	42	51.0	161	19.68	1	Heterosexual
11	1	WC	Female	Single	34	53.0	156	21.78	8	Heterosexual
12	2	ST	Female	Spouse	22	42.0	151	18.42	2	Heterosexual
13	1	SS	Female	Spouse	34	42.9	152	18.57	8	Heterosexual
14	2	BL	Female	Single	27	54.0	163	20.32	1	Heterosexual
15	1	WS	Male	Spouse	26	59.0	165	21.67	4	Heterosexual
16	2	PY	Male	Single	22	55.0	171	18.81	1	Homosexual
17	1	NT	Female	Divorce	44	58.0	156	23.83	5	Heterosexual
18	2	KN	Male	Single	29	50.0	160	19.53	6	Heterosexual
19	1	CS	Female	Spouse	38	59.3	159	23.46	7	Heterosexual
20	2	AY	Female	Spouse	33	44.5	157	18.05	6	Heterosexual
22	2	PT	Female	Single	28	47.0	159	18.59	6	Heterosexual
Mean		-	Male = 6	-	32.25	51.04	158.75	20.26	4.20	-
SD		-	Female = 14	-	6.70	5.17	5.04	2.00	2.65	-
Min.		-	-	-	22	42	150	18	1	-
Max.		-	-	-	44	59	171	24	8	-

Baseline laboratory profiles of the individual patients

Subject No.	*Hematocrit (%)*	*Hemoglobin (g/dL)*	*Platelet ($x10^3$ cell/mm^3)*	*WBC ($x10^3$ cell/mm^3)*	*BUN (mg/dL)*	*Cr (mg/dL)*	*ALP (U/L)*	*SGOT (U/L)*	*SGPT (U/L)*	*Lactate (mmol/L)*
1	40	13.20	218	4.810	11.9	0.73	108	31	32	0.7
2	31	10.40	388	6.790	8.0	NA	101	16	9	1.1
3	32	9.76	206	3.950	10.0	0.76	137	31	19	0.6
4	37	12.70	173	3.670	15.0	0.72	67	27	14	0.6
5	33	11.00	333	4.450	15.0	0.71	73	23	13	1.4
6	41	13.60	300	2.470	15.1	1.18	83	36	55	0.7
7	41	13.90	196	4.640	9.0	0.88	64	57	37	1.3
9	31	10.20	245	2.800	7.0	0.60	162	30	16	1.0
10	33	10.80	266	6.840	14.0	0.72	153	37	30	0.5
11	35	12.10	259	4.920	9.0	0.73	95	37	23	1.2
12	31	9.09	301	3.760	11.0	0.68	59	25	10	0.9
13	34	11.20	300	4.160	11.0	0.82	130	31	13	1.0
14	38	12.90	275	3.410	10.0	0.91	70	19	11	0.3
15	49	16.50	232	5.760	16.0	1.23	103	28	28	1.3
16	43	14.30	321	6.970	9.0	0.93	103	18	19	0.7
17	37	12.60	319	3.120	10.0	0.91	104	20	10	0.9
18	42	13.10	302	5.440	9.0	0.79	62	41	31	1.0
19	34	11.40	334	5.330	12.0	0.60	93	26	16	2.4
20	32	10.90	249	1.680	7.0	0.62	96	36	20	0.5
22	31	9.64	240	2.860	11.0	0.72	83	26	18	1.1
Mean	**36.25**	**11.96**	**272.85**	**4.392**	**11.00**	**0.80**	**97.30**	**29.75**	**21.20**	**0.96**
SD	5.05	1.86	54.23	1.495	2.76	0.17	29.63	9.46	11.46	0.45
Min.	31	9.09	173	1.680	7.0	0.60	59	16	9	0.3
Max.	49	16.50	388	6.970	16.0	1.23	162	57	55	2.4

Laboratory profiles of the individual patients at week 2nd and week 18th

Subject No.	Week 2nd		week 18th									
	SGOT (U/L)	SGPT (U/L)	Hematocrit (%)	Hemoglobin (g/dL)	Platelet ($x10^3$ cell/mm^3)	WBC ($x10^3$ cell/mm^3)	BUN (mg/dL)	Cr (mg/dL)	ALP (U/L)	SGOT (U/L)	SGPT (U/L)	Lactate (mmol/L)
1	21	22	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	NA	NA	32	10.40	352	6.230	11.0	0.77	111	15	16	2.3
3	28	15	38	12.60	241	4.570	10.0	0.94	185	37	35	0.8
4	30	21	39	13.60	258	6.450	11.0	0.76	90	26	30	0.8
5	29	13	35	12.40	275	4.890	11.0	0.80	89	19	18	1.7
6	38	51	43	14.80	316	5.040	11.0	0.91	124	38	26	1.4
7	37	18	38	13.70	190	4.540	13.0	0.71	82	56	27	1.7
9	84	46	35	12.10	204	3.080	19.0	0.64	264	104	75	0.6
10	12	8	31	10.50	325	7.230	17.0	0.77	83	27	27	2.0
11	27	24	34	11.50	281	2.700	11.0	1.08	136	42	42	1.8
12	35	40	32	10.10	348	4.360	11.0	0.43	47	27	23	1.7
13	18	10	33	11.50	279	5.370	12.0	1.41	118	21	8	0.8
14	18	18	37	12.80	287	3.300	14.0	0.70	60	16	15	1.3
15	26	27	46	15.70	228	4.160	13.0	1.59	135	19	20	1.2
16	19	17	45	15.00	283	5.360	8.0	1.07	97	19	14	1.6
17	19	12	34	11.10	310	2.990	11.0	0.81	101	27	22	1.0
18	21	17	42	13.60	257	5.350	5.0	0.81	97	23	18	2.0
19	17	14	35	11.60	296	7.210	14.0	0.69	85	22	24	1.3
20	20	11	33	11.10	313	3.250	7.0	0.79	109	18	12	1.5
22	21	17	42	13.60	317	4.790	14.0	0.72	85	15	12	1.7
Mean	27.37	21.11	37.05	12.51	282.11	4.783	11.74	0.86	110.42	30.05	24.42	1.43
SD	15.49	12.06	4.62	1.63	44.63	1.364	3.23	0.27	47.95	20.82	14.87	0.47
Min.	12	8	31	10.10	190	2.700	5.0	0.43	47	15	8	0.6
Max.	84	51	46	15.70	352	7.230	19.0	1.59	264	104	75	2.3

Virological and immunological profiles of the individual patients at baseline and week 18th

Subject No.	Baseline							Week 18th						
	CD4 (cell/mm^3)	%CD4	CD8 (cell/mm^3)	%CD8	Ratio	VL (copies/mL)	VL (log_{10} copies/mL)	CD4 (cell/mm^3)	%CD4	CD8 (cell/mm^3)	%CD8	Ratio	VL (copies/mL)	VL (log_{10} copies/mL)
1	121	8	877	59	0.19	281,000	5.45	NA	NA	NA	NA	NA	NA	NA
2	122	6	951	49	0.13	115,000	5.06	130	6	1,281	56	0.10	49	1.69
3	55	4	773	61	0.07	>750,000	>5.87	0	0	0	0	0.00	49	1.69
4	133	8	781	49	0.17	67,100	4.83	187	13	689	47	0.27	50	1.70
5	128	7	1,085	57	0.14	121,000	5.08	282	11	1,030	42	0.27	49	1.69
6	58	7	469	60	0.12	423,000	5.63	232	18	544	43	0.43	66	1.82
7	173	12	609	43	0.28	176,000	5.25	146	21	260	38	0.56	49	1.69
9	79	7	725	68	0.11	185,000	5.27	186	12	1,072	71	0.17	49	1.69
10	65	2	1,968	70	0.03	>750,000	>5.87	255	14	881	50	0.29	157	2.20
11	198	11	1,107	62	0.18	215,000	5.33	250	15	984	58	0.25	49	1.69
12	110	11	660	67	0.22	387,000	5.59	366	20	849	47	0.43	49	1.69
13	90	9	811	78	0.13	385,000	5.59	226	15	892	60	0.25	81	1.91
14	122	9	650	48	0.19	128,000	5.11	228	17	596	44	0.38	49	1.69
15	139	10	447	32	0.31	7,470	3.87	188	11	727	43	0.26	49	1.69
16	57	3	1,587	73	0.04	116,000	5.06	107	6	1,344	76	0.08	49	1.69
17	51	4	813	69	0.06	77,900	4.89	118	10	764	66	0.15	49	1.69
18	170	7	1,457	60	0.12	24,700	4.39	270	15	1,060	58	0.25	49	1.69
19	100	9	691	61	0.18	214,000	5.33	252	10	1,394	56	0.18	49	1.69
20	104	13	462	56	0.23	452,000	5.66	110	16	326	48	0.34	49	1.69
22	59	7	445	49	0.13	490,000	5.69	188	19	496	49	0.38	49	1.69
Mean	**106.70**	**7.70**	**868.40**	**58.55**	**0.15**	**214,731.67**	**5.17**	**195.84**	**13.11**	**799.42**	**50.11**	**0.27**	**57**	**1.74**
SD	43.13	2.96	403.78	11.14	0.07	152,716.25	0.47	82.16	5.30	372.73	15.88	0.14	25.46	0.13
Min.	51	2	445	32	0.03	7,470	3.87	0	0	0	0	0.00	49	1.69
Max.	198	13	1,968	78	0.31	>750,000	>5.87	366	21	1,394	76	0.56	157	2.20

APPENDICES D

Formulas:

Mean ($\overline{X}$)

$$(\overline{X}) = \Sigma Xi / n$$

Standard deviation (SD)

$$SD = \sqrt{\Sigma(Xi - \overline{X})^2 / n - 1}$$

Coefficient of variation (CV)

$$CV = SD / Mean$$

Area under the concentration-time curve (AUC_{0-t})

$$[AUC_{0-t}]_0^t = \Sigma \frac{(C_{n-1} + C_n)(t_n + t_{n-1})}{2}$$

Elimination rate constant (K_e)

$$K_e = \frac{\ln C_1 - \ln C_2}{t_2 - t_1}$$

Half-life ($t_{1/2}$)

$$t_{1/2} = 0.693/K_e$$

Apparent clearance (CL/F)

$$CL/F = Dose / [AUC_{0-t}]_0^t$$

Apparent volume of distribution (Vd/F)

$$Vd/F = t_{1/2} \times CL/F / 0.693$$

Average concentration (C_{avg})

$$C_{avg} = [AUC_{0-t}]_0^t / \tau$$

In which

τ = dosing interval

90% Confidence interval (90% CI)

$$90\%\ CI = (\overline{X}_T - \overline{X}_R) \pm t_{0.1,df}\,(SEM)$$

in which

$\overline{X}_T$, $\overline{X}_R$ = Mean value of pharmacokinetic parameters of test product (GPO-vir S30) and reference product, respectively

$t_{0.1,df}$ = Tabulated, two-sided distribution value for 90% confidence interval at degree of freedom for error from ANOVA table

SEM = Standard error of mean = $\sqrt{2\ MSE/n}$

VITAE

Miss Maneerat Luangwattanawilai was born on the 5th of November in 1974 at Amphur Kampaengsaen, Nakhon Pathom. She graduated with Bachelor degree in Pharmaceutical Sciences in 1998 from Faculty of Pharmaceutical Sciences, Silapakorn University. Her current position is a pharmacist in Department of Pharmacy, Makarak Hospital, Amphur Thamaka, Kanchanaburi, Thailand