CHARACTERISATION OF CIRCULATING HIV-1 SUBTYPES AND DRUG RESISTANCE-ASSOCIATED MUTATIONS IN *Protease* AND *Reverse Transcriptase* GENES AMONG HIV-1 PATIENTS IN KELANTAN

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By

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

A_{280}	Absorbance at 280 nm
°C	Degree Celsius
L	Liter
$MgCl_2$	Magnesium cloride
µM	Micromolar
µL	Microliter
µg/mL	Microgram per mililiter
Min	Minute
%	Percentage
pmol/µL	Picomole per microliter
v/v	Volume per volume
w/v	Weight per volume
AIDS	Acquired Immune Deficiency Syndrome
ARV	Antiretroviral
BLAST	Basic Local Alignment Search Tool
CCR5	C-C chemokine receptor type 5
CD4	Cluster of differentiation 4
CDC	Centers for Disease Control and Prevention
COMET	Context based Modeling for Expeditious Typing
CRF	Circulating Recombinant Form
CXCR4	C-X-C chemokine receptor type 4
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked Immunosorbent Assay
EtBr	Ethidium bromide
FDA	Food and Drugs Administration
g	Gravity
GARD	Genetic Algorithm for Recombination Detection
GRID	Gay Related Immunodeficiency Syndrome
HAART	Highly Active Antiretroviral Therapy
HIV	Human Immunodeficiency Virus
HIV-1	Human Immunodeficiency Virus type 1
HIV-2	Human Immunodeficiency Virus type 2
HSV-1	Herpes Simplex Virus type 1
HTLV-III	Human T-lymphotropic Virus Type III
IDU	Intravenous drug use
jpHMM	Jumping profile Hidden Markov Model
kb	Kilobase
IFA	Immunofluorescence assay
IQR	Interquartile range
LAV	Lymphadenopathy-associated virus
LIA	Line Immunoassay
MAC	*Mycobacterium avium* complex
MEGA	Molecular Evolutionary Genetics Analysis
MMT	Methadone Maintenance Therapy
NAT	Nucleic Acid Test
NCBI	National Center for Biotechnology Information
NJ	Neighbour Joining
NNRTI	Non-nucleoside Reverse transcriptase Inhibitor

NRTI	Nucleoside/Nucleotide Reverse transcriptase Inhibitor
NSEP	Needle Syringe Exchange Program
PAUP	Phylogenetic Analysis Using Parsimony
PCP	*Pneumocystis carinii* Pneumonia
PCR	Polymerase Chain Reaction
PHYLIP	Phylogeny Inference Package
PI	Protease Inhibitor
PLHIV	People living with HIV
PLWHA	People living with HIV/AIDS
PR	*Protease*
RAM	Resistance-associated mutation
RIP	Recombination Identification Program
RNA	Ribonucleic Acid
RT	*Reverse transcriptase*
RT-PCR	Reverse transcription Polymerase Chain Reaction
SCUEAL	Subtype Classification Using Evolutionary Algorithms
SD	Standard deviation
SDRM	Surveillance drug-resistance mutation
SimPlot	Similarity plotting
SIV	Simian Immunodeficiency Virus
SIVcpz	Simian Immunodeficiency Virus infecting Chimpanzee
SIVcpzPt	SIV infecting Chimpanzee (*Pan troglodytes troglodytes*)
SIVcpzPts	SIV infecting Chimpanzee (*Pan troglodytes schweinfurthii*)
STAR	Subtype Analyzer
TAM	Thymidine analogue mutation
TB	Tuberculosis
TBE	Tris Borate Ethylenediaminetetraacetic acid
TDR	Transmitted drug resistance
Tm	Melting temperature
TREAT	Therapeutics, Research, Education and AIDS Training
URF	Unique Recombinant Form
UV	Ultra violet
V	Volt
X	Times or multiplication
WHO	World Health Organisation

PENCIRIAN SUBTIP HIV-1 DAN MUTASI BERKAITAN KERINTANGAN UBAT PADA GEN *Protease* DAN *Transkriptase Membalik* DI KALANGAN PESAKIT HIV-1 DI KELANTAN

ABSTRAK

Jangkitan Virus Imunodefisiensi Manusia (HIV) merupakan salah satu penyakit berjangkit yang utama di Malaysia. Banyak kajian berkaitan kepelbagaian genetik HIV dan pengawasan kerintangan ubat telah dijalankan di Malaysia terutamanya di Kuala Lumpur dan Selangor. Walau bagaimanapun, sejak dua dekad yang lalu, masih terdapat kekurangan maklumat berkaitan kepelbagaian genetik dan kerintangan ubat di kalangan penghidap HIV di Kelantan meskipun negeri ini merupakan antara tiga negeri yang mencatatkan bilangan kes jangkitan baru HIV yang tertinggi. Kajian ini menunjukkan terdapat enam subtip HIV-1 yang wujud di kalangan penghidap HIV di Kelantan dalam pelbagai kumpulan berisiko. Subtip tersebut ialah CRF33_01B, subtip B', CRF01_AE, CRF53_01B, CRF54_01B dan URF CRF01_AE/B. Kajian ini turut menunjukkan dominasi CRF33_01B di kalangan pesakit dewasa dan pediatrik serta semua populasi berisiko (penagihan dadah melalui suntikan, hubungan heteroseksual, transmisi vertikal).

Perkongsian jarum suntikan merupakan penyumbang terbesar kepada penyebaran jangkitan HIV dalam populasi kajian, berbeza dengan kebanyakan penyelidikan lain di Malaysia yang melaporkan hubungan seksual sebagai faktor utama penyebaran jangkitan HIV sejak 12 tahun yang lepas. Pesakit lelaki kekal sebagai majoriti penghidap jangkitan HIV dan sebahagian besar jangkitan diperoleh melalui perkongsian jarum suntikan. Walau bagaimanapun, kebanyakan pesakit perempuan pula dijangkiti virus HIV melalui hubungan seksual.

Analisis kerintangan ubat telah dijalankan menggunakan pangkalan data kerintangan ubat HIV Stanford. Daripada 25 pesakit yang tidak menerima rawatan, 40% dijangkiti HIV-1 yang mengandungi sekurang-kurangnya satu Mutasi Berkaitan Kerintangan (RAMs). Berdasarkan garis panduan Pertubuhan Kesihatan Dunia (WHO) pada tahun 2012, bilangan RAMs ini menurun kepada 16%. Dua puluh RAMs diperoleh daripada pesakit yang belum menerima rawatan dengan 60% daripadanya berkaitan dengan Perencat Transkriptase Membalik Bukan Analog Nukleosida (NNRTI) dan 20% masing-masing berkaitan dengan Perencat Transkriptase Membalik Analog Nucleosida/Nukleotida (NRTI) dan Perencat Protease (PI). RAMs yang paling banyak diperoleh adalah V179D/T (16%), yang mampu mengurangkan kerentanan terhadap NNRTI. Sementara itu, menurut pangkalan data kerintangan ubat HIV Stanford, 79.7% daripada pesakit yang sedang menerima rawatan dijangkiti HIV-1 yang mengandungi RAMs berkaitan dengan NRTI, NNRTI dan PI. Antara pesakit yang sedang dirawat, 251 RAMs diperoleh dengan lebih separuh daripadanya berkaitan dengan NRTI (55.4%), diikuti oleh NNRTI (37.1%) dan PI (7.6%). Mutasi-mutasi yang paling kerap ditemui ialah M184I/V (40.6%), K103N/R/S (37.5%), T215F/H/I/V/Y (35.9%) dan D67G/H/N (31.3%). Hanya K103N/R/S yang berkaitan dengan NNRTI, manakala yang selebihnya berkaitan dengan NRTI.

Kajian ini mengesahkan dominasi subtip CRF33_01B di negeri Kelantan, seterusnya mendedahkan kepelbagaian genetik yang tinggi dengan kewujudan subtip HIV-1 Bentuk Rekombinan Beredar (CRF) yang baru dan HIV-1 Bentuk Rekombinan Unik (URFs). Pengawasan molekular berterusan perlu dilakukan untuk memantau kehadiran HIV-1 URFs yang konsisten di kalangan pesakit HIV. Keputusan daripada analisis kerintangan ubat ini mampu meningkatkan pengetahuan berkenaan prestasi ubat Terapi Aktif Antiretrovirus (HAART), membantu merangka strategi pemilihan kombinasi ubat HAART yang berkesan untuk pesakit yang belum menerima rawatan mahupun yang sedang dirawat dan memberi penekanan terhadap keperluan untuk menjalankan ujian kerintangan ubat sebelum memulakan atau menukar rawatan HAART.

CHARACTERISATION OF CIRCULATING HIV-1 SUBTYPES AND DRUG RESISTANCE-ASSOCIATED MUTATIONS IN *Protease* AND *Reverse Transcriptase* GENES AMONG HIV-1 PATIENTS IN KELANTAN

ABSTRACT

Human Immunodeficiency Virus (HIV) infection is one of major infectious diseases in Malaysia. A number of studies pertaining to genetic diversity and drug resistance surveillance have been performed in Malaysia, especially in Kuala Lumpur and Selangor. However, in the past two decades, there is a lack of knowledge on HIV-1 diversity and drug resistance among people living with HIV (PLHIV) in Kelantan although this state is among the top three states reporting the highest number of new HIV cases annually. This study demonstrated that among PLHIV in Kelantan, there were six HIV-1 subtypes circulating in different risk groups. These subtypes were CRF33_01B, B' subtype, CRF01_AE, CRF53_01B, CRF54_01B and URF CRF01_AE/B. Additionally, this study showed the predominance of CRF33_01B subtype among adult and paediatric patients and all risk populations (Intravenous drug use (IDUs), heterosexual contact and vertical transmission).

Sharing needle was found to be the main contributor for HIV dissemination in study population, different than other studies in Malaysia which reported sexual contact as the main factor of HIV transmission since 12 years ago. Majority of HIV patients continued to be male who acquired the infection mostly through sharing needle. In contrast, female patients were infected with HIV via sexual contact.

Drug resistance analysis was carried out based on Stanford HIV drug resistance database. From 25 treatment-naive patients, 40% were infected by HIV-1 containing at least one transmitted Resistance-associated mutations (RAMs). When analysed against World Health Organisation (WHO) consensus, the value was reduced to 16%. Twenty transmitted RAMs

were harvested from treatment-naive patients, with 60% were associated with Non-nucleoside Reverse Transcriptase Inhibitor (NNRTI), and 20% each were associated with Nucleoside/Nucleotide Reverse Transcriptase Inhibitor (NRTI) and Protease Inhibitor (PI). The most commonly observed transmitted mutation was V179D/T (16%), which may reduce drug susceptibility to NNRTI. Meanwhile, according to Stanford HIV drug resistance database, 79.7% of patients currently receiving treatment carrying HIV-1 containing acquired RAMs against NRTI, NNRTI and PI. Among treated patients, 251 acquired RAMs were obtained with more than half were associated with NRTI (55.4%), followed by NNRTI (37.1%) and PI (7.6%). The most frequent mutations observed were M184I/V (40.6%), K103N/R/S (37.5%), T215F/H/I/V/Y (35.9%) and D67G/H/N (31.3%). Only K103N/R/S was associated with NNRTI while the rest of mutations were associated with NRTI.

The present study confirms the high prevalence of CRF33_01B in Kelantan state and reveals a high genetic diversity with the presence of newly described HIV-1 Circulating Recombinant Forms (CRFs) and HIV-1 Unique Recombinant Forms (URFs). Continuous molecular surveillance should be performed to monitor further emergence of HIV-1 URFs. Result from drug resistance analysis enhances the knowledge pertaining to Highly Active Antiretroviral Therapy (HAART) performance, helps in designing effective HAART strategy on treatment-naive and treated patients and subsequently emphasises the need to commence drug resistance testing prior to starting or changing HAART treatment.

CHAPTER ONE

INTRODUCTION

1.1 Overview of HIV/AIDS

1.1.1 Emergence of HIV/AIDS

Acquired Immune Deficiency Syndrome (AIDS) was first reported in 1981 with the name Gay Related Immunodeficiency Syndrome (GRID) before it was replaced with the current name, AIDS in September 1982 by the United States Centers for Disease Control and Prevention (CDC) (Bell *et al*., 2011). Initial report described five cases of *Pneumocystis carinii* Pneumonia (PCP) implicated a group of young gay men in Los Angeles. PCP is a rare opportunistic infection that is known to occur in people with very compromised immune systems (Stolley and Glass, 2009; Bell *et al*., 2011). Soon, some of the infected gay men developed a rare skin cancer called Kaposi's sarcoma (Hoffmann *et al*., 2007; Hoffmann and Rockstroh, 2009; Stolley and Glass, 2009; Zuckerman *et al*., 2009). At that time, CDC had no official name and the causative agent of the disease. One year later, two groups of researchers, Robert Gallo group from United States and Luc Montaigner and Barré-Sinoussi group from France (Hoffmann and Rockstroh, 2009), proposed the name for the virus that causes AIDS based on the different hypothesises in which came out from variable symptoms.

In 1983, Robert Gallo group from the United States had declared that a novel retrovirus named Human T-lymphotropic virus Type III (HTLV-III) may have been infecting AIDS patients since HTLV-III has a similar structure with other HTLVs (Gallo *et al*, 1983). In the same year in France, Luc Montaigner and Barré-Sinoussi group had named the virus Lymphadenopathy-associated Virus (LAV) from their own isolate taken from a patient suffering from swelling lymph nodes at the neck and physical weakness (two common symptoms of AIDS) as a causative agent for AIDS (Barré-Sinoussi *et al*., 1983). As these two viruses proved to be the same, the term Human Immunodeficiency Virus (HIV) was

officially adopted in 1986 and both Gallo and Montagnier groups are credited as co-discoverers (Lerner and Wilmoth, 2003; Hoffmann *et al*., 2007; Zuckerman *et al*., 2009).

In Malaysia, HIV case was first reported in 1986 with a total of three cases while AIDS was first discovered in a patient in 1987, one year after the Ministry of Health established a national AIDS task force. This patient was diagnosed and confirmed HIV positive by Enzyme-linked Immunosorbent Assay (ELISA) and Western Blot tests. The patient was a 45 years old man of Malaysia origin who had been living overseas for the past 30 years and was infected due to multiple sexual partners ten years prior to entering AIDS stage (Goh *et al*., 1987).

From 1986 until December 2011, a total of 94 841 HIV cases were reported, 17 686 people living with HIV had advanced to AIDS and 14 986 people had died from AIDS related diseases making the total number of people living with HIV in this country to 79 855 (Ghani *et al*., 2012; Ngadiman *et al*., 2012). HIV cases peaked in 2002 and then showed a declining pattern, partly due to the harm reduction programmes, particularly Needle and Syringe Exchange Programme (NSEP) and Methadone Maintenance Therapy (MMT) (Figure 1.1) (Ghani *et al*., 2012). In recent years, interestingly, new cases of HIV infection showed shifted trends; an increased of female to male ratio and heterosexual transmission of the virus (Feary, 2011; Ghani *et al*., 2012).The female to male ratio of new HIV infections had shifted tremendously from 1:99 in 1990 to 1:10 and 1:4 in 2000 and 2011, respectively. This change has an impact on the risk factors trend in new HIV cases, where sexual transmission replaces transmission via injecting drug use as the major route of HIV transmission in Malaysia (Ghani *et al*., 2012).

Selangor, Johor and Kelantan are the three states in Malaysia which constantly report high new HIV cases annually. Kelantan is located on the east coast of Peninsular Malaysia and shares border with Thailand, one of the Southeast Asia countries with high

new HIV cases. In the past ten years, injecting drug use was the main mode of HIV transmission in Kelantan, Terengganu and Pahang. However, in some states in northern (Perlis, Kedah, Penang and Perak) and southern (Negeri Sembilan, Melaka and Johore) regions, the major route of HIV infection had shifted to sexual transmission, starting from 2002. Similar observation has been reported in the western region states (Selangor and Kuala Lumpur) (Ghani *et al*., 2012; Ngadiman *et al*., 2012).

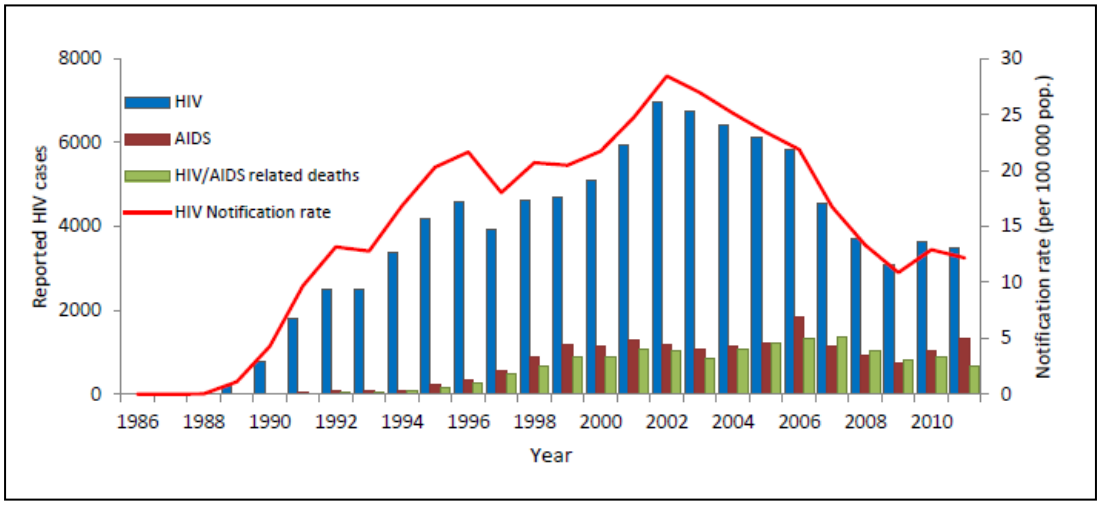

Figure 1.1: Reported new HIV case and AIDS-related deaths in Malaysia (1986 – 2011). Adapted from: Malaysia 2012 Global Aids Response Progress Report, 2012.

1.1.2 Origin of HIV

Scientists believe that HIV-1 was originated from West Africa and subsequently spread to Europe and the United States through Caribbean (Lerner and Wilmoth, 2003). HIV-1 results from multiple cross-species transmissions of Simian Immunodeficiency Viruses (SIVs) which naturally infects African primates. The cross-species transmission of SIV into human occurs through hunting and deforestation activities which increase the contact between humans and SIV infected primates (Kalish *et al*., 2005).

SIV is a weak virus and easily suppressed by human immune system within weeks of infection before the virus has sufficient time to overcome human immune system. However, several transmissions between humans (horizontal transmissions) led to certain viral mutation that could overcome human immune system, resulting in SIV mutated to HIV (Marx *et al*., 2001). HIV-1 is believed to be originated from chimpanzee infected with SIV. Common chimpanzee (*Pan troglodytes*) has been shown to be the true reservoir of SIV (Sharp and Hahn, 2011). There are several types of SIV based on their host origin, but the virus that infects Chimpanzee is known as SIVcpz. The SIVcpz strains fall into two subspecies-specific lineages, termed SIVcpz*Ptt* (from *Pan troglodytes troglodytes*) and SIVcpz*Pts* (*Pan troglodytes schweinfurthii*). SIVcpz*Ptt* is closely related to HIV-1 (Sharp and Hahn, 2011).

1.1.3 Mode of transmission

HIV/AIDS is a bodily fluid-borne infection. The virus could not be transmitted directly via air, water, food and skin touch. HIV/AIDS could only be transmitted to a healthy person via three major routes; unsafe sex with an HIV-infected partner (both homo and heterosexual), sharing injection needle with an HIV-infected partner and vertical transmission of HIV from infected mother to baby during pregnancy, delivery, or through breast milk feeding. All other transmission routes such as blood or blood product transfusion and needle prick injury are rare or extremely rare (Hoffmann and Rockstroh, 2012).

Heterosexual transmission was proposed as a transmission route for AIDS in 1983 by Luc Montagnier. At the beginning of disease dissemination, most healthy individuals were infected following sexual intercourse, especially through heterosexual contact (Bell *et al*., 2011). In Malaysia, half of new HIV cases are contributed by sexual transmission (hetero, homo and bisexual collectively) and has replaced injecting drug use as the major transmission route from 2010 onwards (Ghani *et al*., 2012). Moreover, heterosexual transmission is the major cause of HIV infection among Malaysian women since 87% of them are infected with HIV through their partners (Ngadiman *et al*., 2012).

In Malaysia, males continue to represent the majority (90%) of cumulative HIV cases despite showing a significant decline beginning 2003. In contrast, an opposite trend has been observed in females. Among males, 48% acquired HIV via injecting drug use (IDU) and 47% through sexual transmission (Ghani *et al*., 2012; Ngadiman *et al*., 2012). In 2011, new HIV cases due to injecting drug use had declined significantly from eight years ago (70-80%) to 38.7%. The reduction of newly reported HIV cases caused by injecting drug use is attributed to Needle Syringe Exchange Program (NSEP) and Methadone Replacement Therapy (Ngadiman *et al*., 2012).

The first HIV infection involving children was reported in 1982. The infants acquired HIV from their mother and this vertical transmission at present is recognised as a major mode of HIV transmission in children (Bell *et al*., 2011). In Malaysia, new HIV cases due to vertical transmission have doubled to two percent in 2011 compared with the previous year (Ghani *et al*., 2012).

1.2 AIDS and opportunistic infections

Person who is infected by HIV are recognised as HIV carrier and now, the term has changed to people living with HIV/AIDS (PLWHA or PLHIV) to reduce stigma and discrimination by society. At least 85% of people who are diagnosed with HIV but do not receive treatment

will progress to AIDS within an estimated of eight to 12 years (Stolley and Glass, 2009) and the duration may become shorter depending on patient health care and nutritional status. AIDS is the stage when the immune system is badly damaged, resulting in the body becomes vulnerable to opportunistic infections. PLHIV will progress to AIDS stage if they have CD4 count lower than 200 cells per mm^3 and show at least one of AIDS-defining diseases or symptoms (Stolley and Glass, 2009; AIDS.gov, 2009). However, the criteria for diagnosing AIDS may differ depending on individual country guidelines (Avert.org, 2005b). People who are diagnosed with AIDS usually survive for about three years without treatment, but may only live for one year if they are infected with potentially fatal opportunistic infections such as Kaposi's sarcoma, tuberculosis, toxoplasmosis and *pneumocystis carinii* pneumonia (PCP) (AIDS.gov, 2009).

Opportunistic infections can be caused by bacteria, viruses, fungi, protozoans and parasites that are typically brought under control by a healthy and functional immune system (Holmes *et al*., 2003; Kayser *et al*., 2005). Opportunistic infections related to AIDS or termed as AIDS-defining diseases for HIV patients who have advanced to AIDS. Some of the world's most common HIV-related opportunistic infections and diseases include:

I. Bacterial diseases such as tuberculosis (TB) caused by *Mycobacterium tuberculosis*, *Mycobacterium avium* complex (MAC), bacterial pneumonia and septicaemia (blood poisoning or bacteraemia) caused by *Escherichia coli*, *Pneumococcus*, *Klebsiella*, *Pseudomonas*, *Staphylococcus* and *Streptococcus* (Avert.org, 2005a; Kayser *et al*., 2005; Better Health Channel, 2012),

II. Protozoal diseases such as toxoplasmosis caused by *Toxoplasma gondii*, microsporidiosis, cryptosporidiosis, isopsoriasis and leishmaniasis caused by microsporidian species, *Cryptosporidium*, *Isospora belli* and genus *Leishmania*

protozoa, respectively (Avert.org, 2005a; Kayser *et al*., 2005; Pubmed Health, 2012),

III. Fungal diseases such as PCP caused by *pneumocystis jirovecii*, candidiasis, cryptococcosis and penicilliosis caused by *candida albicans* (most frequent), *Cryptococcus neoformans*, and *Penicillium marneffei*, respectively (Avert.org, 2005a; Kayser *et al*., 2005),

IV. Viral diseases such as those caused by cytomegalovirus, herpes simplex (HSV-1 and HSV-2) and herpes zoster virus (Avert.org, 2005a; Kayser *et al*., 2005), and

V. HIV-associated malignancies such as Kaposi's sarcoma, B-cell lymphoma Epstein-Barr virus (EBV)-positive and squamous cell carcinoma (Avert.org, 2005a; Kayser *et al*., 2005).

TB is the principal cause of death among people living with HIV (Hoffmann and Rockstroh, 2009; UNAIDS, 2010). In 2009, there were an estimated of 380 000 deaths from TB among people with HIV. In some countries, the HIV prevalence among people with TB is as high as 80% (UNAIDS, 2010). In Malaysia, about 20 666 new TB cases were registered in 2011 and about 3.2% of them also have HIV co-infection. From a total of 52 205 people infected with TB until 2011, about eight percent were TB/HIV co-infected (Ngadiman *et al*., 2012). Penicilliosis caused by *Penicillium marneffei* is the most common opportunistic infection in AIDS patients in Southeast Asia and present primarily in lungs (Kayser *et al*., 2005).

1.3 Molecular epidemiology and diversity of HIV

HIV is a positive sense single-stranded RNA virus, a member of *Lentivirus* genus and in the *Retroviridae* family (Carter and Saunders, 2007; Cann, 2011). HIV is classified into two

major groups; HIV-1 and HIV-2. HIV-1 is originated from SIV found in common chimpanzees while HIV-2 is isolated from SIV infected-sooty mangabeys in 1986 (Zuckerman *et al*., 2009). HIV-1 is distantly related to SIV compared with HIV-2 (Chakrabarti *et al*., 1987; Reeves and Doms, 2002). HIV-1 is more virulent than HIV-2 since the former virus has greater infectivity capability and infects host cell more efficiently than HIV-2 (Donnelly *et al*., 1993; Gilbert *et al*., 2003) and thus, responsible for most HIV/AIDS pandemic.

HIV-1 variants can be divided into group M, N, O, and P. Each group arose from a separate transmission of SIVcpz into humans, except for group P which came from SIVgor infecting western gorilla (*Gorilla gorilla*) (Sharp and Hahn, 2011). Group M which means 'major' or 'main', is the first to be discovered and primarily responsible for the global HIV/AIDS pandemic and has been found virtually in every country on the globe (Karim and Karim, 2010; Sharp and Hahn, 2011).

Group M is further divided into subtypes or clades, sub-subtypes, intersubtype recombinant forms, circulating recombinant forms (CRFs) and unique recombinant forms (URFs) (Taylor *et al*., 2008). The subtypes are represented by letters from A to K, aside from E and I. Sub-subtypes are derived from subtype A and F only. CRFs are the outcome from recombination between viruses from different subtypes and are assigned by a number. So far, there are nine subtypes and 58 CRFs identified to cause the HIV/AIDS pandemic (Taylor *et al*., 2008; Los Alamos, 2013). Table 1.1 shows the complete list of CRFs. Recent estimates show that subtype C accounted for almost half (48%) of all worldwide infections, followed by subtypes A (12%), B (11%), CRF02_AG (8%), CRF01_AE (5%), subtype G (5%) and D (2%). Subtypes F, H, J and K together cause less than one percent of global infections. Other CRFs and URFs are each responsible for four percent of worldwide infections. Combined all CRFs and URFs contribute to 20% of global HIV infections (Hemelaar *et al*., 2011).

Table 1.1 Complete list of specific CRFs of HIV-1 (Last update: 14 November 2013).

Name	Reference strain	Subtypes	Author(s)
CRF01_AE	CM240	A, E	J.K. Carr
CRF02_AG	IbNG	A, G	J.K. Carr
CRF03_AB	Kal153	A, B	K. Liitsola
CRF04_cpx	94CY032	A, G, H, K, U	D. Paraskevis
CRF05_DF	VI1310	D, F	T. Laukkanen
CRF06_cpx	BFP90	A, G, J, K	R. B. Oelrichs
CRF07_BC	97CN54	B', C	R. Wagner
CRF08_BC	97CNGX-6F	B', C	F.E. McCutchan
CRF09_cpx	96GH2911	A, G, U	F.E. McCutchan
CRF10_CD	TZBF061	C, D	I.N. Koulinska
CRF11_cpx	GR17	A, E, G, J, U	M. Peeters
CRF12_BF	ARMA159	B, F1	J.K. Carr
CRF13_cpx	96CM-1849	CRF01, A, G, J, U	K. Wilbe
CRF14_BG	X397	B, G	R. Najera
CRF15_01B	99TH.MU2079	CRF01, B	F.E. McCutchan
CRF16_A2D	97KR004	A2, D	U. Visawapoka
CRF17_BF	ARMA038	B, F1	J.K. Carr
CRF18_cpx	CU76	A1, F, G, H, K, U	M. Thomson
CRF19_cpx	CU7	A1, D, G	M. Thomson
CRF20_BG	Cu103	B, G	M. Thomson
CRF21_A2D	99KE_KER2003	A2, D	F.E. McCutchan
CRF22_01A1	02CMLT72	CRF01, A1	J.K. Carr
CRF23_BG	CB118	B, G	M. Thomson
CRF24_BG	CB378	B, G	M. Thomson
CRF25_cpx	02CM_1918LE	A, G, U	J.K. Carr
CRF26_AU	02CD_MBTB047	A, U	M. Peeters
CRF27_cpx	04FR-KZS	A, E, G, H, J, K, U	M. Peeters
CRF28_BF	BREPM12609	B, F1	R. Diaz
CRF29_BF	BREPM16704	B, F1	R. Diaz
CRF30_0206	00NE36	CRF02, CRF06	M. Peeters
CRF31_BC	04BR142	B, C	M. Soares
CRF32_06A1	EE0369	CRF06, A1	M. Adojaan
CRF33_01B	05MYKL007	CRF01, B	K.P. Ng and K.K. Tee
CRF34_01B	OUR2275P	CRF01, B	F.E. McCutchan
CRF35_AD	AF095	A, D	F.E. McCutchan
CRF36_cpx	NYU830	CRF01, CRF02, A, G	R. Powell
CRF37_cpx	NYU926	CRF01, CRF02, A, G, U	R. Powell
CRF38_BF	UY03_3389	B, F1	C. Lopez-Galindez
CRF39_BF	03BRRJ103	B, F1	M.G. Morgado
CRF40_BF	05BRRJ055	B, F1	M.G. Morgado

Adapted from: Los Alamos (http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html). Accessed on: 17 February 2014

Table 1.1 Continued

Name	Reference strain	Subtypes	Author(s)
CRF41_CD	CO6650V1	C, D	S. Tovanabutra
CRF42_BF	luBF_13_05	B, F1	J-C. Schmit
CRF43_02G	J11223	CRF02, G	C. Brennan
CRF44_BF	CH80	B, F1	M. Thomson
CRF45_cpx	04FR.AKU	A, K, U	M. Peeters
CRF46_BF	01BR087	B, F1	S. S. Sanabani
CRF47_BF	P1942	B, F1	M. Thomson
CRF48_01B	07MYKT014	CRF01, B	Y. Takebe
CRF49_cpx	N28353	A1, C, J, K, U	T. de Silva and M. Cotten
CRF50_A1D	-	A1, D	G. Foster
CRF51_01B	HM021	CRF01, B	O.T. Ng
CRF52_01B	M043	CRF01, B	J. Li
CRF53_01B	-	CRF01, B	K.K. Tee
CRF54_01B	-	CRF01, B	K.K. Tee
CRF55_01B	-	CRF01, B	X. Han
CRF57_BC	1439	B, C	L. Li
CRF59_01B	09LNA423	CRF01, B	X. Han
CRF61_BC	JL100010	B, C	X. Li

Adapted from: Los Alamos (http://www.hiv.lanl.gov/content/sequence/HIV/CRFs/CRFs.html). Accessed on: 17 February 2014

Intersubtype recombinant form is a mosaic strains that have segments from two or more subtypes alternating across their genome (Taylor *et al*., 2008). CRF is defined as specific intersubtype recombinants from at least three epidemiologically unlinked variants that are monophyletic spreading in a population and share an identical genetic structure (Taylor *et al*., 2008; Holguin *et al*., 2008). URF variants are widely distributed worldwide, with recombination breakpoints different from those of CRFs and they have been found in not more than three infected persons. Recombination events result from co-infection and/or superinfection of host cells with different HIV variants. URFs occur especially in populations where multiple subtypes co-circulate (Holguin *et al*., 2008).

Group O (outlier) was identified in 1990 from two Cameroonian AIDS patients living in Belgium and is much less prevalent than group M (Leys *et al*., 1990; Gurtler *et al*., 1994). It represents less than one percent of global HIV-1 infections (Mauclère *et al*., 1997), and largely restricted to Cameroon, Nigeria, Gabon, Senegal, Togo, Chad and Niger (Peeters *et al*., 1997). However, the origin of HIV-1 group O remains unknown because there are no ape viruses that are closely related to this group. Therefore, HIV-1 group O could either be of chimpanzee or gorilla origin (Sharp and Hahn, 2011).

Group N (non-M, non-O) was discovered in 1998 from a Cameroonian woman with AIDS (Simon *et al*., 1998). This group of virus is even less prevalent than group O. Until now, only 13 cases of group N infection have been documented, all in individuals from Cameroon (Vallari *et al*., 2010).

Finally, group P was discovered in 2009 in a Cameroonian woman living in France (Plantier *et al*., 2009). In 2011, Vallari *et al.* found another case of group P from extensive screening in Cameroon from 2006 to 2009. Until present, there are only two known cases of HIV group P infections with one person for each case.

1.4 Diagnosis of HIV

The first test for HIV detection was developed by Robert Gallo and co-workers and was approved in 1985 by the Food and Drugs Administration (FDA) in the United States. Sensitivity and specificity are crucial in determining the accuracy of the test. Sensitivity is defined as the ability to correctly identify positive samples as positive, whereas specificity measures its ability to correctly identify negative samples as negative (Hoffmann *et al*., 2007). Generally, diagnosis of HIV could be done via detection of antibody, antigen or viral nucleic acid (Kayser *et al*., 2005).

1.4.1 Antibody detection

HIV antibody testing detects the presence of HIV-specific antibodies and consists of at least two different assays; a screening test and a confirmatory test. Most screening tests are based on the ELISA principle. Screening tests should be highly sensitive (close to 100%) to minimize false negative result (Hoffmann *et al*., 2007).

ELISA is a qualitative test, used to detect the presence of antibodies specific to HIV. Typically, the screening test result is reported as 'reactive' rather than 'positive' to avoid misunderstanding since 'reactive' sample later needs to undergo confirmatory test before a patient is diagnosed as HIV positive (Hoffmann *et al*., 2007; Hoffmann and Rockstroh, 2012).

Western Blot is usually carried out to confirm reactive sample and considered as a gold standard for validation of HIV test results (Zuckerman *et al*., 2009). Interpretation of the result differs in different countries such as United States and Germany. According to WHO recommendation, result of a Western blot is considered positive if at least two *env* proteins (gp41, gp120 and gp160) are observed (Hoffmann *et al*., 2007; Hoffmann and Rockstroh, 2012).

Apart from western blot, other tests that could be used as confirmatory assays are immunofluorescence assay (IFA), line immunoassay (LIA) and second antibody test (Avert.org, 2005c). IFA is a qualitative assay and uses fluorescence microscope to detect HIV antibody. IFA is less expensive compared with Western Blot for mass use and thus, suitable for limited financial resources countries (Van Dyck *et al*., 1999). In LIA, recombinant or synthetic peptide antigens are applied on a nitrocellulose strip, rather than electrophoresed as in the Western blot. Moreover, this assay is commonly used in Europe, reduces sample cross-contamination and the results are as accurate as the Western Blot (Avert.org, 2005c).

HIV antibody testing is only useful two weeks after a patient acquires HIV infection. However, detection rate at this point of time is low. Sensitivity of HIV antibody testing however continues to increase after four, six, eight and 12 weeks post infection in 60-65%, 80%, 90% and 95% of cases, respectively (Hoffmann and Rockstroh, 2012). Most patients develop detectable HIV antibodies within six to 12 weeks of infection but this may take up to six months in auto-immune disorder patients (Avert.org, 2005c).

1.4.2 Antigen detection

In HIV antigen test, a viral protein, usually capsid protein p24 is detected in serum. The p24 antigen is detectable in serum about five days before seroconversion (the first occurrence of specific antibodies), which is about two weeks after transmission. However, antigen detection is not useful after eight to 12 weeks upon transmission since HIV enters stable latency period during this point of time and p24 antigen will be detectable again months or years later (Hoffmann *et al*., 2007; Hoffmann and Rockstroh, 2012).

Simultaneous detection of HIV antigen and antibody is applied in fourth generation diagnosis test and acts as a screening test. Reactive result from this test will be confirmed using Western Blot (Hoffmann *et al*., 2007; Hoffmann and Rockstroh , 2012). The fourth

generation test is highly accurate and capable of diagnosing people exposed to HIV infection 11 days to one month post infection (Avert.org, 2005c).

1.4.3 Nucleic acid test

HIV nucleic acid test (NAT) detects the genetic material of HIV. Similar to HIV antigen assay, the test could detect HIV in the blood within two or three weeks of infection. However, the test is not frequently used since it is very expensive and requires trained personal compared with a standard antibody test. The nucleic acid test usually detects and amplifies at least one of several target sequences located specifically in HIV target genes such as *pol*, *gag* and *env* (Avert.org, 2005c).

For newborns with suspected vertical HIV transmission, HIV NAT is mostly preferred test than antibody based assay since maternal antibodies may remain detectable in infants until around the age of 18 months, resulting in false-positive results. At least two negative NAT results are required to confirm a newborn is free from HIV infection. The first HIV NAT should be performed after the first month of life (sensitivity 96%, specificity 99%), while the second test should be conducted after the baby is three months old (Hoffmann and Rockstroh, 2012).

1.5 Treatment

1.5.1 Drug classes

At present, there is no antiretroviral (ARV) drug that could effectively treat HIV infection. The ARVs currently available in the market could only reduce the viral load but could not completely eliminate them. In 1987, the first antiretroviral agent, Zidovudine (AZT, Retrovir®) was licensed for the treatment of HIV (Hoffmann and Rockstroh, 2009) and was the first tested on humans (Hoffmann and Rockstroh, 2012). Later, between 1991 and 1994, more drugs including Zalcitabine (ddC), Didanosine (ddI) and Stavudine (d4T) were introduced. During early discovery period of ARV drug, physicians used monotherapy as a

sole treatment for HIV patients despite being ineffective due to limited number of drugs available. However, using one nucleoside analogue drug alone could not suppress HIV for long. When more new ARVs were discovered, dual therapy, a combination of two nucleoside analogues was introduced in 1995 and proved to be more effective than monotherapy. Since then, more new antiretroviral agents from other drug classes were introduced and licensed, including Saquinavir, Ritonavir and Indinavir from protease inhibitor drug class and Nevirapine from non-nucleoside reverse transcriptase inhibitor drug class (Hoffmann and Rockstroh, 2012).

ARV acts by targeting various genes responsible for HIV life cycle such as *pol*, *integrase* and *env*. From these three target genes, ARV is divided into five different classes; Nucleoside or nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside and nucleotide reverse transcriptase inhibitor (NNRTI), and protease inhibitor (PI) which target *pol* gene, entry inhibitors that act on *env* gene and lastly, integrase inhibitors, which target *integrase* gene (Hoffmann and Rockstroh, 2012). As of 2012, there are 31 individual or combination ARVs licensed for HIV treatment (Table 1.2).

NRTI targets the HIV enzyme reverse transcriptase by acting as a competitive inhibitor. NRTI competes with natural nucleosides to bind with reverse transcriptase since its structure is similar to natural nucleosides. By binding to reverse transcriptase, NRTI halts elongation of Deoxyribonucleic acid (DNA) strand by preventing formation of phosphodiester bond that bind phosphate group in nucleosides to 3’ end of elongated DNA strand (Men´endez-Arias, 2008; Hoffmann and Rockstroh, 2012). So far, seven ARVs from NRTI class have been approved and used.

Table 1.2 Antiretroviral drugs approved by FDA.

Drug	Abbrev.	Trade name	Manufacturer	Year approved
Nucleoside and Nucleotide Reverse Transcriptase Inhibitors (NRTIs)				
Zidovudine	AZT	Retrovir	GlaxoSmithKline	March 1987
Didanosine	ddl	Videx	Bristol-Myers Squibb	Oct. 1991
Stavudine	d4T	Zerit	Bristol-Myers Squibb	June 1994
Lamivudine	3TC	Epivir	GlaxoSmithKline	Nov. 1995
Abacavir	ABC	Ziagen	GlaxoSmithKline	Dec. 1998
Tenofovir	TDF	Viread	Gilead Sciences	Oct. 2001
Emtricitabine	FTC	Emtriva	Gilead Sciences	July 2003
Non-nucleoside Reverse Transcriptase Inhibitors (NNRTIs)				
Nevirapine	NVP	Viramune	Boehringer Ingelheim	June 1996
Delavirdine	DLV	Rescriptor	Pfizer	April 1997
Efavirenz	EFV	Sustiva	Bristol-Myers Squib	Sept. 1998
Etravirine	ETR	Intelence	Tibotec	Jan. 2008
Rilpivirine	RPV	Edurant	Janssen Pharmaceuticals, Inc	May 2011
Protease Inhibitors (PIs)				
Saquinavir	SQV	Invirase	Hoffmann-La Roche	Dec. 1995
Ritonavir	RTV	Norvir	Abbott Laboratories	March 1996
Indinavir	IDV	Crixivan	Merck	March 1996
Nelfinavir	NFV	Viracept	Agouron Pharmaceuticals	March 1997
Lopinavir/ritonavir	LPV/r	Kaletra	Abbott Laboratories	Apr. 2001
Atazanavir	ATV	Reyataz	Bristol-Myers Squibb	June 2003
Fosamprenavir	FPV	Lexiva	GlaxoSmithKline	Oct. 2003
Tipranavir	TPV	Aptivus	Boehringer Ingelheim	June 2005
Darunavir	DRV	Prezista	Janssen Pharmaceuticals, Inc	June 2006
Integrase Inhibitors				
Raltegravir	RAL	Isentress®	Merck	Oct. 2007
Elvitegravir	EVG	-	Gilead Sciences	Aug. 2012
Entry Inhibitors				
Enfuvirtide	T-20	Fuzeon	Hoffmann-La Roche, Trimeris	March 2003
Maraviroc	MVC	Celsentri®, Selzentry®	Pfizer	Aug. 2007

Adapted from: AIDS info (2012) and Hoffmann and Rockstroh (2012)

Table 1.2 Continued

Drug	Abbrev.	Trade name	Manufacturer	Year approved
Combination drugs				
Lamivudine, Zidovudine	CBV	Combivir	GlaxoSmithKline	Sept. 1997
Abacavir, lamivudine, zidovudine	TZV	Trizivir	GlaxoSmithKline	Nov. 2000
Abacavir, lamivudine	KVX	Epzicom	GlaxoSmithKline	Aug. 2004
Emtricitabine, Tenofovir	TVD	Truvada	Gilead Sciences	Aug. 2004
Efavirenz, Emtricitabine, Tenofovir	ATP	Atripla	Bristol-Myers Squibb, Gilead Sciences	July 2006
Rilpivirine, Emtricitabine, Tenofovir	CPL	Complera	Gilead Sciences	Aug. 2011
Elvitegravir, Cobicistat, Emtricitabine, Tenofovir	-	Stribild	Gilead Sciences	Aug. 2012

Adapted from: AIDS info (2012) and Hoffmann and Rockstroh (2012)

NNRTI also targets HIV reverse transcriptase. However, unlike NRTI, NNRTI binds to an allosteric site of the enzyme. NNRTI binding causes structural change in the active site of reverse transcriptase binding site thus making the enzyme inaccessible for natural nucleoside (Hoffmann and Rockstroh, 2012). There are three first-generation NNRTIs; Nevirapine, Delavirdine and Efavirenz which were introduced between 1996 and 1998. Recently, there are two newly approved NNRTIs, Etravirine (2008) and Rilpivirine (2011). Nevirapine and Efavirenz are the most widely prescribed NNRTI since they are as effective as or better than ARV from PIs group in naive and slightly in treatment-experienced patients and less expensive compared with PIs. Apart from AZT, Nevirapine is also prescribed to pregnant women as an alternative (Page *et al*., 2006).

HIV protease cuts the viral gag-pol polyprotein into functional subunits which are crucial for maturation of new virions. Thus, blocking this stage will be one of the appropriate strategies to inhibit viral replication. PIs bind exactly into active site of HIV protease to block the enzyme function. Initially, PIs were criticised for their high pill burden and side effects but with several PIs improvement in reducing dose taking, they remain as essential component of ARV treatment (Hoffmann and Rockstroh, 2012). Saquinavir is the first protease inhibitor approved by the FDA on 1995. In term of efficacy, Saquinavir is the weakest while Ritonavir is the strongest protease inhibitor. At present, Ritonavir is used as a booster for the other PIs. Boosted PIs are useful in reducing the frequency and number of pills to be taken daily, moreover, some PIs can now be used in once-daily regimens. PIs boosted with Ritonavir are usually indicated by a "/r" after the drug name and are recognised as second generation drugs (Martinez-Cajas and Wainberg, 2007). For example, Lopinavir boosted with Ritonavir is written as "Lopinavir/r" or "(LPV/r)". So far, Lopinavir is the only PI with a fixed boosting dose of Ritonavir and known as Kaletra® (Hoffmann and Rockstroh, 2012). As of 2012, there are eight PIs approved by FDA and are actively used in hospitals (FDA, 2012).

HIV integrase integrates HIV DNA into human genome and is one of the crucial enzymes for HIV replication. Thus, integrase inhibitor was designed to stop integrase activity and prevent further spread of HIV infection. The first integrase inhibitor approved by FDA for treatment of HIV infection was Raltegravir on 2007 (McColl and Chen, 2010; FDA, 2012). Raltegravir is a strand transfer inhibitor that inhibits strand binding of viral DNA to cellular DNA. Moreover, Raltegravir is frequently used in salvage therapy for HIV patient, whose viruses had mutated and acquired resistance toward drugs from other classes. Elvitegravir is the second integrase inhibitor licensed recently in August 2012 by FDA and has the same role as Raltegravir (Hoffmann and Rockstroh, 2012).

Entry inhibitors are the fifth ARV class, divided to three types based on how the drugs prevent HIV enters into CD4 T cells; attachment inhibitors, co-receptor antagonists (CXCR4 or CCR5), and fusion inhibitors. Maraviroc and Enfuvirtide (T-20) are the two currently available ARVs in this class. Maraviroc is an ARV in the co-receptor antagonists CCR5 class, bind to the CCR5 located on human cell surface and thus, blocking HIV protein gp120 from binding to CCR5 and preventing activation of entry mechanism (Hoffmann and Rockstroh, 2012). Co-receptor antagonists are the first ARV drugs which do not target the virus directly, instead they prevent viral entry into target cells. Enfuvirtide attaches to gp41 to prevent the formation of an entry pore for the capsid of the virus, keeping it outside of the cell (Hoffmann and Rockstroh, 2012). HIV gp41 is a viral transmembrane protein that undergoes a conformational change to facilitate viral entry into target cells (Chan *et al*., 1997).

1.5.2 Combination therapy and HAART

In the early period of ARV discovery, only one type of drug was used to treat HIV-infected patients, known as monotherapy. During that period, AZT was used as the sole drug to treat HIV infection and became the standard treatment for several years. However, it has been shown that HIV easily develops resistance following individual ARV therapies than when

combined multiple ARV drugs with varied mechanism of actions are administered. Dual therapy with AZT and (ddI or ddC) combination was later introduced and proven to be more effective than monotherapy (Zuckerman *et al*., 2009).

Combination of different ARV is subject to positive and negative synergies and this consequently limits the number of effective combinations. At present, combination of abacavir and lamivudine (ABC + 3TC) or emtricitabine with tenofovir (FTC + TDF) are the most preferred choice for viral suppression compared with the rest of the NRTI drug combinations. AZT plus 3TC combination which was the most preferred combination in the 1990s is now chosen as an alternative if the most preferred combination is not effective in patients (Hoffmann and Rockstroh, 2012). Gallant and co-workers (2006) claimed that FTC plus TDF is more effective and causing less frequent lipoathrophy but has equal infrequent resistance mutations and virological failure when compared with AZT plus 3TC. Outcomes from a study comparing ABC plus 3TC with FTC plus TDF showed insignificant differences between them in terms of virological efficacy and severe side effects (Gallant *et al*., 2006).

There are several criteria need to be considered to formulate the best drug combination, which is level of efficacy, tolerability toward side effects and time taken for HIV to develop ARV resistance. There are several drug combinations that could result in lower efficacy become antagonistic to each other or have high probability to confer side effects such as mitochondrial toxicity and lipoatrophy, thus are not suggested to be used in the therapy especially for treatment-naive patients. For instance, FTC plus 3TC, ddI plus (d4T or TDF) and AZT plus d4T (Table 1.3) (Hoffmann and Rockstroh, 2012). Combinations of AZT plus d4T and FTC plus 3TC are antagonistic and must be avoided since both drugs compete for the same active site. Combination of ddI plus d4T or TDF also must be avoided due to high toxicity level since ddI is associated with long-term toxicity.

Table 1.3 Interaction between NRTI combinations.

ARV	3TC	ABC	ddI	d4T	FTC	TDF	AZT
3TC		+++	++	+	-	++	++
ABC	+++		0	0	0	0	+
ddI	+	0		-	0	-	0
d4T	+	0	-		0	0	-
FTC	-	0	0	0		+++	0
TDF	++	0	-	0	+++		0
AZT	++	+	0	-	0	0	

+++ preferred combination, ++ recommended as alternative, + other alternative, - pairing should be avoided, 0 insufficient data.
Adapted from: Hoffmann and Rockstroh (2012)

Moreover, using d4T as part of drug combination is more associated with lipodystrophy. Therefore, combination of d4T with AZT or ddI should be used as the last resort for patient experiencing toxicities (Wormser, 2004).

For pregnant women, the most preferred drug combination is AZT plus 3TC with Lopinavir/r (PIs), whereas Nevirapine and Efavirenz are an alternative to replace Lopinavir/r if the most preferred combination is not effective. However, Efavirenz is not recommended during first trimester since it may cause birth defects and should only be used during second and third trimester if it has an advantage over other drugs (Rathbun *et al*., 2012; AIDS info, 2012).

The availability of new drug classes such as NNRTI and PI has led to a more complex combination called "cocktail therapy" or highly active antiretroviral therapy (HAART). HAART was first introduced to the global market and Malaysia in 1999 and 2002, respectively (Tee *et al*., 2006c). HAART usually involves a combination of at least three drugs from two or more classes of ARV treatment such as two NRTIs and one NNRTI (first-line treatment), two NRTIs and one PI (second-line treatment), two NRTIs and one integrase inhibitor (third-line treatment), two PIs and one or two NRTIs, one PI and one NNRTI with one or two NRTIs or other such combinations (Kayser *et al*., 2005).

NNRTIs are extremely effective when combined with nucleoside analogues. Therefore, combination of one NNRTI and two NRTI is chosen as the first line treatment. The immunologic and virologic potency of NNRTIs in treatment-naive patients is almost equal to PIs. However, in treatment-experienced patients, the efficacy of NNRTIs is probably weaker compared with PIs (Hoffmann and Rockstroh, 2012).

1.6 Antiviral drug resistance

HIV drug resistance occurs if the virus could mutate and replicate itself in the presence of ARV drugs. Drug resistance leads to the treatment failure, spread of resistant strains of HIV, the need to develop new anti-HIV drugs and to change treatment strategy to second or third line treatment, resulting in increased of health costs. Using ineffective drugs will cause selective pressure which allows the resistant virus to replicate faster without the need to compete with other viruses that are sensitive to drugs. The mutated virus continues to replicate, able to retain mutations and could produce other mutations associated with drug resistance (Kessler, 2010).

According to Pillay and co-workers (2000), despite good control of HIV infection by HAART, at least 50% of patients show virologic failure within the first year of treatment. There are several factors attributable to development of HIV drug resistance including poor adherence to ARV, infection with a resistance strains of HIV, superinfection and natural mutations during replication (Cane, 2005; Men´endez-Arias, 2008). Drug resistance can be achieved through the accumulation of one or more mutations in the viral target gene-coding region (*pol*, *gag*, *env*, *integrase*) (Men´endez-Arias, 2008).

Poor adherence to drugs is the most common cause of initial drug resistance. This may involve in one particular drug or the entire regimen (Volberding, 2008). Non-compliance of ARV drugs will cause incomplete suppression of viral replication, thus, allowing virus to overcome inhibition by drugs. Side effects of the drug, high burden to take complete drug dose and trouble swallowing pills are among several reasons why patients are not compliant with the therapy (AIDS info, 2012).

Widespread use of HAART has successfully decrease morbidity and mortality of HIV infected patients. However, low ARV adherence has partly contributed to the emergence and spreading of resistance HIV strains to the newly-infected person. Emergence

of drug resistance HIV in treatment-naive patients were reported from several studies including Valle-Bahena *et al.* (2006), Babič *et al.* (2006), Stańczak *et al.* (2007), Toor *et al.* (2011) and Tseng *et al.* (2012). However, the prevalence of resistance to all drug classes is still low.

Superinfection or multiple infections occur due to simultaneous infection with at least two heterologous strains (co-infection) or due to sequential infection, in which a second infection with a heterologous strain occurs after seroconversion to the primary infection has taken place (Hemelaar, 2012). There was a case reported in 2001, where a man with a primary HIV infection and no drug resistance observed had reported PI-resistance HIV from last blood taken. However, the patient denied taking any other antiretroviral medications especially PI, but he did report frequent unprotected anal receptive intercourse with partners of unknown HIV serostatus since his first HIV diagnosis (Smith *et al*., 2005).

Lack of proofreading activity, high replication rate of approximately one billion virions per day and high mutation frequency especially in the HIV *reverse transcriptase* region contribute to natural resistance mutations (Martinez-Cajas and Wainberg, 2007). Previous studies have reported occurrence of mutations conferring resistance due to natural selection in *protease* (Holguin *et al*., 2004) and *reverse transcriptase* regions (Leal *et al*, 2004). A study done by Leal and co-workers (2004) reported mutation A98G was retained in population for at least five years in the absence of continuous drug pressure.

In Malaysia, the earliest drug resistance test was done by Tee *et al*. (2006a and 2006c) for both treatment-naive and treated patient groups in Kuala Lumpur. Among treatment-naive patients, only one percent of them were reported having at least one major mutation conferring drug resistance in which was considered low prevalence (Tee *et al*., 2006a). However, the prevalence of treated patients harboring at least one major mutation conferring resistance to NRTIs, NNRTIs, or PIs was high (77.8%) (Tee *et al*., 2006c), but