CHAPTER I

INTRODUCTION

1.1. Background and rationale

1.1.1. Minority HIV-1 drug resistance

Since the first discovery of the cause of Acquired Immunodefiency Syndrome (AIDS) in 1983 [1-3], it has been estimated that 33 million people worldwide are living with Human Immunodefiency Virus (HIV) [4]. Although the epidemic is stabilizing, it is still at a high level, affecting many people's lives especially in developing countries in Sub-Saharan Africa, Asia, and South America [4].

With the initial introduction of the first antiretroviral (ARV) drug, zidovudine (AZT) of nucleoside reverse transcriptase inhibitors (NRTIs) in 1987 and then protease inhibitors (PIs) in 1995 and non-nucleoside reverse transcriptase inhibitors (NNRTIs) in 1996, the era of Highly Active Anti-Retroviral Therapy (HAART) began, resulting in a dramatic reduction of AIDS deaths, opportunistic infections, and burden for hospitals due to AIDS related-hospitalized patients [5, 6]. Although having such breakthrough achievements in HIV treatment, once gets infected, patients cannot get rid of HIV, and the virus remains archived in cells and organs for the rest of life. Side-effects and drug toxicities can affect treatment results, making it difficult for patients to fully adhere the therapy. Furthermore, drug resistance defined as the phenomenon of viruses resisting activity of drugs leading to increasing concentration of drugs required to suppress viral replication, is an important factor causing treatment failure. Due to the natural diversities of HIV such as rapid replication, lack of proofreading ability of viral reverse transcriptase (RT), accumulation of pro-viral strains during infection period, and recombination ability when viruses with different sequences infect the same cell, a variety of genetically distinct viruses which are called quasispecies

carrying mutations evolve. Variants in viral quasispecies display different fitness in different environments. Before the initiation of treatment, variants with wild-type (WT) sequences are predominant, but in the presence of therapy, variants harboring drug resistance-associated mutations (DRAM) show a strong selective advantage over WT ones and gradually dominate the viral population leading to treatment failure.

Currently, there are 5 classes of antiretroviral drugs, NRTIs, PIs, NNRTIs, entry inhibitors (EIs), and integrase inhibitors (INIs) which target on different stages of viral replication in the host cells. In developing countries, 3 common drug classes widely available for HIV prescription are NRTIs, NNRTIs, and PIs. The combinations of at least 3 drugs from different classes compose HAART that helps to reduce the number of viral particles down to the viral suppressed level, to obtain immunological reconstitution in HIV/AIDS patients, and also to decrease the level of viral mutations leading to drug resistance. However, because of the high rate of mutation development and the variation in penetration of drugs making low level of drug concentrations in some compartments differing from plasma, drug resistance still occurs in different patterns and at different levels. HIV drug resistance (HIVDR) gradually develops following the course of HAART. In one large cohort study with 4,306 participants, the cumulative percentage of virological failure was 38% of patients within 6 years of starting first line-therapy and more than 27% developed HIV-1 resistance to at least one ARV drug [7]. The increasing numbers of HIV-infected people having access to ARV drugs in both developed and developing countries have resulted in an elevated incidence of HIVDR. That subsequently creates a large pool of viral drug resistance variants leading to an increased primary or transmitted resistance occurring when one gets infected by a variant already resistant with one or more ARV drugs [8-11]. Drug resistant mutants, once acquired by developed or transmitted resistance, persist as proviral DNA over a long period of time. In a patient undergoing treatment and harboring drug resistant viruses, the dominant resistant mutants is accompanied by minority quasispecies expressing different sequence mutations

and they evolve in distinct pathways [12, 13]. The initial minority quasispecies can rapidly emerge as the major mutants if their mutations show a higher level of resistance or changes of drug pressure give them a growth advantage over the prior dominant population, subsequently leading to early therapy failure in treatment-naïve individuals [14]. There are evidences that HIV-1 still residually replicates and subsequently selects drug resistant variants although virus is fully suppressed in undetectable level (viral load is below 50 copies/ml) by available commercial kits [15, 16]. Recent studies have shown that minority HIV-1 drug resistant variants, not identified by conventional genotyping, are present in HIV-1 infected ARV therapy (ART)-naïve persons at baseline and lead to the early failure of first line therapy [14, 17]. The drug mutants can emerge rapidly in mothers receiving a single dose of nevirapine (NVP). In one trial, the cross-resistant mutation to NNRTIs K103N was detected in 8 of 9 women after 6-8 weeks of administration of single-dose of NVP and in 4 of 5 their infants and persists as minority isolates for more than 1 year [18]. Therefore, the detection and quantification of minority variants of HIV-1 drug resistance might be important and could have clinical relevance. The conventional genotyping which is currently used to test HIV-1 drug resistance however cannot detect minority variants present less than 20% of total viral population [19, 20].

1.1.2. HIV-1 minority resistant variants have not been studied yet in Thailand

In Thailand, the first cases of AIDS was identified in 1984 [21]. By the end of 2007 the estimated numbers of people living with HIV/AIDS (PLWH) were 700,000 and nearly 50,000 new AIDS cases reported each year [22]; most cases were infected with HIV-1 subtype CRF01_AE [23, 24]. In 2001, the scale-up of access to ARV began with the success of the Thai Government Pharmaceutical Organization (GPO), a state company run by the Thai Ministry of Public Health to produce a number of generic ARVs, including AZT, didanosine (ddI), stavudine (d4T), lamivudine (3TC), NVP, saquinavir (SQV), and nelfinavir (NFV) and

fix-dose combinations of d4T, 3TC, and NVP in one tablet called GPO VIR® S with the prices approximately $1/day [25]. Thanks to the National Access to Antiretroviral Therapy for People with HIV/AIDS Program (NAPHA), there have been 58,133 cases receiving ARV drugs nationwide from February 2001 to December 2004 [26] and the number of people access to ARV is increasing with additional support from the Thailand Social Security Health Care Scheme in late 2004 and the Universal Health Care Scheme in 2005 [25]. In order to ensure the success of expanding HAART in Thailand especially in the time of switching to second-line therapy, besides adherence, monitoring HIV-1 drug resistance is of critical importance. Conventional genotyping has been conducting in many laboratories all over the country with commercial kits such as HIV-1 TrueGene™, Bayer Healthcare Diagnostics/Siemens Medical Solutions Diagnostics; or ViroSeq™, Celera Diagnostics/Abbott Laboratories and some in-house assays. Studies on treatment naïve HIV-1 infected patients in the Bangkok Metropolitan Area and central Thailand with samples collected from 2005-2006 using conventional genotypic assays showed a low prevalence of ARV resistance-associated mutations [27-30] and study on 98 patients with first line failures reported that 95% and 92% of patients had at least one major mutation related to drug resistance to NRTIs and NNRTIs, respectively with median time of 20 months on GPO VIR® S, the most commonly used drug in Thailand [31].

However, as mentioned above, the direct genotyping can only detect majority population of HIV-1 drug resistant viruses accounting for more than 20% of the total population. Up to now, without techniques to detect and quantify minority quasispecies of HIV-1 in Thailand, minority HIV-1 DRAMs have not been studied yet in Thai populations infected with HIV, particularly the mutations M184V and Y181C conferring resistance to 3TC and NVP [32], the most frequently prescribed ARVs in Thailand. Pyrosequencing (PSQ) technology was applied to develop an assay to detect minority Y181C and M184V mutations.

This PSQ technique was then performed on samples collected in two cohorts of patients with previously undetected Y181C and M184V mutations by conventional genotypic test.

1.1.3. Minority N155H mutation in patients failing raltegravir-containing regimen in France

Integration of viral DNA into the host cell DNA, an important step in the HIV life cycle, is facilitated by the viral integrase (IN). Integrase inhibitor (INI) is a new class of antiretroviral (ARV) drugs designed to target the viral enzyme and impair its normal activity. Raltegravir (RAL) is the first drug of INI that was approved by the US FDA and the European Medicines Agency (EMEA) for HIV treatment [33, 34]. RAL has been shown to have a potent antiviral activity in heavily treated patients harboring multiple resistant viruses to reverse transcriptase and protease inhibitors, particularly when combined with at least 1 active ARV drug [35, 36]. However, due to the high mutation rate of HIV, under continuously selective pressure of treatment, drug resistance inevitably emerges, resulting in treatment failure [37]. Most of the DRAMs conferring resistance to RAL are located within the catalytic domain of the IN. RAL-resistant patterns involve 3 common independent pathways associated with the primary mutations N155H, Q148K/R/H and Y143R/C [38-40]. Each pathway consists of one or more secondary mutations that either further reduce susceptibility or compensate the loss of viral replicative capacity caused by primary mutations or both [39]. In clinical studies on patients experiencing prolonged therapy failure with RAL-based salvage regimen, the N155H ± E92Q pathway was selected at the early time of failure and sequentially replaced by the Q148H mutation usually along with the G140S mutation [41, 42] or by the mutations Y143C/R [43]. The double mutant Q148H + G140S displayed an increased resistance level and a higher level of viral replicative capacity than the single N155H mutant [43-45]. The more favorable selective advantage of the double mutant Q148H + G140S *in vitro* could be an explanation of the resistance profile switch from the N155H to

the Q148H pathway [46]. However, the mechanism and dynamic of these evolutions so far have not been completely elucidated. Due to the fact that emergence of RAL resistance usually initiated with the N155H mutant, in the present study we assessed the role of minority N155H-mutated variant in circulating RNA and archived DNA in 5 heavily treated patients experiencing long-term RAL therapy failure and harboring different resistance profiles determined by standard genotyping: 1) beginning with the N155H pathway and remaining with this pathway, 2) beginning with the N155H pathway and switching to the double mutant Q148H + G140S, 3) beginning with the double mutant Q148H + G140S and remaining with this profile.

1.1.4. Genetic barrier to the development of resistance to integrase inhibitors in HIV-1 subtypes CRF01_AE and B

In INIs, besides RAL being the first approved compound for HIV treatment, other drugs which are currently in different stages of clinical development include elvitegravir (EVG) and dolutegravir (S/GSK1349572, DTG), a recently developed compound appeared as next-generation INI [47]. Based on its novel mechanism of action, INI can suppress the viral replication of HIV variants harboring drug resistance mutations to other classes such as NRTIs, NNRTIs, PIs, and EIs [35, 48, 49]. However, like other classes of ARV, emergence of HIVDR to INI still occurs *in vitro* as well as in patients experiencing treatment failure through the selection of DRAMs in IN-coding region of viral polymerase gene. RAL and EVG generally have a resistance profile with primary mutations at the positions T66 (EVG), E92 (EVG), Y143 (RAL), Q148 (both), and N155 (both) and secondary mutations that could play a role in increasing resistance and/or compensating for reduced viral fitness caused by the primary mutations [50]. DTG considered a next generation INI was designed to have a potent antiviral activity, low dosing given once daily without pharmacokinetic boosting, and a non-overlapping resistance profile in particular compared to the other INIs. With mutation

profile at positions E92, L101, A124, Q148, S153 and G193 [51], DTG displays significant antiviral activity against isolates harboring RAL and EVG resistance mutations [52].

The genetic barrier, defined by the accumulative number of DRAMs required for the virus to escape drug-selective pressure, is a crucial factor in the development of drug resistance [53, 54]. Since INI is a new class of ARV and particularly DTG appears as a next generation INI with current promises of potential antiviral activities against virus conferring resistance to both RAL and EVG, it is important to investigate its genetic barrier in the context of the variability of HIV epidemic worldwide [55]. Furthermore, major HIV1 strain predominant in North America and Europe is B subtype. Thus, most of the researches on the development of new drugs, drug resistant pattern in particular have been carried out in studies with HIV-1 subtype B which accounts for only 12% of the HIV infections worldwide [56].

It was showed that the genetic barrier among subtype B, C, A, CRF02_AG are similar in most of the major IN mutations related to RAL and EVG resistance; some mutations V151I, G140C, and especially G140S which is associated with the Q148K/R/H pathway have a lower genetic barrier in subtype B [53, 57]. However, there are limited data on subtype CRF01_AE, a predominant isolate in Southeast Asia which is the cause of a number of people infected with HIV-1 in the region and is also frequently seen in France [58]. We calculated and reported the genetic barrier for INI resistance at 41 amino acid positions corresponding to 66 substitutions [50, 53, 59, 60] related to RAL, EVG, and DTG resistance in 144 IN nucleotide sequences derived from INI-naïve patients. Of these 144 sequences, 109 were HIV-1 subtype CRF01_AE (22 from France and 87 from Cambodia, Thailand, and Vietnam [61] downloaded from the Los Alamos database (http://hiv-web.lanl.gov) and 35 were subtype B from France.

1.2. Studies of the thesis

This thesis includes 3 studies leading to 3 manuscripts:

- Paper 1: Minority HIV-1 resistant variants in recent infection and in patients who failed first-line ARV therapy with no detectable resistance-associated mutations in Thailand
  Hai Le Nguyen, Patrawadee Pitakpolrat, Sunee Sirivichayakul, Constance Delaugerre, Kiat Ruxrungtham
  J Med Virol. 2012 May; 84(5):713-20.
- Paper 2: Longitudinal analysis of minority N155H drug resistance in heavily treated patients failing with raltegravir-based regimens
  Hai Le Nguyen, Charlotte Charpentier, Nga Nguyen, Pierre de Truchis, Jean-Michel Molina Kiat Ruxrungtham, Constance Delaugerre
  Submitted a revision to *HIV Medicine* and being under review
- Paper 3: Genetic barrier to the development of resistance to integrase inhibitors in HIV-1 subtypes CRF01_AE and B
  Hai Le Nguyen, Kiat Ruxrungtham, Constance Delaugerre
  Intervirology. 2012 Mar 23. [Epub ahead of print]

1.3. Objectives

- To determine the prevalence of M184V and Y181C minority variants associated with 3TC and NVP resistance primarily transmitted in a cohort of 104 persons infected with HIV-1, naïve to treatment without resistance mutations found by standard genotypes.

- To investigate the presence of M184V and Y181C minority viruses during first-line ART failure in a second cohort of 22 patients receiving HAART with no DRAMs detected by standard genotypes and their relations to sequential treatment outcomes.

- To assess the minority N155H mutation in a longitudinal follow-up of five heavily treated patients experiencing RAL failure with 3 different RAL resistance profiles.

- To analyze the genetic barrier for INI resistance at 41 amino acid positions corresponding to 66 substitutions related to RAL, EVG, and DTG resistance in 144 IN nucleotide sequences of HIV-1 subtypes CRF01_AE and B.

1.4. Key words

Minority drug resistance, HIV-1 subtype CRF01_AE, ARV-naïve, first-line treatment failure, raltegravir resistance profile, genetic barrier, integrase inhibitor, elvitegavir, dolutegravir, Thailand, France.

1.5. Expected benefits

- Know the prevalence of minority DRAMs through the common mutations M184V and Y181C in drug naïve people recently infected with HIV-1 and in patients with first-line treatment failure in Thailand.

- Understand whether found minority DRAMs have an impact on sequential treatment outcomes.

- Identify whether PSQ could be applied for screening samples to detect point mutations in clinical practice as well as in surveillance. The current assay could be extended to detect and quantify other interested point mutations.

- Understand the role of minority N155H mutation contributing to mechanism of different profiles of RAL resistance.

- Understand genetic barrier to develop resistance to INI particularly DTG, a new compound with potential antiviral activity currently under clinical investigations, in subtypes CRF01_AE and B.