

## **CHAPTER I**

### **INTRODUCTION**

HIV/AIDS remains a public health problem worldwide, including Thailand. According to WHO and UNAIDS estimates, in 2009, the number of people living with HIV was 33.3 million (31.4 million–35.3 million), with 1.8 million deaths from AIDS (1.6 million–2.1 million) (UNAIDS, 2010). In Thailand, as of March 2011, the Bureau of Epidemiology, Ministry of Public Health, Thailand (MOPH) reported 372,874 people living with HIV/AIDS and 98,153 AIDS-related deaths. The use of combination antiretroviral therapy (ART) has become a standard of care in the treatment of HIV infection. At the end of 2009, 36% (about 5.2 million) of the 15 million people in need in low- and middle-income countries were receiving antiretroviral therapy. An additional 1.2 million people received antiretroviral therapy in 2009, bringing the total number of people receiving treatment in low- and middle income countries to 5.2 million, a 30% increase over 2008. Thailand has the policy to provide full coverage of care and treatment for people living with HIV/AIDS throughout the country as part of universal coverage. In 2009, 76 percent of HIV-infected people were receiving ART, according to the WHO/UNAIDS/UNICEF. Due to increased ART coverage, AIDS-related morbidity and mortality are decreasing.

ART has been a major driver for public health in diminishing the HIV-related morbidity and mortality over the past decade (Palella et al., 1998). The aim of ART in HIV infection is to reduce the amount of replicating virus to as low a level as

possible, thereby preventing infection of new cells and preserving the immune system. In 1987, zidovudine (AZT) was the first approved antiretroviral (ARV) (Davies, 2000). To date, almost 30 antiretroviral drugs of 6 classes including; nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors (PI), entry or fusion inhibitors, CCR5 inhibitor and integrase inhibitor have been approved for HIV-1 clinical care. In Thailand, three classes, NRTIs, NNRTIs and the PIs, are widely used drugs in first-line and second-line regimens.

In order to provide HIV/AIDS health care for all, the treatment guideline has been established as national policy in Thailand. According to the national policy in Thailand, antiretroviral therapy should be initiated in adult and adolescent HIV-1 infected patients with a history of HIV-related illness or AIDS or with a CD4+ T-cell count  $< 350 \text{ cell/mm}^3$ . For treatment-naïve patients, the preferred initial therapy is a non-nucleoside reverse transcriptase inhibitor (NNRTI)-based regimen which consists of 2 NRTIs as the backbone plus 1 NNRTI. Following treatment initiation, it is necessary that the CD4+ T-cell count be monitored twice per year and the viral load once per year (Sungkanuparph et al., 2010). Obviously, the proper management of antiretroviral-related toxicity and enhancement of adherence are crucial for the long-term success of ART. In the event that the first-line NNRTI-based regimen has failed, the subsequent therapies employed are the combination of boosted-PI with 2 active NRTIs, as recommended for second-line treatment (Johnson et al., 2008).

Since 2002, the fixed-dose combination of three generic antiviral agents, stavudine (d4T) + lamivudine (3TC) + nevirapine (NVP), in one pill, has been available in Thailand and other developing countries (Cohen, 2003). This formula has

reduced the cost of treatment dramatically and makes antiretroviral therapy more affordable for HIV-1-infected patients in resource-limited areas worldwide. In addition to the cheaper cost, this fixed-dose combination and NVP-based regimen is simple and well tolerated (Podzamczer and Fumero, 2001). Nonetheless, continuing use of ART has led to the emergence of HIV-1 drug resistant (HIV-1 DR) strains. The emergence of ART-resistant viral strains has been a major cause of treatment failure and limits options for alternative antiretroviral regimens (Sungkanuparph et al., 2007). In addition, the transmission of HIV-1 DR variants has been reported with an increasing incidence, which is apparently related to the widespread use of antiretroviral drugs (Weinberg et al., 1998; Yerly et al., 1999; Ballotta et al., 2000; Little 2000). These situations strongly promote the need for the effective tools in HIV-1 DR detection to be in place. HIV-1 DR screening and monitoring would be useful in guiding the choice of therapeutic regimens by identifying drugs that are unlikely to suppress viral replication.

HIV-1 DR variants can be identified based on phenotypic and/or genotypic determination. Phenotypic assays evaluate the ability of HIV-1 replication in the presence of increasing concentrations of antiretroviral agents. Due to time, labor, and proper laboratory infrastructure requirements, the phenotypic assay is inappropriate for routine use in clinical settings. Genotypic assays rely on the detection of mutations, by using nucleotide sequencing or a non-sequencing approach, within the viral gene *pol* known to have an association with phenotypic drug resistance. Genotype-based detection of mutations can be determined using a number of methods that can be performed in most clinical molecular diagnostic laboratories.

Nucleotide sequencing is a commonly used method to determine the presence of drug resistance mutations in clinical settings. This method provides the opportunity to comprehensively evaluate multiple mutations associated with HIV-1 antiretroviral resistance and novel mutation. Although sequencing reaction is simple to perform in research settings and in the universities' hospital, it is difficult to implement this assay in general hospital in many resource-limited areas, due to the requirement of costly equipments, infrastructure, and skilled personnel. Such limitations demand an economical method for HIV-1 DR detection that is less expensive but more simple in relative to the standard nucleotide sequencing. According to a systemic observation between the phenotype and the genotype of HIV-1 variants that are resistant to antiviral drugs, it has been demonstrated that HIV-1 drug resistance may resulted from specific point mutations (Johnson et al., 2008). Therefore, techniques developed to detect single nucleotide polymorphisms or point mutations may be used to detect HIV-1 DR as an alternative method to nucleotide sequencing (Edenstien et al., 1998; O'Meara et al., 2001; Metzner et al., 2003, Nissley et al., 2005; Palmer et al., 2005; Tsongalis et al., 2005; Palmer et al., 2006).

In this study, we propose to develop a probe-based method for detecting point mutations within the HIV-1 *pol* gene that are associated with ART resistance called an oligonucleotide ligation assay (OLA). The OLA is a rapid, specific, and sensitive method for the detection of known point mutations (Landegren et al., 1988; Tobe et al., 1996), and has been applied for HIV-1 DR for subtype B since 1995 (Frenkel et al., 1995). The assay's principle is based on the covalent joining of two adjacent oligonucleotide probes at the specific position where the mutation has occurred. This process is facilitated by a thermostable DNA ligase after hybridization

to a cDNA template, usually a PCR product. The advantages of the OLA are as follows; firstly, the assay can be performed in a high throughput manner that makes it useful for epidemiologic studies or clinical trials that evaluate a large number of specimens for a specific mutation. Secondly, the OLA is highly sensitive in the detection of minor populations of mutant genotypes among wild-type viral sequences. Thirdly, the readouts of the OLA can be inspected either visually or by spectrophotometer machine.

The initial target of the OLA proposed in this study is primarily focused on the detection of M184V mutation of HIV-1 circulating in Thai patients. M184V is characterized by nucleotide substitution, from A to G, at the position 3099 HXB2 in the *pol* region resulting in the change of amino acid from methionine (ATG) to valine (GTG) at codon 184 of the reverse transcriptase (RT) enzyme. This mutation is selected by therapeutic regimens containing lamivudine (2',3'-dideoxy-3'-thiacytidine, 3TC) and emtricitabine (FTC) which confer a loss of drug susceptibility, ranging from 100- to 1,000-fold and >300-fold to these drugs, respectively. Whereas, selection of this mutation by lamivudine occurs rapidly compared to the development of resistance to other drugs.

In this study, the specific-primers and probes for OLA for the detection of M184V drug-resistant mutation in the HIV-1 CRF01\_AE *pol* gene will be developed, optimized, and evaluated. If the study goal is achieved, this method would be a rapid and simple assay for the detection of a specific mutation associated with resistance to lamivudine and emtricitabine. Furthermore, this study would provide a basis for designing additional oligonucleotides to use as specific probes to detect other known point mutations spanning the whole NRTI class or other drug classes. Finally,

because of the ease of the assay and the minimal technical skill required, transformation of this technology into a detection kit for the detection of HIV-1 DR variants circulating in Thai patients may be possible. The use of OLA may be helpful to better understand the occurrence of primary and secondary resistance of HIV-1 DR in Thailand in the era of the widespread use of anti-HIV drug treatments.