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ANALYSIS OF HIV INFECTION DYNAMICS AND
IDENTIFICATION OF OPTIMAL MEDICATION STRATEGIES

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by
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ABSTRACT

Human Immunodeficiency Virus, HIV, is the causative agent of acquired immunodeficiency syndrome, AIDS. The disease has received a lot of attention because of its global presence, various forms of contraction, and long-term fatal effects. Treating HIV infection has been an active research topic for the last two decades. Mathematical modeling is an invaluable tool in this quest to capture different aspects of disease progression, in an attempt to assist research efforts on the development of treatment policies that enhance the life expectancy and quality of lives of patients.

The objective of this research is studying HIV infection dynamics and developing mathematical models to investigate aspects of the diseases which have not been previously mathematically analyzed. The proposed models enhance the current understanding of the disease dynamics and are used to identify optimal treatment strategies. This research consists of four distinct projects which are ultimately connected to build a multiscale model of HIV infection.

The study is initiated by focusing on the early stages of infection. At this stage of the disease, the viral load is low and random fluctuations have significant
effects on the dynamics of the disease progression. Currently, post exposure pro-
phylaxis treatment regimens are advised for patients where high dosages of drug
are prescribed for four weeks. Commonly used deterministic models that describe
the expected progress of the infection can not be employed to predict the proba-
bility of infection establishment or eradication at the early stage. Consequently, a
stochastic model based on Gillespie’s algorithm is derived to determine the prob-
ability of infection establishment. The effect of treatment latency and efficacy on
infection establishment probability is also investigated.

In the next step, toxicity and side effects of HIV drugs are taken into account.
In fact, severe side effects cause early termination of recommended post exposure
treatment regimens among many patients. Hence, an optimization problem is
formulated which employs the developed stochastic model in the first project.
The objective is to minimize the infection establishment probability by utilizing
minimum amount of drug. The proposed optimal strategies are compared with
constant treatment strategies and are shown to be more beneficial in silico, i.e.,
they either decrease the infection establishment probability or the amount of the
drugs.

Drug efficacy is considered as manipulated variable in the developed model
of the early stage. Efficacy is an increasing function of drug amount, but their
exact relationship has not been determined. In order to more accurately predict
the dynamics of infection, it is necessary to improve the estimation of treatment
efficacy based on treatment dosage and prescription protocols. Consequently, the
mechanisms by which NRTIs, the most common class of drugs used in the treat-
ment of HIV-1, exert their antiviral effects are analyzed and a mechanistic model
to estimate drug efficacy in terms of measurable physical values is developed. The
model is employed to compute and compare the effectiveness of four different drugs
and a sensitivity analysis on mutation and resistance is performed.

Finally, a hybrid intracellular/extracellular model that describes the changes in
virus and host populations at the animal level, while having the required accuracy
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to the revised extracellular model which is initially developed to study the early
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relations between medication dosages under HAART (highly active antiretroviral
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INTRODUCTION AND BACKGROUND

1.1 Motivation

Human Immunodeficiency Virus, HIV, is the causative agent of acquired immunodeficiency syndrome, AIDS, in which the immune system of the patient begins to fail and opportunistic infections can become life threatening. The main target of HIV are CD4 T-cells, part of the immune system of the body. HIV can be transmitted sexually, from mother to child, and by blood and blood products transfusion. More than two thirds of new infections are due to unsafe sexual relationships, around 11% are among newborn babies, 10% from drug injection, and...
5-10% occur in healthcare facilities.

AIDS pandemic in the last three decades has been one of the most destructive diseases among humans and has been spread worldwide. Since the beginning of the global spread, almost 60 million people have been infected and 25 million people have died [4]. Figure 1.1 shows the 2008 estimation of HIV/AIDS prevalence [1].

![Figure 1.1](image_url)

**Figure 1.1.** Estimated HIV/AIDS prevalence among young adults (15-49) by country as of 2008. Figure reproduced from [1].

World health organizations estimates that in 2008, 33.4 million people were living with HIV, 2.7 million new infections and 2 million AIDS related deaths occurred [4]. Although antiretroviral drugs have been successful in reducing the mortality among HIV infected people and increase the quality of life of the patients, routine access to treatment is not available in all countries. Due to global attention and procedures, the number of deaths has slightly declined from the 2004 peak. In spite of this fact, the World Health Organization predicts that AIDS will remain a leading cause of death worldwide for the next decades.
As of today, there is no definitive cure for this disease. Although previously reported unsuccessful [5, 6], recently some successful results in developing HIV vaccination with an estimated success rate of around thirty percent have been reported [7].

Highly active antiretroviral therapy, or HAART is the current treatment for HIV infection. In general, HAART options are combination therapy or “cocktails” consisting of at least three drugs belonging to at least two classes of antiretroviral agents. The common classes of drugs are nucleoside analogue reverse transcriptase inhibitors (NRTIs), protease inhibitors (PIs), and non-nucleoside reverse transcriptase inhibitors (NNRTIs). In developed countries, clinical tests such as measuring the viral load and T-cell population are performed before recommending HAART. The most important concerns regarding antiretroviral regimens are the serious associated side effects and onset of resistance to treatment. In the recent years, drug resistance testing is also recommended for all persons with HIV infection when they enter into care [8].

Although eradication of HIV infection cannot be achieved with current antiretroviral therapy, the primary goals of therapy are:

- maximal suppression of plasma HIV viral load,
- reducing morbidity and prolonging survival,
- improving quality of life,
- restoring and preserving immunologic function, and
- preventing HIV transmission [8].

Important queries are faced by scientists in treating HIV infection everyday. Problems such as drug resistance, toxicity and side effects, optimum treatment strategies, standardized global protocols, etc. are the challenges in HIV treatment in the next years. In section 1.4, these issues are discussed in more detail. Consequently, seeking new methods of treatment as well as optimizing current treatment protocols are important fields of today’s research. Mathematical models are a valuable tool in this quest due to their ability to predict the infection dynamics and estimate the response of the average patient to medication. Mathematical modeling has been applied previously to study various biological systems and to predict their behavior and reaction to changes. Examples of application of mathematical modeling in biology include but not limited to single species and interacting species population dynamics, population genetics and evolution, biological motion, and molecular and cellular biology [9].

1.2 History of HIV infection

Scientists believe that HIV has originated from simian immunodeficiency virus, SIV, which infects wild chimpanzees in west central Africa [10]. Researchers have estimated that the virus was transferred to human in the late nineteenth or early twentieth century [11]. There are a few theories explaining the transmission of the virus to humans. The most scientifically validated theory is the “Hunter Theory”
which explains that the virus was most probably transmitted from a chimpanzee to a bushmeat hunter who was bitten or cut while hunting or butchering an infected animal [12]. The colonialism, poor health of workers, and use of unsterile needles probably facilitated the spread of the virus among humans during the first half of the twentieth century in Africa [13].

The increase in the number of national and international travelers to different parts of the globe, unscreened blood donations, and increased availability of disposable plastic syringes in the sixties and seventies further escalated the global infection transmission.

June 5, 1981 is the official recognition of AIDS epidemic when the U.S. Center for Disease Control and Prevention reported unusual clusters of *Pneumocystis carinii* pneumonia (PCP) [14]. Soon, more PCP clusters and other serious opportunistic infections were discovered among homosexuals, men and women who injected intravenous drugs, haemophiliacs, sexual partners of individuals in these risk groups, and infant children born to women at risk [15]. All these patients had almost absent level of CD4 cells which made them vulnerable to opportunistic infections. By August 1982, the disease was referring to by its new name: Acquired Immune Deficiency Syndrome or AIDS. A few years later scientists succeeded in isolating a retrovirus, the causative agent of the infection [16] and in 1986, the name “Human Immunodeficiency Virus” was settled.

By 1995, HIV had become a global issue and had affected around 6 million peo-
ple. In spite of efforts to combat the fatal opportunistic infections and monotherapy of the virus, the disease was progressing inevitably. Although some patients had slower deterioration, nearly all patients died [15]. Eventually, AIDS became the leading cause of death among Americans aged 25 to 44 in 1995.

HAART, Highly Active Antiretroviral Therapy was introduced at the XI international AIDS conference in 1996. Researchers disclosed that a triple-drug combination therapy had been drastically successful in decreasing the viral load. Today, more than 30 antiretroviral drugs in six to seven different regimens are approved for HAART and HIV infection has become a manageable disease to some extent rather than a death sentence. Although HAART has been very successful in increasing the quality of life of patients drastically, treatment disruption results in immediate burst of viral load, decrease in T-cell count, and clinical progression to inevitable death. Hence, a definite cure for the disease has not yet been discovered and is not currently expected to be identified in near future.

The other important problem is the fact that HAART has not reached far beyond the developed countries and only about 20% of patients in low and middle income countries are receiving it [17]. Consequently, there are still challenges to be faced by researchers to identify new viral targets, by clinicians and health care providers to manage treatment and drug resistance, and by the global community to maintain a worldwide front against the spread of the disease.
1.3 Infection cycle

Retroviruses is a large and diverse family of enveloped RNA viruses defined by their structure, composition, and replicative properties. Reverse transcription of the single stranded viral RNA to double stranded DNA and its integration into the genome of the host cell is the distinguishing feature of this family. HIV belongs to lentiviruses class of retroviruses. They have cone-shaped core, complex genome, remarkably complex interactions with the host, and a chronic course of disease [18].

Figure 1.2 shows the intracellular events of the infection cycle. HIV needs to attach to a host cell and enter its contents to the host to replicate. The process of viral entry into a target cell represents the first step in the viral infection cycle. It is characterized by a complex series of events that are initiated through the binding of the viral surface glycoproteins to specific receptor molecules on the host cell’s outer membrane. The primary receptor of the virus is the CD4 protein which is present on the surface of CD4+ T lymphocytes (which will be called T-cells afterwards), macrophage cells and some other cells [18]. T-cells, the main target of HIV infection, are specific type of white blood cells known as lymphocytes and are part of the immune system. Although HIV can bind to CD4 receptor on many cells, presence of a coreceptor is necessary for fusion of viral content into the host cell. CXCR4 and CCR5 are the two chemokine coreceptors of HIV [19].

Reverse transcription, the reverse flow of genetic information from viral RNA
to DNA, is the hallmark of retroviral replication cycle. The reverse transcription protein (RT) uses viral RNA as a template to catalyze the polymerization of a double stranded DNA. This is a nonlinear process where up to three strands, a negative sense strand and two halves of the positive sense strand, may be transcribed at once. In section 4.1 the events following the transfer of viral RNA to the host cytoplasm will be explained in detail.

The next step in HIV growth cycle is integration. Soon after completion of viral DNA synthesis, while still in the cytoplasm, a viral enzyme called integrase, cleaves the viral DNA and provides the sites of attachment of the provirus to the host DNA. Then, the viral complex enters the nucleus of the host cell. Integra-
tion is an essential step in the life cycle of HIV. Integration contributes to viral replication in two important ways. First, since retroviral DNA is ordinarily unable to autonomously replicate, it depends on integration for stable maintenance in dividing cells. Once integrated, the provirus is replicated along with the host cell DNA and genetically transmitted as an integral element of the host genome. Unintegrated DNA is degradable and integration also stabilizes the viral DNA. Second, integration is important for efficient transcription of viral DNA into new copies; thus, integration defines a turning point in the life cycle at which the virus can begin to multiply [18].

Translation of the viral genome results in different transcripts. After viral mRNA has left the nucleus, translation results in the synthesis of different polyproteins which will be cleaved into envelope and other proteins. Viral contents including two copies of the viral RNA genome gather at the surface of the cell and new viral particles are assembled at the plasma membrane and bud. Once formed, the new virus particle has to undergo a maturation step to become infectious. The protease enzyme becomes active and cleaves the gag-pol polyprotein into several smaller proteins. After this, the virus is able to infect new cells.

1.3.1 Disease stages

From a clinical perspective, HIV infection can be assumed to have four stages: incubation period, acute infection, latency stage and AIDS. The first two to four
weeks after the initial transmission is the incubation period and is usually asymptomatic.

The second stage or acute infection lasts an average of four weeks and the patient usually shows flu or mononucleosis like symptoms such as fever, swollen lymph nodes, sore throat, rash, muscle pain, and mouth and esophageal sores. Due to the non specificity of the symptoms, they are not often recognized as signs of HIV even by the doctors or at the hospitals. The viral load starts to grow exponentially upon incubation and reaches to several million particles per milliliter of blood during the acute infection. Meanwhile, T-cell population drops significantly because of the activation of CD8+ T-cells, also part of the immune system, which kill HIV-infected T-cells. CD8+ T-cell response is important in controlling virus levels. This strong immune defense reduces the viral load and rebounds CD4 T-cells to some extent.

The next stage is the clinical latency which can last as short as a few weeks or as long as twenty years. The patient is usually asymptotic in this stage, however, the viral population gradually grows over years and virus particles accumulate within lymphoid and other organs. T-cells population is under continuous suppression and decline steadily.

The final stage is AIDS when the CD4 T-cells number drops below 200 per $\mu L$ of blood and the cell mediated immunity is lost. As a result, various opportunistic infections such as respiratory tract infections, skin rashes, tumors, reactivation of
herpes virus, and etc. appear which result in death of the patient.

1.4 Treatment of HIV infection and antiretroviral drugs

The initial approach to combat the disease was treatment of the opportunistic infections with drugs already in use. It was successful to prolong life by merely a few months and hence, researchers started to focus on therapies to cure AIDS or hinder immune system dysfunction. Although a few antiretroviral drugs emerged slowly in the next decade, they were minimally effective in reducing the viral RNA. The high rate of mutation caused resistance to single therapy and rendered the drug inactive [20]. The burst of infection, fear and confusion, and lack of effective treatment marked the “dark ages” of HIV treatment which lasted until the mid 90’s.

Antiretroviral therapy for treatment of HIV infection has improved continuously since HAART was introduced in 1996 and decreased the mortality rates by 60-80%. In this approach, three drugs from at least two different classes of drugs (usually two NRTIs and one NNRTI or two NRTIs and one PI) are administrated simultaneously. The treatment benefit is estimated to be 13 and 24 years of life when HAART is started at T-cell count of 87 cell/mm$^3$ and 310 cell/mm$^3$, respectively [21, 22].
Antiretroviral drugs can be classified by the phase of the HIV infection cycle that the drug inhibits:

- Nucleotide reverse transcriptase inhibitors (NRTIs) inhibit reverse transcription process by mimicking natural nucleotides and being incorporated into the synthesized viral DNA and preventing further extension.

- Non-nucleoside reverse transcriptase inhibitors (NNRTI) inhibit reverse transcriptase enzyme directly by binding to it and interfering with its function.

- Protease Inhibitors (PIs) block the viral protease and thereby prevent the cleavage of nascent proteins in the maturation step.

- Integrase inhibitors inhibit the integration of viral DNA into the DNA of the host by inhibiting enzyme integrase. Several integrase inhibitors are currently under clinical trial and one has been approved by FDA in 2007 [23].

- Entry inhibitors (or fusion inhibitors) block the receptors and interfere with binding and fusion of the virus to the host cell.

The last two classes are more recent and will soon become part of HIV treatment protocols [15].

1.4.1 Today’s challenges in HIV treatment

In order to have significant effectiveness, HAART has to be administrated continuously which causes difficulties such as drug adherence, psychological problems,
toxicity, and development of drug resistance. Hence, it is crucial to design better treatment strategies, improve detection and prevention programs, and reduce inequalities in having access to HAART [15]. Some of the challenges faced by clinicians and researchers are presented in this section.

**Drug resistance:** The mutation rate of HIV is $3 \times 10^{-5}$ per nucleotide base per replication cycle [24] which can result in the production of resistant strains to different drugs. In addition to the wild type, all variants with one resistance mutation, some with two resistance mutations, and very few with multiple resistance mutations commonly exist before starting treatment. However, the dominant strain is commonly the wild type virus since mutation can have adverse effects on viral growth. The various mutant species are known as quasi-species. This fact was the reason for the rapid failure of monotherapy prior to the HAART era. Although HAART can not eradicate the infection completely, it is successful in keeping the viral load below 50 copies/mL for several years. Unfortunately, because of pre-existing resistant strains, difficulties in long term adherence to the treatment schedule, and viral reservoir, resistance will eventually happen [15]. When resistance appears, other drug combinations are prescribed. However, resistance to one drug often extends to other drugs of the same class. This limits the treatment options and can lead to “multi-drug resistant HIV” or MDR-HIV which is an extremely complicated problem.

**The optimal time to start therapy:** False hopes to eradicate HIV infec-
tion after introduction of HAART and inadequate understanding of viral dynamics resulted in the “hit hard, hit early” doctrine in the mid 90’s. The outcome of this approach appeared a few years later when significant increase in antiretroviral resistance, and serious side effects like fat redistribution syndrome and metabolic toxicities were witnessed. Consequently, treatment guidelines shifted to “hit hard, but only when necessary” when the T-cell count reached as low as 200 cells/mm$^3$. Soon, it became clear that this paradigm can be hazardous to patients and put them in higher risk of developing AIDS-defining events, neurocognitive impairment, and immune system destruction [15]. As of today, scientists still have divided opinions about “when to start” therapy. While some suggest starting therapy when the T-cell count drops below 350 cells/mm$^3$ to avoid earlier onset of drug resistance, reduction of quality of life because of side effects, and increased cost, others believe that therapy should not be delayed and recommend a T-cell count of 500 cells/mm$^3$ in order to prevent other complications such as co-infection (hepatitis B and/or hepatitis C), cardiovascular disease, and malignancies [8].

**Structured treatment interruptions:** “Drug holidays” or intermittent therapy is another example of uncertainty regarding the optimized method of prescription. In [25], it is suggested that therapy interruptions had no benefit and was even harmful. While in another study [26], patients in the scheduled treatment interruption group reached a lower viral load and experienced less frequent side effects than the patients in the continuous treatment group.
1.5 Mathematical models of HIV infection

In order to describe the dynamics of the disease, numerous mathematical models of varying detail have been proposed in the open literature to capture different aspects of the disease progression. These models can be very beneficial to test hypotheses before making crucial decisions, such as investigating “the optimal time to start therapy” which was discussed in section 1.4.1. A historical example of such models is the paper published in NATURE in 1995 where the replication rate of the virus was estimated for the first time to be around $10^9$ to $10^{10}$ virion per day [27]. Before that, it was commonly believed that HIV has a very slow dynamics. This breakthrough in understanding the dynamics of the disease had an enormous effect on therapeutic approaches and paved the way for foundation of combination therapy [15]. Soon, other mathematical analysis of the disease began to appear and enhanced the overall understanding of HIV infection dynamics.

Previous work on modeling includes [28], in which an extracellular deterministic model was proposed to describe the concentration of virus, T-cell, and infected T-cell of an average patient. The basic model was subsequently revised and analyzed for a number of cases to estimate various biological coefficients. Other research results include [29, 30, 31, 32] where various dynamic models of infection are proposed. In [33], an intracellular level model was developed which captures important steps of the intracellular HIV infection cycle. In [34], the dynamics of viral infection by incorporating both intracellular and extracellular infection level
descriptions were investigated.

Such mathematical models have been also employed to control disease and optimize medication schedules [35, 36, 37]. In [38], a model predictive control based method for determining optimal treatment interruption of HAART was developed. A simulation model was developed in [39] to evaluate the clinical outcomes and life expectancy projections for three primary HIV infection treatment strategies: Immediate, interrupted, and delayed ART therapy. Recently, the role of mathematical modeling on the optimal control of HIV-1 pathogenesis was reviewed in [40].

Although many models account for the effects of antiretroviral therapy, the majority include drug efficacy as time-invariant constants [28, 41, 36, 42, 43], and they tend to be far less reflective of the actual mechanisms by which antiretroviral drugs achieve their desired results. Other studies such as [44, 45] use empirical models, such as those summarized in [46], to estimate the correlation between drug concentration and efficacy. However, no model has attempted to reflect the class-specific mechanisms by which the major types of antiretroviral drugs exert their inhibitory effect. This is possibly due to the complexity of interactions between pharmaceuticals and patients. Nucleoside analogue reverse transcription inhibitors (NRTI), the most commonly used class of antiretroviral medications, seem especially daunting in this regard. NRTIs are prodrugs, which must undergo three enzyme-catalyzed phosphorylations before reaching their active anabolite form.
Once in active form, the drugs must compete with natural deoxynucleotides to exert their effects. This prevents easy correlation of plasma concentrations with instantaneous efficacy. However, since the emergence of NRTIs as the workhorses of HAART, techniques for quickly and directly measuring the intracellular concentration of NRTI triphosphates and natural dNTPs [47, 48, 49, 50] have been refined and demonstrated numerous times. With the ability to directly measure the concentrations of active metabolite, a mechanistic model to predict NRTI concentration may be feasible. The challenge still remains of translating knowledge of the NRTI mechanism into a concise mathematical model.

Mathematical models of chemical and biological processes traditionally take the form of deterministic differential-algebraic equation systems, however for many processes of current interest, accurate descriptions need to account for phenomena at the molecular level. In [51] the authors implicitly accounted for such phenomena by considering structured and parametric uncertainty and using the dissipative nature of diffusion reaction systems and results on finite dimensional nonlinear control theory to develop a class of nonlinear feedback controllers that are able to ensure stabilization of moving fronts for specific biological system. In [52], molecular phenomena are also implicitly analyzed by considering mode transitions in biological networks by modeling them as discrete event systems. Recently, a computational superstructure was developed to allow such descriptions to perform system-level tasks [53, 54, 55], while efficient multiscale models were derived for
material processing [56, 57]. In [58], a hybrid stochastic method was developed that partitions the system into subsets of fast and slow reactions, approximating the fast reactions as a continuous Markov process and describing slow reactions as discrete/jump Markov process, using “next reaction” algorithm. Stochastic models to describe HIV infection have also been developed, such as a stochastic model for early HIV population dynamics [59]. A basic viral growth model based on a time dependent continuous-time branching process is used in [60] to describe the early growth of HIV infected cells in the macrophages and lymphocyte populations. In [61], by investigating stochastic chemical kinetics and employing the quasi-steady state assumption, a new application to Gillespie algorithm [62] was proposed and the method was applied to study gene expression [61]. In [63], stochastic versus deterministic modeling of intracellular kinetics was compared and it was shown that for low viral concentrations, stochastic and deterministic modeling leads to different predictions. In [64, 65], the Monte Carlo method was employed to estimate the natural variation of HIV infection and CD8 T-cells. These results are based on the model proposed in [66] where it was shown that there is a positive probability of viral infection clearance. The proposed models in [59] and [66] were subsequently compared in [67].
1.6 Current work

The objective of this research is analysis of HIV infection dynamics and identification of optimal treatment strategies. This is achieved by developing models of HIV infection which address aspects of the diseases which have not been previously mathematically analyzed and employ the developed models to improve the current understanding of the disease as well as refining treatment protocols. This research consists of four distinct, yet related projects.

Modeling of resistant virus populations and stochastic simulation of the early stage of infection

This project is focusing on the analysis of HIV infection dynamics during the initial stages of infection when the viral load is low and random fluctuations have significant effects on the dynamics of the disease. Deterministic models that describe the expected progress of the infection can not be employed to predict the probability of infection establishment at the primary stage. Consequently, stochastic simulations are used to determine the probability of successful infection in an average patient. A stochastic model based on Gillespie’s algorithm is derived which considers two distinct subpopulations of virus, one resistant to NRTI and NNRTI and the other one resistant to PI. The model is employed to determine the probability of infection establishment and its sensitivity to different treatment strategies with various efficacy values. The effect of treatment latency on virus clearance probability is also investigated.
Scheduling optimal treatment for the early stage of infection

After the sensitivity analysis of treatment strategies, the problem of scheduling optimal treatment strategies for patients at the early stage of HIV infection is considered. Since complete eradication of the infection is still possible at this stage, treatment can further increase the probability of eradication. However, high dosages of drugs should be avoided, if possible, because of toxic side effects. Consequently, to obtain acceptable treatment strategies, an optimization problem is formulated, employing the developed stochastic model in the first project to predict the response of an average patient to treatment. Optimal treatment strategies for prompt and also a few days latency in treatment initiation are computed. These strategies are compared with constant treatment strategies and are shown to be more beneficial in silico, i.e., they either decrease the infection establishment probability or the dosage of the drugs.

Estimation of efficacy of NRTIs using known mechanisms of actions

Similar to the majority of other models, drug efficacy is considered as constants in the developed model of the primary stage. In order to more accurately predict the dynamics of infection, it is necessary to improve the estimation of treatment efficacy. Consequently, the mechanisms by which NRTIs, the most common class of drugs used in the treatment of HIV-1, exert their antiviral effects are analyzed and ways in which those known mechanisms can be employed to generate mathematical models for drug efficacy in terms of measurable physical values are identified.
It is demonstrated that the probability a NRTI instead of a natural nucleotide is included can be expressed in terms of intracellular drug concentrations, natural nucleotide concentrations, and relevant rate constants derived from reverse transcriptase’s mechanism of nucleotide addition. In order to determine the ultimate effect, the resistance of the NRTI to removal from the genome is also considered, which is achieved via stochastic modeling. The model is employed to determine the relationship between efficacy and drug concentration and administration time, as well as other drug characteristics like half life. The model is used for four different drugs and a sensitivity analysis on mutation and resistance is performed.

Intracellular dynamics of HIV infection and Multiscale model of infection at the latency stage

Finally, a hybrid intracellular/extracellular model that describes the infection progression at the animal level, while having the required accuracy at the cellular level is derived. This model circumvents to a large degree the phenomenological values of process parameters that are currently used in mathematical models, and provide quantitative correlations between HAART medication dosages and the effect on the viral load and resistant populations. The hybrid model is used for the simulation of the latency stage with the goal of controlling the resistant populations. The developed model can be easily visualized by experimental researchers, because the description is based on the enumeration of events and the quantity of modeled species. As a result the proposed model can be easily employed to
investigate “what if” scenarios and provide experimentalists with a mathematical tool to test hypotheses for experimental results.
EXTRACELLULAR MODEL OF HIV INFECTION DYNAMICS AND RESPONSE TO TREATMENT AT THE EARLY STAGE

HIV is usually diagnosed after the establishment of infection in the body because as mentioned in section 1.3.1, initially the patients have indistinguishable flu-like symptoms. However, few groups of people, such as medical staff, might be aware of contamination with virus promptly after occurrence. The importance and strategies of treating these patients have been investigated in several studies [68, 69, 70].
If treatment starts immediately after viral entry into the body, the chance of complete eradication of infection increases dramatically [71]. Because of the toxicity of the currently used drugs, avoiding high dosages of medication is favorable [72]. In [73], it is reported that 20% of the health-care workers receiving postexposure prophylaxis after occupational exposure discontinued treatment because of developing symptoms (nausea, fatigue, vomiting, etc.).

Because of the importance of the problem, an extracellular stochastic model to describe the initial stages of infection is developed. Similar to many other models [28, 35, 59], populations of healthy T-cells, latently and actively infected T-cells, and virus are considered and in addition, the model explicitly accounts for resistance effects. To achieve this, the probability of resistance to medication based on pharmaceutical data [18] is calculated and two different resistant subpopulations to the two different class of drugs, i.e., RTI and PI, are included in the model. Subsequently, the sensitivity of the expectation of the infection evolution to medication type, efficacy, and treatment time variations is analyzed.

2.1 Extracellular infection cycle

The infection cycle is illustrated in Figure 2.1a. The process of virus attachment to a host cell and transfer of the viral genetic information to the host cell is the first step in the infection cycle. Virus particles and T-cells are represented by V and T, respectively. The successful “attachment” of a virus particle to a host
T-cell is shown by “+” sign. After successful attachment, the T-cell may become actively infected or latently infected, denoted by I and L, respectively. Latently infected cells will become actively infected at a later time. Inside the actively infected T-cell, the reverse transcription enzyme converts the virus single-stranded RNA genome to double-stranded DNA. The produced DNA is integrated into the host cell DNA and subsequently HIV proteins are produced and spliced. The viral components then accumulate on the inner surface of the host cell, they aggregate and commence assembly to form new virus particles. Subsequently, the new virus particles bud from the surface of the host cell. Here, it is assumed that all the virus particles emerge instantaneously causing lysis of the T-cell. After maturation, these new virus particles repeat the infection cycle. Death events are considered in the model for both virus particles and healthy and infected T-cells, but are not presented in the figure. Virus replication is an error prone process, thus mutation in gene expression is quite probable. If the gene expression of the infecting virus changes, it produces mutant virus particles; the associated events that take place are elaborated in section 2.1.2.

2.1.1 Medication effects

As discussed in section 1.4, the commonly used classes of antiretroviral drugs are NRTI, NNRTI, and PI. Since the effects of NRTI and NNRTI on the extracellular model are similar, the general term RTI (reverse transcriptase inhibitor) is
Figure 2.1. Extracellular events during virus infection cycle. Solid lines present regular infection cycle. Dashed lines present events due to therapy. (a) wild type virus, (b) mutant virus resistant to RTI, (c) mutant virus resistant to PI. (Death events are not presented).

RTI blocks the reverse transcription of viral RNA to DNA and consequently viral genetic information cannot be transferred to the host DNA, i.e., the virus replication cycle is ceased. The efficacy of RTI is denoted by $\eta_R$. PI inhibits the
cleavage of proteins and consequently noninfectious virus particles are produced. Noninfectious virus particles are denoted by $V_N$ and efficacy of PI is shown by $\eta_p$. It should be noted that the amount of drug does not appear explicitly in this mathematical model. Instead, efficacy of the drug, which is a complex function of concentration and effectiveness of the drug is usually used in mathematical modeling [74, 28]; in this chapter the same definition of efficacy is used. In [75], efficacy of Ritonavir, a common PI, was estimated to be around 0.65. In this chapter, values of 0.5 and 0.75 are investigated. Similar to PI, efficacy values of 0.5 and 0.75 are considered for RTI. The effects that these two drugs have on the infection cycle are shown in Figure 2.1 with dashed lines.

### 2.1.2 Mutation effects

An important characteristic of HIV limiting the therapeutic usefulness of RTI and PI is the appearance of viral variants which are resistant to inhibition due to mutation. Resistance has been associated with specific nucleotides substitutions at different parts of virus genome. There are four kinds of nucleotides which form the DNA: Adenine, Guanine, Cytosine, and Thymine denoted by A, G, C, and T, respectively. Mutation happens when a nucleotide is erroneously transcribed into one of the other three nucleotides. During transcription of DNA in the cell nucleus, the nucleotides are transcribed sequentially. Transcription of each of the nucleotides takes place independently and mutation is a result of error in copying
each specific nucleotide. Hence, it is assumed that mutations in different parts of genome are independent of each other since the transcription errors happen independently. The mutation rate in HIV is high because the enzyme responsible for copying the genetic code of the virus to DNA, makes many errors [18]. However, few of all the possible mutations in viral genome will lead to resistance to medication.

The onset of resistance can be seen in Figure 2.1a since the actively infected T-cell can produce either wild type viruses or mutant viruses that are resistant to drugs. The subpopulations of mutant virus particles which are resistant to RTI and PI are represented by $V_{mR}$ and $V_{mP}$, respectively. The effects of resistance are also presented in Figures 2.1b and 2.1c. The resistant virus particles can attach to new host T-cells and initiate infection cycle. In Figure 2.1b it is shown that when a $V_{mR}$ particle attaches to a host T-cell, the resistant gene will be transferred to the host. The produced actively and latently infected T-cell are denoted by $I_{mR}$ and $L_{mR}$, respectively. Also in Figure 2.1c infection cycle with a $V_{mP}$ particle is shown. The produced actively and latently infected T-cell are represented by $I_{mP}$ and $L_{mP}$, respectively.

It should be noted that the new virus populations, $V_{mR}$ and $V_{mP}$, merely present small fractions of mutant population which are resistant to PI and RTI, respectively. Since they are mathematically indistinguishable, the mutant virus particles which are not resistant to medication have been considered as part of the wild
type population. Also, the probability of mutating back to non resistant virus is assumed to be negligible. Furthermore, a mutation that leads to both RTI and PI resistance is also not considered because of the same reason.

In order to include the new populations in the proposed mathematical model, the probability of developing resistant virus particles need to be quantified. The mutation rate for HIV genome has been estimated as $3 \times 10^{-5}$ per base (nucleotide) per replication cycle [24]. As mentioned before, certain mutations in specific parts of the genome lead to resistance to medication. In [18], the necessary mutations for developing resistant virus particles to specific drugs are reported and they are used here to calculate the probability of resistance to AZT (zidovudine), a commonly used RTI. According to [18], mutation in eight different parts of genome will cause resistance to RTI. As a result of above discussion, these mutations were considered as eight independent events, denoted by $A_i$'s, that may lead to resistant subpopulation.

In five out of the eight events, change of one base into one other specific base is sufficient to lead to resistance, e.g. in amino acid D67N, if GAC mutates to AAC, the resulting virus particles will be resistant to AZT. Consequently, for each of these five cases, the probability of the specific mutation to occur is equal to the mutation rate per base multiplied by the probability of changing into the other specific nucleotide: $(3 \times 10^{-5}) \times \left(\frac{1}{3}\right) = 10^{-5}$. It should be emphasized that it is assumed that change of the same base to a base other than the specified one will
not cause resistance (i.e., if in D67N, GAC mutates to TAC, the virus particle will
not be resistant.) In the sixth event, change of one base into either of two other
specific bases is sufficient for resistance, i.e., in amino acid M41L, if ATG mutates
to either TTG or CTG, the virus particle will be resistant. Consequently, for this
case, the probability of the specific mutation to occur equals to mutation rate per
base multiplied by the probability of changing into two other specific proteins:

\[(3 \times 10^{-5}) \times \left(\frac{2}{3}\right) = 2 \times 10^{-5}.\]

In the remaining two events, change of two bases
is necessary for developing resistance, e.g., in amino acid T215F, if ACC mutates
to TTC, the virus particle will be resistant. For each of these two cases, the
probability of the specific mutation to occur equals to mutation rate per base for
two bases multiplied by the probability of changing into another specific protein
for two bases:

\[(3 \times 10^{-5})^2 \times \left(\frac{1}{3}\right)^2 = 10^{-10}.\]

The probability that one of the eight independent events happens (under the
assumption of independent events) is equal to one minus the compliment of any
of them taking place. Consequently the probability of developing resistance virus
particle equals to:

\[P\left(\bigcup_{i=1}^{8} A_i\right) = 1 - P\left(\bigcup_{i=1}^{8} A_i^c\right) = 1 - P\left(\bigcap_{i=1}^{8} A_i^c\right) = 1 - \prod_{i=1}^{8}(1 - P(A_i)) = 7 \times 10^{-5}.\]

A similar analysis is performed for PI medication. A-BT538 (ritonavir) is a
commonly used protease inhibitor. According to [18], mutation in thirteen different
parts of genome will cause resistance to PI. Similar to RTI, these mutations are
considered as thirteen independent events. In ten out of the thirteen events, change of one base into one other specific base is sufficient to lead to resistance. In the eleventh event, change of one base into either of two other bases and in the remaining two events, change of two bases to two other bases is necessary to develop resistance. Similar calculations as above lead to a value for resistance probability of $1.2 \times 10^{-4}$.

2.2 Deterministic modeling

The majority of HIV models in literature are deterministic models in the form of differential equations describing the dynamics of healthy and infected T-cells and virus particles. In the current model, two populations of mutant viruses, one resistant to RTI and the other resistant to PI, were considered for the first time.

The focus of this project is on the primary stage of infection when the size of virus population is small and random fluctuations are important in the dynamics of the system. Deterministic models give reliable predictions when large populations are studied and fluctuation effects are negligible. To present the significance of stochastic modeling, for result comparisons, and to assist the model description, initially a deterministic representation of the model is presented in the form of differential equations (1)-(11).
\[
\begin{align*}
\frac{dT}{dt} &= \lambda - \mu T - (1 - \eta_R)kTV - kTV_{mR} - (1 - \eta_R)kTV_{mP} \\
\frac{dL}{dt} &= (1 - \eta_R)kpTV - \mu L - \alpha L \\
\frac{dI}{dt} &= (1 - \eta_R)k(1 - p)TV + \alpha L - aI \\
\frac{dV}{dt} &= (1 - \eta_P)(1 - M_P)(1 - M_R)cI - \gamma V - (1 - \eta_R)kTV \\
\frac{dV_N}{dt} &= \eta_P(1 - M_P)cI + \eta_Pc'I_{mR} - \gamma V_N \\
\frac{dL_{mR}}{dt} &= pTV_{mR} - \mu L_{mR} - \alpha L_{mR} \\
\frac{dI_{mR}}{dt} &= (1 - p)TV_{mR} + \alpha L_{mR} - aI_{mR} \\
\frac{dV_{mR}}{dt} &= (1 - \eta_P)(1 - M_P)M RcI + (1 - \eta_P)c'I_{mR} - \gamma V_{mR} - kTV_{mR} \\
\frac{dL_{mP}}{dt} &= (1 - \eta_R)kpTV_{mP} - \alpha L_{mP} - \mu L_{mP} \\
\frac{dI_{mP}}{dt} &= (1 - \eta_R)k(1 - p)TV_{mP} + \alpha L_{mP} - aI_{mP} \\
\frac{dV_{mP}}{dt} &= cIM_p + c'I_{mP} - \gamma V_{mP} - (1 - \eta_R)kTV_{mP}
\end{align*}
\]

Equation (1) is explained here in detail as an example. It describes the rate of change of T-cells with time. The first three terms are common in basic HIV models: \( \lambda \) (which is a constant) represents the number of the T-cells produced by the body, \( \mu T \) (which is proportional to the current number of T-cells) represents the number of death events in the population, and the number of T-cells which become infected by wild type virus (RTI reduces this term by \( 1 - \eta_R \)) is represented by \( (1 - \eta_R)kTV \). The two other terms were added to capture the resistant mutant
virus particles effect on T-cell population. The number of T-cells which become infected by mutant virus resistant to RTI is equal to \( kTV_{mR} \), while the number of T-cells which become infected by mutant virus resistant to PI is \((1 - \eta_R)kTV_{mP}\) (RTI is assumed to be effective on \( V_{mP} \)).

A host T-cell will become a latently infected cell (\( L, L_{mR}, \text{ or } L_{mP} \), depending on the type of infecting virus and probabilities of resistance) with probability \( p \) or an actively infected cell (\( I, I_{mR}, \text{ or } I_{mP} \)) with probability \( 1 - p \). A latently infected cell will change to an actively infected cell with rate of \( \alpha \). An actively infected cell will produce new virus particles with rate of \( c \) for \( I \) and \( c' \) for \( I_{mR} \) and \( I_{mP} \). The death rates of actively infected, latently infected, and \( V \) are denoted by \( a, \mu, \text{ and } \gamma \), respectively. The model does not include virus populations that are resistant to both RTI and PI. This is due to the fact that the probability of such a virus population becoming significant during the initial stage of infection is negligible. Values of these parameters are shown in Table 2.1 and chosen from relevant literature (referenced in section 1.5).

When no drug is in the system, \( \eta_R = \eta_P = 0 \). Furthermore, the resistant populations can be considered as part of the wild population. Then, the steady states of the system can be found analytically. There are two steady states:

- Infection eradication:
  \[
  T_{ss} = \frac{\lambda}{\mu},
  \]
  \[
  V_{ss} = 0,
  \]
Table 2.1. HIV infection parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda$</td>
<td>production rate of T-cell</td>
<td>$10^8,\text{cell/(5 liter blood.day)}$</td>
</tr>
<tr>
<td>$\mu$</td>
<td>death rate of T and L</td>
<td>0.02 /day</td>
</tr>
<tr>
<td>$k$</td>
<td>infection rate</td>
<td>$4.8 \times 10^{-12}/(\text{cell.day})$</td>
</tr>
<tr>
<td>$p$</td>
<td>probability of becoming L</td>
<td>0.1</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>rate of change of L to I</td>
<td>0.0055</td>
</tr>
<tr>
<td>$a$</td>
<td>death rate of I</td>
<td>0.5 /day</td>
</tr>
<tr>
<td>$c$</td>
<td>number of V per I</td>
<td>100 /day</td>
</tr>
<tr>
<td>$c'$</td>
<td>number of V per $I_{mR}$ and $I_{mP}$</td>
<td>80 /day</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>death rate of virus</td>
<td>2 /day</td>
</tr>
<tr>
<td>$M_{R}$</td>
<td>probability of resistance to RTI</td>
<td>$7.0 \times 10^{-5}$ per cycle</td>
</tr>
<tr>
<td>$M_{P}$</td>
<td>probability of resistance to PI</td>
<td>$1.2 \times 10^{-4}$ per cycle</td>
</tr>
</tbody>
</table>

$L_{ss} = 0$, and

$I_{ss} = 0$;

which is the condition for a healthy person with $5 \times 10^9$ T-cells in the whole volume of blood, equivalent to 1000 cells per one $mm^3$ of blood.

- Infection establishment:

\[
T_{ss} = \frac{a\gamma}{k(c - \frac{c\mu p}{\mu + \alpha} - a)},
\]

\[
V_{ss} = \frac{\lambda}{a\gamma}[c - a - \frac{c\mu p}{\mu + \alpha}] - \frac{\mu}{k},
\]

\[
L_{ss} = \frac{p}{\mu + \alpha}[\lambda - \frac{\mu\alpha\gamma}{k(c - a - \frac{c\mu p}{\mu + \alpha})}], \quad \text{and}
\]

\[
I_{ss} = \left[\frac{1}{a} - \frac{\mu p}{a(\mu + \alpha)}\right][\lambda - \frac{\mu\alpha\gamma}{k(c - a - \frac{c\mu p}{\mu + \alpha})}];
\]

substituting the parameter values from table 2.1, the steady state values are

$T_{ss} = 454\,\text{cells/mm}^3$ and $V_{ss} = 10^6\,\text{particles/mL}$ of blood which are in range of the reported values of untreated patients [18].

The system of differential equations (1)-(11) is solved numerically to study
the populations’ dynamics without any medication for a 120 day period (before reaching infection establishment steady state) and provides a comparison base for the stochastic model (which is presented in section 2.3). Figure 2.2 shows the trajectories of wild type and resistant types virus population and 2.3 shows the trajectories of T-cell population (the results are in good agreement with current literature).

It is observed that immediately after infection the amount of virus particles rises dramatically and reaches the peak value usually after seven to nine weeks. Then, because of the immune response to the infection and the reduced number of host T-cells, the virus concentration falls to a lower level. After the primary infection period, the virus concentration deviates little from the set-point level over a relatively long period of time, however, the concentration of T-cells slowly declines leading to AIDS when it drops below 200 particles/mL [28].

The wild type and resistant virus populations and T-cell trajectories for three different initial viral loads, 10, 100, and 1000 virus particles per 5L of blood are shown in Figures 2.2 and 2.3, respectively. The initial number of virus particles entering the body is shown by V(0). Assuming that the average volume of a droplet is 0.05 milliliters, 1000 virus particles in the body is equivalent to 1 mm$^3$ or 0.02 of a drop of blood of a person with AIDS entering the bloodstream of the patient (A person at the established stage of infection is assumed to have $10^6$ virus particles per milliliter of blood). Similarly, 100 and 10 virus particles per 5 liters of blood
corresponds to 0.1mm$^3$ and 0.01mm$^3$ of blood of a person with AIDS entering the bloodstream of the patient (it is assumed that all the virus particles entered the body were wild type and infectious). These calculations are valid under the assumption of blood being a homogenous fluid, i.e., homogeneous concentration of the virus particles in the patients’ blood. This assumption is a result of fast blood
circulation system, not the amount of virus particles. Studies in the relevant open literature state that the whole volume of blood circulates through the body in less than one minute. This implies that a concentrated drop of infected blood will be diluted in just a few minutes.

![T-cell population profiles](image)

**Figure 2.3.** T-cell population profiles with time for initial viral load of 1000 particles/5L (dashed line), 100 particles/5L (solid line), and 10 particles/5L (dotted line). (deterministic model simulation).

As it can be observed in Figure 2.2, the trajectories for the three different initial viral loads differ only in when the respective virus population attains the highest value, but as it is discussed in section 2.3, there is a considerable difference in the chance of developing successful infection between the three cases.

### 2.3 Stochastic modeling

When deterministic models are used, the chance of establishment of successful infection in an average patient cannot be determined. Specifically, at the early
stage of infection, the number of virus particles is considerably low and the body immune response in combination with medication may be able to eradicate the infection thoroughly. Other factors (e.g., other infections in the body at the time or the patient immune system strength) also play a role for a successful infection. The per act risk for acquisition of HIV-1 is different by exposure route [76, 77, 78]. In other words, entrance of Human Immunodeficiency Virus will not always lead to establishment of infection in the body and similar initial number of virus particles entering the body of different patients can lead to noticeable differences in the chance of infection establishment. Considering these factors, random fluctuations that might affect the dynamics of primary infection are important.

The proposed model to capture the stochasticity of the system draws significantly upon the principles of stochastic modeling, as originally proposed by D. T. Gillespie [62]. Based on the fact that macroscopic rates for chemical reactions are averages resulting from individual molecular collisions which constitute a system that is random and memoryless, Gillespie proposed that the overall process may be represented as a chain of random events in time, each with exponential probability distribution. He formulated the problem as follows: if a volume $V$ contains molecules of $N$ species $S_i$ ($i = 1, ..., N$), and molecules of several inert species as well, then $X_i$ represent current number of molecules of the $i^{th}$ species. These $N$ chemical species $S_i$ can participate in $M$ chemical reactions $R_\mu$, ($\mu = 1, ..., M$). Each reaction is characterized by a numerical reaction parameter $c_\mu$, where $c_\mu \delta t$
is the average probability to first order in $\delta t$, that a particular combination of $R_\mu$ reactant molecules will react accordingly in the next time interval $\delta t$. The relationship between $c_\mu$ and the familiar rate constant $k_\mu$ is discussed in section 2 of [62]. For monomolecular and bimolecular reactions which are of interest to the current work, $k_\mu = c_\mu$, and $k_\mu = Vc_\mu$, respectively. Then, the probability profile of any single reaction $R_\mu$ becomes:

$$P(\tau, \mu) = H_\mu c_\mu \exp\left[-\sum_{\nu=1}^{M} H_\nu c_\nu \tau\right]$$  \hspace{1cm} (2.12)$$

where $H_\mu$ is defined as the “number of distinct molecular reactant combinations for reaction $R_\mu$, found to be present in $V$ at time $t$” [62]. Given the memoryless property of the exponential distribution, it becomes clear that at any time the marginal probability that the next reaction to occur is reaction $\mu$ is:

$$P(\mu) = \frac{H_\mu c_\mu}{\sum_{\nu=1}^{M} H_\nu c_\nu}$$  \hspace{1cm} (2.13)$$

Gillespie initially applied his method only to simple chemical reaction systems, but the information gained from applying stochastic simulation to micro-scale and nano-scale systems makes it desirable for the analysis of cellular events as well. Investigations such as [79, 58] have demonstrated the approach’s applicability in
such systems.

For the stochastic model, the populations of T-cell (T), infected cells (I, L, $I_{mR}$, $L_{mR}$, $I_{mP}$, $L_{mP}$), and virus particles ($V$, $V_{mR}$, $V_{mP}$, $V_N$) are considered. It is assumed that the infection is a Markov process which implies the change of a system from the current state to a future state depends on the current state of the system only. To accurately compute the probability of infection establishment, the whole volume of blood (5 liters in an average person) is simulated for 120 days and the initial values are: $5 \times 10^9$ T-cells (average value for a healthy person), and zero for all latently and actively infected cells. For $V(0)$, three initial values, 10, 100, and 100, are investigated. Initial values of $V_{mR}$, $V_{mP}$, and $V_N$ are assumed to be zero.

One problem of the Gillespie's algorithm for a system with a large number of particles is the computational cost of simulations. Hence, the algorithm chosen to evolve the processes is the improved leap-size selection for accelerated stochastic simulation by Gillespie and Petzold [80]. In this algorithm, the number of times that reaction channel $R_j$ will take place during the period $(t; t + \tau)$ can be approximated by a Poisson random variable.

For each of the investigated cases 1,000,000 simulation runs are used to obtain the expectation of the process. At the end of each simulated run, if the number of any of the infectious virus populations ($V$, $V_{mP}$, or $V_{mR}$) is greater than zero, that simulated run is considered as a successful infection case. The infection probability
is then reported as the percentage of successful infection cases among the total number of simulations. The infection probability percentage is denoted by IPP. If each simulation is considered to represent one patient, then IPP can be interpreted as the ratio of patients who develop infection to the total number of people initially contaminated. In Figure 2.4(a), the snapshot of the first four days of wild type virus trajectories from four different simulations with initial viral load of 100 is shown. It can be observed that two of the trajectories reach zero after some time while the other two grow. Consequently, the effect of random fluctuations is well captured by the stochastic simulations. Figure 2.4(b) shows the trajectories of 100 simulations for initial viral load of 100 particles. It is important to note that the average of successful infection cases has the same trend as predicted by the deterministic model. To have a basis for comparison, initially no treatment is assumed in the system to investigate the ability of the body immune system to eradicate the infection. The computed IPP is 5.8%, 44.8%, and 99.8% for $V(0)=10$, 100, and 1000 respectively. As expected, the IPP depends highly on initial value of virus particles. The simulation results imply that one cubic millimeter of infected blood entering the body will result in infection establishment with a very high probability close to a hundred percent, whilst one tenth of that value results in less than half of the probability of the former case.

Investigating the simulation results, cases are found where the population of all kinds of viruses are equal to zero at the end of simulated time horizon, however,
there are either actively or latently infected T-cells present in the system which can be considered as a source of later infection establishment. Consequently, another variable is defined to account for these cases. The residual infection probability, RIP, is defined as the percentage of these cases among the total number of simulations. RIP is the result of the finite time horizon of the stochastic simulations and
implies that it can not be concluded if the specific case will lead to a successful infection establishment or not based on the available trajectory. For instance, for \( V(0)=100 \) and 5 days latency, IPP and RIP are equal to 10.5% and 6.8%, respectively. This means that based on the available trajectories at the end of simulation time horizon, for 6.8% of the cases it can not be determined if the infection is established or eradicated. As a result, the simulation time horizon is extended to 240 days if at the end of 120 days, potential source of infection (RIP > 0) is observed in the system. This change in the simulation reduces RIP to less than 1% for most of the cases; in other words, RIP becomes negligible in comparison to IPP. RIP of no treatment case for \( V(0)=10, V(0)=100, \) and \( V(0)=1000 \) are 0.005%, 0.028%, and 0.001%, respectively.

Currently, as discussed in 2.1.1, RTI and PI are the most commonly employed drugs for HIV patients. Separate simulations are run to investigate the effect of combination therapy with constant efficacy. \( \eta_R = \eta_P = 0.5 \) is refereed to as case (I), and \( \eta_R = \eta_P = 0.75 \) is refereed to as case (II) in the rest of this chapter and the next chapter. The initiation time of treatment has a significant effect on IPP. It has been suggested by U.S. Public Health Service [71] that treatment should be initiated as soon as possible, preferably within hours of exposure rather than days. However, starting treatment immediately after the incident may not be always possible even for medical staff. As a result, the effect of treatment latency is also investigated. Table 2.2 shows IPP and RIP of cases (I) and (II)
Table 2.2. Effect of treatment initiation latency on IPP for constant combination therapy. RIP is presented in parentheses.

<table>
<thead>
<tr>
<th>treatment period (days)</th>
<th>V(0)</th>
<th>case(I) η_R = η_P = 0.5</th>
<th>case(II) η_R = η_P = 0.75</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-28</td>
<td>10</td>
<td>0.092 (0.004)</td>
<td>0.021 (0.001)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.946 (0.034)</td>
<td>0.223 (0.008)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>9.033 (0.300)</td>
<td>2.193 (0.088)</td>
</tr>
<tr>
<td>1-29</td>
<td>10</td>
<td>0.235 (0.007)</td>
<td>0.098 (0.005)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.200 (0.063)</td>
<td>1.001 (0.044)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>19.99 (0.63)</td>
<td>9.83 (0.40)</td>
</tr>
<tr>
<td>2-30</td>
<td>10</td>
<td>0.370 (0.011)</td>
<td>0.19 (0.11)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>3.47 (0.13)</td>
<td>1.770 (0.079)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>30.10 (0.92)</td>
<td>16.70 (0.64)</td>
</tr>
<tr>
<td>5-33</td>
<td>10</td>
<td>1.180 (0.035)</td>
<td>0.642 (0.032)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>11.02 (0.35)</td>
<td>6.51 (0.27)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>68.7 (1.2)</td>
<td>49.3 (1.4)</td>
</tr>
<tr>
<td>10-38</td>
<td>10</td>
<td>3.890 (0.078)</td>
<td>3.000 (0.078)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>32.61 (0.49)</td>
<td>26.19 (0.66)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>97.96 (1.15)</td>
<td>95.26 (0.39)</td>
</tr>
<tr>
<td>15-43</td>
<td>10</td>
<td>5.540 (0.022)</td>
<td>5.120 (0.038)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>42.55 (0.13)</td>
<td>40.94 (0.22)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>99.600 (0.008)</td>
<td>99.490 (0.019)</td>
</tr>
</tbody>
</table>

for prompt initiation of treatment and also for 1, 2, 5, 10, and 15 days latency in treatment initiation for V(0)=10, V(0)=100, and V(0)=1000. Following the recommendations in [71], the treatment period is assumed to be 28 days (four weeks). Figure 2.5 shows the importance of latency graphically (dashed lines and dotted lines represent the IPP of case (I) and case (II), respectively). The presented results in table 2.2 and figure 2.5 help to deduce the following:

1. If treatment initiates immediately upon the entrance of infection into the body, the IPP will be very small for V(0)=10 and V(0)=100 under both
Figure 2.5. Effect of treatment initiation latency on IPP for (a) $V(0)=10$, (b) $V(0)=100$, and (c) $V(0)=1000$. The solid horizontal black lines specify IPP for untreated infection. Dashed blue lines: $\eta_R = \eta_P = 0.5$ and dotted red lines: $\eta_R = \eta_P = 0.75$. 
treatment cases. The reason is that the ratio of virus particles to the T-cells is very small and immediate treatment suppresses the virus population and prevents viral growth. However, for $V(0)=1000$, the initial amount of virus is relatively higher and IPP becomes considerable.

2. The virus populations grows exponentially within the very first few days. Consequently, if treatment initiates even after one day of virus entrance, the IPP increases significantly. As expected, the increase in the IPP is more dramatic the higher the initial viral load entering the body is. This is because of the exponential growth of the virus population. In other words, the larger the initial number of virus particles is, the increase in IPP over the time becomes more noticeable. For $V(0)=10$, treatment is still somewhat effective after 15 days, e.g., the IPP of both case (I) and case (II) are less than no treatment case. However, for $V(0)=1000$, if treatment starts after 10 days, the IPP of both case (I) and case (II) is very close to IPP of no treatment case.

3. The IPP of case (II) is always less than IPP of case (I) because of the higher efficacy of both RTI and PI. However, the difference among the two treatment cases is small for immediate treatment initiation and becomes more significant as treatment initiation time increases. However, for 10 days latency and more, this difference starts to decrease. The reason is that the affectivity of the drugs become less significant over time due to the high number of virus
particles and the efficacy value does not make a considerable difference on IPP.

2.4 Challenges in developing the model

- Obviously, the dynamics of the infection and the outcome of stochastic modeling (IPP specifically) depend on the initial conditions chosen for simulation. As stated earlier, it is assumed the initial population of virus to be only wild type. In different studies, [81, 82, 83], genotypic resistance among patients with no history of treatment is reported to be between 3% to 14% to specific drugs. If the source of infection is a person naive to treatment, the probability of having resistance type virus is low. On the other hand, if the source of infection is a person who is already under treatment, there is a significant possibility to have virus strands resistant to that specific kind of drug. Consequently, different drugs (of the same class) should be prescribed to the newly infected person for which the current population exhibits resistance. For medical personnel specifically, the types of drugs through essays of the person that had the infected blood are identifiable.

To further investigate the first this possibility, it is assumed that the initial viral population consisted of 90% wild type virus, 5% resistance type virus to RTI, and 5% resistance type virus to PI and then it is investigated if the successful infection establishment cases are due to growth of the wild type
or resistant types. The results show that when the initial population is only wild type, a very small number of established infection cases (from zero for immediate treatment to less than 0.02% for 10 days latency in treatment) are due to resistant types growth. When resistant populations assumed initially, the rates were increased to up to 2% for 10 days latency. Since small initial populations of resistant types do not have a significant increase in these rates, and also considering the low genotypic reported resistance, only wild type population to initiate simulation is chosen.

- Deterministic models have been previously employed to simulate a finite volume of blood, e.g., one milliliter is a commonly used volume in literature [28, 35]. Consequently, a finite volume of blood (one milliliter of blood) was initially used to calculate the infection probability. It was assumed that virus-cell interactions in each milliliter of blood is independent from the rest of the blood volume. The simulations initially involved 10 and 100 particles per milliliter (which is equivalent to $5 \times 10^4$ and $5 \times 10^5$ per 5 liter of blood), respectively. However, upon further investigation, it became apparent that the IPP of a finite volume of blood is not an appropriate representation of the IPP of the whole body for stochastic simulation under the investigated conditions. The IPP for the whole body would be $1 - (1 - IPP_{1mL})^{5000} \rightarrow 1$. As a result, the whole volume of blood assuming fewer number of initial virus particles than initial numbers are assumed for the simulations. The
new initial values (10, 100, or 1000 particles per 5 liter of blood) is actually a more accurate estimate for a needlestick incident and also in accordance with other stochastic models [67].

In the next chapter, the issue of drug toxicity is considered and an optimization problem is formulated to schedule treatment regimens that decrease IPP with minimum possible drug efficacy.
In chapter 2, the sensitivity analysis results with regard to drug efficacy, initial viral load, and treatment latency are presented. In this chapter, the objective is to seek optimal treatment strategies which minimize the infection establishment probability, IPP, and simultaneously minimize the dosage of medication over time. This objective is mathematically formulated as a dynamic optimization problem. To solve this problem, a direct search method, Hooke-Jeeves algorithm, is employed. The optimal treatment strategy is scheduled for prompt treatment and
also for a few days latency in treatment initiation. To show the advantage of optimal treatment, the results are compared with constant treatment strategies. It is also demonstrated that optimal treatment schedules can be more beneficial for the patients, i.e., either the achieved infection establishment probability or medication dosage is lower than constant medication strategy.

### 3.1 Problem formulation

To obtain the optimal treatment strategy for patients in the early stage of HIV infection, two important issues should be considered. First, it is intended to decrease the infection probability percentage, IPP, to the lowest possible value given a certain total amount of drug. RIP is also included in the optimization problem.

Second, the optimal schedule should utilize the minimum possible amount of drug over the period of treatment. Because of serious side effects of drugs such as liver failure, lower dosages of drugs are more desirable if treatment is still effective for the patients. A four-week combination treatment with RTI and PI is assumed and unlike constant treatment (Table 2.2), efficacy of each medication can change weekly. Further changes in medication dosage may prove to cause a treatment schedule that is too involved for the patient to follow correctly. The maximum efficacy of both drugs, \( \eta_{R_{\text{max}}} \) and \( \eta_{P_{\text{max}}} \) are assumed to be 0.8 since considering very high efficacy for current drugs would be unrealistic [75].
The optimization problem can then be mathematically formulated as:

$$\min_{\eta_{R_k}, \eta_{P_k}} \omega_1 \sum_{k=1}^{K} \frac{\eta_{R_k} + \eta_{P_k}}{4(\eta_{R_{max}} + \eta_{P_{max}})} + \omega_2 IPP(t_f) + \omega_3 RIP(t_f)$$  \hspace{1cm} (3.1)$$

subject to:

$$0 \leq \eta_{R_k} \leq \eta_{R_{max}}$$  \hspace{1cm} (3.2)$$

$$0 \leq \eta_{P_k} \leq \eta_{P_{max}}$$  \hspace{1cm} (3.3)$$

$$IPP(t_f) = \frac{1}{N} \sum_{j=1}^{N} SI(j)$$  \hspace{1cm} (3.4)$$

$$RIP(t_f) = \frac{1}{N} \sum_{j=1}^{N} SRI(j)$$  \hspace{1cm} (3.5)$$

where

$$SI(j) = \begin{cases} 1 & \text{if } VL_j(t_f) > 0, \forall j \in \{1, ..., N\} \\ 0 & \text{otherwise} \end{cases}$$  \hspace{1cm} (3.6)$$

$$VL_j(t_f) = V_j(t_f) + V_{mR,j}(t_f) + V_{mP,j}(t_f)$$  \hspace{1cm} (3.7)$$

$$SRI(j) = \begin{cases} 1 & \text{if } VL_j(t_f) = 0 \& RL_j(t_f) > 0, \forall j \in \{1, ..., N\} \\ 0 & \text{otherwise} \end{cases}$$  \hspace{1cm} (3.8)$$

$$RL_j(t_f) = I_j(t_f) + I_{mR,j}(t_f) + I_{mP,j}(t_f) + L_j(t_f) + L_{mR,j}(t_f) + L_{mP,j}(t_f)$$  \hspace{1cm} (3.9)$$

$$[VT(t_{i+1}), RT(t_{i+1})] = \Pi(VT(t_i), RT(t_i), \eta_{R_k}(t_i), \eta_{P_k}(t_i))$$  \hspace{1cm} (3.10)$$

Here \(\Pi(\cdot)\) denotes the KMC-based integrator and \(N\) is the total number of
simulations. \( \eta_{R_k} \) and \( \eta_{P_k} \) show the efficacy of RTI and PI, and \( k = 1, \ldots, K \) specifies the corresponding week of treatment. Since a four week treatment period is considered, \( K = 4 \) here. \( \omega_1 \), \( \omega_2 \), and \( \omega_3 \) are weight factors on drug amount, IPP, and RIP, respectively. The weights define the relative importance of the three (conflicting) objectives; the higher the value of the weight, the more important that quantity becomes. The sensitivity of the identified treatment strategies to the relative values of \( \omega_1 \), \( \omega_1 \), and \( \omega_3 \) and the procedure to choose their values is discussed in section 3.3.

\( VT \) is the vector of number of virus particles, i.e., \( V \), \( V_{mR} \), and \( V_{mP} \), at each time step of KMC simulation: \( VT(t_i) = [V(t_i) \ V_{mR}(t_i) \ V_{mP}(t_i)] \), \( t_i = t_0, \ldots, t_f \) and the initial values are: \( VT(t_0) = [100 \ 0 \ 0] \). Viral load, denoted by \( VL(t_f) \), is the sum of all types of virus particles at the end of each simulation time horizon in equation 3.7. \( IPP \) is defined as the number of times when the viral load is greater than zero, (which is called successful infection, denoted by \( SI \) in equation 3.6 and determined by \( VL(t_f) \)) at the end of simulation horizon divided by the total number of simulations, \( N \), in equation 3.4. \( RIP \) is determined similarly by \( RT \), \( RL \), and \( SRI \). \( RT \) is the vector of actively and latently infected cells: \( RT(t_i) = [I(t_i) \ I_{mR}(t_i) \ I_{mP}(t_i) \ L(t_i) \ L_{mR}(t_i) \ L_{mP}(t_i)] \), \( t_i = t_0, \ldots, t_f \) and the initial values are: \( RT(t_0) = [0 \ 0 \ 0 \ 0 \ 0 \ 0] \). \( RL(t_f) \), is the sum of all types of infected cells at the end of each simulation time horizon in equation 3.9. \( RIP \) is defined as the number of times when the number of infected cells is greater than
zero, (which is denoted by $SRI$ in equation 3.8 and determined by $VL(t_f)$) at the end of simulation horizon divided by the total number of simulations, $N$, in equation 3.5. In order to avoid treatment schedules being a mathematical artifact of the truncated time horizon, the simulation time is extended to 120 days ($t_f = 120$ days) after the four weeks of treatment and IPP is computed at the end of 120 days, not the four weeks of treatment.

100,000 simulation runs are used for each investigated case. The case of $V(0)=100$ is chosen for the optimization problem since the amount of blood is the closest to a needlestick incident. For $V(0)=10$ the amount of infected blood entering the body is very small and consequently, the IPP is a relatively small value; hence optimization may decrease the IPP only slightly. For $V(0)=1000$, the amount of infected blood entering the body is higher than a needlestick incident and as a result the IPP of 10 and 15 days latency are very close to IPP of no treatment case, i.e., treatment does not have an considerable effect after 10 days latency.

Similar to chapter 2, prompt and also latent combination therapy are considered for the optimization problem. As it can be seen in Table 2.2, combination therapy for four weeks at a constant efficacy is successful in decreasing the IPP. These results are motivating to seek medication strategies with less amount of drug or lower IPP, which are considered to be more beneficial for the patients.
3.2 Solution algorithm

In this optimization problem, calculation of the objective function involves KMC simulations which are unavailable in a closed form. Moreover, due to the stochastic nature of KMC simulations, gradient based optimization algorithms are not directly applicable. As a result, direct search algorithms, which do not evaluate gradients to compute search direction, are more suitable for the current problem. Such methods include Hooke-Jeeves, Nelder-Mead, MCS, etc. which are discussed in details in [84, 85].

Hooke-Jeeves algorithm to obtain optimal dosage strategies is used. Similar to coordinate descent, Hooke-Jeeves is a stencil based method, however, it is a more aggressive search [84]. Similar to all stencil-based methods, a set of search directions and also an array of scales which determine the allowed step size to be investigated from the current point should be provided. The array of scales used for the solution of the current formulation is [0.2, 0.1] since it is impossible to quantify the efficacy more precisely in practice as discussed in 3.1.

3.3 The “optimal” treatment strategy

In this section the simulation results are discussed and compared to the results in section 2.3.

Choosing the weights of the objective function: The choice of the weights
\( \omega_1, \omega_2 \) and \( \omega_3 \) in equation 3.1 has a considerable effect on the optimization results.

It is important to notice that the relative amount of \( \omega_1, \omega_2 \) and \( \omega_3 \) creates the difference in optimization results, not the absolute values of them.

Since the ultimate goal is to decrease IPP not the RIP, the ratio of \( \omega_2 \) to \( \omega_3 \) was chosen to be 100 to 1 for all simulations. The choice of \( \omega_2 \) to \( \omega_1 \) ratio hinges on the decision to put more weight on IPP or efficacy, i.e, determine which quantity needs to be penalized more. Table 3.1 presents the effect of the relative value of these weights. The last two columns of the Table show the average amount of RTI (\( \eta_{R_{avg}} \)) and PI (\( \eta_{P_{avg}} \)), respectively. For one day latency, initially the ratio of \( \omega_2 \) to \( \omega_1 \) is 1 to 2 (more weight on efficacy than on IPP) is examined which results in IPP of 0.997\%. If the ratio of \( \omega_2 \) to \( \omega_1 \) is further decreased, lower IPP is not achievable with lower efficacy. On the other hand, when the ratio of \( \omega_2 \) to \( \omega_1 \) is equal to 4 to 1, (i.e., put more weight on IPP and less weight on efficacy) in the third line of the Table it can be seen that IPP drops from 0.997\% to \( \%0.917 \). This implies IPP has been diminutively reduced for 8 patients out of 10000 with the cost of higher efficacy, i.e, higher dosages of the drugs. Hence, the ratio of \( \omega_2 \) to \( \omega_1 \) is chosen to be 1 to 2 for one day latency.

Table 3.1. Effect of weights, \( \omega_1 \) and \( \omega_2 \), on computed values of IPP and efficacy.

<table>
<thead>
<tr>
<th>treatment</th>
<th>( \omega_2 ) to ( \omega_1 ) ratio</th>
<th>IPP</th>
<th>( \eta_{R_{avg}} )</th>
<th>( \eta_{P_{avg}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-29</td>
<td>1 to 2</td>
<td>0.997</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>1-29</td>
<td>4 to 1</td>
<td>0.917</td>
<td>0.65</td>
<td>0.75</td>
</tr>
<tr>
<td>10-38</td>
<td>1 to 1</td>
<td>25.9</td>
<td>0.7</td>
<td>0.65</td>
</tr>
<tr>
<td>10-38</td>
<td>1 to 10</td>
<td>27.0</td>
<td>0.5</td>
<td>0.6</td>
</tr>
</tbody>
</table>
For ten day latency, when the ratio of $\omega_2$ to $\omega_1$ is equal to 1 to 1, IPP is 25.9%. If we the ratio of $\omega_2$ to $\omega_1$ is decreased to 1 to 10, (i.e., put more weight on efficacy and less weight on IPP) in the fifth line of Table 3.1 the average efficacy of both drugs drops considerably. However, the infection establishment risk has been significantly increased for 109 patients out of 10000. On the other hand, further increase in the ratio of $\omega_2$ to $\omega_1$ is not successful in lowering IPP with less amount of drug. Hence, the ratio of $\omega_2$ to $\omega_1$ is chosen to be 1 to 1 for ten day latency. Similar analysis is performed to identify optimal values of weights depending upon latency. The weights are shown in table 3.2.

**Optimal treatment scheduling:** The results of optimization simulations are shown in Tables 3.2 and 3.3. In Table 3.2, the IPP of optimal treatment strategies are shown for the proposed treatment in column three and comparison with the constant efficacy medication strategies, case (I) and case (II) of Table 2.2, can be seen in the forth and fifth columns, respectively. It is observed that with respect to case (I), the optimal treatment IPP is always significantly lower (up to 57% decrease) which implies that the first optimization goal, decreasing IPP, is always achieved. With respect to case (II), IPP of optimal strategy is slightly lower (up to 4.0% decrease) except for the prompt initiation of treatment. Although the optimal treatment IPP is 82% higher than case (II) for prompt initiation, it should be considered that IPP is still a very small number, 0.405%, i.e., less than one patient in one hundred. Consequently, the first optimization goal, decreasing
IPP, is also achieved with respect to case (II) except for one case.

Table 3.2. IPP of optimal treatment strategies (RIP is presented in parentheses); percent of change of IPP with respect to constant treatment, cases (I) and (II) of Table 2.2.

<table>
<thead>
<tr>
<th>treatment</th>
<th>$\omega_2$ to $\omega_1$ ratio</th>
<th>IPP (RIP)</th>
<th>$%$ change of IPP w.r.t. case(I)</th>
<th>case(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-28</td>
<td>1 to 2</td>
<td>0.405 (0.018)</td>
<td>-57.2</td>
<td>81.6</td>
</tr>
<tr>
<td>1-29</td>
<td>1 to 2</td>
<td>1.000 (0.040)</td>
<td>-54.5</td>
<td>-0.1</td>
</tr>
<tr>
<td>2-30</td>
<td>1 to 2</td>
<td>1.700 (0.082)</td>
<td>-51.0</td>
<td>-4.0</td>
</tr>
<tr>
<td>5-33</td>
<td>1 to 1</td>
<td>6.32 (0.27)</td>
<td>-42.6</td>
<td>-2.9</td>
</tr>
<tr>
<td>10-38</td>
<td>1 to 1</td>
<td>25.90 (0.64)</td>
<td>-20.6</td>
<td>-1.1</td>
</tr>
<tr>
<td>15-43</td>
<td>1 to 1</td>
<td>40.92 (0.22)</td>
<td>-3.8</td>
<td>-0.04</td>
</tr>
</tbody>
</table>

In Table 3.3, the weekly schedule of each drug and the average efficacy over the treatment period are shown. In the last column, the average efficacy has been compared with case (I) and case (II) of Table 2.2. The average efficacy in optimal treatment strategy is always higher (up to 50%) than in case (I) but lower (up to 20%) than in case (II). Hence, it is concluded that with respect to case (II), the second optimization goal is also achieved. Figure 3.1 shows the graphical image of the treatment schedules. Prompt initiation, 1, 2, 5, 10, and 15 days latency schedules are shown in figures 3.1(a), 3.1(b), 3.1(c), 3.1(d), 3.1(e), and 3.1(f), respectively. The efficacy of RTI is shown by solid blue line and efficacy of PI is shown by dashed green line. It is observed that both RTI and PI are scheduled to start at the maximum level and then generally decline during the following weeks, although maximum efficacy at third or forth week is still observed in some cases. It is interesting to note that if the sum of both efficacies is considered (shown
Figure 3.1. Trajectories of $\eta_R$ (solid blue lines) and $\eta_P$ (dashed green lines) of optimal treatment strategies. Dotted red lines show the sum of $\eta_R$ and $\eta_P$. Subfigures present: (a) prompt initiation, (b) 1 day latency, (c) 2 days latency, (d) 5 days latency, (e) 10 days latency, and (f) 15 days latency.
Table 3.3. Weekly schedule of efficacy for optimal treatment strategy and the average efficacy of RTI and PI over time; percent of change of average efficacy with respect to constant treatment, cases (I) and (II)

| treatment period | 
|------------------|------------------|
|                  | efficacy | week1 | week2 | week3 | week4 | average | % change of average w.r.t case(I) | case(II) |
| 0-28             | \( \eta_R \) | 0.8   | 0.6   | 0.2   | 0.4   | 0.5     | 0        | -50       |
|                  | \( \eta_P \) | 0.8   | 0.8   | 0.8   | 0.6   | 0.75    | 50       | 0         |
| 1-29             | \( \eta_R \) | 0.8   | 0.8   | 0.4   | 0.4   | 0.60    | 20       | -20       |
|                  | \( \eta_P \) | 0.8   | 0.4   | 0.8   | 0.8   | 0.70    | 40       | -6.67     |
| 2-30             | \( \eta_R \) | 0.8   | 0.6   | 0.8   | 0.4   | 0.65    | 30       | -13.33    |
|                  | \( \eta_P \) | 0.8   | 0.8   | 0.4   | 0.7   | 0.675   | 35       | -10.00    |
| 5-33             | \( \eta_R \) | 0.8   | 0.8   | 0.6   | 0.7   | 0.725   | 45       | -3.33     |
|                  | \( \eta_P \) | 0.8   | 0.6   | 0.6   | 0.5   | 0.625   | 25       | -16.67    |
| 10-38            | \( \eta_R \) | 0.8   | 0.8   | 0.6   | 0.6   | 0.7     | 40       | -6.67     |
|                  | \( \eta_P \) | 0.8   | 0.6   | 0.6   | 0.6   | 0.65    | 30       | -13.33    |
| 15-43            | \( \eta_R \) | 0.8   | 0.8   | 0.4   | 0.6   | 0.65    | 30       | -13.33    |
|                  | \( \eta_P \) | 0.8   | 0.6   | 0.6   | 0.4   | 0.6     | 20       | -20       |

with dotted red lines in Figure 3.1), for all the six cases, it either decreases from each week to the next one or remains constant. The high initial efficacy of drugs and the gradual decline during the next few weeks can be better understood if the infection cycle is considered. HIV infection starts with a few number of virus particles and a dramatic change in number of virus particles and infected cells happens during the first few days. After about five days, the virus particles have been either eradicated or have been increased to such large population that their eradication becomes improbable. In the optimization formulation the efficacy of the drugs are to be minimized. Hence, the identified optimal treatment schedules recommend high dosages of drugs upon start of treatment to eradicate infection in
as many cases as possible. In other words, the efficacy of the drugs are scheduled at higher values when they are more effective and at lower values when they have limited affectivity.

To show the significance of efficacy scheduling (weekly change), the optimization problem is considered without weekly change, i.e., finding a constant optimal efficacy for 28 days. The optimal efficacy for almost all of the cases for both drugs is then 0.8 (for 15 days latency, $\eta_R = 0.8$ and $\eta_P = 0.6$). These results are predictable: employing maximum efficacy to decrease the IPP. In other words, the importance of weekly drug scheduling is to decrease the IPP while also minimizing the efficacy of drugs.

Also to further investigate if the optimal treatment strategy leads to lower IPP values, separate simulations for each of the six investigated cases are run with similar period of treatment and the same (constant) efficacy of drugs equal to the average of optimal treatment strategy (the average shown in the seventh column of Table 3.3), i.e., same amount of drug over the time. The results are shown in Table 3.4 and it can be observed that IPP of constant treatment efficacy is always significantly higher (between 1% to 33%) compared to when optimal treatment is employed. These results prove that given a certain amount (efficacy) of drugs, lower IPP is achievable by weekly scheduling the efficacy.

To decide whether to apply the optimal strategy or not, it can be concluded that if it is possible to start treatment immediately after the incident, it is more
Table 3.4. Comparison of IPP of optimal treatment strategies with constant efficacy medication with the efficacy equal to the average of optimal strategy. RIP is presented in parentheses.

<table>
<thead>
<tr>
<th>treatment</th>
<th>optimal strategy</th>
<th>constant strategy</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-28</td>
<td>0.405 (0.018)</td>
<td>0.540 (0.020)</td>
<td>33.3</td>
</tr>
<tr>
<td>1-29</td>
<td>1.000 (0.040)</td>
<td>1.250 (0.057)</td>
<td>25.0</td>
</tr>
<tr>
<td>2-30</td>
<td>1.700 (0.082)</td>
<td>2.055 (0.078)</td>
<td>20.9</td>
</tr>
<tr>
<td>5-33</td>
<td>6.32 (0.27)</td>
<td>7.01 (0.29)</td>
<td>10.9</td>
</tr>
<tr>
<td>10-38</td>
<td>25.90 (0.64)</td>
<td>27.22 (0.59)</td>
<td>5.1</td>
</tr>
<tr>
<td>15-43</td>
<td>40.92 (0.22)</td>
<td>41.47 (0.20)</td>
<td>1.3</td>
</tr>
</tbody>
</table>

beneficial for the patient to have a constant efficacy medication strategy at a high efficacy, e.g., case (II). For all other cases, the optimal treatment strategy will be more beneficial since it will increase the chance of survival. The most important outcome of these simulation is the urgent need to start medication immediately after contamination with virus happens. Even one day latency in medication initiation increases the chance of infection establishment considerably. It is concluded that treatment should be available for immediate consumption and optimal treatment strategy should be followed if treatment does not start immediately.
CHAPTER FOUR

ESTIMATION OF EFFICACY OF NRTIS

In this chapter, a mechanistically informed model for the intracellular interaction of HIV-1 and NRTIs is developed. As discussed in section 1.5, the majority of HIV models include drug efficacy as time-invariant constants (such as what is used in the models of chapters 2 and 3) which is far less reflective of the actual mechanisms by which antiretroviral drugs achieve their desired results. In order to have a more accurate description, the knowledge of the NRTI mechanism should be translated into a concise mathematical model.

Such models could elucidate the efficacy profiles that might be expected for various intracellular conditions. The aim is to provide necessary tools to more
accurately simulate the progression of the HIV infection and its response to treat-
ment. The development of this type of mechanistic model can help guide future
experimental investigations by highlighting the key parameters that ultimately
determine a drug’s efficacy. In this chapter, efficacy is linked with time-varying
NRTI intracellular triphosphate concentration from physiologic data on plasma
concentration maximums and half lives, such as that collected in [49] and [86].

4.1 Reverse Transcription, the hallmark of HIV
infection cycle

Following fusion with a victim T-cell, HIV injects its inner protein capsid into
the cell’s cytoplasm. This capsid contains two copies of the viral genome in the
form of single strands of (+) RNA, along with multiple copies of the viral reverse
transcription (RT) protein [87]. Once in the intracellular environment, RT begins
using viral RNA as a template to catalyze the polymerization of a (-) DNA strand
from free deoxynucleotides in the host cell. As it transcribes, the RT complex also
functions as an RNAase, cleaving the backbone of the RNA genome and promoting
its dissociation from the newly transcribed DNA. In turn, additional RT complexes
use the nascent (-) DNA as a template for formation of complimentary (+) DNA.
The (+) DNA is synthesized as two strands, referred to as the Upstream (U)
and Downstream (D) segments [3]. The process of reverse transcription is not
linear; up to three strands, a negative sense strand and two halves of the positive sense strand, may be transcribed at once. Initially, only the (-) strand begins transcription, using the viral RNA as a template. The site of initiation is only around 200 bases away from the 5′ terminus of the RNA. Once the (-) strand grows to the 5′ terminus site, it must undergo a strand transfer process that shifts it to the 3′ end of the RNA where it may continue elongation until it reaches the 5′ end of the RNA once more [3]. After the (-) strand reaches approximately 650 bases in length, a purine-rich segment of the viral RNA can act as a primer, initiating the simultaneous transcription of the U(+) strand. An identical purine rich segment can be found in the RNA at the approximate halfway point of the genome. This segment too acts as a primer, initiating the transcription of the D(+) strand [88]. The U(+) and D(+) strands elongate using the nascent (-) strand as a template [3]. If the U(+) strand has reached the 5′ end of the (-) strand and the (-) strand has reached the 5′ end of the viral RNA, a second strand transfer event may occur. This results in the formation of a temporary loop structure, in which the (-) actually begins to use the U(+) strand as a template. Transcription of the (-) strand ends when it reaches the terminus of the U(+) strand. Transcription of the D(+) strand ends when it reaches the terminus of the (-) strand. The U(+) strand ultimately displaces the D(+) strand and extends for approximately 100 more bases before terminating. Rates of nucleotide addition, (+) strand initiation, and strand transfer processes have been reported in [89, 90] and the process is
illustrated in figure 4.1.

Figure 4.1. An outline of critical reverse transcription events: (1) Host-provided tRNA binds to the primer binding site (PBS) and (-) strand transcription begins. (2) Upon reaching the 5’ end of the RNA template, the (-) strand is transferred to the 3’ end. (3) Initiation of the U(+) and D(+) strands begins as the (-) strand reaches the two purine-rich primer sites. (4) After the U(+) and the (-) strand reach the 5’ end of their respective templates, another strand transfer occurs. This allows each to use the other as a template and continue transcription. (5) The (-) strand continues to the 5’ end of the U(+) strand and the D(+) strand continues to the 5’ end of the (-) strand. (6) Transcription is complete once the U(-) strand displaces 100 bases of the D(+) strand to reach the central termination site (CTS). (Figure and caption reproduced from [3].)

The complete transcription of the (-) strand and both (+) segments results in a complete double-stranded copy of viral DNA called a provirus. At this point,
reverse transcription stops and the provirus is prepared for nuclear import and integration into the host genome. The completed HIV provirus contains approximately 9700 base pairs \[91\], the result of almost 20000 reverse-transcriptase mediated nucleotide polymerization events.

NRTIs are drugs that structurally mimic natural deoxynucleosides, the building blocks of DNA. A comparison between a commonly used NRTI, AZT, and natural nucleotide Thymidine is shown in figure 4.2, illustrating structural and anabolic similarities. Unlike their natural congeners, NRTIs lack the 3’ hydroxyl group necessary for the formation of a 3’-5’ phosphodiester linkage. As such, NRTIs are chain terminators: while included in a strand of DNA, they prevent strand extension. Just as nucleosides must be triphosphorylated into nucleotides prior to inclusion in DNA, NRTIs must pass through the same cellular enzymatic pathways to achieve their active triphosphorylated forms. Once triphosphorylated, NRTIs compete with natural nucleotides for addition into the HIV reverse transcription complex, where they inhibit proviral production through chain termination.

Unfortunately, addition of an NRTI molecule to the growing HIV genome does not guarantee permanent chain termination in the intracellular environment. In addition to its function as a polymerase, HIV RT can also catalyze the reverse reaction: phosphorolysis of the last 3’-5’ linkage and removal of the terminal nucleotide. This can be achieved through two pathways: pyrophosphorolysis and nucleotide-mediated phosphorolysis. In pyrophosphorolysis, a pyrophosphate ion
Figure 4.2. Comparison of chemical structure of AZT and Thymidine. is used as a substrate, resulting in the removal of the chain-terminating nucleotide by converting it back to nucleoside triphosphate [92, 93].

Mechanistically, pyrophosphorolysis occurs as the reverse reaction of standard RT catalyzed DNA polymerization. In nucleotide-mediated phosphorolysis, a free nucleotide triphosphate is used as a pyrophosphate analog, removing the chain terminating nucleotide and producing a dinucleotide polyphosphate (two nucleotide bases connected by a series of three to four phosphate groups) [94, 95]. Both processes occur appreciably at physiological concentrations of their respective substrates. Phosphorolytic removal will not occur immediately following every chain termination. Although no further deoxynucleotides can be bound to NRTI terminated DNA, the RT-template complex may still assume a configuration in which it can accept an additional dNTP. The addition of a free nucleotide induces a configurational change in the RT protein that produces a stable ternary product. This would normally be the immediate precursor to 3'-5' linkage formation.
Table 4.1. Description of model parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P(\mu)$</td>
<td>probability of reaction $\mu$ to occur in the next step</td>
</tr>
<tr>
<td>$p$</td>
<td>instantaneous probability of NRTI addition</td>
</tr>
<tr>
<td>$p_{TDEC}$</td>
<td>probability of stable dead end complex formation</td>
</tr>
<tr>
<td>$\alpha$</td>
<td>relative affinity of NRTI addition to natural nucleotide</td>
</tr>
<tr>
<td>$B$</td>
<td>number of vulnerable bases in the genome</td>
</tr>
<tr>
<td>$\epsilon_{WN}$</td>
<td>efficacy percentage from [45]</td>
</tr>
<tr>
<td>$\epsilon_{DC}$</td>
<td>efficacy percentage due to formation of dead-end complex</td>
</tr>
<tr>
<td>$\bar{\epsilon}_{DC}$</td>
<td>estimated time averaged efficacy percentage from equation 4.16 due to formation of dead-end complex</td>
</tr>
<tr>
<td>$\epsilon_{si}$</td>
<td>overall efficacy percentage of successful inhibition</td>
</tr>
<tr>
<td>$[RTP]$</td>
<td>intracellular concentration of phosphorylated drug</td>
</tr>
<tr>
<td>$[dNTP]$</td>
<td>intracellular concentration of natural nucleotide</td>
</tr>
<tr>
<td>$k_{rl}$</td>
<td>dissociation constant of the natural nucleotide in the rate limiting step</td>
</tr>
<tr>
<td>$k_{rl}$</td>
<td>dissociation constant of the drug in the rate limiting step</td>
</tr>
<tr>
<td>$K_D$</td>
<td>dissociation constant of the natural nucleotide</td>
</tr>
<tr>
<td>$K_i$</td>
<td>dissociation constant of the drug</td>
</tr>
<tr>
<td>$K_M$</td>
<td>Michaelis dissociation constant of natural nucleotide</td>
</tr>
<tr>
<td>$IC_{50}$</td>
<td>plasma concentration of drug which has a 50% inhibitory effect</td>
</tr>
<tr>
<td>$C(t)$</td>
<td>plasma concentration of drug as a function of time</td>
</tr>
<tr>
<td>$C_{max}$</td>
<td>maximum drug concentration</td>
</tr>
<tr>
<td>$t_p$</td>
<td>time needed for the concentration to peak</td>
</tr>
<tr>
<td>$T$</td>
<td>dosing interval</td>
</tr>
<tr>
<td>$a_t$</td>
<td>drug administration time in simulation</td>
</tr>
<tr>
<td>$T_{1/2}$</td>
<td>plasma half life of drug</td>
</tr>
<tr>
<td>$h$</td>
<td>plasma half life of drug in simulation</td>
</tr>
</tbody>
</table>

[96, 97]. Without the ability to form this bond, the complex becomes “stuck”: the forward process cannot continue, the reverse conformational shift is extremely kinetically unfavorable (the forward reaction has been estimated to occur approximately 300 times faster than the reverse reaction for natural nucleotides), and the conformational shift has resulted in extremely tight binding of the RT to the template-primer complex [97, 98]. This product is referred to as a Ternary Dead End Complex (TDEC). The exact rate of formation and stability of TDECs appears to vary based on many factors, including the type of NRTI acting as the
terminator, mutations in the RT protein, and the sequence of nearby sections of the template [99, 90]. Thus, of all the steps of the reverse transcription process it appears that two major factors contribute to NRTI effectiveness and merit close investigation. First, the probability that an NRTI triphosphate will be added in lieu of the natural dNTP each time RT transcribes a “vulnerable” base. Second, once NRTI is added, the overall stability of the NRTI complex and the probability that it will form an indefinitely stable complexes. Since both of these events are affected by the enzyme-level polymerase mechanism of the HIV RT, a quick summary of RT structure and kinetics follows. Table 4.1 shows the parameters used in this chapter and Table 4.2 lists some of the NRTIs currently recommended for HIV therapy.

**Table 4.2.** Commonly used NRTIs, their literature abbreviations, the natural deoxynucleotides they imitate, and the number of cellular enzyme-catalyzed phosphorylations they must undergo to become active.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Abbreviation</th>
<th>Base imitated</th>
<th>Cellular phosphorylations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tenofovir</td>
<td>TDF</td>
<td>A</td>
<td>2</td>
</tr>
<tr>
<td>Didanosine</td>
<td>ddI</td>
<td>A</td>
<td>3</td>
</tr>
<tr>
<td>Zidovudine</td>
<td>AZT</td>
<td>T</td>
<td>3</td>
</tr>
<tr>
<td>Stavudine</td>
<td>d4T</td>
<td>T</td>
<td>3</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>3TC</td>
<td>C</td>
<td>3</td>
</tr>
<tr>
<td>Zalcitabine</td>
<td>ddC</td>
<td>C</td>
<td>3</td>
</tr>
<tr>
<td>Emtricitbine</td>
<td>FTC</td>
<td>C</td>
<td>3</td>
</tr>
<tr>
<td>Abacavir</td>
<td>ABC</td>
<td>G</td>
<td>3</td>
</tr>
</tbody>
</table>
4.2 Kinetics of HIV-1 RT polymerase activity

As with most polymerase proteins, RT must initially form a complex with an existing nucleic acid primer and template. It may then begin to add nucleotides in a processive manner. The nucleotide addition reaction catalyzed by HIV-1 RT occurs kinetically as a multi-step, reversible process: A nucleotide or nucleotide analogue reversibly binds to a location on the RT protein referred to as the Nucleotide Binding site, or N-site [98]. This triggers a conformational change in the RT protein, which aligns and brings together the nucleotide and the primer; this conformational change is the rate-limiting step of the process [96, 97]. This is followed by the actual chemical reaction that forms the $3'\rightarrow 5'$ phosphodiester linkage between primer and nucleotide, liberating a pyrophosphate ion. The pyrophosphate ion then dissociates from the complex, which allows the RT protein to resume its original configuration and the nucleoside -now the leading end of the growing primer- to translocate from the N-site to the neighboring primer binding site, or P-site. This translocation is an equilibrium process. When the leading end of the primer is in the P site, DNA synthesis may continue; when it is in the N site, it is vulnerable to phosphorolysis [98]. This process is summarized in figure 4.3. In addition, like most polymerases, RT is liable to dissociate from the transcription complex entirely.
Figure 4.3. Kinetics of Processive HIV-1 Polymerase Activity. The mechanism by which RT adds nucleotides or NTRIs involves numerous reversible steps. A: Natural deoxynucleotide reversibly binds to the N-site of RT while the end of the primer occupies the P site, with a dissociation constant of $K_D$. B: Addition of NTRI triphosphates follows the same mechanism as addition of dNTPs, with dissociation constant $K_i$. Since the mechanism is reversible, we may revert from step 6i back to 1i, albeit at the cost of time. The presence of RTI prevents further extension of the primer and if another nucleotide adds to the terminated complex, step 7i, a Ternary Dead End Complex is formed.

4.3 The concept of efficacy

The term “efficacy” itself may appear somewhat ambiguous. When treating HIV, there are several measures used to determine the efficacy of therapy: changes in T-cell counts, log-reductions in viral titers, and $IC_{50}$ values are all reported as methods of assessing the effect that a particular therapy will have [45, 46, 47, 86].

For this discussion, the concept of efficacy outlined by Perelson et al. [28] will be used. In this definition, the efficacy of an NRTI is the percentage by which it reduces the apparent rate of conversion of healthy T-cells to infected T-cells over the untreated case. This definition correlates most closely with drug $IC_{50}$, which
is defined as the plasma concentration of drug that results in a 50% reduction in the appearance of infected cells over a set incubation period in an *in vitro* culture.

### 4.4 Analysis of key aspects of NRTI mechanisms

From the mechanisms of NRTI/HIV interactions, it appears that two major factors should contribute to efficacy: the ability of the drug to be added to the growing HIV genome, and the stability of the potential terminated complexes. If these elements of the mechanism could be mathematically modeled, an overall method for mathematically quantifying efficacy becomes possible. In the following sections methods by which elements of the NRTI mechanism can be quantified in terms of measurable physical values are explained. Since these methods rely on the principles of stochastic modeling, initially it is discussed how to employ Gillespie’s method to compute relative quantities.

#### 4.4.1 Computing Competing Rates as Probabilities

The Gillespie’s method is explained in section 2.3. Equation 2.13 which shows the probability of reaction $\mu$ to occur is repeated here:

$$P(\tau, \mu) = H_\nu c_\nu \exp \left[ - \sum_{\nu=1}^{M} H_\nu c_\nu \tau \right]$$  

(4.1)
and based on the discussion in section 2.3, for a bimolecular reaction $H_\mu = X_iX_j$. In the case of $M = 2$ and $N = 3$ (two reactions and three species) where the first reaction is between molecules of species 1 and 3 and the second reaction is between molecules of species 2 and 3, the probability of reaction 1 to occur is:

$$P(1) = \frac{c_1H_1}{c_1H_1 + c_2H_2} \quad (4.2)$$

Substituting the relationships for $H$ and $c$ (from section 2.3) results in:

$$P(1) = \frac{k_1X_1X_3}{k_1/k_2X_1X_3 + k_2/k_2X_2X_3} = \frac{k_1X_1X_3}{k_1X_1X_3 + k_2X_2X_3} \quad (4.3)$$

It is important to note that $k_1X_1X_3$ and $k_2X_2X_3$ are the rates of reaction 1 and 2, respectively. In order to better compare the two reactions, equation 4.3 can be also written as:

$$P(1) = \frac{k_1/k_2X_1X_3}{k_1/k_2X_1X_3 + X_2X_3} = \frac{\alpha X_1}{\alpha X_1 + X_2} \quad (4.4)$$

$\alpha$ is defined here as the relative affinity of reaction 1 taking place compared to reaction 2. Further elaboration on $\alpha$ in the context of the problem at hand is
given in section 4.4.2.2.

4.4.2 NRTI Triphosphate addition: a propensity perspective

As previously outlined, addition of NRTI triphosphate occurs by the same mechanism as addition of deoxynucleotides, with NRTI triphosphate and the dNTP it mimics acting as competing substrates of the RT complex. Hence, two possible “reactions” are assumed in the system: natural nucleotide addition and NRTI addition. Then, the first step in determining efficacy is to quantify the average probability that an NRTI triphosphate is added at each “vulnerable” nucleotide in the genome. This probability is expected to be dependent upon three factors: the intracellular concentration of NRTI triphosphate, the concentration of the deoxynucleotide with which it competes, and the relative selectivity ($\alpha$) of the RT-Genome complex for the NRTI. The immediate problem, then, becomes one of describing these three quantities in the intracellular environment.

4.4.2.1 Intracellular concentrations of NRTI and dNTP

It would be ideal to be able to determine drug effects from traditionally measured values such as blood plasma AUC or, preferably, dose. Unfortunately, the cellular triphosphorylations necessary to convert NRTIs to their active form prevent easy correlation between the plasma concentration of NRTI prodrug and the effective
concentration of anabolite in the intracellular environment. Generally, one of these phosphorylation events is slow due to poor affinity of the modified nucleotide to the natural kinase. In the case of AZT, for example, there is near-total intracellular conversion of the nucleoside form to the monophosphate form. On the other hand, concentrations of the diphosphate and triphosphate forms remain two to three orders of magnitude lower due to inhibition of nucleoside monophosphate kinases by high concentrations of AZT-MP [47] and weak interaction between AZT-DP and nucleoside diphosphate kinase active site [100]. These phosphorylation steps are potentially sensitive to drug interactions and may be a source of inter-patient variability, as well. Further compounding this is the fact that the plasma half-lives of NRTIs are up to an order of magnitude shorter than the intracellular half-lives of their anabolites [101]; these anabolites have charged phosphate groups that prevent them from easily diffusing out of the cell. Thus, the intracellular concentration of NRTI triphosphate (NRTI TP) cannot be easily determined from measurement of plasma drug concentrations. However, numerous experiments have directly measured the in vivo concentration of the triphosphate products for a variety of NRTIs [47, 48, 49] including experimental therapies in which dosing was set to achieve target intracellular NRTI TP concentrations [102, 103, 104]. Combined HPLC/mass spectrometry is the main technique used to determine intracellular triphosphate concentrations. Therefore, data on therapeutic-dose associated intracellular concentrations of NRTI TPs as well as expected profiles of concentration vs. time
can be found in literature or directly determined experimentally. In the proposed model, therefore, intracellular concentration is set as a time varying quantity and calculated directly from the plasma time varying concentration. It is important to emphasize on the significant impact of the intracellular concentration on the computed results. This matter is elaborate more in section 4.4.4.

The same techniques that are used to determine intracellular NRTI TP concentrations are also used to determine natural deoxynucleotide concentrations [50, 105, 104]. It is worth noting that, since NRTIs rely on the same cellular pathways for phosphorylation as the natural nucleotides, the presence of an NRTI may lower concentrations of its equivalent natural nucleotide and alter concentrations of other nucleotides through various biological feedback mechanisms [104, 106, 107]. This effect has been investigated as a potential cause of the lower-than-expected efficacy of triple NRTI regimens. Thus, being able to quantify the effect of natural nucleotide concentration on NRTI efficacy could greatly assist in predicting and quantifying potential drug interactions.

4.4.2.2 Relative affinity for the RT complex, $\alpha$

The question still remains of how to quantify the affinity correction factor $\alpha$, and what exactly it represents. Analysis of the mechanism of nucleotide addition can be used to reveal the physical significance of $\alpha$. As mentioned in section 4.2, the rate limiting step in RT catalyzed nucleotide addition is a conformational change in
the RT protein [96, 97]. This process can be considered first order in nucleotide-RT complex, identified as intermediate states 2 and 2i in Figure 4.3. The mechanism by which RT adds nucleotides or NRTIs involves numerous reversible steps:

A. Natural deoxynucleotide reversibly binds to the N-site of RT while the end of the primer occupies the P site, with a dissociation constant of $K_D$. A rate-limiting conformational change of the RT protein then occurs with rate constant $k_{rl}$. The reverse conformational change is also the rate-limiting step for chain-shortening phosphorolytic reactions. The formation of the 3'-5' bond and subsequent dissociation of pyrophosphate ion allows the reverse transcriptase to resume its original conformation, at which point the newly-lengthened primer may relocate to the P pocket, returning the complex to its original state.

B. Addition of NRTI triphosphates follows the same mechanism as addition of dNTPs, with dissociation constant $K_i$ and conformational change rate constant $k_{irt}$. However, the presence of NRTI prevents further extension of the primer. If another nucleotide adds to the terminated complex, a Ternary Dead End Complex (TDEC) is formed. In turn, the binding of nucleotide to the complex is a reversible process. Since the conformational change is significantly rate limiting, the concentration of nucleotide-RT complex can be expressed in terms of its dissociation constant, as well as the concentrations of free nucleotide and RT transcription complex. As such, the overall rates of nucleotide and NRTI addition could be expressed as:
rate(+dNTP) = k_{irl} \left( \frac{1}{K_D} [dNTP][RT.DNA] \right) \quad (4.5)

rate(+RTP) = k_{irl} \left( \frac{1}{K_i} [RTP][RT.DNA] \right) \quad (4.6)

In which $K_D$ and $K_i$ are the dissociation constants for the natural nucleotide and the drug, and $k_{irl}$ and $k_{irl}$ are the respective rate constants for the rate limiting step. We can now use equation 4.3 and the computed rates (equations 4.5 and 4.6) to calculate the probability of NRTI triphosphate addition:

\[
p = \frac{\text{rate}(+RTP)}{\text{rate}(+RTP) + \text{rate}(+dNTP)} = \frac{k_{irl}[RTP]}{\frac{k_{irl}}{K_i}[RTP] + \frac{k_{irl}}{K_D}[dNTP]} \quad (4.7)
\]

which is equivalent to:

\[
p = \frac{\frac{K_D k_{irl}}{K_i k_{irl}} [RTP]}{\frac{K_D k_{irl}}{K_i k_{irl}} [RTP] + [dNTP]}, \quad (4.8)
\]

Since the intracellular concentration is a function of time, $p$ is time dependent as well and will attain a different value at each vulnerable base during the reverse
transcription process. Comparing equation 4.8 with equation 4.4, it is clear that 

\[ \alpha = \frac{K_D k_{irl}}{K_i k_{rl}} \]  

(4.9)

Unfortunately, this does not prove immediately helpful in determining actual values for \( \alpha \), as the various rate constants are not widely available from literature, and may vary considerably between the different NRTIs. However, an upper bound for \( \alpha \) may be set using data available in literature. First, although the two rates are generally expected to be similar, the rate limiting conformational change of RT when an NRTI triphosphate is bound is thought to be slower due to the difference in its interactions with the binding pocket [41]. Therefore, \( \frac{k_{irl}}{k_{rl}} \) should be close to but always less than one. Additionally, although \( K_D \) values for the natural nucleotides are not widely reported, the Michaelis dissociation constants are available. By definition, the Michaelis dissociation constants, \( K_M \), is larger than the dissociation constant. Therefore, we can conclude that

\[ \frac{K_M}{K_i} > \frac{K_D k_{irl}}{K_i k_{rl}} \]  

(4.10)

Values of \( K_M \) and \( K_i \) for dNTPs and NRTIs with respect to HIV RT have been
widely reported in literature [97, 108, 109, 110, 111]. Thus, an expected upper bound for the probability of NRTI additions may be derived from the current literature. The value of $\frac{K_M}{K_i}$ for the four investigated drugs are calculated based on the data from [109]. These values are reported in Table 4.3.

<table>
<thead>
<tr>
<th>drug</th>
<th>nucleotide analogue</th>
<th>$K_M$</th>
<th>$K_i$</th>
<th>relative affinity</th>
<th>$k_{ext} [1/s]$</th>
<th>$p_{TDEC} [%]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZT</td>
<td>T</td>
<td>0.80</td>
<td>0.08</td>
<td>10.0</td>
<td>$1.76 \times 10^{-3}$</td>
<td>8.1</td>
</tr>
<tr>
<td>d4T</td>
<td>T</td>
<td>0.80</td>
<td>0.03</td>
<td>26.7</td>
<td>$7.24 \times 10^{-5}$</td>
<td>0.4</td>
</tr>
<tr>
<td>3TC</td>
<td>C</td>
<td>0.61</td>
<td>1.16</td>
<td>0.5</td>
<td>$5.03 \times 10^{-4}$</td>
<td>51.3</td>
</tr>
<tr>
<td>ddC</td>
<td>C</td>
<td>0.61</td>
<td>0.14</td>
<td>4.3</td>
<td>$6.28 \times 10^{-4}$</td>
<td>62.1</td>
</tr>
</tbody>
</table>

It can thus be concluded that the probability of NRTI addition can be expressed in terms of measurable physical constants. Consequently, if the concentration vs. time curves for the intracellular concentrations can be predicted, then trends in probability of NRTIs addition over time should be likewise predictable. The effect of this probability on overall efficacy depends upon many additional factors, most importantly the stability of the NRTI once included in the HIV genome.

### 4.4.3 Stability of NRTI as a chain terminator

In order to determine the effect of an NRTI once added, its behavior within the RT complex must be known as well. Unfortunately, this is an involved task. RT can catalyze two phosphorolytic reactions to remove chain-terminating NRTIs; at the same time, as shown in Figure 4.3B, the addition of the next complimentary
nucleotide to the complex can result in the formation of a relatively stable complex. While all NRTIs have been shown to undergo these basic reactions, the relative rates of each reaction and the stability of the Ternary Dead End Complex that forms varies enormously between drugs.

As reported by Isel et al. [99], HIV RT complexes terminated by different NRTIs demonstrated varied results when incubated in the presence of the next incoming nucleotide. Isel et al. kindly provided the data of +1 rescue (shown in [99], figure 3) for the four drugs studied here: AZT, d4T, ddC, and 3TC. The raw data is used to evaluate the parameters to equation 

\[ A(1 - \exp(-k_{ext}t)) \]

where \( A \) is the amplitude of the reaction and \( k_{ext} \) is the apparent repair rate constant.

The amplitude of reaction, \( A \), represents the probability of NRTI dissociation from the chain in percentage. Hence, the probability of forming TDEC, \( p_{TDEC} \), i percent is \( 100 - A \). If TDEC is not formed, the rate of repair or extension \( k_{ext} \) determines the time needed for NRTI dissociation. Computed values of \( p_{TDEC} \) and \( k_{ext} \) are shown in table 4.3.

Based on computed values of \( A \) and \( k_{ext} \), it is concluded that among AZT, 3TC, and ddC, AZTTP TDECs are the most unstable (smallest \( p_{TDEC} \)) once formed, followed by 3TCTP, ddCTP. d4T has a very small \( p_{TDEC} \) which means the included NRTI will be dissociated from the chain almost all the time. However, d4T also has the smallest \( k_{ext} \) which means it may take hours for the included NRTI to dissociate from the chain. Despite this variance, all NRTIs achieve the same in
vivo effect which is the reduction in the number of new cells that HIV can infect in a given period of time. This is achieved through two mechanisms:

**Mechanism 1:** T-Cells have a set lifespan in vivo, and express certain natural defense factors that may potentially degrade the HIV RT complex. If an NRTI is stable in the chain, then the reduction in infection rate might be the result of a certain fraction of HIV fusions being effectively arrested during reverse transcription. The apparent rate of infections decreases by the percentage reduction in completed reverse transcriptions.

**Mechanism 2:** If the included NRTI is not stable, each addition of an NRTI to the HIV genome would still represent a period of holdup in the reverse transcription phase, extending the time between viral fusion and active infection. When multiple NRTI additions occur, the holdup time may exceed the time it would take for the host cell to die or clear the RT complex. This, also will reduce the number of cells that become infected in a given period of time. Two types of time delay: time delay which exceeds the life span of RNA in cytoplasm, which is defined as mechanism 2.I, and time delay which does not exceed the life span of the RNA in cytoplasm, which is defined as mechanism 2.II.

In other words, the source of NRTI efficacy is likely due to a combination of both mechanisms (1) and (2): NRTI can potentially cease the reverse transcription and causing a viral fusion failing to produce an actively infected T-cell, or causing those viral fusions that do succeed in reverse transcription to take longer in doing
so. Such a process defies reduction to a simple mathematical description, but is approachable via in silico stochastic modeling of the individual events that occur during reverse transcription.

4.4.4 Estimation of NRTI efficacy

A general simple model used for drug inhibition in the absence of detailed mechanistic information is presented in [45]:

\[
s(t) = 1 - \frac{C(t)}{C(t) + IC_{50}}
\]  

(4.11)

When the drug concentration is zero, \( s = 1 \) and when the drug concentration becomes very large, \( s \to 0 \). By definition, \( IC_{50} \) is the concentration required for 50\% efficacy and estimation of \( s(t) \) is highly dependent on this value. The plasma time-course of drug concentration is also given in [45]:

\[
C(t) = \begin{cases} 
C(T) + \frac{1}{t_p}(C_{max} - C(T)) & 0 \leq t \leq t_p \\
C_{max}\exp(-\omega(t - t_p)) & t_p < t < T 
\end{cases}
\]

(4.12)

where \( t_p \) and \( T \) represent dosing interval (administration time) and time to peak, respectively. \( \omega = \frac{\log(2)}{T_{1/2}} \) where \( T_{1/2} \) is the plasma half life of the drug. It is assumed that the drug concentration peaks instantaneously when the dose is
taken, i.e., $t_p = 0$. To estimate the average efficacy based on the model in [45], a new variable $\epsilon_{WN}$ is defined:

$$\epsilon_{WN} = 1 - \bar{s}(p)$$

where

$$\bar{s}(p) = \frac{1}{T} \int_0^T s(t)dt$$

$$= \frac{1}{T} \int_0^T (1 - \frac{C_{max}e^{-\omega t}}{C_{max}e^{-\omega t} + IC_{50}})dt$$

$$= 1 + \frac{1}{T\omega}(\log(C_{max}e^{-\omega T} + IC_{50}) - \log(C_{max} + IC_{50})).$$

$\bar{s}(p)$ is the average of $s(t)$ after one dosing interval ($p = 1$) and the calculations are adopted from equation (A1) in [45]. With this definition, $\epsilon_{WN} = 0\%$ when the drug has no effect and $\epsilon_{WN} = 100\%$ when inhibition is complete.

In this chapter, it is attempted to develop a more accurate model which considers not only changes in drug concentration, but also specific drug properties like the probability of forming dead end complex. Each time the idealized NRTI is added to the growing HIV provirus, it is either removed by pyrophosphorolysis or successfully forms a stable dead-end complex. Under cellular conditions, both phosphorolysis and TDEC formation can be considered first-order reactions that compete for NRTI-terminated RT complexes. Thus, the formation of a stable
complex can again be calculated as a propensity function in terms of these rates, yielding some probability $p_{TDEC}$ that a dead end complex will form. The overall probability of permanent elimination of the RT complex, which is equivalent to efficacy in this case, can be expressed as:

$$1 - \epsilon_{DC} = (1 - p \cdot p_{TDEC})^B$$

(4.15)

In which $\epsilon_{DC}$ is the efficacy due to dead end complex formation, $p$ is the probability of NRTI addition each time a vulnerable base is transcribed (expressed in equations 4.8 and 4.9) and $B$ is the number of vulnerable bases in the complete genome. It is important to note that only formation of indefinitely stable TDECs (mechanism 1) contributes to the calculation of efficacy in the above equation. The effect of time delay (mechanisms 2.I and 2.II) on efficacy needs detailed simulation which is presented in section 4.6.

As previously discussed, $p_{TDEC}$ has been quantified based on Isel et al. results [99]. Thus, any NRTI that follows Mechanism 1 will have the above efficacy profile at normal concentrations. As a result, the only information one would need to explicitly determine the efficacy is $p$, which is a function of [$RTP$], the intracellular drug concentration, shown in equation 4.8. It should be noted that $C(t)$ used in literature and equation 4.11 represents drug plasma concentration, while [$RTP$] in equation 4.8 is the intracellular concentration. Consequently, the ratio of 0.0012
(intracellular concentration of the tri-phosphorylated drug to its plasma concentration) from [47] is used for AZT and equation 4.12 is employed to calculate $[\text{RTP}]$ as a function of time. As discussed earlier in section 4.4.2.1, calculation of $\epsilon_{DC}$ is very sensitive to the intracellular concentration. Since intracellular concentration varies with time, the value of $p$ will be different at each vulnerable base. To obtain a closed-form approximate expression, the time averaged efficacy, $\overline{\epsilon}_{DC}$ over time is calculated by integrating equation 4.15 over time. Hence, the estimated efficacy is derived as:

$$\overline{\epsilon}_{DC} = \int_{0}^{T} 1 - (1 - p(t) \cdot p_{TDEC})^R dt$$  \hspace{1cm} (4.16)

A comparison between the results of equations 4.13 and 4.16 for AZT is shown in figure 4.4. It is observed that the ratio of 0.0012 resulted in a quite close efficacy prediction to previous estimations by [45] for AZT with $IC_{50} = 10$. The results are in good agreement specially at lower drug concentrations, smaller half lives, and larger administration times. It is important to note that because of employing equation 4.12, drug administration time (dosing interval) and half life are considered indirectly in calculation of $[\text{RTP}]$. As it can be seen in figure 4.4, three different values for half-life (one, two, and four hours) and two different values for administration time (four and eight hours) are investigated. The intracellular concentration and subsequently efficacy increase with longer half life and shorter
administration time.

There are a number of shortcomings to expression 4.16. As discussed earlier, the delay of reverse transcription (mechanism 2), might be as important as permanent termination of the RT complex (mechanism 1) which can not be included in the proposed equation 4.16 in a straightforward manner. Fortunately, stochastic simulation provides us with the ability of calculating the time delay associated with a certain concentration as well as the permanent termination. Furthermore, since addition of NRTI is a discrete event, and $p(t)$ depends on the concentration of NRTI which changes with time, stochastic simulation can provide a more accurate
estimation of the probability of NRTI addition over the transcription process. The results of stochastic simulation is discussed in detail in section 4.6.

4.5 Stochastic model of the reverse transcription process

An investigation of the problem might persuade one to wonder if the overall delay in reverse transcription resulting from NRTI could be calculated simply by taking the expected number of NRTI additions per genome and multiplying by the expected time that each chain termination will last. Unfortunately, as discussed in section 4.1, the reverse transcription is a nonlinear process. The inhibition of one strand will not necessarily prevent the others from being elongated. As such, the location and time when NRTI addition occurs affects just how long it delays the entire process. The proposed stochastic model accurately simulates the entire process of reverse transcription on an event-by-event basis, “building” the genome one base at a time with probabilistic addition of NRTIs followed by stochastic analysis of the ultimate path to chain-terminator removal. As discussed earlier in section 4.4.2, there are two possible reactions in the system and their rate constants can be computed based on kinetics data as shown in equations 4.5-4.9. A number of simulations of this model are used to produce an expected profile for the time delay associated with certain concentrations of drug and natural nucleotide. Figure
4.5 shows the algorithm of the simulation. Specifically this algorithm is based on Gillespie’s next reaction algorithm to the RT process. The “nonlinear guideline” in figure 4.5 eludes to choosing which strand of the DNA the simulation is currently transcribing. It is a sequence of events which is described in section 4.1 and graphically presented in figure 4.1.

A random sequence, HXB2-K03455 is chosen from the HIV sequence database for simulation [91]. For each drug one million simulation runs are used to compute the time distributions as well as percentage of TDEC formation. In order to investigate the sensitivity of the presented results to the sequence chosen, another sequence (WR27-AF286365) is also simulated for one case (AZT, $h = 2$) and produced similar results (within half percent of the main sequence chosen for simulations). The similarity in the results shows that the presented results represent the expected values for wild HIV sequences.

In this study, four different NRTIs are analyzed: AZT and d4T are T-analogues while ddC and 3TC are C-analogues. Table 4.3 shows the relative affinities and excision times of the four investigated drugs.

Among these drugs, d4T has the highest relative affinity followed by AZT and ddC. 3TC has the lowest affinity, less than two percent of d4T’s. Although the affinity values (except for 3TC) are significant, the concentrations of drug and the natural nucleotide play an important role in determining the probability of inclusion of NRTI in the chain. Once included in the chain, it is more beneficial if
Figure 4.5. Algorithm of the stochastic simulation. $p$ and $p_{\text{T DEC}}$ show the probability of NRTI addition to the chain and the probability of forming a dead end complex, respectively. “Nonlinear guideline” refers to which strand of the DNA is currently being considered as graphically shown in figure 4.1.

1) the extension time is long (small $k_{\text{ext}}$) and 2) the probability of forming TDEC, $p_{\text{T DEC}}$ is high. d4T has the longest extension time, however with a very small probability of forming TDEC. In comparison, the other T analogue, AZT, has the
shortest extension time and a much larger $p_{TDEC}$, which is still not significant comparing to $p_{TDEC}$ of C analogue drugs. The probability of forming TDEC is higher for C analogue drugs, ddC and 3TC. Also, both of them have extension times larger than AZT and smaller than d4T. Consequently, it seems that all of the four drugs have advantages and disadvantages when compared and it is difficult to judge which one is a better drug. Stochastic simulation is helpful in identifying the most beneficial drug.

Half-life and the initial drug concentration are two very important inputs for the simulations. Because of the variety in reported half-life values in literature, the average of reported values for each drug is chosen. Similarly to half-life, the reported values of $IC_{50}$ range widely. As a result an average $IC_{50}$ for each drug is used. Since reported $IC_{50}$ values in literature for AZT are quite similar, and hence more reliable; AZT results are discussed in more detail. Finally, to show the significance of $IC_{50}$, three different values of $IC_{50}$ are used for d4T simulation. It is important to emphasize here that $C_{max}$, which is the maximum drug concentration at the time of administration, is the input for the simulation, not the $IC_{50}$. However, $C_{max}$ should be chosen considering the $IC_{50}$. For AZT, six different ratios of $C_{max}$ to $IC_{50}$ are simulated: (0.1, 0.25, 0.5, 1, 2, 4). Ratios of (0.1, 0.5, 1) are simulated for 3TC and ddC and d4T is simulated with the ratio of (1).

Table 4.4 shows the literature values [112] and values used in simulations. It
should be noted that $C_{\text{max}}$ reported in the tables is the highest drug concentration at the time of drug administration; the actual concentration at each time point is calculated during the simulation based on equation 4.12. Eighteen different cases were simulated for AZT and three cases for d4T, 3TC, and ddC.

Table 4.4. Reported values of half-life and $IC_{50}$ in open literature and values of half-life and NRTIs’ concentrations used in simulations; their combinations lead to the number of investigated cases.

<table>
<thead>
<tr>
<th>drug</th>
<th>half-life [hr]</th>
<th>$IC_{50}$ [nM]</th>
<th>simulated $C_{\text{max}}$ $IC_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>reported</td>
<td>simulated</td>
<td>reported</td>
</tr>
<tr>
<td>AZT</td>
<td>0.5 to 4</td>
<td>1, 2, 4</td>
<td>10</td>
</tr>
<tr>
<td>d4T</td>
<td>0.8 to 1.5</td>
<td>1</td>
<td>9-400</td>
</tr>
<tr>
<td>3TC</td>
<td>5 to 7</td>
<td>6</td>
<td>3-1500</td>
</tr>
<tr>
<td>ddC</td>
<td>1-2</td>
<td>1.5</td>
<td>30-500</td>
</tr>
</tbody>
</table>

4.6 Simulation Results

In this section, the simulations’ results are discussed. The reverse transcription (without considering the effect of inhibitors) is a stochastic process as explained in section 4.2. As a result, the reverse transcription process acquires a time distribution rather than a precise time. Figure 4.6 shows the time distribution of reverse transcription in the absence of medication where the vertical line shows the mean: $t_{RT} = 221.14$ min with variance of 5.48 min.
Figure 4.6. Distribution of time to complete RT process in the absence of NRTIs (solid line). The dashed line represents the average time of the process. The dotted line represents the average life span of viral RNA in the intracellular host environment. Note that the RT process concludes on average well before the average life span of RNA in cytoplasm.

4.6.1 Effect of reverse transcription inhibitors on reverse transcription time

As discussed in section 4.5, for each of the investigated cases, one million simulation runs are used to obtain the expectation of the process. Investigating the one million runs for each case, four different outcomes can be identified: NRTI additions which lead to dead end complex formation (mechanism 1 or $\epsilon_{DC}$), NRTI additions which delay the process so that the overall time exceeds the life span
of RNA (mechanism 2.I), NRTI additions which delay the overall process but not enough to exceed the life span of RNA (mechanism 2.II), and finally no NRTI addition. Although mechanism 2.II results in delaying the reverse transcription process, viral DNA will be produced and eventually integrated and transcribed to generate new virus particles. In other words, mechanism 2.II and no NRTI addition can be considered as “unsuccessful inhibition” cases and mechanism 1 and 2.II as “successful inhibition” cases. Consequently, the successful inhibition or the overall efficacy of stochastic simulation, $\epsilon_\text{si}\%$, is defined as the sum of mechanism 1 $\epsilon_\text{DC}\%$ and mechanism 2.I. In Table 4.5 and Table 4.6 summary of the simulations results are presented. $\epsilon_\text{si}\%$ is shown in the last column in Table 4.5, Table 4.6, and Table 4.7. Before explaining the presented results in Tables 4.5-4.7, first the difference between the calculated efficacy of AZT based on equation 4.16 and the actual outcome of the simulation which is presented in figure 4.7 is explained.

The efficacy (due to mechanism 1), $\epsilon_\text{DC}\%$, can be simply calculated as the percentage of dead-end terminated runs among the total number of runs. As it can be seen in figure 4.7 there is a difference between the two trajectories which is more significant at higher concentrations. The efficacy calculated based on stochastic simulation is more accurate because of considering the changes of concentration in time. In order to further investigate the reliability of the stochastic simulation, simulations with constant concentrations over time are also run and the results are in very good agreement with the outcome of equation 4.16.
Table 4.5 shows the results for AZT. The administration time is every eight hours, and two different half lives, 2 and 4 hours, are investigated. As mentioned before in section 4.4.4, a longer half life means higher $p$ and consequently higher efficacy. Efficacy also increases with drug concentration as expected.

In order to show the effect of drug administration time, $a_t$, as well a value for maximum concentration (here $\frac{C_{\text{max}}}{IC_{50}} = 2$) is considered and six cases are simulated: administration time of 4 and 8 hours; and half lives of 1, 2, and 4 hours. The results are shown in figure 4.8. It is interesting to note that when the half life is very short (comparing to $a_t$), $\epsilon_{st}$ is slightly larger than $\epsilon_{DC}$; however, when the half
Table 4.5. Effect of concentration and half life on AZT inhibitory effect for wild type virus. The overall efficacy $\epsilon_{si}$% in the last column is the sum of $\epsilon_{DC}$% and the percent of time delay exceeding life span cases. No NRTI addition percentage and time delay not exceeding life span percentage represent the failure of the NRTI to inhibit reverse transcription.

<table>
<thead>
<tr>
<th>$\frac{C_{\text{max}}}{IC_{50}}$</th>
<th>$h$</th>
<th>no NRTI addition %</th>
<th>dead-end formation ($\epsilon_{DC}$%)</th>
<th>time delay exceeding life span %</th>
<th>not exceeding life span %</th>
<th>$\epsilon_{si}$%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2</td>
<td>69.02</td>
<td>3.78</td>
<td>1.0E-4</td>
<td>27.20</td>
<td>3.78</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>61.98</td>
<td>5.05</td>
<td>4.0E-4</td>
<td>32.97</td>
<td>5.05</td>
</tr>
<tr>
<td>0.25</td>
<td>2</td>
<td>39.67</td>
<td>9.12</td>
<td>1.4E-3</td>
<td>51.21</td>
<td>9.12</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>30.25</td>
<td>11.96</td>
<td>4.3E-3</td>
<td>57.79</td>
<td>11.96</td>
</tr>
<tr>
<td>0.5</td>
<td>2</td>
<td>15.75</td>
<td>17.12</td>
<td>7.2E-3</td>
<td>67.12</td>
<td>17.13</td>
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<td></td>
<td>4</td>
<td>9.11</td>
<td>22.35</td>
<td>0.03</td>
<td>68.51</td>
<td>22.38</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2.50</td>
<td>30.20</td>
<td>0.08</td>
<td>67.22</td>
<td>30.28</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.82</td>
<td>38.89</td>
<td>0.31</td>
<td>59.98</td>
<td>39.20</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.06</td>
<td>48.66</td>
<td>0.83</td>
<td>50.45</td>
<td>49.49</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>7.8e-3</td>
<td>60.81</td>
<td>2.90</td>
<td>36.28</td>
<td>63.71</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1.0e-4</td>
<td>68.89</td>
<td>5.95</td>
<td>25.16</td>
<td>74.84</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0</td>
<td>81.70</td>
<td>10.19</td>
<td>8.11</td>
<td>91.89</td>
</tr>
</tbody>
</table>

life is larger, the difference between $\epsilon_{si}$ and $\epsilon_{DC}$ becomes significant.

Table 4.6 shows the results of simulations for 3TC, ddC, and d4T. In all of the presented simulations, the administration time is $a_t = 8$ hours. In order to compare the different drugs, let’s focus on a medication schedule based on $\frac{C_{\text{max}}}{IC_{50}} = 1$ (for AZT, $h = 2$ and $a_t = 8$ case in table 4.5 should be considered). It can be observed that ddC has the highest $\epsilon_{DC}$ percentage (mechanism 1), followed by 3TC and AZT, while d4T is last. However, although d4T has the lowest TDEC percentage, it also has the highest probability of exceeding the RNA life span (mechanism 2.I). Thus, if $\epsilon_{si}$% is analyzed, the most beneficial drugs are ordered as d4T, ddC, 3TC,
Figure 4.8. $\varepsilon_{DC}$ and $\varepsilon_{si}$ of AZT as a function of administration time ($a_t = 4$ and $a_t = 8$) at different half lives ($h = 1$, $h = 2$, $h = 4$).

In practice, medication strategies rely on experimental values of $IC_{50}$ to determine drug dosage. However, as mentioned earlier, there is large variability in $IC_{50}$ values that are reported in the literature. To investigate the effect of $IC_{50}$ on $\varepsilon_{si}$, d4T is focused on. In the last three rows of table 4.6, results based on three different $IC_{50}$ values are shown. It is observable that the medication outcome, $\varepsilon_{si}$, is very sensitive to the choice of $IC_{50}$ since in the three simulations $C_{max} = IC_{50}$. Furthermore, increasing the drug dosage beyond a certain value, will not increase $\varepsilon_{si}$ significantly since it has reached saturation. These results exemplify the need
Table 4.6. Comparison of 3TC, ddC, and d4T inhibitory effects at different concentrations (half life of each drug reported in Table 4.4) for wild type virus.

<table>
<thead>
<tr>
<th>drug</th>
<th>$\frac{C_{max}}{IC_{50}}$</th>
<th>no NRTI addition %</th>
<th>dead-end formation ($\epsilon_{DC}$%)</th>
<th>time delay exceeding life span %</th>
<th>time delay not exceeding life span %</th>
<th>$\epsilon_{si}$ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3TC</td>
<td>0.1</td>
<td>84.33</td>
<td>9.42</td>
<td>0.15</td>
<td>6.10</td>
<td>9.57</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>42.55</td>
<td>38.87</td>
<td>0.73</td>
<td>17.85</td>
<td>39.60</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>18.06</td>
<td>62.20</td>
<td>1.22</td>
<td>18.52</td>
<td>63.42</td>
</tr>
<tr>
<td>ddC</td>
<td>0.1</td>
<td>58.76</td>
<td>29.79</td>
<td>0.13</td>
<td>11.32</td>
<td>29.92</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>7.04</td>
<td>80.85</td>
<td>0.47</td>
<td>11.64</td>
<td>81.32</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.49</td>
<td>95.05</td>
<td>0.56</td>
<td>3.90</td>
<td>95.61</td>
</tr>
<tr>
<td>d4T</td>
<td>1; $IC_{50} = 10$</td>
<td>27.59</td>
<td>0.45</td>
<td>69.40</td>
<td>2.56</td>
<td>69.85</td>
</tr>
<tr>
<td></td>
<td>1; $IC_{50} = 50$</td>
<td>0.16</td>
<td>1.31</td>
<td>98.03</td>
<td>0.50</td>
<td>99.34</td>
</tr>
<tr>
<td></td>
<td>1; $IC_{50} = 200$</td>
<td>0</td>
<td>2.64</td>
<td>97.35</td>
<td>1E-3</td>
<td>99.99</td>
</tr>
</tbody>
</table>

for accurate $IC_{50}$ data so that the optimum dosages which produce higher $\epsilon_{si}$ can be determined and obtain upper limits so that prescribing high dosages which marginally increase $\epsilon_{si}$ at the cost of toxic side-effects can be avoided.

The drug effectiveness is also very sensitive to the ratio of intracellular tri-phosphorylated concentration to the plasma concentration. Due to the unavailability of such quantitative data for ddC, in the results presented in 4.6 the same ratio as 3TC is used. To investigate the sensitivity of $\epsilon_{si}$ to this ratio for ddC, simulations for half of this value are performed and $\epsilon_{si}$ decreases to 14.13, 52.42, and 76.44 for $\frac{C_{max}}{IC_{50}}$ equal to 0.1, 0.5, and 1, respectively. This is a significant reduction in effectiveness which is more prevalent at low drug concentrations. Based on these results, again the need for accurate experiments that provide this ratio is emphasized.

Finally, it is also interesting to investigate the time distribution of reverse tran-
scription process under treatment and compare it with figure 4.6. It is important to note that the TDEC forming cases are not considered in time distribution graphs. No NRTI addition cases also are not included in order to see the effect of time delays more vividly. It is necessary to consider the TDEC and no NRTI addition percentages with the graphs to gain an overall insight of each drug. Data with \( \frac{C_{\text{max}}}{IC_{50}} = 0.5 \) are chosen to create figure 4.9. Comparing figure 4.9 with figure 4.6, it is apparent that the distributions are shifted to the right towards longer times. This is the effect of (single or multiple) NRTI inclusions into the chain and the time needed for their dissociation. It is important to note that the excision constant of 3TC and ddC are one order of magnitude smaller than AZT. In other words, it takes longer for 3TC and ddC to dissociate from the chain once included. As a result, 3TC and ddC distributions are “wider” and reach to around 800 minutes. d4T’s excision constant is the smallest of all, being two orders of magnitude smaller than that of AZT. Consequently, it has the widest distribution which reaches to more than 40,000 minutes and the peak of the distribution is 50 times smaller than the peak of the AZT distribution (the d4T distribution is not presented in figure 4.9 because of the very small peak and the significantly different range).
Figure 4.9. Distribution of time to complete reverse transcription process under treatment (only mechanisms 2.I and 2.II are considered). The dotted vertical line represents the life span of RNA in cytoplasm.

4.6.2 Mutation and emergence of resistant viral strains to NRTI

HIV has a significantly high rate of mutation which can lead to production of resistant genome to drugs. As Isel et al. has discussed in [99]: “In principle, the resistance mutations could act either by (i) decreasing the incorporation efficiency of the triphosphate form of NRTI or (ii) increasing the removal of the incorporated NRTI”. Obviously, quantitative data to simulate resistant genome is needed to calculate the efficacy. Fortunately, values of $K_m$ and $K_i$ are reported in [109] and
therefore, the relative affinity for K65R mutant is calculated based on equation 4.10 and used in the simulations. The affinity is smaller than the affinity of wild type (shown in table 4.3) and constant for all the simulated cases in table 4.7. Unfortunately, quantitative data to calculate $k_{ext}$ and $p_{TDEC}$ are not available. As a result, a sensitivity analysis for AZT, assuming $p_{TDEC}$ of the resistant RNA to be 25%, 50%, 75%, and 100% of the wild type is performed. Table 4.7 shows the result of the sensitivity analysis simulations for $h = 2$ and $a_t = 8$. In the second row, $p_{TDEC}$ is assumed to be similar to wild type and it can be seen that the probability of no NRTI addition has increased considerably from two and a half percent (table 4.5) to more than forty one percent which is due to smaller affinity. The effect of decreasing the $p_{TDEC}$ to 75, 50, and 25 percent of the initial value can be seen in third, fourth, and fifth rows, respectively. As expected, the probability of forming TDEC decreases accordingly. There is no significant change in the probability of no NRTI addition because the affinity is the same.

**Table 4.7.** Sensitivity analysis of AZT inhibitory effect on K65R mutant’s reverse transcription process with respect to $p_{TDEC}$.

<table>
<thead>
<tr>
<th>$p_{TDEC}$ [%]</th>
<th>$p_{TDEC}$ w.r.t. $p_{TDEC_{-wild}}$</th>
<th>no NRTI addition [%]</th>
<th>dead-end formation $\epsilon_{DC} [%]$</th>
<th>time delay exceeding life span [%]</th>
<th>not exceeding life span [%]</th>
<th>$\epsilon_{si} [%]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1</td>
<td>100</td>
<td>41.21</td>
<td>8.79</td>
<td>1.1e-3</td>
<td>49.99</td>
<td>8.79</td>
</tr>
<tr>
<td>6.07</td>
<td>75</td>
<td>41.44</td>
<td>6.70</td>
<td>2.0e-3</td>
<td>51.85</td>
<td>6.70</td>
</tr>
<tr>
<td>4.05</td>
<td>50</td>
<td>41.70</td>
<td>4.52</td>
<td>2.9e-3</td>
<td>53.77</td>
<td>4.52</td>
</tr>
<tr>
<td>2.02</td>
<td>25</td>
<td>41.91</td>
<td>2.26</td>
<td>1.8e-3</td>
<td>55.83</td>
<td>2.26</td>
</tr>
</tbody>
</table>
4.7 Challenges in model development

Stochastic simulation can be employed to investigate the mechanism of inhibition of the RT process. It facilitates the investigation of the formation of TDEC as well as time delay associated with NRTI inclusion. Advantages and disadvantages of different drugs can be easily compared and the treatment outcomes can be predicted as a function of drug concentration and also administration time. The developed model can be easily employed for other types of NRTIs and combination of NRTIs such as in HAART.

The most important challenge in widely employing this model is the lack of various necessary quantitative data for different drugs. The proposed model’s predictions can be updated once more accurate data are provided. Specifically, experimental studies to provide more accurate kinetics data such as $K_M$ and $K_i$ for different types of drugs can facilitate simulation and comparison of other drugs. It is also important to improve the experiments to quantify the stability and excision time of TDECs, $IC_{50}$, and intracellular phosphorylated concentrations of different NRTIs. Such in vitro experiments could be employed to decipher what will take place in vivo.

Due to the fact that mutation rate of HIV is quite high and resistance to treatments decreases the effect of treatment, it is also important to quantify the mentioned parameters of resistant strains. It is qualitatively known that mutation can lead to decrease in incorporation rate of NRTIs and also increase in the re-
moval of the incorporated NRTI. Currently, quantitative data which can be used in mathematical simulations are scarce and limited to a few types of currently used drugs.
HYBRID MULTISCALE MODEL
OF HIV DYNAMICS AND
CONTROLLING RESISTANCE
UNDER HAART

A known shortcoming of the extracellular models in general is that the values of
the parameters that describe the infection dynamics are obtained by curve fitting
sparsely available hospital data. To increase the reliability of the predictions,
developing more accurate models that consider infection dynamics at both extra
and intracellular levels is necessary. Multi-scale modeling platforms that span
several biological levels have been previously developed and employed to study cancerous tumor growth [113].

In this chapter, a hybrid model is created in a sequential manner. First, a stochastic intracellular model is developed. Then the extracellular model of chapter 2 is revised to incorporate intracellular dynamics. The developed model enhances the understanding of infection cycle and is useful in predicting the overall infection dynamics while considering the intracellular infection events. Specifically, the hybrid model is instrumental in correctly quantifying the effect of drug concentration on the progression of resistant populations.

5.1 Intracellular model development

There are fewer intracellular models which describe the dynamics of viral components inside the infected host in comparison to extracellular models of virus-host interactions. The reason is the fact that the interaction dynamics of intracellular components are very complicated and quantitative information from experimental studies is not available for all parts of the cycle. These obstacles make developing intracellular mathematical models of HIV very difficult. In [33], a model for the entire intracellular infection cycle is developed. However, the model does not have the necessary level of detail. In other works such as [114, 115, 116], more precise models have been proposed, yet for a specific part of the cycle. The model constructed here includes the complete intracellular cycle and is based on reported
physiological data collected through experiments.

The intracellular events are explained in sections 1.3 and 4.1. Based on the explained mechanisms, a stochastic model is developed that predicts the expected behavior of each infected cell as a function of time and the amount of available medication inside the cell. This molecular description of the events following the binding of the virus to the host cell provides the necessary information to develop a model of the intracellular HIV infection dynamics. In Figure 1.2 the infection cycle is shown and the intracellular events are discussed in section 1.3. Figure 5.1 shows the algorithm of the intracellular model. The model initiates when successful binding of the virus to the host occurs and the viral contents enter the host cytoplasm. The viral RNA is then reverse transcribed to create DNA. This process is explained in detail in chapter 4. The diamond labeled “reverse transcription” is actually a multi-step algorithm itself which is shown in figure 4.5. As it is explained in section 4.6, the probability of “successful inhibition” or $\epsilon_{si}$ can be computed for a specific drug with known dosage as the sum of “dead-end formation” probability and “delay exceeding RNA lifespan” probability. Moreover, the probability of “no NRTI addition” and “delay not exceeding RNA lifespan” are available and the time distribution is also accessible for the latter case. Hence, when the “reverse transcription” step is executed in the simulation, a uniform random number is generated to determine the fate of the RNA among the three possible outcomes: successful inhibition, no NRTI addition, and time delay. If the
first outcome is chosen, then the simulation ends in “Failure”. For the other two cases, the time needed to generate the complete genome is determined from the relevant time distributions (Figures 4.6 and 4.9). When no drug is prescribed, the “no NRTI addition” case is always selected.

**Figure 5.1.** Block diagram of the intracellular model

The produced DNA in cytoplasm is unstable and can be either degraded or
transferred to the nucleus. The rate of degradation and transfer are reported in [33] and [114]. Then, based on equation 2.13 and a generated uniform random number, it is decided if the DNA in cytoplasm is degraded (“failure”) or if it is transferred to the nucleus. Similarly, the fate of the DNA in the nucleus is determined which can be degradation (“failure”) or integration into the host genome. After integration, the provirus becomes stable and deterministic description can be used.

Once integrated, the provirus is replicated along with the host cell DNA. During transcription, the genetic information contained within the DNA is copied to RNA. Still in the nucleus, the HIV transcripts undergo a series of post-transcriptional splicing events. This results in the generation of over 20 different mRNAs that can be grouped into 3 size classes [18]: unspliced or full length viral RNA (9kb) for synthesis of gag and gag-pol polyproteins, singly spliced viral RNA (4kb) for synthesis of Vif, Vpr, Vpu, and Env proteins, and fully (or doubly) spliced viral RNA (2kb) which encodes the regulatory proteins such as Tat, Rev, and Nef. The viral RNAs are produced in a sequential manner. The full length RNA is the initial product of transcription which is then translated into singly spliced which itself is spliced to generate fully spliced RNA. Among all the viral proteins, a few have significant role in the transcription of other proteins and the formation of new virus particles. These proteins are Tat, Rev, and Gag which are explicitly included in the model.

Rev, the regulator of viral gene expression, binds to the Rev response element
(RRE). Rev’s function is to facilitate the export of full length and singly spliced viral RNA from the nucleus to the cytoplasm. RNA transcripts that are not fully spliced are generally retained inside the nucleus and only fully spliced RNA can be exported from nucleus to the cytoplasm where it is synthesized to Rev and Tat. Rev can be imported back to the nucleus where several copies of it associate on the RRE with the nuclear export protein. This association makes the export of unspliced and singly spliced RNAs possible. Rev levels are regulated by a feedback mechanism. The high level of Rev means high export levels of unspliced and singly spliced RNA. This means that the amount of RNA left for fully splicing is reduced and Rev expression is subsequently decreased.

Tat, the transactivator of transcription, is essential for HIV-1 replication. Tat binds to a short-stem loop structure, the transactivation response element (TAR). Binding of Tat to TAR activates transcription of HIV up to a thousand fold. Tat promotes completion of initiated transcriptional activity. In the absence of Tat only short transcripts (less than 100 nucleotides) are produced [18]. Figure 5.2 shows unspliced, singly spliced, fully spliced RNAs and regulatory proteins Rev and Tat, and structural proteins such as Gag.

The full length RNA in the nucleus is translated to several polyproteins, among them is Gag, the structural protein of HIV. Gag alone can form virus like particles [117]. It is determined that an average HIV particle contains $\sim 5000$ Gag proteins [118]. Other proteins as well as two copies of viral genome are also packed in the
particle. Another important role of Gag is mediating interactions between Gag itself and the viral genome. In other words, Gag “recruits” viral RNA during assembly process [2]. Once the particle is formed, the new virus has to undergo a maturation step to become infectious. The protease enzyme becomes active and cleaves Gag and other polyproteins into several smaller proteins.

In [114], an intracellular model in the form of ODEs is proposed which focuses on the events after integration of provirus and describes the production and dynamics of full length, singly spliced, and fully spliced RNAs, Rev-bounded RNAs, and regulatory proteins Rev and Tat, in both nucleus and cytoplasm. This model is used in [119] where Gag is also considered in the model which makes the es-
imation of virion production possible. An ODE model is constructed based on these two models. The equations and some details are provided in Appendix A. This model represents the “Transcription, Translation, Budding” block in figure 5.1. Figure 5.3 shows the number of produced virions after the integration of viral DNA.

![Graph showing number of produced virions as a function of post integration time](image)

**Figure 5.3.** Number of produced virions as a function of post integration time

The life span of an infected cell is assumed to be two days [31]. The time needed for pre-integration steps should be considered along with Figure 5.3 to compute the number of produced virions. Figure 5.4 shows the distribution of pre-integration time for cases not ended in “failure”. The mean of the distribution is
12.04 hours with standard deviation of 0.09.

![Distribution of pre-integration events time](image)

**Figure 5.4.** Distribution of pre-integration events time

For each case which successfully reaches the “Transcription, Translation, Budding” step, the pre-integration time is deducted from 48 hours (“available” time for virion production) to calculate the remaining time for post-integration events before the infected cells is cleared. Then, the corresponding number of virions is identified from Figure 5.3. The average number of produced virions is 313 in two days with standard deviation of 1.6. In other words, the full cycle completion time is 48 hours for all cases, but the number of virions varies for each run.

Since the infection has the potential to fail at three steps (the pink diamond
blocks in Figure 5.1) during the cycle, the success rate or probability can be determined through a large number of simulations: the success rate will be the number of simulations concluded at “success” block divided by the total number of simulations. Hence, the outcome of the intracellular model is two important parameters: success rate and the rate of virion production. These parameters depend on the chosen treatment (types of drugs, dosage, etc) and are inputs to the multiscale model. The results shown in Figure 5.4 and the calculated value of 313 virions in two days are based on one million simulations with no treatment considered. In section 5.2 the incorporation of these two parameters in the model is explained.

The success rate and the rate of virion production are reported in Table 5.1 for no treatment and also under treatment cases. These values depend on drug dosage which is shown by $x = \frac{C_{\text{max}}}{IC_{50}}$. Success rate of the intracellular cycle initiating by virus attachment and concluding by virion production is shown by $\epsilon_c$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>success rate %</th>
<th>number of virions</th>
<th>production rate n (virions per day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No drug</td>
<td>51.18</td>
<td>313</td>
<td>156</td>
</tr>
<tr>
<td>AZT, x=1</td>
<td>30.97</td>
<td>234</td>
<td>117</td>
</tr>
<tr>
<td>3TC, x=0.5</td>
<td>30.82</td>
<td>292</td>
<td>146</td>
</tr>
<tr>
<td>3TC, x=1</td>
<td>18.56</td>
<td>271</td>
<td>135</td>
</tr>
<tr>
<td>AZT, x=1 &amp; 3TC, x=0.5</td>
<td>22.06</td>
<td>233</td>
<td>116</td>
</tr>
<tr>
<td>AZT, x=1 &amp; 3TC, x=1</td>
<td>13.10</td>
<td>223</td>
<td>113</td>
</tr>
</tbody>
</table>

The reason that AZT and 3TC are chosen is the fact that they are part of a three drug HAART regimen Zidovudine (AZT), Lamivudine (3TC), and efavirenz (a PI drug) which is commonly prescribed for patients [120, 121]. The effect of PI is
seen in the multiscale model (section 5.2) since the intracellular model defined here concludes at the virion production step and PI acts after the particle is formed.

5.2 Multiscale model of infection

The extracellular model of chapter 2 is based on other proposed models for HIV infection such as [28, 59]. The drawback of these widely used models is the phenomenological parameters which are determined by fitting available data to the proposed model. Including treatment efficacy, commonly shown by $\eta$, is also inaccurate and its relationship with drug dosage and dosing interval is not clearly stated.

The model proposed in this section tries to circumvent this problems by incorporating the effect of treatment more accurately and also employing physiologically meaningful parameters whenever possible.

\[
\frac{dT}{dt} = \lambda - \mu T - k_b T V_{ex} + k_{dis} T_b + k_{endo} T_b + (1 - \epsilon_c) T_f \quad (5.1)
\]

\[
\frac{dV_{ex}}{dt} = -k_b T V_{ex} - \gamma V_{ex} + n \epsilon_c T_f + k_{dis} T_b \quad (5.2)
\]

\[
\frac{dT_b}{dt} = k_b T V_{ex} - \mu T_b - k_{endo} T_b - k_{dis} T_b - k_y T_b \quad (5.3)
\]

\[
\frac{dT_f}{dt} = k_y T_b - a T_f - (1 - \epsilon_c) T_f \quad (5.4)
\]

The model variables are $T$, defining healthy T-cell, $V_{ex}$, free virus, $T_b$, virus-T-
cell complex, and $T_f$, which is the T-cell containing fused virus. The infection rate $k$ between T-cells and viruses which is used in equation 2.2 is a coarse parameter not reflective of the events happening inside the infected T-cell. Hence, $k_b$ the binding rate between virus and host, is employed here. $k_b$ represents the binding rate between CD4 receptors on the surface of the host and the virus [122, 115]. This attachment is not enough for fusion. In fact, the virus may dissociate from the $T_b$ complex by the rate of $k_{dis}$, it may be endocytosed by cellular traps and degrades [116] with the rate of $k_{endo}$, or it may form a second bound with coreceptor as shown in figure 5.5 with the rate of $k_y$. Binding to coreceptors is necessary for fusion of viral content. The outcome of viral content fusion into the host is formation of $T_f$. However, as it is discussed in section 5.1, formation of $T_f$ does not always result in production of virions. The parameters $\epsilon_c$ and $n$ from the intracellular model are used here. These values can be easily changed during simulation based on the treatment regimen under investigation.

In this chapter, the proposed model is used to study the evolution of resistance virus strands under HAART for the latency stage of infection when the infection is established. One milliliter of blood is considered for simulation. The infection establishment steady state of the model without treatment is $T_{ss} \simeq 500$ per $1mm^3$ of blood and $V_{ex_{ss}} \simeq 3.2 \times 10^5$ per $1mL$ of blood.

The HAART regimen with AZT, 3TC, and efavirenz (a PI drug) is chosen for this study. Unlike the early stage of the disease, the probability of existence
of resistance strain to multiple drugs is considerable for the established infection. Since there are three drugs, eight virus populations should be considered. In Table 5.2 the resistance (R) or vulnerability (V) of the viral populations to drugs are shown. \( p_1, p_2, \) and \( p_3 \) are the probability of resistance to 3TC, AZT, and efavirenz, respectively. They are calculated based on pharmaceutical data as explained in

**Table 5.2.** Virus populations and their resistance (denoted by R) or vulnerability (presented by V) to 3TC, AZT, and efavirenz

<table>
<thead>
<tr>
<th>population index</th>
<th>drug</th>
<th>index</th>
<th>probability of production ( (q_i) )</th>
<th>virus reduction coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>V</td>
<td>V</td>
<td>((1 - p_1)(1 - p_2)(1 - p_3))</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>R</td>
<td>V</td>
<td>( p_1(1 - p_2)(1 - p_3) )</td>
<td>( r_1 )</td>
</tr>
<tr>
<td>3</td>
<td>V</td>
<td>R</td>
<td>((1 - p_1)p_2(1 - p_3))</td>
<td>( r_1 )</td>
</tr>
<tr>
<td>4</td>
<td>R</td>
<td>R</td>
<td>( p_1p_2(1 - p_3) )</td>
<td>( r_2 )</td>
</tr>
<tr>
<td>5</td>
<td>V</td>
<td>V</td>
<td>((1 - p_1)(1 - p_2)p_3)</td>
<td>( r_1 )</td>
</tr>
<tr>
<td>6</td>
<td>R</td>
<td>V</td>
<td>( p_1(1 - p_2)p_3 )</td>
<td>( r_2 )</td>
</tr>
<tr>
<td>7</td>
<td>V</td>
<td>R</td>
<td>( (1 - p_1)p_2p_3 )</td>
<td>( r_2 )</td>
</tr>
<tr>
<td>8</td>
<td>R</td>
<td>R</td>
<td>( p_1p_2p_3 )</td>
<td>( r_3 )</td>
</tr>
</tbody>
</table>
section 2.1.2. The other factor which should be considered is the fact that mutation causes reduction in virus fitness [18]. Hence, a coefficient is commonly considered to show reduction in the number of produced virions [28]. The virus populations 2, 3, and 5 have only one resistance causing mutation and as a result, $r_1 = 1$ is considered for them. Populations 4, 6, and 7 have two resistance causing mutations, consequently $r_2 = 0.9$ is assumed for them. Finally, $r_3 = 0.8$ is used for population 8 with three mutations. The model of equations 5.1-5.4 is revised to include all the virus populations and respective $T_b$ and $T_f$:

$$\frac{dT}{dt} = \lambda - \mu T$$

$$-k_bTV_1 + k_{dis}T_{b,1} + k_{endo}T_{b,1} + (1 - \epsilon_{c,1})T_{f,1}$$

$$-k_bTV_2 + k_{dis}T_{b,2} + k_{endo}T_{b,2} + (1 - \epsilon_{c,2})T_{f,2}$$

$$-k_bTV_3 + k_{dis}T_{b,3} + k_{endo}T_{b,3} + (1 - \epsilon_{c,3})T_{f,3}$$

$$-k_bTV_4 + k_{dis}T_{b,4} + k_{endo}T_{b,4} + (1 - \epsilon_{c,4})T_{f,4}$$

$$-k_bTV_5 + k_{dis}T_{b,5} + k_{endo}T_{b,5} + (1 - \epsilon_{c,5})T_{f,5}$$

$$-k_bTV_6 + k_{dis}T_{b,6} + k_{endo}T_{b,6} + (1 - \epsilon_{c,6})T_{f,6}$$

$$-k_bTV_7 + k_{dis}T_{b,7} + k_{endo}T_{b,7} + (1 - \epsilon_{c,7})T_{f,7}$$

$$-k_bTV_8 + k_{dis}T_{b,8} + k_{endo}T_{b,8} + (1 - \epsilon_{c,8})T_{f,8}$$

$$\frac{dV_1}{dt} = -k_bTV_1 - \gamma V_1 + k_{dis}T_{b,1} + q_1(1 - \eta_P)n_1\epsilon_{c,1}T_{f,1}$$

(5.5)

(5.6)
\[
\begin{align*}
\frac{dT_{b,1}}{dt} &= k_b TV_1 - \mu T_{b,1} - k_{\text{endo}} T_{b,1} - k_{\text{dia}} T_{b,1} - k_y T_{b,1} \\
\frac{dT_{f,1}}{dt} &= k_y T_{b,1} - a T_{f,1} - (1 - \epsilon_{c,1})T_{f,1} \\
\frac{dV_2}{dt} &= -k_b TV_2 - \gamma V_2 + k_{\text{dia}} T_{b,2} + r_1 q_{22}(1 - \eta_P)n_2 \epsilon_{c,2} T_{f,2} \\
&\quad + r_1 q_2(1 - \eta_P)n_1 \epsilon_{c,1} T_{f,1} \\
\frac{dT_{b,2}}{dt} &= k_b TV_2 - \mu T_{b,2} - k_{\text{endo}} T_{b,2} - k_{\text{dia}} T_{b,2} - k_y T_{b,2} \\
\frac{dT_{f,2}}{dt} &= k_y T_{b,2} - a T_{f,2} - (1 - \epsilon_{c,2})T_{f,2} \\
\frac{dV_3}{dt} &= -k_b TV_3 - \gamma V_3 + k_{\text{dia}} T_{b,3} + r_1 q_{33}(1 - \eta_P)n_3 \epsilon_{c,3} T_{f,3} \\
&\quad + r_1 q_3(1 - \eta_P)n_1 \epsilon_{c,1} T_{f,1} \\
\frac{dT_{b,3}}{dt} &= k_b TV_3 - \mu T_{b,3} - k_{\text{endo}} T_{b,3} - k_{\text{dia}} T_{b,3} - k_y T_{b,3} \\
\frac{dT_{f,3}}{dt} &= k_y T_{b,3} - a T_{f,3} - (1 - \epsilon_{c,3})T_{f,3} \\
\frac{dV_4}{dt} &= -k_b TV_4 - \gamma V_4 + k_{\text{dia}} T_{b,4} + r_2 q_{44}(1 - \eta_P)n_4 \epsilon_{c,4} T_{f,4} \\
&\quad + r_2 q_4(1 - \eta_P)n_1 \epsilon_{c,1} T_{f,1} + r_2 q_{24}(1 - \eta_P)n_2 \epsilon_{c,2} T_{f,2} \\
&\quad + r_2 q_{34}(1 - \eta_P)n_2 \epsilon_{c,2} T_{f,3} \\
\frac{dT_{b,4}}{dt} &= k_b TV_4 - \mu T_{b,4} - k_{\text{endo}} T_{b,4} - k_{\text{dia}} T_{b,4} - k_y T_{b,4} \\
\frac{dT_{f,4}}{dt} &= k_y T_{b,4} - a T_{f,4} - (1 - \epsilon_{c,4})T_{f,4} \\
\frac{dV_5}{dt} &= -k_b TV_5 - \gamma V_5 + k_{\text{dia}} T_{b,5} + r_1 q_{55}n_5 \epsilon_{c,5} T_{f,5} \\
&\quad + r_1 q_5n_1 \epsilon_{c,1} T_{f,1} \\
\frac{dT_{b,5}}{dt} &= k_b TV_5 - \mu T_{b,5} - k_{\text{endo}} T_{b,5} - k_{\text{dia}} T_{b,5} - k_y T_{b,5} \\
\frac{dT_{f,5}}{dt} &= k_y T_{b,5} - a T_{f,5} - (1 - \epsilon_{c,5})T_{f,5}
\end{align*}
\]
\[
\frac{dV_6}{dt} = -k_bTV_6 - \gamma V_6 + k_{dis}T_{b,6} + r_2q_6n_6\epsilon_{c,6}T_{f,6} + r_2q_6n_1\epsilon_{c,1}T_{f,1} + r_2q_2(1 - \eta_P)n_2\epsilon_{c,1}T_{f,2} + r_2q_5n_5\epsilon_{c,5}T_{f,5}
\]

\[
\frac{dT_{b,6}}{dt} = k_bTV_6 - \mu T_{b,6} - k_{endo}T_{b,6} - k_{dis}T_{b,6} - k_yT_{b,6}
\]

\[
\frac{dT_{f,6}}{dt} = k_yT_{b,6} - aT_{f,6} + (1 - \epsilon_{c,6})T_{f,6}
\]

\[
\frac{dV_7}{dt} = -k_bTV_7 - \gamma V_7 + k_{dis}T_{b,7} + r_2q_7n_7\epsilon_{c,7}T_{f,7} + r_2q_7n_1\epsilon_{c,1}T_{f,1} + r_2q_5n_5\epsilon_{c,5}T_{f,5}
\]

\[
\frac{dT_{b,7}}{dt} = k_bTV_7 - \mu T_{b,7} - k_{endo}T_{b,7} - k_{dis}T_{b,7} - k_yT_{b,7}
\]

\[
\frac{dT_{f,7}}{dt} = k_yT_{b,7} - aT_{f,7} + (1 - \epsilon_{c,7})T_{f,7}
\]

\[
\frac{dV_8}{dt} = -k_bTV_8 - \gamma V_8 + k_{dis}T_{b,8} + r_3n_8\epsilon_{c,8}T_{f,8} + r_3q_8n_1\epsilon_{c,1}T_{f,1} + r_3q_8(1 - \eta_P)n_2\epsilon_{c,2}T_{f,2} + r_3q_8(1 - \eta_P)n_3\epsilon_{c,3}T_{f,3} + r_3q_8(1 - \eta_P)n_4\epsilon_{c,4}T_{f,4} + r_3q_8n_5\epsilon_{c,5}T_{f,5} + r_3q_8n_6\epsilon_{c,6}T_{f,6} + r_3q_8n_7\epsilon_{c,7}T_{f,7}
\]

\[
\frac{dT_{b,8}}{dt} = k_bTV_8 - \mu T_{b,8} - k_{endo}T_{b,8} - k_{dis}T_{b,8} - k_yT_{b,8}
\]

\[
\frac{dT_{f,8}}{dt} = k_yT_{b,8} - aT_{f,8} + (1 - \epsilon_{c,8})T_{f,8}
\]

At the steady state without any treatment, the viral population mostly consists of the wild population, \(V_1\), which is approximately equal to \(3.2 \times 10^5\). Figure 5.6 shows the trajectories of the eight viral populations. The figure is in log scale to show the difference between viral populations. It can be seen that populations
resistant to one drug exist in the order of $10^3$, populations resistant to two drugs exist in the order of $10^1$ and the all resistant population is negligible. The various mutant species are known as quasi-species as discussed in section 1.4.1.

Figure 5.6. Viral populations trajectories without treatment

When HAART starts after one year, the wild population $V_1$ and other populations which are vulnerable to at least one drug start to decrease rapidly. When populations $V_1 - V_7$ become very small, $V_8$ which is resistant to all three drugs but has the lowest fitness gains the opportunity to grow. Figure 5.7 shows the total viral load (sum of $V_1$ to $V_8$) and the all-resistant population $V_8$. It can be observed that populations $V_1$ to $V_7$ become extinct in a few months and $V_8$ starts
to grow. Although it takes a considerable time (more than three years) for $V_8$ until it reaches detectable levels, the resistant population will eventually establish in the patient’s body. The appearance of resistance strains is one of the most important concerns in HAART therapy [15].

Figure 5.7. Total viral load and $V_8$ trajectories after start of HAART

### 5.2.1 Structured HAART schedule

The appearance of the all-resistant population is the result of extinction of other populations. Interrupted therapy has been studied before [39] where treatment stops completely for some time and starts again. There are different perceptions
The positive or destructive effect of interrupted HAART. While in [25] it is concluded that therapy interruptions had no benefit and was even harmful, in another study [26] it is reported that patients in the scheduled treatment interruption group reached a lower viral load and experienced less frequent side effects than the patients in the continuous treatment group.

In this section three different scheduled interruptions are compared. Figure 5.8 shows the results of the simulations. In schedule 1, all three drugs are administrated for one week followed by one week of drug holiday and this order is repeated. The outcome of schedule 1 is shown by dashed red line in Figure 5.8. It can be observed that the evolution of total viral load under schedule 1 is very close to regular HAART. The reason is that the amount of drug is still high and can successfully suppress other populations other than the all-resistant population similar to regular HAART. Hence, the growth of total viral load is similar to regular HAART. This result inspired the investigation of a schedule with longer drug holiday periods. Schedule 2 has one week of treatment followed by four weeks of drug holiday which is repeated afterwards. Schedule 2 is shown by green line and it can be seen that long drug holidays prevent populations $V_1 - V_7$ to become extinct. These populations are more “fit” and reproduce faster. As a result, the total viral load reaches the detection limit more than one year earlier than regular HAART and schedule 1. Although less amount of drug is used in schedule 2 which is desirable, the overall progress of the disease is faster. Consequently, schedule 3 is proposed
where treatment period is always one week, but the holiday period starts at one week and is increased gradually to a maximum of six weeks. This order is repeated, but after a few repetitions, the shortest holiday period is increased to two weeks and the longest holiday period is reduced to four weeks. The evolution of virus population under this schedule is shown by blue line in Figure 5.8. It is observed that under this schedule, populations $V_1 - V_7$ survive for a few years and although the viral load is higher than regular HAART and schedule 1, it is still under the detection limit. After about two and a half years, only the all-resistant population survives and evolves similarly to regular HAART. The population reaches the detection limit less than two months earlier than regular HAART.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig5.8}
\caption{Total viral load under regular HAART and scheduled HAART}
\end{figure}
Finally, it is interesting to compare the percentage of days under treatment during the investigated horizon. The less number of days under treatment means the less amount of drug in patient’s body which potentially causes fewer side effects [26]. Hence, the lower percentage of days under treatment can be considered better for patients. For regular HAART, this percentage is 100%. Schedule 2 has treatment days percentage of 50%. For schedule 2 and 3, this percentage is equal to 20% and 23.6%, respectively. Although schedule 2 has the lowest percentage, the infection evolution is significantly faster under this regimen. Consequently, schedule 3 is the most successful among the three investigated regimens. It utilizes $\simeq 76\%$ less amount of drug compared to regular HAART and still the infection progress is quit similar to regular HAART. Table 5.3 shows the summary of results.

<table>
<thead>
<tr>
<th>treatment schedule</th>
<th>treatment On-Off time to reach viral detection limit</th>
<th>% of “on” days during treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAART</td>
<td>always on</td>
<td>3 y* &amp; 48 d**</td>
</tr>
<tr>
<td>schedule 1</td>
<td>1 w on - 1 w off</td>
<td>3 y* &amp; 42 d**</td>
</tr>
<tr>
<td>schedule 2</td>
<td>1 w on - 4 w off</td>
<td>2 y* &amp; 15 d**</td>
</tr>
<tr>
<td>schedule 3</td>
<td>variable</td>
<td>3 y*</td>
</tr>
</tbody>
</table>

### 5.3 Conclusion

In this section the focus is on investigating the evolution of various viral populations under HAART. The model proposed here is a first step towards developing
more accurate description of HIV dynamics which considers resistant virus populations and computes the effect of drugs on the evolution of these populations. These types of models can help researchers test various hypothesis and predict the outcome of different approaches for treating HIV. Here, three hypothetical interrupted HAART schedules are studied and it is shown that there is a considerable difference between the results. Designing experiments to measure relevant parameters and also comparing the model predictions with data from patients under HAART can help refining the proposed model.
CONCLUSION AND FUTURE DIRECTION

The objective of this research is developing models of HIV infection dynamics to study infection evolution and treatment effects on disease progression. While there are numerous models of HIV dynamics in literature, the models presented here are focused on aspects of the process not investigated before in mathematical analyses.

In chapter 2 it is shown that stochastic simulation is the proper tool for studying the early stage of HIV infection where random fluctuations are important and infection eradication is possible. In addition to the commonly considered wild virus population, the proposed extracellular model includes two distinct populations of virus, one resistant to PI and one resistant to RTI. The probability of infection
establishment for different initial viral loads is computed. The model is subse-
quently employed in chapter 3 in an optimization problem. The objective of the
optimization algorithm is to simultaneously minimize the probability of infection
establishment and the amount of drug. The results show that the common treat-
ment protocol (constant high amount of drug) is not the most beneficial option
and the optimized schedule is capable of reducing the amount of drug and/or the
probability of infection establishment.

One of the most important shortcomings of HIV models is that the effect of
treatment is commonly considered by the term efficacy. Efficacy is an increasing
function of drug amount, but the exact relationship is not clearly stated and the
few available studies on estimation of efficacy are empirical models. Consequently,
a mechanistic model is constructed in chapter 4 to estimate the efficacy of NRTI
drugs based reverse transcription process and dosage, dosing interval, and other
characteristics such as half life of the drug. This is the first attempt in devel-
op ing mechanistic models to compute HIV drugs efficacy from drug measurable
quantities.

The development of a multiscale model is the ultimate goal of the study which
aligns individual parts of this research. This goal is achieved by initially building
a stochastic intracellular model. The efficacy model of section 4 is part of this
model where the fate of an infected cell is determined based on success or failure
of intracellular events and the medication schedule.
This model is used to revise and extend the applicability of the extracellular model of chapter 2. Instead of two resistant populations, eight virus populations are then considered to account for resistant strains to multiple drugs. The developed multiscale model is useful in predicting the overall infection dynamics and evolution of resistant virus populations under regular HAART and hypothetical interrupted HAART.

6.1 Future direction

**Model refinement and collaborative research:** The predicted results of this study depend on the accuracy of the developed models. In this study, it is tried to propose models which are as accurate as possible and reflective of the relevant biological processes. Hence, the extracellular model is built based on other models such as the model in [28] which has been validated by experimental data. Then, parameters from published experimental studies are used to extend the models. In spite of these efforts to employ accurate data, there are still biological processes which have been described merely qualitatively. Collaborative research can help circumvent this problem to a large extent. Mathematical analysis can mutually help experimentalists to design more efficient experiments, discover the crucial parameters of a system, evaluate and compare drugs, and find the optimal administration regimes of drugs. In return, the developed models can be evaluated by the experiments’ data and refined to produce more accurate predictions.
HIV treatment is improving constantly and new drugs are synthesized and tested everyday. Fusion inhibitors are becoming part of HAART and other classes of drugs are under way for commercial use. Hence, development of models similar to the model of chapter 4 for estimation of efficacy of other classes of drugs is going to be a necessity. Ultimately, the synergic effect of various drugs should be evaluated to help designing more efficient treatment protocols for patients.

**Optimized HAART schedule:** The preliminary results of chapter 5 are promising in designing more efficient HAART protocols. Therefore, the multiscale model of HIV infection can be used in an optimization problem where the goal is to keep the viral load under a determined threshold as long as possible, cause the largest possible delay in emergence of multi drug resistant population, and employ the least amount of drugs to reduce side effects. In order to achieve this aim, several pre steps are necessary to be taken. For instance, methods to quantify drug toxicity in a way which is suitable for use in mathematical models should be created. Medical data of patients under regular and interrupted HAART should be gathered and studied to identify the probability of appearance of opportunistic infections if the viral load becomes large for a short period of time. Enhancing the current understanding of infection cycle to further refine the developed models is also crucial in achieving the overall goal. This will not be possible without effective collaboration between medical society, experimentalist and pharmaceutical industry, and the mathematical and optimization experts.
APPENDIX

A

INTRACELLULAR ODES

\[
\begin{align*}
\frac{dF_N}{dt} &= (TCb + TCaddKTatT_N/(1 + KTatT_N))PV \\
&\quad + kd1F_N^1 - (kspF + kdegNRNA + ka1R_N)F_N \\
\frac{dS_N}{dt} &= kspFF_N + kd1SR_N^1 - (kspS + kdegNRNA + ka1R_N)S_N \\
\frac{dM_N}{dt} &= kspSS_N + (1 - dS1)kspSSR_N^1 + (1 - dS2)kspSSR_N^2 \\
&\quad + (1 - dS3)kspSSR_N^3 + (1 - dS4)kspSSR_N^4 \\
&\quad + (1 - dS5)kspSSR_N^5 + (1 - dS6)kspSSR_N^6 \\
&\quad + (1 - dS7)kspSSR_N^7 + (1 - dS8)kspSSR_N^8 \\
&\quad + (1 - dS9)kspSSR_N^9 + (1 - dS10)kspSSR_N^{10}
\end{align*}
\]
\[(1 - dS11)kspSSR_{N}^{11} + (1 - dS12)kspSSR_{N}^{12} - (kexpM + kdegNRNA)M_{N}\]
\[
dF_{C}/dt = kexpF1FR_{N}^{1} + kexpF2FR_{N}^{2} + kexpF3FR_{N}^{3} + kexpF4FR_{N}^{4} + kexpF5FR_{N}^{5} + kexpF6FR_{N}^{6} + kexpF7FR_{N}^{7} + kexpF8FR_{N}^{8} + kexpF9FR_{N}^{9} + kexpF10FR_{N}^{10} + kexpF11FR_{N}^{11} + kexpF12FR_{N}^{12} - kdegCRNAF_{C}\]
\[
dS_{C}/dt = kexpS1SR_{N}^{1} + kexpS2SR_{N}^{2} + kexpS3SR_{N}^{3} + kexpS4SR_{N}^{4} + kexpS5SR_{N}^{5} + kexpS6SR_{N}^{6} + kexpS7SR_{N}^{7} + kexpS8SR_{N}^{8} + kexpS9SR_{N}^{9} + kexpS10SR_{N}^{10} + kexpS11SR_{N}^{11} + kexpS12SR_{N}^{12} - kdegCRNAS_{C}\]
\[
dM_{C}/dt = kexpMM_{N} - kdegCRNAM_{C}\]
\[
dFR_{N}^{1}/dt = ka1R_{N}F_{N} + kd2FR_{N}^{2} - (kd1 + ka2R_{N} + kexpF1 + (1 - dF1)kspF + kdegNRNA)FR_{N}^{1}\]
\[
dFR_{N}^{2}/dt = ka2R_{N}FR_{N}^{1} + kd3FR_{N}^{3} - (kd2 + ka3R_{N} + kexpF2 + (1 - dF2)kspF + kdegNRNA)FR_{N}^{2}\]
\[
dFR_{N}^{3}/dt = ka3R_{N}FR_{N}^{2} + kdAFR_{N}^{4} - (kd3 + ka4R_{N} + kexpF3 + (1 - dF3)kspF + kdegNRNA)FR_{N}^{3}\]
\[
\begin{align*}
\frac{dFR_1^4}{dt} &= ka_4R_NFR_1^3 + kd_5FR_1^5 - (kd_4 + ka_5R_N + kexpF4 + (1 - dF4)kspF + kdegNRNA)FR_1^4 \\
\frac{dFR_1^5}{dt} &= ka_5R_NFR_1^4 + kd_6FR_1^6 - (kd_5 + ka_6R_N + kexpF5 + (1 - dF5)kspF + kdegNRNA)FR_1^5 \\
\frac{dFR_1^6}{dt} &= ka_6R_NFR_1^5 + kd_7FR_1^7 - (kd_6 + ka_7R_N + kexpF6 + (1 - dF6)kspF + kdegNRNA)FR_1^6 \\
\frac{dFR_1^7}{dt} &= ka_7R_NFR_1^6 + kd_8FR_1^8 - (kd_7 + ka_8R_N + kexpF7 + (1 - dF7)kspF + kdegNRNA)FR_1^7 \\
\frac{dFR_1^8}{dt} &= ka_8R_NFR_1^7 + kd_9FR_1^9 - (kd_8 + ka_9R_N + kexpF8 + (1 - dF8)kspF + kdegNRNA)FR_1^8 \\
\frac{dFR_1^9}{dt} &= ka_9R_NFR_1^8 + kd_{10}FR_1^{10} - (kd_9 + ka_{10}R_N + kexpF9 + (1 - dF9)kspF + kdegNRNA)FR_1^9 \\
\frac{dFR_1^{10}}{dt} &= ka_{10}R_NFR_1^9 + kd_{11}FR_1^{11} - (kd_{10} + ka_{11}R_N + kexpF10 + (1 - dF10)kspF + kdegNRNA)FR_1^{10} \\
\frac{dFR_1^{11}}{dt} &= ka_{11}R_NFR_1^{10} + kd_{12}FR_1^{12} - (kd_{11} + ka_{12}R_N + kexpF11 + (1 - dF11)kspF + kdegNRNA)FR_1^{11} \\
\frac{dFR_1^{12}}{dt} &= ka_{12}R_NFR_1^{11} - (kd_{12} + kexpF12 + (1 - dF12)kspF + kdegNRNA)FR_1^{12} \\
\frac{dSR_1^3}{dt} &= ka_1R_NS + kd_2SR_1^2 + (1 - dF1)kspFFR_1^3
\end{align*}
\]
\[-(kd_1 + ka_2 R_N + kexp S_1 + (1 - dS_1) ksp S + kdeg NRNA)\]

\[SR^1_N\]

\[dSR^2_N/dt = ka_2 R_N SR^1_N + kd_3 SR^3_N + (1 - dF_2) ksp F FR^2_N\]

\[-(kd_2 + ka_3 R_N + kexp S_2 + (1 - dS_2) ksp S + kdeg NRNA)\]

\[SR^2_N\]

\[dSR^3_N/dt = ka_3 R_N SR^2_N + kd_4 SR^4_N + (1 - dF_3) ksp F FR^3_N\]

\[-(kd_3 + ka_4 R_N + kexp S_3 + (1 - dS_3) ksp S + kdeg NRNA)\]

\[SR^3_N\]

\[dSR^4_N/dt = ka_4 R_N SR^3_N + kd_5 SR^5_N + (1 - dF_4) ksp F FR^4_N\]

\[-(kd_4 + ka_5 R_N + kexp S_4 + (1 - dS_4) ksp S + kdeg NRNA)\]

\[SR^4_N\]

\[dSR^5_N/dt = ka_5 R_N SR^4_N + kd_6 SR^6_N + (1 - dF_5) ksp F FR^5_N\]

\[-(kd_5 + ka_6 R_N + kexp S_5 + (1 - dS_5) ksp S + kdeg NRNA)\]

\[SR^5_N\]

\[dSR^6_N/dt = ka_6 R_N SR^5_N + kd_7 SR^7_N + (1 - dF_6) ksp F FR^6_N\]

\[-(kd_6 + ka_7 R_N + kexp S_6 + (1 - dS_6) ksp S + kdeg NRNA)\]

\[SR^6_N\]

\[dSR^7_N/dt = ka_7 R_N SR^6_N + kd_8 SR^8_N + (1 - dF_7) ksp F FR^7_N\]

\[-(kd_7 + ka_8 R_N + kexp S_7 + (1 - dS_7) ksp S + kdeg NRNA)\]
\[ SR_N^7 \]

\[ \frac{dSR_N^8}{dt} = ka8RN bulk + kd9SR_N^9 + (1 - dF8) kspF FR_N^8 - (kd8 + ka9RN bulk + kexpS8 + (1 - dS8) kspS + kdegRNA) \]

\[ SR_N^8 \]

\[ \frac{dSR_N^9}{dt} = ka9RN bulk + kd10SR_N^{10} + (1 - dF9) kspF FR_N^9 - (kd9 + ka10RN bulk + kexpS9 + (1 - dS9) kspS + kdegRNA) \]

\[ SR_N^9 \]

\[ \frac{dSR_N^{10}}{dt} = ka10RN bulk + kd11SR_N^{11} + (1 - dF10) kspF FR_N^{10} - (kd10 + ka11RN bulk + kexpS10 + (1 - dS10) kspS + kdegRNA) \]

\[ SR_N^{10} \]

\[ \frac{dSR_N^{11}}{dt} = ka11RN bulk + kd12SR_N^{12} + (1 - dF11) kspF FR_N^{11} - (kd11 + ka12RN bulk + kexpS11 + (1 - dS11) kspS + kdegRNA) \]

\[ SR_N^{11} \]

\[ \frac{dSR_N^{12}}{dt} = ka12RN bulk + (1 - dF12) kspF FR_N^{12} - (kd12 + kexpS12 + (1 - dS12) kspS + kdegRNA)SR_N^{12} \]

\[ dR_C/ dt = fRevTrRev frev MM_C + kexpRR \]

+1(\exp F1MR_N^1 + \exp S1MR_N^1)

+2(\exp F2MR_N^2 + \exp S2MR_N^2)

+3(\exp F3MR_N^3 + \exp S3MR_N^3) \]
\[ +4(k_{exp} F N + k_{exp} S N) \]
\[ +5(k_{exp} F N + k_{exp} S N) \]
\[ +6(k_{exp} F N + k_{exp} S N) \]
\[ +7(k_{exp} F N + k_{exp} S N) \]
\[ +8(k_{exp} F N + k_{exp} S N) \]
\[ +9(k_{exp} F N + k_{exp} S N) \]
\[ +10(k_{exp} F N + k_{exp} S N) \]
\[ +11(k_{exp} F N + k_{exp} S N) \]
\[ +12(k_{exp} F N + k_{exp} S N) \]
\[ - (k_{imp} + k_{deg} C Pro) R C \]
\[ dT_C/dt = fTat Trtat (f tat S S_C + f tat M M_C) + k_{exp} T T_N \]
\[ - (k_{imp} T + k_{deg} C Pro) T_C \]
\[ dR_N/dt = k_{imp} R R_C + k d1(F N + S N) + k d2(F N + S N) \]
\[ + k d3(F N + S N) + k d4(F N + S N) \]
\[ + k d5(F N + S N) + k d6(F N + S N) \]
\[ + k d7(F N + S N) + k d8(F N + S N) \]
\[ + k d9(F N + S N) + k d10(F N + S N) \]
\[ + k d11(F N + S N) + k d12(F N + S N) \]
\[ + k_{deg} N R N A 1(F N + S N) \]
\[ + k \text{deg}NRNA2(FR^2_N + SR^2_N) \]
\[ + k \text{deg}NRNA3(FR^3_N + SR^3_N) \]
\[ + k \text{deg}NRNA4(FR^4_N + SR^4_N) \]
\[ + k \text{deg}NRNA5(FR^5_N + SR^5_N) \]
\[ + k \text{deg}NRNA6(FR^6_N + SR^6_N) \]
\[ + k \text{deg}NRNA7(FR^7_N + SR^7_N) \]
\[ + k \text{deg}NRNA8(FR^8_N + SR^8_N) \]
\[ + k \text{deg}NRNA9(FR^9_N + SR^9_N) \]
\[ + k \text{deg}NRNA10(FR^{10}_N + SR^{10}_N) \]
\[ + k \text{deg}NRNA11(FR^{11}_N + SR^{11}_N) \]
\[ + k \text{deg}NRNA12(FR^{12}_N + SR^{12}_N) \]
\[ +(1 - dS1)kspS1SR^1_N + (1 - dS2)kspS2SR^2_N \]
\[ +(1 - dS3)kspS3SR^3_N + (1 - dS4)kspS4SR^4_N \]
\[ +(1 - dS5)kspS5SR^5_N + (1 - dS6)kspS6SR^6_N \]
\[ +(1 - dS7)kspS7SR^7_N + (1 - dS8)kspS8SR^8_N \]
\[ +(1 - dS9)kspS9SR^9_N + (1 - dS10)kspS10SR^{10}_N \]
\[ +(1 - dS11)kspS11SR^{11}_N + (1 - dS12)kspS12SR^{12}_N \]
\[ - ka1(F_N + S_N)R_N - ka2(FR^1_N + SR^1_N)R_N \]
\[ - ka3(FR^2_N + SR^2_N)R_N - ka4(FR^3_N + SR^3_N)R_N \]
\[-ka5(\text{FR}_N^4 + \text{SR}_N^4)R_N - ka6(\text{FR}_N^5 + \text{SR}_N^5)R_N\]

\[-ka7(\text{FR}_N^6 + \text{SR}_N^6)R_N - ka8(\text{FR}_N^7 + \text{SR}_N^7)R_N\]

\[-ka9(\text{FR}_N^8 + \text{SR}_N^8)R_N - ka10(\text{FR}_N^9 + \text{SR}_N^9)R_N\]

\[-ka11(\text{FR}_N^{10} + \text{SR}_N^{10})R_N - ka12(\text{FR}_N^{11} + \text{SR}_N^{11})R_N\]

\[-k_{\text{exp}}RR_N - k_{\text{deg NP}}R_N\]

\[
\frac{dT_N}{dt} = k_{\text{imp}}TT_C - (k_{\text{exp}} + k_{\text{deg NP}})T_N
\]

\[
\frac{dG_C}{dt} = f_{\text{gag}}TrGagF_C - k_{\text{deg Gag}}G_C - k_{\text{bud}}G_C
\]

\[
\frac{dvirion}{dt} = k_{\text{bud}}G_C/nGV
\]
NOTES ON ODE SOLUTION

The software used to numerically solve the various ODE systems throughout this study was Matlab. The specific computational mathematics software was used because there are a few built-in functions to solve both stiff and nonstiff initial value problems for ODEs with user-defined accuracy. The commonly used function is *ode45* which uses an explicit Runge-Kutta formula. When the system is stiff causing the simulation to become unstable or unduly slow (due to extremely small numerical integration time step), other ODE solvers such as *ode15s* can be used. The code *ode15s* uses a quasi-constant step size implementation of the numerical differentiation formulas in terms of backward differences [123].

Through “option” input, various parameters such as error tolerance and step size can be changed from the default integration numerical values. When dealing
with stiff systems, it is necessary to investigate if the solver is not neglecting the dynamics during the numerical integration. This can happen if the time step is large compared to the time scale of variables. The default value of the maximum time step is one tenth of the simulated time horizon. When necessary, an upper bound on the magnitude of the step size that the solver uses can be employed.

In this study, *ode15s* was used to solve the ODE system of the intracellular translation, transportation, and budding of section 5.1 (the differential equations are given in Appendix A) and the multiscale model of section 5.2 where the differential equations are given in equations 5.5 to 5.29. The dimension of time is “day” in equations 5.5 to 5.29 and the simulation time horizon is a few years. Hence, the default maximum time step is in the order of months which is extremely large for this system and can lead to disregardance of important dynamics. Also, as it can be observed in figure 5.8, viral trajectories show oscillatory behavior. Although the rises and drops in viral load are expected because of the on-off periods of treatment, it needs to be investigated if this behavior is true to the dynamics or an artifact of the integration error.

In order to address the above issues, a maximum time step of one day, one tenth of a day, and one hundredth of a day were investigated. The simulation results were similar in all cases, however, the simulation time was increased from a few seconds for maximum time step of one day to a few minutes for time step of one hundredth of a day. No difference was observed in the results by reducing the
maximum time step. Furthermore, the observed oscillations were in accordance with the treatment schedule and the period was insensitive to the maximum time step. Usually when oscillatory behavior in numerical simulation is observed due to numerical instabilities, the periodicity changes with the chosen time step (and in adaptive simulators with the relative accuracy). Considering the above and the computational cost, it was concluded that the maximum time step of one day provides the desired accuracy for this system with.
BIBLIOGRAPHY


[42] DeJong, M. D., J. Veenstra, and et al. (1996) “Host-parasite dynamics and outgrowth of virus containing a single K70R amino acid change in reverse transcriptase are responsible for the loss of human immunodeficiency virus type 1 RNA load suppression by zidovudine,” PNAS, 93(11), pp. 5501–5506.


to the validation of the triphosphorylated anabolite of antiretrovirals in peripheral blood mononuclear cells,” *J. Mass Spect.*, 38, pp. 879–890.


[91] “HIV sequence database,”
www.hiv.lanl.gov/content/sequence/HIV/mainpage.html.


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