CHAPTER III

METHODS

Study Design and Scope

This was a single centre, cohort study The participants were from the HIVNAT 006 long-term follow-up study that enrols patients who have completed other HIVNAT studies. After consenting to enrol in HIVNAT 006, these patients attend for clinical and laboratory assessment at least twice per year and receive free or highly subsidised ART. The sub-set of this cohort eligible for his study had o have been ARV-naïve when they enrolled into their first study at HIV-NAT.

This study had two main time intervals. The first period of interest was the time from baseline (study enrolment) to the time of virological suppression, in patients from the HIV-NAT 006 cohort initiating HAART between January 1, 2000, and December 31, 2006. As patients were ARV-naive at study commencement, it was possible to examine the effect of time from starting treatment (the date of enrolment) to virological suppression. It is important to be able to study naïve patients. Data from these patients is not confounded by the previous use of other therapies. Information obtained from naïve patients can contribute to clinical practice at a time when many new patients are commencing therapy due to the scaling up of the availability of ART in the developing world.

Additionally, this study investigated virological failure following initial virological suppression, to ascertain the numbers of patients who failed virologically

in each year and to investigate some determinants of virological failure, in particular time from commencing therapy.

Participants were HIV positive with a detectable pVL and were ART naive at study entry. After commencing a HAART regimen, either NNRTI- or PI-based, eligible participants must have achieved virological suppression, measured as pVL <50 copies per mL, for at least two months to be included in the study. Subsequently, some participants failed virologically, measured as a pVL above the limit of detection (i.e., pVL >50 copies per mL). At this point, the participant experienced the event under study and dropped out of the study.

The Viral Load Test

PVL testing is the gold standard in monitoring the success of HAART and in detecting early treatment failure. Successful combination antiretroviral therapy should give a fall in pVL of 1.5 to 2.0 logs (30-100 fold) within six weeks, with the pVL falling below the limit of detection within twelve to 24 weeks. (DHHS, 2006).

There are various assays commercially available. Each assay has a different upper and lower limit of detection. (NAM, 2007) In this study, pVL is measured using a branched DNA (bDNA) assay, a test for specific nucleic acid chains typically used to detect reverse transcriptase viruses such as HIV. (Murphy et al., 1999) The assay mixes bDNA with a sample to be tested. If the bDNA binds to viral RNA, then the luminescent compound will react, permitting measurement of the result. The assay used in the majority of HIV-NAT's clinical trials that provided participants for this study is the bDNA assay manufactured by Chiron/Bayer, marketed under the tradename Quantiplex 3.0 in Europe or Versant 3.0 in the United States. This assay

has a lower detection limit of 50 HIV RNA copies per mL of blood (copies/mL) and an upper limit of 500 000 copies/mL.

Another assay, the Roche Amplicor HIVRNA PCR assay, is used in some studies. This assay also has a lower limit of detection of 50 copies/mL. The two assays should not be used in comparing levels of detectable pVL as they will yield slightly different results. However, they are equivalent in detecting whether the pVL is undetectable or not.

Study Site

The study was conducted at HIV-NAT, a Division of the Thai Red Cross AIDS Research Centre in Bangkok, Thailand.

Participants

HIV-NAT has been conducting HIV-related clinical trials since 1996 and trials using HAART regimens since 1998. HIV-NAT is committed to both clinical research and the continued well-being of its study volunteers, and provides antiretroviral medications and monitoring for these participants via a system of study extensions or rollover study protocols and long-term follow-up protocols. (Safreed-Harmon et al., 2004).

One particular issue with HIV clinical trials in Thailand is that the majority of participants, especially participants with antiretroviral resistant virus, have limited options to continue their treatment with the limited second-line antiretroviral medications available in the country's health care system. After the completion of a HIV-NAT study, the investigator is obliged to ensure that participants included in the study receive appropriate treatment and follow up. Currently, more than 1 000

individuals are actively followed-up at HIV-NAT. Participants in clinical trials at HIV-NAT receive life-long antiretroviral medications for as long as they receive benefit, as part of the research commitment from some pharmaceutical and other sponsors. Other post-study participants are receiving their drugs via the National Social Security Office (SSO), National Health Security Office (NHSO) and the HIV-NAT Drug Fund. (Ananworanich et al., 2004; HIV-NAT, 2006, 2007).

In addition to time and based on previous studies (Dragsted et al., 2004; Fournier et al., 2005; Manegold et al., 2004; Oette et al., 2006; Paredes et al., 2000), this study investigated several candidate prognostic variables; gender, age and mode of HIV transmission, CDC stage of disease, baseline (pre initiation of HAART) pVL and baseline CD4+ count, co-infection with hepatitis B or hepatitis C and baseline ART. The year of commencing HAART was included as an indirect indication of different initial ARV regimens that were available in the different years when subjects commenced ART.

Sample Size

The target population was the HIV-positive people in Bangkok who are being treated with a HAART regimen, estimated at 50 000. The World Bank in 2006 estimated the numbers of HIV-positive people on ART in Thailand in 2000 as almost negligible, and projected the numbers in 2002 as approximately 15 000, in 2003 as approx. 20 000, in 2004 as approx. 30 000 and in 2007 as approx. 130 000. (Revenga et al., 2006) The extremely low figure for the year 2000 is because HAART was not commercially available in Thailand at that time. Research centers like HIV-NAT in Bangkok were able to use the new therapies as part of research programs. The years of interest for this study are from January 2000 until December 2006 inclusive. The

median year is 2003. According to the World Bank estimate, the figure for the year 2003 is approximately 20 000. However, if one selects the figure for 2007 and divides this by 7 (years) then a figure of 18 571 is arrived at. Using this figure cumulatively for the years 2000 to 2003 (4 years) one arrives at an estimated total population sample of 74 284. Overs and colleagues estimate the number of adults taking ART in Thailand in 2006 to be 88 457 (from a total number of 556 848 adults living with HIV/AIDS). (Over et al., 2007) Therefore, the sample size calculations are based on an estimated lower level of 50 000 and an upper level of 90 000. Current literature suggests that the number of failures in this population, the primary endpoint for this study, will be 22-25% (van Leth et al., 2004). A confidence level of 95% and an acceptable error of 5% have been used in the calculations.

The sample size for this study was estimated at 383, applying the formula in Epi Info 3.4.1 (Centers for Disease Control and Prevention, USA) StatCalc module for a population survey or descriptive study using random sampling.

Table 5: Sample size calculations

	Population Size	Expected frequency	Expected frequency
	90 000	25%	50%
Sample size		287	383
	50 000	25%	50%
Sample size		286	381

Table 5 shows that the sample sizes can range from 286 individuals to 383. We added a margin of 20 for safety, resulting in a requirement for 403 participants. Initially, 413 individuals fulfilled the criteria for inclusion in this study. There was no random selection of participants. This study included as many subjects from the HIV-

NAT 006 cohort who fulfilled the inclusion criteria and had sufficient available data. Nine participants who never achieved virological suppression were excluded from the analysis, leaving a cohort of 404 individuals.

Inclusion/Exclusion Criteria

Patients from the HIV-NAT 006 cohort who entered a study on or after 1 January 2000 through 31 December 2006 were included. They must have been HIV positive with a detectable pVL, ARV naive at study enrolment and have subsequently achieved virological suppression, measured as pVL <50 copies, for at least two months. Individuals who did not achieve defined virological suppression by the end of the study were not included in the analysis. The cut-off point for data collection was 31 December 2007.

Data Collection, Processing and Analysis

Data was sourced from HIV-NAT 006 Microsoft Access databases and transferred (via Microsoft Office Excel) to SPSS 15.0 (SPSS Inc, Chicago, Illinois, USA), SAS 9.1 (SAS Institute Inc. Cary, North Carolina, USA) and STATA 8.0 (Stata Corporation, College Station, Texas, USA) for analysis. Data are available from original enrolment of the eligible participants (January 2000 until June 2007). SPSS was used mainly for descriptive analysis. SAS was used for logistic and Poisson regression. STATA was used for some graphical output.

Baseline characteristics analysed included date-of-birth (age), gender, weight, CDC category, transmission risk category, hepatitis B and hepatitis C status, and baseline antiretroviral regimen. A positive hepatitis B surface antigen or (HBsAg)

indicates Hepatitis B (HBV) positivity. Hepatitis C (HCV) positivity is indicated by a positive hepatitis C antibody.

CD4+ count (absolute cells/mm^3) and pVL (viral copies per mL of plasma) laboratory results were available at baseline and at several other subsequent time points.

Year commencing HAART – this may be relevant as an indicator of the antiretroviral regimen commenced. Tolerance of some initial HAART regimens was poor and could result in severe side effects that affected adherence. Some of these regimens were not potent for individual patients. By examining the year commencing ART, it is possible to determine if this factor has an influence on the outcome.

Descriptive statistics were used to describe baseline characteristics, such as gender, age and weight at enrolment, disease stage (Centers for Disease Control and Prevention [CDC], 1993), first ARV regimen, hepatitis co-infection, baseline CD4+ count and pVL, and mode of HIV transmission. All of the subjects in this analysis were from Southeast Asia and most from Thailand. It is possible that some participants may have been from bordering countries, such as Myanmar, Cambodia or Laos, but these data were not available. All subjects were included in the analysis without regard to race or ethnicity. For variables that were not normally distributed, medians and interquartile ranges (IQR) were reported.

The models were fit by using person-time logistic regression and were verified using Poisson regression. Two techniques were used to enable comparison of the techniques and to verify the stability of the time variable. The effects of variables other than time were analysed using the Kaplan-Meier (product limit) method and univariate and multivariable Cox proportional hazards regression. These techniques

are useful in the evaluation of effects that covariates have on risk of the event of interest and were used to check the consistency and stability of person-time regression results. Similar effects' estimates for independent variables using different techniques increases confidence in the person-time regression results.

Person-time logistic regression requires expanding the data set to create a separate observation for each person-time unit. (Abbott, 1985; Halpern et al., 1993) For example, if the time unit was weeks and a given subject was in the study for 50 weeks, then that subject would contribute 50 observations to the analytical data set, one for each week in the study. This procedure creates an independent variable for time, which can be analysed in the same fashion as any other independent variable in the analysis. This is an important difference from Cox regression in which it is generally not possible to derive an estimation of the effects of the time variable. Person days were used in the analysis as this resulted in the most accurate analysis.

As previously mentioned, the study was divided into two time periods, the time from enrolment to virological suppression and the time from virological suppression to virological failure.

All statistical tests were 2-sided. A P value ≤ 0.05 represents statistical significance.

Time to Virological Suppression

The Kaplan-Meier survival distribution function was used in the unexpanded data set to describe the interval for time to virological suppression. The Kaplan-Meier curve showed a distinct inflection point. This suggested that a polynomial transformation of the time variable from the expanded data set might achieve a better fit in the logistic regression model. For time to virological suppression, initial

quadratic transformation increased the likelihood ratio χ^2 of the model from 11.6215 (P=0.0007) to 25.3992 (P<0.0001), a clear indication of a better fit to the model. The addition of cubic transformation of the variable to the model produced an even better fit, the likelihood ratio χ^2 of the model was 124.5334 (P<0.0001). Although further polynomial transformation is possible, it is not customary. (Allison, 1999).

Person-time logistic regression was used to identify predictors of virological suppression and the binary outcome variable was virological suppression after initiating a HAART regimen. For the interval time to virological suppression, the original data set of the 404 eligible patients was expanded to 71 113 person days.

The eleven candidate prognostic variables that were included in the univariate model were: gender, baseline age, baseline weight, mode of transmission (modelled as homosexual vs. heterosexual and other vs. heterosexual, and always modelled together), baseline CDC category, year of entry, hepatitis B or C infection, CD4+ count, pVL and baseline regimen. Covariates with a P value $\leq$0.2 were included in the multivariable model. All significance tests were two-sided with significance at the 0.05 level. Analyses were 'intention to continue treatment', ignoring subsequent changes to treatment regimens, treatment interruptions and terminations.

A multivariable model was used to determine odds ratios (ORs) for various independent variables that might have been associated with time to virological suppression in this study.

Time from Virological Suppression to Virological Failure

An identical process was followed for time to virological failure as for time to virological suppression.

The Kaplan-Meier survival distribution function was used in the unexpanded data set to describe the interval for time to virological failure. The Kaplan-Meier curve showed two distinct inflection points, suggesting that a polynomial transformation of the time variable from the expanded data set might achieve a better fit in the logistic regression model. When modelled by itself including first quadratic transformation of the time variable, the likelihood ratio χ^2 of the model was 33.2781, $P<0.0001$, a clear indication of a better fit of the model. The addition of cubic transformation of the variable to the model produced an even better fit, the likelihood ratio χ^2 of the model was 44.6527, $P<0.0001$, demonstrating a steady increment in the model χ^2.

Person-time logistic regression was used to identify predictors of virological failure and the binary outcome variable was virological failure following initial virological suppression. For the interval time to virological failure, the original data set of the 404 eligible patients was expanded to 553 083 person days.

As well as time, the candidate prognostic variables that were included in the univariate model were: gender, age at entry, CDC disease staging, baseline CD4+ count and viral load, mode of HIV transmission, year of entry, infection with hepatitis B or hepatitis C, baseline regimen and whether or not suppression was achieved within the first 12 weeks. Covariates with a P value ≤ 0.2 were included in the multivariable model. All significance tests were two-sided with significance at the 0.05 level. Analyses were 'intention to continue treatment', ignoring subsequent changes to treatment regimens, treatment interruptions and terminations.

A multivariable model was fit by using person-time logistic regression to determine ORs for various independent variables that might have been associated with time to virological failure in this study.

All participants achieved initial virological suppression. For participants not reaching the end-point of virological failure following defined suppression, data censoring occurred at the last date of follow-up. Some patients were lost to follow-up before the end of the study and were censored at the time of their last available pVL measurement.

Ethical Considerations

This study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University, which approves all studies at HIV-NAT, and by the Ethical Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University (Appendix A and B). All participants provided signed, informed consent before entering the study (Appendix C).