Chapter 1

Introduction

ACCORDING to the United States Department of Health and Human Services (USDHHS) [1] guidelines on the use of antiretroviral agents in HIV infected adults and adolescents, HIV therapy is considered effective if it can reduce the viral load by 90% in less than 8 weeks and continue to suppress it to below 50 copies per mL of plasma in less than 6 months. The primary goals of such an effective therapy regimen are stated as: "maximal and durable suppression of the viral load, restoration and/or preservation of immunologic function, improvement of quality of life, and reduction of HIV related morbidity and mortality" [1]. Furthermore, the tools that are available for the attainment of these goals are: maintenance of high adherence to potent antiretroviral therapy, rational sequencing of drugs in order to maximize the benefits of antiretroviral therapy and preserve future treatment options, testing for drug resistance and adequate monitoring for predictors of virologic success .

There is no doubt that Highly Active Antiretroviral Therapy (HAART) is capable of suppressing the viral load of infected individuals to levels that are below detection by the current assays, can maintain an acceptable $CD4^+$ T cell count and consequently, prolong the life of the infected person. What is not yet clear though, and the guidelines do concede, is when, during the HIV infection progression, is the optimal time to initiate therapy.

HIV can and has been initiated during all the stages of the infection. Therapy in most cases entails the use of antiretroviral drugs that interfere with the replication cycle of the virus. Other therapies that reconstruct the immune system are also available. The issues of when best to initiate therapy have been studied in [2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12]. Some authors, for example, [2, 8, 10] believe that early therapy, when the $CD4^+$ T cell count is still high, is best. The recent argument being put forth is that initiating therapy during the acute infection stage while the immune response to the virus is not

yet compromised, could most likely lead the individual to attain immunologic control of the virus with the use of structured treatment interruptions (STI) [13, 14, 15, 16, 17, 18]. However, in many cases, initiating therapy early during the infection is no guarantee for attaining this so called long term non-progressor (LTNP) status [19, 20, 21].

Other authors, for example [3] believe that late therapy during the final decline of the $CD4^+$ T cells is best. The arguments being put forth for delayed therapy are that the drugs are toxic and exposure should be delayed for as long as possible. Furthermore, care should be taken not to exhaust the regimen options in case resistance emerges, and as stated before, not all who initiate therapy early manage to attain LTNP status. Yet some studies [4, 5, 6] have shown that there is a higher mortality rate for patients who start therapy in the advanced stages of the disease as opposed to those who started early.

The main reason for the lack of consensus on when best to initiate therapy, as well as other HIV issues, is that the chemotherapy of HIV has multiple objectives and the studies that have been carried out had different objectives. So, the current position is that some individuals could benefit from early therapy, while for some it is better to defer therapy. How then can one predict in advance if an individual will benefit from early or late therapy?

Once the decision to initiate therapy has been made, the starting regimen should then be prescribed. It is apparent that there is variability in response to therapy among individuals. Some individuals have virologic failure on therapy that is highly effective on others. Furthermore, many experience viral load rebounds, known as blips after periods of effective suppression [22, 23, 24, 25]. Some rebounds are transient, while others lead to virologic failure. How then, can one predict in advance who will experience a rebound, and whether the rebound signals virologic failure or will be short lived? In other words, what then, are the prognostic indicators of virologic success?

The HAART regimen in most cases will manage to suppress the viral load to below detection in 3-4 months from when therapy was initiated [1]. However, the virus persistently replicates in body compartments [26, 27, 28, 29, 30, 31, 32, 33]. Furthermore, there is differential drug penetration into different compartments and target cells [34, 35, 36, 37, 38] and some latent compartments act as virus reservoirs [39, 40]. This has rendered virus eradication impossible with the currently available antiretroviral drugs.

Durable suppression of the viral load has also proven to be difficult because of the problems associated with HAART. Antiretroviral drugs are toxic, instantaneously and cumulative. There is therefore a need to design dosage regimens that can attain maximal and durable suppression of the viral load with minimal drug exposure. However, maximal

viral load suppression and minimal dosing are conflicting objectives. The objectives of therapy must therefore, be prioritized and the dosage should be designed to strike a balance between aggressive therapy and minimal side effects, now that the focus has shifted from virus eradication to managing a chronic infection.

1.1 Motivation

Millions of people world wide are infected by the HI virus. This HIV/AIDS pandemic has placed a heavy burden on medical workers. It is therefore, fitting that all who can assist should do so.

One of the motivations for this study is that there is a need to find plausible explanations to some currently observed clinical responses from individuals on antiretroviral therapy. These observations can be summarized as follows:

There is variability in response to therapy, as some individuals experience virologic failure on therapy that is highly effective on others [41].

For the majority who do attain viral load suppression, the maximality and duration of such suppression varies.

Persistent virus replication [28, 31, 32, 33, 40] and transient rebounds of plasma viremia (viral load blips) under HAART have also been reported [22, 23, 24, 25].

Furthermore, the optimal time to initiate therapy during the course of the HIV infection is currently not very clear.

Vigorous research in the medical field and clinical trials on HIV/AIDS and related issues mentioned above is currently ongoing. Affirmative and contradicting conclusions on most of the issues are drawn from one study to the other. This has paved the way for mathematicians and control engineers to be of assistance to medical practitioners by providing some insights and suggestions on how the above problems can be approached and solved.

The other motivating factor for this thesis is that there is a need to individualize therapy, or at least, derive dosage schedules that will apply to most HIV infected individuals. This need can be summarized as follows:

Eradication of the virus is not attainable with the currently available drugs, and now the focus has shifted from virus eradication to management of a chronic infection.

Given that antiretroviral dugs are generally toxic, the general objectives of therapy are therefore, to maximally suppress the viral load with minimal use of drugs.

Once therapy is initiated, the individual can not later choose to indefinitely discontinue therapy without undesirable effects.

1.2 HIV/AIDS Therapy: A Control Engineering Problem

The role that control engineering has played, and continues to play in control of biomedical systems is legendary. Examples include the automation of anaesthetic delivery during surgery, optimization of drug dosages in the management of bacterial infections and cancer chemotherapy, control of robotic endoscopic surgery systems, and so on. There is an IEEE Transaction on Automatic Control issue that addressed some of the applications of control theory in medicine. The goal of this issue, according to the guest editorial, was to "showcase some of the applications of control theory to medicine with two goals in mind. The first goal was to interest the control theory community in the idea of developing applications in medicine, and the second goal was to demonstrate to the medical community that control theory had solid applications in the medical field" [42].

Current research on HIV therapy is diverse and multi-disciplinary. Engineers however, were late in joining the research movement and as such, engineering literature related to HIV chemotherapy is limited. Control engineers in particular, should have risen to the challenge as it is apparent that chemotherapy scheduling and control engineering have a lot in common.

It is acknowledged though that HIV/AIDS models, like many biological and industrial systems, are not well defined mathematically. The main reason for the HIV/AIDS models' limitations is a lack of a good understanding of the immunology of the body against HIV. Biological systems tend to exhibit multi-compartmental interactions that are usually not well understood and as a result, can not be accurately modelled mathematically. The accuracy of the models is increasing though with new medical discoveries.

From a control theoretic perspective, HIV chemotherapy is control of a time varying nonlinear dynamical system with constrained controls. Administering an antiretroviral agent is equivalent to introducing a control signal that perturbs the current state of the HIV dynamics. The adopted control approach or strategy depends primarily on the control objectives, performance specifications and the control constraints. Obtaining

measurements of the controlled variables though, has the potential to hinder effective control.

Applying control engineering concepts to the analysis of HIV/AIDS models and therapy design is however, gaining momentum. Examples are works on viral load controllability and timing the initiation of therapy [43], optimized treatment interruptions with relation to controllability [44, 45], feedback control of the viral load [46, 47], as well as optimal control and multi-drug therapy scheduling for multiple viral strains [48, 49, 50, 51].

For the enhancement of the immune response to the virus, [52] compared continuous dose control with receding horizon control, [53] used model predictive control to derive structured treatment interruption cycles, while [54] proposed a gradual reduction of drug dosage approach that drives the patient to attain the long tern non-progressor status. [55] also used predictive control to derive a dosage sequence for combination therapy. [56] presented a globally stable nonlinear control approach to HIV therapy, while [57] presented a strategy for structured treatment interruption protocol design for the chronically infected patient.

Other analytical works such as bifurcation [58], stability of the steady states [54, 59] and viral load time response analysis under therapy are also available [60]. These works are model based, and this puts an emphasis on the need to obtain, as early as possible, estimates of the individual's viral and host cell model parameters. Works related to system identifiability [61, 62, 63] and parameter estimation from clinical data [64, 65, 66, 67] are also available. [68, 69] have gone the extra mile to assess and incorporate HIV/AIDS education into the control engineering curriculum.

Most of these HIV control works are aimed at suppressing the plasma viral load of infected persons, while some focus on maintaining the $CD4^+$ T cell count within a given range or above a specified level. There is a general view though, that treatment should vary with time and depend on the individual patient's response to treatment. This calls for frequent measurements of the controlled variables for effective feedback control.

All the foregoing call for the derivation of a control strategy for HIV therapy that can meet the goals/control objectives of therapy and simultaneously minimize the toxicities associated with the use of antiretroviral agents. This creates an ideal opportunity for control engineers to derive such control strategies. There are various approaches that the control engineer can take towards designing a control strategy for HIV therapy. A control engineering approach to HIV therapy would involve the following steps:

- Model development: This entails the translation of clinical observations into a mathematical formulation. This model should be able to adequately explain the

interaction of the virus, the host cells, and the immune system. Mathematical Biologists have paved the way by building these models and to date, such models that describe different aspects of this virus, host cell and immune systems are available in for example [70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87]. Most of these models are deterministic and based on balancing the population dynamics of the virus and target cells in plasma, while some are stochastic and take into account the random variations in the HIV dynamics.

- Model validation: This entails the collection of clinical data that can be used to determine the model parameters and verify that the model is representative of what is observed clinically. Model validation in this context can therefore, be considered as part of model development. There are numerous clinical trials that have been carried out and data has been collected for the determination of model parameters [64, 65, 66, 88, 89, 90, 91, 92, 93, 94]. However, the focus has been mostly on obtaining parameter estimates related to the replication competency of the virus. For these estimates, there is very wide variability between individuals, within a study and between studies. None of the above mentioned models can completely exhibit all that is observed clinically and account for the full course of the HIV infection. Most of the models though, can accurately model the dynamics from initial infection to the clinical latency stage. A point to consider is that these models do not take into account other extenuating environmental, social and welfare factors that may affect the progression of the disease.
- Model analysis: Extensive mathematical analysis of the model can be carried out in order to see how different drug regimens will affect viral response and preempt the type of response one can expect. To date, model parameters that are affected by various drugs that are used to treat the HIV infection have been identified and such extensive mathematical analysis has been carried out in works such as [43, 60, 61, 71, 72, 73, 75, 78, 84, 85, 86] and [95, 96, 97, 98, 99, 100]. However, very few of these works have a control theoretic analytic perspective.
- Controller design: Once the model is validated and analyzed, then in principle, it can be used to design suitable drug dosage regimens for HIV therapy. Such controller design related works have been presented in for example [44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 55, 56, 57]. Compared to other control design works for other medical conditions, those related to HIV are few. There is therefore, a need to derive optimal control strategies for the chemotherapy of HIV, given the multiple control objectives and constraints on the available treatment options.

- Control system simulation: Simulations can be used to gain insight into the type of response one can expect before a particular drug regimen is tried out on a patient. System simulations can also be used to guide the design of some clinical trials.

1.3 Thesis Objectives and Scope

In this thesis, analysis of the HIV/AIDS models will be carried out using various mathematical and control systems analytical tools. The intention is to gain some insights into the HIV infection dynamics from a control theoretic perspective. Such insights can help explain why individuals on antiretroviral therapies respond the way they do, as well as give the individual or practitioner the ability to preempt future responses. The problems or issues addressed by each analysis will be stated and the usefulness of such an analysis to clinicians will be made apparent.

The primary objective of this thesis is to analyze models for the eventual control of the infection. The intention of the proposed control strategy is to produce practical solutions to the current antiretroviral problems, and not necessarily to postulate on solutions that can not currently be implemented. This however, does not mean that issues that could enhance the solution to the problems will not be highlighted. That is, suggestions on which areas to investigate in order to improve the attained solution will be made.

The scenario is such that, given the observed responses from individuals on antiretroviral therapy and the toxicity problems associated with this therapy, what can possibly be done to alleviate these problems? A solution should then be prescribed. The next question will then be, is such a solution implementable? The answer to this last question should be in the affirmative - Yes. And finally, areas that need attention in order to further enhance the solutions will be highlighted.

The first part of this research focuses on the application of control system analytical tools to HIV/AIDS models in order to obtain insight into the following issues:

- Variability in response to therapy between individuals on the same regimen.
- Rebounds of plasma viremia after periods of suppression.
- The attainment of maximal and durable suppression of the viral load.
- The prognostic markers of virologic success.
- Timing the initiation of antiretroviral therapy such that the benefits of therapy are maximized.
- The possibility of individualizing therapy.

The second part of the research focuses on the following control issues:

- Modelling antiretroviral drugs as control inputs.

- Sampling for effective control given the invasive nature of drawing blood samples.
- Drug dosage design to strike a balance between maximal suppression (aggressive therapy) and toxicity (drug exposure) reduction.
- Design of other drug dosage schemes to further reduce total drug intake, once viral load suppression to below detectable levels is attained and maintained.
- The practicality of implementing such a dosage scheme.

1.4 Contribution

Contribution of this research can be found in the following published works:
Journal papers [43, 60], a book chapter [61], an educational CD [101], and conference papers [102, 103, 104, 105, 106, 107]. Additional contributory works have been submitted to journals [55, 57].

1.5 Organization of Thesis

The thesis is arranged as follows: The thesis has a total of six chapters. Except for the concluding chapter, each chapter is concluded with a summary and other related matters or supplemental information can be found in the appendix.

Chapter 2 presents a brief background on immunology and how the virus replicates within the host cells. The interaction between the immune system and the HI virus is explained, as well as how HIV infection compromises the immune system. The chapter presents the different classes of drugs that are used to treat the infection and matters related to drugs resistance are discussed. The guidelines views and advice on the treatment of HIV/AIDS infections then follow, and the case for the need to individualize therapy is presented. Finally, the logistics and reasons for interrupting therapy are presented.

Chapter 3 gives an over view of some HIV/AIDS mathematical models and the aspects of the immune system that each model illustrates are discussed. At the end of the chapter, the models that were adopted for this thesis are presented. The validity of these adopted models and the effect of therapy on model parameters is discussed.

In chapter 4, a detailed analysis of the selected models is carried out in order to gain some insights into the HIV infection dynamics. The analysis starts with the lower order model and is then extended to the higher order model. At the end of the analysis, the usefulness of the analysis and the HIV/AIDS issues that have been addressed are stated.

Chapter 5 presents drug dosage design control strategies for HIV therapy. The chapter first presents the system to be controlled and highlights points that need to be considered

before hand. A way of modelling antiretroviral drugs as control inputs is presented. Sampling, the prioritization of objectives and Model Predictive Control as a control strategy of choice, are discussed. A sequential perturbation approach to dosage design for the treatment naïve patient is implemented. This is followed by the design of structured treatment interruption protocols.

Chapter 6 presents a summary of all the major work that was carried out in this research. This is followed by the conclusions that were drawn from this study as well as recommendations on future work.

A reference list follows and other related information is appended thereafter.

Chapter 2

Background

2.1 HIV and the Immune System

2.1.1 Virus Replication

EACH human immunodeficiency virus (HIV) particle has a glycoprotein (gp) called gp120 on its surface. This gp120 glycoprotein can precisely fit the protein marker called cluster designation 4 (CD4), that is found on the surfaces of most immune system cells. Cells with this marker are referred to as being CD4 positive/plus ($CD4^+$). When the HI virus enters the body, it *directly* seeks out the immune system cells because the virus can recognize the CD4 receptor on their surfaces. When a virus comes into contact with such a recognizable cell, it attaches to the CD4 receptor. However, the virus needs to attach to a second co-receptor in order to facilitate entry of the CD4-gp120 complex into the cell, or for the virus to pull itself across the cell membrane [108]. This secondary co-receptor could be CXCR4 or CCR5. After the virus attaches to the co-receptor, the host cell and virus membranes then fuse, and the virus enters the cell, as illustrated in figure 2.1.

HIV is a retrovirus. This is a type of virus that, when outside the target cell, stores its genetic information on a single-stranded ribonucleic acid (RNA) molecule instead of the more usual double-stranded molecule (DNA). However, once inside the cell cytoplasm, the virus sheds its coat and proceeds to construct a complementary DNA version of its genes. The virus enzyme called reverse transcriptase facilitates the synthesizing of this complementary double strand of viral DNA. The double stranded DNA can then proceed into the cell nucleus, where it integrates itself into the host cell's DNA. This integration process is facilitated by another viral enzyme called integrase. Once integrated, the viral DNA is called a provirus. The viral DNA then hijacks the host cell, and directs the cell to produce multiple copies of viral RNA. These viral RNA are translated into

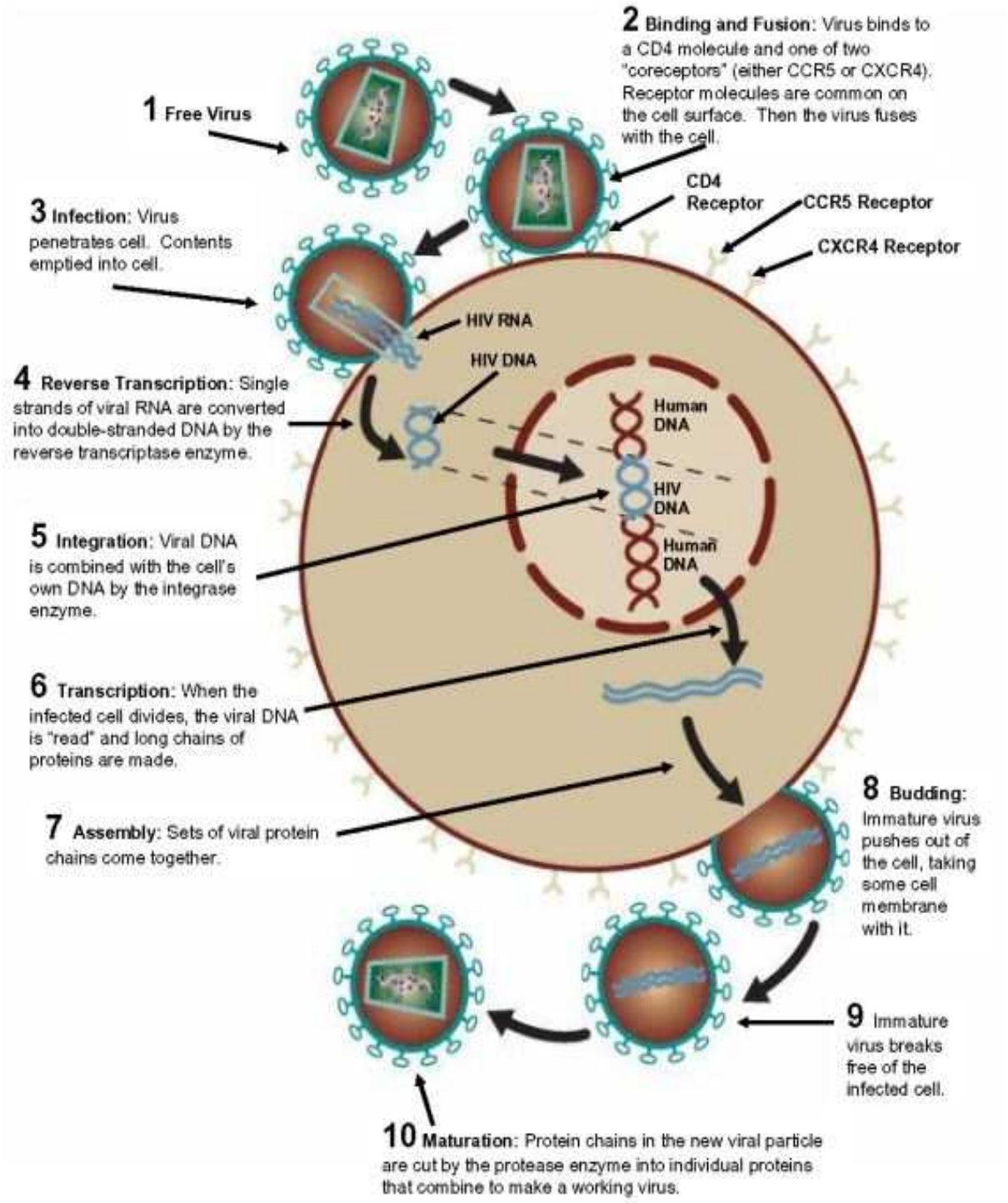

Figure 2.1: Virus Replication cycle. A reproduced picture [109].

viral proteins to be packaged with other enzymes that are necessary for viral replication. Viral core proteins, enzymes, and RNA gather just inside the cells membrane, while the viral envelope proteins aggregate within the membrane. An immature viral particle is formed and then pinches off from the cell, acquiring an envelope and the cellular and HIV proteins from the cell membrane. The immature viral particle then undergoes a maturation process. A viral enzyme called protease facilitates maturation by cutting the protein chain into individual proteins that are required for the production of new infectious viruses.

Eventually, multiple copies of the virus are released, and in the process, the immune cells are destroyed in large numbers, and certain cell pools are even depleted. This leaves the body with little or no defence against disease causing invaders. Furthermore, the replication process is error prone, and consequently, some of the virus particles released are mutants that can also replicate. This gives rise to resistance to antiretroviral drugs.

2.1.2 The Immune System

The immune system is made up of different types of white blood cells (lymphocytes), antibodies and some active chemicals [108, 110], whose responsibility it is to defend the body against any disease causing foreign invaders. The immune cells work together to defend the body by identifying, disabling and destroying the invader. White blood cells are present in the blood, lymph, and lymphoid tissue and can be categorized as B cells (B lymphocytes) or T cells (T lymphocytes). B cells are derived from the bone marrow and spleen, whereas T cells are derived from the thymus gland. There are three different types of T cells that participate in a variety of cell-mediated immune reactions, namely, helper, killer, and suppressor T cells.

$CD4^+$ T cells:
$CD4^+$ T cells are also known as the helper T cells and are crucial to the immune response. In an uninfected individual, $CD4^+$ T cells constitute 60%-80% of the circulating T cells. While circulating in plasma, $CD4^+$ T cells can come across foreign invades, or can be summoned to the infection site by macrophages. It is the $CD4^+$ T cell's responsibility to recognize viral, fungal and parasitic invaders [110]. When $CD4^+$ T cells spot a foreign antigen, they begin to proliferate or multiply and secrete a chemical alarm (lymphokines) that alerts and triggers other immune cells into action. However, once infected by the HIV, the cell does not function normally. The infected cell often does not trigger an alarm, but instead, secretes a soluble suppressor factor that blocks other T

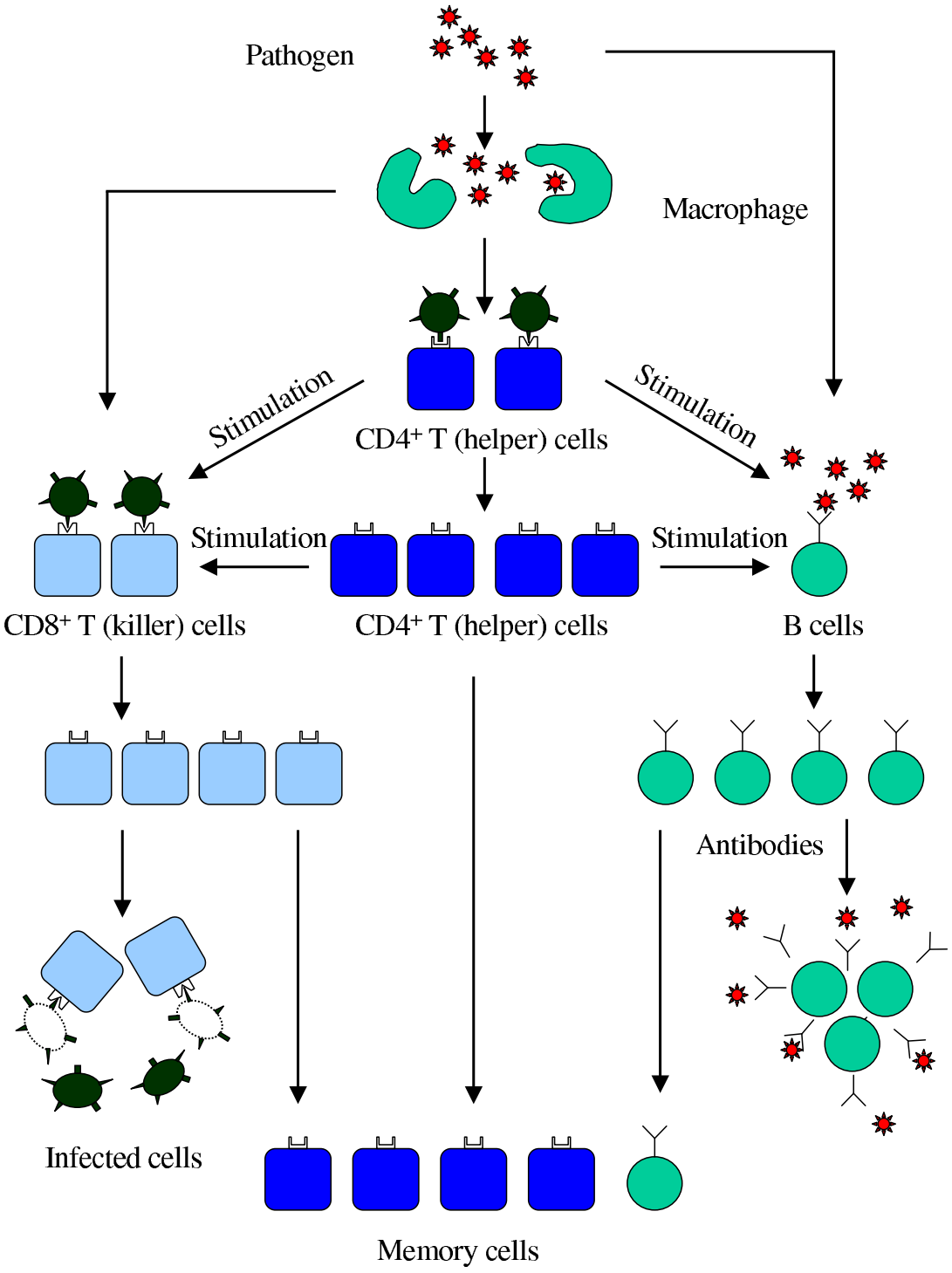

Figure 2.2: Cells of the immune system. Each cell has a receptor and co-receptor(s) on its surface that facilitate virus attachment and entry into the cell. A reproduced picture [77].

cells from responding to the HIV antigen. When the infected cell gets activated, it starts producing viruses and release of the virus destroys the cell. Infection of these $CD4^+$ T cells by the virus therefore, leads to a drastic reduction in their numbers, and eventually, even complete depletion.

B cells:
During infections, B cells are transformed into plasma cells that produce large quantities of antibodies directed at specific pathogens or antigens. Antibodies are chemicals that lock onto the virus or foreign antigens, and thus marking the foreign antigen or virus and making it easier for other cells of the immune system to destroy it. This transformation of B cells to plasmas cells and to the production of antibodies, occurs through interactions with various types of T cells and other components of the immune system. However, in HIV infection or AIDS, the functional ability of both the B and the T lymphocytes is compromised or damaged. Furthermore, the production of antibodies takes time, and in the mean time, the virus multiples and goes on to infect other cells.

$CD8^+$ T cells:
These are a subset of T cells that carry a cluster designation 8 (CD8) marker on their surfaces, and are also known as killer T cells or cytotoxic T cells. Because viruses replicate inside host cells where antibodies cannot reach them, the other way viruses can be eliminated is by killing the infected host cell. It is the $CD8^+$ T cells' responsibility to kill cells infected by intracellular pathogens and some cancer cells. However, $CD8^+$ T cells can act only when they encounter an infected cell that carries on its surface, a distress signal or marker that links the infected cell to a foreign antigen, that being the invading virus.

Alerted by the helper $CD4^+$ T cells, these killer $CD8^+$ T cells likewise proliferate and their receptors then bind to an infected cell's distress signal and releases a potent chemicals that destroys the infected cell. So, while antibodies are marking free floating viruses in the blood for destruction by other cells of the immune system, $CD8^+$ T cells are destroying cells that are infected by the virus. When the foreign antigen has been vanquished, the $CD8^+$ T cells produces a signal that suppresses or halts antibody production and other immune responses. $CD8^+$ T cells, are also suppressor T cells.

Macrophages:
These are large cells that devour invading pathogens and stimulates other immune cells by displaying the pathogen's antigen or body shape for the other immune cells to see. Macrophage infection during the primary phase of the infection may be essential for HIV to be successfully established [111]. Macrophages live longer than $CD4^+$ T cells and are chronic virus producers that can harbour large quantities of the virus without being killed, acting as reservoirs of the virus. Thus, they facilitate virus evolution towards more replication competent strains and away from recognition by the immune system.

Unlike in $CD4^+$ T cells, HIV replication in macrophages does not require cell activation and division [111], and macrophages have been shown to continue producing virus after $CD4^+$ T cells have been depleted [112]. Macrophage role in virus replication and spreading of the infection is well accepted, but under-appreciated and poorly defined.

Follicular Dendritic Cells - FDC:
These cells are found in the germinal centers of lymphoid organs such as tonsils, lymph nodes, spleen, thymus, and other tissues. These organs act as the body's filtering system. FDCs have thread-like tentacles that form a web-like network to trap invaders and present them to other cells of the immune system that congregate there for destruction. FDCs can trap large quantities of virus, and the disassociation or release of this virus has been shown to affect the virus dynamics in plasma [113, 114].

Memory T Cells:
After an immune response has been successful at abating the invading pathogen, the $CD8^+$ T cells shuts down the immune response. However, a few of each type of immune cells and antibodies remains in circulation. This subset of immune cells that have been exposed to specific antigens can then quickly proliferate on subsequent immune system encounters with the same antigen [108, 115].

2.1.3 Compromised Immune Response

Figure 2.3 shows a typical course of HIV infection. The course has 3 main stages, namely the acute or primary infection stage; the asymptomatic or clinical latency or chronic infection stage; and lastly the advanced or AIDS stage. The following summary for the acute and asymptomatic stages is directly extracted from [108].

- **Acute HIV Infection**: This is the period of rapid viral replication immediately following exposure to HIV. An estimated 80 to 90 percent of individuals with pri-

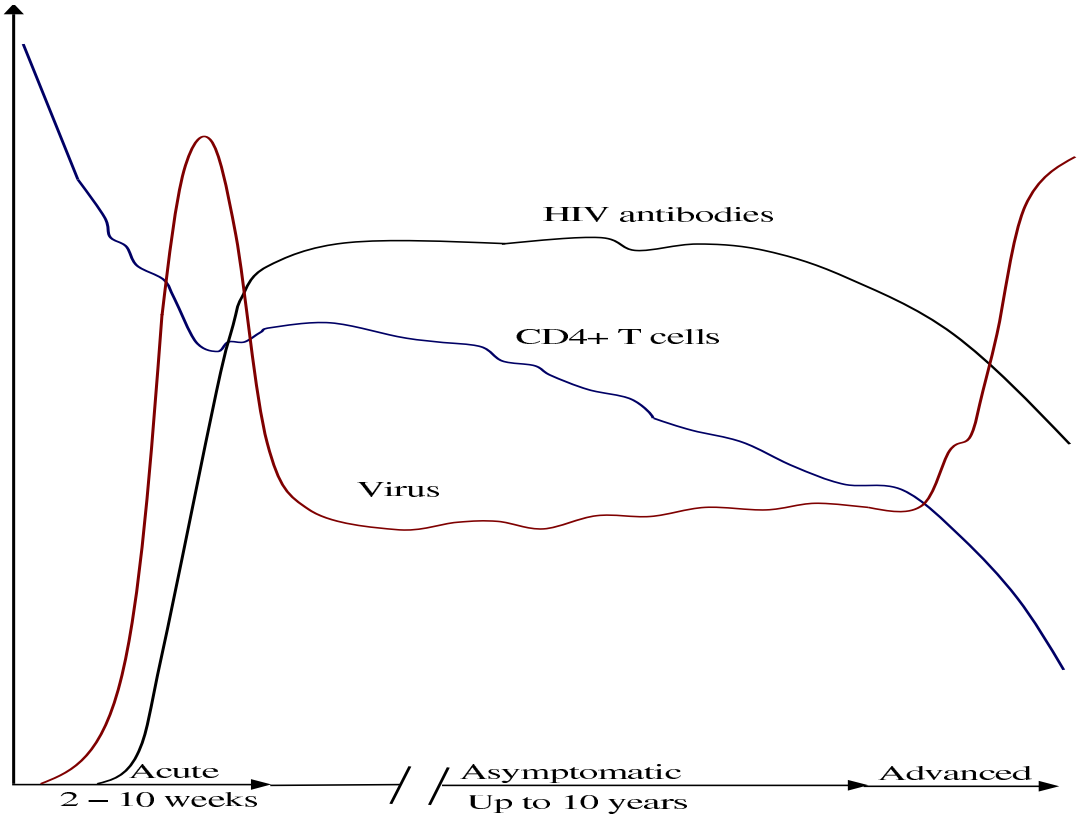

Figure 2.3: Typical HIV infection progression and stages [115].

mary HIV infection develop an acute syndrome characterized by flu-like symptoms of fever, malaise, lymphadenopathy, pharyngitis, headache, myalgia, and sometimes rash. Following primary infection, seroconversion occurs. When people develop antibodies to HIV, they seroconvert from antibody-negative to antibody-positive. It may take from as little as 1 week to several months or more after infection with HIV for antibodies to the virus to develop. After antibodies to HIV appear in the blood, a person should test positive on antibody tests [108].
It was previously thought that HIV was relatively dormant during this phase. However, it is now known that during the time of primary infection, high levels of plasma HIV RNA can be documented, as illustrated in figure 2.3.

- **Asymptomatic**: Asymptomatic means "without symptoms", and this period in infection is also known as the clinical latency period. During this period of time, a person with HIV infection does not exhibit any evidence of disease or any clinically noticeable ill effects, even though HIV is continuously infecting new cells and actively replicating. The virus is also, during this period, active within lymphoid organs where large amounts of virus become trapped in the follicular dendritic cell

network [108].

The period of clinical latency varies drastically in length from one individual to another. There are reports of this latency period lasting only 2 years, while others report it lasting for more than 15 years [115]. But normally, the duration in untreated individuals ranges from 7 to 10 years.

- **Advanced - AIDS**: After a normally long asymptomatic period, the virus eventually gets out of control and the remaining immune cells are destroyed. When the $CD4^+$ T cell count has dropped lower than 200 per μL (mm^{-3}) of plasma, the individual is said to have AIDS, and will start to succumb to opportunistic infections, because of the loss of immune competence [115].

So, HIV is also a lentivirus. This is a subclass of seemingly "slow" viruses characterized by a long interval between infection and the onset of symptoms. That is why most people are HIV positive but not aware that they are infected. However, the $CD4^+$ T cell counts are gradually decreasing towards the 200 cells per μL AIDS cutoff during this period. This destruction of $CD4^+$ T cells is the major cause of the immunodeficiency observed in AIDS, and decreasing $CD4^+$ T cells levels appear to be the best indicator for developing opportunistic infections.

There are some individuals who progress from initial infection to AIDS within 2-3 years (fast progressors), while yet others are characterized as long term non-progressors (LTNP). These are individuals who have been infected with HIV for at least 9 to 15 years (different authors use different time spans) and have stable $CD4^+$ T cell counts of 600 or more cells per cubic millimeter of blood. Furthermore, long term non-progressors have low viral loads and no HIV-related diseases, even though they have no previous antiretroviral therapy. Data suggest that this LTNP phenomenon is associated with the maintenance of the integrity of the lymphoid tissues and with less virus trapping in the lymph nodes than is seen in other individuals living with HIV.

Besides the depletion of $CD4^+$ T cells during HIV infection, the way the immune system responds to the infection is impaired on multiple levels. There are two aspects of the immune system's response to disease: innate and acquired. The innate part of the response is mobilized very quickly in response to infection and does not depend on recognizing specific proteins or antigens foreign to an individual's normal tissue. It includes macrophages and dendritic cells. The acquired, or learned, immune response arises when dendritic cells and macrophages present pieces of antigen to lymphocytes, which are genetically programmed to recognize very specific amino acid sequences. The ultimate result is the creation of cloned populations of antibody-producing B cells and

cytotoxic T lymphocytes primed to respond to a unique pathogen. (Extracted from [108]).

In HIV infection, both the innate and acquired immune responses are compromised. There is a breakdown in immunocompetence and certain parts of the immune system no longer function and certain cells types are even depleted. HIV infection has been shown to lead to increased rates of cellular turnover and ultimately to deterioration of the immune system. In particular, HIV-1 infection is known to increase the turnover rates of both the $CD4^+$ and $CD8^+$ T cells, and to deplete the populations of naïve CD4+ T cells, naïve $CD8^+$ T cells, and memory $CD4^+$ T cells [116]. However, the rates of turnover for these cells (even during health) are poorly characterized and this limits our understanding of the infection. Current estimates for the turnover rates of $CD4^+$ and $CD8^+$ T cells vary between 1 and 2% in normal individuals and by up to 10% in HIV- 1 infected patients [116].

The reasons for the increased turnover of T cells have been disputed widely. However, it is clear that there is over stimulation of the immune system. In any case, when follicular dendritic cells present the virus to the $CD4^+$ T cells, these cells are stimulated to proliferate. This means that the FDCs bring the virus in contact with the $CD4^+$ T cells at the time when these cells are responding to the antigen [115]. Furthermore, activated $CD4^+$ T cells are prone to apoptosis, or programmed cell suicide. This leads to the depletion of a subset of cells with specific immune response to the HI virus.

As discussed before, $CD8^+$ T cells shut down the immune response after it has wiped out invading pathogen. $CD8^+$ T cells are sensitive to high concentrations of lymphokines in circulation, and release their own lymphokines when an immune response has achieved its goal, thus signaling to all other components of the immune system to cease their coordinated attack. With HIV infection, the immune systems response coordination is impaired. $CD4^+$ T cells do not function properly and there is an over supply of lymphokines in the bloodstream. $CD8^+$ T cells then compound the problem by erroneously interpreting the oversupply of lymphokines to mean that the immune system has effectively eliminated the virus.

So while HIV is multiplying in infected $CD4^+$ T cells and macrophages, $CD8^+$ T cells are simultaneously attempting to further shut down the immune system. The stage is set for infectious agents that could normally be suppressed, to proliferate unhindered and to cause disease.

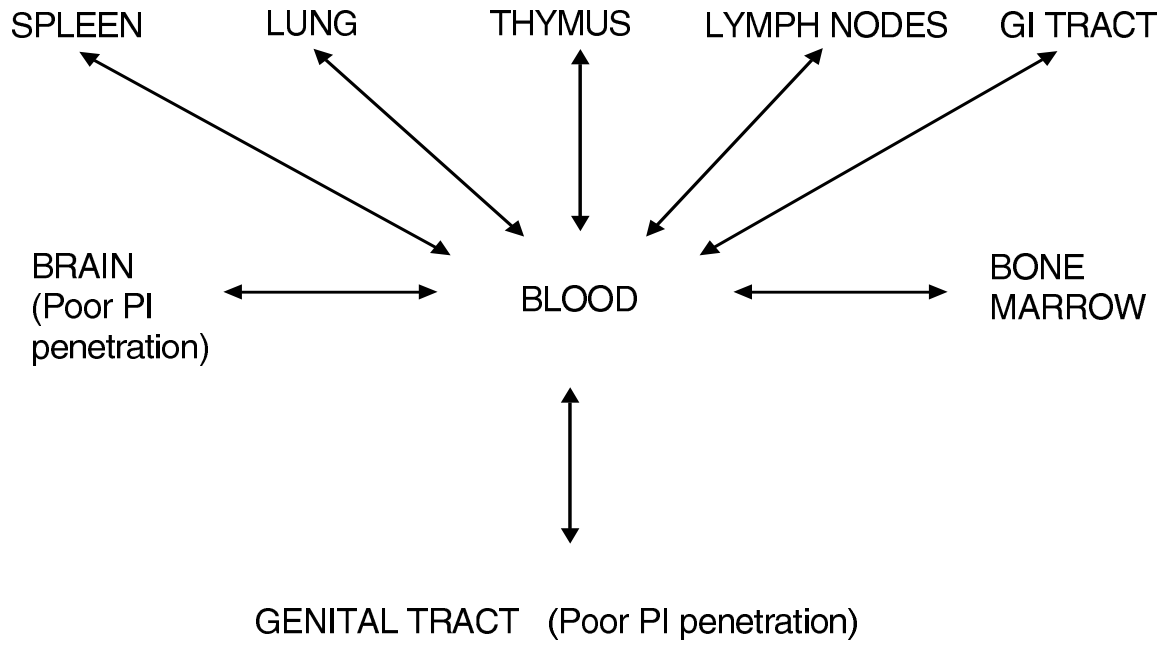

Figure 2.4: HIV Compartmentalization. Some compartments act as virus reservoirs or sanctuary sites [117].

2.1.4 HIV Compartmentalization

The human body is made up of different compartments as illustrated in figure 2.4. Some immune cells have the freedom to circulate in plasma or reside in any of the other compartments. Consequently, these cells not only facilitate virus replication, but its dissemination as well. Macrophages are particularly notorious for trafficking virus between compartments. Macrophages have been likened by [110] to a "Trojan horse which hides the invader and carries it to protected places", and infected macrophages have been shown to be responsible for transporting the virus to the brain.

The release of the virus from other cells and other infected compartments has been shown to affect the virus kinetics in plasma [113]. This situation is problematic because some compartments are not easily penetrated by some drugs used to treat the HIV infection [34, 38, 117]. Furthermore, there is differential drug penetration into different cell types, even within a compartment [35, 37]. This poses a problem for virus eradication.

2.2 Drugs Used to Treat HIV Infection

There is a variety of antiretroviral agents that are currently being used for the treatment of HIV infection and to enhance the immune response to the virus. The antiretroviral drugs can generally be classified depending on whether they are virus replication cycle based, or are based on the immune system's response to the virus infection.

2.2.1 Replication Cycle Based Antiretroviral Therapies

Figure 2.1 showed the various steps of how virus replication takes place within a host cell. If any stage of the replication process is disrupted, then technically, virus replication can be halted. To this end, various antiretroviral agents that interfere or disrupt one stage or another, have been developed and/or are being currently used. These replication cycle based antiretroviral drugs are classified depending on the point in the virus replication cycle that they disrupt.

Entry Inhibitors (EI) are an emerging class of antiretroviral drugs. These inhibitors prevent virus replication at the very early stages of the replication cycle. These drugs are designed to disrupt the interactions between the HI virus and the potential host cell surface, and their focus is on preventing the virus from entering the target cell.

The entry inhibitor class encompasses attachment (binding) inhibitors and fusion inhibitors. Attachment inhibitors are drugs that prevent attachment of the virus gp120 protein to either the target cells CD4 receptor, the CCR4 or CXCR5 co-receptors. If the virus manages to evade the attachment inhibitors (or in the absence thereof, as is the current case) and attaches to the target cell, then the fusion inhibitors can prevent the virus and target cell membranes from fusing together. Fusion inhibitors bind to the gp41 envelope protein and blocks the structural changes necessary for the virus to fuse with the host CD4 cell. This can effectively prevent the virus from entering the target cell. The problem with current entry inhibitors is that they have short half lives and require intravenous administration.

Reverse Transcriptase Inhibitors (RTI) work inside the infected cell. These compounds are designed to bind to the reverse transcriptase enzyme, thus preventing nucleosides from binding to the enzyme active sites [108]. This binding interferes with the reverse transcription process and effectively reduces the chances of successful infection of the cell by the virus by halting the transcription of viral RNA into viral DNA. RTIs can further be sub-categorized as nucleoside (NRTI), nucleotide (NtRTI) or non-nucleoside (NNRTI) analogues, depending on the active enzyme site to which they bind to.

Integrase Inhibitors: Integrase is not a well understood viral enzyme that however, plays a vital role in the HIV infection process. After reverse transcriptase has transcribed the viral RNA to viral DNA, integrase inserts or integrates the HIVs genes into the cells normal DNA. Once integrated, the HIV DNA is called the provirus. Integrase Inhibitors are a class of currently experimental antiretroviral drugs that prevents the HIV integrase enzyme from inserting viral DNA into a host cells normal DNA.

Zinc Finger Inhibitors. Zinc fingers are chains of amino acids found in cellular protein, and play important roles in a cells life cycle. Zinc fingers are involved in binding and packaging viral RNA into new viruses budding from an infected host cell. There are two zinc fingers in HIVs nucleocapsid. Zinc finger inhibitors are drugs which prevents the nucleocapsid part of the gag protein of HIV, which contains the zinc finger amino acid structures, from capturing and packaging new HIV genetic material into newly budding viruses. These drugs are still experimental, and the major problem with them is that zinc fingers are found in other cells of the body. Interfering with zinc fingers in the HI virus consequently interferes with other cells' life cycles.

Protease Inhibitors (PI) also work within the host cell as new virus particles are budding off the cell membrane. Protease is the first HIV protein whose three-dimensional structure has been characterized. PIs inhibit the viral protease enzyme from cleaving or cutting the long protein chains into structural proteins and enzymes that make up the viral core. If the larger HIV proteins are not broken apart, they cannot assemble themselves into new functional HIV particles. This results in the production of mostly immature noninfectious virus particles. There are therefore two types of virus particles when protease inhibitors are used. The first type are the infectious virus particles that still continue to infect target cells and the other is the noninfectious type that is not capable of causing new infections, but just circulates until it is cleared from the body.

The currently approved drugs can be classified as entry inhibitors (fusion type), reverse transcriptase inhibitors and protease inhibitors. Multi-drug therapies primarily use a combination of protease and reverse transcriptase inhibitors. Entry inhibitors are also used, but not as widely as reverse transcriptase and protease inhibitors.

Antiretroviral drugs are generally toxic, and the reader is referred to the guidelines [1] for the characteristics of the FDA approved antiretroviral drugs, as well as their toxicity and resistance profiles.

2.2.2 The Development of Drug Resistance

HIV has nine genes. Pol is one of these nine HIV genes and codes for the enzymes protease, reverse transcriptase and integrase [108]. This pol gene is prone to mutations or sudden changes in its structure. This leads to the emergence of mutated strains that generally differ in their ability to infect and kill different cell types, as well as in their rate of replication.

The genetic mutations also lead to drug resistance, where resistance occurs when the sensitivity of the virus to a drug is reduced. In HIV, mutations can change the structure of viral enzymes and proteins so that an antiviral drug can no longer bind with them as well as it used to. Often in HIV infection, when an individual's virus strain develops resistance to a particular drug in the regimen, it also turns out to have resistance to some drug or even drugs that the virus has never been previously exposed to. This is referred to as cross-resistance, and is one of the many problems facing antiretroviral therapy.

In many instances, an individual has one or more mutants by the time they start antiretroviral therapy. This pre-existence of mutant virus strains has been cited as the primary reason for the emergence of resistance [118], and even high levels of adherence to therapy will fail to prevent the accumulation of some of these mutant strains [119]. After the initial decline in viral load as the responsive wild type viral stain is cleared, resistance emerges as the mutant strain now thrives in the absence or reduction of the wild type virus. Individuals who have only the wild type strain when they initiate therapy have better therapeutic results as they take longer to develop resistance. This is because the probability of developing resistance if there was no pre-existence of resistant strains is much lower than when mutants are present before therapy is initiated.

There are two aspects to resistance. The first is genotypic resistance and can be detected by searching the virus genetic makeup for mutations that could confer lower susceptibility to a particular drug. In essence, tests for genotypic resistance, known as genotypic assays, are used to determine if HIV has become resistant to the antiviral drug(s) by analyzing a sample of the virus from the patients blood to identify any mutations in the virus that are associated with resistance to specific drugs.

The other aspect of resistance, referred to as phenotypic resistance, is detected by successfully growing laboratory cultures of the virus in the presence of a drug. Likewise, phenotypic assays are resistance tests whereby sample DNA of a patients HIV is tested against various antiretroviral drugs to see if the virus is susceptible or resistant to these drugs. Table 2.1 has recommendations on resistance testing in HIV infection.

Table 2.1: Recommendations for using drug-resistance assays

Clinical Setting / Recommendation	**Rationale**
Drug-resistance assay recommended:	
Virologic failure during combination antiretroviral therapy (BII)	Determine the role of resistance in drug failure and maximize the number of active drugs in the new regimen, if indicated.
Suboptimal suppression of viral load after antiretroviral therapy initiation (BIII)	Determine the role of resistance and maximize the number of active drugs in the new regimen, if indicated.
Acute human immunodeficiency virus (HIV) infection, if decision is made to initiate therapy (BIII)	Determine if drug-resistant virus was transmitted to help design an initial regimen or to change regimen accordingly (if therapy was initiated prior to test results).
Drug-resistance assay should be considered:	
Chronic HIV infection before therapy initiation (CIII)	Available assays might not detect minor drug-resistant species. However, should consider if significant probability that patient was infected with drug-resistant virus (i.e., if the patient is thought to have been infected by a person receiving antiretroviral drugs).
Drug resistance assay not usually recommended:	
After discontinuation of drugs (DIII)	Drug-resistance mutations might become minor species in the absence of selective drug pressure, and available assays might not detect minor drug-resistant species. If testing is performed in this setting, the detection of drug resistance may be of value, but its absence does not rule out the presence of minor drug-resistant species.
Plasma viral load $<$ 1,000 HIV RNA copies/mL (DIII)	Resistance assays cannot be consistently performed because of low copy number of HIV RNA; patients/providers may incur charges and not receive results.

Reproduced from [1].

2.2.3 Immune Based Therapies

Immune based therapies for HIV control entail the direct targeting of the immune system as a therapeutic strategy. Immune based therapies, as opposed to replication cycled based HAART, are not sensitive to virus mutations, and as such, are attractive as they offer the potential to minimize the emergence of drug resistance [120]. However, there is an unavoidable overlap between immune based therapy and replication cycle based therapy.

Immune based therapies can be used to augment the replication cycle based therapies. The proposed strategies include:

- Expansion of the $CD4^+$ T cell pool by direct lymphocyte transfer or Interleukin-2 [121].
    (a) Interleukin-2 (IL-2): (Extracted from [108]) A cytokine secreted by Th1 $CD4^+$ T cells to stimulate $CD8^+$ T cytotoxic T lymphocytes. IL-2 also increases the proliferation and maturation of the $CD4^+$ T cells themselves. During HIV infection, IL-2 production gradually declines. Recent data suggest that therapy with subcutaneous IL-2, in combination with replication cycle based antiretroviral drugs, has the potential to halt the usual progression of HIV disease by maintaining an individuals $CD4^+$ T cell count in the normal range for prolonged periods of time. Long term cell expansions with its use have been recorded in clinical trials.
    (b) Lymphocyte transfers: This entails the direct transferring of $CD4^+$ T cells to the infected individual. Transients effects in clinical trials have been recorded with this procedure.
- Enhancement of HIV specific immunity by structured treatment interruptions, therapeutic immunization or passive immunotherapy [121].
    (a) Structured treatment interruptions (STI): These are planned interruption of treatment by discontinuation of all antiretroviral drugs. The is no evidence of enhanced antiviral activity with this approach. However, there are reports of some individuals who sustain viral control and attain LTNP status with STI, especially when HAART was initiated during the acute stage of the infection. This strategy also reduces drug exposure and the cost of treatment.
    (b) Passive immunotherapy: (Extracted from [108]) Process in which individuals with advanced disease (who have low levels of HIV an-

tibody production) are infused with plasma rich in HIV antibodies or an immunoglobulin concentrate (HIVIG) from such plasma. The plasma is obtained from asymptomatic HIV-positive individuals with high levels of HIV antibodies.

- Suppression of immune activation by the use of immunosuppressive drugs such as hydroxyurea or cylosporin [120].

  (a) Hydroxyurea: This is an inexpensive prescription drug used for the treatment of sickle-cell anemia and some forms of leukemia. Hydroxyurea has been used investigationally for the treatment of HIV infection. Hydroxyurea does not have direct antiretroviral activity, rather, it inhibits immune activation. Some results of the use of hydroxyurea with HAART are promising [120], while others show no enhanced efficacy with its concomitant use with HAART [122, 123, 124].

  (b) Cylosporin: This drug also reduces cell activation. However, clinical trials data with its use are disappointing.

- Short-term accelerated depletion of the $CD4^+$ T cells by induced apoptosis [125].

  Apoptosis: (Extracted from [108]) Also referred to as "cellular suicide," or programmed cell death. Normally when $CD4^+$ T cells mature in the thymus gland, a small proportion of these cells is unable to distinguish self from nonself. Because these cells would otherwise attack the body's own tissues, they receive a biochemical signal from other cells that results in apoptosis. HIV infection can also induce apoptosis in both infected and uninfected immune system cells. The adoption of this approach as a form of therapy is based on the fact that high viral loads in HIV infection are a result of an abundant supply of cells that the virus can replicate in. However, the use of drugs that induce apoptosis as a form of therapy is controversial.

2.3 Guidelines on the Use of Antiretroviral Agents

The guidelines referred to in this section are "Guidelines for the Use of Antiretroviral Agents in HIV-infected Adults and Adolescents" [1]. These guidelines are published by the United States Department of Health and Human Services, and are available online.

Prolonged suppression of plasma viral load is attainable with the available antiretroviral agents. However, eradication of HIV infection has proven to be elusive. The reason

for this is primarily because there is a pool of latently infected $CD4^+$ T cells that is established very early during the acute HIV infection stage and persists with a long half-life, even with suppression of plasma viral load to below detectable levels.

Now that the focus has shifted from virus eradication to managing a chronic infection, the primary goals of antiretroviral therapy, according to the guidelines [1] are:

- reduce HIV-related morbidity and mortality,
- improve quality of life,
- restore and preserve immunologic function, and
- maximally and durably suppress viral load.

Adoption of treatment strategies recommended in these guidelines has resulted in substantial reductions in HIV-related morbidity and mortality.

Plasma viral load is considered as a strong prognostic indicator of HIV disease progression. Reductions in plasma viral load achieved with antiretroviral therapy account for substantial clinical benefits. Therefore, suppression of plasma viral load as much as possible, for as long as possible, is a critical goal of antiretroviral therapy. This goal, however, must be balanced against the need to preserve effective treatment options in patients who do not achieve undetectable viral load due to extensive viral resistance or persistent medication non-adherence.

Viral load reduction to below limits of assay detection in a treatment-naïve patient usually occurs within the first 16-24 weeks (4-6 months) of therapy [1]. However, maintenance of excellent treatment response is highly variable. Predictors of long-term virologic success are stated as [1]:

- potency of antiretroviral regimen,
- adherence to treatment regimen,
- low baseline viral load,
- higher baseline $CD4^+$ T cell count, and
- rapid (i.e. $>$ 1 log 10 in 1-4 months) reduction of viral load in response to treatment.

Successful outcomes have not been observed across all patient populations. However, achieving treatment goals requires a balance of sometimes competing considerations . The guidelines suggests the following strategies to achieve treatment objectives.

- **Selection of Combination Regimen**.
  Several preferred and alternative antiretroviral regimens are recommended for use. These regimens vary in efficacy, pill burden, and potential side effects. A regimen tailored to the patient may be more successful in fully suppressing the virus with

fewer side effects. Individual tailoring is based on such considerations as lifestyle, co-morbidities, and interactions with other medications.

- **Preservation of Future Treatment Options**.
  Multiple changes in antiretroviral regimens, prompted by virologic failure due to drug resistant virus or patient non-adherence, can rapidly exhaust treatment options. While these are valid reasons to prompt a change in therapy, they should be considered carefully.
- **Drug Sequencing**.
  Appropriate sequencing of drugs for use in initial and subsequent salvage therapy preserves future treatment options and is another tool to maximize benefit from antiretroviral therapy. Currently recommended strategies spare at least two classes of drugs for later use and potentially avoid or delay certain class-specific side effects.
- **Improving Adherence**.
  The reasons for variability in response to antiretroviral drugs are complex but may include inadequate adherence due to multiple social issues that confront patients. Patient factors clearly associated with the risk of decreased adherence such as active substance abuse, depression, and lack of social support need to be addressed with patients before initiation of antiretroviral therapy. Strategies to improve medication adherence can improve outcomes.

2.3.1 Recommended Regimens

There is a variety of combinations of antiretroviral drugs that are recommended for the treatment of HIV infection. These regimens are rated as illustrated in Table 2.2. A typical start up regimen could for example be a combination of Efavirenz + lamivudine + tenofovir. This combination has a rating of AII, meaning it is strongly recommended (A) and this recommendation is supported by clinical trials with laboratory results (II). These regimens are presented in Table 2.3, and all utilize replication cycle based antiretroviral agents.

2.3.2 The Need to Individualize Antiretroviral Therapy

The following is an extraction from the top part of table 5 in the guidelines

> Regimens should be individualized based on the advantages and disadvantages of each combination such as pill burden, dosing frequency, toxicities,

Table 2.2: Rating scheme for clinical practice recommendations.

Strength of Recommendation	**Quality of Evidence for Recommendation**
A: Strong	I: At least one randomized trial with clinical results
B: Moderate	
C: Optional	II: Clinical trials with laboratory results
D: Should usually not be offered	
E: Should never be offered	III: Expert opinion

Reproduced from [1].

drug-drug interaction potential, co-morbid conditions, and level of plasma HIV-RNA ...

Regimens are designated as "preferred" for use in treatment naïve patients when clinical trial data suggest optimal and durable efficacy with acceptable tolerability and ease of use.

Alternative regimens are those where clinical trial data show efficacy, but it is considered alternative due to disadvantages compared to the preferred agent, such as antiviral activity, durability, tolerability, drug interaction potential, or ease of use.

In some cases, based on individual patient characteristics, a regimen listed as alternative in this table may actually be the preferred regimen for a selected patient ...

From the guidelines perspective, therapy should be individualized because some regimens are tolerable and/or have better efficacy than others. To this end, some regimens are designated as "preferred", while others are considered alternatives. On the same note, the guidelines acknowledge that there will be variability in the way individuals respond to a particular regimen, preferred or alternative, and in a clinical environment or otherwise.

The underlying cause for this variability in response is complex. In clinical trial conditions where poor adherence has been ruled out, this variability in response has been linked to inter-individual variations in drug uptake, because HIV drug pharmacodynamics, pharmacokinetics and adverse reactions are genetically predisposed [41, 126].

Table 2.3: Antiretroviral regimens recommended for treatment of HIV-1 infection in antiretroviral naïve patients.

	Regimens	**No. of pills**
Preferred Regimens		
NNRTI-based	**efavirenz** + **(lamivudine or emtricitabine)** + **(zidovudine or tenofovir DF) (AII)**	2−3
PI-based	**lopinavir/ritonavir** (co-formulation) + **(lamivudine or emtricibatine)** + **zidovudine (AII)**	8−9
Alternative Regimens		
NNRTI-based	**efavirenz** + (lamivudine or emtricitabine) + (abacavir or didanosine or stavudine) **(BII)**	2−4
	nevirapine + (lamivudine or emtricitabine) + (zidovudine or stavudine or didanosine or abacavir or tenofovir) **(BII)** -	3−6
PI-based	**atazanavir** + (lamivudine or emtricitabine) + (zidovudine or stavudine or abacavir or didanosine) or (tenofovir + ritonavir 100mg/d) **(BII)**	3−6
	fosamprenavir + (lamivudine or emtricitabine) + (zidovudine or stavudine or abacavir or tenofovir or didanosine) **(BII)**	5−8
	fosamprenavir/ritonavir† + (lamivudine or emtricitabine) + (zidovudine or stavudine or abacavir or tenofovir or didanosine) **(BII)**	5−8
	indinavir/ritonavir† + (lamivudine or emtricitabine) + (zidovudine or stavudine or abacavir or tenofovir or didanosine) **(BII)**	7−12
	lopinavir/ritonavir + (lamivudine or emitricitabine) + (stavudine or abacavir or tenofovir or didanosine) **(BII)**	7−10
	nelfinavir + (lamivudine or emtricitabine) + (zidovudine or stavudine or abacavir or tenofovir or didanosine) **(CII)**	5−8
	saquinavir (sgc or hgc)/**ritonavir**† + (lamivudine or emtricitabine) + (zidovudine or stavudine or abacavir or tenofovir or didanosine) **(BII)**	13−16
3 NRTI-based	**abacavir** + zidovudine + lamivudine - *only when a preferred or an alternative NNRTI- or a PI-based regimen cannot or should not be used* **(CII)**	2

Preferred regimens are in bold type † Low-dose (100 − 400 mg) ritonavir per day
Reproduced from [1]

2.4 Treatment Interruption

2.4.1 Reasons for Interrupting Therapy

Interruption of therapy can be for the following reasons [1]:

- Reduce drug exposure and related toxicities
- Autoimmunization or Re-immunization
- Salvage therapy

The problem with HAART interruption is that the virus starts to rebound immediately following HAART cessation, and there is an associate decline in $CD4^+$ T cell counts. The reason for this rebound of plasma viral load is because viral load suppression with HAART does not necessarily imply a reconstitution of HIV specific immune responses [127]. Other reasons for the rapid viral load rebound when HAART is interrupted are the over stimulation of the immune system during infection [92] and the availability of new target cells due to $CD4^+$ T cell gains incurred during HAART.

There are fears that therapy interruption has a similar effect on the virus as does not adhering to therapy in that they cause the virus to become resistant [128, 129, 130, 131]. Some authors argue that even high levels of adherence do not prevent the emergence of drug resistant mutants [119], and that the pre-existence of resistant mutants is the primary cause of drug resistance [118]. The later is supported by the outcome of many clinical trials where treatment interruption did not lead to drug resistance. In any case, the associated viral rebounds can also increase the transmission of the virus.

Structured treatment interruptions - STI, for the purpose of reducing the total time on HAART and drug exposure is getting a lot of attention because of the growing concern over the adverse side effects of HAART. This has been tried out primarily on patients with previous viral suppression [132]. The intention is to reduce the toxicity associated with antiretroviral drugs, given that one has to use them indefinitely. One such study on Strategies for the Management of Antiretroviral Therapies [133] aimed to "strike a balance between adequately aggressive treatment and minimal side effects". This was a long term study that was intended to cover a period of up to nine years with some patients on either STI or continuous HAART.

STI as an immune based therapy for autoimmunization, is meant to allow short bursts of viral replication to augment HIV specific immune responses. The general intention is to use STI to shift the infected individual to a state where one can attain a degree of viral load control without antiretroviral drugs, or hopefully, attain the long term non-progressor status. Clinical studies have been carried out using this approach on patients

who had initiated therapy during the acute and chronic infection stages and had a record of sustained viral suppression to below detectable levels [15, 16, 132, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146]. Results indicate that autoimmunization has more benefit in the acute infection stage and less in the chronic stage. Individuals who initiate therapy during the acute infection stage are therefore more likely to attain long term non-progressor status, than those who wait to initiate therapy in the asymptomatic and advanced stages. Unfortunately, this favours a small percentage of HIV infected persons because the majority are already in the chronic infection stage.

One of the objectives of STI should be to improve the ability, or find ways, to determine immune competence in chronically infected patients, and to develop tests that may then predict which patients will be the best candidates for STI [127]. The general idea is that STI, just like therapeutic drug monitoring (TDM), is not for every body. This poses the question: is it possible then, for one to tell in advance, whether or not the individual will benefit from STI?.

For those patients who have virologic failure, treatment interruption can be employed for salvage purposes. The intention of this is to allow the re-emergence of the virus strain that responds to therapy. This has been tried out in [147, 148, 149] and results show that more harm than good is done in most cases, as the $CD4^+$ T cell count often drops to very low levels while the resistant virus strain remains.

2.4.2 Structured Treatment Interruption Protocols

One approach to treatment interruption strategies is to monitor either the viral load or $CD4^+$ T cell count. Therapy is interrupted or resumed when the monitored variable rises above or below predetermined upper and lower bounds. In essence, it entails keeping the variable between an upper and lower bound by on/off control. Another approach to treatment interruption is to have predetermined time periods for when therapy is on and when it is interrupted. The former approach is more difficult to implement when compared to treatment interruption with predetermined on/off periods, as it requires more frequent measurements in order to check if the variable is above or below the cut off points.

Tables 2.4, 2.5 and 2.6 are summaries of clinical STI trials that had positive, neutral and negative outcomes, respectively. The criteria used to determine the success or failure of the trials is rather subjective. The trials generally had varying objectives and determinants of success or failure. Furthermore, the STI protocols that were adopted vary.

Table 2.4: Summary of clinical trials with a positive outcome

Entry Criteria		**No of**	**Treatment**	**Results**	**Reference**
CD4$^+$	**VL**	**Patients**	**Schedule**		
>300	<50	10	7 days on/7 days off for up to 68 weeks	Viral control maintained ↓ Side effects	[132]
$\frac{CD4}{CD8}$ >1	<20	12	Off until VL > 3000 copies mL^{-1} or max. 30 days off	Viral control maintained	[150]
$\frac{CD4}{CD8}$ >1	<50	12	Off until VL > 3000 copies mL^{-1} or max. 30 days off	Progressive ↓ in viral replication ↑ HIV-1 specific T-cell response	[145]
>500	<20	10	1 month off / 6 months on or VL > 200 copies mL^{-1}	↓ Viral setpoint ↑ HIV-1 specific T-cell response	[139]
Varied	Varied	3	3 weeks on/1 week off	No emergence of drug resistance ↓ Viral setpoint	[14]
>500	<50	8	Off until VL > 5000 copies mL^{-1}	↑ HIV-1 specific T-cell response	[16]
<200	<50000*	68	8 weeks off	↑ CD4$^+$ T cell count ↓ Viral load	[147]

[151].
∗ Salvage therapy

Table 2.5: Summary of clinical trials with a neutral outcome

Entry Criteria		No of	Treatment	Results	Reference
$CD4^+$	VL	Patients	Schedule		
>400	<400	8	30 days on/30 days off for 7 months	↑ HIV-1 specific T-cell response No viral control	[142]
Varied	<500	14	Range: 14-196 days off	Viral rebound to pre-HAART levels	[152]
Varied	Varied	5	Varied	↓ Latently infected cells	[153]
>150	≥5000**	10	28 days on/28 days off	No ↑ drug resistance	[154]
>400	<200	3	Median 7 days off for 11 cycles	Transient ↑ in T-cell response Virus rebound in all patients	[155]
Varied	<50	11	Varied	Resistance ≠ interruptions	[156]
>350	<50	18	Off until VL > 5000 copies mL^{-1} or CD4 decline by 25% from baseline	Viral rebound within $2-3$ weeks	[135]
>300	<50	97	2 weeks off/8 weeks on for 4 cycles	No change in setpoint	[143]
>300	<50	133	2 weeks off/8 weeks on for 4 cycles	Viral load similar to pre-HAART levels	[136]

[151].
** Therapy naïve patients

Table 2.6: Summary of clinical trials with a negative outcome

Entry Criteria		No of	Treatment	Results	Reference
$CD4^+$	VL	Patients	Schedule		
>300	<60	14	2 weeks off/8 weeks on for 4 cycles	Viral rebound within 8 days	[137]
>300	<50	52	4 weeks off/8 weeks on	↑ Drug resistance	[157]
>350	<50	600	7 days on/7 days off or CD4 < 350	The majority of patients exhibit viral rebound > 500 copies mL^{-1}	[20]
Varied	Varied	40	Median 214 days off	↑ AIDS events	[158]
Varied	Varied	2	Varied	↓ HIV-1 specific T-cell response	[159]

[151].

2.5 Chapter Summary

The immune system is made up of different types of white blood cells (lymphocytes), antibodies and some active chemicals [108], whose responsibility it is to defend the body against any disease causing foreign invader. When the HI virus enters the body, it *directly* seeks out the immune system cells because the virus can recognize the CD4 receptor on their surfaces. Some immune cells have the freedom to circulate in plasma or reside in any of the other body compartments. Consequently, these cells not only facilitate virus replication, but its dissemination as well. Macrophage cells are particularly notorious for trafficking virus between compartments.

Besides the depletion of $CD4^+$ T cells during HIV infection, the way the immune system responds to the infection is impaired on multiple levels. There is a breakdown in immunocompetence and certain parts of the immune system no longer function and certain cells types are even depleted. There is over stimulation of the immune system, which is characterized by an over supply of lymphokines in the bloodstream. So while HIV is multiplying in infected $CD4^+$ T cells and macrophages, $CD8^+$ T cells are simultaneously attempting to further shut down the immune system. Furthermore, activated

$CD4^+$ T cells are prone to apoptosis. This leads to the depletion of a subset of cells with specific immune responses to the HI virus.

There are many antiretroviral agents that have been approved for the treatment of HIV. These agents can be categorized as replication cycle based, or immune based. Replication cycle based drugs are the reverse transcriptase, protease and entry inhibitors, so named depending on the stage of the stage of the replication cycle that they disrupt. Immune based drugs modulate the immune system.

Treatment interruptions can be for autoimmunization purposes, for salvage therapy, or to have drug holidays. Whatever the reason, viral load rebounds will be observed with each treatment interruption cycle for many patients, especially those in the asymptomatic and advanced stages of the HIV infection. Furthermore, $CD4^+$ T cell declines during HAART interruption are considered as undesirable, especially when the T cell count did not rebound adequately while one was still on HAART. To this end many clinical trials have been carried out in an attempt to come up with treatment interruption protocols that will work for most individuals.

Many individuals with chronic HIV infection will fail to attain long term non-progressor status with structured interruptions of therapy. For these individuals, who represent the majority of HIV infected persons, there is need to focus on estimating the time before the viral load rebounds, as well as monitoring both the viral load and $CD4^+$ T cell count so that therapy can be resumed before the viral rebound occurs, or T cell counts drop to dangerous levels. There is also need to explore other therapeutic options that will slow down the viral rebound and/or $CD4^+$ T cell decline during HAART interruption.