Mathematical modelling studies of the role of superinfection and non-adherence to antiretroviral therapy on HIV disease progression and viral blips

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DECLARATION

I declare that the work presented in this thesis is the candidate’s own work.

____________________
Chun Hai FUNG
Dedicated to my parents,

Mr. Wai Shek FUNG and Ms. Manna Kwan Fong HONG,

Who in their words and deeds,

Act justly, love mercy and walk humbly with God in their daily lives.
Abstract

This thesis examines the impact of HIV superinfection (infection of HIV-positive individuals by a heterologous HIV strain after immune responses have been established against the first strain) upon HIV disease progression and viral blips, and the relationship between non-adherence to cART and the occurrence of viral blips. For these purposes, a mathematical model of HIV within-host dynamics with two strains has been developed.

My results suggest: firstly, HIV superinfection in and of itself was found not leading to faster progression to AIDS; it is only superinfection with strains of a higher replication capacity that does. Secondly, it was found that superinfecting strains susceptible to the existing cART regimen cannot establish themselves in patients, while those resistant to the regiment will lead to treatment failure. Superinfection in either scenario will not lead to viral blips. Thirdly, the choice of sampling frame was found to have a significant impact upon the observed number and incidence of viral blips. Instead of calculating the incidence of blips from their observed number over a period of time, one should take into account the sampling frame and calculate the proportion of blips among the measurements made over that period. Fourthly, increased drug adherence three days before a clinic visit does not mask poor
adherence; regular consecutive non-adherence results in more blips than a random non-adherence pattern; and dose-timing variation around the regimen-prescribed time leads to more blips. Fifthly, the non-linear relationship between the proportion of measurements with detectable viral blips and the probable drug adherence of a patient, and how this relationship varies with the viral replication rate, are studied.

This thesis improves our understanding of anti-HIV immune responses, refines our public health messages and provides us with indications of drug adherence through observation of viral blips with different sampling frames.
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My girlfriend, Chi-Ngai, has given me great support since we first met online on Xanga, and began our relationships in Hong Kong in January 2008. Being my soulmate who shares with me a great passion in knowledge and faith and an interest in many a discipline from the humanities to social sciences to natural sciences, she is the one who can really understand me, given the fact that most of my friends are confined to the narrow vision of one or two disciplines or professions dictated by the division of labour in the modern academia. Chi-Ngai’s support in my final months of writing my PhD thesis is invaluable and indispensable.

For many of us Chinese, academic achievement of one individual is the glory of the whole extended family or clan. My cousins, aunts and uncles are always proud of me. Here I take the opportunity to thank my grandparents, three of four have by now passed this world to the next, who never received anything more than a primary education and yet were firm believers in the value of education, and who sacrificed
everything for the education of my parents and their siblings, and therefore enabled their grandchildren to have a comfortable life and to make doing a PhD in England a tangible goal for me.

My parents are always an inspiration for me. Without their spiritual, moral and financial support, I could never have accomplished what I have achieved today. Their diligence and perseverance enabled them to achieve much since their childhood amidst dire poverty in Hong Kong in the 1950s and 60s. Their dedication to my education enables me to have a good start in this marathon of academic study. Though I have never been as devout as they are, in their own words and deeds, they taught me the values to act justly, to love mercy, and to walk humbly with God in one’s daily life. Like rivers of ever-flowing water nourishing the hills and plains, they are channels of God’s grace into my life. It is to my parents that this PhD thesis is dedicated.
List of acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Ab</td>
<td>antibody</td>
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<tr>
<td>Ag</td>
<td>antigen</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immunodeficiency syndrome</td>
</tr>
<tr>
<td>AOC</td>
<td>autonomy oriented computing</td>
</tr>
<tr>
<td>ART</td>
<td>anti-retroviral therapy</td>
</tr>
<tr>
<td>ATHENA</td>
<td>AIDS Therapy Evaluation Project</td>
</tr>
<tr>
<td>cART</td>
<td>combinational anti-retroviral therapy</td>
</tr>
<tr>
<td>CA</td>
<td>cellular automaton (or automata)</td>
</tr>
<tr>
<td>CAF</td>
<td>CD8+ T-cell antiviral factor</td>
</tr>
<tr>
<td>CAFISS</td>
<td>Complex Adaptive Framework for Immune System Simulation</td>
</tr>
<tr>
<td>CI</td>
<td>confidence intervals</td>
</tr>
<tr>
<td>CSV</td>
<td>common separated values</td>
</tr>
<tr>
<td>CSW</td>
<td>commercial sex worker</td>
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<tr>
<td>CTL</td>
<td>cytotoxic lymphocyte</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>DPI</td>
<td>days post infection</td>
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<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<tr>
<td>IDU</td>
<td>injecting drug user</td>
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<tr>
<td>IQR</td>
<td>interquartile range</td>
</tr>
<tr>
<td>LLOQ</td>
<td>lower limit of quantification</td>
</tr>
<tr>
<td>MA</td>
<td>Multi-Agent</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean corpuscular volume</td>
</tr>
<tr>
<td>Mo</td>
<td>month(s)</td>
</tr>
<tr>
<td>MEMS</td>
<td>Medication Event Monitoring System</td>
</tr>
<tr>
<td>MMAS</td>
<td>Massively Multi-Agent System</td>
</tr>
<tr>
<td>MSM</td>
<td>Men who have sex with men</td>
</tr>
<tr>
<td>Nab</td>
<td>neutralising antibody</td>
</tr>
<tr>
<td>NNRTI</td>
<td>non-nucleoside reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>ODE</td>
<td>ordinary differential equation</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>PI</td>
<td>protease inhibitor</td>
</tr>
<tr>
<td>PY</td>
<td>person-year(s)</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RTI</td>
<td>reverse transcriptase inhibitor</td>
</tr>
<tr>
<td>VL</td>
<td>(plasma) viral load</td>
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Introduction
Introduction

Acquired Immunodeficiency Syndrome (AIDS) is caused by infection with the Human Immunodeficiency Virus (HIV) which infects CD4 positive T cells leading to their depletion. Since the initial recognition of AIDS in 1981 and the identification of the virus in 1984, work on the interaction of the virus and the immune system has explored the precise mechanisms of pathogenesis of AIDS. The major advance in both treatment and understanding of the interaction of the virus and the immune system came in 1996 with the use of combinational antiretroviral therapy (cART) which demonstrated the rapid turnover of CD4 cells and virus. Subsequent virological, immunological and theoretical work has greatly advanced our detailed understanding of viral dynamics and the mechanisms of pathogenesis. However, further details of viral population biology and the observed behaviour of the virus in treated patients remain to be resolved. This thesis starts from an understanding of CD4 cell activation and viral replication to ask very specific questions about the nature of superinfection and transient viraemia. Having developed a theoretical framework to explore superinfection, we then use this framework to address other questions arising out of observations in a patient cohort.
The aims of this thesis are therefore to explore the relationship between (a) HIV superinfection and HIV disease progression, (b) HIV superinfection and episodes of transient viraemia (commonly known as ‘viral blips’) and (c) drug adherence and viral blips, through mathematical models and computer simulations.

HIV positive individuals may be infected with heterologous strains of HIV after they have seroconverted. Such a phenomenon, known as HIV superinfection, is suspected to be associated with a faster progression to AIDS. However, the mechanism leading to a faster progression is unclear. Viral blips are observed among HIV positive individuals receiving combinational anti-retroviral therapy with previously suppressed viral loads. Questions arise about the nature of these observed increases in viral load: What are these viral blips? What are their causes? Is HIV superinfection one of the causes?

Adherence to cART is an important issue, as inadequate compliance may lead to unsuppressed viral replication and viral rebound, facilitating within-host viral evolution, treatment failure and leading to the emergence of drug-resistant strains. What is the relationship between drug adherence and viral blips? Does the choice of sampling frame – the time between observations of viral load – matter to the observed
incidence of viral blips? How often do you expect viral blips to be observed in a given sampling frame for a given drug adherence?

The evidence for viral superinfection will first be reviewed in Chapter 1, followed by a description and discussion of viral blips and drug adherence patterns as reported in the literature. The development of mathematical models of within-host dynamics of HIV infection will then be reviewed in Chapter 2: agent-based models are described, followed by ordinary differential equation models, as two different approaches that were considered. The model developed in this project is then introduced in Chapter 3, with analytical solutions of the equilibrium values of some variables and the basic reproductive ratio of the virus. Computer simulations of the model and their numerical results will be presented in the subsequent chapters. Chapter 4 studies how superinfection of cells affects the outcome of superinfection of an individual and whether superinfection leads to faster progression to AIDS in the absence and presence of cART. It also addresses whether superinfection leads to viral blips. The impact of the choice of sampling frame and the definition of a viral blip upon the observed viral blip incidence is considered in Chapter 5. This is followed by a study of drug adherence patterns and their impact in Chapters 5 and 6. Variation of the time of taking the prescribed dose, ‘white coat compliance’ and weekend ‘drug holidays’
are also studied. The possibility of inferring patients’ drug adherence from observed viral blip numbers is then investigated. The final discussion (Chapter 7) pulls together the different threads of questions answered in this thesis, and sets them against the wider context of HIV research, with an appreciation of their limitations, to shed light upon possible directions of future work.
Chapter 1

Superinfection, viral blips and drug adherence: a literature review
1. Superinfection, viral blips and drug adherence: a literature review

This chapter first gives a brief overview of HIV progression and antiretroviral therapy (section 1.1) and provides a literature review of recent research on three topics: HIV superinfection (section 1.2), viral blips (section 1.3) and drug adherence (section 1.4).

1.1. Overview of HIV progression and treatment

1.1.1. HIV clinical stages and AIDS

The clinical progress of HIV infection is usually divided into three stages: primary infection, chronic asymptomatic infection and the late stage infection, which is also known as AIDS (Weber, 2001) (Fig 1.1).

Figure 1.1 Typical HIV disease progression

This figure is taken from http://upload.wikimedia.org/wikipedia/commons/a/a4/Hiv-timecourse.png. “Permission is granted to copy, distribute and/or modify this document under the terms of the GNU Free Documentation License, Version 1.2 or any later version published by the Free Software Foundation.”
Within 2 to 6 weeks of infection, patients experienced an elevated viral load (viraemic peak) with a concurrent decrease in CD4 cell count. This is known as primary HIV infection. Within 2 to 4 weeks, the peak viraemia resolves. Both the viral load and the CD4 cell count reached quasi-steady states, and for the former, this is also known as the ‘viral set point’. This chronic infection phase can last for a number of years in the absence of cART, and does not involve manifestation of any symptoms. Viral replication continues rapidly and there is a quick turn-over of activated CD4 cells (Weber, 2001). Poor replication fidelity leading to frequent mutations allows the virus to evade immune control through changing epitopes. Both target cell activation and cytotoxic lymphocyte (CTL) control are believed to play important roles in determining the rate of disease progression. For the majority (70 – 80%) of patients, after 6 to 10 years of infection, the immune system is no longer able to control the disease and AIDS commences. A significant minority (10 – 15%) of patients who are known as ‘rapid progressors’, developed AIDS two or three years following primary infection, while for a minority (5%) of HIV-infected people (‘late progressors’), it takes many more than 10 years before AIDS develops (Langford et al., 2007, Pantaleo and Fauci, 1996). In the absence of cART, the AIDS patient is subject to different
opportunistic infections and was initially expected to live for 14 to 26 months in
industrialised countries (Kitahata et al., 1996). However, as experience increased
cART, those effectively treated seem to have a normal life expectancy.

Historically, before CD4 cell count was widely available, a case definition for AIDS
mainly based on symptoms was used by the World Health Organisation (WHO) and
the Centers for Disease Control and Prevention (CDC) of the United States of
America. It was first developed by the CDC in 1982 and was accepted by the WHO in
December 1985 (World Health Organisation, 1986a). Later the CDC definition was
revised (Centers for Disease Control, 1986, 1987), with a “greater emphasis on HIV
infection status”, and to “include additional indicator diseases, and to accept
presumptive diagnosis of some of the indicator diseases”, this too was subsequently
accepted by the WHO (World Health Organisation, 1988). Earlier in 1986, in order to
facilitate AIDS reporting in non-industrialised countries, a WHO case definition was
devised, known as Bangui definition (World Health Organisation, 1986b), because the
CDC case definitions were designed and appropriated for use in industrialised
countries. Later in 1986, the WHO case definition was formalised and again in 1989,
it was modified to include HIV serological testing. It was further modified in 1994 to
incorporate the 1993 CDC revised definition (cf. World Health Organisation, 2007).
This CDC revised standard incorporated CD4 cell count into the definition. The cut off point for CD4 cell count of AIDS was taken as 200 cells / µl (Centers for Disease Control, 1992). This was carried over to the revised case definition produced by CDC in December 2008 (Schneider et al., 2008). However, a patient is considered as having AIDS if AIDS-defining symptoms develop even if the CD4 cell count is above 200 cells / µl (Schneider et al., 2008). A list of these conditions can be found in Appendix C of Centers for Disease Control (1992). The inclusion of CD4 cell count in the definition of AIDS took into account the fact that the increasing use of antiretroviral therapy prevented seriously immuno-suppressed patients from developing AIDS-definition symptoms. This enabled “AIDS surveillance to reflect more accurately the number of persons with severe HIV-related immunosuppression and those at highest risk for severe HIV-related morbidity” (Centers for Disease Control, 1992).

The current version of the WHO case definition was published in 2007 (World Health Organisation, 2007) and the CDC one in 2008 (Schneider et al., 2008). The current WHO case definition for HIV infection employs serological and / or virological tests (for adults and children 18 months or older). It categorises HIV disease progression into four clinical stages: stage 1, asymptomatic; stage 2, mild symptoms; stage 3, advanced symptoms; and stage 4, severe symptoms. The current WHO case definition
for AIDS involves “presumptive or definitive diagnosis of any stage 3 or stage 4 conditions”, and / or CD4 cell count < 350 cells / µl (for 5 years of age or older)

(World Health Organisation, 2007). The current CDC definition incorporated the AIDS definition into the definition of HIV infection and its progression stages to provide a single case definition: For adults and adolescents, in HIV infection stage 1, CD4 count is ≥ 500 cells / µl; in stage 2, 200-499 cells / µl; and in stage 3 (AIDS), <200 cells / µl (Schneider et al., 2008). It is important to note that the immunological cut-off point for AIDS is different between the two standards: WHO, 350 cells / µl, and CDC, 200 cells / µl.

1.1.2. Causes of HIV progression

How does HIV influence the CD4 cell count? There have been a number of different theories proposed to explain the gradual decline of CD4 cells. Direct killing is the predominate explanation, but others such as indirect killing have also been proposed. Historically, direct killing was more in doubt and theories like the graft-versus-host-like disease (Habeshaw et al., 1992) have been proposed to explain the seemingly latent phase of HIV infection. It was only in 1995 when the discovery of the rapid turn over rate of CD4 cells (Ho et al., 1995, Wei et al., 1995) proved that the virus is

1 If the patient is younger than the age of five, the immunological criteria will be using CD4 cell percentage: <30% (<12 months), <25% (aged 12-35 months), and <20% (aged 36-59 months) (World Health Organisation, 2007).
actively killing CD4 cells even during the chronic phase, that people realised that the virus is not really ‘latent’. The virus load is maintained in a quasi-equilibrium in which activated CD4 cells are rapidly turned-over. Cytotoxic lymphocyte action is believed to be important in modulating disease progression, but the extent of CTL influence was disputed. It was recently shown that CTLs do kill a lot of infected cells (around $10^7$) every day but they are not responsible for the death of majority of infected cells (Asquith et al., 2006).

In recent years, the importance in HIV pathogenesis of the gastro-intestinal tract (GI tract) and the depletion of localised CD4 cells within it has been highlighted. It is known that the GI tract suffers a heavy loss of CD4 cells during primary infection and they are not fully reconstituted even after the commencement of cART. The damage inflicted upon the GI tract, esp. its mucosal tissues, by HIV facilitates the establishment of generalised immune activation by lipopolysaccharide through microbial translocation through the GI tract. This further enhances the availability of target cells for HIV infection. Furthermore, the discovery in recent years of polyfunctional T cells and their correlation with better viral control led to the idea that the quality of T cell response towards HIV infection was important, probably more so than its magnitude (Douek et al., 2009).
### 1.1.3. Antiretroviral therapy

The first antiretroviral drug, zidovudine (AZT), was discovered in 1985 and was first introduced into clinical practice in 1986 (Rachlis, 1990). The first non-nucleoside reverse transcriptase inhibitor (NNRTI), nevirapine, was discovered in 1990 (Merluzzi et al., 1990) and was introduced into clinical practice in 1996. The first protease inhibitor (PI), saquinavir, was introduced into clinical practice in 1995 (Noble and Faulds, 1996). As antiretroviral monotherapy is prone to drug resistance arising very quickly, cART has been the standard treatment for HIV patients in the mid-1990s (Ho, 1996). It was demonstrated in 1998 that combinational antiretroviral therapy (cART) had had a profound effect in reducing morbidity and mortality among HIV patients (Palella et al., 1998).

The 2008 Recommendations of the International AIDS Society – USA Panel (Hammer et al., 2008) were for the commencement of cART for symptomatic patients or those patients who, though asymptomatic, experienced a CD4 cell count < 350 cells / μl. For asymptomatic patients with ≥ 350 cells / μl, such decisions should be ‘individualised’. The drugs commonly used in cART fall into three categories: nucleoside reverse transcriptase inhibitor (NRTI), non-nucleoside reverse transcriptase inhibitor (NNRTI) and protease inhibitor (PI). While NRTI and NNRTI
interrupt viral genome replication, PI prevents infectious virions from being properly assembled. Common combinations for cART involves two NRTIs plus either an NNRTI or a PI (NNRTI-based or PI-based regimens) (Hammer et al., 2008). In most cases, if patients adherence to the prescribed cART regimens, their viral load will be suppressed to <50 copies/ml, a level considered to be ‘undetectable’ by routine viral load measurements. There are also new classes of antiretroviral drugs available in clinical practice, e.g. integrase inhibitors, and cell membrane fusion inhibitors (also known as fusion inhibitors) (Hammer et al., 2008, Hughes et al., 2008), and some others still under investigation, e.g. maturation inhibitors.

1.2. HIV superinfection by heterologous strains

1.2.1. Definitions

The nomenclature for viral ‘double infections’, ‘dual infections’, ‘co-infections’, ‘super-infections’ and ‘re-infections’ needs to be clarified in the first place, so that it is clear what is being described. Double infections are those infections that involve two strains. Within this category, there are four scenarios:

(a) Dual infections are double infections of unknown timing;

(b) Co-infections are double infections where the second infection takes place before seroconversion, i.e. antibodies being detectable in the blood;
(c) Superinfections are double infections where the second infection takes place after seroconversion;

(d) Re-infections are double infections where the second infection takes place after the first infection has been cleared (van der Kuyl and Cornelissen, 2007).

Given that, up to now, HIV has not been cleared from infected individuals, the category of re-infection does not apply here.

Heterologous HIV strains are defined as quasi-species that are not derived from a single ancestor strain through evolution within an individual. One of the criteria to establish double infections is to establish that the two quasi-species detected could not have been evolved from one ancestor strain within an individual. Throughout this thesis, and especially in the description of the mathematical model of superinfection, we will use the term ‘strain’ to distinguish HIV virus that are genetically different enough to exclude the possibility of having evolved from the same ancestor virus in an individual.

1.2.2. Importance

Super-infection with heterologous strains of HIV (referred to simply as ‘HIV superinfection’ hereafter) is of great interest to both clinicians and scientists, as there
had been reports of an association with a more rapid progression to AIDS and death (Gottlieb et al., 2004), and if super-infection with HIV is common, it indicates that immune responses to infection by an HIV strain are not protective against infection by another one, which has serious implications for HIV vaccine development (Smith et al., 2005a). If it can be proven that super-infection with HIV has a negative health consequence, it has consequences for current sexual practice among HIV positive people and public health promotion strategies. The practise of sero-sorting, currently common among men who have sex with men (MSM), cannot prevent super-infection, and it has been argued that protected anal sex should be practised even among sero-concordant relationships (Poudel et al., 2007).

There are a few reviews on HIV-1 super-infection (Allen and Altfeld, 2003, Blackard et al., 2002, Smith et al., 2005a, van der Kuyl and Cornelissen, 2007) that give a good summary of the case reports and cohort studies documenting HIV superinfection and the clinical and public health implications that can be drawn from these studies.

Up to January 2009, there have been at least 40 studies of HIV superinfection in humans (Table 1.1). Studies of HIV superinfection usually involve sequence analysis of HIV strains present in peripheral blood, and the identification of different strains
present in a temporal sequence. This sequence is firmly established through comparison of sequence data of viral samples taken at different times. Differences of sequence that are beyond what could be due to HIV evolution within the human host, and the absence of the second strain in earlier samples, have to be established to exclude the possibilities of within-host evolution from a single infecting strain and co-infection of two or more strains at the same time. Fourteen of these 40 papers are case reports – nine reports of a single superinfection case, two reports of two cases of single superinfection, one report of one dual superinfection case (Pernas et al., 2006), one report of serial superinfection (van der Kuyl et al., 2005), and one report that annuls a previous superinfection claim (Angel et al., 2004). Using such strict criteria to define the occurrence of superinfection, episodes of reinfection with similar strains may be missed. However, this is a moot point since a similar strain is unlikely to differ much biologically from the original infection. As we shall see, only if the quantity of virus differs due to the reinfection could biologically identical strains influence progression, which seems unlikely.

There are a number of questions that need to be addressed:

(a) How often does superinfection take place in a population of HIV positive individuals? i.e. What is the incidence and prevalence of superinfection?
(b) Is superinfection more frequent during certain stages of HIV progression?

(c) Will superinfection lead to faster HIV progression to AIDS?

(d) Is superinfection one of the possible causes of viral blips as observed in HIV positive individuals whose viral loads have been suppressed to an undetected level by cART?

1.2.3. Incidence rate of superinfection

How often does superinfection take place in a population of HIV positive individuals? There is some debate about how frequently super-infection occurs. Some have reported that it is rare, while others have argued that it is common (Table 1.1).

1.2.3.1. Reports where the incidence of superinfection was zero

In a retrospective cohort of 3155 HIV-1 positive individuals in Northern California, no cases of superinfection were identified during 1072 person-years of observation (Gonzales et al., 2003). Similarly, another study suggested that existing infection conferred a 21 - 100% protection against super-infection with an HIV-1 strain of the same subtype. The actual estimated relative risk was 0.0 [95% confidence interval (CI): 0.00-0.79] which is the same as 100% protection (Tsui et al., 2004). In a retrospective study of two cohorts of early HIV-1 seroconverters in France, with a total sample size of 660 individuals, no cases of HIV superinfection were found
(prevalence = 0 [0,0.5%]) (Courgnaud et al., 2007). In Spain, fourteen HIV-seroconcordant couples (i.e. each partner was infected independently with different HIV-1 strains) and two HIV-infected couples where one patient infected his partner were selected as a prospective cohort and were observed for one to four years. No superinfection case was identified (Chakraborty et al., 2004b). In a group of 13 individuals with established, untreated HIV-1 infections, no case of superinfection was identified (Diaz et al., 2005). In the Gladstone study at California, one case of suspected superinfection (but not confirmed) was observed in 46 couples and 34 single individuals out of a total of 243 person-years (379880 episodes of unprotected sexual intercourse) (Grant et al., 2004). No cases of superinfection were identified among 31 recently infected female sex workers in Durban, South Africa, over the course of 24 months of follow-up (Grobler et al., 2004). Among 101 patients who experienced virological failure in the Dutch AIDS Therapy Evaluation Project (ATHENA) cohort, no cases of superinfection were found (Bezemer et al., 2008).

1.2.3.2. Reports where the incidence of superinfection is greater than zero

In contrast to these studies, where the incidence of superinfection was zero, two cases of super-infection among injection drug users in Bangkok, Thailand, were observed during an observation of 92.5 person-years, i.e. an incidence rate of 2.2 per 100
person-years [95% CI: 0.3-7.8] (Hu et al., 2005). In Mombasa, Kenya, three cases of superinfection were identified among 20 HIV positive women in 70 person-years of observation, i.e. an incidence rate of 4.29 cases per 100 person-years of observation (Chohan et al., 2005). Among another 36 high-risk HIV positive women in the same Mombasa cohort (188.7 person-years of observation), seven cases of superinfection were found (an incidence of 3.7 per 100 person-years) (Piantadosi et al., 2007). In a further study of Chohan et al. (2005), two more superinfection cases were identified among 14 of the individuals previously not identified as superinfected (Piantadosi et al., 2008). Taken together in these three studies, 12 cases were observed in a follow-up time of 253.2 total person-years (an incidence of 4.7 per 100 person-years) (Piantadosi et al., 2008). In a study of 78 individuals recently infected with HIV, three cases of superinfection were identified. It was suggested that within six to twelve months of initial infection, the incidence rate of HIV-1 clade B superinfection was 5% (Smith et al., 2004). As a further note, three cases of co-infection were identified among 58 recently infected injecting drug users (IDUs) and one superinfection case was observed in 40 of these 58 individuals where there was a median follow-up of 14.5 months (range, 6-60 months) (Yerly et al., 2004).
1.2.3.3. Other reports of superinfection that do not provide incidence rate

There are reports of superinfection having occurred within a group of individuals studied, but where the incidence rate was not reported since the follow-up time of the cohort was not clearly reported or the studies were not longitudinal (Table 1.1): In a retrospective cohort of 64 HIV positive patients, one case of superinfection and four cases of coinfection were identified (Gottlieb et al., 2004, Gottlieb et al., 2007). In a sub-cohort of 31 patients prospectively followed in the Quebec primary HIV infection cohort, one case of superinfection was identified (Brenner et al., 2004). In a retrospective cohort of 94 HIV positive individuals from a specimen repository at Ohio (plus two clinical cases on suspicion of HIV-1 superinfection), one case of superinfection was identified (Chakraborty et al., 2004a). Out of seven untreated long-term survivors of HIV-1 infections in a cohort of female sex workers in Kenya, one case of superinfection was identified (Fang et al., 2004). Two cases of superinfection were identified among 14 HIV positive individuals who experienced viral blips that were not due to treatment interruption (Jurriaans et al., 2008). Among a group of eight HIV “controllers”, one dual infection (co- or super-infection) was identified (Lamine et al., 2007). Two cases of superinfection were identified among 130 seroconverters who were all IDUs in Bangkok, Thailand (Ramos et al., 2002).
1.2.3.4. Conclusion

There is no consensus on the incidence of HIV superinfection. It appears to lie between zero and two per 100 person-years across the studies published. However, in addition to within-host virus-immune system dynamics, the incidence rate of superinfection is connected with two important factors: (1) HIV prevalence of the population or sub-group therein and (2) frequency of risk behaviours. These together determine the exposure of infected individuals to the risk of acquiring a second infection. It is likely that exposure varies between different groups of individuals who have different risk factors, e.g. commercial sex workers who have more risky sexual behaviours may experience a higher incidence of HIV superinfection than the general public. Furthermore, most of these cohort studies were small in size. A bigger cohort is needed in order to raise the power of the analysis.

1.2.4. Time frame for superinfection and neutralising antibodies

A case-control study of HIV positive individuals with and without HIV superinfection found that individuals who experienced HIV superinfection had no detectable neutralising antibody (NAb) response to heterologous laboratory strains at baseline, and a weaker response to autologous HIV-1, while NAb was detected among those
who were not superinfected (Smith et al., 2006). The results suggest that cross-
protective NAb may play a role in protecting HIV individuals from being 
superinfected. It was also found that compared to individuals with chronic HIV 
infections, acutely infected individuals had a weaker NAb response against both 
autologous virus and heterologous viruses (Deeks et al., 2006). Moreover, in a cohort 
study, among 104 individuals of acute or recent HIV infections, four cases of 
superinfection were identified, while none was identified among 35 chronically 
infected couples. It was also found that those who were superinfected had a weaker 
NAb response (McConnell et al., 2006). Nevertheless, in a recent case-control study 
of 6 superinfected females, the potency and breadth of their NAb responses were 
found to be similar to those found in their matched controls and other chronically 
infectected patients (Blish et al., 2008).

It is now generally believed that HIV superinfection in general takes place in the first 
two years of HIV infection, although the possibility of a later superinfection should 
not be excluded. A recent case report described a dual superinfection (i.e. 
superinfection by two different HIV-1 strains, in other words, a triple infection) and 
recombination within HIV-1 subtype B twelve years after infection by the first strain.
This proves that superinfection can happen many years after infection by the first strain, however low its frequency might be (Pernas et al., 2006).

1.2.5. Superinfection of cells affects outcome of Superinfection

Superinfection of cells has recently been reviewed (Nethe et al., 2005). Whether or not, and to what extent, infected target cells are susceptible to superinfection by a heterologous HIV virus is under study. Among reported cases of HIV superinfection, there were cases in which both the initial and the superinfecting strains co-exist in the individuals (Altfeld et al., 2002, Chohan et al., 2005, Jurriaans et al., 2008, Lamine et al., 2007, Manigart et al., 2004, Piantadosi et al., 2007, Plantier et al., 2004, Ramos et al., 2002, van der Kuyl et al., 2005, Yerly et al., 2004), while there have also been cases of displacement of the first strain by the second (superinfecting) strain in the individuals (Brenner et al., 2004, Chohan et al., 2005, Gottlieb et al., 2007, Jost et al., 2002, Jurriaans et al., 2008, Koelsch et al., 2003, McCutchan et al., 2005, Piantadosi et al., 2007, Piantadosi et al., 2008, Rachinger et al., 2008, Smith et al., 2005b, Yang et al., 2005, Yerly et al., 2004). In addition, there were also cases in which a transient viraemia of the second strain was observed and then disappeared soon afterwards (Chakraborty et al., 2004a) Pernas et al., 2006, Yerly et al., 2004). In a recent paper (Piantadosi et al., 2007), it was reported that replacement of the first strain by the
superinfecting strain is associated with a great increase in viral load (two of seven cases), while co-existence is associated with no significant changes in viral load (five of seven cases). The study suggested a role of viral fitness in determining the clinical consequences of superinfection: a much fitter superinfecting strain will replace the first strain and lead to a higher viral load while a superinfecting strain with similar fitness will lead to co-existence and no changes in viral load. If a superinfecting strain is much less fit than the established strain, it will fail to establish itself in the host. Of course, superinfection of cells could lead to the emergence of a recombinant strain as in a recent case report where the recombination took place at two of the immunodominant CD8 epitopes that were targeted before superinfection (Streeck et al., 2008).

1.2.6. Superinfection and progression to AIDS

Early reports of HIV superinfection suggested that HIV superinfection may hasten the progression to the onset of AIDS (Jost et al., 2002, Gottlieb et al., 2004). This suggestion is usually based on the temporal association between superinfection events and increased viral loads. Temporal association between high viral load and superinfection event has been observed in a number of cases (Altfeld et al., 2002, Brenner et al., 2004, Chakraborty et al., 2004a, Chohan et al., 2005, Gottlieb et al., 2004, Gottlieb et al., 2007, Jost et al., 2002, Jurriaans et al., 2008, Koelsch et al., 2003,
Manigart et al., 2004, McCutchan et al., 2005, Pernas et al., 2006, Piantadosi et al., 2007, Rachinger et al., 2008a, Ramos et al., 2002, Smith et al., 2006, Smith et al., 2004, Smith et al., 2005b, Streeck et al., 2008, van der Kuyl et al., 2005, Yang et al., 2005, Yerly et al., 2004). While, as it was noted before (Smith et al., 2005a), some cases might be associated with recent treatment interruptions (Altfeld et al., 2002, Chakraborty et al., 2004a, Jost et al., 2002, Pernas et al., 2006, Smith et al., 2005b), a number of superinfection events happened in the absence of cART (Brenner et al., 2004, Casado et al., 2007, Chohan et al., 2005, Fang et al., 2004, Gottlieb et al., 2007, Grobler et al., 2004, Hu et al., 2005, Jurriaans et al., 2008, Koelsch et al., 2003, Lamine et al., 2007, Piantadosi et al., 2007, Piantadosi et al., 2008, Plantier et al., 2004, Rachinger et al., 2008, Ramos et al., 2002, Smith et al., 2006, Smith et al., 2004, van der Kuyl et al., 2005, Yang et al., 2005, Yerly et al., 2004). The increase in viral load associated with a superinfection event may be a consequence of ‘viral burst’ as seen in primary infection where the virus has not yet been controlled by the immune system. Given that a high viral load is an indicator for faster progression to AIDS, these patients were predicted to experience an accelerated disease progression. However, this is not always the case. There have also been cases where the superinfection event took place without any significant changes in viral load (Casado et al., 2007, Chohan et al., 2005, Piantadosi et al., 2007, Plantier et al., 2004, van der
Kuyl et al., 2005). In a case of two consecutive superinfection events in an individual (van der Kuyl et al., 2005), in which the first superinfection event was intra-clade, no clinical symptoms or decline of CD4 cell count were reported, whereas for the second superinfection that was inter-clade, the manifestation of clinical symptoms, a significant increase in viral load, and a decrease in CD4 cell count were observed, suggesting that the genetic and hence antigenic distance between viral types determines their ability to escape from immune control.

However, recent studies of long-term survivors of HIV found that superinfection could have happened without hastening the rate of disease progression (Casado et al., 2007, Lamine et al., 2007). There is a recent report of an ‘elite controller’ whose viraemia was once again under control even though the same superinfecting strain led to more progressive disease in two other individuals (Rachinger et al., 2008); this is evidence that host factors play an important role in determining clinical outcomes of superinfection and it raises the important question of what host factors enable the elite controller to control the superinfecting strains. There have been discussions of the problem of reverse causality. Is HIV superinfection leading to a faster progression to AIDS? Or, are individuals with a weaker immune response (esp. neutralising antibody response) more susceptible to HIV superinfection (van der Kuyl and Cornelissen,
2007)? This is a question that has yet to be answered and the study design which could distinguish between the two would need prospective sampling and analysis to identify the temporal sequence of superinfection and immune decline.
<table>
<thead>
<tr>
<th>Study</th>
<th>Study genre</th>
<th>Sample size</th>
<th>No. of case of super-infection</th>
<th>Time to super-infection since 1st infection/incidence/prevalence</th>
<th>Follow-up Time</th>
<th>HIV sub-types</th>
<th>Co-existence/displacement after superinfection/transient 2nd strain viraemia?</th>
<th>Risk</th>
<th>On cART?</th>
<th>Accelerated disease progression after superinfection</th>
<th>Drug resistance (DR) or drug sensitive (DS)</th>
<th>Clinical trial?</th>
<th>Region/Country</th>
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<tbody>
<tr>
<td>Altfeld et al. (2002)</td>
<td>Case report</td>
<td>n/a</td>
<td>1</td>
<td>&lt;32 Mo</td>
<td>~ 4 years</td>
<td>1st: B; 2nd: B</td>
<td>Co-existence but 2nd strain dominated after superinfection</td>
<td>MSM</td>
<td>Yes</td>
<td>Supervised treatment interruption</td>
<td>Not reported</td>
<td>Supervised treatment interruption</td>
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<td>Angel et al. (2004)</td>
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<td>n/a</td>
<td>0</td>
<td>n/a</td>
<td>~ 10 years</td>
<td>n/a</td>
<td>MSM</td>
<td>n/a</td>
<td>n/a</td>
<td>DS</td>
<td>No</td>
<td>Ottawa, Canada, Netherlands</td>
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<td>Bezemer et al. (2008)</td>
<td>Retrospective cohort</td>
<td>101</td>
<td>0</td>
<td>Incidence = 0</td>
<td></td>
<td>n/a</td>
<td>MSM</td>
<td>n/a</td>
<td>n/a</td>
<td>DR (virological failure)</td>
<td>No</td>
<td>Montreal, Quebec, Canada</td>
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<td>Blish et al. (2008)</td>
<td>Follow-up immunological study of Chohan et al., 2005, and Pastradossi et al., 2007, 2008</td>
<td>n/a</td>
<td>56 previously studied</td>
<td>n/a</td>
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<td>n/a</td>
<td>CSW</td>
<td>No</td>
<td>n/a</td>
<td>n/a</td>
<td>Montreal, Quebec, Canada</td>
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<tr>
<td>Brenner et al. (2004)</td>
<td>Retrospective cohort</td>
<td>31</td>
<td>1</td>
<td>10 Mo</td>
<td>2 years or more</td>
<td>1st: B; 2nd: B</td>
<td>Displacement</td>
<td>MSM</td>
<td>No</td>
<td>1st: DR; 2nd: DR</td>
<td>No</td>
<td>Montreal, Quebec, Canada</td>
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<tr>
<td>Casado et al. (2007)</td>
<td>Case report</td>
<td>n/a</td>
<td>2 (or 1)</td>
<td>20 or more</td>
<td></td>
<td>1st: B; 2nd: B</td>
<td>IDU</td>
<td>No</td>
<td>long-term, nonprogressive (i.e. remained asymptomatic without ART and with CD4 levels &gt;500 cells/mL)</td>
<td>Not reported</td>
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<tr>
<td>Chakraborty et al. (2004a)</td>
<td>Retrospective cohort</td>
<td>94+2</td>
<td>1</td>
<td>Not reported</td>
<td>~ 7 years</td>
<td>1st: B; 2nd: B</td>
<td>Transient viraemia of superinfecting strains</td>
<td>MSM</td>
<td>Yes</td>
<td>1st: DR; 2nd: DR</td>
<td>No</td>
<td>USA (a)</td>
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<tr>
<td>Study</td>
<td>Study genre</td>
<td>Sample size</td>
<td>No. of case of super-infection</td>
<td>Time to super-infection since 1st infection/incidence/prevalence</td>
<td>Follow-up Time</td>
<td>HIV sub-types</td>
<td>Co-existence/displacement after superinfection/ transient 2nd strain viraemia?</td>
<td>Risk</td>
<td>On cART?</td>
<td>Accelerated disease progression after super-infection</td>
<td>Drug resistance (DD) or drug sensitive (DS)</td>
<td>Clinical trial?</td>
<td>Region/Country</td>
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<tr>
<td>Chakraborty et al. (2004b)</td>
<td>Prospective cohort</td>
<td>14</td>
<td>0</td>
<td>n/a</td>
<td>1 – 4 years</td>
<td>Not reported</td>
<td>n/a</td>
<td>14 heterosexual couples (7 couples also IDU); two MSM couples</td>
<td>Yes</td>
<td>Yes</td>
<td>n/a</td>
<td>No</td>
<td>Hospital Carlos III, Madrid, Spain</td>
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<tr>
<td>Chohan et al. (2005)</td>
<td>Prospective cohort</td>
<td>20</td>
<td>3</td>
<td>355 days post-infection (on average)</td>
<td>70 PY</td>
<td>Not reported</td>
<td>1st: Dr, 2nd: A; 1st: C; 2nd: C/A; 1st: D; 2nd: A; Mostly B in the cohort</td>
<td>Co-existence: Displacement; Co-existence</td>
<td>CSW</td>
<td>No</td>
<td>No</td>
<td>Not reported</td>
<td>No</td>
</tr>
<tr>
<td>Cornelissen et al. (2007)</td>
<td>Retrospective cohort</td>
<td>37</td>
<td>1 (and 15 other dual infections)</td>
<td>For the superinfection case report, see van der Kayl (2005) prevalence = 0 [0, 0.5%]</td>
<td>Not reported</td>
<td>95% SEROCO cohort: B; 77% of PRIMO cohort: B</td>
<td>n/a</td>
<td>n/a</td>
<td>Yes</td>
<td>n/a</td>
<td>n/a</td>
<td>No</td>
<td>Amsterdam, the Netherlands.</td>
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<tr>
<td>Courgnaud et al. (2007)</td>
<td>Retrospective cohort</td>
<td>660</td>
<td>0</td>
<td>(2003) prevalence = 0 [0, 0.5%]</td>
<td>7 – 8 years</td>
<td>Not reported</td>
<td>Most B; one C</td>
<td>9: IDU; 4: transfusion (2: IDU; 1: haemophilia)</td>
<td>CSW</td>
<td>No</td>
<td>No</td>
<td>n/a</td>
<td>No</td>
</tr>
<tr>
<td>Diaz et al. (2004)</td>
<td>Retrospective cohort</td>
<td>13</td>
<td>0</td>
<td>n/a</td>
<td>Not reported</td>
<td>Most B; one C</td>
<td>n/a</td>
<td>9: IDU; 4: transfusion (2: IDU; 1: haemophilia)</td>
<td>CSW</td>
<td>No</td>
<td>Yes (CD4 count rapid decline)</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Fang et al. (2004)</td>
<td>Retrospective cohort</td>
<td>7</td>
<td>1</td>
<td>unsure, at least 1 year [range 1 – 12 years]</td>
<td>14 years</td>
<td>1st: A; 2nd: C; resulting in an A/C recombinant, recombinant strain dominant after superinfection</td>
<td>n/a</td>
<td>n/a</td>
<td>-</td>
<td>n/a</td>
<td>No</td>
<td>No</td>
<td>Nairobí, Kenya</td>
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<tr>
<td>Goncales et al. (2003)</td>
<td>Retrospective cohort</td>
<td>718</td>
<td>0</td>
<td>n/a</td>
<td>1072 PY</td>
<td>approx. 99% of isolates: B 1st: B; 2nd: B</td>
<td>n/a</td>
<td>n/a</td>
<td>-</td>
<td>n/a</td>
<td>No</td>
<td>No</td>
<td>California, USA, Multi-countries (b)</td>
</tr>
<tr>
<td>Gottlieb et al. (2004)</td>
<td>Retrospective cohort</td>
<td>64</td>
<td>1</td>
<td>1.3 years</td>
<td>up to 15 years</td>
<td>Ditro</td>
<td>Displacement</td>
<td>Super-infection before ART</td>
<td>Ditro</td>
<td>Ditro</td>
<td>Ditro</td>
<td>No</td>
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<td>Gottlieb et al. (2007)</td>
<td>Retrospective cohort</td>
<td>n/a</td>
<td>Follow-up of Gottlieb et al. (2004) 0 days after seroconversion n/a</td>
<td>0.8 to 1.3 years after seroconversion</td>
<td>3.3 years</td>
<td>Ditro</td>
<td>Displacement</td>
<td>Super-infection before ART</td>
<td>Ditro</td>
<td>Ditro</td>
<td>Ditro</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Guent et al. (2004)</td>
<td>Prospective cohort</td>
<td>28</td>
<td>(c)</td>
<td>(d)</td>
<td>(d)</td>
<td>MSM or heterosexual couples</td>
<td>CSW</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>No</td>
<td>No</td>
<td>North Carolina, USA, South Africa</td>
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<td>Gobber et al. (2004)</td>
<td>Longitudinal/prospective cohort</td>
<td>31</td>
<td>0 (6 co-infections)</td>
<td>n/a (e)</td>
<td>25 of 31 for 18-24 Mo</td>
<td>C (all 31 CSWs)</td>
<td>n/a</td>
<td>Microbiocide phase 3 trial</td>
<td>Ditro</td>
<td>Ditro</td>
<td>Ditro</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Study</td>
<td>Study genre</td>
<td>Sample size</td>
<td>No. of case of super-infection</td>
<td>Time to super-infection since 1&lt;sup&gt;st&lt;/sup&gt; infection/ incidence/ prevalence</td>
<td>Follow-up Time</td>
<td>HIV sub-types</td>
<td>Co-existence/ displacement after superinfection/transient 2&lt;sup&gt;nd&lt;/sup&gt; strain viraemia?</td>
<td>Risk</td>
<td>On cART?</td>
<td>Accelerated disease progression after super-infection</td>
<td>Drug resistance (DR) or drug sensitive (DS)</td>
<td>Clinical trial?</td>
<td>Region/Country</td>
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<tr>
<td>Hu et al. (2005)</td>
<td>Prospective cohort</td>
<td>126</td>
<td>2</td>
<td>Incidence = 2.2 per 100 PY [95% CI, 0.3-7.8]</td>
<td>92.5 PY</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;; 2&lt;sup&gt;nd&lt;/sup&gt;; CRF01 AE; 1&lt;sup&gt;st&lt;/sup&gt;; CRF01 AE; 2&lt;sup&gt;nd&lt;/sup&gt;; B</td>
<td>Not reported</td>
<td>IDU</td>
<td>No (f)</td>
<td>Not reported</td>
<td>Not reported</td>
<td>No</td>
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<td>Jost et al. (2002)</td>
<td>Case report</td>
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<td>&lt;28 Mo</td>
<td>~ 3 years</td>
<td>Displacement</td>
<td>MSM</td>
<td>Yes, intermittent</td>
<td>Yes</td>
<td>Not reported</td>
<td>No</td>
<td>Europe/Brazil/Amsterdam, Netherlands</td>
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<tr>
<td>Harriens et al. (2008)</td>
<td>Case report</td>
<td>n/a</td>
<td>Retrospective cross-sectional</td>
<td>14</td>
<td>Displacement</td>
<td>MSM and 1 heterosexual female</td>
<td>Yes for MSM and 1 heterosexual female</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<td>Koelsch et al. (2003)</td>
<td>Case report</td>
<td>n/a</td>
<td>1</td>
<td>&lt;4 Mo</td>
<td>12 Mo</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;; B; 2&lt;sup&gt;nd&lt;/sup&gt;; B</td>
<td>Displacement</td>
<td>MSM</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>California, USA.</td>
<td></td>
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<td>Kozaczynska et al. (2007)</td>
<td>Follow-up of van der Kuyl et al. (2005)</td>
<td>-</td>
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<td>No</td>
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<td>Lamine et al. (2007)</td>
<td>Case report</td>
<td>1</td>
<td>8</td>
<td>Undetermined</td>
<td>&gt; 10 years</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;; B; 2&lt;sup&gt;nd&lt;/sup&gt;; B</td>
<td>Co-existence</td>
<td>MSM</td>
<td>No</td>
<td>No (HIV controllers)</td>
<td>No</td>
<td>No</td>
<td>Paris, France</td>
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<td>Manigart et al. (2004)</td>
<td>Retrospective study in a prospective cohort</td>
<td>147</td>
<td>2</td>
<td>Not reported</td>
<td></td>
<td>1&lt;sup&gt;st&lt;/sup&gt;; CRF02 AG; 2&lt;sup&gt;nd&lt;/sup&gt;; a divergent virus not significantly related any known sub-type</td>
<td>Co-existence</td>
<td>CSW</td>
<td>Not reported</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Bobo-Dioulasso, Burkina Faso.</td>
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<tr>
<td>Case 1</td>
<td>30 Mo</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;; CRF02 AG; 2&lt;sup&gt;nd&lt;/sup&gt;; CRF01 AG</td>
<td>Co-existence</td>
<td></td>
<td>Co-existence</td>
<td></td>
<td></td>
<td>No (f)</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td></td>
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<tr>
<td>Case 2</td>
<td>12 Mo</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;; CRF02 AG; 2&lt;sup&gt;nd&lt;/sup&gt;; CRF06-cpx</td>
<td>Co-existence</td>
<td></td>
<td>Co-existence</td>
<td></td>
<td></td>
<td>No (f)</td>
<td>No</td>
<td>No</td>
<td>No</td>
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<td>Study</td>
<td>Study genre</td>
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<td>Time to super-infection since 1st infection/ incidence/ prevalence</td>
<td>Follow-up Time</td>
<td>HIV sub-types</td>
<td>Co-existence / displacement after superinfection / transient 2nd strain viraemia?</td>
<td>Risk</td>
<td>On cART?</td>
<td>Accelerated disease progression after super-infection</td>
<td>Drug resistance (DR) or drug sensitive (DS)</td>
<td>Clinical trial?</td>
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<td>McCutchan et al. (2005)</td>
<td>Case report (in a cohort)</td>
<td>n/a</td>
<td>1</td>
<td>3 -- 9 Mo</td>
<td>30 Mo</td>
<td>1*: ACD; 2*: AC (g)</td>
<td>Displacement by superinfecting strain and recombinants of initial and superinfecting strain</td>
<td>female bar workers</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td>No</td>
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<td>Pernas et al. (2006)</td>
<td>Case report</td>
<td>n/a</td>
<td>1</td>
<td>~ 12 years</td>
<td>10 years (b)</td>
<td>1*: B; 2*: 2 B strains</td>
<td>Transient viraemia of superinfecting strains</td>
<td>IDU/ heterosexual</td>
<td>On ART since 1996</td>
<td>Not reported</td>
<td>1*: DS; 2*: (DS + DR) (i)</td>
<td>No</td>
<td>Madrid, Spain</td>
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<td>Piantadosi et al. (2007)</td>
<td>Prospective cohort</td>
<td>36</td>
<td>7</td>
<td>Incidence=3.7% per person-year</td>
<td>188.7 person-years</td>
<td>1*: A-D recombinant: env (A) / gag (D); 2*: A</td>
<td>Co-existence</td>
<td>CSW</td>
<td>No; No ART was reported</td>
<td>Not reported</td>
<td></td>
<td>No</td>
<td>Mombasa, Kenya</td>
</tr>
<tr>
<td>Case 1</td>
<td>749-1031 DPI (days post-infection)</td>
<td></td>
<td></td>
<td>1*: A-D recombinant: env (A) / gag (D); 2*: A</td>
<td></td>
<td>1*: A-D recombinant: env (A) / gag (D); 2*: A</td>
<td>Co-existence</td>
<td>CSW</td>
<td>No; No ART was reported</td>
<td>Not reported</td>
<td></td>
<td>No</td>
<td>Mombasa, Kenya</td>
</tr>
<tr>
<td>Case 2</td>
<td>1680-2048 DPI</td>
<td></td>
<td></td>
<td>1*: D/A2 recombinant: env (A2) / gag(D/A2); 2*: A1</td>
<td></td>
<td>1*: D/A2 recombinant: env (A2) / gag(D/A2); 2*: A1</td>
<td>Recombinant in gag region between initial and superinfection strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>Mombasa, Kenya</td>
</tr>
<tr>
<td>Case 3</td>
<td>52-73 DPI</td>
<td></td>
<td></td>
<td>1*: A; 2*: D</td>
<td></td>
<td>1*: A; 2*: D</td>
<td>1st strain predominant; recombinant in env region between initial and superinfection strain detected twice (4.5 yr apart)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>Mombasa, Kenya</td>
</tr>
<tr>
<td>Case 4</td>
<td>1832-1957 DPI</td>
<td></td>
<td></td>
<td>1*: A; 2*: C</td>
<td></td>
<td>1*: A; 2*: C</td>
<td>Displacement; recombinant gag (A) and env (C).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>Mombasa, Kenya</td>
</tr>
<tr>
<td>Case 5</td>
<td>714-1007 DPI</td>
<td></td>
<td></td>
<td>1*: A; 2*: A</td>
<td></td>
<td>1*: A; 2*: A</td>
<td>Recombinant: gag (1st strain); env (2nd strain)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>Mombasa, Kenya</td>
</tr>
<tr>
<td>Case 6</td>
<td>303-1453 DPI</td>
<td></td>
<td></td>
<td>1*: A; 2*: A</td>
<td></td>
<td>1*: A; 2*: A</td>
<td>Displacement; recombinant (no 2nd strain in env)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>Mombasa, Kenya</td>
</tr>
<tr>
<td>Case 7</td>
<td>58-152 DPI</td>
<td></td>
<td></td>
<td>1*: C/A recombinant: env (A) / gag (C); 2*: A</td>
<td></td>
<td>1*: C/A recombinant: env (A) / gag (C); 2*: A</td>
<td>Recombinant at gag; 2nd strain not detected at env.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td>Mombasa, Kenya</td>
</tr>
<tr>
<td>Study</td>
<td>Study genre</td>
<td>Sample size</td>
<td>No. of case super-infection</td>
<td>Time to super-infection since 1st infection/ incidence/ prevalence</td>
<td>Follow-up Time</td>
<td>HIV subtypes</td>
<td>Co-existence / displacement after superinfection/ transient 2nd strain viraemia?</td>
<td>Risk</td>
<td>On cART?</td>
<td>Accelerated disease progression after super-infection</td>
<td>Drug resistance (DR) or drug sensitive (DS)</td>
<td>Clinical trial?</td>
<td>Region/Country</td>
</tr>
<tr>
<td>-------</td>
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<td>---------------------------------------------------------------</td>
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<td>-----------------</td>
<td>---------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Plantadosi et al. (2006)</td>
<td>Case report</td>
<td>2</td>
<td>14 of 21, previously not detected in Chohan et al. (2005)</td>
<td>1046-1487 DPI</td>
<td>19 Mo (j)</td>
<td>1st: D; 2nd: A</td>
<td>Displacement</td>
<td>No</td>
<td>Yes (increase viral load)</td>
<td>Not reported</td>
<td>No</td>
<td>Mombasa, Kenya</td>
<td></td>
</tr>
<tr>
<td>Plantier et al. (2004)</td>
<td>Case report</td>
<td>n/a</td>
<td>1</td>
<td>341-449 DPI</td>
<td>3 years</td>
<td>1st: A; 2nd: A; 1st: HIV-1 O highly divergent variant 2nd: HIV-1 M(CFR02_AG)</td>
<td>Displacement</td>
<td>No</td>
<td>Yes (diato)</td>
<td>Not reported</td>
<td>No</td>
<td>France (Cameronian origin)</td>
<td></td>
</tr>
<tr>
<td>Rachinger et al. (2008), Ramos et al. (2002)</td>
<td>Case report</td>
<td>n/a</td>
<td>1</td>
<td>at least 14 years</td>
<td>Not reported 3 years</td>
<td>1st: B; 2nd: B</td>
<td>Displacement</td>
<td>MSM</td>
<td>No</td>
<td>Elite controller</td>
<td>Not reported</td>
<td>No</td>
<td>Netherlands</td>
</tr>
<tr>
<td>Case report</td>
<td>n/a</td>
<td>130</td>
<td>in a prospective cohort</td>
<td>&lt;3 Mo</td>
<td>1st: CRF01_AE; 2nd: B; 1st: B; 2nd: CRF01_AE</td>
<td>Co-existence</td>
<td>IDU</td>
<td></td>
<td>Yes</td>
<td>Not reported</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith et al. (2004)</td>
<td>Case report</td>
<td>n/a</td>
<td>8</td>
<td>1st: C; 2nd: C</td>
<td>min. 19 Mo</td>
<td>1st: B; 2nd: B</td>
<td>Displacement</td>
<td>MSM</td>
<td>Yes (intermittent adherence)</td>
<td>Yes</td>
<td>1st: DS; 2nd: DR.</td>
<td>Yes</td>
<td>AIDS Clinical Trials Group 5095</td>
</tr>
<tr>
<td>Smith et al. (2005b)</td>
<td>Case report</td>
<td>n/a</td>
<td>130</td>
<td>&lt;11 Mo</td>
<td>B</td>
<td>Co-existence</td>
<td></td>
<td></td>
<td></td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smith et al. (2005b)</td>
<td>Retrospective cohort</td>
<td>78</td>
<td>3; see Smith et al. (2006)</td>
<td>Incidence ~ 5% within 6 – 12 Mo of initial infection</td>
<td>~1 year</td>
<td>B</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
<td>No</td>
<td></td>
<td>California, USA</td>
</tr>
<tr>
<td>Smith et al. (2006)</td>
<td>Case control</td>
<td>n/a</td>
<td>3 cases (same as Smith et al., 2004a; 11 controls S1 S2 S3)</td>
<td>&lt;1.5 year</td>
<td>-</td>
<td>-</td>
<td>Not reported</td>
<td>Not reported</td>
<td>Not reported</td>
<td></td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shreek et al. (2008)</td>
<td>Case report</td>
<td>n/a</td>
<td>1</td>
<td>1160 days</td>
<td>Not reported</td>
<td>Recombination in Gag and Env</td>
<td>Not reported</td>
<td>No</td>
<td>No</td>
<td>No (probably no from the report)</td>
<td>Yes</td>
<td>No</td>
<td>Boston, USA</td>
</tr>
<tr>
<td>Study</td>
<td>Study genre</td>
<td>Sample size</td>
<td>No. of cases of superinfection</td>
<td>Time to superinfection since 1st infection/ incidence/ prevalence</td>
<td>Follow-up Time</td>
<td>HIV subtypes</td>
<td>Co-existence/ displacement after superinfection/ transient 2nd strain viraemia?</td>
<td>Risk</td>
<td>On cART?</td>
<td>Accelerated disease progression after superinfection</td>
<td>Drug resistance (DR) or drug sensitive (DS)</td>
<td>Clinical trial?</td>
<td>Region/ Country</td>
</tr>
<tr>
<td>-----------------------------</td>
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<td>-----------------</td>
</tr>
<tr>
<td>Tsui et al. (2004)</td>
<td>Retrospective cohort</td>
<td>37</td>
<td>0</td>
<td>Incidence = 0</td>
<td>215 PY</td>
<td>Not reported, likely to be B. n/a</td>
<td>mainly IDU</td>
<td>n/a</td>
<td>&lt;5% of the total follow-up time taking HARRT</td>
<td>n/a</td>
<td>Not reported</td>
<td>No</td>
<td>San Francisco Bay area, California, USA</td>
</tr>
<tr>
<td>van der Kuyl et al. (2005)</td>
<td>Case report</td>
<td>n/a</td>
<td>1</td>
<td>16-20 Mo</td>
<td>-31-32 Mo</td>
<td>1st: B, 2nd: B, 3rd: CRF01_AE</td>
<td>Co-existence MSM</td>
<td>Not reported; probably no No</td>
<td>Not reported (m)</td>
<td>Not reported</td>
<td>No</td>
<td>The Netherlands</td>
<td></td>
</tr>
<tr>
<td>Yang et al. (2005)</td>
<td>Case report</td>
<td>n/a</td>
<td>1</td>
<td>&lt;5 Mo</td>
<td>&lt;12 Mo</td>
<td>1st: B, 2nd: B</td>
<td>Displacement MSM</td>
<td>Yes; increased viral load</td>
<td>1st: DR; 2nd: DS</td>
<td>No</td>
<td>Los Angeles, California, USA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yarly et al. (2004)</td>
<td>Retrospective cohort</td>
<td>40 of 55</td>
<td>patients with recent infection</td>
<td>18-24 Mo</td>
<td>156 IDUs</td>
<td>1st: CRF-11; 2nd: B</td>
<td>Transient viraemia of superinfecting strain</td>
<td>IDU</td>
<td>No</td>
<td>No</td>
<td>Not reported</td>
<td>No</td>
<td>Switzerland</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 out of 156 IDUs</td>
<td>156 IDUs followed for a total of 346 PY</td>
<td>~36 Mo</td>
<td></td>
<td>Co-existence IDU</td>
<td>Yes; increased viral load, decreased CD4 count</td>
<td>Not reported</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: (a) Samples from the specimen repository at the Center for AIDS Research, Case Western Reserve University, Cleveland, Ohio, USA. (b) The multicentre AIDS cohort study, the Seattle primary infection cohort, and the South African female sex worker cohort. (c) 33 seroconcordant couples were analysed at baseline, of which 28 had distinguishable viruses in spite of having reported frequent unprotected sex with enrollment partners. Of the other five couples who had indistinguishable virus at baseline, three reported infection histories that suggested of transmission within the relationship while two might experience superinfection prior to enrolment that could not be ruled out. 32 singles were also studied. (d) Couples: Prospective: 58 persons-years (3725 episodes of unprotected anal or vaginal intercourse) Retrospective: 160 persons-years (37341 episodes) Singles: Prospective: 24 persons-years (2744 episodes) Grand Total: 243 persons years (379880 episodes). (e) Dualy infected in the first 3 months: 6 (19%), but no evidence of superinfection was detected over the course of 24 months of follow-up. (f) No ART was reported for any of the seroconvertors during the 1st year following seroconversion (1995-98). (g) AC recombinant after a complex ACD recombinant. (h) Follow-up from Oct 1994 to Oct 2003. (i) Clones belonging to the main A group did not show any resistance mutation except for the April 1999 sample in which the mutation M184V was detected in all clones after a 3-month period of lamivudine (3TC) + stavudine (d4T) + nelfinavir (NFV) treatment. Virus B showed in all clones analyzed the azidothymidine (AZT) resistance mutation T215Y together with the M184V, 3TC-associated resistance mutation. The other superinfecting virus (strain C) presented the V179D polymorphism that was not present in the other 2 viruses. The distinct mutation patterns displayed by the 3 viruses supported the identification of 3 different strains in the patient. (j) Diagnosed in 1999. Routine monitoring between Oct 2002 (month 0) and May 2004 (month 19). (k) Time to first superinfection: At least 16-20 Mo. (m) No symptoms when superinfected with a second B-strain, but clinical symptoms when superinfected with CRF01_AE strain. *PRIMO cohort participants were HIV-positive patients of whom the vast majority were infected via heterosexual or homosexual contacts (Goujard et al., 2001). SEROCO cohort participants include HIV-positive patients infected via homosexual or heterosexual contacts, blood transfusion and intravenous drug use (Carre et al., 1996; Hubert et al., 2000). § MSM (n=68); hetero-sexual (n=21); IDU (n=6); transfusion (n=2); unknown (n=4). ‡ Reported use of a syringe previously used by another IDU (n=2; 79.6%); MSM (n=1; 30.8%); received money or drugs in exchange for sex (n=15; 4.1%).

CSW: (female) commercial sex worker(s); DPI: days post infection; DR: drug resistance; DR: drug sensitive; IDU: intravenous drug user(s); Mo: months; MSM: men who have sex with men; n/a: Not applicable; PY: person year(s).
1.3. **Episodes of transient viraemia (viral blips)**

1.3.1. **What are viral blips?**

A viral blip is defined as a single, low-level, plasma viral load (VL) measurement of > 50 copies/ml after successful suppression of viral load below detectable level and followed by undetectable viral load measurements (Gallant, 2007). Viral blips have been suggested as measurement artefacts (Stosor et al., 2005) or as random biological and statistical variation around mean HIV level (Nettles et al., 2005). Stosor et al. (2005) found that a higher incidence of viral blips was observed using plasma preparation tubes (PPTs) than EDTA tubes. Previous observation in a cross-sectional assessment in a clinical trial also found large differences in HIV ribonucleic acid (RNA) levels between these two tubes. These suggest that at least some viral blips were products of measurement errors using PPTs (Squires et al., 2004). Nettles et al. (2005) closely monitored ten patients (with VL measurements every two or three days) and found no factors that are statistically significantly associated with viral blips. However, Percus et al. (2003) found that such blips cannot be explained solely by assay variation. They examined the dynamics of viral blips in 123 patients receiving cART monitored for a mean of 2.6 years and found that no common probability distribution of blip amplitude is shared among them. Further analyses of the frequency and amplitude of blip distributions in these 123 patients suggested that for two blips less than 22 days apart, there is a significant possibility that both belong to a single episode of viraemia (Di Mascio et al., 2003). These intermittent episodes had common amplitude profiles and the VL decay from the peak of a blip might follow two phases (Di Mascio et al., 2004b). In a subsequent analysis of the time series of
blips from these 123 patients, it was found that such a transient episode of viraemia on average lasted for three weeks (Di Mascio et al., 2005).

While the importance of consistency in measurement procedures when comparison across studies is made was highlighted by Stosor et al. (2005), the findings of Nettles et al. (2005) are compatible with that of Percus et al. (2003) and Di Mascio et al. (2003, 2004b, 2005). What they represent may be two tiers of blips with two different underlying causes: one of random variations around a mean VL and another of ‘genuine’ increase in VL as a result of other factors. The frequency of VL sampling matters, as the inherent stochasticity of VL will only be observed via frequent sampling (every two or three days) but not in a sampling frame similar to that of clinical routine (e.g. every three months).

1.3.2. With what factors are viral blips associated?

While some have argued that viral blips are of little clinical significance (Lee et al., 2006), some have raised genuine clinical concern. It is well established that a low viral load below detectable level is associated with a longer period of virologic suppression (Raboud et al., 1998, Raboud et al., 1999). An association between viral blips and increased risk of virological failure were previously identified in some studies (Easterbrook et al., 2002, Greub et al., 2002, Masquelier et al., 2005), but were absent in others (Cohen Stuart et al., 2001, Garcia-Gasco et al., 2008, Havlir et al., 2001, Macias et al., 2005, Martinez et al., 2005, Mira et al., 2002, Nettles et al., 2005, Podsadecki et al., 2007, Sklar et al., 2002, Sungkanuparph et al., 2005) (Table 1.3). Havlir et al. (2001) identified an association between viral blips and higher levels of viral replication, but not
virological failure. Martinez et al. (2005) reported an association between viral blips and impairment of CD4 cell recovery but no virological failure is observed during the follow-up period of their study. In a retrospective cohort study, García-Gascó et al. (2008) found that low-level viral rebound are rarely (<10%) followed by virological failure. However, the viral load at the time of rebound is associated with risk of subsequent virological failure. Drawing from their data, they proposed a critical viral load threshold of 120 copies/ml for selection of drug-resistant mutants. It has been suggested that the diverse clinical outcomes associated with viral blips were to do with two different processes that underline viral blips – clonal expansion of long-lived infected cells and continuous viral replication (Tobin et al., 2005).

Emergence of resistant strains is an indicator of continuous viral replication. It has been suggested that viral blips are associated with the emergence of resistant strains (Cohen Stuart et al., 2001, Macias et al., 2005), but this was not supported by other studies (Garcia-Gasco et al., 2008, Masquelier et al., 2005, Nettles et al., 2005, Podsadecki et al., 2007).

It has also been suggested that poor adherence to cART is associated with viral blips (Masquelier et al., 2005, Podsadecki et al., 2007) and VL rebound (Raboud et al., 2002). Easterbrook et al. (2002) identified poor adherence, drug interruption or change, in the medical records for 44 (42.6%) of 103 episodes of intermittent viraemia among patients in their cohort. Masquelier et al. (2005) measured protease inhibitor (PI) concentration in plasma that suggested poor adherence in 50% of patients with blips. Electronic pill bottle
caps that recorded each time the bottle was opened (Medication Events Monitoring System, MEMS) were used by both Podsadecki et al. (2007) and Miller et al. (2004) to monitor patients’ drug adherence and an association was present in the former but absent in the latter (which also measured drug adherence by pill count and patients’ self-reports). Association with self-reported poor adherence was also marginal in a cohort study in which Nettles et al. (2005) measured viral loads and drug concentration very frequently (every 2-3 days usually) during the study and found no association between drug concentration and viral blips. These pieces of evidence did not exclude the possibility that non-adherence to cART can lead to viral blips, but demonstrate that viral blips can arise despite good adherence.

It has also been shown that the choice of cART regimen, PI-based or non-nucleoside reverse transcriptase inhibitor (NNRTI)-based, made no difference to the frequency or prognostic significance of viral blips; it was ART experience (not necessarily non-optimal) that predicted a higher risk for blips (Sungkanuparph et al., 2005).

It has also been reported that a low CD4 cell count (<200 cells/µL) predicts a higher risk for blips (Di Mascio et al., 2003, Sungkanuparph et al., 2005). Patients with intermittent viraemia were reported to have a lower median CD4 cell count than those without (Easterbrook et al., 2002). It has been suggested that early treatment might help preserve an anti-HIV immune response which would in turn suppress viral blips; this was supported by Di Mascio et al. (2004a) who confirmed that viral blips were twofold more frequent in patients who started therapy during chronic infection than in patients who
commenced treatment during primary infection. However, viral blip frequency (sample\(^1\)) did not increase with longer periods of observation in both groups. The occurrence of a viral blip was not associated with a recent change in CD4+ T-cell count. Amongst the primary HIV patients, the VL at the start of therapy was found to be a significant predictor of blip frequency during treatment (Di Mascio et al., 2004a). Taking these pieces of evidence together, duration of HIV infection may predict the frequency of viral blips and some host-specific factors may play a role in them.

For detailed information on each study of viral blips, please refer to Table 1.2 and 1.3.

1.3.3. Superinfection and viral blips

Superinfection has been proposed as a possible causal mechanism leading to viral blips among patients on cART, since superinfection can lead to increased viral load in untreated patients. However, to date, very few clinical data have been collected on this. This PhD project employs mathematical models to explore whether this is a tenable hypothesis.
### Table 1.2: Association of viral blips with different outcomes / factors.

<table>
<thead>
<tr>
<th>Study</th>
<th>Virological failure</th>
<th>Resistant strains</th>
<th>Poor adherence to cART</th>
<th>Other associations / comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Cohen Stuart et al., 2001)</td>
<td>N</td>
<td>Y</td>
<td>n/a</td>
<td>Self-reported full adherence to therapy. No statistically significant difference in CD4 cell count was found during viral blips and the time immediately before and after them.</td>
</tr>
<tr>
<td>(Easterbrook et al., 2002)</td>
<td>Y (HR=3.15, 95%CI, 1.72-5.77, \textit{P}&lt;0.001)</td>
<td>n/a</td>
<td>n/a</td>
<td>Of 103 episodes, 44 (42.6%): poor adherence, drug interruption or change; 27 (26.2%): intercurrent infection or vaccination; 4 (6.8%): drug interaction.</td>
</tr>
<tr>
<td>(Garcia-Gasco et al., 2008)</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Higher viral load at the time of rebound associated with risk of subsequent virological failure (\textit{P} = 0.003, in a multivariate analysis). Drug resistance developed in most cases.</td>
</tr>
<tr>
<td>(Greub et al., 2002)</td>
<td>Y (HR=2.01, 95%CI, 1.51-2.91; \textit{P}&lt;0.0001)</td>
<td>n/a</td>
<td>n/a</td>
<td>See Table 1.3 for this study’s special definition of viral ‘blips’. For ‘bumps’, HR=5.08 (95%CI, 4.26-7.90; \textit{P} &lt; 0.0001)</td>
</tr>
<tr>
<td>(Havlir et al., 2001)</td>
<td>N (HR=1.28, 95%CI, 0.59-2.79)</td>
<td>n/a</td>
<td>n/a</td>
<td>Viral blips are associated with higher levels of viral replication</td>
</tr>
<tr>
<td>(Macias et al., 2005)</td>
<td>N</td>
<td>Y</td>
<td>n/a</td>
<td>Only patients with self-reported therapy adherence ≥ 95% to all prescribed drugs at all visits were eligible to this study.</td>
</tr>
<tr>
<td>(Martinez et al., 2005)</td>
<td>N</td>
<td>n/a</td>
<td>N</td>
<td>Viral blips are associated with impairment of CD4 cell recovery. Patients with blips had a higher viral load than those who did not (using sensitive assay with a detection limit of 3 copies/ml). Blips occurred despite efficient plasma levels of NNRTI drugs at different times. All enrolled patients had past experience to PI but all switch to NNRTI-based regimen for at least 6 months. PI concentration suggested poor adherence in 50% of patients with blips</td>
</tr>
<tr>
<td>(Masquelier et al., 2005)</td>
<td>Y (\textit{P}=0.03)</td>
<td>N</td>
<td>Y</td>
<td>Monitoring patients’ drug adherence through pill count, self-report and electronic pill bottle cap (Medication Event Monitoring System).</td>
</tr>
<tr>
<td>(Miller et al., 2004)</td>
<td>n/a</td>
<td>n/a</td>
<td>N</td>
<td>Viral blips were not associated with decline of CD4 cell count. Drug adherence was not associated with virological failure in this study either.</td>
</tr>
<tr>
<td>(Mira et al., 2002)</td>
<td>N</td>
<td>n/a</td>
<td>n/a</td>
<td>This study showed that rebound of VL after successful suppression of virus is more often transient (blips) than leading towards virological failure. However, this study is not designed to study viral blips and their associated factors.</td>
</tr>
<tr>
<td>(Moore et al., 2002)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>Intense sampling (every 2-3 days). Self-reported drug adherence. Viral blips were not associated with drug concentrations. No virological failure observed. No other associations were found.</td>
</tr>
<tr>
<td>(Nettles et al., 2005)</td>
<td>N</td>
<td>N</td>
<td>Marginal (\textit{P} = 0.08)</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Virological failure</td>
<td>Resistant strains</td>
<td>Poor adherence to cART</td>
<td>Other associations / comments</td>
</tr>
<tr>
<td>------------------------------</td>
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<td>-------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>(Podsadecki et al., 2007)</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>Adherence monitored by electronic pill bottle cap (Medication Event Monitoring System). LPV/r-based regimen.</td>
</tr>
<tr>
<td>(Raboud et al., 2002)</td>
<td>N for an isolated blip, but Y for two consecutive measurements</td>
<td>n/a</td>
<td>Y for VL rebound (OR=2.11, 95%CI, 1.06-4.22, P=0.03)</td>
<td>This paper focused on VL rebound and its associated factors.</td>
</tr>
<tr>
<td>(Sklar et al., 2002)</td>
<td>N</td>
<td>n/a</td>
<td>n/a</td>
<td>CD4 cell count response to ART was correlated with transient viraemia. No association was found between transient viraemia and (i) ART experience or (ii) composition of ART regimen (PI-based or not). Only 16 of 122 patients who developed viral blips later developed persistent viraemia (&gt;50 copies/ml)</td>
</tr>
<tr>
<td>(Stosor et al., 2005)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>This study compared viral load measurements using two different types of tubes and identified a high incidence of viral blips using plasma preparation tubes (PPTs) than EDTA tubes.</td>
</tr>
<tr>
<td>(Sungkanupaph et al., 2005)</td>
<td>N</td>
<td>n/a</td>
<td>n/a</td>
<td>No association with choice of ART regimen (PI-based and NNRTI-based)</td>
</tr>
<tr>
<td>(van Sighem et al., 2008)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>There is an association between the probability of viraemia and a higher VL at the most recent measurement. If VL: 50-1000 copies/ml OR = 2.6 (95% CI, 2.4 – 2.8) per 1-log increase; if VL: &gt;1000 copies/mL OR = 3.3 (95% CI, 3.1 – 3.5)</td>
</tr>
</tbody>
</table>

Statistical association: Y, with association; N, without association; n/a, not applicable.

Other acronyms: ART, antiretroviral therapy; CI, confidence interval; HR, hazard ratio; LPV/r: ritonavir-boosted lopinavir; NNRTI, non-nucleoside reverse transcriptase inhibitor; OR: odds ratio; PI, protease inhibitor; VL: viral load.
<table>
<thead>
<tr>
<th>Study</th>
<th>Study type</th>
<th>Definition of viral blips</th>
<th>Definition of virological failure</th>
<th>Sample size</th>
<th>Prevalence, n (%)</th>
<th>Incidence of blips (blips/100 person-years)</th>
<th>Measurement frequency</th>
<th>Follow-up period, median</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Cohen Stuart et al., 2001)</td>
<td>case series</td>
<td>≥ 50 copies/ml, preceded and followed by a measurement &lt; 50 copies/ml</td>
<td>&gt; 400 copies/ml at two consecutive time points</td>
<td>15</td>
<td>n/a</td>
<td>n/a</td>
<td>Various across patients. In weeks.</td>
<td>27 months after 'relapse'</td>
</tr>
<tr>
<td>(Easterbrook et al., 2002)</td>
<td>retrospective cohort</td>
<td>≥ 400 copies/ml, followed by a measurement &lt; 400 copies/ml</td>
<td>sustained virological rebound of ≥ 400 copies/ml</td>
<td>765</td>
<td>122 (16%) of all patients initiating cART; 27% of patients who initially attained an undetectable VL</td>
<td>-</td>
<td>At approximately 3-month interval</td>
<td>27.9 (IQR 22.6-31.3) months for sustained undetectable VL group and 29.5 (IQR 25.2-32.5) months for intermittent viraemia group (P=0.003) from PI/NRTI initiation</td>
</tr>
<tr>
<td>(Garcia-Gasco et al., 2008)</td>
<td>retrospective cohort</td>
<td>51,500 copies/ml, preceded and followed (after 12 weeks) by measurements viral load ≤ 50 copies/ml ‘blips’: 51-500 copies/ml followed next VL measurement of ≤ 50 copies/ml; ‘bumps’ two consecutive VL measurements of 51-500 copies/ml. Period under analysis began with at least two measurements of ≤ 50 copies/ml.</td>
<td>&gt; 500 copies/ml</td>
<td>2720</td>
<td>458 (17%) developed blips</td>
<td>-</td>
<td>On regular follow-up at hospital</td>
<td>17.7 months, after first VL measurement</td>
</tr>
<tr>
<td>(Greub et al., 2002)</td>
<td>retrospective cohort</td>
<td>&gt; 500 copies/ml</td>
<td>&gt; 500 copies/ml</td>
<td>2055</td>
<td>704 ‘blips’ = 490; ‘bumps’ = 155. *Also 71 of 176 patients who experienced rebound to &gt; 500 copies/ml return to ≤ 50 copies/ml.</td>
<td>37.4; see (Sklar et al., 2002; Sungkanuparph et al., 2005)</td>
<td>Within 24 weeks</td>
<td></td>
</tr>
<tr>
<td>(Havlir et al., 2001)</td>
<td>retrospective study in a clinical trial</td>
<td>&gt; 50 copies/ml, followed by a measurement &lt; 50 copies/ml</td>
<td>two consecutive measurements of &gt; 200 copies/ml</td>
<td>241</td>
<td>40% (20% if ≥ 200 copies/ml)</td>
<td>-</td>
<td>?</td>
<td>84 weeks; and 46 weeks after first intermittent viraemia episode 120 (range 36-150) weeks after the blip</td>
</tr>
<tr>
<td>(Macias et al., 2005)</td>
<td>retrospective cohort</td>
<td>50-1000 copies/ml, preceded by two consecutive measurements and followed by one measurement that are &lt; 50 copies/ml</td>
<td>n/a</td>
<td>330</td>
<td>37 (11%)</td>
<td>-</td>
<td>Baseline; 4 and 12 weeks afterwards; and every 12 weeks thereafter</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Study type</td>
<td>Definition of viral blips</td>
<td>Definition of virological failure</td>
<td>Sample size</td>
<td>Prevalence, n (%)</td>
<td>Incidence of blips (blips/100 person-years)</td>
<td>Measurement frequency</td>
<td>Follow-up period, median</td>
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<tr>
<td>(Martinez et al., 2005) retrospective cohort</td>
<td>&gt; 50 copies/ml with a subsequent measurement of &lt; 50 copies/ml</td>
<td>two consecutive measurements of &gt; 200 copies/ml</td>
<td>43</td>
<td>8 (19%)</td>
<td>-</td>
<td>Baseline (the day switching from PI- to NNRTI-based therapy); 6, 12, 18 months after NNRTI substitution</td>
<td>Baseline; month 1, and then every two months</td>
<td>18 (range 6-24) months</td>
</tr>
<tr>
<td>(Masquelier et al., 2005) prospective cohort</td>
<td>'intermittent viremia': &gt; 500 copies/ml on a single specimen preceded and followed by measurements &lt; 500 copies/ml</td>
<td>(i) two consecutive measurements of &gt; 500 copies/ml after an initial suppression below 500 copies/ml, (ii) persistently ≥ 500 copies/ml during the first year of follow-up.</td>
<td>219</td>
<td>20 (9%)</td>
<td>-</td>
<td>Baseline; month 1, and then every two months</td>
<td>2 years</td>
<td></td>
</tr>
<tr>
<td>(Miller et al., 2004) case-control</td>
<td>40-1000 copies/ml, preceded and followed by a measurement (per month) of VL &lt; 40 copies/ml each, in the course of three months; only the first blip is considered if &gt; 1.</td>
<td>n/a</td>
<td>128</td>
<td>32 (25%); of which only 28 had complete drug adherence data and were used in the analysis</td>
<td>-</td>
<td>Every 4 week (altogether three times)</td>
<td>12 weeks</td>
<td></td>
</tr>
<tr>
<td>(Mira et al., 2002) retrospective case-control in a prospective cohort</td>
<td>50-1000 copies/ml preceded by two consecutive measurements and followed by one measurement of &lt; 50 copies/ml</td>
<td>at least two consecutive measurements of &gt; 200 copies/ml</td>
<td>same cohort as in (Macias et al., 2005)</td>
<td>same cases as in (Macias et al., 2005)</td>
<td>-</td>
<td>Baseline; 4 and 12 weeks afterwards; and every 12 weeks thereafter</td>
<td>120 (range 36-150) weeks after the blip</td>
<td></td>
</tr>
<tr>
<td>(Moore et al., 2002) retrospective cohort</td>
<td>'raised viral load': &gt; 50 copies/ml</td>
<td>two consecutive measurements of &gt; 400 copies/ml</td>
<td>553</td>
<td>192 (35%); experienced at least one measurement of &gt; 50 copies/ml of 154 who had had a single measurement of &gt; 50 copies/ml and had not altered their therapy, 54% returned to &lt; 50 copies/ml, while 46% was &gt; 50 copies/ml.</td>
<td>-</td>
<td>Every 14 weeks (median)</td>
<td>56 (range 4 – 174) weeks</td>
<td></td>
</tr>
<tr>
<td>(Nettles et al., 2005) prospective cohort</td>
<td>≥ 50 copies/ml, preceded and followed by measurements &lt; 50 copies/ml</td>
<td>n/a</td>
<td>10</td>
<td>9 (90%)</td>
<td>-</td>
<td>Every 2 or 3 days</td>
<td>99.4 days (range, 12 weeks – 127 days)</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Study type</td>
<td>Definition of viral blips</td>
<td>Definition of virological failure</td>
<td>Sample size</td>
<td>Prevalence, n (%)</td>
<td>Incidence of blips (blips/100 person-years)</td>
<td>Measurement frequency</td>
<td>Follow-up period, median</td>
</tr>
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</tr>
<tr>
<td>(Podsadecki et al., 2007)</td>
<td>retrospective studies of 2 clinical trials</td>
<td>50-1000 copies/ml, immediately preceded and immediately followed by a measurement &lt; 50 copies/ml</td>
<td>(i) two consecutive measurements of ≥ 50 copies/ml or a final measurement of ≥ 50 copies/ml; (ii) a measurement of &gt; 1000 copies/ml at the end of the study or the last study visit; (iii) two consecutive measurements of &gt; 200 copies/ml or a final measurement of &gt; 200 copies/ml</td>
<td>223</td>
<td>60 (27%)</td>
<td>-</td>
<td>Baseline; Weeks 4, 8, 12, 16, 20, 24, 32, 40, 48, 60, 72, 84, 96 (excluding week 12 and 20 in one of the two cohorts)</td>
<td>96 weeks</td>
</tr>
<tr>
<td>(Raboud et al., 2002)</td>
<td>retrospective study of 3 clinical trials</td>
<td>LLOQ for INCAS and AVANTI-2 trials, 20 copies/ml; LLOQ for AVANTI-3 trials, 50 copies/ml, ≥ 50 copies/ml, preceded by two consecutive measurements &lt; 50 copies/ml (minimum, 2 months apart), and subsequently &lt; 50 copies/ml</td>
<td>&gt; 500 copies/ml at 52 weeks of follow-up; 165 achieved undetectable VL in the first place, of which 35 became undetectable again in the next measurement</td>
<td>358</td>
<td>85 of 165 experienced VL rebound; of which 122 (27.2%)</td>
<td>-</td>
<td>Baseline; Weeks 2, 4, 8, 12, 16, 28, 40 &amp; 52.</td>
<td>52 weeks</td>
</tr>
<tr>
<td>(Sklar et al., 2002)</td>
<td>retrospective cohort</td>
<td>lasting viraemia of &gt; 400 copies/ml</td>
<td></td>
<td>448</td>
<td>22.5</td>
<td>-</td>
<td>Outpatient visits; frequency not available in the paper.</td>
<td>485 days (69 weeks)</td>
</tr>
<tr>
<td>(Stosor et al., 2005)</td>
<td>retrospective study in a prospective cohort</td>
<td>50–2000 copies/ml after 3 consecutive measurements of &lt; 50 copies/ml, and followed by at least 1 measurement of &lt; 50 copies/ml</td>
<td>&gt; 2000 copies/ml after 3 consecutive measurements of &lt; 50 copies/ml</td>
<td>56</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>(Sungkanupark et al., 2005)</td>
<td>retrospective cohort</td>
<td>50–1000 copies/ml, preceded and followed by another measurement of &lt;50 copies/ml</td>
<td>two consecutive measurements of &gt; 1000 copies/ml</td>
<td>244</td>
<td>53 (21.7%) of NNRTI group; 34 (25.0%) of PI group</td>
<td>19.4 (overall); 19.2 (NNRTI group); 19.7 (PI group)</td>
<td>Every 2 – 3 months</td>
<td>NNRTI group: 24.0 (IQR 15.0-42.3); PI group: 23.0 (IQR 16.4-33.7) total 11187 person-years after success</td>
</tr>
<tr>
<td>(van Sighem et al., 2008)</td>
<td>retrospective study in a prospective cohort</td>
<td>low-level viraemia: 50–1000 copies/ml; high-level viraemia: &gt; 1000 copies/ml</td>
<td>two consecutive measurements of &gt; 1000 copies/ml</td>
<td>136</td>
<td>1281 (28.8%)</td>
<td>-</td>
<td>Average 3.30 (95% CI, 3.27-3.34) measurements per person-year</td>
<td>69</td>
</tr>
</tbody>
</table>

CI: confidence interval; IQR, interquartile range; LLOQ, lower limit of quantification; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; VL, viral load.
1.4. Drug adherence and its impacts

1.4.1. Different means of measurements of drug adherence

There are many ways of measuring adherence to cART and each has its own strengths and weaknesses (Paterson et al., 2002, Bangsberg, 2008b). Self-reports of drug adherence tend to over-estimate adherence in practice, but they are the easiest way to measure adherence in a routine clinical setting. Biological parameters or therapeutic drug monitoring can be used as objective proxy measures of adherence but are subject to increased drug adherence immediately prior to clinic visit, a phenomenon commonly known as ‘white coat compliance’ or the ‘toothbrush effect’ in the medical literature and well-known amongst many clinicians. Health care providers may not predict adherence well and the ‘pill identification test’ – where photos of pills are shown to patients who will then be asked to identify their regimen and their daily doses from the photos – awaits validation against a rigorous measure of adherence. Unannounced pill counts, by the patients themselves under instruction over telephone, performed better than routine clinic-based pill counts. Pharmacy refill monitoring is the only applicable method of adherence monitoring in resource-limited settings that has been demonstrated to be a good predictor of virological failure. Electronic monitoring devices, like MEMS, record adherence patterns more accurately than all the other methods, but may be subject to ‘pill dumping’ (taking the pill out of the bottle but without ingestion) or ‘curiosity events’ (cap opening without actually taking the pill). A new generation of electronic monitoring devices, compatible with pill boxes, are now available and real-time monitoring is made possible through internet or mobile phone connections (Bangsberg et al., 2008a, 2008b;
Bisson et al., 2008; Duong et al., 2001a, 2001b; Parienti et al., 2001; Paterson et al., 2000, 2002; Ruskin et al., 2003; Simoni et al., 2006; Singh et al., 1996, 1999). For detailed descriptions of these measurement strategies, and their advantages and disadvantages, please refer to Table 1.4.

**Table 1.4 Methods of measuring drug adherence to cART (including methods applied in other health contexts with future potentials of application to cART)***

<table>
<thead>
<tr>
<th>Category</th>
<th>Descriptions of method(s), examples or references</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological parameters</td>
<td>Mean corpuscular volume (MCV) – marker of adherence to zidovudine or stavudine</td>
<td>An objective measure independent of self-report of drug adherence.</td>
<td>Vitamin B12 deficiency, folic acid deficiency, alcoholism and liver disease might lead to increase in MCV (Paterson et al., 2002). Only reflect recent treatment adherence; subject to “white coat compliance”; affected by drug interaction and diet (Paterson et al., 2002). Recall bias; inaccurate memory; social desirability bias; therefore, over-estimation of adherence (Simoni et al., 2006); no real-time monitoring (Bangsberg, 2008b).</td>
</tr>
<tr>
<td>Therapeutic drug monitoring</td>
<td>Monitoring plasma concentrations of antiretroviral medications</td>
<td>An objective measure independent of self-report of drug adherence.</td>
<td></td>
</tr>
<tr>
<td>Patient self-report</td>
<td>Face-to-face interview</td>
<td>Low cost; minimal participant burden; ease and speed of administration; flexibility in terms of mode of administration and timing of assessment; potential to yield specific information about timing of doses and adherence to food requirements; high specificity of self-reported non-adherence (Simoni et al., 2006); compatible with pill-box organizers (Bangsberg, 2008b).</td>
<td>Web-based interview not accessible to those without internet connection; recall bias; inaccurate memory; no real-time monitoring (Bangsberg, 2008b).</td>
</tr>
<tr>
<td>Patient-completed questionnaires (Duong et al., 2001a, Duong et al., 2001b) Computer-assisted self-interview or Web-based interview, e.g. West Portal Software: <a href="http://www.westportal.com/">http://www.westportal.com/</a> (Bangsberg et al., 2002a, Bangsberg et al., 2002b)</td>
<td>Appear more neutral and private than face-to-face interview or questionnaire; increased willingness to disclose true adherence (Bangsberg et al., 2002) Cost-efficient; improved precision and frequency of self-reporting; continuous monitoring; more detailed assessment and intervention; feasible in routine clinical practice; compatible with pill-box organizers (Bangsberg, 2008b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Provider prediction of adherence</td>
<td>Patient diary of adherence</td>
<td>Inexpensive and accurate (Paterson et al., 2002)</td>
<td>Potential non-adherence to taking diary; patient burden (Paterson et al., 2002)</td>
</tr>
<tr>
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<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Predictions made by clinicians or other healthcare providers (Paterson et al., 2000, Bangsberg et al., 2001)</td>
<td>Part of routine clinical practice (Paterson et al., 2002)</td>
<td>Poor estimate of adherence (Paterson et al., 2000, Bangsberg et al., 2001)</td>
<td></td>
</tr>
<tr>
<td>Pill recognition</td>
<td>‘Pill identification test’ (Parienti et al., 2001)</td>
<td>Easy to administer; well-accepted by patients; unaffected by the identity of person administering it (Parienti et al., 2001).</td>
<td>Not yet validated against a rigorous measure of adherence (Paterson et al., 2002).</td>
</tr>
<tr>
<td>Pill count</td>
<td>Clinic-based pill counts</td>
<td>Compatible with pill-box organizers (Bangsberg, 2008b).</td>
<td>Overestimation of adherence due to “pill dumping”, i.e. patients disposing of pills to make their adherence appear better (Paterson et al., 2002); not precise, unable to detect adherence pattern; no real-time monitoring; not cost-efficient (Bangsberg, 2008b).</td>
</tr>
<tr>
<td>Home-based pill count</td>
<td>Data on interruption collected by means of patient interview during pill count; compatible with pill-box organizers; can be adapted to incorporate telephone interview (Bangsberg, 2008b).</td>
<td>Not feasible in routine clinical practice; not cost-efficient; no real-time monitoring (Bangsberg, 2008b).</td>
<td></td>
</tr>
<tr>
<td>Unannounced pill counts by telephone (Bangsberg, 2001)</td>
<td>Data on interruption collected by means of patient interview during pill count; compatible with pill-box organizers (Bangsberg, 2008b).</td>
<td>Some patients may regard these as an invasion of privacy (Paterson et al., 2002).</td>
<td></td>
</tr>
<tr>
<td>Pharmacy refill monitoring</td>
<td>Pharmacy refill records were used to calculate adherence (Singh et al., 1999, Singh et al., 1996). Pharmacy refill adherence was defined as the number of months of cART claims submitted divided by the number of complete months between cART initiation and the last refill prior to the endpoint of interest, expressed as a percentage (Bisson et al., 2008)</td>
<td>Prediction of virological failure as good as or even better than CD4 count changes; can be realistically used in resource-limited settings (Bisson et al., 2008); cost-efficient; feasible without active patient participation; compatible with pill-box organizers (Bangsberg, 2008b).</td>
<td>Drug hoarding, i.e. receipt of drugs from a pharmacy does not guarantee that the drugs are actually taken (Paterson et al., 2002, Bisson et al., 2008). Use of multiple pharmacies by the same person; no real-time monitoring; unable to detect adherence pattern; for medications regularly delivered by mail, unable to calculate patient drug-possession ratios (Bangsberg, 2008b).</td>
</tr>
</tbody>
</table>
1.4.2. ‘White coat compliance’

In a recent clinical trial, it was found that 66% of 178 subjects exhibited ‘white coat compliance’ (Podsadecki et al., 2008), while in another cohort study no such effect is observed (Levine et al., 2006). Both studies employed MEMS.

1.4.3. Adherence variation in weekdays and weekends

Patterns of drug adherence may differ between weekdays and weekends as daily life patterns differ. In a pilot study of supervised therapy of zidovudine (AZT) using multiple measures of adherence including MEMS, adherence was higher during weekdays when it was supervised than in the weekends when it was not (Wall et al., 1995). In a cohort study that examined longitudinal adherence pattern data using MEMS, a distinct group of patients who achieved relatively poorer adherence at the weekends than during weekdays

* Adapted from Paterson et al., 2002, and Bangsberg, 2008b; with information from various other papers.
was identified. Compared to other groups in the cohort, there was a higher proportion of participants in this group who had global cognitive impairment or specific impairment in the attention domain (Levine et al., 2005). To get around this problem of uneven adherence pattern within a week in self-reports of adherence, recollection of pill taking can be of a 7-day period to take into account within-week variation (Paterson et al., 2002) and yet the possibility of recall bias calls any recollection of more than 4 days into doubt (Levine et al., 2006). Alternatively, additional questions should be asked with respect to pill taking in the weekends (Paterson et al., 2002).

1.4.4. Drug adherence, resistance and virological failure

The general assumption that poor adherence leads to the rise of resistant strains and subsequent virological failure was found to be over-simplistic. Bangsberg and colleagues (2004) identified a more complex relationship that is not linear and in which the association varies by drug class. Selection pressure is maximised when drug is around for a long time at low levels rather than around at high levels all the time or not around. This is related to the fitness of mutant organisms in the presence and absence of drug. The risk of resistance is the highest around 70-80% of adherence for single PI. This is because residual viral replication is still observed in many patients with a high adherence to PI-based cART, while fitness costs are high for PI-resistant virus at a low adherence level. However, when PI is ritonavir-boosted, the risk of resistance peak becomes lower and shifted towards a narrow middle range of adherence which is sufficient to allow selection for resistant strains with a heavier fitness cost but insufficient to suppress viral replication. For NNRTI, the risk of resistance is high at the low and moderate levels of adherence due to little fitness costs conferred to the mutant viruses, but it is low at high adherence level.
as viral replication is suppressed. Similar yet different patterns were observed in two retrospective longitudinal studies, one of a cohort of HIV-positive, antiretroviral-naive adults commencing cART (using monthly prescription refill data and 3-monthly ‘untimed’ plasma drug concentrations as measures of drug adherence) (Tam et al., 2008) and another of a cohort of illicit drug users under a directly observed therapy program (using daily methadone adherence as a surrogate for cART adherence) (Raffa et al., 2008). Taking these studies together, a different concave adherence-resistance curve for each class of drug was supported.

Patterns of drug adherence are composite of three different and yet equally important measures: proportion of doses taken, standard deviation of the random dose-timing error and any systematic dose-timing bias (Ferguson et al., 2005). If a patient has a high level of dose frequency adherence, it does not necessarily mean that their adherence to dose timing is good. A study that made use of electronic monitoring of pill bottle opening revealed the complex heterogeneity of drug adherence patterns among HIV-positive patients and concluded that summary measures of short-term adherence that incorporate pharmacodynamic and pharmacokinetic data on the regimen under monitoring, would predict suboptimal VL and CD4 cell count trends better than measures using adherence data alone (Ferguson et al., 2005).

1.4.5. Drug adherence, viral load and infectiousness

A recent debate on the infectiousness of HIV-positive individuals on effective cART with undetectable viral load indirectly demonstrates the relevance of the issue of drug adherence. Early in 2008, the Swiss Commission on Aids-related Issues concluded that
HIV-positive individuals are sexually non-infectious if and only if (a) they have a good adherence to cART with the treating physician’s regular evaluation of the effects of the treatment; AND (b) their VL suppressed below detectable level (<40 copies/ml) for at least six months; AND (c) they had no additional STI (Vernazza et al., 2008). This conclusion was criticised by an Australian team (Wilson et al., 2008b) whose modelling exercise of HIV transmission among 10000 discordant couples (heterosexual and homosexual) based on the Rakai data highlighted a substantial number of seroconversions over 10 years. Garnett and Gazzard (2008) highlighted the key difference in assumptions between these two papers: Vernazza et al. (2008) assumed a threshold of VL below which no HIV transmission is possible, while Wilson et al. (2008b) extrapolated the data to the range of undetectable VL in which they assumed instead a log-linear relationship between VL and transmission risk. Residual viraemia persists in patients with successful cART (Shen and Siliciano, 2008). A recent longitudinal study of 40 patients for seven years identified low-level viraemia (≥ 1 copy per ml) in every patient (Palmer et al., 2008). The crucial question is whether such a threshold for infectiousness exists and if it does, at what VL? If patterns of drug adherence influence VL and therefore affect infectiousness, understanding this hypothetical or real threshold will be of importance for our understanding of how different drug adherence patterns affect HIV transmission from an epidemiological perspective. Furthermore, given the growing attention towards employing viral load monitoring to improve HIV positive patients’ drug adherence (Wilson et al., 2008a), the relationship between the three factors: drug adherence, viral load and infectiousness, would be of interest to clinical practice as well.
1.5. Summary

This chapter has reviewed our current understanding of HIV superinfection, viral blips and adherence to cART. HIV superinfection has been reported in a number of studies. However, consensus has yet to be reached with regard to its incidence in different HIV-positive populations and its clinical consequences. Viral blips have been observed in different groups of patients. However, different studies used different definitions and different sampling frames, making cross-study comparison difficult. The biological causes and clinical consequences of viral blips are yet to be fully understood. The question of whether or not superinfection is one of the causes of viral blips will be addressed in this PhD thesis. Though it is well-known that adherence to cART is crucial to viral suppression in most patients, its non-linear relationship with the probability of emergence of drug resistance is only beginning to be understood. Measurement of adherence is still a research problem in spite of technological advances with instruments like the electronic pill bottle caps. This thesis will help elucidate the relationship between drug adherence and viral blips through computer simulations. Having reviewed the clinical and behavioural data that informs the study, the next chapter will review the theoretical frameworks that have been developed to synthesise our biological understanding with data.
Chapter 2

Development of Mathematical model of within-host dynamics of HIV infection
2. Development of Mathematical model of within-host dynamics of HIV infection

2.1. Introduction

HIV pathogenesis is a complex phenomenon which is yet to be fully understood and agreed upon. Mathematical and computational models enable scientists and clinicians to make their assumptions explicit, in a logical and quantitative way, and to study and test hypotheses analytically and numerically (often through computer simulations). This chapter is a review of the development of mathematical and computational models of the within-host dynamics of HIV infection, and in particular, HIV disease progression.

There have been two different approaches described in the scientific literature, developed by two separate scientific communities largely independent of each other. The first approach employs agent-based models or Multi-Agent (MA) models and the second employs ordinary differential equations (ODEs). The former is usually based on the concept of cellular automata (CA) and has been primarily developed by computer scientists with little connection with clinicians. The latter employs ODEs to model the virus-host interaction and is mainly developed by mathematical biologists, who are more aware of the necessary clinical contributions to test and parameterise their models. These models will be discussed in turn below.
2.2. **Agent-based models**

2.2.1. **Cellular automata (singular: automaton)**

According to Celada and Seiden (1992), the ‘requirements’ for a cellular automaton are:

1. They consist of a discrete lattice of sites.
2. They evolve in discrete time steps.
3. Each site takes on a finite set of possible values.
4. The value of each site evolves according to the same ‘deterministic’ rules.
5. The rules for the evolution of a site depend only on ‘a local neighbourhood of sites around it’.

This is a very specific type of a more general set of lattice or meta population models where longer range influences are possible. In their model, Celada and Seiden (1992) replaced ‘deterministic’ in rule 4 with ‘probabilistic’ and ‘a local neighbourhood of sites around it’ in rule 5 with ‘the site itself’ and added rule 6:

6. Entities move from site to site. At the end of each time step the entities are allowed to diffuse to adjacent sites.

On the other hand, automata with particles that can move on the lattice are known as “lattice gases” (Kleinstein and Seiden, 1999).

Early CA models of HIV infection were categorised as follows (cf. review by Zhang et al., 2005):

(a) **Physical space model (1)** (Feng et al., 2004, Hecquet et al., 2007, Liu and Ruskin, 2002, Mannion et al., 2000a, Mannion et al., 2000b, 2002, Mielke and Pandey, 1998,
Pandey, 1989, 1991, 1998, Pandey et al., 2000, Ruskin et al., 2002, Zhang et al., 2005); (b) **Physical space model (2)** (Benyoussef et al., 2003, Ormerod, 2004, Sloot et al., 2002, Strain and Levine, 2002, Strain et al., 2002, Zorzenon dos Santos and Coutinho, 2000, 2001); (c) ‘**Shape**’ space model (Hershberg et al., 2001); and (d) **Sequence space model** (Kamp and Bornholdt, 2002a, b). Both ‘shape’ space and sequence space are spaces constructed by the HIV genome (Zhang et al., 2005).

Later, more sophisticated agent-based models were developed: (e) **bit-string space model** or PARIMM (Bernaschi and Castiglione, 2001, 2002, Castiglione et al., 2004), (f) **Massively Multi-agent System** (Zhang and Liu, 2005, Zhang et al., 2005, Zhao et al., 2007), (g) **Complex Adaptive Framework for Immune System Simulation** (CAFISS) (Guo et al., 2005, Tay and Jhavar, 2005), and (h) **Conformon-P system** (Corne and Frisco, 2008).

Among the four categories of CA model reviewed by Zhang et al. (2005), only the physical space models type 2 created by Zorzenon dos Santos and Coutinho (2000, 2001) and the ‘shape’ space model created by Hershberg et al. (2001) “reproduced the typical three stages HIV dynamics”, namely the stages of primary infection, chronic infection and AIDS. However, both are over-simplified and would seem to be deficient in many relevant details.

2.2.2. **Physical space model (1)**

Physical space models are those that explicitly represent spatial dimensions with cellular automata. The first type of physical space model represents physical spaces in the body
with cells of a CA lattice. Each lattice site represents a local space of the biological system (e.g. thymus) in which different types of cells meet (Pandey, 1989). Therein three types of immune cells were introduced in the early models (CD4 cells, CD8 cells and viral antigen or virus-infected cells) (Pandey, 1989) while macrophages were introduced in later models (Pandey, 1991). No immune cells of the same type can occupy a lattice site at the same time, but immune cells of different types can (Pandey, 1989). The Pandey (1989) model was three-dimensional and a two-dimensional adaptation was developed too (Kougias and Schulte, 1990). In both of Pandey’s papers (1989, 1991), two scenarios were studied: ‘mean-field-like interactions’ and ‘nearest neighbour interactions’ (i.e. proper CA). In the former, each immune cell interacts with every other immune cell (and therefore for each cell type, all cells behave in the same manner, and spatial distribution of immune cell types is ignored), while in the latter, the spatial effect is introduced and immune cells only interact with those next to them spatially. In both cases, the state of an immune cell is denoted in a binary fashion (zero for low concentration and one for high concentration) and its state in the next time step will be determined by the current states of the cells in contact with this cell. The states of all the cells are updated each time step. Pandey (1989) proposed two sets of interaction between cells in different sites. In Interaction set 1, when the concentration of virally infected cells is low at a particular site, CD4 cells at that site will become high in the next step. The concentration of CD8 cells will be high if either CD4 or CD8 cells are high. The concentration of virally infected cells will be high if either the concentration of CD4 cells or virally infected cells are high, and the concentration of CD8 cells is low. In Interaction set 2, if the concentrations of CD4 cells and virally infected cells are not high simultaneously, CD4 cells will be in high
concentration the next step. The concentration of CD8 cells follows the same rule as that of the first set. The concentration of virally infected cells will be high if the concentrations of CD4 cells, CD8 cells and virally infected cells are not high at the same time. For the ‘nearest neighbour interactions’ scenario, the temporary state of an immune cell type at a given site for the next time step is first assigned by adding the sum (logical ‘OR’) of the states of that site and its six adjacent neighbours (a cube with six adjacent cubes). If that sum is greater than zero, the state of the immune cell type at that site would be temporarily assigned as high concentration; otherwise, its concentration will be low. This is known as a CA sum rule (cf. Pandey, 1998). Once every immune cell type of every lattice site is assigned a temporary state, it will take into account its interaction with its adjacent sites, following either interaction set 1 or set 2 (according to a probability $B$ and $1 - B$, that may be fixed at the beginning or changed each time step). One round of lattice site update is now completed.

A two-dimensional version of this model (Pandey, 1998) was further developed to incorporate random sequential moves with immunological motility (macrophages and cytotoxic cells moving towards viral infected sites). For a given mobility probability ($P_{mob}$), an immune cell can be moved from site $j$, to one of its neighbouring sites $k$. Both $j$ and $k$ are randomly selected. Some restrictions apply: (a) the cell can only move to $k$ if its concentration is low there; (b) macrophages and cytotoxic lymphocytes (CTLs) can only move there if there is a high concentration of virally infected cells; and (c) virions (or virally infected cells) can only move to $k$ if either macrophages or CD4 cells are in high concentration there (Pandey, 1998). More sets of interactions between the different
immune cell types were further introduced (and one of which would be chosen randomly for each cell type in each site in each time step). A viral mutation probability ($V_{\text{mut}} = 0$, when $p = P_{\text{mut}}$; and $V_{\text{mut}} = V$, when $p = 1 - P_{\text{mut}}$) was used to study the impact of viral mutation (Mielke and Pandey, 1998). Another variation of this model that also studied the impact of viral mutation on cellular dynamics was developed using a direct Monte Carlo simulation (Mannion et al., 2000a). A Monte Carlo step is defined as the sum of three procedures (a) random sequential update of cellular state (as described above, in Pandey, 1989), (b) mutation (as described above, in Mielke and Pandey, 1998) and (c) mobility (as described above, in Pandey, 1998). This model can be extended to include different states (intermediate state and stimulated state) to “incorporate the effects of stimuli factors via inter-cellular inter-site interaction” (Mannion et al., 2000b). The results of the model depend critically on the probability of mutation ($P_{\text{mut}}$) and mobility ($P_{\text{mob}}$). Mannion et al. (2000b) pointed out that “the range of numerical value[s] of $P_{\text{mut}}$ is relatively large and should not be compared with [the] clinical mutation rate”. These models were used to study the effect of cellular mobility on immune response (Pandey et al., 2000). This two-dimensional model can be extended to three-dimensional (Mannion et al., 2002). It was further developed to study the impact of growth factors (cellular growth factor probability plus growth probability due to mutation) (Ruskin et al., 2002). The model was further developed, following a ‘mesoscopic approach’, to incorporate immunological memory and B cells (nine entities: macrophages, TH1 cells, TH2 cells, cytotoxic T cells, memory T cells, B cells, memory B cells, antibodies and antigens) (Liu and Ruskin, 2002). This ‘mesoscopic’ immunological memory model was further modified and parameterised to study human immune response against HIV by comparing
a basic four-entities model – CD4 cells, CD8 cells, macrophages and virally infected cells (Mannion et al., 2002) – with that of eight – the nine entities aforementioned minus B cells (Feng et al., 2004). Strategies for optimisation and parallelisation of this type of HIV model were further developed (Hecquet et al., 2007).

However, these models have not yet managed to create a three-stage AIDS progression nor correspond the time steps of their model to real time. Mannion et al. (2002) claimed that “real changes in latency periods depend on changes in the mutation rate” and that their results are in agreement with that of the physical space model type two (see below) (Zorzenon dos Santos and Coutinho, 2001) and ‘shape’ space model (Hershberg et al., 2001). However, as we shall see below, both of these models are problematic.

**2.2.3. Physical space model (2)**

While in the first type of physical space model, a CA lattice site represents a physical site in the body where immune cells of different types meet, in the second type of physical space model, a CA lattice site represents an immune cell in time and space. Zorzenon dos Santos and Coutinho (2001) created a physical space model of HIV infection by representing immune cells (CD4 cells or monocytes that are targets of HIV infection) in the lymphoid tissues with a two-dimensional square lattice of ‘cells’. The cells can be in either one of the four states: (a) healthy cells; (b) ‘infected-A1’: productively infected cells; (c) ‘infected-A2’: infected cells about to die as a result of immune control; and (d) dead cells. A healthy cell will become an ‘infected-A1’ cell in the next step, if it has an ‘infected-A1’ neighbour, or if it has at least n (2 < n < 8) ‘infected-2’ neighbours. After x time steps, an ‘infected-A1’ cell turns into an ‘infected-A2’ cell, which in turn will die in
the next time step. The site of a dead cell will be replaced in the next time step with a healthy cell with a probability $P_{\text{repl}}$ (representing replenishment of target cells) or will remain dead with a probability $(1 - P_{\text{repl}})$; and each newly introduced healthy cell may be replaced by an ‘infected-A1’ cell with a probability $P_{\text{infect}}$ (representing the influx of infected cells from other body compartments).

Zorzenon dos Santos and Coutinho (2001) claimed that they produced a three-stage AIDS progression with this model. However, the patterns formed in the model were a direct consequence of the chosen rules and how infected cells were re-introduced into the system. The three-phase dynamics obtained in the model were in fact artefacts. Beauchemin (2002) commented that “The fact that the authors chose to have each site represent a cell and have no cell diffusion led to patterns that are not to be expected in the real system.” What they did was to have a quick wipe-out of CD4 cells by viral infection and then slowly and randomly reintroduce a number of healthy cells into the lattice, which resulted in large error bars of the data point of the third stage of AIDS progression (Beauchemin, 2002). Strain and Levine (2002) commented that the results of Zorzenon dos Santos and Coutinho (2001) were highly dependent on two parameters (the initial fraction of cells infected and the fraction of cells that are infected). Despite its drawbacks, this model was furthered developed by incorporating drug therapy (Sloot et al., 2002) or by changing the shapes of a cellular automaton and the way it is linked to its neighbour.\(^2\)

\(^2\) The shape of the lattice cell can be varied and neighbouring cells can share a vertex, an edge or a face. Choices of lattice cell shape include square, triangle, cube, hexagon and pentagon. This will generate CA with different numbers of nearest neighbours. For example, a square which shares an edge has 4 neighbours while a square which shares a vertex has 8 neighbours; a cube which shares a face has 6 neighbours, a cube which shares an edge has 18 neighbours and a cube which shares a vertex has 26 neighbours (Ormerod, 2004).
Another two-dimensional physical model that incorporated the impact of antiretroviral therapy was described by Benyoussef and colleagues (2003). Strain and colleagues (2002) modelled the within-host spatiotemporal dynamics of HIV, comparing the ODE and the CA approaches.

2.2.4. ‘Shape’ space model

The ‘shape’ space model tried to represent viral mutations by “propagation of the multiplying virions in the structure-less infinite dimensional shape space” and viral confrontation with the immune cells which results in the ‘disappearance’ of both cells and virions (Hershberg et al., 2001). Hershberg et al. (2001) used a random lattice in which every site has a fixed number of neighbours to represent the viral shape space. An adjacent site represents a ‘shape’ such that by a mutation of single base, virus can ‘travel’ from one to the other. For each site, there is an occupation number that represents “the number of virions existing with that shape in the organism” and another occupation number that represents the immune cells that recognise that particular shape. Hershberg et al. (2001) emphasised that “the existence of a virus and an immune cell on same lattice site does not imply their proximity in real space.” They might be distant. However, there is a small probability that they do meet and interact in real space. Diffusion rates of virus and immune cells on the lattice represent their mutation. Immune cells were supposed to be able to diffuse on the ‘shape’ space (i.e. can mutate). Zhang et al. (2005) criticised the model and claimed that immune cells cannot mutate. What Hershberg et al. (2001) probably meant to suggest, was that they had included clonal selection of immune cells.
If an immune cell and an HIV virion happen to be at the same site, the immune cell will replicate with a rate $r_c$ and will destroy the virus with a probability $d_v$. An HIV virion replicates with a rate that is proportion to the total immune cell population ($r_vC_{tot}$) and an immune cell dies with a rate that is proportional to the total population of virus ($d_vV_{tot}$). There is a continual replenishment of new immune cells and with a probability $\lambda$, a new immune cell will be created in a random lattice site. There is a slow diffusion of immune cells and HIV across the shape space (i.e. hypermutation and clonal selection of immune cells and mutations of HIV) at a rate of $D_c$ and $D_v$ respectively (Hershberg et al., 2001).

Hershberg et al. (2001) claimed that the main success of their model was the “natural emergence of a hierarchy of very different dynamical time scales” (original italics) that does not require the fine tuning of parameters that was required in the models of Zorzenon dos Santos and Coutinho (2001). They claimed that the AIDS progression (or evolution to use their term) is due to “the spread of the virus strains across the shape space” (Hershberg et al., 2001). Although the virus is losing ground on an individual shape space basis, it eventually filled the shape space “with a multitude of small but numerous strain populations” and thus killed “more immune cells than it activates” (Hershberg et al., 2001). This is the same idea as Nowak’s antigenic diversity threshold (Nowak et al., 1991).

Burns and Ruskin (2004) combined model categories (b) and (c) and created a model of both ‘shape’ space and physical space to represent the first six days of primary immune response.
Box 2.1 Shape-space

The concept of shape-space was first introduced by Perelson and Oster (1979) “to quantitatively describe the interactions between molecules of the immune system and antigens” (de Castro and Timmis, 2002):

“Assume that it is possible to adequately describe the generalized shape of an antibody by a set of $L$ parameters (e.g., the length, width, and height of any bump or groove in the combining site, its charge, etc.). Thus, a point in an $L$-dimensional space, called shape-space $S$, specifies the generalized shape of an antigen binding region of the molecular receptors on the surface of immune cells with regard to its antigen binding properties. Also, assume that a set of $L$ parameters can be used to describe an antigenic determinant, though antigens and antibodies do not necessarily have to be of the same length. The mapping from the parameters to their real biological counterparts is not important from a computational standpoint but will be basically dictated by the application domain of the AIS [Artificial Immune System].

“If an animal has a repertoire of size $N$, i.e., $N$ antibodies, then the shape-space for that animal contains $N$ points. These points lie within some finite volume $V$ of the shape-space since there is only a restricted range of widths, lengths, charges, etc. that a combining site can assume. Similarly, antigens are also characterized by generalized shapes whose complements lie within the same volume $V$. If the antigen (Ag) and antibody (Ab) shapes are not quite complementary, then the two molecules may still bind, but with lower affinity.” (de Castro and Timmis, 2002)

There are four types of shape-space:

1. *Real-valued shape-space*: the attribute strings are real-valued vectors;
2. *Integer shape-space*: the attribute strings are composed of integer values;
3. *Hamming shape-space*: composed of attribute strings built out of a finite alphabet of length $k$;
4. *Symbolic shape-space*: usually composed of different types of attribute strings where at least one of them is symbolic, such as a “name”, a “color”; etc. (de Castro and Timmis, 2002).
**2.2.5. Sequence space model**

The sequence space model (Kamp and Bornholdt, 2002b) represents immune cells of different receptor coding sequences with a CA lattice. If a site’s sequence matches a virus with the same epitope, that site will be infected. If a viral sequence matches an immune response, it will be removed. The CA steps are as follows. A random site is first chosen. Then if an active immune receptor is represented by this site, any bit will be mutated with a probability of \(1 - q_0\); and if as a result, a novel immunological ‘strain’ emerges, and it matches a site that is infected, the viral status of that site will become ‘recovered’ and that site will be occupied with an immune receptor. If the randomly chosen site is an infected one, any bit will be mutated with a probability of \(1 - q_v\), and if as a result, a novel strain emerges that matches a susceptible site, that site will become infected. At every step, there is a probability of \(\rho_\alpha(t)\) of a meeting with a random immunological clone for every viral strain, that is followed by its destruction with a probability \(p\). It is basically a CA representation of the coevolution of quasi-species and it was based mainly on Martin Nowak’s theory of antigenic diversity threshold (Nowak et al., 1991). However, this theory has long been under dispute (Miedema and Klein, 1996); for more on this theory, see section 2.3.1.2.

**2.2.6. Bit-string space model (PAR\textsc{imm})**

The bit-string space model, known as PAR\textsc{imm}, (Bernaschi and Castiglione, 2001, 2002, Castiglione et al., 2004), was based on IMMS\textsc{im}, an immune system simulation model developed by Celada and Seiden (1992) (for a review of bit-string space models, see
Beauchemin, 2002). PARIMM modelled the immune system on a diamond lattice\(^3\), with six biological cell types (B cells, plasma B cells, T helper cells / CD4 cells, cytotoxic lymphocytes / CD8 cells, macrophages / antigen processing cell (APC), and epithelial cell / generic target cells) in different states\(^4\), and five molecule types (interferon-\(\gamma\), danger signal, antigen or virus, antibody, immune complex / antibody-antigen binding). Both biological cells and molecules can move from a lattice site to one of its six nearest neighbours (Bernaschi and Castiglione, 2001). This model was extended to incorporate HIV infection by turning T helper cells into target cells of viral infection (Bernaschi and Castiglione, 2002). The model is also known as a bit-string space model because two binary strings of \(l\) bit-length (\(l = 12\), in Castiglione et al., 2004) are used to represent a virus – one for its epitope or the binding site of B cell receptor and one for its peptide or the binding site of MHC class I and II molecules. Mutations are introduced into the model as follows. Either of the two binary strings is randomly selected and a point mutation of any of its bits occurs with a constant probability, \(p_m\). If the viral epitope mutates, it will elicit a different humoral response. If the viral peptide mutates, a different cellular immune response will be elicited\(^5\). However, the crux of the model lies in the ‘transcription rate’, \(p_w\), a probability of activation of transcription of viral deoxyribonucleic acid (DNA) in an infected cell, which is different for each viral strain in

\(^3\) A diamond lattice is a two-dimensional lattice, made up of the corners of equilateral triangles. Each lattice site has six nearest neighbours (Bernaschi and Castiglione, 2001).

\(^4\) Initially, all cell types are in their normal state and are active (ACT). When an antigen presenting cell (B cell or APC) interacts with an Antigen, phagocytosis occurs and the antigen is internalised (the cell becomes INT). When an epithelial cell is infected by an Antigen (virus), it is infected (INF). When a B cell or APC has internalised the antigen, and if there is a successful binding between the antigen and the MHC class II molecule, the MHC II – Antigen complex is being exposed (EXP) by the cell. If their MHC class I molecule loaded with antigen peptide is exposed, APC and epithelial cells become LOA. APC can become inactive and resting (RES). B cells, T helper cells and cytotoxic lymphocytes can be stimulated (STI) and divide. If an infected epithelial cell is killed by a cytotoxic lymphocyte, it become dead (DEA) (Bernaschi and Castiglione, 2001).

\(^5\) In Castiglione et al. (2004), instead they were the three parameters \(p_{mw}, p_m\), and \(p\), that were subject to changes due to mutation in epitopes.
the model. The HIV progression time scale in the simulations is based on the model assumption that an optimal ‘transcription rate’ will be selected such that it will not be too high (and therefore the patient progresses to AIDS rapidly) or too low (and therefore the patient progresses very slowly as the long-term non-progressors) (Bernaschi and Castiglione, 2002). At a rate of $p_r$, virions are produced and accumulated in progressively infected cells. Each viral strain is identified with its unique combination of its two bit strings, and its parameters $p_m$, $p_w$, and $p_t$ (Castiglione et al., 2004). It is important to note that the success of the model is contingent to the range of values of the parameters $p_m$, $p_w$, and $p_t$ ($10^{-4} < p_m < 10^3$; $10^{-5} < p_w < 10^{-4}$; and $10^{-5} < p_r < 10^{-4}$), for they were chosen as such to fit the simulation outputs to the timescales of the HIV progression as observed in real patients (Castiglione et al., 2004). Moreover, the model also depends on the assumption that only about 1% of virions released from infected cells are fully assembled. If more infectious virions are made, the HIV progression time scale will be much shortened (Bernaschi and Castiglione, 2002). Nonetheless, Bernaschi and Castiglione (2002) found that it was necessary to introduce one more mechanism before a reasonable time scale is achieved: the replenishment rate of mature CD4 cells into the lymphatic system will be lowered if infected cells outnumber a predefined threshold. Castiglione et al. (2004) claimed to follow the hypothesis of Wolinsky and colleagues (1996a) and criticized the antigenic diversity threshold theory. They found that “the number of escape mutants ‘alone’ is a poor indicator of disease progression”. Instead, a better indicator would be “the ratio of escape mutants to total viral diversity” (Castiglione et al., 2004).
2.2.7. Massively Multi-Agent System

Zhang and Liu (2005) devised a model that incorporated mathematical equations into a CA model by treating entities in a given lattice site that belong to the same type as homogenously distributed and calculating their values with ODEs. This Massively Multi-Agent System or MMAS model, involves a two-dimensional circular lattice of size 20 × 20, in which three entities (HIV, T cells, and other cells (O)) are found. An integer [0 ~ 1024] is used to represent an HIV ‘gene’. For each step, every lattice cell will undergo the following steps in the sequence as follows: (a) T cells and O cells at a given site are replenished if the density of immune cells at that site is below a threshold, $T_{cell}$, and that the viral density is below a threshold, $T_{hiv}$. The new cell created at that site will be a T cell with a probability of $P_{type}$; otherwise, it will be an O cell. (b) If an HIV is recognised by a T cell with the same ‘gene’, the virus will be killed and the four neighbouring sites will be ‘stimulated’ in which new T cells with the same ‘gene’ will be produced. (c) If a virus is present in a lattice site, a T cell or O cell therein might be infected with a probability of $P_{inf}$, and the cell will die with a burst size of $N_{cellhiv}$ of virions produced. (d) These virions would have the same ‘gene’ as the infecting virion, unless it mutates with a probability $P_{mutation}$, when a new sequence is randomly chosen when the virus mutates. (e) Diffusion of HIV and immune cells is allowed, depending on their density at the lattice sites. (f) Immune cells and HIV may die a natural death with density-dependent probabilities, $P_{cellnd}$ and $P_{hivnd}$, respectively (Zhang and Liu, 2005).

Zhang et al. (2005) expanded the above model and into which they incorporated the ‘sequence representation of the HIV genome’, ‘immune memory’ and ‘remote diffusion’.

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The probability of an HIV strain being recognised by T cells is determined by the similarity between the viral genome and the complementary ‘shape’ of T cells. The ‘distance’ between the virion and the T cell is the sum of the difference for each bit of the sequence, and the recognition probability is the difference between the sequence length and the ‘distance’, divided by the sequence length. Zhang et al. (2005) added a ‘global memory repertory’ to the model that takes records of all HIV strains that have been recognised by T cells already. During the creation of new T cells, for a constant probability $P_c$, the new cells would be ones that can recognise the HIV strains stored in the repertory; otherwise, they will be randomly selected from the remainder of sequence space. This mechanism ensures that a higher proportion of T cells created will recognise those HIV strains and therefore ‘immune memory’ is represented. ‘Remote diffusion’ allows immune cells and virions to migrate to a remote lattice site at a probability $P_j$, in addition to their nearest neighbour sites. “Agents adopt remote diffusion with a certain probability for the transport effect of humoral liquid or capillary vessel.” (Zhang et al., 2005) Zhang et al. (2005) claimed that this ‘remote diffusion’ forms a ‘dynamical small world network’. While a mechanism to decrease the replenishment of T cells at a later stage of HIV progression was incorporated in the early MMAS by Zhang and Liu (2005) in order to simulate AIDS, it is absent in the ‘enhanced’ version by Zhang et al. (2005). Even though success was claimed in replicating the three-stage dynamics of HIV progression (Zhang et al., 2005), their model was contingent to the chosen HIV mutation rate as shown in their own sensitivity analysis. The onset of AIDS in this model, if any, was a result of an expansion of number of HIV strains in the model and the failure of the immune system to handle therein.
The MMAS model was further developed by using a so-called ‘autonomy oriented computing (AOC)-by-self-discovery’ approach (Zhao et al., 2007), where an automated algorithm replaced the manual trial-and-error process of identifying the correct parameter values. Zhao et al. (2007) also incorporated an antiretroviral therapy component that could be turned on and off in their model.

### 2.2.8. CAFISS

CAFISS was created by Tay and Jhavar (2005) specifically to model the human immune system with HIV infection. It is a two-dimensional rectangular lattice of squares, each of which can accommodate a number of different immune cells and antibodies. They are T helper cells (uninfected CD4 cells), HIV infected cells, B cells and antibodies. This model employs bit string patterns to model immune recognition between these entities. T helper cells, once stimulated by an antigen, will be activated and divide (clonal selection). HIV infected cells will infect other CD4 cells, but may also be killed by other immune cells. B cells, when stimulated by an antigen that matches their receptors (bit string recognition) and co-stimulated by T helper cells, will release antibodies and divide. If the bit strings of the antibodies match those of certain infected cells, those cells will be destroyed.

Extending CAFISS (Tay and Jhavar, 2005), Guo et al. (2005) added to the model CTLs that could detect HIV infected T helper cells and destroy them (by releasing ‘death signals’). However, they also allowed HIV to infect B cells and CTLs, an assumption that is biologically unsound. They first created a null HIV infection model against which four different hypotheses are tested: (a) direct effect on CD4+ cell, (b) rapid viral mutation, (c)
syncytium formation (an infected T cell form a syncytium with a healthy T cell) and (d) filling of CD4+ receptor site (decreasing the activation signals that T helper cells give B cells). They claimed that their preliminary simulation results indicate that AIDS is more likely to be caused by either rapid viral mutation or syncytium formation. Although there is a genuine advantage of having a ‘null model plus hypothesis’ framework compared to the other CA models, we need to be aware that the null model itself rests on the CA assumptions. The CAFISS model is still in its infancy and I suspect that much more work is needed to be done before a proper HIV progression model based on CAFISS is created.

2.2.9. Conformon-P system

Corne and Frisco (2008) developed a ‘conformon-P model’ of HIV within-host dynamics. The ‘Conformon-P system’ (Frisco, 2004) is a variant of what computer scientists called ‘P systems’ or ‘membrane systems’ in ‘membrane computing’ (Păun, 2000, 2004). As computer science jargon, ‘membrane computing’ refers to ‘a framework for devising compartmentalized models’ (original italics, Păun, 2000). ‘P systems’ or ‘membrane systems’ in ‘membrane computing’ are a biologically-inspired way of computing to create ‘objects’ or ‘agents’ which interact in a system. The computer ‘membranes’ separate what are ‘inside’ and ‘outside’ such ‘objects’ and these ‘objects’ can move on or across these ‘membranes’ according to certain rules (Păun, 2004)6. ‘Conformon’ as a concept was first introduced in the early 1970s as ‘conformational deformation of (macro)molecules present in a cell’ (Frisco, 2004, Corne and Frisco, 2008). Frisco (2004)

6 For more information about ‘P systems’, please refer to http://psystems.disco.unimib.it/.
combined both concepts and created a ‘Conformon-P system’\(^7\) in which ‘conformons’ interact with each other and move across ‘membranes’.

In their paper, Corne and Frisco (2008) simulated HIV progression in a patient using a grid of cells, of which each is a ‘conformon-P system’ and each can be in one of five states (1-healthy, A-infected, AA-infected, pre-dead and dead)\(^8\). They compared their ‘conformon-P system’ simulation results with that of their replication of Zorzenon dos Santos and Coutinho’s (2001) physical space CA model. They suggested that the former is better because it is less contingent to initial conditions than the latter. However, the ‘conformon-P system’ simulation method is relatively new and its application in modelling biological systems is nascent. More development is needed before its usefulness in computational biology can be properly evaluated.

\(^7\) In a ‘conformon-P system’, a conformon is defined as ‘an element of the relation name-value: \(V \times N_0\)’ (where \(N_0\) is the set of non-negative natural numbers), ‘denoted by \([X,x]\)’ (where \(X\) is the name of the conformon, and \(x\), its value) (Frisco, 2004). For example, ‘if \(V = \{A, B, C, \ldots\}\)’, Then \([A, 2], [G, 26], [K, 0]\) are conformons (Corne and Frisco, 2008). There are rules that define interaction between conformons and their movements across ‘membranes’ (i.e. from one compartment to another). ‘An interaction rule is of the form: \(r: \alpha \rightarrow \beta\), where \(r\) is the label of the rule, \(\alpha, \beta \in V\) and \(n \in N_0\)’ (Corne and Frisco, 2008). For example, conformons \([V, 15]\) and \([W, 19]\) interact with a rule \(r: V \rightarrow W\), \(V\) will transfer a value of 5 to \(W\) and the outcome will be \([V, 10]\) and \([W, 24]\). Two compartments are linked with predicates that are either \(\geq n\) or \(\leq n\) (where \(n\) is a non-negative natural number), e.g. \(\geq 3\) or \(\leq 15\). If a predicate of \(\leq 15\) governs the movement of conformons between compartments \(R_1\) and \(R_2\), a conformon that is \(\leq 15\) (e.g. \([A, 2]\)) can move from \(R_1\) to \(R_2\). A number of ‘membranes’, together with conformons and interaction rules, are then grouped together as ‘modules’ to perform specific tasks (Corne and Frisco, 2008).

\(^8\) Each of these states are represented by the following conformons: ‘\([H, 1]\), [A, 1], [AA, 1], [PD, 1] and [D, 1]’ (Corne and Frisco, 2008). For example, if the cell is in the pre-dead state, it will have conformons \([H, 0]\), [A, 0], [AA, 0], [PD, 1], and [D, 0]. There are also conformons that denote ‘food’ (\(R\)) or ‘waste’ (\(W\)) for the cell, conformon that denotes virus (no virus, \([V, 10]\); virus, \([V, 11]\)) and a conformon that deals with stages of infection of a cell (E): ‘When two \([E, 1]\) are present in the same cell they can interact to create \([E, 2]\)’ and when ‘two \([E, 2]\) present in the same cell’, they ‘can interact to create \([E, 4]\)’ (Corne and Frisco, 2008). If the creation of \([E, 4]\) occurs in a healthy cell, the cell becomes infected (Corne and Frisco, 2008).
2.2.10. Some comments on agent-based models of HIV disease progression

As presented above, the results of some of the existing agent-based models are computational artefacts and the claims that they successfully simulate the HIV disease progression are unwarranted. The benefit to use agent-based models to model a system as complex as the immune system has yet been demonstrated. The rationale for developing agent-based models of HIV progression is that given such a system as complex as the human immune system, to model classes of entities (e.g. different types of cells or virus) as compartments assuming homogenous mixing therein may miss out important local interactions that are instrumental to our understanding of the HIV progression process. Henceforth, modellers adopt a bottom-up approach and build agent-based models made of individual basic entities. However, without enough prior understanding of what mechanisms through which HIV leads to AIDS, it is impossible to decide the level of details in which a model needs to be: the choice of entities and their ‘local’ interactions to be modelled and the parameters that governs such interactions. These prior knowledge and assumptions determine the model structure and, in turn, pre-determine the model outputs. As agent-based models become more and more complex, as we see above in bit-string space models, MMAS, CAFISS and conformon-P systems, models become ‘black boxes’, in which the mechanisms leading to HIV disease progression are difficult to decipher. Therefore, even though these models may have captured a lot of the immune and viral entities and their interactions, they shed little light upon our current understanding of HIV disease progression. Adding more entities to the models only
makes them even more difficult to understand. It appears that agent-based models of HIV progression are still in their infancy and their promise has yet to be fulfilled. Now, in the following section, I turn to the development of ODE models of HIV within-host dynamics.
2.3. **Differential equation models**

The main question we ask here is why were ODE models of HIV within-host dynamics built the way they were. The underlying biological assumptions of these models will be emphasised in this section. As far as the aforementioned purpose is concerned, the ‘anatomy’ of these models can be divided into the following parts: (a) CD4 cells (b) CD8 cells and (c) virions. With respect to CD4 cells, they are both target cells of HIV infection as well as helper cells facilitating CTL action. Their activation plays an important role in HIV progression (as found in target-cell-limited models). Latently infected target CD4 cells and long-lived infected cells (e.g. macrophages) facilitate the persistence of HIV infection amidst intense cART. These factors determine how many variables (and therefore how many separate ODEs) there are in a given model. CD8 cells function as CTLs and they can be sub-divided as precursor (quiescent / resting) and effector cells. Their role is important in immune control models. Virus (as free virions in circulation) can be explicitly modelled as separate ODEs or implicitly modelled as a function of infected cells. The latter case assumes a quasi-equilibrium between infected cells and virions.

The following account of ODE models will focus primarily on those of HIV progression. We will first follow a chronological framework to review their early development, from the earliest models developed in 1987 to the discovery of a quasi-equilibrium of the HIV reservoir in 1996. Models estimating the rate of viral decline following monotherapy will then be reviewed. A ‘basic’ model of HIV within-host dynamics emerged by then which later became the basis of many models. Subsequent models on HIV progression were
grouped into two major categories: target-cell-limited models and immune cell models. We will then review in brief various models addressing a number of different issues.

2.3.1. Early models

2.3.1.1. The earliest models

The earliest published ODE models of HIV within-host dynamics were those presented in the theoretical immunology workshop, June 1987, at the Sante Fe Institute (Intrator et al., 1988, McLean, 1988, Merrill, 1988). The next one was that of Anderson and May (1989), which described the HIV within-host dynamics and tackled the possibility of opportunistic infection activating CD4 cells leading to faster progression to AIDS. They demonstrated with the model the potential for highly oscillatory or chaotic HIV within-host dynamics. Some more models were soon developed (McLean and Kirkwood, 1990, Nowak and May, 1992, Perelson et al., 1993).

2.3.1.2. Within-host evolution: Antigenic diversity threshold theory

Nowak and colleagues proposed the antigenic diversity threshold “theory” and suggested that the development of AIDS was the result of a continual increase in antigenic diversity of HIV within the host leading to a threshold above which the human immune system could not cope and subsequently collapsed (Nowak et al., 1990, Nowak et al., 1991, Nowak and May, 1991). Some studies followed this model, e.g. (de Boer and Boerlijst, 1994). However, Nowak et al.’s modelling results were later criticised as being too dependent on the initial conditions, including mutation rate, initial virus population size, initial viral diversity and the antigenic diversity threshold itself and its composition.
Furthermore, the antigenic diversity threshold did not capture the full picture of the model dynamics, and therefore its significance was challenged by Stilianakis and colleagues (1994). There were empirical observations in patients that were inconsistent with Nowak et al.’s hypothesis (Wolinsky et al., 1996a). Nowak et al. (1996) disputed Wolinsky et al. arguing that they had mis-interpreted their model and that Wolinsky et al.’s results were actually consistent with predictions of their model. However, Wolinsky et al. (1996b) argued that the antigenic diversity threshold hypothesis did not explain the limited diversity observed among those HIV positive individuals who experienced a rapid decline in CD4 cell count after primary infection and that for those whose decline in CD4 cell count was slow, the existence of an “antigenic diversity threshold” was yet to be demonstrated empirically (Wolinsky et al., 1996b).

2.3.2. Dynamic equilibrium & basic models

2.3.2.1. Dynamic equilibrium

In 1995, both Wei et al. (1995) and Ho et al. (1995) found that the HIV viral load in human plasma after acute infection was in a dynamic equilibrium, or a quasi-steady state, in which virions were rapidly produced through rapid CD4 cell death, which was compensated through a massive regeneration of lymphocytes. This discovery revolutionised our understanding of HIV pathogenesis and the quantitative findings on HIV and CD4 cell turnover rates were fed into later developments of HIV within-host models.
2.3.2.2. Basic model(s)

Perelson (2002) described a basic model of HIV within-host dynamics with only three equations that has served as the basis of many models (the equations below follow the notations of Nowak and Bangham (1996)):

\[
\frac{dx}{dt} = \lambda - dx - \beta xv \\
\frac{dy}{dt} = \beta xv - ay \\
\frac{dv}{dt} = ky - uv
\] (2.1-2.3)

Uninfected cells, \(x\), die at a rate of \(dx\) and are replenished at a constant rate of \(\lambda\). They are infected at a rate of \(\beta xv\). Infected cells, \(y\), die at a rate of \(ay\). Virus, \(v\), is produced at a rate of \(ky\) and is cleared at a rate of \(uv\). The basic model can be extended to incorporate different assumptions. The equation for free virus can also be removed if we assume that it is at equilibrium and is proportional to the virus-producing cell population before and during therapy (Bonhoeffer et al., 1997a). If we incorporate the actions of reverse transcriptase inhibitor (RTI) and PI into the model (Callaway and Perelson, 2002), we have (following my own notation),

\[
\frac{dx}{dt} = \lambda - dx - (1 - drug_{RT}) \beta xv \\
\frac{dy}{dt} = (1 - drug_{RT}) \beta xv - ay \\
\frac{dv_{RT}}{dt} = (1 - drug_{RT}) ky - uv \\
\frac{dv_{PI}}{dt} = drug_{PI} ky - uv
\] (2.4-2.7)
With RTIs preventing productive infection of cells and PIs the production of infectious virions. One specific challenge modellers faced was how to develop a model that replicates the observed sustained quasi-equilibrium of viral load that eventually leads to AIDS and the sustained suppression of viral load to a very low level by cART that will rebound very soon after treatment is interrupted (Bonhoeffer et al., 1997a, Callaway and Perelson, 2002).

Variations on the basic model exist and one such is adding an extra term to equation 2.4, \(-q_{xy}\), whereby \(q\) represents virus-induced killing of uninfected cells and differential effects of drugs on various cell types (Bonhoeffer et al., 1997a).

2.3.3. Viral decay in the presence of ART

2.3.3.1. Early estimations of rate of viral decay in the presence of drug

Perelson et al. (1996) employed the above basic model and estimated the rate of viral decay in the presence of a PI, assuming that the drug is 100% effective and there is no delay in effect. They fitted the model to data obtained from ritonavir-treated HIV patients and obtained an estimation of virion clearance rate (\(c\), mean \(3.07 \pm 0.64\) day\(^{-1}\)), rate of loss of infected cells (\(\delta\), mean \(0.49 \pm 0.13\) day\(^{-1}\)) and average viral generation time (\(\tau\), mean \(2.6 \pm 0.8\) day\(^{-1}\)).
In order to capture the second-phase decay of plasma virus concentration, Perelson et al. (1997) extended the model to include latently infected T cells and long lived infected cells (using my own notations):

\[
\frac{dy}{dt} = kvx + ay_L - \delta y \\
\frac{dy_L}{dt} = fkvx - \mu_M y_L \\
\frac{dy_M}{dt} = k_M vx_M - \mu_M y_M \\
\frac{dv}{dt} = N\delta y + py_M - cv
\]  

(2.8)

CD4 cells, \(x\), are infected at a per cell rate of \(kv\) and become \(y\) and \(x\) become latently infected CD4 cells, \(y_L\), at a per cell rate \(fkv\) (\(f<<1\)). Infected cells, \(y\), die at a per cell rate of \(\delta\) and produce \(N\) virus per cell at this point. The latently infected cells, \(y_L\), are activated at a per cell rate of \(a\) and die at a per cell rate of \(\delta L\), and \(\mu_M = a + \delta L\). There are also cells, \(y_M\), which upon infection at a per capita rate of \(k_Mv\), become long-lived infected cells, \(y_M\), which die at a per cell rate of \(\mu_M\) and produce virus at a per cell rate of \(p\). Virus is cleared at a per capita rate of \(c\). In both models, the time scale is short and therefore the uninfected cell pool is assumed to be constant.

### 2.3.3.2. Intracellular delay in viral decay

An intracellular delay term was first introduced to the basic model in Herz et al. (1996) to achieve a better estimate of viral decay. It was a discrete and fixed time lag of which the infection term in the infected cells equation is a function. Mittler et al. (1998) made the delay term continuous according to a gamma distribution. While the intracellular delay – from infection of cells to virus production – was modelled through an exponential decay
for the infection term in the infected cell equation in Herz et al. (1996), it was modelled in Tam (1999) through a delay term in the virus equation (instead of the infected cells equation) – a virus production term that was a function of the value of infected cells a finite period in the past.

2.3.3.3. More models on viral decay and loss of productively infected cells

The efficacy of protease inhibitor in the modelling of Herz et al. (1996) and Mittler et al. (1998) was assumed to be perfect. By explicitly modelling non-perfect drug potency, Ding and Wu (1999) found that the viral decay rates at the early stage of cART are related directly to the efficacy of cART. Nelson et al. (2000) found too that the effect of the intracellular delay on the exponential decline of viral load was masked by this assumption which, if relaxed, allowed a more realistic estimation of the loss rate of productively infected T cells and the rate of viral decay to be obtained. Nelson and Perelson (2002) further extended this to compare a continuous delay term with a discrete one under an imperfect drug and found that the discrete one fit data better. They also studied RTI with a delay and argued that because RTI would lead to a decrease in concentration of productively infected T cells produced, it would generate a higher estimate of their loss rate than from the use of PIs which allow for some viable virus. They also studied the possibility of relaxing the assumption of setting uninfected T cells as a constant (quasi-equilibrium), so that the model could be generalised to be applied in other scenarios.
2.3.4. Immune control models – Cytotoxic lymphocytes and other mechanisms

2.3.4.1. Basic CTL control models

Nowak and Bangham (1996) (and also Nowak and May (2000), Section 3.1, Ciupe et al. (2006), Section 2.2) extended the basic model in section 2.3.2.2 to capture a self-regulating CTL response, a nonlinear CTL response or a linear CTL response, by first adding a term $-pyz$ to the equation for infected cells, representing CTL cells, $z$, destroying infected cells, $y$, at a rate $pyz$:

$$\frac{dy}{dt} = \beta x v - ay - pyz \quad (2.9)$$

A fourth equation describing CTL response was then added. For a self-regulating CTL response (Nowak and May, 2000, Section 6.1), where CTL growth is constant, assuming that $c = 0$ in the absence of infected cells and $c > 0$ if $y > 0$:

$$\frac{dz}{dt} = c - bz \quad (2.10)$$

For a non-linear CTL response (Nowak and Bangham, 1996, Nowak and May, 2000, Section 6.3) where the growth of CTLs is a function of concentrations of both infected cells and CTLs:

$$\frac{dz}{dt} = cyz - bz \quad (2.11)$$
However, Bonhoeffer et al. (1997a) found that this model extension did not work as viral load did not change under treatment.

They argued that there could be a linear CTL response (Bonhoeffer et al., 1997a, Ciupe et al., 2006, Section 2.1, Nowak and May, 2000, Section 6.4) where the growth of CTLs is a function of concentration of infected cells:

\[
\frac{dz}{dt} = cy - bz
\]  

These early models of HIV within-host dynamics are the foundations upon which many recent models are built. Here I will examine further models that capture different aspects of HIV virology and human immunology.

### 2.3.4.2. More complex CTL control models

De Boer and Perelson (1998) developed an immune control model based on CTL control. In order to distinguish it from the target-cell-limited model, they assume that virus replication is not limited by the density of target cells:

\[
\frac{dy}{dt} = \beta v - pyz
\]  

However, they also indicated that it is possible to combine both target-cell-limited and immune control models by making the assumption that the target cell infection rate is a saturating function of the density of target cells:

\[
\frac{dy}{dt} = \beta v \frac{x}{\theta + x} - pyz
\]
If the constant $\theta >> x$, it becomes a target cell limited model; if $\theta << x$, it becomes an immune control model.

Taking equations 2.9 and 2.11 together, it will yield a “structurally non-robust Lotka-Volterra predator-prey model with perpendicular nullclines” (De Boer and Perelson, 1998). One can create a more realistic model by changing equation 2.9 and using an activation function that saturates,

$$\frac{dz}{dt} = \frac{czy}{1+\varepsilon_y} - b_z$$  \hspace{1cm} (2.15)

This yields a non-robust perpendicular nullcline (De Boer and Perelson, 1998). Based on their earlier T cell proliferation model (De Boer and Perelson, 1995), they developed a structurally robust immune-control model that allows for competition between CTLs when interacting with infected cells:

$$\frac{dz}{dt} = \frac{czy}{1+\varepsilon_y+\varepsilon_z} - b_z$$  \hspace{1cm} (2.16)

### 2.3.4.3. CTL precursors

Wodarz and Nowak (1999) extended the CTL control model to include CTL precursors, $w$, while assuming the viral load is in equilibrium with infected cells (for a version of this model with an explicit ODE for virus concentration, see Wodarz and Nowak (2002)):  

110
\[
\begin{align*}
\frac{dx}{dt} &= \lambda - dx - \beta xy \\
\frac{dy}{dt} &= \beta xy - ay - pyz \\
\frac{dw}{dt} &= cxyw - cqyw - bw \\
\frac{dz}{dt} &= cqyw - hz
\end{align*}
\] (2.17)

CTL precursors differentiate into effector CTLs, \(z\), at a rate of \(cqyw\). CTL precursors, \(w\), die at a per cell rate of \(b\) while effectors, \(z\), die at a per cell rate of \(h\). Wodarz and Nowak (2000b) argued that it was when memory CTL precursors fall below a critical threshold that HIV grows uncontrolled leading to AIDS. They suggested that the breadth of CTL response is important in efficient virus control (Wodarz and Nowak, 2000a). They advocated that early ART will boost memory CTL precursors and, among chronic patients, structured treatment interruptions might lead to re-establishment of CTL memory (Wodarz and Nowak, 1999). However, a recent study found that structured treatment interruptions of more than six months are detrimental to patients leading to increased treatment failure (Leon et al., 2009).

### 2.3.4.4. Immune control in primary infection

To model primary infection, Stafförd et al. (2000) compared two possible immune response mechanisms developing from the basic model (equations 2.1 to 2.3). They modelled CTL-mediated cytolysis with a composite death rate of infected cells (i.e. \(a\) in equation 2.2) which equals \(\delta_0 + \delta_f(V)\) where \(\delta_f(V)\) is 0 if \(t < t_f\), and \(f(t)V\) when \(t \geq t_f\) – where

\[
f(t) = \frac{\beta}{1 + \kappa e^{-(t-t_f)/\Delta t_1}} - \frac{\beta}{1 + \kappa e^{-(t-t_f)/\Delta t_2}}
\] (2.18)
to mimic expansion of effector cells between \( t_1 \) and \( t_2 \) and their decline after \( t_2 \). They modelled CD8+ T-cell antiviral factor (CAF) by reducing the virus production rate coefficient (i.e. \( k \) in equation 2.3) to 35% of its original value shortly after the first minimum in viral load. They were unable to tell whether one mechanism was better than the other in representing the course of primary infection.

Ciupe et al. (2006) compared an effector model (basic model plus equation 2.14), its extension with time delay (delay model 1), and a logistic model with time delay (delay model 2). The CTL equation of both delay models 1 and 2 is (using my own notation):

\[
\frac{dz}{dt} = cy(t - \tau) - bz
\]  

(2.19)

The equation of uninfected CD4 cells for delay model 2 (using my own notation):

\[
\frac{dx}{dt} = \alpha x \left(1 - \frac{x + y}{T_{max}}\right) - kvx
\]  

(2.20)

They found that their delay model 1 fits better to patients’ data than Stafford’s immune response model; but both their delay models 1 and 2 fit the data equally well.

### 2.3.4.5. CTL control – limited attributable results

It was found that there is a delay in CTL response to HIV and it is too little to prevent HIV from establishing a persistent infection (Davenport et al., 2004, Davenport et al., 2005). Asquith et al. (2006) and Ganusov and De Boer (2006) studied the role of CTLs in controlling HIV-1 infection. Borrowing from models of previous viral resistance (see section 2.3.7), both their models are essentially the same and are as follows (using my own notation instead of original):
There are two types of infected CD4 cells. One is infected with wild-type virus, \( w \), and the other with a variant virus \( m \), with an escape epitope. Both are killed by CTLs that recognise epitopes other than the escape epitope at a rate of \( d \) (assuming \( d' = d \)), but the ones with wild-type virus are also killed at a per cell rate of \( k \) by CTLs that recognise the wild-type but not the variant. The cells, \( w \) and \( m \), replicate at a net per cell rate of \( r \) and \( r' \) respectively. Ganusov and de Boer (2006), defined \( r' = r(1-c) \), assuming a reduced growth rate of the mutant variant, and they also considered two possibilities: (1) increase death rate of mutant due to cost of mutation: \( d' = d + k \) while \( r' = r \); (2) noncytolytic CD8 cell response:

\[
\frac{dw}{dt} = \frac{rw}{1+E+k} - dw \text{ wild-type}
\]
\[
\frac{dm}{dt} = \frac{r(1-c)m}{1+E} - dm \text{ variant}
\] (2.22)

Asquith et al. (2006) estimated the selection pressure exerted by CTL responses that drove “the emergence of immune escape variants, thereby directly quantifying the efficiency of HIV-1-specific CTLs in vivo”. Their estimation of productively infected CD4 cell death attributable to CTLs recognising a single epitope is only 2%. They proposed that CTLs kill about \( 10^7 \) (i.e. 10% of) productively infected cells per day but are not responsible for the majority of infected cell death (Asquith et al., 2006). Using this model, Asquith and McLean (2007) also found that CTLs kill infected cells faster and escape variants bear higher fitness costs in macaques than in humans. Also, Ganusov and
de Boer (2006) found that to get a minimal estimate of the fitness cost of the escape mutation, one has to assume unlimited virus growth, and that to get a minimal estimate of the average killing rate, one has to assume no virus growth during the escape. Althaus and de Boer (2008) developed a multiple CTL response model with escape and compensatory mutations to explain the sequential and late occurrence of CTL escape mutants.

### 2.3.5. Target-cell-limited models – CD4 cells as targets and helpers

#### 2.3.5.1. Basic target-cell-limited models

In 1996, a target-cell-limited model was proposed that, through limiting target cells, viral load could be controlled in the model (De Boer and Boucher, 1996). De Boer and Perelson (1998) categorised target-cell-limited models into two categories: logistic models and activated T cell models.

The logistic model’s equation for uninfected target cells are as follows (following my own notation) (De Boer and Perelson, 1998):

\[
\frac{dx_A}{dt} = \alpha_A x_A \left(1 - \frac{x_{built}}{x_{max}}\right) - (\beta + \gamma) x_A y
\]  

(2.23)

In the above equation, \(\beta\) represents a true infection rate and \(\gamma\) represents “all other virus induced depletion” of uninfected target cells, \(x_A\) (De Boer and Perelson, 1998). In some models, an additional term (usually plus a constant) was added to indicate a constant
supply of CD4 cells from some sources in the body, e.g. thymus, and differentiate it from proliferation of CD4 cells from existing cells (Perelson and Nelson, 1999).

For the activated T cell model, an equation for quiescent cells, \( x \), was added and that for uninfected (activated) target cells, \( x_A \), became (following my own notation) (De Boer and Perelson, 1998):

\[
\frac{dx}{dt} = \frac{2rx_A}{1 + \frac{x_{total}}{x_{max}}} - \left( \alpha_{\theta} + \delta_{\theta} \right)x
\]

\[
\frac{dx_A}{dt} = \alpha_{\theta}q - rx_A - \left( \beta + \gamma \right)x_A
\]

The concept that the target cells of HIV infection are predominantly activated CD4 cells is an important part of later model development and will be discussed in Section 2.4.4.

### 2.3.5.2. CD4 helper cells as mediator of CTL response

CD4 helper cells are important in the CTL response to control viral infection and yet they themselves are targets of HIV infection. Korthals Altes et al. (2002) extended the model in equation 2.17 (Wodarz and Nowak, 1999) to include a separate equation for T helper cells (using my own notation):
\[
\frac{dx}{dt} = \lambda - dx - \beta xy
\]
\[
\frac{dx_{H}}{dt} = \frac{ry_{x_{H}}}{x_{H} + \gamma} - dx_{H} - \beta x_{H}y
\]
\[
\frac{dy}{dt} = \beta y\left(x + x_{H}\right) - ay - pyz
\]
\[
\frac{dw}{dt} = cx_{H}yw - cqw - bw
\]
\[
\frac{dz}{dt} = cqw - hz
\]  \hspace{1cm} (2.25)

Where \(x_{H}\) denote HIV-specific CD4 helper cells while \(x\) represents all other target cells (others the same as in equation 2.17). They found that unless a sustained CTL response is maintained over the long term, in a target-cell-limited scenario, increasing the CD4 T helper proliferation rate is detrimental to HIV+ individuals.

Korthals Altes et al. (2003) extended the above model to explain different viral set points by the abundance of HIV-specific CD4 and CD8 response. The three main biological assumptions of the model are that (a) the immune response is directly proportional to the number of HIV-specific CD4 cells (no separate CTL equations); that (b) there are lytic and non-lytic components of the immune response, assuming that the latter protects a cell against infection and the former is responsible for the direct killing of infected cells; and that (c) there is a density-dependent death rate of T-helper cells (representing intra-cell type competition). Comprising of target cells not involved in the immune response (\(x\)), HIV-specific CD4 T cells (\(x_{H1}\) and \(x_{H2}\)) and infected cells (\(y\)), the model is as follows (in my own notation):
\[ \frac{dx}{dt} = \lambda - dx - \beta xy \]
\[ \frac{dx_{H1}}{dt} = \lambda_{H1} + \frac{ry_{H1}}{\gamma_1 + y} - \varepsilon x_{H1}^2 - \beta_{H1} x_{H1} y N(x_{H1}, x_{H2}) \]
\[ \frac{dx_{H2}}{dt} = \lambda_{H2} + \frac{ry_{H2}}{\gamma_2 + y} - \varepsilon x_{H2}^2 - \beta_{H2} x_{H2} y N(x_{H1}, x_{H2}) \]
\[ \frac{dy}{dt} = y \left[ \beta x + \beta_{H1} x_{H1} + \beta_{H2} x_{H2} \right] N(x_{H1}, x_{H2}) - ay - K(x_{H1}, x_{H2}) y \]

(2.26)

where

\[ N(x_{H1}, x_{H2}) = \frac{1}{1 + n_1 x_{H1} + n_2 x_{H2}} \quad \text{Non-lytic response} \]

\[ K(x_{H1}, x_{H2}) = k_1 x_{H1} + k_2 x_{H2} \quad \text{Lytic response} \]

\[ \gamma_2 = g \gamma_1 \quad \text{and} \quad g > 1 \]

As both targets of infection and mediators of the CTL response, Korthals Altes et al. (2003) suggested that the number of HIV-specific CD4 cell clones was the key to understand the difference in viral set points of patients whose viral and immune parameters are otherwise similar, and that they are in turn determined by their initial abundance and viral inoculum at the time of infection. However, in a later model, which was an extension of equation 2.24, but can represent a potential of \( n \) clones of HIV-specific CD4 cells of different avidity instead of two, Korthals Altes et al. (2006) rejected their previous conclusion and argued that it was the quality (avidity) more than quantity (breadth) of the immune response, that determine viral set points and time to AIDS. However, it appears that their conclusion is at least partially inherent in their model assumptions, given that \( n \) was set to 200 for every patient, and it was obviously that for a lower avidity average, the time to AIDS would be shorter. While the antigenic diversity threshold theory (section 2.3.1.2) proposes that as HIV becomes more diverse and above a critical threshold in the host, immune control of virus fails, the reciprocal is Korthals Altes et al.’s (2006) model where the virus is assumed to be uniform (or a fast succession
of viral variants in a different interpretation) while as disease progresses, the immune response becomes less and less diverse because escape mutants render clones of HIV-specific immune cells useless. I concur that the model output is consistent with its assumptions, but it cannot prove that the assumptions are right.

### 2.3.5.3. Activation heterogeneity within CD4 cell sub-populations

While the major focus of Korthals Altes et al.’s (2006) model was the diversity of avidity of HIV-specific CD4 cell clones, Ferguson et al.’s (1999) was activation heterogeneity (heterogeneous distribution of cell turnover rates) within CD4 cell sub-populations caused by past and present exposure to a diverse array of antigens. They did not explicitly model the CD4 cell activation process, but they obtain a bimodal distribution of proliferation rate of non-naive CD4 cells, and effectively generate the quiescent and active cell pools.

By neglecting individual antigen responses and activation/deactivation process and by approximating the dynamically generated proliferation distribution with a static bimodal parametric form, Ferguson et al. (1999) modelled density-dependent proliferation of CD4 cells that described the short-term post-therapy dynamics.

Ferguson et al. (1999) claimed that their model explained (a) a rapid multiphase decline in HIV viral load after cART, (b) a rapid initial rebound in CD4 cell count after treatment, followed by a slower recovery phase, (c) a low prevalence of infected CD4 cells in both lymph nodes and peripheral blood, (d) a viral load rebound after vaccination, and (e) a higher rate of CD4 cell replication after treatment than before therapy. They predicted
that the outcome of treatment that stimulated CD4+ T cell activation critically depends on the level of residual viral replication under the accompanying antiretroviral therapy. They also highlighted the redistribution of CD4 cells between the lymphatic system and peripheral blood as an explanation of the rebound of CD4 cell counts in the first few weeks of therapy (Ferguson et al., 1999). Ghani et al. (2002) employed Ferguson et al.’s (1999) model to compare four different cART regimens’ effects at reducing viral replication.

2.3.5.4. CD4 cell activation as major driving force for pathogenesis

Fraser and colleagues (2001b) developed a model within which the driving force for AIDS development was antigen-driven CD4 cell activation and therefore it was a target-cell-limited model. The model was based on the basic equations in section 2.3.2.2 but without explicitly modelling viral load, assuming that viral load is in equilibrium with infected cells. They explicitly modelled the activation process of spatially localized invasion of non-HIV pathogens through a stochastic mechanism, with separate equations for quiescent and activated CD4 cells, and likewise for CD8 cells. This stochastic antigenic stimulation reproduced stochastic viral bursts under treatment when the usual lower-than-unity $R_0$ becomes greater than 1 as a result of raised antigenic stimulation. An unspecific homeostatic regulation of T cells that controls the total number of CD4 and CD8 cells is introduced into the equations that contribute to a shift of balance towards CD8 cells (as CD4 cells are disadvantaged due to infection). The model also contains equations for infected cells, latently infected cells and HIV-specific CTLs. They also developed a surrogate measure for CD4 helper cell response. It was found that once treatment is sufficiently potent to reduce $R_0$ to below unity, CTL cytolytic activity will
rapidly cease, thus increasing the infected cell lifetime, sustaining the lower viral load under treatment (Fraser et al., 2001b).

### 2.3.5.5. Vaccination and CD4 cell activation

Jones and Perelson (2002) introduced a model of CD4 cell activation by ‘vaccination with a common recall antigen (tetanus toxoid)’ on chronic HIV positive individuals who were untreated. This model was an extension of Perelson et al. (1996) and Nowak and May (2000) (using my own notations):

\[
\begin{align*}
\frac{da}{dt} &= -\gamma ax \\
\frac{dx}{dt} &= \lambda + \alpha\left(\frac{a}{a + K}\right)x - dx - \beta vx \\
\frac{dy}{dt} &= (1 - f_c)\beta vx - \delta y \\
\frac{dy_c}{dt} &= f_c\beta vx - \mu y_c \\
\frac{dv}{dt} &= N\delta y + N_c\mu y_c - cv
\end{align*}
\]

The decline of vaccine antigen, \(a\), is CD4 cell dependent with a rate constant \(\gamma\). The activation of CD4 cells, \(x\), is dependent on antigen concentration, with a maximum rate \(\alpha\) and a half saturation constant \(K\). In the absence of virus, \(x\) dies at a per cell rate of \(d\). When being infected, \(x\) becomes actively infected, \(y\), or chronically infected, \(y_c\), which in turn die at per cell rates of \(\delta\) and \(\mu\) respectively. Virus, \(v\), is cleared at a per virus rate of \(c\).

It is important to note that this model assumes that both actively and chronically infected cells will produce virions, in numbers \(N\) and \(N_c\) per cell respectively.

With this model, Jones and Perelson (2002) showed that antigen activation of CD4 cells by vaccination can explain the small increases in viral load after tetanus booster
vaccination of HIV patients (Stanley et al., 1996), but the long-term impact on disease progression is small.

2.3.5.6. **Blood-lymph transport and CD4 cell activation**

To correct for the inaccurate timescale of early CD4 cell increase after commencement of cART in a previous model (Ferguson et al., 1999), Griffin (James T. A. Griffin, PhD thesis, Imperial College London, November 2006) and colleagues (Griffin et al., 2006) extended Fraser et al.’s models (Fraser et al., 2001a, Fraser et al., 2001b) to include blood-lymph transport of quiescent target CD4 cells. These cells were categorised as four variables (one ODE each) – marked or not by contact with HIV; lymph or blood. “Marked” here refers to raised expression of L-selectin or other cell-surface proteins that lead to an increased speed of a cell moving to the lymphatic system.

2.3.6. **Steady low viral load and latent viral reservoir**

Compared to the constraints suggested by Bonhoeffer et al. (1997a), Callaway and Perelson (2002) argued that the constraints on HIV within-host dynamics models are actually more stringent, due to the potency of cART when it is compared to monotherapy. They proposed three models that reproduced a low steady state of viral load on treatment. In the first model, the death of infected cells is density-dependent, rather than constant. The second and third model feature heterogeneous drug efficacy (either spatial or phenotypic).
Kim and Perelson (2006) investigated the relationship between latently infected cells and the persistence of HIV in the presence of cART by extending previous published models (Callaway and Perelson, 2002, Perelson et al., 1997, Perelson and Nelson, 1999, Perelson et al., 1996). Their model consists of seven ODEs – target cells (uninfected, infected, latently infected); long-lived CD4 cells (uninfected and infected); and virus (infectious and non-infectious). One new feature of this model is the introduction of the concept of “bystander” proliferation – occasional proliferation of latently infected CD4 cells without being activated into clonal expansion and therefore viral production. Another interesting assumption is the decline of activation of cells to a minimum level as patients undergo cART, as latently infected cells specific to common antigens have been activated and die. They confirmed that the persistence of low-level activation of target CD4 cells is a major factor that influences the persistence of the virus and the latent reservoir, and that the increase of CD4 cells under cART paradoxically hastens disease progression unless cART remains effective through the whole time course. They suggested that possible reasons for persistence of HIV under cART (real efficacy below critical efficacy) included, (a) over-estimation of the efficacy of current cART regimens, (b) long-lived infected cells, and (c) a higher regeneration rate constant (i.e. rate of bystander proliferation minus rate of death) of latently infected cells. Even if the real efficacy is above the critical efficacy, it may still take a very long time to clear the virus. Their results suggested that the intrinsic stability of latently infected cells, maintained by possible mechanisms like bystander proliferation, rather than ongoing viral replication, is the key factor in maintaining the persistence of HIV under cART. If this is true, Kim and Perelson argued that therapy should be designed to flush out the infected cells rather than
suppress their activation, assuming that effective suppression of viral replication is in place. They concluded that the key factors for maintaining HIV persistence under cART are (a) bystander proliferation and (b) persistence activation of latently infected cells, and (c) ongoing viral replication to be determined by (d) cART efficacy.

Another model was developed by Yate and his colleagues (2007) to test whether the ‘runaway’ hypothesis – activation-induced cell death of immune activated CD4 cells – explains the gradual decline of CD4 cells during the chronic phase of HIV progression (De Boer, 2007). However, they found that CD4 cells deplete too fast in their model and therefore reject this hypothesis (Yates et al., 2007).

2.3.7. Selection of the fittest

2.3.7.1. The reproduction number increase hypothesis

Schenzle (1994) proposed an alternative model that connected HIV pathogenesis with HIV within-host mutations. It hypothesised that HIV would evolve within-host towards a higher cell infection rate and therefore a higher reproduction number and would eventually lead to the collapse of the immune system and therefore the development of AIDS. Its results were further analysed in Stilianakis et al. (1997b) and were extended in Stilianakis and Schenzle (2006). The results were independent of initial dose but highly dependent on the evolutionary speed of HIV, the initial infection rate and the initial CD4 cell count (Stilianakis and Schenzle, 2006).
2.3.7.2. Viral fitness and burst size

Through an invasion analysis, based on a cell age-structured model of viral dynamics, Gilchrist et al. (2004) derived the within-host relative viral fitness and suggested that HIV replication rates would vary between cells with different life spans. They found that “for chronic infections, in the absence of trade-offs between viral life history stages, natural selection favours viral strains whose virion production rate maximises viral burst size,” (Gilchrist et al., 2004) while natural selection favours production rates lower than the one that maximizes burst size in the presence of trade-off events like that between virion production and immune system recognition and clearance of virally infected cells.

2.3.8. Dynamics of resistance

There are a number of models that were developed to model explicitly different strains of HIV in order to elucidate problems associated with antiretroviral resistance (Althaus and Bonhoeffer, 2005, Bonhoeffer et al., 1997b, Bonhoeffer and Nowak, 1997, Bretschger et al., 2004, Fraser, 2005, Ribeiro and Bonhoeffer, 1999, 2000, Rong et al., 2007a, Rong et al., 2007b, Stilianakis et al., 1997a). A model of a pulsing (‘impulsive’) drug effect was also employed to study drug resistance (Smith and Wahl, 2005). However, since the major question that these models addressed was not HIV progression, they are not included in this literature review. For a review of their basic concepts, see section 5 in Müller and Bonhoeffer (2003).
2.3.9. Multi-infected cells

Dixit and Perelson (2004) found that the distribution of proviral genomes in HIV-1-infected splenocytes can be captured quantitatively by two mechanisms: 1) in a series of sequential infectious contacts of a target cell with free virions and infected cells, multiple genomes are acquired one at a time; and 2) a single infectious contact of a target cell with an infected cell leads to cell-to-cell transmission of multiple virions or genomes. “The two mechanisms imply different genetic diversities of proviruses within an infected cell and therefore different rates of emergence of drug resistance via recombination” (Dixit and Perelson, 2004).

In their next paper, Dixit and Perelson (2005) found that “the experimentally observed scaling law, that the number of cells coinfected with two distinctly labelled viruses is proportional to the square of the total number of infected cells, can be generalized so that the number of triply infected cells is proportional to the cube of the number of infected cells, etc.”, but this scaling relationship holds only under certain conditions (Dixit and Perelson, 2005). They also found that “multiple infections do not influence viral dynamics when the rate of viral production from infected cells is independent of the number of times the cells are infected, a regime expected when viral production is limited by cellular rather than viral factors” (Dixit and Perelson, 2005).

2.3.10. Viral blips

As discussed in chapter 1, viral blips may arise due to a number of different possible mechanisms, of which four have been studied by Perelson and his colleagues: (a)
opportunistic infection, (b) target cell pools with heterogeneous drug penetration, (c) activation of latently infected cells and (d) asymmetric division of activated latently infected cells.

(a) **Opportunistic infection:** In this case, to create viral blips of reasonable amplitude and duration, a model was developed that incorporated separate antigen-specific and non-specific target pools, and programmed proliferation of both CD8 effector cells and antigen-specific target CD4 cells. Opportunistic infection stimulated the proliferation of antigen-specific target cells and therefore viral replication (and thus blips) (Jones and Perelson, 2005).

(b) **Target cell pools with heterogeneous drug penetration:** This model consisted of two target cell pools subject to different drug penetration, but no density-dependent cell death was invoked. Blips arose out of the target cell pool where there was reduced drug penetration – the pool is known as a drug ‘sanctuary’ (Jones and Perelson, 2005), and it was shown that blip amplitude is a linear function of baseline VL on a log-log plot (Jones and Perelson, 2007).

(c) **Activation of latently infected cells:** This model incorporated a cell pool of latently infected cells (memory cells). Activation of these cells also produced viral blips for which amplitude was also a linear function of baseline VL on a log-log plot (Jones and Perelson, 2007).

(d) **Asymmetric division of activated latently infected cells:** The idea that latently infected cells, upon activation by antigen, may divide and differentiate according to certain probabilities, into (i) two latently infected cells, (ii) one latently infected cell and one activated infected cell, and (iii) two activated infected cells, was hypothesized as a
possible mechanism for both viral blips and the replenishment of latent reservoir. A model that featured this mechanism was developed and it generated viral blips of reasonable amplitude and duration as well as various decay profiles of the latent reservoir (Rong and Perelson, 2009).

2.3.11. Drug adherence

Adherence to antiretroviral therapy and its relationship with the emergence of resistant strains also caught the attention of mathematical modellers. A model with two virus strains (one sensitive and one resistant) were used to predict treatment outcome for different patterns of adherence (Wahl and Nowak, 2000). This theme was further taken up by Krakowska and Wahl who developed a model that examined the costs and benefits of drug treatment in different scenarios. It was found that tolerance for drug-resistant mutations (Krakovska and Wahl, 2007a) and patterns of adherence (Krakovska and Wahl, 2007c) had major impacts upon the ‘optimal’ regimen. Congruent with clinical observations, structured treatment interruptions were found to be rarely optimal in their treatment effect and ‘drug coasting’ – very short drug-free periods in which drug concentrations are still adequate due to the long half-life of certain drugs – was proposed instead (Krakovska and Wahl, 2007b).

Another line of model development was to assess the distinct effects of PI and RTI (Smith, 2008, Smith and Wahl, 2004). Smith and Wahl (2004) developed a model with an ‘impulsive’ drug effect; Smith (2008) simplified it to a continuous version to avoid discontinuities in the model and to examine its underlying dynamics. Interestingly, by
comparing two models, with and without explicit modelling of T cell infection dynamics, Smith (2008) found that PI-only therapy, but not RTI-only therapy, would lead to treatment failure.

A third line of model development took account of pharmokinetics. It started with a simple 4-ODE dynamics model of logistic target cell growth and infection, with separate equations for infectious and non-infectious virions. Drug adherence was modelled as different patterns of missing doses (Huang et al., 2003). A simplified version (3-ODE; no logistic target cell growth and no differentiation between RTI and PI and therefore one equation for virions only) was then fitted to data from a clinical trial, using a composite measure for drug adherence, taking into account the impact of adherence on IC\textsubscript{50} and EC\textsubscript{50} (Wu et al., 2005b). A hierarchic Bayesian modelling approach was used in Wu et al. (2005) for parameter estimation. This was further explored in two subsequent papers (Huang et al., 2006, Huang and Wu, 2006). The model was tested against short-, middle-, and long-term virological responses and its parameters were found to be robust in all three cases (Huang, 2007). Huang (2007) suggested that short-term data may be useful to predict long-term responses through modelling. Further studies using subject-specific data on pharmacokinetics, drug susceptibility (IC\textsubscript{50}) and drug adherence (measured by pill counts) found that drug adherence adds little to prediction of virological outcome in the models (Wu et al., 2006). Wu et al. (2006) suspected that it was because pill count as a measurement was not accurate enough. The model was later reparameterised where parameters were estimated through model fitting to drug adherence data measured using MEMS (Labbé and Verotta, 2006). This model was also used in another study where data
from both MEMS and questionnaires were used (Huang, 2008). While Labbé and Verotta (2006) computed a weekly average out of the daily adherence data, Huang (2008) computed an average between study visits (week 0, 2, 4, 8, 16, and every 8 weeks till week 72). For a discussion of reparameterisation and selection of models that incorporated drug adherence data and the use of Bayesian methods, see (Verotta, 2005). For a study of the different aspects of observed adherence behaviour, see (Ferguson et al., 2005).

The fourth group of models are those using computer simulations of a probabilistic, second-order Monte Carlo process, to incorporate explicitly drug adherence and HIV genome mutations through HIV progression. These were used to estimate the proportion of HIV positive patients who will die of co-morbidity (Braithwaite et al., 2005) and the rate of accumulating resistance mutations (Braithwaite et al., 2007). Such a model was employed to explain the heterogeneity of the adherence-resistance curve between two cohorts of patients (Braithwaite et al., 2006).

### 2.3.12. Some concluding remarks

The purpose of this section is to review the basic biological concepts that underlie ODE models of HIV within-host dynamics, with a focus on disease progression models. Since the discovery in 1995-6 of the nature of HIV ‘latency’ as a dynamic quasi-equilibrium of viral replication and latently infected cells, ODE models of various degrees of complexity have been developed aiming at different levels of verisimilitude. For disease progression models, two major categories exist: target-cell-limited models and immune control
models. After reviewing in greater detail some representatives of these models, we move on to discuss in brief mathematical models that were developed to address issues like, latency and the reservoir of virus when on treatment, selection of mutants, emergence of resistance, multi-infected cells, viral blips and drug adherence. These models demonstrate the great versatility of mathematics to address these complicated biological questions. However, as these models become more and more complex, analytical solutions become less possible and computer simulations are to be relied upon to produce numerical solutions.

None of the ODE models described in this section captured all aspects of HIV disease progression. Many model simulations start with the quasi-equilibria reached by CD4 cells and viral load after primary infection. Even if the model simulation starts before the infection event, viral load change in primary infection is difficult to capture. The AIDS phase is the most difficult of all to simulate. Up to now, there is no definitive consensus on the biological mechanisms through which HIV leads to AIDS. Therefore, it is not surprising that each and every ODE model that tries to capture AIDS fails in certain aspects. Models based on the idea that AIDS is a result of antigenic diversity leading to the failure of immune control usually model within-host viral evolution explicitly. Initial parameters for such models effectively determine the time scale of disease progression. In such simulations, the onset of AIDS becomes a sudden collapse of immune control at the critical point at which the dynamical balance tips to the other side, as seen in Korthals Altes et al. (2006). Models based on the idea that CD4 activation is the determining factor on disease progression (target-cell-limited models) simulates AIDS as a result of
the exhaustion of the CD4 cell pool. Fraser et al. (2001b) successfully simulated the gradual decline of CD4 cells. However, as virions were represented in the model as a factor of productively infected cells, the gradual decline of CD4 cells led to a decline in viral load. Therefore, the model was unable to simulate the general rebound of viral load as observed in AIDS patients. From a different angle, the fact that the present models are unable to capture all features of the AIDS phase highlights the reality that none of the existing hypotheses is sufficient to explain the AIDS phenomenon. The recent understanding of the role of the GI tract in HIV primary infection and chronic immune activation highlights the need to develop ODE models that take into account of the within-host spatial dynamics of HIV, esp. the GI tract.

Those who advocate agent-based models based on cellular automata like to highlight the limits of existing ODE models while acknowledging their strengths, as in (Guo and Tay, 2005). One of the advantages of ODE models is that much is known about ODEs. If we can solve ODEs analytically, much of the characteristics of the system can be predicted by using stability analysis (Beauchemin, 2002). This method is also much less demanding, computationally, than discretely integrating the ODEs. However, if the system becomes complex, to obtain analytical solutions becomes very difficult and models have to be solved numerically. Sometimes mathematical simplifications are employed. However, they may be of a mathematical nature rather than based on the real system. Furthermore, if no analytical solutions can be obtained, one of the benefits of using ODEs is lost, namely “being able to obtain the entire landscape of the dynamics in the parameter space that comes from analytical solutions” (Beauchemin, 2002). Therefore advocates for
agent-based models argue that when “the differential equations can no longer be solved analytically, CA are a preferable modelling approach” (Beauchemin, 2002). Moreover, the large number of populations involved and their continuously changing numbers will mean that ODE models can become very complicated by giving each idiotype of lymphocytes a separate ODE.

A counter-argument is that using ODEs is good enough for a model to capture the essential features of the immune system as it is tailored to solve a particular problem. Agent-based models are much more computationally demanding than ODEs and may not be necessary. In this case an ODE model will be a better option; even advocates for agent-based models have conceded this point (Beauchemin, 2002).

The claims that agent-based models represent the biological reality of HIV progression better than ODE models are, in my view, not necessarily true. On the contrary, I believe that ODE models do better than agent-based models in the representation of the within-host dynamics of HIV infections. While agent-based models may have captured a lot of entities and their interactions, their representations of disease progression mechanisms are poorly understood. To the contrary, ODE models shed light upon our understanding of the immune reactions against HIV infections and are simple enough to allow simple hypotheses to be tested.
2.4. Summary

This chapter reviews the development of computational and mathematical models of within-host dynamics of HIV over two decades. These models fall into two major categories: agent-based models and differential equations models. After reviewing the various agent-based models, it is argued that these models are still far from helpful in providing insights to clinicians and biologists in their work. Next, the historical development of differential equations models was reviewed, with a specific focus on HIV disease progression. From the simple models of the early days, these models have become much more complicated, trying to encompass greater details of the interaction between HIV and the human immune system. While a greater verisimilitude is achieved, it is no longer possible to analytically solve many of these models. Computer simulations are useful to help us understand the outcomes of the non-linear dynamics, but it is sometimes difficult to determine whether the simulation outputs genuinely represent the biological reality or they are just artefacts. Generally data are available to distinguish between models, although the comparison of model structures has been unsystematic to date. None of the ODE models capture all aspects of HIV disease progression and the AIDS phase appears to be the most difficult stage to model. A possible future direction for HIV pathogenesis model development is to construct compartmental ODE models that explicitly model the involvement of the GI tract in the within-host spatial dynamics of HIV disease progression. Parsimonious descriptions that capture key features and focus on areas of interest seem most appropriate. Prudence has to be exercised when we try to interpret outputs of these mathematical models.
Chapter 3

The model
3. The model

3.1. Introduction

HIV dual infection, including both coinfection and superinfection, is an important issue, as it may lead to recombination of HIV genomes that in turn facilitates the emergence of resistant strains (Smith et al., 2005a). It also affects our understanding of the human immune response against HIV infection, as the occurrence of superinfection events implies that the anti-HIV immune responses in the HIV-positive individual are insufficient to protect against a second infection (Blackard et al., 2002, Fultz, 2004, Smith et al., 2005a, Smith et al., 2006), which has substantial implications for the development of an HIV vaccine (Fultz, 2004). For a recent review on HIV dual infection, see van der Kuyl and Cornelissen (2007).

Early reports of HIV superinfection suggested that it may hasten the progression to AIDS (Gottlieb et al., 2004, Jost et al., 2002). However, some patients with long-term non-progressive HIV-1 infection have experienced co-infection or superinfection without clinical manifestations (Casado et al., 2007, Lamine et al., 2007). Thus it is uncertain whether superinfection *per se* is associated with disease progression. This has public health implication since there is concern over whether those already infected with HIV need to protect themselves from superinfection when in contact with known HIV infected partner (Blackard and Mayer, 2004, Smith et al., 2005a). Transmission of drug-resistant strains in the event of superinfection has also been documented (Blick et al., 2007, Brenner et al., 2004, Hecht et al., 2007, Smith et al., 2005b).
The implication of HIV dual infections are to some extent dependent upon the ability of the virus to co-infect cells, which is a prerequisite to HIV genome recombination at a cellular level. Mechanisms of down-modulation of CD4 receptors by HIV protein *nef*, *vpu* and *env* have been discovered (Piguet et al., 1999, Wildum et al., 2006). Studies also found that co-infection of cells was more frequent than expected in random events (Chen et al., 2005, Dang et al., 2004, Jung et al., 2002). A scaling law kinetics has been identified regarding HIV superinfection of cells and it was suggested that most infected cells are being infected and reinfected simultaneously, as a consequence of infection kinetics and target cell depletion ((Levy et al., 2004), erratum in (Levy et al., 2005)). To what extent an HIV-infected target cell is resistant to being superinfected with another virus genome will have an impact, not only upon the frequency of genome recombination, but on the whole viral and cellular profile of HIV dual infection (reviewed in Nethe et al. (2005)).

Mathematical models have been used to help elucidate the mechanisms of HIV pathogenesis (Nowak and May, 2000, Perelson and Nelson, 1999, Perelson et al., 1996). Some of them were tailored to focus on specific problems, e.g. residual replication (Ferguson et al., 1999), viral latent reservoirs (Kim and Perelson, 2006) and transient viraemia (Jones and Perelson, 2005, 2007). As both clinical and epidemiological data on HIV superinfection are still limited (e.g. incidence rate of superinfection and data on which to base an estimate of the long-term prognosis of superinfected patients),
mathematical models can help elucidate the within-host dynamics of HIV in the case of superinfection with heterologous HIV virus.

In order to investigate how the cellular and viral profiles of HIV superinfection would be affected by a varying degree of HIV superinfection of cells, and to test whether superinfection per se leads to faster progression to AIDS, a mathematical model was developed and will be presented in this chapter. It will be followed by an analysis of the model behaviour – quasi-equilibrium values and basic reproductive number.

### 3.2. The Model

The model described below is an extension of Fraser et al.’s (2001b) model of long-term HIV within-host dynamics that encompasses both primary infection and the long-term progression to AIDS. Additional insights from the extended version of Fraser et al.’s (2001b) model by Griffin and colleagues (James T. A. Griffin, unpublished PhD thesis, Imperial College London, 2006, and Griffin et al., 2006), Fraser et al.’s (2001a) model of short-term HIV within-host dynamics and Kim and Perelson’s (2006) model on viral and latent reservoir persistence were added to the model. Figure 3.1 is a flowchart representing the model. The model parameters and variables are described in Tables 3.1 and 3.2 respectively. The parameter values Fraser et al. (2001b) and Griffin et al. (2006) employed were explored in the modelling exercise. The sources of these parameters in the previous works were identified and the justifications or initial references are listed in the comments for table 3.1.
Table 3.1  Parameters of the model.
Those values that follow Fraser et al. (2001b) are marked with * and those follow Griffin et al. (2006) are marked with †. Those values that are expressed as a fraction of the total resting T cell pool at steady state are marked with ‡. Those values that are used only in experiments described in Chapter 4 are marked with §.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Value in simulation</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_d ) and ( \lambda_h )</td>
<td>daily rate of thymic production of new CD4 and CD8 T cells *‡</td>
<td>( \lambda_d + \lambda_h = 10^{+4} ) and ( \lambda_d = 5 \lambda_h )</td>
<td>chosen so that the pre-infection CD4/CD8 ratio = 2</td>
</tr>
<tr>
<td>( a_0 )</td>
<td>average rate of T cells activation per antigenic exposure *†</td>
<td>( 10^{-5} ) day(^{-1} )</td>
<td>chosen to match the observed rate of disease progression (Collaborative Group on AIDS Incubation and HIV Survival including the CASCADE EU Concerted Action, 2000)</td>
</tr>
<tr>
<td>( \mu )</td>
<td>daily rate of non-antigen-driven homeostatic T cell division *‡</td>
<td>0.01</td>
<td>chosen to match turn-over rates directly observed in (Hellerstein et al., 1999)</td>
</tr>
<tr>
<td>( x_0 )</td>
<td>relative T cell pool size below which T cell activation fails due to exhaustion of repertoire *‡</td>
<td>0.05</td>
<td>value at which T helper competence starts to decline (Fraser et al., 2001b)</td>
</tr>
<tr>
<td>( \mu_A )</td>
<td>activated T cell division rate *</td>
<td>1 day(^{-1} )</td>
<td>(Fraser et al., 2001b)</td>
</tr>
<tr>
<td>( p_{12} )</td>
<td>average probability of an activated T cell successfully dividing in an individual free of HIV *</td>
<td>0.55</td>
<td>(Fraser et al., 2001b)</td>
</tr>
<tr>
<td>( \beta_1 ) and ( \beta_2 )</td>
<td>average infection rate of an activated CD4 T cell per virion (virus 1 and virus 2) *</td>
<td>754 (to be varied in the experiment)</td>
<td>The same value as that in Fraser et al. (2001b) was chosen so that the results of both models would be comparable. Cf. (Fraser et al., 2001b)</td>
</tr>
<tr>
<td>( a_F )</td>
<td>activated infected cells become virally productive †</td>
<td>1 day(^{-1} )</td>
<td>(Klenerman et al., 1996)</td>
</tr>
<tr>
<td>( \alpha )</td>
<td>death rate of infected cells in the absence of CTL †</td>
<td>1 day(^{-1} )</td>
<td>(Klenerman et al., 1996)</td>
</tr>
<tr>
<td>( \gamma )</td>
<td>death rate of productively infected cell in the absence of CTL †</td>
<td>1 day(^{-1} )</td>
<td>(Klenerman et al., 1996)</td>
</tr>
<tr>
<td>( a_L )</td>
<td>rate of reactivation of latent infected cells *</td>
<td>0.01 day(^{-1} )</td>
<td>assuming this process is similar to the non-antigen-driven homeostatic T cell division and therefore assuming ( a_L = \mu ) (Fraser et al., 2001b, Hellerstein et al., 1999)</td>
</tr>
<tr>
<td>( f_c )</td>
<td>proportion of successful infections that result in latency *</td>
<td>( 10^{-5} )</td>
<td>(Fraser et al., 2001b)</td>
</tr>
<tr>
<td>( a_Z )</td>
<td>rate of CTL activation per productive infected cell</td>
<td>( 1.3334 \times 10^{-5} ) per CTL per infected cell per day</td>
<td>(Fraser et al., 2001a)</td>
</tr>
<tr>
<td>( p_z )</td>
<td>maximum proliferation of anti-HIV CTLs †</td>
<td>1 day(^{-1} )</td>
<td>1% of CTL are activated (Klenerman et al., 1996)</td>
</tr>
<tr>
<td>( d_z )</td>
<td>death rate of resting CTLs †</td>
<td>0.01 day(^{-1} )</td>
<td>(Ogg et al., 1998)</td>
</tr>
<tr>
<td>( z_0 )</td>
<td>pre-infection frequency of anti-HIV CTL *‡</td>
<td>( 10^{-6} )</td>
<td>(Fraser et al., 2001b)</td>
</tr>
<tr>
<td>( y_0 )</td>
<td>threshold value of infected cells for the logistic proliferative response of CTL to HIV *</td>
<td>( 10^{+3.5} )</td>
<td>(Fraser et al., 2001b)</td>
</tr>
<tr>
<td>( b/c )</td>
<td>ratio of viral production rate in productively infected cells and viral clearance rate †</td>
<td>292</td>
<td>The same value as that in Griffin et al. (2006) was chosen so that the results of both models would be comparable. Cf. (Griffin et al., 2006)</td>
</tr>
<tr>
<td>( \sigma )</td>
<td>maximum rate of CTL killing of HIV-infected cells *</td>
<td>( 10^{+1} ) day(^{-1} )</td>
<td>(Fraser et al., 2001b)</td>
</tr>
<tr>
<td>( N_{PB} )</td>
<td>typical pre-infection T cell count *</td>
<td>( 1500 ) mm(^{-3} )</td>
<td>the peripheral blood (PB) CD4 count is ( N_{PB}(x + x_d + z_0) ) and the PB CD8 count is ( N_{PB}(x + x_d + z) )</td>
</tr>
<tr>
<td>( \theta_1 ) and ( \theta_3 )</td>
<td>average clearance rate in antigenic exposure model *</td>
<td>( 0.02 ) day(^{-1} )</td>
<td>(Fraser et al., 2001b)</td>
</tr>
<tr>
<td>( \tau_1 ) and ( \tau_2 )</td>
<td>average exposure rate in antigenic exposure model *</td>
<td>( 0.1 ) day(^{-1} )</td>
<td>(Fraser et al., 2001b)</td>
</tr>
<tr>
<td>( \epsilon_{12,2} )</td>
<td>the ratio of the rate of infection by virus 2 of a cell already infected by virus 1 that of an uninfected cell by virus 2</td>
<td>( [10–0.00001] )</td>
<td>It is set to vary within this range or fix at a particular value.</td>
</tr>
<tr>
<td>( \epsilon_{23,1} )</td>
<td>the ratio of the rate of infection by virus 1 of a cell already infected by virus 2 that of an uninfected cell by virus 1</td>
<td>( [10–0.00001] )</td>
<td>Ditto</td>
</tr>
<tr>
<td>( \zeta )</td>
<td>the ratio of CTL action against doubly infected cells to that against singly infected cells</td>
<td>1</td>
<td>Assumed that CTL action against infected cells regardless of whether they are singly or doubly infected</td>
</tr>
<tr>
<td>( d_{vHIV} )</td>
<td>efficacy of reverse transcriptase against virus 1</td>
<td>0.9</td>
<td>Set at this value to achieve suppression of virus 1</td>
</tr>
<tr>
<td>( d_{vHIV} )</td>
<td>efficacy of reverse transcriptase against virus 2</td>
<td>0.9</td>
<td>It is set to vary within this range or fix at a particular value.</td>
</tr>
<tr>
<td>( d_{vPI} )</td>
<td>efficacy of protease inhibitor against virus 1</td>
<td>0.9</td>
<td>Set at this value to achieve suppression of virus 1</td>
</tr>
<tr>
<td>( d_{vPI} )</td>
<td>efficacy of protease inhibitor against virus 2</td>
<td>0.9</td>
<td>It is set to vary within this range or fix at a particular value.</td>
</tr>
<tr>
<td>( t_{vHIV} )</td>
<td>half-life of reverse transcriptase inhibitor</td>
<td>0.5 day §</td>
<td>Chosen to match the dose interval</td>
</tr>
<tr>
<td>( t_{vPI} )</td>
<td>half-life of protease inhibitor</td>
<td>0.5 day §</td>
<td>Ditto</td>
</tr>
<tr>
<td>( t_{d} )</td>
<td>interval between each dose of drug</td>
<td>0.5 day §</td>
<td>Two doses per day</td>
</tr>
<tr>
<td>Symbols</td>
<td>Definitions</td>
<td>Initial values (start of simulation and before infection)</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td>--------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>$x_4$</td>
<td>CD4 cells – Quiescent</td>
<td>$1/(1+10^{-0.3013})$ †</td>
<td></td>
</tr>
<tr>
<td>$x_8$</td>
<td>CD8 cells – Quiescent</td>
<td>$1-1/(1+10^{-0.3013})$ †</td>
<td></td>
</tr>
<tr>
<td>$x_{4A}$</td>
<td>CD4 cells – Activated</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>$x_{8A}$</td>
<td>CD8 cells – Activated</td>
<td>0.0005</td>
<td></td>
</tr>
<tr>
<td>$y_1$</td>
<td>CD4 cells infected by virus 1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$y_{1L}$</td>
<td>CD4 cells infected by virus 1 – latent</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$y_{1P}$</td>
<td>CD4 cells infected by virus 1 – virally productive</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$y_2$</td>
<td>CD4 cells infected by virus 2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$y_{2L}$</td>
<td>CD4 cells infected by virus 2 – latent</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$y_{2P}$</td>
<td>CD4 cells infected by virus 2 – virally productive</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$y_{12}$</td>
<td>CD4 cells infected by virus 1 and 2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$y_{12L}$</td>
<td>CD4 cells infected by virus 1 and 2 – latent</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$y_{12P}$</td>
<td>CD4 cells infected by virus 1 and 2 – virally productive</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$z_0$</td>
<td>CTL – Resting</td>
<td>$10^6$</td>
<td></td>
</tr>
<tr>
<td>$z_A$</td>
<td>CTL – Activated</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>$y_0$</td>
<td>Inoculum – number of infected cells in the first place</td>
<td>$10^{-10}$</td>
<td></td>
</tr>
<tr>
<td>$a_4$</td>
<td>Rate of CD4 cell activation</td>
<td>$4.6512 \times 10^{-4}$</td>
<td></td>
</tr>
<tr>
<td>$a_8$</td>
<td>Rate of CD8 cell activation</td>
<td>$4.6512 \times 10^{-4}$</td>
<td></td>
</tr>
<tr>
<td>$k_4$</td>
<td>Number of concurrent antigenic exposures of CD4 cells</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>$k_8$</td>
<td>Number of concurrent antigenic exposures of CD8 cells</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>$v_1$</td>
<td>Virus 1 – infectious virions</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>$v_2$</td>
<td>Virus 2 – infectious virions</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>$v_{1NI}$</td>
<td>Virus 1 – non-infectious virions</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>$v_{2NI}$</td>
<td>Virus 2 – non-infectious virions</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

† $x_4 + x_8 = 1$

Figure 3.1 Flowchart representing the mathematical model.

Adapted and re-drawn from Griffin’s PhD thesis (2006).
3.2.1. Healthy T cells

The model describes uninfected quiescent T cells, $x_m$, where $m = 4$ represents CD4 cells and $m = 8$ represents CD8 cells,

$$\frac{dx_m}{dt} = \lambda_m - a_m + 2p_A\mu_A x_{mA} + \mu x_m - \Theta x_m(x_4 + x_8)$$ \hspace{1cm} (3.1)

CD4 and CD8 cells are supplied from a thymic source at a constant rate, $\lambda_4$ and $\lambda_8$ respectively, and go through non-antigen-driven homeostatic cell division at a rate of $\mu_9$. Their deaths are density-dependent, at rates of $\Theta x_4(x_4 + x_8)$ and $\Theta x_8(x_4 + x_8)$ respectively, where $\Theta = \lambda_4 + \lambda_8 + \mu + 2a_0(2p_A + 1)$, chosen to ensure at steady state $x_4 + x_8 = 1$ on average. They are activated at rates $a_4$ and $a_8$, a stochastic process that will be discussed below, and become activated cells, $x_{4A}$ and $x_{8A}$:

$$\frac{dx_{4A}}{dt} = a_4 - \mu_A x_{4A} - \beta_1(1 - drug_{RT1})v_1 x_{4A} - \beta_2 (1 - drug_{RT2})v_2 x_{4A}$$ \hspace{1cm} (3.2)

$$\frac{dx_{8A}}{dt} = a_8 - \mu_A x_{8A}$$ \hspace{1cm} (3.3)

Activated cells undergo divisions at a per cell rate of $\mu_4$ and become quiescent cells at a per cell rate of $2p_A\mu_A$, where $p_A$ is the average probability of an activated T cell successfully dividing in an HIV-negative individual. In the presence of virus, where $v_1$ and $v_2$ denoting the first strain and the superinfecting strain respectively, activated CD4 cells are infected at a rate of $\beta_1 v_1$ and $\beta_2 v_2$, where $\beta_1$ and $\beta_2$ denote fitness of the two viral strains respectively. In the presence of RTI, the rate of infection will be reduced by a proportion, the RTI drug efficacy with respect to each virus strain, denoted by $drug_{RT1}$ and $drug_{RT2}$ respectively.

\footnote{An alternative way of model construction is to combine homeostatic cell division with cell activation. As I am not aware of evidences that strongly favour one way or the other, I decide to follow the example of Fraser et al. (2001b).}
3.2.2. Infection of CD4 cells

Infected cells are divided into categories according whether they are activated ($y_i$), latent infected ($y_{Li}$) or virally productive ($y_{Pi}$) and with which virus are the cells infected ($i = 1$ or $2$): 

$$\frac{dy_i}{dt} = (1 - f_i)\beta_i(1 - drug_{RT})v_i x_{A4} + a_i y_{Li} - a_p y_i - \alpha y_i - \omega_{\nu_i} \beta_i(1 - drug_{RT}) y_j y_i$$ (3.4) 

$$\frac{dy_{Li}}{dt} = f_i \beta_i(1 - drug_{RT})v_i x_{A4} - a_L y_{Li}$$ (3.5) 

$$\frac{dy_{Pi}}{dt} = a_p y_i - \gamma y_{Pi} - \epsilon \sigma z_{A} y_{Pi}$$ (3.6)

Where $i, j = 1$ or $2$, and $i \neq j$, representing two strains of virus.

When activated CD4 cells are infected by virus 1 and 2, a small fraction, $f_i$, of them become latently infected cells $y_{Li}$ and $y_{L2}$, while the majority of them, $1 - f_i$, become activated infected cells, $y_i$ and $y_{2i}$. Latently infected cells are activated at a per cell rate of $a_L$. Activated infected cells die at a per cell rate of $\alpha$ and become productively infected cells, $y_{Pi}$ and $y_{P2}$, at a per cell rate of $a_p$. Productively infected cells die at a per cell rate of $\gamma$, and are killed by cytotoxic lymphocyte (CTL) action at a rate of $\epsilon \sigma z_{A}$, where $\sigma$ is the maximum per cell rate of CTL killing of HIV-infected cells and $\epsilon = \hat{Y}_p / (\hat{Y}_p + y_T)$ where $\hat{Y}_p = (y_{P1} + y_{P2} + y_{P12})$ and $y_T$ is the threshold value of productively infected cells for the logistic proliferative response of CTL to HIV (cf. Fraser et al, 2001b). When the total number of productively infected cells ($\hat{Y}_p$) is very small, we can assume $y_{Pi} \approx \hat{Y}_p$, and $\hat{Y}_p \ll y_T$. Then, $\epsilon \approx \hat{Y}_p / y_T \approx y_{Pi} / y_T$, and therefore, $\epsilon \sigma z_{A} y_{Pi} = \sigma z_{A} (y_{Pi})^2 / y_T$. As we shall see below, because the growth of the activated CTLs ($z_A$) depends on the total number of productively infected cells: $z_A$ will be very small. As both $y_{Pi}$ and $z_A$ are very small, the
term $\epsilon \sigma_{\text{A}}y_{\text{P}}$ tends towards zero, i.e. CTL action is minimal. The death of productively infected cells will then depend solely on $\gamma$. When the total number of productively infected cells is big, $\dot{Y}_p >> y_T$. Then, $\epsilon \approx 1$ and the term $\epsilon \sigma_{\text{A}}y_{\text{P}}$ becomes $\sigma_{\text{A}}y_{\text{P}}$, which is a mass action term for CTL action.

### 3.2.3. Superinfection of CD4 cells

Activated infected cells may be infected a second time by a heterologous virus at a per cell rate of $\omega_{y_{i}v_{j}}\beta(1-\text{drug}_{RT})v_{0}$, where $\omega_{y_{i}v_{j}}$ denote the cellular and viral resistance of $y_{i}$ to superinfection by $v_{j}$ respectively ($i, j = 1$ or $2$, and $i \neq j$), and become doubly infected cells,

$$
\frac{dy_{12}}{dt} = \omega_{y_{1}v_{2}}(1-f_{L})\beta(1-\text{drug}_{RT})v_{2}y_{1} + \omega_{y_{2}v_{1}}(1-f_{L})\beta(1-\text{drug}_{RT})v_{1}y_{2} + a_{L}y_{L12} - a_{p}y_{12} - \alpha y_{12}
$$

(3.7)

$$
\frac{dy_{L12}}{dt} = \omega_{y_{1}v_{2}}f_{L}\beta(1-\text{drug}_{RT})v_{2}y_{1} + \omega_{y_{2}v_{1}}f_{L}\beta(1-\text{drug}_{RT})v_{1}y_{2} - a_{L}y_{L12}
$$

(3.8)

$$
\frac{dy_{p12}}{dt} = a_{p}y_{12} - \gamma y_{p12} - \epsilon \xi \sigma_{\text{A}}y_{p12}
$$

(3.9)

Likewise, a small fraction of these doubly infected cells becomes latent, $y_{L12}$, while the rest are activated, $y_{12}$. The latter will become virally productive, $y_{p12}$. $\xi$ is a parameter that allows for the intensity of CTL action against doubly infected cells to be different from that against singly infected cells. As viral production is limited by cellular factors rather than number of viral genomes present in a cell, a cell co-infected by two virus of the same strain would be the same from the point of view of viral production as that infected by only one virus. Therefore, no separate equations are made for cells superinfected by the same viral strain.
3.2.4. Cytotoxic lymphocyte

CTLs play a role in clearing productively infected cells (Asquith et al., 2006) (but not the activated or latently infected cells in the model). They are either resting, \( z_R \), or activated, \( z_A \):

\[
\frac{dz_R}{dt} = d_Z (z_0 - z_R) + 2p_z z_A - a_z (y_{p1} + y_{p2} + y_{p12}) z_R \tag{3.10}
\]

\[
\frac{dz_A}{dt} = a_z (y_{p1} + y_{p2} + y_{p12}) z_R - p_z z_A \tag{3.11}
\]

The homeostasis of resting CTLs is maintained by a supply of new resting CTLs at a constant rate of \( d_Z \cdot z_0 \) and a death rate of \( d_Z \cdot z_R \). Resting CTLs are activated at a per cell rate of \( a_Z(y_{p1} + y_{p2} + y_{p12}) \) dependent on the density of CD4 cells. An activated CTL will undergo division and become two resting CTLs at a per cell rate of \( p_Z \).

3.2.5. Virus

The creation and destruction of virus is not explicitly followed in the model but the population of virus is a function of the numbers of infected cells. This assumes that this process is rapid and at equilibrium coupled to the cell dynamics.

\[
v_i = \frac{b}{c} (1 - \text{drug}_{p_i}) (y_{p_i} + \frac{y_{p_{ij}}}{2}) \tag{3.12}
\]

\[
v_{iNI} = \frac{b}{c} \text{drug}_{p_j} (y_{p_i} + \frac{y_{p_{ij}}}{2}) \tag{3.13}
\]

Where \( i, j = 1 \) or \( 2 \), and \( i \neq j \).

Virions are produced from infected cells at a per cell rate of \( b \), and have a viral lifetime of \( 1/c \). The proportion of infectious virions and non-infectious virions, denoted \( v_i \) and \( v_{iNI} \),
are given by $1 - drug_{PI_i}$ and $drug_{PI_i}$ respectively, where $i = 1$ or 2, and $drug_{PI_1}$ denotes the efficacy of PI against virus 1 and 2 respectively. Doubly infected cells were assumed to produce an equal number of virions to that produced by singly infected cells, among which half are virus 1 and half are virus 2.

3.2.6. T cell activation and antigenic exposure

Following Fraser et al. (2001b) and their hypothesis that the occurrence of bursts of activation is predominantly localised, the rate of T cell activation is limited by the availability of antigen presenting sites and thus occurs at an approximately constant rate. The rates of activation of CD4 and CD8 cells, denoted by $a_4$ and $a_8$ respectively, were given as,

$$a_i = a_i k_i \frac{x_i}{x_i + x_S}$$

where $a_0$ is the average rate of T cell activation per antigenic exposure and $x_S$ is the relative T cell pool size below which T cell activation fails due to exhaustion of repertoire, and $k_i$ (a non-negative integer) is the number of concurrent antigenic exposures experienced by the ‘patient’ at a particular instance.

A stochastic element was introduced into the model through $k_i$:

$$k_i(t + h) = k_i(t) + Poisson(\tau h) - binomial(\theta h, k_i)$$

Where $Poisson(\tau h)$ denotes the probability of which $k_i$ will increase by 1 (Poisson distribution) and $binomial(\theta h, k_i)$ denotes the probability of which $k_i$ will decrease by 1 (binomial distribution), given $i = 4$ or 8 (cf. Griffin et al., 2006). The average exposure rate, $\tau$, and the average clearance, $\theta$, of antigenic exposures are 0.1 and 0.02 per day. As
the product of $\tau$ and $h$ (the size of a time-step) is very small, therefore we have $\text{Poisson}(\tau h) = e^{(\tau h)} \cdot \tau \cdot h = \tau h$; and $\text{binomial}(\theta h, k_i) = k_i \theta h$. The stochastic processes for CD4 and CD8 cells activation are separate. The initial value for both $k_4$ and $k_8$ is 5.

### 3.3. Analytical Results

Analytical solutions for the quasi-equilibrium states of the system were obtained to identify major drivers of the level of each state. The model can be solved to calculate a set of quasi-equilibrium values of immune cell levels and viral loads after primary infection and before the onset of AIDS. This allows us to understand the factors that influence the relationship between the immune cell levels and viral loads and the potential spread of virus within the host. Expressions for the basic reproductive ratios in the absence and presence of CTL response can also be derived which identify the factors that drive the initial growth of HIV infection in the host. Numerical solutions to the model will be presented in Chapter 4.

#### 3.3.1. Equilibrium values

Following the example of Griffin’s PhD thesis (2006), by setting the above equations to zero, equilibrium values of T cells and virions can be found. The only exception is the equation for quiescent CD4 cells that will not reach equilibrium within the time scale of the model representing a decade of infection. In the following equations, let $\tilde{A}$ denote the equilibrium value of any variable $A$ and let $\tilde{Y}$ and $\tilde{Y}_{\rho}$ be the equilibrium values of $(y_1 + y_2 + y_{12})$ and $(y_{P1} + y_{P2} + y_{P12})$ respectively.
For $dz_R / dt = 0$ and $dz_A / dt = 0$, we can solve the two simultaneous equations – equations 3.10 and 3.11 – for $\hat{z}_A$, and by assuming that $z_0 << \hat{z}_R$, we get,

$$\hat{z}_A = \frac{d_z z_R}{p_z}$$  \hspace{1cm} (3.16)

Substituting equation 3.11 with equation 3.16 gives,

$$\hat{Y}_P = \frac{d_z}{a_z}$$  \hspace{1cm} (3.17)

Assuming that no cART is taken, adding equations 3.12 for $i = 1$ and $i = 2$, gives,

$$v_1 + v_2 = \frac{b}{c} \hat{Y}_P$$  \hspace{1cm} (3.18)

Substituting equation 3.17 into equation 3.18, we get the equilibrium viral load,

$$\hat{v}_1 + \hat{v}_2 = \frac{d_z}{a_z} \cdot \frac{b}{c}$$  \hspace{1cm} (3.19)

Equation 3.19 implies that the equilibrium viral load is determined by (i) the CTL death rate, (ii) the CTL activation rate per productive infected cells, (iii) the viral production rate in productively infected cells and (iv) the viral clearance rate.\(^\text{10}\)

Taking equation 3.14 and let $\bar{k}_q$ be the mean number of CD4-specific antigens present, and let $\bar{x}_q$ be the number of quiescent CD4 cells in the absence of infection and $\bar{a}_q$ be the rate of activation of CD4 cells in the absence of infection, gives,

\(^\text{10}\) The examiner pointed out that equation 3.19 has the same expression as equation 2.11, and suggested that “the steady state viral load should also be independent of almost anything except $d_z$ and $a_z$.” I want to highlight the fact that here, it is assumed that both resting and activated CTLs are in equilibrium. When the patient commences cART, the system is no longer in equilibrium.
Provided that the CD4 count is not at a low level, \( x_4 >> x_S \), equation 3.20 can be simplified as,

\[
\tilde{a}_4 \approx a_0 k_4
\]  

(3.21)

Assuming the rate of activation of CD4 at equilibrium is similar to that in the absence of infection (i.e. \( \tilde{a}_4 \approx \tilde{a}_4 \)), equation 3.2 gives the equilibrium of activated CD4 count as,

\[
\tilde{x}_{4,e} \approx \frac{\tilde{a}_4}{\mu_4 + \beta_1 \tilde{v}_1 + \beta_2 \tilde{v}_2}
\]  

(3.22)

Equation 3.22 implies that the equilibrium activated CD4 count is determined by (i) the pre-infection CD4 activation rate (\( \tilde{a}_4 \)), (ii) the activated T cell division rate (\( \mu_4 \)), and (iii) the average infection rate of an activated CD4 T cell (i.e. \( \beta_1 \tilde{v}_1 + \beta_2 \tilde{v}_2 \)). However, \( \tilde{x}_{4,e} \) will become low at low \( x_4 \) levels, i.e. \( x_4 \approx x_S \), as equation 3.21 does not hold.

At equilibrium and in the absence of drugs, equation 3.5 gives,

\[
\tilde{y}_{i,0} = \frac{f_i \beta_{ij} \tilde{v}_j \tilde{x}_{4,e}}{\tilde{a}_L}
\]  

(3.23)

where \( i = 1 \) or 2.

Substituting equation 3.23 into equation 3.4, gives,

\[
\tilde{y}_i = \frac{\beta_{ij} \tilde{v}_j \tilde{x}_{4,e}}{a_p + \alpha + \omega_0 \beta_j \tilde{v}_j}
\]  

(3.24)
where \( i, j = 1 \) or \( 2 \) and \( i \neq j \).

The equilibrium value of target cells infected by one virus only is determined by (i) the infection event (\( \beta_i \hat{V}_i \hat{X}_{4,i} \)), (ii) the activated infected cells becoming virally productive (\( a_P \)), (iii) the death rate of infected cells in the absence of CTL (\( \alpha \)), and (iv) the rate of second infection of infected cells by heterologous virus (\( \omega_{\nu \nu} \beta_i \hat{V}_j \)).

Let \( \zeta \) be 1 (i.e. CTL kills doubly infected cells at the same rate as singly infected cells). For \( dy_{p_i} / dt = 0 \) and \( dy_{p_2} / dt = 0 \), we can solve equations 3.6 (for \( i = 1 \) or \( 2 \)) and 3.9 together and get,

\[
\hat{z}_A = \frac{a_P \hat{Y} - \gamma \hat{Y}_P}{\epsilon \sigma \hat{Y}_P} \quad \text{where} \quad \epsilon = \frac{\hat{Y}_P}{\hat{Y}_P + y_T}
\]

Equation 3.25 implies that the equilibrium value of activated CTL is determined by (i) the rate of activated infected cells become virally productive (\( a_P \)), (ii) the death rate of productively infected cells in the absence of CTL (\( \gamma \)), and (iii) the maximum rate of CTL killing of HIV-infected cells (\( \sigma \)).

### 3.3.2. Basic reproductive ratios

The definition of the basic reproductive ratio for a virus within a host, \( R_0 \), is the number of virions produced by each virion at initial infection. This value determines whether, and
how fast, virus will establish itself in the host. Its calculation below follows the examples in Griffin’s PhD thesis (2006). In the absence of infection, the equilibrium number of activated CD4 cells is $\frac{\alpha_q}{\mu_A}$ (see equation 3.22). Virus 1 infects these cells at a per cell rate of $\beta_1v_1$ and virus 2 infects cells at a per cell rate of $\beta_2v_2$. Virions are produced at a per cell rate $b$ by virally productively infected cells. At a rate of $c$ they are cleared from the blood. (In our model, $b/c$ is a composite parameter, see Table 3.1.) In other words, for a mean time of $1/c$ each virion persists in the blood. During this time period, the virion infects $\frac{\beta\alpha_q}{c\mu_A}$ or $\frac{\beta\alpha_q}{c\mu_A}$ cells (virus 1 or virus 2 respectively). These cells then become virally productive. If at the initial stage of infection, anti-HIV CTL response is not present and the virally productive cells die at a per cell rate of $\gamma$. A number of $b/\gamma$ virions are produced by each virally productive cell. Therefore, the basic reproductive ratio of virus $i$, where $i = 1$ or 2, is calculated as,

$$R_0 = \frac{\beta\alpha_q}{\gamma\mu_A} \cdot \frac{b}{c} \text{ where } i = 1 \text{ or } 2$$

(3.27)

If at the initial infection there was a steady state CTL response, the virally productive cells will die at a per cell rate of $(\gamma + \epsilon\sigma\tilde{z}_A)$. Applying equation 3.25,

$$\gamma + \epsilon\sigma\tilde{z}_A = \gamma + a_p\hat{Y} - \epsilon\hat{Y}_p = a_p\hat{Y} - \hat{Y}_p$$

(3.28)

Assuming that $\hat{Y}_p \approx \hat{Y}$, and applying equation 3.28, the basic reproductive ratio will become:

$$R_{0\text{CTL}} = \frac{\beta\alpha_q}{\mu_A(\gamma + \epsilon\sigma\tilde{z}_A)} \cdot \frac{b}{c} = \frac{\beta\alpha_q}{\mu_Aa_p} \cdot \frac{\hat{Y}_p}{c} \cdot \frac{b}{c}$$

(3.29)

$$\approx \frac{\beta\alpha_q}{\mu_Aa_p} \cdot \frac{b}{c} \text{ where } i = 1 \text{ or } 2$$
Equation 3.29 implies that the initial growth of infection is determined by the following factors: (i) viral fitness (\(\beta_i\)), (ii) the rate of activation of CD4 cells in the absence of virus (\(\tilde{a}_d\)), (iii) the rate of activated T cell division (\(\mu_A\)), (iv) the rate at which activated infected cells become virally productive (\(a_p\)), (v) the viral production rate in productively infected cells (\(b\)) and (vi) the viral clearance rate (\(c\)). Factors iii to vi are fixed in the model, based on the assumption that these parameters do not vary according to the virus strain or the individual. That implies that it is the interplay between the given fitness of a viral strain and the activation of CD4 cells before infection (the latter being stochastic in the model) that determines the initial course of infection.

What difference does it make having two viruses? The calculation of the basic reproductive numbers for each virus is not influenced by the existence of the other when invading a treatment naïve patient and the only viral type specific parameter is \(\beta_i\). However, if already infected with one virus, the equilibrium number of activated cells will be reduced and hence the basic reproductive ratio lower, assuming a very low rate of co-infection of cells. Therefore, assuming \(\omega_{ij}v_{ij} \ll 1\) (where \(i, j = 1\) or \(2\), and \(i \neq j\)) and therefore \(y_{12} \ll y_1\) or \(y_2\), the basic reproductive ratio of virus 2 in the presence of virus at equilibrium will become:

\[
R_{02} = \frac{\beta_1 \cdot b \cdot \tilde{a}_d}{\gamma \cdot c \cdot (\mu_A + \beta_1 \tilde{v}_l)}
\] (3.30)

The prevalence of one virus is influenced by the other through the infection rate of cells that are no longer susceptible to infection (depending on \(\omega_{ij}\)). It looks like equilibrium number of activated CD4 cells is reduced by the presence of another virus from
\[ \frac{\tilde{a}_i}{\mu_i + \beta_i \tilde{v}_i} \text{ to } \frac{\tilde{a}_i}{\mu_i + \beta_1 \tilde{v}_1 + \beta_2 \tilde{v}_2} \]. However, since there is conservation of viral population with an infection having one virus, \( v_i = \frac{d_i}{a_z} \cdot \frac{b}{c} \), and two virus, \( v_i + v_j = \frac{d_i}{a_z} \cdot \frac{b}{c} \), the viral numbers stay constant and only if \( \beta_i \neq \beta_j \) does activated cell numbers change.

### 3.4. Reliability of the model

Simulations with only one virus were run and their results were checked against Fraser et al.’s model (2001b) and were found to be consistent with the outcomes of the latter. The model is more successful modelling CD4 cell decline (Fig. 3.2 and 3.3) than viral load (Fig. 3.4). In the example shown in Figure 3.2, CD4 cell decline to < 200 cells / µl (i.e. AIDS) in about seven years. The mechanism that drove the decline of CD4 count was the same as that of Fraser et al. (2001b): “Homeostatic regulartion of the overall T cell pool size that is blind to CD4 and CD8 surface markers, leading to gradual competitive exclusion of CD4+ T cells by CD8+ T cells under the fitness disadvantage caused by HIV infection”. One of the limitations of the model is that the primary infection phase was not reproduced in the model. The model does not include the B cell-associated antibody response which is believed to control the initial viraemia. Therefore it is a model of the long latent decline of CD4 through direct killing of infected cells, but is not a full representation of the interaction of the virus and the immune system. Another limitation of the model is that it was unable to reproduce the accompanied viral load increase following CD4 cell decline < 200 cells / µl (i.e. AIDS).
Figure 3.2  Relative quantity of quiescent CD4 and CD8 T cells in a simulation example, with only one virus and no treatment.

Figure 3.3  Five examples of decline of quiescent CD4 cell count. There is only one virus in each simulation with no treatment.
3.5. Summary

This chapter describes a new ODE model of the within-host dynamics of HIV that incorporates two viruses and therefore enables me to perform in silico experiments of superinfection of two heterologous HIV strains. Equilibrium values of some variables and basic reproductive ratios of the virus were also calculated and their implications analysed.
Chapter 4

Superinfection, disease progression and viral blips
4. Superinfection, disease progression and viral blips

In the previous chapter, the mathematical model and the analytical results were presented. In this chapter, numerical solutions of the model, obtained through computer simulations, are presented. Here I address the issue on the relationship between superinfection and disease progression, and address the issue on the relationship between superinfection, resistance and viral blips.

4.1. Research questions

The followings are the questions that the two experiments in this chapter is set out to address.

4.1.1. Superinfection and disease progression (experiment A)

To investigate (a) how viral fitness and cellular susceptibility to co-infection affects the outcome of superinfection of a heterologous HIV strain in an HIV positive individual, which can be defined as co-existence of both strains or competitive exclusion of either strain, and (b) whether HIV superinfection leads to faster progression to AIDS.

4.1.2. Superinfection, resistance and viral blips (experiment B)

How far does drug resistance confer a superinfecting strain the ability to establish itself in a HIV positive individual? Can a superinfecting strain establish itself without drug resistance towards the existing cART regimens? Can superinfection give rise to viral blips?
4.2. Methods

Numerical solutions to the model described in full in Chapter 3 were obtained through computer simulations to elucidate the impact of viral fitness and co-infection of cells upon the infection outcome, the impact of superinfection upon progression to AIDS, and the relationship between drug resistance and the establishment of the superinfecting strains.

4.2.1. Computer programming

The model was written in C++ programming language and was compiled using Microsoft® Visual Studio .NET 2003 programming environment. Outputs of simulation were generated by the programme in comma separated files and were then analysed with Microsoft® Office Excel 2003 and SAS 9 for Windows (SAS Institute Inc., Cary, N.C., USA., 2002-03).

4.2.2. Fixed or flexible time steps

Two versions of the basic model were developed with regard to the stochastic process in the system. In the first version, the simulations were generated using fixed time-steps. In the second version, the simulations were generated using flexible time-steps. In both cases, the stochastic system is coupled to the deterministic model, by numerically integrating the deterministic equations over the same time-step. Flexible time-steps were used in all simulations discussed in this thesis.

4.2.3. Latin Hypercube Sampling

Latin Hypercube Sampling (McKay, 1992) was used to draw numbers without replacement from the parameter space. This procedure ensured that the whole parameter
space will be evenly explored without the need of sampling every point across the parameter space. This is implemented by first drawing a value in a random sequence without replacement for each given parameter within a range input by the investigators and then matching them together for the sequence of simulations.

4.2.4. Simulation conditions

For both experiments, all simulations start on day -730 (year -2) to allow the system to equilibrate before the introduction of infection. Infection by virus 1 takes place on day 0. Simulations end on day 3650 (year 10). In Experiment A, superinfection by virus 2, if there is any, takes place on day 365 (year 1). The reason behind the choice of year 1 is to allow the first strain to establish before superinfection event occurs and to allow enough time for the influence of the superinfection event upon progression to AIDS to manifest. In Experiment B, cART commences on day 2920 (year 8) and superinfection by virus 2 takes place on day 3285 (year 9). The choice of year 8 for the start of cART reflects the fact that the majority of HIV-positive patients start cART at the late stage of HIV infection (usually at the onset of AIDS) and the choice of year 9 for the superinfection event allows me to test the impact of drug resistance of the superinfecting strain on the outcome of superinfection. An example of a time series of viral load is given in Figure 4.1.

4.2.5. Parameter space for Experiment B

100 sets of parameters\textsuperscript{11} were drawn from a parameter space of efficacy of RTI and PI against the superinfecting strain using Latin Hypercube Sampling method (McKay, 1992).

\textsuperscript{11} Since Latin Hypercube Sampling method is used, 100 sets of parameters are good enough to cover the parameter space representatively.
The efficacies of RTI and PI against strain 1 were both 0.9 (assuming a half-life of 0.5 day and an interval of 0.5 day between each dose), while that against strain 2 (i.e. the reciprocal of drug resistance against RTI and PI of strain 2) varied and were drawn using Latin Hypercube Sampling from a uniform distribution between 0 and 1 for both RTI and PI efficacies. Fitness of strain 1 equals that of strain 2. The probability of infection by a second HIV strain of an already infected cell is 1% of that of infection of a healthy cell by an HIV virus in the first place. Ten simulations were performed per set of parameters and the log median of the ratio between virus 2 and virus 1 of the ten simulations were obtained.

Figure 4.1   An example of a time series of viral load
An example of a simulation used in Experiment B. Black line represents virus 1 infectious virions [V1 (I)]; red line represents virus 2 infectious virions [V2 (I)]; yellow dotted line represents virus 1 non-infectious virions [V2 (NI)]; and blue line represents virus 2 non-infectious virions [V2 (NI)]. It shows that when cART starts (day 2920), some non-infectious virions were made as a result of protease inhibitor action. When superinfection took place in day 3285, virus 2 (resistant to reverse transcriptase inhibitor) soon displace virus 1 to be the dominant virus (competitive exclusion). In this example, the parameter values are: $\beta_1 = \beta_2 = 754; \text{drug}_{\text{RT1}} = \text{drug}_{\text{PI1}} = 0.9; \text{drug}_{\text{RT2}} = 0.465; \text{drug}_{\text{PI2}} = 0.985; \text{half-lives of all drugs} = 0.5 \text{ days}; \text{dose interval} = 0.5 \text{ days}; \omega_{y2v1} = \omega_{y1v2} = 0.01; \xi = 1.$

---

12 Ten simulations per set of parameters are sufficient because 10 simulations give a good idea of variation, and we would not get additional information with more simulations.
4.3. Results

4.3.1. Experiment A

4.3.1.1. Viral fitness and co-infection of cells determine the outcome of the simulations in terms of viral success

It was found that the susceptibility of cells to superinfection by a heterologous HIV virion is crucial to the outcome of HIV superinfection of the host with respect to the relative fitness of the two strains involved. Figure 4.2 shows that if target CD4 cells that are already infected with one strain of HIV are less susceptible to the infection of a heterologous strain than their uninfected counterparts, competitive exclusion between the two strains will arise. In this scenario, the relative fitness of the two strains determined which strain will prevail in the host after the superinfection event. However, if cells are equally (or more) susceptible to the virus infection, regardless of whether they are infected by another strain, then co-infection may arise given that the superinfecting strain is sufficiently fit with respect to the established strain.
**Figure 4.2**  Competitive exclusion or co-existence of two strains of HIV.

The vertical axis is the log ratio of virus 2 and virus 1 viral RNA numbers (median of 10 runs per set of parameters). It is set against a parameter space of the log rate of infection of infected cells by a heterologous virus relative to that of healthy cells (x-axis), and the log ratio of $\beta_2$ and $\beta_1$ (y-axis). Latin hypercube sampling were used to draw numbers without replacement from the parameter space of $\beta_2 / \beta_1$ (within a range of -2 and 2, by varying $\beta_2$ while keeping $\beta_1$ constant at 754) and the infection rate ratio (by varying the rate of superinfection of infected cells, $\omega$, from 0.001 to 1000, assuming $\omega y_2 = \omega y_1 v_2$). For each combination of parameters, 10 simulations were performed. The median of $\log_{10}$ of the ratio between the final viral load of virus 2 and that of virus 1, i.e. $\log_{10} \left( \frac{V_2}{V_1} \right)$ of the 10 simulations were obtained and using the software SAS, performing procedures g3GRID and g3D, a three-dimensional interpolated smooth surface was created as shown. It is evident that when an infected cell is very unlikely to be re-infected by a second strain, a scenario of competitive exclusion – either virus 1 or virus 2 persists – occurs with varying degree of exclusiveness depending on the ratio between $\beta_2$ and $\beta_1$. Take for example, when $\log_{10}$ (infection rate ratio) = -3, with log $\left( \beta_2 / \beta_1 \right)$ between 0 and 0.5, $\log_{10} \left( \frac{V_2}{V_1} \right)$ falls between -7.1 and 11.4. Given that the simulation viral load is of the order $10^4$, beyond this range, one is certain that only one strain prevails. If an infected cell is equally or more likely to be infected by a second strain, a scenario of co-existence of two strains can occur, depending on the fitness of the second strain. For example, when $\log_{10}$ (infection rate ratio) = 0, $\log_{10} \left( \frac{V_2}{V_1} \right)$ varies between -24.1 and 3.4; and when $\log_{10}$ (infection rate ratio) = 3, $\log_{10} \left( \frac{V_2}{V_1} \right)$ varies only between -1.7 and 0.4.

### 4.3.1.2. Superinfection and progression to AIDS

Figure 4.3 shows the Kaplan-Meier curves for 1500 simulated HIV positive individuals showing the time to CD4 cell count <200 cells / $\mu$l in the absence or presence of superinfection of a heterologous HIV strain respectively. It was found that only superinfection with a fitter strain (green line; $\beta_2 > \beta_1$) leads to a faster progression to a
status of low CD4 cell count when compared with simulations without superinfection (black line). Superinfection with a weaker strain (red line; $\beta_2 < \beta_1$) does not lead to a faster progression, assuming that no recombination takes place or such recombination events do not lead to a mutant strain with a higher $\beta$.

**Figure 4.3** Kaplan-Meier curve showing the time (months) to achieve CD4 count ≤ 200 cells per ml among three categories of simulated patients.

Each category consists of 500 simulations. Black: No superinfection; Red: $\beta_2 \leq \beta_1$; Green: $\beta_2 > \beta_1$. Only patients superinfected with a fitter strain (Green: $\beta_2 > \beta_1$) experienced a faster progression to AIDS. The data for both categories with superinfection (Red and Green) are the same as that used for the analysis in Figure 4.2, with a range of $\beta_2$ from 0.01 to 100 times of $\beta_1$. For each combination of $\beta_2$ and $\beta_1$, 10 simulations were performed. Because Latin hypercube sampling was used to draw number across the parameter space, the uncertainty of the rate of superinfection of infected cells is averaged out between the two categories (Red and Green) and its influence upon the aggregated outcomes therefore is minimised$^{13}$.

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$^{13}$ A sensitivity analysis of parameters used in the model was performed and is presented in Chapter 5. The majority of parameters are not sensitivity to changes. Those that are sensitive are those related to CD4 T cells activation. Please refer to section 5.4.
4.3.2. Experiment B

If an HIV positive individual is on cART which successfully suppresses the viral load, a superinfecting strain may be able to establish itself in the patient if it is resistant to the existing cART regimen. Figure 4.4 illustrates that in the absence of cART, a superinfection with a resistant strain made little difference in total viral load while in the presence of cART only a superinfecting strain that is drug-resistant can successfully establish itself in the patient. In this example, the superinfection event took place on day 2436 after first infection. While the fitness of the first strain equal that of the second strain, drug efficacy against the second strain is 0.5 of that of the first strain.

The results are shown in Figure 4.5, which illustrates that if the resistance of superinfecting strain against either RTI or PI or both is above a threshold level (i.e. the drug efficacy against the superinfecting strain is below the threshold level), it can establish itself in an individual and displaces the initial strain (demonstrated by a positive log ratio between the superinfecting strain and the initial strain), when co-infection of cells is uncommon.

If a patient with a well-suppressed viral load is super-infected with a drug-resistant strain, even if that is of the same fitness with the first strain, the super-infecting strain will emerge and the patient will experience a rebound in viral load and treatment failure.
Figure 4.4  Viral load profile comparison.
(a) No cART: (i) single virus infection [dashed grey line with diamond] and (ii) superinfection with a resistant strain [black solid line]. (b) With cART: (i) single virus infection [dashed grey line with diamond] and (ii) superinfection with a resistant strain [black solid line]. Viral load shown is the total viral load of both strains in the case of superinfection.
Figure 4.5 The log ratio between the superinfecting HIV strain (virus 2, V2) and the initial HIV strain (virus 1, V1) against a parameter space of efficacy of reverse transcriptase inhibitor (RTI) and protease inhibitor (PI) against the superinfecting strain at the end of simulation (10 years since first infected; 1 year since superinfection).

4.4. Discussion

4.4.1. Experiment A

Through the analysis of a mathematical model incorporating superinfection into previously published within-host models of HIV (Fraser et al., 2001a, Fraser et al., 2001b, Griffin et al., 2006, Kim and Perelson, 2006), I have shown in Chapter 3 the variables influencing the quasi-equilibrium levels of CD4 cells and viral loads and the criteria for the establishment of infection. In this chapter, through numerical analysis of computer simulations of the model (Experiment A), I have shown that susceptibility of cells to HIV
superinfection is of paramount importance to the outcome of superinfection as it determines whether competitive exclusion or co-existence prevails. The relative viral fitness between the two strains determines the final outcome, only along the course set by the cellular susceptibility to superinfection in the parameter space. We also found that whether superinfection leads to faster progression to AIDS depends on whether the superinfecting strain is a fitter strain than the first strain. Only if the superinfecting strain is fitter will superinfection result in a higher viral load that in turn leads to a faster progression to AIDS. In this chapter this result is derived numerically but is consistent with our analytical findings in the previous chapter where I found the total virus population to be the same with one and two virus types leading to similar activated and infected CD4 cell numbers and progression to disease. Even in the case of fitter superinfecting virus, the difference in the pace of progression to AIDS is small. This explains the observation that not all known cases of superinfection are associated with a faster progression to AIDS.

There are examples of co-existence of the initial and superinfecting strains (Altfeld et al., 2002, Chohan et al., 2005, Jurriaans et al., 2008, Manigart et al., 2004, Plantier et al., 2004, Ramos et al., 2002, van der Kuyl et al., 2005), and also examples of competitive exclusion between two strains in the event of superinfection. Most of the latter were displacement of the first strain by the second strain (Brenner et al., 2004, Chohan et al., 2005, Gottlieb et al., 2007, Jost et al., 2002, Jurriaans et al., 2008, Koelsch et al., 2003, McCutchan et al., 2005, Smith et al., 2005b, Yang et al., 2005, Yerly et al., 2004), but there were also two cases in which a transient viraemia of the second strain was observed
and then disappeared soon afterwards (Pernas et al., 2006, Yerly et al., 2004). The relationship between model results and observations of superinfection suggest that co-infection of cells is rare and that the outcome is driven by the relative fitness of virus. With similar viruses co-existing, viruses with moderate differences in fitness take some time to move to exclusion; and with extreme differences, there can be no invasion or rapid replacement.

In a report of seven superinfection cases (Piantadosi et al., 2007), two patients had their original strain replaced by the superinfecting strain in association with a great increase in viral load, while the other five experiences no significant changes in viral load when both strains co-existed. In another report of three superinfection cases (Chohan et al., 2005), while one experienced the displacement of the first strain that had a very low level of replication, the other two experienced co-existence of two strains after superinfection in the presence of a high level of replication of the first virus. In another report of three cases of superinfection (Yerly et al., 2004), a patient, who had high viral loads for years that included the time of the superinfection event, experienced a transient viraemia of the second strain that was 1000-fold lower than that of the first strain and that was not detectable in later samples, while the other two cases had low viral load before the superinfection event and experienced a displacement of the first strain by the second strain after superinfection. These three reports point to the dynamics between the two strains which reflect the relative replicative fitness of the two strains that determine whether competitive exclusion or co-existence prevails. Furthermore, there was a case of a wild type first strain being displaced by a drug resistant second strain (Smith et al.,
cases of a drug resistant first strain being displaced by a wild-type second strain after superinfection (Koelsch et al., 2003, Yang et al., 2005). However, in two of such cases, the replicative capacity as conferred by pol of the second strain was lower than that of the first strain (Smith et al., 2005b, Yang et al., 2005), suggesting a possible difference in immune containment between the two viruses or the genetic basis of HIV viral fitness lying somewhere other than the pol coding region.

Mathematical models of multiple infections of target cells have been proposed (Dixit and Perelson, 2004, 2005, Suryavanshi and Dixit, 2007). Dixit and Perelson’s model (Dixit and Perelson, 2004) captured two mechanisms of multiple infections of cells, one being a series of sequential infectious contacts of a target cell with infected cells and free virions, and one being a single contact between a target cell and an infected cell leading a transfer of multiple viral genomes. With an extended model (Dixit and Perelson, 2005), they found that they could replicate the scaling law as observed in experiments by Levy et al. (2004), and generalise it so that number of cells infected by three viruses and the cube of the number of infected cells are proportional to each other. They also found that if the rate of production of virus was independent of the number of times the cells are infected, viral dynamics is not influenced by multiple infections of target cells. This model was further extended to study the emergence of recombinant HIV (Suryavanshi and Dixit, 2007). In essence what these three models have in common is an exponential decay of susceptibility of cells. However, all three models were based on the hypothesis that CD4 down-modulation was the underlying mechanisms of ‘Superinfection resistance’, which is unlikely to be case as argued by Nethe and colleagues (Nethe et al., 2005). Given that
Levy et al. (2004) observed that little inhibition to multiple infection events was there during HIV-1 replication, this feature was not incorporated into our model in order to keep the model simple. However, there was a recent report that most of the CD4 cells in the blood of HIV-positive patients are only infected with one copy of viral DNA (Sarah Palmer et al., ‘Single-cell Analysis of HIV DNA from Infected Patients’, Abstract 442, 16th Conference on Retroviruses and Opportunistic Infections 2009, Montreal, Canada, http://www.retroconference.org/2009/Abstracts/35935.htm). This may help parameterise the model in the future.

It has also been suggested that superinfection is associated with an accelerated progression to AIDS. Temporal association between high viral load and superinfection event has been observed in a number of cases (Altfeld et al., 2002, Brenner et al., 2004, Chakraborty et al., 2004, Chohan et al., 2005, Gottlieb et al., 2004, Gottlieb et al., 2007, Jost et al., 2002, Jurriaans et al., 2008, Koelsch et al., 2003, Manigart et al., 2004, McCutchan et al., 2005, Pernas et al., 2006, Piantadosi et al., 2007, Ramos et al., 2002, Smith et al., 2006, Smith et al., 2004, Smith et al., 2005b, van der Kuyl et al., 2005, Yang et al., 2005, Yerly et al., 2004). While as it was noted before (Smith et al., 2005a) that some cases might be associated with recent treatment interruptions (Altfeld et al., 2002, Chakraborty et al., 2004a, Jost et al., 2002, Pernas et al., 2006), a majority of reported superinfection events took place in the absence of cART (Brenner et al., 2004, Chohan et al., 2005, Gottlieb et al., 2004, Gottlieb et al., 2007, Jurriaans et al., 2008, Koelsch et al., 2003, Manigart et al., 2004, McCutchan et al., 2005, Piantadosi et al., 2007, Ramos et al., 2002, Smith et al., 2006, Smith et al., 2004, Smith et al., 2005b, van der Kuyl et al., 2005,
Yang et al., 2005, Yerly et al., 2004). The increase in viral load associated with superinfection event may be a consequence of viral burst as seen in primary infection. Given that a high viral load is an indicator for a faster progression to AIDS, these patients were predicted to experience an accelerated disease progression. However, this is not always the case. There have also been cases where the superinfection event took place without any significant changes in viral load (Casado et al., 2007, Chohan et al., 2005, Piantadosi et al., 2007, Plantier et al., 2004, van der Kuyl et al., 2005). In a case of two consecutive superinfection events in an individual (van der Kuyl et al., 2005), the first superinfection event that was intra-clade, no clinical symptoms or decline of CD4 cell count were reported whereas the second superinfection that was inter-clade, clinical symptoms, significant increase in viral load and decrease in CD4 cell count were observed.

In addition to increased viral load, decreased CD4 count has also been reported to be associated with superinfection events. Superinfection may be associated with an abrupt decline in CD4 cell count observed in an untreated long-term HIV survivor (Fang et al., 2004). An individual experienced superinfection around one year after seroconversion, had his CD4 cell count dropped to below 200 cells/µl in 2.4 years after seroconversion, and his first clinical AIDS-defining illness in 3.4 years after seroconversion (Gottlieb et al., 2004, Gottlieb et al., 2007). However, this individual’s rapid CD4 decline was not associated with the superinfection event in a regression analysis (Gottlieb et al., 2007). In addition, there have been reports of superinfection identified retrospectively among individuals with long-term non-progressive HIV infection (Casado et al., 2007).
Therefore, there is evidence that that HIV superinfection does not necessarily lead to high viral load or accelerated decline in CD4 count.

This experiment is limited in a number of ways. It is impossible to capture every details of a biological system with a mathematical model without making it too complicated. To keep the model simple, humoral responses are not incorporated in this model though neutralising antibodies are known to play a role against HIV superinfection (Smith et al., 2006). Within-host evolution of viral strains was not captured by this model either. Therefore, we should not exclude the possibility that superinfection of a less fit virus can also lead to the emergence of a mutant strain through recombination leading a faster progression to AIDS, if and only if, such a recombined strain has a higher fitness. As the possibilities of recombination and mutations are not included in the model, the model outputs should be carefully interpreted in the light of these simplifications.

On a different note, some might suggest that a simpler model will suffice, at least for the results presented in Figure 4.2. However, it is reasonable to construct a model with details good enough to test the implication of superinfection upon disease progression and simultaneously analyse the relationship between the rate of infection of infected cells by a second virus ($\omega$), the rate of infection of healthy cells ($\beta$) and viral load.

HIV superinfection is an important issue in relation to public health intervention and vaccine development. This mathematical model shows that HIV superinfection in and of itself is not a problem but a fitter virus could lead to faster progression. Acquisition of
resistant virus, and the possibility of cellular superinfection and therefore recombination leading to emergence of mutant strains are two other possibilities that needed to be taken into account. A less fit superinfecting strain will not establish itself if cellular superinfection is not possible. Superinfection can only go one way – either making things worse or generating no difference – but treatment is available. Such model findings are consistent with observation of viral competition and co-existence in cases studies. It warrants future studies including fitting the model outputs to empirical data from observational cohort where superinfection is carefully monitored should such become available.

4.4.2. Experiment B

In experiment B, with computer simulations of a within-host mathematical model, I demonstrated that in the presence of cART, superinfection with a heterologous HIV strain will not lead to viral blips. If the superinfecting strain is resistant to the existing cART regimen, superinfection will lead to treatment failure; if the superinfecting strain is susceptible to the existing regimen, it is difficult to establish itself in the patient and lead to a viral blip. In other words, viral blips observed in patients on cART with sustained viral suppression are not the result of superinfection.

In most of the reported cases of HIV superinfection, the superinfection event happened when the individuals were not on cART (Brenner et al., 2004, Chohan et al., 2005, Gottlieb et al., 2004, Gottlieb et al., 2007, Jurriaans et al., 2008, Koelsch et al., 2003, Manigart et al., 2004, McCutchan et al., 2005, Piantadosi et al., 2007, Ramos et al., 2002, Smith et al., 2006, Smith et al., 2004, Smith et al., 2005b, van der Kuyl et al., 2005, Yang
et al., 2005, Yerly et al., 2004), or experienced interruptions in their cART (Altfeld et al., 2002, Chakraborty et al., 2004a, Jost et al., 2002, Pernas et al., 2006). Pernas et al. (2006) was the only superinfection case report in which the superinfection event probably took place when the individual was on cART. The patient had been on various anti-retroviral regimens, with frequent changes and interruptions. The superinfection event took place within a 3-month period in which the patient was on cART (didanosine + stavudine (d4t) + nelfinavir (NFV)) for two months. However, the drug-resistant mutations of one of the two superinfecting strains were against azidothymidine (AZT) and lamivudine (3TC) (Pernas et al., 2006). Therefore, currently there were not yet any reports of superinfection in the presence of a cART regimen by a superinfecting strain that is resistant to that regimen. I predict that given transmission of resistant virus to uninfected individuals, transmission to treated infected individuals also occurs. That such cases have not been observed may be because they are not distinguished from evolution of the treated infection within the host.

Non-adherence to cART is probably a major cause for viral blips, and in the next chapter, I have demonstrated that a three-monthly sampling frame of viral loads will miss a certain proportion of viral blips caused by non-adherence to cART.

In conclusion, the results of this experiment have eliminated the possibility of superinfection with HIV heterologous strain as an explanation of viral blips in HIV positive patients with sustained suppressed viral loads. If viral blips are observed in
patients, alternative hypotheses need to be looked at for explanations, e.g. non-adherence to cART.

**4.5. Summary**

Through numerical analysis of computer simulations of the mathematical model described in Chapter 3, it was found that (a) both viral fitness and susceptibility of target cells to superinfection of heterologous HIV strains determine the outcomes of superinfection events experienced by HIV positive individuals; that (b) superinfection, in and of itself, does not lead to faster progression to AIDS, and that (c) superinfection is not an explanation for viral blips observed in HIV positive individuals whose viral loads were previously suppressed through cART.
Chapter 5

Viral blips and drug adherence: definition and sampling frame
5. Viral blips and drug adherence: definition and sampling frame

5.1. Introduction

Episodes of transient viraemia, or ‘viral blips’, have been a topic for concern to clinicians and HIV patients alike. Their nature, their causes and their clinical consequences have all been topics of debate. While some argued that they were just measurement artefacts (Stosor et al., 2005) or random biological and statistical variations around mean HIV viral loads (Nettles et al., 2005), some argued that they were genuine biological phenomena that require alternative explanations (Percus et al., 2003). I believe that one contributing factor to the diverse outcomes of the various studies on viral blips is that there has been no consensus on the definition of viral blips and the choice of sampling frame to detect them (see Table 1.3). This made comparison across studies difficult as we may not be comparing like with like.

In this chapter I explore this issue by applying the mathematical model of HIV within-host dynamics described in Chapter 3. By using different definitions of viral blips and three separate sampling frames (<1 week, monthly and 3-monthly), I can explore how the number of blips observed is affected.

5.1.1. Viral blip definition

Table 1.3 lists a number of papers that have studied viral blips. A definition of viral blips can be categorised in the following dimensions:
(a) the lower limit of HIV RNA detection offered by the assay: most studies used 50 copies/ml as the cut off point, but there were other values used;
(b) whether there was an upper viral load limit: some studies considered any measurements above a certain threshold (e.g. 500 or 1000 copies/ml) as an indication of treatment failure and therefore a ‘blip’ must be something below that threshold, while other studies might not have any upper viral load limit for a blip;
(c) any requirement for preceding and/or subsequent measurements that were below the detectable level: some studies made explicit such a requirement, e.g. preceded by two consecutive measurements that were <50 copies/ml and followed by one that was <50 copies/ml;
(d) whether consecutive measurements above the detection threshold count as blips or treatment failure: e.g. Podsadecki et al. (2007) defined two consecutive measurements that were ≥ 50 copies/ml as ‘treatment failure’.

The variety of definitions of viral blips makes comparison of their prevalence or incidence across studies difficult. Later, I will discuss how it affects the number of viral blips identified in a study.

5.1.2. Choice of sampling frame

Table 1.3 also lists the frequency of measurement used in these studies. Most studies, especially those among routine clinical cohorts, had a sampling frame of around every three months (or 12 weeks). Some studies using data from clinical trials had a sampling frame of unevenly spaced measurements over a matter of weeks. The only exception is Nettles et al. (2005), in which measurements were taken every two or three days. We
argue that Nettles et al. (2005) needs to be treated in a separate category and its results should not be compared with those of other studies. Viral blips as found in Nettles et al. (2005) were likely to be of a different nature than those found in other studies.

The difficulty in defining a ‘true’ viral blip coincides with the problem of the choice of sampling frame. As will be demonstrated later in this chapter, both definition of viral blips and measurement sampling frame are of great importance in determining the number and frequency of viral blips as observed in a cohort of patients. In a clinical setting, where a clinic visit is usually scheduled every three or four months, consecutive viral load measurements above the detection threshold, after achieving viral suppression, is often considered as an indication of treatment failure. However, as it had been demonstrated (Di Mascio et al., 2005), a transient episode of viraemia usually lasted for three weeks: two consecutive detectable viral load measurements could well be two viral blips. Nevertheless, as will be shown below, poor drug compliance clearly correlates with a high number of positive detectable viral load measurements, regardless of whether they were ‘true’ blips or not.

5.1.3. Mathematical modelling

A number of mathematical modelling studies have been performed to study the phenomenon of viral blips (Jones and Perelson, 2005, 2007, Rong and Perelson, 2009) and different patterns of drug adherence respectively (Huang, 2008, Huang et al., 2003, Krakovska and Wahl, 2007c, Labbé and Verotta, 2006, Wahl and Nowak, 2000, Wu et al., 2005a, Wu et al., 2006). Mathematical models enable researchers to examine different hypotheses by generating simulated data sets when clinical data are scarce and usually
inadequate. In this chapter, data sets generated through computer simulations can be treated as the ‘real’ data that are usually absent in clinical studies because of the infrequency of the sampling frame and against which the monthly and quarterly sampling frames are tested. In the following sections, I will present my mathematical modelling results.

5.2. Methods

The model has been described in Chapter 3. It was developed in C++ using the Microsoft® Visual Studio .NET 2003 programming environment. In this chapter, the drug adherence pattern was added to the flexible time-step version of the model. There was only one viral strain; no superinfection was simulated. Simulations started on day -730 (two years before infection); HIV infection happened on day 0; cART began on day 2920 (year 8 since infection) and simulations finished on day 3650. For a given drug adherence level or pattern, we have either 10 or 100 simulations (depending on the experiment), each representing an individual patient.

5.2.1. Simple drug adherence pattern

A simple drug adherence pattern was achieved by fixing the dose interval at 12 hours. Every time the designated time passes, a random number is drawn from a uniform distribution between 0 and 1. If the number is above the designated adherence probability for that given simulation, then that dose will be missed. In other words, the taking of drug is a Bernoulli distribution of a probability of the designated drug adherence. Ten simulations were performed for each drug adherence level between 0.3 and 0.8 (inclusive).
5.2.2. More realistic drug adherence pattern

Three parameters were identified as important for a model of drug adherence: 1) proportion of doses taken \( (p) \), 2) standard deviation of the random dose-timing error and 3) any systematic dose-timing bias (Ferguson et al., 2005). We incorporated the first two into our model. At the beginning of every day, a random number was drawn from a binomial distribution with a proportion of average doses taken \( (p) \) and possible outcomes of 0, 1 or 2, i.e. Bin \((2, p)\). If a number 1 or 2 was drawn, then, a random number would be drawn from a normal distribution with a mean of 0.5 day and a standard deviation of 2.5 hours \( (0.1042 \text{ day}) \). The exact timing of the dose was then the random number minus 0.25 day. If number 2 was drawn from the binomial distribution (i.e. two doses on that day), a second random number would be drawn from the normal distribution, again \( N (0.5, (0.1042)^2) \). The exact timing of the second dose was that of the first dose plus the second random number. In other words, if we follow the example of Ferguson et al. (2005) and take 3 a.m. as the boundary between each 24-hour period (a day), then the timing of the two doses would be \( N (9 \text{ a.m., (2½ hours)}^2) \) and \( N (9 \text{ p.m., (2½ hours)}^2) \). One hundred simulations were performed for each drug adherence level from 0 to 1 (inclusive).

5.2.3. Pharmacokinetics

The half-life for RTI and PI used in the model are 0.75 day and 0.16667 day respectively. The former is the half-life of Abacavir (18 hours) and the latter is that of Ritonavir (4 hours). The prescribed dose interval for both Abacavir and Ritonavir is 12 hours, which is also the prescribed dose interval in this modelling study (Krakovska and Wahl, 2007c).
The plasma concentrations of RTI and PI ([RTI] and [PI]) are converted into their effectiveness against the virus according to the following equations:

\[
drug_{RTI} = \frac{[RTI]}{[RTI]+1} \quad (5.1)
\]

\[
drug_{PI} = \frac{[PI]}{[PI]+1} \quad (5.2)
\]

### 5.2.4. Sampling frame

We use three sampling frames for counting the number of events of transient viraemia:

1. Less than one week;
2. Monthly;
3. Quarterly (3-monthly).

For the <1-week sampling frame, the viral load outputs (that were outputted every 100 flexible time steps, corresponding to every three to seven days) were plotted in Microsoft® Office Excel 2003 (Microsoft Corporation©) and the number of events of transient viraemia (or viral blips, defined below), was counted by eye. For the monthly and quarterly sampling frames, a separate common separated values (CSV) file with monthly viral load measurement was outputted for each set of simulations. Viral blips were counted from month 97 onwards. The quarterly measurements were counted from the same set of monthly measurements, from month 97 onwards (month 97, 100…).

### 5.2.5. Definition of viral blips

#### 5.2.5.1. Definition set A

**< 1-week sampling frame.** An episode of transient viraemia or a viral blip was defined as a continuous series of viral load measurements ≥ 50 copies/ml after suppression of
viral load below detectable level and before the next measurement of undetectable viral loads as observed by eye in the time series for the patient (a definition used in Nettles et al., 2005).

**Monthly and quarterly sampling frame.** Each viral load measurement $\geq 50$ copies/ml is considered to be an independent event of transient viraemia (i.e. an individual blip).

### 5.2.5.2. Definition set B

**Monthly and quarterly sampling frame.** A viral load measurement $\geq 50$ copies/ml, preceded and followed by viral load measurement $< 50$ copies/ml, is defined as an independent event of transient viraemia (i.e. a ‘single’ blip). If it is consecutively preceded or followed by a viral load measurement $\geq 50$ copies/ml, it is defined as a ‘treatment failure’.

### 5.2.6. Cumulative viral load (Area under the viral load curve)

The cumulative viral load was calculated from the output data (every 100 flexible time steps, i.e. $<1$ week sampling frame) using Microsoft® Office Excel 2003 (Microsoft Corporation©). The area under the curve for each flexible time step was calculated using the trapezium rule. The data reported in Figure 5.8 are the cumulative areas under the curve from the last measurement before commencement of antiretroviral therapy (RTI-only) (day 2920) to the end of simulations (day 3650).
5.3. Results

5.3.1. Simple adherence pattern

First, I simulated a simple drug adherence pattern in which the timing of doses is fixed (every 12 hours, see Methods). Whether or not the patient takes drug at the designated time is drawn from a binomial distribution, where \( p \), the probability of each particular dose being taken equals the adherence, i.e. the proportion of doses taken. Definition set A was used. Two examples of viral load time series are given in Figures 5.1 and 5.2.

Figure 5.1 An example of viral load time series (drug adherence = 0.6; simple adherence pattern)
This is an example of time series of viral load in a simulation, with protease inhibitor, simple adherence pattern and a drug adherence of 0.6. The black line represents infectious virions \([V(I)]\) and the grey dotted line with squares represents non-infectious virions \([V(NI)]\). This example assumes that \( drugRTI = drugPII = 0.99\) when a dose is taken; half-life of reverse transcriptase inhibitors = 0.75 day; half-life of protease inhibitors = 0.16667 day; and there is only one virus.
Figure 5.2 An example of viral load time series (drug adherence = 0.3; simple adherence pattern)
This is an example of time series of viral load in a simulation, with protease inhibitor, simple adherence pattern and a drug adherence of 0.3. The blue line represents infectious virions [V (I)] and the grey line with square represents non-infectious virions [V (NI)]. The red line highlights the level of detectable viral load (50 copies/ml). This example assumes that $drug_{RT1} = drug_{PI1} = 0.99$ when a dose is taken; half-life of reverse transcriptase inhibitors = 0.75 day; half-life of protease inhibitors = 0.16667 day; and there is only one viral strain.

Figure 5.3 shows that if both PI and RTI are taken, the number of events of transient viraemia is lower than the case when only RTI is taken. In the RTI-only case, when drug adherence is 0.4 or lower, the number of blips observed in the sampling frame of < 1 week is lower than that of every month (Figure 5.3 top). That is because very long periods of viraemia (longer than one month) were observed which made the assumption in Definition set A for monthly and quarterly sampling frames untenable.

It is clear that more episodes are seen when adherence is low and when detection frequent. Given that patients normally see their doctors once every three months (as in the case of
the ATHENA cohort of the Netherlands), a substantial number of blips would be missed. It is notable that very poor adherence (e.g. \( p < 0.45 \)) is required before viral blips are observed frequently (e.g. \( \geq 10 \) in two years).

**Figure 5.3** Comparison of number of blips between the presence and absence of protease inhibitor in a simple adherence pattern.
(Top) Total number of episodes of transient viraemia (blips) (y-axis) as observed under different sampling frequencies over a period of 2 years under anti-retroviral therapy and in a set of 10 simulations per drug adherence level (x-axis). Reverse transcriptase inhibitors are taken in all cases. For definition of viral blips, Definition set A was used. (Below) Same as (Top), but showing results for drug adherence \( \geq 0.45 \) only.

### 5.3.2. More realistic adherence pattern

In real life, timing of dose taking is seldom exact and it varies across time and between patients. A more realistic adherence pattern was later simulated (see Methods) and its results were compared with that of the simple drug adherence pattern (Figure 5.4). It was found that more blips were observed if the timing of every dose is not fixed exactly, but
varied around the designated time. To achieve no blips observed when sampling occurs every week or more, a drug adherence of 0.75 is needed for the more realistic pattern rather than 0.6 in the simple adherence scenario. This result indicated that in addition to the proportion of doses taken \((p)\), the standard deviation of the random dose-timing error is also important (cf. Ferguson et al., 2005). Therefore, for further analysis, we focused on the data set of this more realistic adherence pattern. Furthermore, the difference in number of blips observed across different sampling frames is rather obvious in both adherence patterns.

**Figure 5.4** Comparison of number of blips between a more realistic drug adherence pattern [R] and the simple drug adherence pattern [S].
The latter are the results [RTI] shown in Figure 5.1. Results shown are total number of period of transient viraemia (blips) \((y\text{-axis})\) as observed under different sampling frequencies over a period of 2 years under anti-retroviral therapy of 10 simulations per drug adherence level \((x\text{-axis})\). Only reverse transcriptase inhibitors were taken; no protease inhibitors. This is to simplify the scenario in order to illustrate the point. Definition set A was used.

We then repeated the simulations using the more realistic sampling frame, but with both RTI and PI, and for a total of 100 simulations. Again, it was found that fewer blips were observed when both drug classes were used (Figure 5.5). In the RTI-only scenario, more than half of the observations were \(\geq 50\) copies/ml if drug adherence \(\leq 0.35\), but in the RTI+PI scenario, less than 40% of observations were \(\geq 50\) copies/ml even if drug
adherence was 0.3. The figure shows the proportion of observations that are ≥ 50 copies/ml from all observations, taken while cART was being administered, in a given sampling frame. It was found that for a given scenario, the proportion is similar in both monthly and quarterly sampling frames. This shows that rather than calculating the incidence of blips directly from the number of blips observed in a given period of time, it is better to report the proportion of observations in a given period of time that are ≥ 50 copies/ml. Therefore, as the denominator, the number of observations in a given period of time is important. This further draws our attention to the question how the choice of sampling frame affects the results of studies on viral blips.

Figure 5.5  Comparison of proportion of observations that are ≥50 copies/ml between the presence and absence of protease inhibitor in a more realistic drug adherence pattern. Proportion of observations that were ≥50 copies/ml as observed under monthly (left) and quarterly (right) sampling frames over a period of 2 years under anti-retroviral therapy. RTI-only (same data set as [S] in Figure 5.2): 10 simulations per drug adherence level; PI+RTI: 100 simulations per drug adherence level.
5.3.3. Definition matters

Next, we further analysed the quarterly sampling frame data of the more realistic adherence pattern, to see how big a difference would be observed if there was a change of viral blip definition. It is interesting to note that in the RTI-only scenario (Figure 5.6), for \( p \leq 0.4 \), the majority of \( \geq 50 \) copies/ml measurements were preceded and/or followed by another \( \geq 50 \) copies/ml measurement (excluding the first and last measurement under cART), but it is for \( p \leq 0.3 \) in the RTI+PI scenario (Figure 5.7). Figure 5.7 also shows that a fraction of the ‘blips’ that were identified previously were the first post-treatment viral load measurement (month 97) that was \( \geq 50 \) copies/ml, of which many are followed by viral suppression. They were more likely an indication of viral load yet to be fully suppressed with therapy than an independent blip after successful suppression. Figure 5.7 also shows that some previously identified ‘blips’ were the last measurements before the end of the observation period and we are therefore unable to determine whether they would be followed by viral suppression (and hence ‘blips’) instead of continual rebound (‘failure’). Using the RTI-only scenario as an example, if we adopt definition set B for viral blips and treatment failure (Figure 5.8), there were more ‘blips’ observed (Figure 5.8a) and more patients (8 out of 10) experienced ‘blips’ (Figure 5.9 top), when \( p = 0.4 \). There were more events of treatment ‘failure’ as drug adherence is lower (Figure 5.8b) with the vast majority of patients with \( p \leq 0.35 \) experiencing failure (Figure 5.9 bottom).

At a lower adherence, more patients experienced slower viral decline (more ‘first’ measurements being \( \geq 50 \) copies/ml; Figure 5.8c) and more patients experienced ‘last’ measurements being \( \geq 50 \) copies/ml (Figure 5.8d).
Figure 5.6 Analysis of viral load measurements of 3-monthly sampling frame of a more realistic drug adherence pattern (RTI-only).

Percentage of quarterly observations over a period of two years under anti-retroviral therapy; 10 simulations per drug adherence level (from 0.3 to 0.8). Re-analysis of the same data set in Figure 5.5, that are classified as: Consecutive: ≥ 50 copies/ml measurement preceded and/or followed immediately by another ≥ 50 copies/ml measurement. Single: ≥ 50 copies/ml measurement both preceded and followed immediately by a < 50 copies/ml measurement (Definition Set B). Last: ≥ 50 copies/ml measurement in the last measurement. First: first measurement being ≥ 50 copies/ml. ‘Consecutive’ and ‘Single’ are counted only from the second measurement under cART to the second measurement. ‘First’, ‘Last’ and ‘Consecutive’ can be grouped together as one category – ≥ 50 copies/ml observations that are not categorised as ‘blips’ according to Definition Set B.
Figure 5.7 Analysis of viral load measurements of 3-monthly sampling frame of a more realistic drug adherence pattern (RTI and PI).
Percentage of quarterly observations over a period of two years under anti-retroviral therapy; 100 simulations per drug adherence level (from zero to 1). Re-analysis of the same data set in Figure 5.5, that are classified as: Consecutive: ≥50 copies/ml measurement preceded and/or followed immediately by another ≥50 copies/ml measurement. Single: ≥50 copies/ml measurement both preceded and followed immediately by a <50 copies/ml measurement (Definition Set B). Last: ≥ 50 copies/ml measurement in the last measurement. First: first measurement being ≥50 copies/ml. ‘Consecutive’ and ‘Single’ are counted only from the second measurement under cART to the second measurement. ‘First’, ‘Last’ and ‘Consecutive’ can be grouped together as one category – ≥ 50 copies/ml observations that are not categorised as ‘blips’ according to Definition Set B.
Figure 5.8 Number of events as observed in a 3-monthly sampling frame of a more realistic drug adherence pattern.

Data were the same as [S] in Figure 5.4 and were re-analysed; they were over a period of 2 years under anti-retroviral therapy of 10 simulations per scenario. (a) Top left corner: ‘single’ refers to a single ≥ 50 copies/ml measurement both preceded and followed immediately by a < 50 copies/ml measurement (Definition set B) (b) Top right corner: ‘failure’ refers to events of ‘treatment failure’ defined as a period of consecutive measurements that are ≥ 50 copies/ml (Definition set B). One ‘failure’ event can be of 2 to 8 measurements here. (c) Bottom left corner: ‘first’ refers to the first post-treatment viral load measurement being ≥ 50 copies/ml regardless of whether it is followed immediately by a < 50 copies/ml measurement. (d) Bottom right corner: ‘last’ refers to the last measurement being ≥ 50 copies/ml with a preceding < 50 copies/ml measurement (therefore not ‘single’ nor ‘failure’).
Figure 5.9  Number of patients with (top) ‘blips’ and (bottom) ‘failures’, of a given number in years, out of 10 patients as observed in a quarterly sampling frame at each drug adherence level. Data same as that of the more realistic sampling frame in Figure 5.4. Blips and failures were defined here according to Definition set B.

5.3.4. Cumulative viral load (Area under the viral load curve)

Given the time under treatment is the same for all simulations (two years), by calculating the area under the viral load curve, i.e. cumulative viral load (days * copies/ml), from the
commencement of cART until the end of simulations, one can compare across drug adherence level the amount of virus present in two years under treatment, and therefore, the impact of the treatment. Figure 5.10 shows that in the presence of RTI only, for \( p \geq 55 \), the time-viral load is around 200,000 days*copies/ml for two years, and the exact time for dose taking matters little in the impact of the treatment. For \( 35 \leq p \leq 50 \), it is clear that variation in dose-taking time affects treatment efficacy, with the difference at \( p = 45 \) the greatest (11 times higher than strict timing). When \( p = 30 \), as drug adherence is low, the timing of dose taking makes little impact on the overall outcome.

Figure 5.10  Cumulative viral load (area under the viral load curve) since commencement of cART for each drug adherence level (RTI only).

Ten simulations per drug adherence level. The data shown was the area under the viral load curve from the last measurement before cART commencement in the < 1 week sampling frame to the end of simulations. Simple (black line): Simple drug adherence pattern with time of dose taking fixed; More realistic (grey line): More realistic drug adherence pattern with exact time of dose taking drawn from a normal distribution with a mean (prescribed time) and a standard deviation (2.5 hours). Median (Diamond for Simple; Square for more realistic), and 25th and 75th percentile (lower and upper error bars) for 10 simulations. Note the log-scale for time-viral load (y-axis).
5.4. Model validation

5.4.1. Sensitivity analysis (Beta)

We performed a sensitivity analysis by varying $\beta$. It was found that by decreasing $\beta$ by 10-fold (from 754 to 75.4), CD4 cell count in month 120 (the last monthly sample) doubles or triples, in the presence or absence of cART respectively (Figure 5.11). If $\beta$ was increased by 10-fold (from 754 to 7540), CD4 cell count would decrease by two- or three-fold if drug adherence is 0.5 or 0.25 respectively, but it would stay roughly the same if drug adherence is 0 or 0.75.

The variation in viral load at month 120 with respect to $\beta$ was great (Figure 5.12). The major variation was found with drug adherence 0.25 and 0.5. If $\beta$ was reduced by 10-fold, even a drug adherence as low as 0.25 can achieve viral suppression. If $\beta$ was increased by 10-fold, a drug adherence was unable to control viral replication. Therefore, the success or failure of the range of drug adherence levels tested in this study to suppress viral replication is highly contingent to the choice of $\beta$, which is chosen to be 754, following a previously established model (Fraser et al., 2001b) on which our model is based\(^\text{14}\).

\(^{14}\) As a response to the examiners, the viral load is dependent on $\beta$. However, it appears that their relationship is non-linear. Between the range of $\beta$ values of 75.4 and 7540, in the absence of drugs, the variation in viral load is negligible on a log scale. However, as suggested by Prof. Rob de Boer, the infection constant is the multiple of $\beta$ and drug effectiveness (which in turn is a non-linear function of drug concentration). The non-linear relationship shown in Figure 5.12 shows that the range at which a huge change in viral load (at the end of simulation) is found to be between $377 (=754*(1-0.5))$ and 754.
Figure 5.11  Variation of β on CD4 T cell count in month 120. Drug adherence levels: (black) 0, (blue) 0.25, (red) 0.5 and (green) 0.75; (broken line with diamond): 25% quartile range (QR), (line with square) median and (broken line with triangle): 75% QR.

Figure 5.12  Variation of β on viral load in month 120. Drug adherence levels: (black) 0, (blue) 0.25, (red) 0.5 and (green) 0.75; (broken line with diamond): 25% quartile range (QR), (line with square) median and (broken line with triangle): 75% QR.
5.4.2. Sensitivity analysis (Initial value for antigenic stimulation)

We performed a sensitivity analysis on the initial value for antigenic stimulation. The initial value of $k_4$ and $k_8$ were set to 5 in all previous simulations. We changed this value to 1, 3, 7, or 9. We found that these changes made no difference to the outcomes, both in terms of CD4 cell count or viral load, measured in month 120 (the last monthly measurement) (Figures 5.13 and 5.14).

Figure 5.13 Variation of initial value of $k_4$ and $k_8$ on CD4 T cell count in month 120. Drug adherence levels: (black) 0, (blue) 0.25, (red) 0.5 and (green) 0.75; (broken line with diamond): 25% quartile range (QR), (line with square) median and (broken line with triangle): 75% QR.
Sensitivity analysis for the other parameters (Table 5.1 and Figures 5.15 and 5.16) found that the parameters that influence the CD4 cell activation process are the most influential; these are: average rate of T cells activation per antigenic exposure ($a_0$), relative T cell pool size below which T cell activation fails due to exhaustion of repertoire ($x_S$), average probability of an activated T cell successfully dividing in an individual free of HIV ($p_A$), average clearance rate in antigenic exposure model ($\theta$), and average exposure rate in antigenic exposure model ($\tau$). It is important to point out that the choice of values used in the sensitivity analysis is judged according to biological plausibility and therefore the percentage change is not uniformed across the parameters (see Table 5.1). Figures 5.15 and 5.16 show the change in proportion of the CD4 cell count and viral load at month 120.
if a particular parameter is changed (to an extent stated in Table 5.1). More detailed sensitivity analysis results are tabulated in the Appendix.

Table 5.1  Sensitivity analysis as shown in percentage change in CD4 count and Viral load

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Description</th>
<th>Model values</th>
<th>Sensitivity analysis values</th>
<th>% change</th>
<th>Value (25QR, 75 QR) as a percentage of the median of the control values</th>
</tr>
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<tbody>
<tr>
<td>Median CD4 count when all parameters follow the model values</td>
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</tr>
<tr>
<td>$a_0$</td>
<td>average rate of T cells activation per antigenic exposure</td>
<td>$10^{-4}$</td>
<td>$10^{-2}$</td>
<td>1000%</td>
<td>18703 (15258, 21615) cells /ml i.e. 100% (82%, 116%)</td>
</tr>
<tr>
<td>$\mu$</td>
<td>daily rate of non-antigen-driven homoeostatic T cell division</td>
<td>0.01</td>
<td>0.001</td>
<td>1000%</td>
<td>5420% (5394%, 5440%)</td>
</tr>
<tr>
<td>$x_s$</td>
<td>relative T cell pool size below which T cell activation fails due to exhaustion of repertoire</td>
<td>0.05</td>
<td>0.01</td>
<td>2000%</td>
<td>100% (21%, 51%)</td>
</tr>
<tr>
<td>$\rho_s$</td>
<td>average probability of an activated T cell successfully dividing in an individual free of HIV</td>
<td>0.55</td>
<td>0.3</td>
<td>55%</td>
<td>75% (40%, 63%)</td>
</tr>
<tr>
<td>$\beta$</td>
<td>average infection rate of an activated CD4 T cell per virion</td>
<td></td>
<td></td>
<td></td>
<td>81% (70%, 103%)</td>
</tr>
<tr>
<td>$a_r$</td>
<td>become virally productive death rate of infected cells in the absence of CTL</td>
<td>1</td>
<td>2</td>
<td>200%</td>
<td>97% (81%, 114%)</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>death rate of productively infected cell in the absence of CTL</td>
<td>1</td>
<td>2</td>
<td>200%</td>
<td>107% (87%, 127%)</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>rate of reactivation of latent infected cells</td>
<td>1</td>
<td>0.5</td>
<td>50%</td>
<td>137% (103%, 170%)</td>
</tr>
<tr>
<td>$a_z$</td>
<td>proportion of successful infections that result in latency</td>
<td>0.01</td>
<td>0.001</td>
<td>10%</td>
<td>100% (80%, 123%)</td>
</tr>
<tr>
<td>$f_s$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>96% (78%, 120%)</td>
</tr>
<tr>
<td>$\beta_{ae}$</td>
<td>rate of CTL activation per productive infected cells</td>
<td>1.3334 * $10^{-5}$</td>
<td>1.3334 * $10^{-6}$</td>
<td>1000%</td>
<td>98% (79%, 120%)</td>
</tr>
<tr>
<td>$\rho_z$</td>
<td>maximum proliferation of anti-HIV CTLs</td>
<td>0.05</td>
<td>0.01</td>
<td>99% (86%, 114%)</td>
<td></td>
</tr>
<tr>
<td>$d_z$</td>
<td>death rate of resting CTLs</td>
<td>0.01</td>
<td>0.001</td>
<td>10%</td>
<td>96% (78%, 114%)</td>
</tr>
<tr>
<td>$\psi$</td>
<td>pre-infection frequency of anti-HIV CTL</td>
<td>10^{-5}</td>
<td></td>
<td></td>
<td>96% (78%, 114%)</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>threshold value of infected cells for the logistic proliferative response of CTL to HIV</td>
<td></td>
<td></td>
<td></td>
<td>97% (78%, 114%)</td>
</tr>
<tr>
<td>$\gamma_T$</td>
<td>ratio of viral production rate in productively infected cells and viral clearance rate</td>
<td></td>
<td></td>
<td></td>
<td>97% (78%, 114%)</td>
</tr>
<tr>
<td>$b/c$</td>
<td>maximum rate of CTL killing of HIV-infected cells</td>
<td>10^{-6}</td>
<td>10^{-2}</td>
<td>1000%</td>
<td>96% (84%, 123%)</td>
</tr>
<tr>
<td>$\theta$</td>
<td>average clearance rate in antigenic exposure model</td>
<td>0.02</td>
<td>0.004</td>
<td>20%</td>
<td>16% (13%, 18%)</td>
</tr>
<tr>
<td>$\tau$</td>
<td>average exposure rate in antigenic exposure model</td>
<td>0.1</td>
<td>0.02</td>
<td>50%</td>
<td>15% (14%, 17%)</td>
</tr>
</tbody>
</table>
Figure 5.15  Variation of initial value of parameters on CD4 cell count in month 120. Note the log scale on the x-axis.
Figure 5.16 Variation of initial value of parameters on viral load in month 120.
5.4.4. Comparison with ATHENA cohort of the Netherlands

Earlier in this chapter, I suggest that the ATHENA cohort data (van Sighem et al., 2008) corresponds to a drug adherence level around 40%. I then further analysed the data for a more detailed comparison. Figure 5.15 shows that the great majority of measurements ≥ 50 copies/ml were > 1000 copies/ml. If we consider the proportion of single blips (defined according to Definition set B) among those measurements that were 50-1000 copies/ml (Figure 5.16), we found that it gradually increase from zero at drug adherence between 0 and 0.1, to over 0.7 at drug adherence of 0.5. This is due to the decreasing number of measurements that are >50 copies/ml and therefore decreasing number of measurements that are ‘consecutive’. When \( p > 0.6 \), all measurements were <50 copies/ml.

![Figure 5.17](image)

**Figure 5.17** Number of viral load measurements that were (black) 50-1000 copies/ml and (grey) >1000 copies/ml, out of 100 patients as observed in two years in a quarterly sampling frame at each drug adherence level.

Re-analysis of data of Figure 5.7.
In van Sighem’s (2008) studies, with a total follow-up of 11,187 person-years after viral suppression of a study population of 4447 patients, there were 36,940 viral load measurements made, of which 2216 were between 50 and 1000 copies/ml. There were 1711 episodes of low-level viraemia (50-1000 copies/ml), of which 81.8% consisted of only one measurement (i.e. ‘Single blip’ according to Definition set B). Therefore, 63% of measurements between 50 and 1000 copies/ml were ‘single blips’. This falls between the range of drug adherence 0.45 and 0.55 in Figure 5.16. This is not too far away from our ‘prediction’ of drug adherence 40%. However, given the absence of drug adherence data in the ATHENA cohort, we are unable to test our model predictions against empirical data.

Figure 5.18 Proportion of single blips among measurements that were 50-1000 copies/ml at each drug adherence level, out of 100 patients as observed in two years in a quarterly sampling frame at each drug adherence level.

Re-analysis of data of Figure 5.7. Single blips were defined here according to Definition set B and therefore exclude those measurements classified as ‘first’, ‘last’ and ‘consecutive’ (cf. Figure 5.7 legend). As the number of measurements that were 50-1000 copies/ml varies across drug adherence levels (cf. Figure 5.15), the denominator for each drug adherence is different. The reason for low proportion at the low end of the drug adherence spectrum is that most measurements were > 1000 copies/ml, and the reason for zero proportion at p ≥ 0.65 is the absence of measurements that were ≥ 50 copies/ml.
5.5. **Discussion**

This chapter highlights the problem of the use of a variety of definitions of viral blips and choices of sampling frame that renders comparison across published studies difficult. I use the mathematical model described in Chapter 3 to demonstrate both the impact of varying drug adherence upon viral blips and the impact of the choice of definition and sampling frame upon the results.

The model showed that by using different sampling frames, a different number of blips will be observed. I chose three sampling frames: $< 1$ week, monthly and quarterly. My model output results every 100 flexible time steps, which represents a time of two to seven ‘days’. Therefore, data from this sampling frame are the most detailed data for each ‘patient’ that we were able to generate and they formed the ‘standard’ against which we understood the outputs of the other two sampling frames. The $< 1$ week sampling frame is similar to that which Nettles et al. (2005) used in their study.

Ten patients were analysed intensively in Nettles et al. (2005) which is the only study with a very frequent sampling frame (every 2-3 days). In their study, 78% of 18 blips observed in 10 patients in less than 8 months, occurred when drug levels were above the trough concentrations provided by drug manufacturers and therefore there was no association between low drug concentrations and blips ($P = 0.22$, $X^2$ test). My model specifically tested the situation with the hypothesis that non-adherence to antiretroviral treatment leads to viral blips. It does not exclude other potential causes of viral blips. Nettles et al. (2005) argued that viral blips were just random variations around an
undetectable mean viral load. It is possible that the blips they observed were of a different category to those of other studies, given its small sample size (713 measurements in 359 observations in 10 patients). As my simulations incorporated both the element of stochasticity and various levels of drug adherence, and given that the same seed for the random number generator was used for each batch of simulations, it is possible to exclude random variation as an explanation for the different outcomes observed in simulations of different levels of drug adherence.

The other two sampling frames – monthly and quarterly – were used by most other studies, although studies using clinical trial data usually had more frequent sampling at the beginning of the trial and less frequent as the trial went on (Table 1.3). My modelling results indicated that results in terms of incidence or prevalence of blips would be highly influenced by the choice of sampling frame as a 3-monthly sampling frame might miss some blips. In both cases, but especially so in the case of a monthly sampling frame, the \textit{a priori} definition of blips determines whether consecutive measurements $\geq 50$ copies/ml are considered as independent viral blips, treatment failure (Podsadecki et al., 2007) or ‘bumps’ (Greub et al., 2002), and therefore would significantly affect the incidence of blips that a study reports. I suggest that a better way of representing the frequency of viral blips is the proportion of blips of all observations in a given period of time. In my monthly and quarterly scenarios, I make eight and 24 observations in two years under cART, and it is the proportion of these classified as ‘blips’ that we should be measuring. As seen in Figure 5.5, the proportion of observations that are blips are similar in both
sampling frames. Therefore, this measure removes the bias introduced by the choice of sampling frame.

Of all the 36,940 RNA measurements among patients with suppression of viral load after commencement of cART in the ATHENA cohort of the Netherlands, 8% were > 50 copies/ml (Van Sighem et al., 2008). This roughly corresponds to what we found in our simulations at drug adherence around 0.4 (Figure 5.7). We might infer that the ‘average adherence’ in the ATHENA cohort could be around 40%. However, this has to be interpreted with caution as it is shown that for a given number of blips observed under a given sampling frame in a given period of time, this can be the result of a range of drug adherence levels (see Chapter 6, also cf. section 5.5). Furthermore, within-host viral evolution has not been incorporated into the model. Taken account of the likely emergence of resistant strains among patients with low drug adherence, the number of viral load measurements that are ≥ 50 copies/ml among patients with drug adherence around 0.5 is likely to be higher in reality than that was found in my simulations (cf. Fig 5.7).

My mathematical model also allows me to calculate and compare the amount of virus produced in two years under treatment for different drug adherence levels and patterns. It is clear from Figure 5.10 that to achieve a reasonable viral suppression, a minimum drug adherence of 0.55 is required. It is also interesting to observe that variation in dose-taking time around the prescribed timing is important in so far as a relative low range of drug adherence (0.35 – 0.5) is concerned. A higher drug adherence renders the effect of taking
drugs a couple of hours earlier or later negligible. As it is known that the higher the amount of virus produced, the higher is the possibility of the emergence of drug resistance, mathematical modelling allows us to examine the impact of drug adherence levels and patterns and identify a particular adherence threshold below which the emergence of drug resistance is likely.

These results are contingent upon the assumptions of our model, and are open to further scrutiny. Variations in pharmacokinetic profiles within a particular drug class were not simulated. Nevertheless, these results provide us with important insights that may not be obtained otherwise. By using a mathematical model that generates viral blips with low drug adherence patterns, I highlighted the importance of the definition of viral blips and sampling frame in the existing literature on viral blip incidence and prevalence. Given such a variety of definitions and sampling frames used across the studies, it is not surprising that little consensus has arisen regarding to the nature and incidence of viral blips across HIV patient populations. I therefore suggest that we should standardise our definitions of viral blips and the choice of sampling frame in our studies, so that comparable data can be generated across different populations.

5.6. **Summary**

A consensus is yet to be reached with regard to the association between viral blips observed in HIV patients and their compliance to antiretroviral therapy. This chapter addresses this issue by asking if the variations across the published studies on the definition of viral blips and the choice of sampling frame confound the issue that they set out to address. By applying three sets of sampling frame (<1 week, monthly and quarterly)
to simulated patient data that was generated by a mathematical model, I demonstrate the difference in the number of viral blips observed between different sampling frames. I apply a different underlying assumption, and therefore a different definition of viral blips to the quarterly sampled data, to demonstrate the importance of the definition of a viral blip when comparison across studies is made. By comparing the amount of virus produced in two years under treatment across simulated patients with different drug adherence levels and patterns, I also demonstrate drug adherence levels at which the exact timing of dose taking is important. By employing mathematical models to create simulated patient data, this chapter provides important theoretical and clinical insights into the relationship between viral blips and drug adherence that would otherwise be unable to obtain.
Chapter 6

Viral blips: an indicator of poor drug adherence
6. Viral blips: an indicator of poor drug adherence

6.1. Introduction

The importance of compliance to antiretroviral therapy to prevent the emergence of resistant human immunodeficiency virus (HIV) strains has long been recognised (Bangsberg, 2008a). It has been noted that different classes of drugs have different drug adherence-resistant profiles (Bangsberg et al., 2004); and it has been shown that adherence as measured by pharmacy claim data is a good predictor for viral suppression as well as viral rebound afterwards (Bisson et al., 2008). However, accurate measurements of drug adherence are by no means simple. A substantial literature exists that discusses the strengths and liabilities of each measurement method; for reviews, see section 1.3.1 and Table 1.4, and also Bangsberg (2008b) and Paterson et al. (2002). The association between poor drug adherence and viral blips has long been debated, with studies demonstrating its existence (Masquelier et al., 2005, Podsadecki et al., 2007), non-existence (Garcia-Gasco et al., 2008, Martinez et al., 2005, Miller et al., 2004) or marginality (Nettles et al., 2005). In this chapter I employ a mathematical model of HIV within-host dynamics to illuminate the relationship between drug adherence and viral blips: (1) what is the impact of ‘white coat compliance’ and weekend ‘drug holiday’ on viral blips? and (2) can one tell the drug adherence level of a patient from the number of viral blips observed over a certain period of time with a certain sampling frame of viral load measurements?
6.2. **Methods**

The model used in this chapter has been described in Chapter 3. It is an ordinary differential model with equations for variables representing healthy T cells (CD4 or CD8; quiescent or activated), infected CD4 cells (activated or latent; by either one virus or by both viruses), and anti-HIV cytotoxic lymphocyte (resting or activated). Virus (infectious or non-infectious) is represented as a function of the numbers of infected cells. In this study, only one HIV strain was present; there was no superinfection. The model is deterministic with a stochastic element of T cell activation by antigenic exposure.

The components of drug adherence and pharmacokinetics were added to the model and the details were presented in Chapter 5. Here I briefly restate my work, for completeness. The drug adherence level \( p \) was defined as the proportion of drug taken and was supplied to the model for each set of simulations. Even though it has been shown that this is only one of the three components of an adequate measure of drug adherence (Ferguson et al., 2005), this is what the term ‘drug adherence level’ usually represents in many papers. Therefore I followed this convention. I first followed a simple pattern in which dose timing was fixed (every 12 hours). A dose is either taken or missed. Dose missing can be simulated as random events, or can be scheduled following a specific pattern, e.g. missing consecutive doses every weekend. I also simulated the phenomenon known as ‘white coat compliance’, by raising the drug adherence to 0.9 (i.e. 90% chance of taking a prescribed dose) three days before their three-monthly clinic visits and falling back to the baseline drug adherence after clinic visits. Then, I took into account the fact that there is usually some deviation in time around the prescribed dose-taking time and I simulated a
more realistic pattern when the time of taking a dose varied stochastically, according to a normal distribution with a standard deviation (2.5 hours in this case).

Every simulation started on day -730 and HIV infection took place on day 0; cART commenced on day 2920 (year 8 since infection) and simulations terminated on day 3650. For each given drug adherence level or pattern, I ran 10 simulations in the RTI-only scenario and 100 simulations in the scenario of both PI and RTI, each representing an individual patient. The half-life for RTI and PI are 0.75 day and 0.16667 day respectively. The conversion of drug concentration into its effectiveness follows equations 5.1 and 5.2.

Three sampling frames were used for measuring the number of events of transient viraemia: (a) less than one week; (b) monthly; and (c) quarterly (3-monthly). For the <1-week sampling frame, viral load outputs were produced every 100 flexible time steps (corresponding to every two to seven days) and were plotted in Microsoft® Office Excel 2003 (Microsoft Corporation©) with the number of viral blips counted by eye. For the monthly and quarterly sampling frames, a separate CSV file with monthly viral load measurement was produced for each set of simulations. Viral blips were counted by eye from month 97 onwards. The quarterly measurements are counted from the same set of monthly measurements, from month 97 onwards (month 97, 100…).

A viral blip is defined according to the sampling frame as follows:

<1-week sampling frame. A viral blip was defined as a continuous series of viral load measurements ≥ 50 copies/ml after suppression of viral load below detectable level and
before the next measurement of undetectable viral loads as observed by eye (a definition used in Nettles et al., 2005).

**Monthly and quarterly sampling frame.** Each viral load measurement \( \geq 50 \) copies/ml is considered to be an independent event of transient viraemia (i.e. an individual blip). This is based on the observation that transient episodes of viraemia on average lasted for three weeks (Di Mascio et al., 2005).

### 6.3. Results

**6.3.1. ‘White coat compliance’ and weekend ‘drug holiday’**

I first used the simple adherence pattern with fixed dose-taking time (see Methods) to simulate random dose-missing, ‘white coat compliance’ and ‘drug holiday’ every weekend. An example of a set of longitudinal measurements of viral load and drug effectiveness for a single patient is shown in Figure 6.1. I hypothesized that patients might tend to achieve a high drug adherence a few days before they visit their doctors, so-called ‘white coat compliance’ previously reported in the literature (Podsadecki et al., 2007, 2008). To test whether such behaviour could mask poor adherence during clinic visits, I assumed that, no matter what the baseline drug adherence, patients’ drug adherence will increase to 0.9 three days before their three-monthly clinic visits and fall back to the baseline drug adherence after clinic visits. There was no significant difference in the results observed (Figure 6.2). I then tested the possibility that some patients regularly missed their doses every weekend. I tested the range of missing doses between 1 day (2 consecutive doses) to 3.5 days (7 consecutive doses). It is observed that missing more than 4 consecutive doses every week \( p < 71\% \) will lead to a significant increase in
number of periods of transient viraemia (Figure 6.3). As shown in Figure 6.3, regular cumulative failure of adherence is more harmful than random failure of adherence. If doses are missed on a regular basis with multiple missed doses (e.g. every weekend), a much higher drug adherence is required to prevent transient viraemia.

Figure 6.1  Viral load (black line) and drug effectiveness (grey broken line) profile in a computer-simulated patient after the commencement of antiretroviral therapy. This simulation follows the simple (fixed-time) drug adherence pattern with a drug adherence level of 0.5. The line at 50 HIV RNA copies / ml indicates the assay detectable threshold. Two blips were observed in the <1 week sampling frame after the initial viral suppression, but only one was observed if monthly or quarterly sampling frame was used.
Figure 6.2  Comparison between results of increased drug adherence three days before clinic visit [I] and that without [S].

Results shown are total number of period of transient viraemia (blips) as observed under different sampling frequencies over a period of 2 years under anti-retroviral therapy of ten simulations per scenario (y-axis) with respect to different drug adherence level (p). Only reverse transcriptase inhibitors were taken. There was no variation in the exact time of taking a dose.

Figure 6.3  Patient missing doses every weekend [W] compared with random missing doses [S].

The latter are the same results [S] as shown in Figure 6.1. Results shown are total number of period of transient viraemia (blips) as observed under different sampling frames (<1 week, monthly, 3-monthly) over a period of 2 years under anti-retroviral therapy of ten simulations per scenario (y-axis) with respect to different drug adherence level (p). Only reverse transcriptase inhibitors were taken. There was no variation in the exact time of taking a dose.
6.3.2. Prediction of drug adherence from observed viral blips

I then simulated a more realistic drug adherence pattern (see Methods) where the dose-taking time was varied stochastically according to a normal distribution around the mean (prescribed dose-taking time) with a standard deviation of 2.5 hours (see Chapter 5). Here I tried to present the simulation outcomes in a different way and to ask an important research question: Can we infer a patient’s drug adherence level from the number of observed viral blips?

Figure 6.4 The possibility of drug adherence as indicated by percentage of viral load measurements ≥50 copies/ml observed in monthly (blue) and quarterly (red) sampling frames. Observation period of two years under anti-retroviral therapy (reverse transcriptase inhibitor and protease inhibitor) of 100 simulations per drug adherence level (p) from zero to one with an interval of 0.05. The drug adherence pattern was the more realistic one with stochastic time of taking a dose, with a normal distribution with a mean of the prescribed time and a standard deviation of 2.5 hours.

Figure 6.4 shows the relationship between the percentage of observations with ≥ 50 copies/ml against drug adherence, from the results of 2100 simulated patients (100
simulations per drug adherence level, \( p = 0, 0.05, 0.1 \ldots 1 \) in the presence of cART (RTI and PI) for two years. It shows that if >10% of observations made are \( \geq 50 \) copies/ml, it is likely that drug adherence of that patient is <0.5; if the percentage of observations \( \geq 50 \) copies/ml is very low (<5%), possible drug adherence ranges from 0.4 to 0.9. For the distributions of number of simulations across the range of percentages of observations \( \geq 50 \) copies/ml as presented in Figures 6.4 and 6.5, please refer to Table 6.1.

**Table 6.1 Number of simulations per data point in Figures 6.4 and 6.5.**

<table>
<thead>
<tr>
<th>Percentage of observations ( \geq 50 ) copies/ml</th>
<th>Monthly sampling frame ( \beta = 754 )</th>
<th>3-monthly sampling frame ( \beta = 754 )</th>
<th>( \beta = 75.4 )</th>
<th>( \beta = 7540 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>1156</td>
<td>1245</td>
<td>1639</td>
<td>614</td>
</tr>
<tr>
<td>4.2</td>
<td>85</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.3</td>
<td>52</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12.5</td>
<td>32</td>
<td>105</td>
<td>93</td>
<td>79</td>
</tr>
<tr>
<td>16.7</td>
<td>28</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>20.8</td>
<td>28</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>25.0</td>
<td>18</td>
<td>69</td>
<td>44</td>
<td>70</td>
</tr>
<tr>
<td>29.2</td>
<td>17</td>
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</tr>
<tr>
<td>33.3</td>
<td>26</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>16</td>
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<td>47</td>
<td>61</td>
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<td>45.8</td>
<td>16</td>
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<td>-</td>
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<tr>
<td>50.0</td>
<td>26</td>
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<td>78</td>
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<td>54.2</td>
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<td>21</td>
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<td>17</td>
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<td>66.7</td>
<td>18</td>
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</tr>
<tr>
<td>70.8</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>75.0</td>
<td>26</td>
<td>76</td>
<td>39</td>
<td>113</td>
</tr>
<tr>
<td>79.2</td>
<td>24</td>
<td>-</td>
<td>-</td>
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<tr>
<td>83.3</td>
<td>32</td>
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<td>87.5</td>
<td>43</td>
<td>109</td>
<td>49</td>
<td>198</td>
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<td>48</td>
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<td>-</td>
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<tr>
<td>95.8</td>
<td>82</td>
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</tr>
<tr>
<td>100.0</td>
<td>235</td>
<td>330</td>
<td>116</td>
<td>796</td>
</tr>
<tr>
<td>Total</td>
<td>2100</td>
<td>2100</td>
<td>2100</td>
<td>2100</td>
</tr>
</tbody>
</table>

I performed a sensitivity analysis in which the average infection rate of an activated CD4 T cell per virion (\( \beta \)) was increased or decreased by 10-fold. Figure 6.5 shows that with an increase in \( \beta \), the same percentage of viral load observations being \( \geq 50 \) copies/ml
indicates a higher drug adherence level while with a decrease in $\beta$, it indicates a lower drug adherence level.

**Figure 6.5** Variation in the average infection rate of an activated CD4 T cell per virion ($\beta$) leads to a shift in the possibility of drug adherence as indicated by the percentage of viral load measurements $\geq$50 copies/ml observed in the quarterly sampling frame. Red: $\beta = 754$ (as in Figure 6.4); Black: $\beta = 75.4$; and Grey: $\beta = 7540$.

### 6.4. Discussion

Complementary to the question of viral blip definition and the choice of sampling frame addressed in Chapter 5, this chapter addresses some clinical aspects of the viral blip phenomenon using mathematical models. Mathematical models allow data which are not normally collected at such frequencies in routine clinical settings to be generated through computer simulations. Hypotheses can be tested against these simulated data and insights from mathematical models can be very helpful to further clinical research.
‘White coat compliance’ has been observed in a clinical trial using electronic pill bottle caps (MEMS) (Podsadecki et al., 2008). In that study, this behaviour – defined as perfect drug intake one to three days before pharmacokinetic sampling but ≤ 95% otherwise – was found among 66% of subjects. However, one should note that ‘white coat compliance’ only happened among 31% of visits in Podsadecki et al.’s study. In our study where increased adherence to cART (90%) just three days before the 3-monthly clinic visit and sampling was found to have little impact upon the number of blips observed and therefore cannot mask the underlying poor drug adherence. A possibility was that 90% adherence was not high enough for that purpose. One should be reminded that ‘white coat compliance’ is not observed in every population (Levine et al., 2006).

Next, I found that consecutive dose-missing incurs far more damage than random dose-missing, given the same overall adherence level (proportion of doses taken). This phenomenon has previously been observed in a cohort of HIV patients, in whom among those who achieved poorer compliance in the weekends than on weekdays, a higher proportion of patients with global cognitive impairment or specific impairment in the attention domain of the brain was found (Levine et al., 2005). This highlights the need to understand whether and how the life-styles of patients affect their drug adherence pattern during weekends or other holidays in order to provide adequate counselling and care to minimise the possibility of having consecutive doses missed.
Finally, by analysing the results of my ‘more realistic’ model that incorporated variations in timing of dose-taking, I suggested a relationship possibly existed between drug adherence and viral blips. On the one hand, if very few viral blips are observed (e.g. <5% of measurements ≥ 50 copies/ml in Figure 6.4), it does not imply that a good drug adherence being achieved. On the other hand, if a certain number of viral blips are observed over a period of time (e.g. > 10% of measurements in 2 years are ≥ 50 copies/ml in Figure 6.4), then it is likely that the patient’s compliance to antiretroviral treatment is poor, provided that other reasons of viral blips have been excluded. The alarming observation is that by observing one blip in two years in a quarterly sampling frame (akin to normal clinical practice), a median adherence level of 0.4 (RTI and PI, Figure 6.4) is indicated. Even if the association between viral blips and virological failure is still under debate, viral blips are very likely one of those indicators of poor adherence that signal the clinicians to provide more counselling with respect to drug compliance.

As I have previously shown in Chapter 5 that the percentage of measurements ≥ 50 copies/ml with a given sampling frame in a given period of time is a better indication of the extent of viral blips than incidence rate without taking account of sampling frame. The simple calculation of incidence rate by counting the number of ‘blips’ in a given period of time without taking into account the choice of sampling frame as in many published papers is misleading and makes comparison across studies with different sampling frames flawed.
There are certain limitations to this study. I did not include in our model some other potential causes for viral blips, e.g. opportunistic infections (Jones and Perelson, 2005), target cell pools of heterogeneous drug penetration (Jones and Perelson, 2005), activation of latently infected cells (Jones and Perelson, 2007), and asymmetric division of activated latently infected cells (Rong and Perelson, 2009). The drug adherence patterns that I simulated might not be able to capture the variety of more complicated patterns that exist in the real world. The shift in the curve of the relationship between the percentage of viral load measurements being $\geq 50$ copies/ml (Figure 6.5) demonstrates the inherent uncertainty of the relationship between observed percentage of viral load measurements being detectable and drug adherence level due to variation in fitness between different viral strains. Given that the average infection rate of an activated CD4 T cell per virion ($\beta$) for a given strain is hardly ascertained in the clinical setting, there is still more research to be done before we can apply this relationship to the clinical setting to determine the average drug adherence level of a patient in a given period of time. However, the model captured the essence of the phenomenon of drug adherence variation and its relationship with the emergence of viral blips. The results draw our attention to this important issue and shed light upon future directions in clinical research.

6.5. Summary

Poor compliance to antiretroviral therapy is known as one of the possible causes for viral blips observed in HIV patients after successful viral suppression. By analysing the numerical outputs of a mathematical model of HIV within-host dynamics, this chapter studies the effect of ‘white coat compliance’ and regular consecutive dose-missing behaviour upon number of viral blips observed. It was found that an increased drug
adherence (90%) three days before a 3-monthly clinic visit did not affect the number of viral blips observed. However, regular consecutive dose-missing over weekends had a bigger impact of viral load than random dose-missing. The quantitative relationship between viral blips observed and the possible average drug adherence level of a patient was studied too. It was found that if no viral blips were observed, it could indicate a range of drug adherence from 0.45 (or 0.4 if sampling every three months) to 0.8. However, when more viral blips were observed, it is very likely that drug adherence of the patient is poor.
Chapter 7

Discussion
7. Discussion

This thesis studies the relationship between HIV superinfection, viral blips and drug adherence in the context of patients who had previously experienced successful viral suppression by cART. In an era of universal accessibility to cART, at least in industrialized countries, these issues become increasingly important in both clinical and public health settings. The clinical consequences of events of HIV superinfection and viral blips and various patterns of drug adherence are of concern to both patients and clinicians alike. The possibility of superinfection and the increased possibility of transmission during viral blips also raise public health concerns. It is in this context that these mathematical modelling studies presented bring forth fresh perspectives and insights that will help clinicians and scientists alike to understand better the within-host dynamics of HIV and therefore to improve clinical care for HIV patients through improved interventions. Here, I will recapitulate the contents of the previous chapters, discuss the overall implications of the findings of this thesis and its limitations, and finally indicate the directions which future research may pursue.

Computational and mathematical models have long been developed to help elucidate the within-host dynamics of HIV. In general, they fall into two categories: agent-based models and ordinary differential equation models. Most agent-based models of HIV within-host dynamics employ the concept of cellular automata and its variations. The benefits of using agent-based models include the emergence of macroscopic phenomena through an understanding of microscopic rules of the ‘agents’ and the incorporation into the models of dimensions from physical spaces to ‘shape’ spaces and sequence spaces.
However, to date, few clinically relevant insights have been generated by these models. In contrast, ordinary differential equation models of HIV within-host dynamics have been of great use to biologists and clinicians. Since the early models of the late 1980s, various lines of subsequent development have led to many models, based on different assumptions and hypotheses, tailored to address different aspects of the general topic. The immune control models and the target-cell-limited models are but two major categories. Many models were developed to elucidate viral reservoir, strain selection, resistance, viral blips, and drug adherence.

This thesis introduces readers to a mathematical model that is a further development of several previously developed models (Fraser et al., 2001a, Fraser et al., 2001b, Griffin et al., 2006) with a basic assumption that activation of CD4 target cells by antigens is the key to understand HIV progression to AIDS. The model comprises both deterministic and stochastic components. The former are ordinary differential equations with variables representing various categories of T cells and the latter is the stochastic activation of quiescent T cells. Virus, both infectious and non-infectious, is represented as a function of infected cells. Effects of reverse transcriptase inhibitors and protease inhibitors, and their pharmacokinetics, are incorporated. The equilibrium values and the basic reproductive ratio of the model are then analysed. Through simulations under different conditions, numerical results can be obtained and different hypotheses can be tested.

The merit of the model – a simplified mathematical representation of the biological reality for the purpose of elucidating the dynamics of certain phenomena – is also its
liability. One of the limitations of this model is that it excludes the possibility of compartmental virus production and the movement of cells between thymus and blood, which have been studied before in a related model (Griffin et al., 2006). Recently, there has been an increased understanding of the role the gastro-intestinal tract (GI tract), esp. its mucosal tissue, play in HIV pathogenesis (Brenchley and Douek, 2008, Brenchley et al., 2006). CD4 cells in GI tract were 10 times more likely to be infected than those in peripheral blood (Mehandru et al., 2007). Upon cART treatment, the reconstitution of CD4 T cells in GI tract is poor (Guadalupe et al., 2006). The latest hypothesis for immunopathogenesis of AIDS is that when an individual is first infected with HIV, there is a rapid drain of the CD4 cell pool at the gut that is never fully reconstituted even after cART commences. The histopathology of the gut leads to an increased microbial translocation and the presence of lipopolysachride and other molecules elicits a sustained immune activation that further drain away CD4 cells from their quiescent pool, leading to AIDS eventually (Douek et al., 2009). This is a limitation of the model, in that it does not incorporate the compartmental difference between peripheral blood and GI tract. However, given the purpose of the modelling in this thesis, a model without compartmental division between different organs / systems was chosen to prevent over complication.

Furthermore, recently it has been argued that it is the quality, rather than quantity, of CD8 cell response that is important. The discovery of polyfunctional T cells (Betts et al., 2006) led to the recognition that the proportion of polyfunctional T cells is correlated with control of viral replication (Turk et al., 2008). However, in the model, for the sake of
simplicity, diversity among anti-HIV cytotoxic lymphocytes is not incorporated, and therefore it is impossible to incorporate this observation into the model.

This thesis did not address the issue of how superinfection relates to viral evolution. Both recombination of the viral genome (as a consequence of co-infection of cells by heterologous viral strains) and mutations during the process of viral replication contribute to viral evolution. However, both processes were excluded from the assumptions of the model in order to keep the model simple. The absence of within-host evolution of HIV is, of course, an over-simplification as HIV evolves rapidly within the human body. The results of this thesis should therefore be interpreted in light of this limitation. Nevertheless, the potential of the model as a stepping stone for further research into which the evolutionary process can be incorporated should not be underestimated.

HIV superinfection is defined in this thesis as the infection of an HIV positive individual by a heterologous HIV strain after the establishment of immune response against the first strain (i.e. sero-conversion). The literature review on this topic in this thesis reveals the diversity of opinion about it. The incidence of superinfection reported across different studies varies hugely and depends upon the HIV prevalence of the (sub-)population concerned and their frequency of risky behaviours. My opinion about the incidence, based on my literature review in this thesis, is that it lies between 0 and 2 per 100 person-years.
Neutralising antibodies have been suggested as (potentially and partially) protective against superinfection as shown in the results of three studies (Deeks et al., 2006, McConnell et al., 2006, Smith et al., 2006), but this was not observed in another study (Blish et al., 2008). One of the limitations of the mathematical modelling presented in this thesis is the absence of the humoral immune response. This is largely because with the introduction of a humoral immune response component, the model would have to be extensively expanded to incorporate multiple strains and viral evolution. An alternative is to introduce a parameter representing the protection offered by neutralising antibodies across time. However, as data for such a parameter are limited at the moment, it was considered better to leave it out. Further mathematical models can incorporate this component into its structure. However, as this involves antibody-antigen recognition and therefore selection of strains that will evade neutralising antibody response, this will heavily complicate the model. Perhaps a completely different model should be employed to tackle this question.

In the Croonian Lecture 1994 (Anderson, 1994), Anderson highlighted that superinfection can lead to competitive exclusion or co-existence of both parasite strains in the host, depending on different parameters. In this thesis, the susceptibility of cells to HIV superinfection is found to be of great importance to the outcome of superinfection of individuals as it determines whether competitive exclusion or co-existence prevails.

It was also found in this thesis that only superinfection with a fitter strain (i.e. one with a higher rate of reproduction) will lead to faster progression to AIDS. In other words,
superinfection in and of itself is not detrimental to the health of HIV positive individuals, but a fitter virus could lead to faster progression. Acquisition of resistant virus is also possible. Therefore, the focus of public health concern over superinfection should be directed towards the prevention of (super-)infection of strains that have strong replicative potentials and, more importantly, that are drug-resistant; and the public health message can be revised and become: using condoms when having sex between HIV-positive individuals to avoid superinfection with fitter or resistant strains. Superinfection per se should not be of worry but it does provide an opportunity for researchers to understand better the interplay between neutralising antibodies and other immune responses and HIV infection and the insights obtained therein will be important for HIV vaccine research.

It was also found that superinfection does not lead to viral blips in the presence of cART. If the superinfecting strain is resistant to the existing antiretroviral therapy regimen, it will lead to treatment failure. If it is susceptible to the regimen, it fails to establish itself in the host. In either case, viral blips among HIV patients on cART are unlikely to be the result of superinfection. Therefore, research projects intending to study the incidence or prevalence of superinfection among such a population are advised not to use viral blips as potential markers for superinfection cases.

The definition of viral blips, or transient episodes of detectable viral load measurements among patients who previously experienced successful virological suppression, is found to vary across different studies, and its nature and implications debated, as revealed by the author’s literature review on existing literature on viral blips in this thesis. The
confusion with respect to the nature of viral blips is in part due to the diversity of definitions and sampling frames used in research projects. The infrequent sampling frame used in most clinical settings (3-4 months in industrialised countries) makes it unfavourable to use these data to study viral blips. Clinical trial data with a fairly frequent sampling frame may be better but their variability in sampling frequency (i.e. the longer into the trial, the less frequent the sampling is) makes calculation of incidence difficult. The very frequent sampling frame, as used in Nettles et al. (2005), generates good data but this is expensive and intensive, and cannot be done in a large scale over a long period of time. I advocate that an international consensus should be reached on viral blips’ definition as well as sampling frame employed in research projects in order to make comparison across studies on viral blips meaningful.

The issues of diverse definitions of viral blips and sampling frame used in studies on viral blips were further pursued in this thesis. By applying the mathematical models with various level of drug adherence (defined as proportions of doses taken), computer-simulated ‘patient’ data were generated and were sampled using three different sampling frames – less than one week, monthly and quarterly. It was found that the choice of sampling frame does matter, in terms of number of blips observed. Furthermore, by applying two different definitions of viral blips – each according to a different assumption – to the quarterly sampled data, the numbers of blips observed will be different. This thesis also demonstrates that viral replication, as measured as the area under the viral load curve since the commencement of cART, is negatively proportional
to drug adherence level. It was also found that taking doses at the prescribed time matters especially if the proportion of doses taken is quite low (35 - 50%).

I argue that ‘viral blips’ as presented in the literature may in fact represent two different virological phenomena. The first one is what Nettles et al. (2005) suggests – random variation around a mean viral load. The second one is what other papers suggest – a biological phenomenon that has an extrinsic cause. In the latter case, poor compliance to prescribed antiretroviral therapy regimens is one among a few potential causes.

Drug adherence is important as poor compliance to antiretroviral therapy may increase the risk of the emergence of viral strains. However, its measurement is always subject to the limitations of the methods chosen and reliable data are difficult to obtain. A few studies reveal the complex relationship between risks of resistance emergence and adherence to different classes of drugs (Bangsberg et al., 2004, Raffa et al., 2008, Tam et al., 2008). One of the limitations of the model presented in this thesis is that it cannot represent the relationship as found in the concave adherence-resistance curve for each class of drug as found in those studies, as the model does not incorporate the possibility of mutation.

Another limitation is that of the three measures that compose patterns of drug adherence (Ferguson et al., 2005), the model presented in this thesis can only take into account the proportion of doses taken and the standard deviation of the random dose-timing error (assuming a normal distribution) and ignore any systematic dose-timing bias. The
complexity of patients’ drug adherence pattern requires more complex mathematical modelling. We need better statistical and mathematical tools to delineate these patterns and some studies have taken us towards this direction (Ferguson et al., 2005). Future research should take into account this complexity and incorporate it into the mathematical models.

A third limitation is that in the experiments using a simple sampling frame, for each drug adherence level, only ten simulations were performed. A bigger sample may provide a better average. However, it should be noted that the seed used for random number generator is the same for each batch of simulations under a single drug adherence level. Therefore, the influence of randomness introduced through the stochastic element of the model is cancelled out and the batch of ten simulations for each drug adherence level can be compared with other batches for other drug adherence levels.

In this thesis, it was found that ‘white coat compliance’ makes little difference in terms of number of blips observed. One possibility was that the choice of 90% adherence in the three days preceding a quarterly clinic visit is not high enough. However, taking into account the fact that ‘white coat compliance’ is not observed in every patient in real life, the discounted 90% adherence level is a reasonable proxy for the real life phenomenon. The implication for this result is unclear. However, the finding that a ‘drug holiday’ every weekend has a negative impact upon virological outcomes is important, as it shows that given the same proportion of doses taken, missing consecutive doses regularly is more detrimental than missing doses randomly. It is obvious that missing consecutive doses
will make the drug concentration in the blood too low to control the virus effectively and therefore create windows of viral replication that generate blips. It is of clinical relevance to understand weekly pattern of patient life and help patients to maintain a good adherence both in weekdays and in the weekend.

It is of interest to clinicians whether they can find out patients’ drug adherence through the number of blips observed over a period of time. In this thesis, it is found that if there are no blips observed in a quarterly sampling frame over two years of cART, it does not necessarily imply good drug adherence, but if there are quite a number of blips observed, it is quite likely that the patients concerned fail to comply to the prescribed regimen.

The so-called Swiss Consensus (Vernazza et al., 2008) suggested that HIV-positive individuals are non-infectious if and only if they comply well with the prescribed cART under regular evaluation, and they experience continual suppression of viral load before detectable level, and they have no other sexually-transmitted infections. This thesis highlights the difficulty in monitoring patients’ drug adherence through measurements of viral loads in a routine clinical setting. The possibility that viral blips may be missed in a quarterly sampling frame needs to be taken into account. Fortunately, in a relatively well-adhered sub-population, episodes of transient viraemia are not frequent (van Sighem et al., 2008) and therefore the risk of transmission is limited.

Currently no drug adherence data are collected in the ATHENA cohort in the Netherlands. In the future, if such data are to be collected, perhaps in a sub-set of participants in the
cohort, further research could compare the observed and simulated data with regard to the relationship between viral blips and drug adherence.

To conclude, this thesis adds much to our knowledge and understanding of the relationships between HIV superinfection, viral blips and drug adherence. Future research that takes account of the results and conclusions drawn in this thesis will certainly benefit the HIV-positive communities and the general population at large.
Appendix
Appendix

In Chapter 5, the results of the sensitivity analysis of model parameters are presented. Here in this Appendix, the results are tabulated in details. Median CD4 counts were shown in Table A1 and median viral load were shown in Table A2. Apart from the ‘no treatment’ scenario presented in Chapter 5, sensitivity analysis of scenarios of drug adherence at 25%, 50% and 75% were also performed. It is worthy to note that for median viral load, the scenario of drug adherence level of 25% saw the greatest variation across parameters (Table A2).
<table>
<thead>
<tr>
<th>Parameters</th>
<th>Model values</th>
<th>Sensitivity analysis values</th>
<th>Drug adherence level</th>
</tr>
</thead>
<tbody>
<tr>
<td>a0</td>
<td>$10^4$</td>
<td>$10^5$, $10^6$</td>
<td>0</td>
</tr>
<tr>
<td>$\mu$</td>
<td>0.01</td>
<td>0.01</td>
<td>25</td>
</tr>
<tr>
<td>$\beta$</td>
<td>754</td>
<td>75.4</td>
<td>50</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>1</td>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>1</td>
<td>0.5</td>
<td>0.15</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>0.01</td>
<td>0.001</td>
<td>0.1</td>
</tr>
<tr>
<td>$f_L$</td>
<td>$10^5$</td>
<td>$10^6$</td>
<td>0</td>
</tr>
<tr>
<td>$a_z$</td>
<td>$1.3334 \times 10^8$</td>
<td>$1.3334 \times 10^9$</td>
<td>0</td>
</tr>
<tr>
<td>$p_z$</td>
<td>$10^5$</td>
<td>$10^6$</td>
<td>0</td>
</tr>
<tr>
<td>$d_z$</td>
<td>$10^6$</td>
<td>$10^7$</td>
<td>0</td>
</tr>
<tr>
<td>$z_o$</td>
<td>$10^6$</td>
<td>$10^7$</td>
<td>0</td>
</tr>
<tr>
<td>$\gamma_T$</td>
<td>$10^{3.5}$</td>
<td>$10^{3.5}$</td>
<td>0</td>
</tr>
<tr>
<td>b/c</td>
<td>292</td>
<td>320</td>
<td>0.1</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>$10^4$</td>
<td>$10^5$</td>
<td>25</td>
</tr>
<tr>
<td>$\theta$</td>
<td>0.02</td>
<td>0.04</td>
<td>50</td>
</tr>
<tr>
<td>$\tau$</td>
<td>0.1</td>
<td>0.5</td>
<td>75</td>
</tr>
</tbody>
</table>

**Table A.1: Sensitivity analysis: Median CD4 count, cells/ml (25QR, 75QR)**
10-4

0.01

0.05

1

0.55

754

1

1

1

0.01

10-5

1.3334 *
10-8

1

0.01

10-6

10-3.5

292

104

0.02

0.1

a0

µ

xS

µA

pA

β

aP

α

γ

aL

fL

aZ

pZ

dZ

z0

yT

b/c

σ

θ

τ

10
10-5
0.1
0.001
0.1
0.01
2
0.5
0.8
0.3
7540
75.4
2
0.5
2
0.5
2
0.5
0.1
0.001
10-4
10-6
1.3334 * 10-7
1.3334 * 10-9
2
0.5
0.1
0.001
10-5
10-7
10-2.5
10-4.5
320
265
105
103
0.1
0.004
0.5
0.02

-3

35928 (32490, 39561)
14359 (8254, 20487)
36397 (27821, 49444)
36700 (27908, 46705)
48328 (33829, 66425)
35754 (27694, 41947)
38522 (25428, 46384)
36085 (28530, 47996)
33155 (25918, 47945)
196516 (151234, 248753)
36240 (30182, 46281)
61882 (37140, 106457)
54589 (41314, 67808)
24394 (17951, 30598)
22767 (16334, 33939)
51926 (37919, 62681)
16606 (12014, 23364)
63980 (80462, 49390)
39307 (25376, 47306)
36765 (30454, 47260)
39234 (27050, 48967)
39351 (28032, 53850)
38691 (27800, 56411)
36500 (26472, 45845)
37292 (26342, 51875)
35140 (24508, 48027)
38822 (27214, 47661)
34302 (25725, 46878)
38258 (27724, 45990)
36966 (28401, 48694)
38607 (28522, 46022)
39884 (30232, 49405)
44027 (30585, 55135)
38391 (28058, 49028)
34819 (25371, 44865)
39314 (27000, 46739)
29632 (211, 46299)
36462 (35084, 37892)
36746 (33693, 38606)
36368 (0.481267, 77376)

19320 (4055, 80015)
0.000195 (0.000136, 0.001041)
1289 (36, 27314)
547 (24, 15680)
4762 (100, 34716)
654 (1.528353, 14265)
1.841985 (0.103289, 543)
14332 (658, 65817)
1953 (29, 43886)
24017 (1014, 101113)
31974 (11722, 54580)
0.001231 (0.000806, 0.002533)
13878 (608, 52942)
87 (0.425039, 3170)
21 (0.378833, 3861)
6951 (242, 37219)
0.553038 (0.055311, 71)
45245 (10116, 95824)
306 (2.016655, 16769)
1796 (36, 18561)
2312 (139, 27718)
961 (9.828723, 26663)
4468 (32, 20874)
1715 (4.798633, 21639)
2407 (10, 12690)
600 (12, 13214)
1639 (17, 25114)
1890 (18, 18254)
844 (12, 11024)
384 (2.752213, 11178)
3061 (45, 25136)
1522 (18, 24154)
1801 (39, 22217)
5509 (9226, 0.000417)
974 (6.11591, 31190)
1506 (19, 23039)
0.024014 (0.002408, 0.178227)
17337 (3849, 70019)
16739 (2384, 71770)
0.028471 (0.005236, 0.556132)

1578 (16, 45368)
0.00012 (9.45 · 10-5 , 0.000157)
0.001169 (0.000697, 0.002221)
0.000393 (0.000307, 0.000975)
0.000662 (0.000394, 0.006212)
0.001038 (0.000804, 0.001701)
0.000778 (0.000521, 0.001087)
0.007454 (0.001323, 0.060693)
0.000445 (0.000317, 0.001471)
0.003936 (0.002045, 0.019956)
8248 (145, 41529)
0.000779 (0.000632, 0.001052)
0.003143 (0.000885, 0.026458)
0.000455 (0.000316, 0.000664)
0.000453 (0.000311, 0.000746)
0.001938 (0.000915, 0.004371)
0.000324 (0.00218, 0.000467)
0.015835 (0.002381, 0.3453)
3.14· 10-32 (1.56· 10-32, 1.15· 10-31)
0.829539 (0.708741, 1.20517)
0.009085 (0.005168, 0.021507)
7.61· 10-5 (5.55· 10-5, 0.000232)
0.000764 (0.000575, 0.001548)
0.000786 (0.000534, 0.00133)
0.000872 (0.000598, 0.001594)
0.000923 (0.000603, 0.002205)
0.000952 (0.000627, 0.001738)
0.000861 (0.000575, 0.001719)
0.000784 (0.000553, 0.001707)
0.000872 (0.000589, 0.001716)
0.000934 (0.000571, 0.00167)
0.000917 (0.000611, 0.002424)
0.001037 (0.00674, 0.002868)
0.000672 (0.000417, 0.001727)
0.000848 (0.000602, 0.001567)
0.000856 (0.000518, 0.002065)
0.000229 (0.000187, 0.000302)
722 (15, 38070)
2863 (19, 42919)
0.000258 (0.000145, 0.000428)

Sensitivity analysis: Median viral load, copies/ml (25QR, 75QR)
Sensitivity
Drug adherence level
ParaModel
analysis
0
25
50
meters
values
values
Median viral load when all
39955 (25600, 51260)
1203 (3.72, 18401)
0.00086 (0.000583, 0.001519)
parameters follow the model values

Table A.2

238

0.033492 (0.001806, 1.145378)
0.000126 (9.61 · 10-5, 0.000141)
0.00076 (0.000517, 0.000972)
0.000287 (0.000249, 0.000325)
0.000294 (0.000273, 0.000342)
0.00077 (0.000665, 0.00095)
0.000612 (0.000431, 0.000892)
0.000584 (0.000386, 0.000882)
0.000298 (0.000254, 0.000332)
0.001506 (0.001158, 0.001812)
0.004565 (0.000953, 0.097449)
0.000832 (0.00062, 0.00103)
0.000738 (0.000519, 0.000938)
0.000346 (0.000264, 0.000476)
0.000358 (0.000262, 0.000443)
0.000737 (0.000513. 0.001096)
0.000277 (0.000213, 0.00037)
0.001137 (0.000838, 0.001646)
1.43· 10-32 (9.79· 10-33, 2.05· 10-32)
0.675614 (0.617063, 0.721061)
0.004043 (0.005249, 0.007191)
5.19· 10-5 (3.83· 10-5, 6.91· 10-5)
0.000486 (0.000399, 0.000735)
0.000477 (0.000388, 0.000711)
0.000504 (0.000385, 0.000684)
0.000518 (0.000379, 0.000687)
0.000523 (0.000373, 0.000741)
0.000488 (0.000351, 0.000728)
0.000508 (0.000365, 0.000736)
0.000482 (0.000375, 0.000685)
0.000491 (0.000381, 0.000722)
0.00053 (0.000389, 0.000739)
0.000584 (0.000457, 0.000823)
0.000435 (0.000335, 0.000574)
0.000575 (0.000385, 0.000799)
0.000523 (0.000414, 0.00071)
0.000234 (0.000193, 0.000303)
0.000398 (0.000311, 0.000803)
0.000363 (0.000314, 0.000597)
0.000265 (0.000143, 0.000355)

0.000517 (0.000381, 0.000732)

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