Agent based modeling of viral infections: an investigation across several spatio-temporal scales
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1. Introduction

The past century has been characterized by the fastest growth of both scientific and technological knowledge in human history. This quest to understand the laws of nature and its complex phenomena has led, among other things, to the formulation of quantum physics, the birth of informatics, and the sequencing of the human genome. The scientific progress of the 20th century turned into technological applications that, for better or for worse, reshaped life on the planet. Although the 20th century vastly increased the quality of life in many countries, it also brought us new and more challenging problems. The technological and scientific growth has strongly influenced our society and has been accompanied by dramatic changes, such as the exponential growth in the world population and energy consumption [1,2], the doubling of the average life expectancy [3], the growth of large metropolitan areas and world airline traffic, and globalization. The latest change in our society has been Internet and the widespread use of electronic devices that have exponentially increased the communications and connections among individuals across the planet [4].

The major problems we have to face today and the evolution of our society under the pressure of new technologies are strongly correlated [5]. Some of these challenges are closely correlated to the exponential growth of the population worldwide and of the growth of metropolis and megacities, e.g. traffic and pollution in cities, worldwide energy consumption and the increased risk of pandemics. It is not surprising that as a society becomes more complex also its problems grow in complexity. Luckily new knowledge and tools are developed to study and understand these new challenges as soon as they are identified.

1.1 Background and Motivation

Among all the complex problems of modern society, the study of infectious diseases is clearly one of the most important. Both ethical and economical issues demand solutions to cure and prevent the
devastating effects that viral infections have on a society so tightly connected and densely populated as ours. One of the most studied viruses is Human immunodeficiency virus (HIV), a virus of the Retroviridae family. In the past 10 years HIV-1 has caused about 2 million deaths per year and the latest UNAIDS report [6] estimates that about 35.3 million people are currently infected. Although the development of the antiretroviral therapy has greatly increased the life expectancy of HIV infected individuals, the number of AIDS related deaths was still 1.6 million in 2012. A huge amount of resources has been invested globally on studying all the different aspects of the HIV infection: from the molecular and intracellular level, to the dynamics of its spread in patients and in countries. Several other viruses have been studied due to their fast spreading mechanisms (airborne transmission) like the influenza viruses and the SARS coronavirus (SARS-CoV) responsible for the severe acute respiratory syndrome. The “Spanish flu” caused over 50 million deaths worldwide in 1918 and the H1N1 influenza pandemic of 2009 was a reminder of their continued health risk and significant economic impact [7]. In 2003 an outbreak of SARS caused 774 deaths over 8098 reported cases, with a high fatality rate of 9.6%. New research projects are being funded to investigate the different scales of threat, from the epidemiological spread of a virus in a population to its early phases of the infection in a cell culture.

The emergence of drug resistant viral strains and the high genetic variability of both HIV and influenza viruses have shown that these solutions need to be dynamic, since any static solution risks to be weakened and eventually nullified by the evolution of the viruses under the selective pressure of drugs. The knowledge that modern biology and medicine have of the complex interplay between viruses, our immune system and drugs has shown the need to understand this adaptive complex system in order to provide long-term dynamic solutions. An example of this complexity is the emergence of HIV mutants resistant to anti-retroviral drugs and the ability of the influenza virus to mutate and reassort its genome that force us to constantly work on drugs and new vaccines. Only a system able to
simulate the biological mechanisms of this complex system and predict how viruses react to our strategies against them will allow our society to minimize the risk of future pandemics.

1.2 Complex adaptive systems

Scientific research in the 20th century has proven that nature, despite its relatively simple fundamental laws, is too complex to be understood by only examining its individual constituents. The complexity hidden in many natural phenomena has shown the limitations of a purely reductionist approach and the rise of a new philosophical approach to science well summarized by a quote from Aristoteles: “the whole is more than the sum of its parts”.

Despite more than 60 years have passed since Warren Weaver’s definition of disorganized and organized complexity [8], the definition of complexity is still controversial. A universal measure of complexity has not been defined yet, and complexity is often well defined in relation to a system or a specific field. The reason is that when considering a specific measurable quantity it is easier to first intuitively grasp and later define when one system is more complex than another. All complex systems consist of a large set of interconnected, mutually (and typically nonlinearly) interacting parts [9] and their macroscopic behavior is an emergent property of the individual interactions. John Holland defined complex adaptive systems as “systems that have a large numbers of components, often called agents, that interact and adapt or learn” [10]. For each complex system it is correct to say that the whole is more than the sum of its parts: conscience cannot be understood by the electrical impulses of a single neuron, a single water molecule cannot be perceived as “wet” and the immune response cannot be described by the interaction of two individual immune cells. Thus emergent properties are possessed by the whole system and not by any of its individual parts. Due to this characteristic it is necessary to study the system both in terms of its components and in terms of their interactions.
1.2.1 The new challenge

In order to study complexity and to create tools for managing complex problems the reductionist approach used so far to study nature and its fundamental laws would not allow for an appropriate level of understanding. According to reductionists any system, no matter how complex, is nothing but the sum of its parts and by examining its individual constituents even emergent phenomena could be understood. Historically using a minimal set of simple principles has succeeded in understanding and predicting relatively simple systems. This top-down approach studies complex systems in terms of its microscopic constituents and was sufficient to drive our understanding of nature from the birth of science until the 20th century. Recently the need to understand more complex problems led to the development of new approaches: connectionism, a bottom-up, data-driven, approach for which a system is a synthesis of its constituent parts (e.g. artificial neural-networks) and collectivism which merges the understanding of the constituent parts of a system and their interactions that characterize the system as a whole [ref. book]. It is in this collectivist and deeply interdisciplinary approach that rests the key to understand complexity and complex systems. A good example of the connectionist approach is the work of Soren Brunak and Ole Lund on the use of artificial neural networks applied to the prediction of T-cell epitopes [11]. An artificial neural network is a computational model in which simple artificial nodes called “neurons” are connected together to form a network. The neurons are the processing elements and the strength of the connections between neurons is represented by a set of adaptive weights. Such weights are tuned using a learning algorithm and, together with the interconnection pattern between neurons, determine the machine learning and pattern recognition properties of artificial neural networks. In this approach neural networks are used as a black box representing the complexity of the binding of a T cell receptor with an antigen. Huge amount of data of known binding events between different sequences is used to train the model to make predictions on the binding affinity of new epitopes. The amino acid sequences are
used as input for the model with no attempt at understanding or representing the complexity of the protein folding that is instrumental to the binding of receptor and antigen. This approach does not aim at understanding the complexity of a given problem. Instead it maps that complexity into another complex system that is easier to handle, namely a complex set of neural connections. This new complex system is then treated as a black box to be trained with known data of inputs and outputs, initial conditions and outcomes. Once the training is successful and the mapping of a given complex problem is complete, this black box will be able to predict with good accuracy the behavior of that specific complex problem without the need to understand it.

1.3 Complexity of viral infections

Viral infections are caused by viruses that interact at molecular level with the receptors of a host cell. They hijack the molecular machinery of the infected cell, replicate, spread to other cells in the host organism, and then, depending on the specific type of virus, infect others organisms by different transmission methods (sexual transmission, direct contact, airborne transmission, etc.). Thus, the infection takes place at various spatio-temporal scales and it can be investigated at each of these scales.

The grand challenge is to understand infectious diseases across all these spatio-temporal scales: from the intracellular events happening within a few milliseconds at the molecular level up to the transmission of the infection within cities and countries at the epidemiological level. One of the most studied viruses is the human immunodeficiency virus (HIV). HIV is a positive-sense single-stranded RNA virus belonging to the Retroviridae family. HIV is a lentivirus responsible for the acquired immunodeficiency syndrome (AIDS). It damages the adaptive compartment of the immune system by slowly wiping out the CD4+ T helper lymphocytes, disabling the adaptive immune response and leaving the infected patients without protection from opportunistic diseases. The nature of HIV infection, as that of all viral infections, is multiscalar, spanning from the molecular interactions
within the host cell to the replication dynamics in the infected individual immune system up to its transmission between individuals. HIV and its interactions with the immune system have the characteristics of a complex system at each spatio-temporal scale and as such it can be studied at each of these levels.

1.4 Modeling of viral infections

The human body is a complex adaptive system that includes an enormous amount of unique, discrete and hierarchical components: millions of molecules form a single cell, thousands of cells are combined into tissues, various tissues form organs, and all the organs function together to keep a human alive. Additionally human beings constantly interact with their environment and with each other in a society of more than 6 billion individuals. This hierarchical system of nonlinearly interacting components crosses many orders of magnitude in temporal and spatial scales. One of the greatest scientific challenges of 21st century is to understand, quantify and eventually replicate in silico this complex multiscale system and is the goal of the Virtual Physiological Human project. The progress done within this program on several organs, for example the heart and blood vessels, is now leading to clinical applications of the in silico models and to personalized, evidence-based medicine.

Despite the success in some areas, modeling of the immune system lags behind. Much still needs to be done, both in terms of biological experiments and computational modeling, before a realistic multi-scale in silico model of the immune system can be integrated in the clinical practice. There are several reasons for such delay: on one hand there is a general lack of experimental data on several components of the immune system. Much information is still missing on the interactions between all the cells and molecules of the immune system, no map of the lymphatic vessels is available, and little is known on the trafficking of immune cells between the complex network of lymph nodes, blood vessels and tissues; on the other hand the human immune system and its interactions with pathogens are, together with
the nervous system, the most complex system in our body, so even if all the information would be available, it would still be a challenge to develop a complete computational model.

The immune system is composed by a large number of different, non-linearly interacting, discrete microscopic constituents that through self-organization lead to the emergence of a macroscopic behavior: the immune response. Agent-based modeling, mathematical modeling, medical research, and systems biology have to join their individual efforts to achieve a complete understanding of the immune response, and computational science is the tool needed to successfully integrate all these different fields.

1.4.1 From mathematical modeling to cellular automata

Mathematical modeling as a tool for the study of viral infections started in the early 1960s, if we neglect few exceptions. The first models focused on epidemics more than on the infection dynamics within the host with the hope to eradicate all infectious diseases with the use of the newly discovered antibiotics and vaccinations. [12]. Several years later the emergence of new infectious diseases like hepatitis and HIV and the progress in the understanding of molecular biology and immunology led to the development of models for the infection dynamics within host [13,14] and inside the infected cells [15].

The most commonly used models to investigate the disease dynamics are still based on ordinary differential equations. In models using ordinary differential equations discrete entities like cells and viruses and their individual properties are homogenized into parameters and variables that describe entire populations. They assume the system to be spatially homogeneous neglecting spatial characteristics that could play a non-trivial role in the disease dynamics. Models using partial differential equations take into account the spatial component but still represent discrete entities as a continuum [9].
The development of computer science and the diffusion of computers as tools for scientific investigation allowed cellular automata and agent-based models to become an established modeling paradigm to investigate viral infections at all spatio-temporal scales. Cellular automata models are spatially and temporally discrete. The first research on cellular automata dates back to 1948 when von Neumann introduced self-reproducing automata as an alternative approach to the study of biology. During the following 50 years research on CA slowly progressed until the late 80s and the foundation of the Santa Fe Institute, a center for interdisciplinary study of complex systems. Several different types of cellular automata have been developed with different characteristics depending on the problem in analysis. In the list below Ilachinski [9] reported the characteristic possessed by most CA models:

- **Discrete lattice of cells**: the system substrate consists of a one-, two- or three-dimensional lattice of cells.
- **Homogeneity**: all cells are equivalent.
- **Discrete states**: each cell takes on one of a finite number of possible discrete states.
- **Local interactions**: each cell interacts only with cells that are in its local neighborhood.
- **Discrete dynamics**: at each discrete time unit, each cell updates its current state according to a transition rule taking into account the states of cells in its neighborhood.

Among the applications of CA modeling to biology there are models on plant growth [16], on the propagation of infectious diseases [17], tumor growth [18], and on the dynamics of HIV infection [19]. A famous CA model from Pytte et al. [20] was used to model a region of the hippocampus with up to 10,000 neurons. This model gives qualitative and quantitative data that otherwise would require the numerical solution of about 250,000 coupled differential equations.
1.4.2 Agent-based models

Agent-based models (ABMs) are an extension of CA. The main characteristics that differentiate ABMs from CA are the potentially asynchronous interactions triggered by discrete events. Agents do not necessarily perform actions simultaneously as happens in CA. In addition in agent-based models the agents do not tile the simulated environment and are not necessarily grid-based.

Agent-based simulations aim at studying both quantitatively and qualitatively complex systems for which the microscopic, simpler rules of the interacting constituents are well known. ABMs are appropriate to investigate hypotheses on the sources of emergent behaviors in the macroscopic system and are an ideal framework for interdisciplinary research, allowing, for example, the integration of physical properties on the diffusion of cells in a media with biological knowledge on the intracellular biochemical processes. The possibility to take into account the detailed knowledge on specific cellular processes makes this methodology appropriate for the simulation of biological systems, where the heterogeneity of the interacting components are not safely reducible to a simplified categorization. For example modeling unique receptors on the surface of immune cells, a key element in the dynamics of the immune response, is relatively simple using agent-based modeling, whereas to simulate thousand of sub-species of the same cellular type would be impractical if not unattainable using differential equations.

In the past 20 years several agent-based models were developed to simulate the immune system as a complex adaptive system whose global behavior emerged from the collective microscopic interactions of its constituent agents. The first attempt at modeling the immune response using ABM is the famous IMMSIM by Franco Celada and Philip Seiden published in 1992 [19]. This initial attempt paved the way to the development of several other models simulating the complex interplay between pathogens and specific mechanisms of our immune system. Some focused on simulating the immune dynamics of
specific infectious diseases [21] or tumor growth [22]. Other agent-based models simulated specific processes involved in viral infections, like the dynamics of the diffusion in tissues [23] or intracellular chemical reactions [24]. Agent-based models have also been extensively used to simulate the immune dynamics of other infectious diseases [25, 26]. One of the most complex immune system simulators is C-ImmSim, an evolution of the original Celada-Seiden model. C-ImmSim and its extension to cancer simulation SimTriplex have been used to run in silico experiments in virtual patients.

1.4.3 C-ImmSim

Thanks to the scientific research in Immunology and Biology there is enough knowledge on the elementary components and their interactions to develop in silico frameworks and models of the immune response.

C-ImmSim is one of the most complex simulators of the immune response. This model has been developed over more than 10 years and its latest version is able to simulate in detail both the cellular and humoral immune response in a three-dimensional volume of a simplified lymph node [27]. It belongs to the class of bit-string models [28]. Most of the entities in the system are characterized by specific strings of bits that represent the binding sites of their receptors. Interactions among the cells and pathogens occur as a function of the matching between such binary strings. C-ImmSim is able to simulate several microliters of lymphatic tissue and all the main cellular entities normally present in it. The cellular entities modeled are macrophages and dendritic cells, responsible of presenting the antigens to activate the adaptive immune response, T helper (CD4+) lymphocytes, the immune cell playing a central role in both humoral and cellular immune response, cytotoxic lymphocytes able to kill infected cells, B cells responsible for the humoral response by the production of pathogen-specific antibodies and epithelial cells. Each cell in the system can be considered as a stochastic finite state machine that changes its state according to the stochastic interactions with other
cells and molecules in the system.

Another key characteristic of C-ImmSim is the possibility to run experiments on different virtual patients. The use of bit-strings to simulate the major histocompatibility complexes (MHC) class I and class II expressed on the cells surface allows the simulation of the variability of immune systems present in different individuals. Thus the parameter that describes the MHC defines the specific virtual patient whose immune response will be simulated in the model.

C-ImmSim has several free parameters that were tuned over the years to replicate the biological functions of the immune system. The basic mechanisms of the immune response, such as the timing of primary and secondary immune response to bacteria and inert antigens, were used to tune the parameters that regulate the formation of immunological memory and the binding affinity thresholds for the triggering of the immune response. Disease specific parameters, for example mutation rates of HIV epitopes and HIV slow damage to the hematopoiesis mechanism, and the variability of immune response to HIV in different individual were tuned using clinical data on the time-to-AIDS of untreated patients and on the proportion of rapid progressors, long-term non progressors and normal progressors. The latest C-ImmSim version also models the effect of antiretroviral treatment on the HIV infection. The parameters that control the effect of combined antiretroviral treatment have been tuned using clinical data from twenty-two patients from the Spallanzani hospital in Rome [29].

In terms of spatio-temporal scales C-ImmSim simulates the immune response with a time step of 8 hours, although in one experiment the model parameters were tuned to work with a time step of 10 minutes [30]. Spatially it models individual cells in a volume of several microliters of lymphatic tissues, whereas temporally it can simulate the whole HIV infection, which lasts several years.
1.4.4 Modeling the different spatio-temporal scales

The complex interplay between the immune system, pathogens, and drug therapy is a hierarchical complex system and as such it happens at several spatio-temporal scales: the intracellular processes that lead to the infection of host cells and the release of new viral particles; the diffusion of viral particles and cells in tissues and the cellular interactions that constitute the immune response; the transmission of infectious viral particles within cities and countries. From the modeling point-of-view the challenge lies in the complexity of the model, which might require extensive programming work, the computational resources necessary to simulate the system in analysis and more often the lack of biological knowledge or experimental data for modeling key processes and validating the results. Agent-based models rely on experimental data to validate their results. Since ABM simulates the microscopic dynamics and its spatial properties, it often requires additional experimental data compared to traditional ODE models. Such additional data usually refers to specific biological processes that are only indirectly related to the problem in analysis, for example the diffusion coefficients of all the entities involved.

1.4.4.1 Microscale, intracellular processes

Although HIV life cycle might appear relatively simple, the complexity of the HIV infection at this spatio-temporal scale lies in its interaction with the host cell machinery and its ability to evade intracellular immune response. These complex phenomena happen at a temporal scale of a few hours within the host cell, about 8 μm in diameter, and the basic constituents are the different molecules of the virus and of the host cell. HIV infects the cells of the immune system, mostly T lymphocytes and macrophages, by binding its viral surface protein gp-120 to the CD4 membrane protein present on both cell types. After the fusion of the viral membrane with the cellular one, the HIV capsid is released in the cell cytoplasm. HIV relies on the reverse transcriptase, a viral enzyme that reverse transcribes the RNA genome into a double-stranded DNA. During this stage, viral protein reverse
transcriptase is likely to make mistakes in the genome transcription and is responsible for the high mutation rate of the HIV and its ability to develop resistance to antiretroviral drugs. After the viral DNA is transported into the host cell nucleus, another viral enzyme, the integrase, integrates the viral genome in the DNA of the infected cell. The integrated DNA may either lie dormant in the host cell genome or actively produce new copies of the virus using the host cell machinery. The intracellular processes that lead to the viral infection of a host cell have been studied using different modeling techniques from deterministic to stochastic \[31\] and from mathematical to cellular automata and agent-based models \[14, 32-37\]. In a mathematical model based on a system of coupled ordinary differential equations (ODEs) \[14\] each sub-process of the viral replication cycle is represented by one or more equations. Although mathematical models represent the overall behavior of the system, they tend to neglect the role of spatial and topological dependencies. The advantage of ABM at this scale is that proteins and molecules are modeled as individual agents and the complex behavior of the system emerges from their interactions. ABM has been widely used in modeling phenomena at cellular and molecular levels \[32-37\]. Some models include cellular details such as membrane with lipid bilayers, substrate molecules and enzymes with reaction rules and metabolic pathways.

1.4.4.2 Mesoscale, intercellular dynamics

At this spatio-temporal scale we can observe the dynamics of the diffusion of viral particles from infected cells and their interactions with neighboring cells. The temporal scale of the events observed ranges from minutes to days. The spatial scale is in the order of a few hundreds micrometers or a few hundreds of cells. The microscopic interactions are those of the infectious viral particles with the cells, including diffusion of the virus, receptor binding and viral entry in the cells, while the macroscopic effect is the outcome of the infection, usually the time to the death of the whole population of cells. At this scale the physical processes like the virus diffusion and cellular growth play a major role and can be studied in detail.
The first models studying the infection dynamics at intercellular level were ODE mathematical models that aimed at extracting key parameters of the infections dynamics [12,38,39]. As the importance of the spatial component on the infection dynamics became evident the modelling approaches shifted to cellular automata (CA) models [26,40-42]. A good example of the advantages of CA modeling is the comparison between Bocharov’s [39] and Beauchemin’s models [26] for the influenza infection. Bocharov uses an ODE model with 13 variables and 60 parameters to fit the experimental data, while Beauchemin’s model manages to reproduce the infection dynamics using only 7 variables and 12 parameters.

1.4.4.3 Macroscale, individual patients

The dynamics of the complex interaction between HIV and the immune adaptive response can be studied at larger temporal (weeks, months and years) and spatial (lymph nodes of a few microliter, several thousands of cells) scales. At this spatio-temporal scales the basic constituents are the immune cells and viral particles that diffuse within lymph nodes of several microliters. Scientific research aims to understand how the complex dynamics of the adaptive immune response emerges from the simple interactions among the different cells of the immune system and their interactions with an antigen. At this spatial scale there are several phenomena that take place at different temporal scales: the primary immune response, which usually lasts between a few days to a few weeks, following the first exposure to a foreign antigen, the secondary immune response, lasting just a few days, due to the presence of memory cells [43] and slower phenomena like the emergence of drug resistant strains of HIV over period of years due to the selective pressure of antiretroviral treatment [44]. Its ability to mutate, that takes place at intracellular level, leads to macroscopic effects such as evading the immune response and developing resistance to antiretroviral drugs. The effect of HIV reservoirs on host survivability and the effect of combined
antiretroviral treatment (cART) on the infection dynamics also occur at this scale.

On first approximation the minimum subset of cells involved in the adaptive immune response are T helper lymphocytes, macrophages, dendritic cells, cytotoxic lymphocytes, and B cells. For each cell type there are sub-populations characterized by their specificity toward given antigens. Each specific sub-population of cells in an immune system co-evolves with sub-populations of a different cell type according to a fitness function that is, in part, itself a function of the emerging immune system. For example a B cell with the right specificity toward a given antigen is activated by a T helper cells able to recognize that antigen. The activation of an immune cell is often followed by its clonal expansion and the formation of memory cells that permanently change the immune system.

The specific sub-populations of cells define the co-evolving immune system, which, in turn, determines the fitness function according to which its sub-populations of immune cells, the constituent parts, evolve. The uniqueness of the entire immune system is the result of its temporal evolution. Such evolution is determined by the nonlinear feedback between the sub-population of immune cells (the microscopic level) and the immune system defined collectively by those sub-populations (the macroscopic level).

A number of mathematical models describe the HIV infection dynamics and the related immune response [refs]. The most common mathematical models represent only the dynamics of the HIV virus and T cells, making only a distinction between T cells in different states (Healthy, Infected, Dead). A few mathematical models considered the role of additional immune cell types in the infection dynamics adding either macrophages [45] or cytotoxic T lymphocytes [46]. Some ODE based models take into account the use of cART [47,48]. Still results of mathematical models tend to be generic, giving high-level assessments of the infection dynamics. The study of HIV through cellular automata and agent-based models is also common
due to the discrete and stochastic nature of the biological entities involved in the phenomena.

1.4.4.4 Population scale

Although in this thesis we have not studied this spatio-temporal scale it is worth mentioning that this is the largest scale at which viral infections can be studied, neglecting the even larger effects of evolution across centuries. The infectiousness of the virus, the types of transmission (droplet contact, direct contact, vector borne, sexual transmission etc.), and the fatality rate represent and approximate the complexity of the lower scales. At this scale the challenge is hidden in both the complexity of the social network that links all the individuals in a community and in the dynamics of the spread of infections between individuals. Epidemics have been studied extensively using mathematical models, the first model dating back to since 189x [52]. After the discovery of the properties of complex networks, epidemiological studies have been performed using this new modeling approach [53-55]. The most evolved models of epidemics are the complex agent networks, which combine characteristics from both agent-based and complex network modeling.

1.5 Thesis

The challenges our society will face in the coming century have surpassed the need of descriptive models or simple predictive models. The next scientific challenge is not just to predict the behavior of a single virus under specific conditions; it is to anticipate their reaction to our strategies and actions against them. A multi scale system able to simulate in silico the evolution of the complex interplay between viruses, the immune system and the eventual antiviral drugs is necessary in order to develop such a dynamically predictive model.

Our thesis is that agent-based modeling offers an appropriate tool for a real multi-scale in silico experimental environment, a “virtual patient”, and that critical holes in our understanding of both the
modeling paradigm and the biology at each spatio-temporal scale need to be addressed. We argue that this challenging goal requires a collectivist and interdisciplinary approach and that agent-based modeling (ABM), due to its characteristics, provides one of the most appropriate tools for this new scientific approach.

This thesis consists of two parts:

- The models developed in this thesis provide information about the missing biological knowledge needed to reproduce in silico the natural interaction between viruses and the immune system at each spatio-temporal scale;
- The new insights on the dynamics of HIV infection prove that ABM is more suitable to predict the dynamics of virus-immune system-drugs interactions than other traditional descriptive models.

1.6 Outline of the Dissertation

In Chapter 2 we investigate the dynamics of the first cycle of HIV intracellular replication at the level of a single cell. A cell is modeled as an individual entity with certain states and properties. The model is stochastic and keeps track of the main viral proteins and genetic materials inside the cell. We evaluate and compare two different simulation approaches: the first is a rate-based approach in which, like in an ordinary differential equations model, discrete viral proteins are treated as continuous entities and rates dictate the physical transformations of the viral proteins during HIV life cycle; the second is a classic agent-based modeling approach in which discrete viral proteins and genome are represented as entities moving in a two-dimensional space representing the cell cytoplasm and nucleus. In this agent-based modeling approach events are driven by the interactions between the agents when they collide in the simulated space. The results of the simulations are compared based on the number of integrated viral cDNA and the number of viral mRNA transcribed after a single round of replication. Both approaches are validated with
available experimental data. Simulation results and their validation give insights about the details of HIV replication dynamics inside the cell at the protein level. In addition the model highlights the strengths and weaknesses of the two different approaches.

In Chapter 3 we focus on the complexity of an in vitro infection of SARS-CoV in a population of cells, increasing the spatial scale by two orders of magnitude. We present a cellular automata model describing critical aspects of in vitro viral infections taking into account spatial characteristics of virus spreading within a culture well. The aim of the model is to understand the key mechanisms of SARS-CoV infection dynamics during the first 24 h post infection. We use data from the infection of human lung epithelial cells to tune the free parameters in the model. We also perform a sensitivity analysis of key parameters using a Latin Hypercube sampling to identify which mechanisms are critical to the observed infection of host cells and the release of measured virus particles.

In Chapter 4 we increase both spatial and temporal scales of several orders of magnitude to simulate HIV infection in a cohort of 250 virtual patients. At this level we model the complex interplay between the human immune system, antiretroviral drugs and HIV using C-ImmSim, an agent-based model that tracks individual immune cells and viral particles inside a single lymph node of 4 microliters. We use this complex model to find the main cause of the failure of structured treatment interruptions observed in several clinical trials. Although continuous antiretroviral therapy is currently the most effective way to treat HIV infection, unstructured interruptions are quite common and cannot be prevented. Several attempts to structure these interruptions failed due to an increased morbidity compared to continuous treatment but its causes are poorly understood and often attributed to the emergence of drug resistance. Here we show that structured treatment interruptions would fail regardless of the emergence of drug resistance. Our computational model of the HIV infection dynamics in lymphoid tissue inside lymph nodes, demonstrates that HIV reservoirs and evasion from immune surveillance themselves are
sufficient to cause the failure of structured interruptions. We validate our model with data from a clinical trial and show that it is possible to optimize the schedule of interruptions to perform as well as the continuous treatment in the absence of drug resistance. Our methodology enables studying the problem of treatment optimization without having impact on human beings.

In Chapter 5 we investigate the effect that temporary antiretroviral therapy has during primary HIV infection on the disease progression in individual patients. The current evidence is insufficient and the understanding of the dynamics of temporary combination antiretroviral treatment (cART) during primary HIV infection is still controversial. Here we quantitatively predict the sustained response of the immune system to a temporary cART based on the temporal correlations between CD4+ cells and HIV viral particles during the natural course of the HIV infection. We calculate the temporal correlations as a function of time post-infection from in silico generated patient data. The in silico experiments are performed with the same clinically validated agent-based model used in the previous chapter. Our analysis predicts that after the acute phase, during which cART is known to have a long-term beneficial effect on the immune system, there is a secondary phase of about ten months during which temporary cART still has a relatively long-lasting effect on a patient’s immune system compared to the asymptomatic phase. We validate our results using recent clinical trial data from Primo-SHM and provide an explanation to the unexpected beneficial effect of cART observed in the Primo-SHM study. The discovery of a secondary phase of sustained response may lead to significant changes to the HIV treatment guidelines.
References:

14. another mathematical model of viral infection


2. Modeling HIV-1 Intracellular Replication: two simulation approaches

2.1 Introduction

In the field of virology and infectious diseases, mathematical and computational models are developed to provide a better understanding of the underlying biological phenomena and medical processes. In recent years, many computational models have been developed to investigate the complexity of HIV dynamics, immune system response, and drug therapy [1-5]. Simulation of HIV infection can vary from modeling the entire immune response (under virus infection and drug therapy) [30] to simulating the intracellular processes at a molecular level. A concrete example of this wide-range multi-scale modeling is *HIV Decision Support: From Molecule to Man* by Sloot et al. (2009) [6, 7]. Despite the wide range of modeling for HIV dynamics, more and more attention is being paid toward single cell analysis to understand intracellular processes.

At cellular and molecular level different modeling techniques, from deterministic to stochastic [12] and from mathematical to agent-based models [8, 9, 11], have been used to simulate the intracellular process of viral replication. A mathematical model by Reddy et al. (1999) [8] was used to study intracellular kinetics of HIV-1 replication. Reddy’s model is based on a system of coupled ordinary differential equations (ODEs) that is solved numerically. Each sub-process of the viral replication cycle is represented by one or more equations. The model provides concentration-based insights on how the overall replication cycle depends on its constituent reactions. Although mathematical models are able to represent the overall behavior of the system, a disadvantage is that they are less helpful in capturing the individual interactions of the system components, ignoring spatial and topological dependencies. On the other hand Agent-Based Simulation (ABS), an emerging field in modeling microbiological phenomena, has been widely used in modeling at cellular and molecular levels [3, 9, 13, 14, 15]. In ABS cells and molecules are modeled as individual agents and the complex behavior of the system emerges from their
interactions. A model using ABS principles was CellAK by Webb et al. (2004) [9]. It includes cellular details such as membrane with lipid bilayers, substrate molecules and enzymes with reaction rules and metabolic pathways. The cellular entities are modeled as agents and the cell behavior emerges from the interaction between agents. Although agent-based modeling has advantages in modeling biological and microbiological systems, such models are computationally more expensive than ODE mathematical models. This problem is becoming less important nowadays with fast supercomputers and distributed computing. In modeling HIV infection, agent-based models are generally large-scale and no intracellular agent-based model exists that specifically concerns HIV.

In this paper, we have modeled the HIV-1 intracellular replication and the virus kinetics inside the cell. The rest of this article is organized as follows: In Section 2 we describe the HIV replication process and present the general model design. Section 3 introduces the two simulation approaches used for implementation. Section 4 presents the simulation results, which are comparable with the experimental data. Section 5 is the conclusions and future work.

2.2 Modeling HIV-1 intracellular replication

Despite having a simple structure, HIV has a complex dynamics inside the cell. HIV replication first requires the virus to enter an uninfected host cell such as CD4+ cells or macrophages. After the virus enters the cell, it utilizes the cell machinery for replication and release of new virions from the cell surface. Figure 1 (a), taken from Gene Therapy Journal in ref. [31], illustrates the intra-cellular replication process of HIV-1 from entering the cell to releasing new virus particles from the cell. For simplicity, we have only simulated the major steps that are critical in the viral replication process. Figure 1 (b) shows the general model design and the main replication steps in the model marked in red. The following 7 steps are considered in the model: Reverse transcription, nuclear transport, integration, cell activation, mRNA transcription, transport to the cytoplasm and translation.
Figure 1. (a) HIV intracellular replication cycle: Entry (Binding to CD4 receptor (1), Co-receptor binding (2), Fusion (3)), Reverse transcription(4,5), Transfer to the nucleus(6), Integration (7), Transcription and regulation(8,9,10), Export to the cytoplasm (11), Translation (12), Assembly, budding and maturation(13,14,15) [31]; (b) 7 main steps of viral replication in the model.
Virus entry to the host cell is not simulated in our model and initiation of infection is modeled as a stochastic process explained in Section 2.1. The cell is modeled as an individual entity with certain states and properties. Over the course of simulation time, we assume the cell to be non-dividing and non-interacting. In what follows we describe the cell infection and different cell states.

2.2.1 Cell infection

Cell entry is an important part of the HIV host infection and is target of many drug treatments. In our simulation we decided not to model the entry in detail because a simple description was not enough to represent such a complex mechanism and we preferred to investigate this aspect in a later study [33]. Moreover, the experimental data used to validate the model is based on quantitative methods that use multiplicity of infection (MOI) as a measure of infection. MOI is the ratio of virus particles to cells or the average number of virus particles per cell. In real experiments the MOI is determined by dividing the number of viruses per ml by the number of cells per ml. Therefore, we used the MOI as a measure of infection, rather than measuring the percentage of infected cells by explicitly modeling the cell entry, until we have the chance to access data with details on the entry process. Cell infection is thus simulated as a stochastic process. For each run the cell may get infected (with 1 or more viruses) or remain uninfected based on the MOI. Although the MOI represents the average number of viruses per cell, the specific number of viruses that infect any given cell follows a Poisson distribution [16]. In a population, the proportion of cells infected by a specific number of viruses is $P(n)$,

$$P(n) = \frac{m^n e^{-m}}{n!}$$

where $m$ is the MOI and $n$ is the specific number of viruses infected the cell. For example if $m = 1$, a higher proportion of cells will get infected with one virus or will not get infected at all ($n = 0$), while some cells may get infected with two or more viruses. We have used this approach to substitute the entry process and initiating the cell
infection. The value of MOI is an input to the model and is specified at the start of the simulation.

2.2.2 Cell states and transitions

The life cycle of HIV inside the cell is arbitrarily divided into two distinct phases: The early phase refers to the steps of replication from cell entry to integration of viral cDNA into the cell DNA, whereas the late phase refers to the post-integration steps from cell activation and mRNA transcription to budding and release of new viruses from the cell [18]. In this model we have defined 6 states for the cell and each cell has associated a specific state at a time. At each time point the cell can be in one of the following states: Uninfected (\(T_{UI}\)), early infected (\(T_{EI}\)), latently infected (\(T_{LI}\)), and actively infected (\(T_{AI}\)), productively infected (\(T_{PI}\)), and dead (\(T_{D}\)). \(T_{UI}\) refers to a healthy cell, \(T_{EI}\) is an infected cell before the viral genome is integrated into its DNA, \(T_{LI}\) is the state of a cell which has an integrated provirus in its genome. \(T_{AI}\) is the state of an activated cell which produces new viral mRNAs transcripts, and \(T_{PI}\) is the state of an activated cell that starts to produce new viral particles from its surface. Hence, the state of the cell may change during its lifespan. Figure 2 shows the state transitions of a cell during the viral replication process.

\[ \text{Figure 2. Cell internal states and transitions} \]

The death rate of the cell might change during its lifespan. It is possible that the infected cell death rate is low initially and increases
during cell state transitions. However, the dependency on cell-death rate on the cell age is still not clear [17]. So, we assumed the death rate of the cell to be independent of its age. Hence, the rate of cell dying is stochastic for an individual cell and constant over a population of cells. An Uninfected cell dies with a constant rate $\mu_{UD} = 0.03$ per day, while an infected cell dies with a higher rate of $\mu_{ID} = 0.5$ per day [18]. The death rates are rescaled to the time step of the simulation and used as a probability for stochastic cell death.

2.3 Model implementation: two different approaches

Two simulation approaches have been used to implement the virus replication model: Rate-based approach and diffusion-based approach. In the rate-based approach the quantities inside the cell are defined as a set of variables and their rates of change are inferred from literature. On the other hand, the diffusion-based approach defines the internal state of the cell by simulating the molecular quantities inside the cell as agents. The movement of these agents is described by random walk and events occur based on rules defined upon collision between agents. Cell infection and viral replication are stochastic processes and are modeled as such in both approaches. The simulation is time-driven in both approaches and the update scheme is by time advance. The simulation time advances uniformly by a constant time-step at each iteration. We chose a time step of 0.2 minute (12 seconds) in the simulation. This time is small enough to capture the level of detail we are looking at and long enough for completion of occurring chemical reactions such as DNA synthesis and transcription.

2.3.1 Stochastic rate-based approach

In the stochastic rate-based approach the cell is an individual entity and the intracellular quantities are a set of variables known as internal variables. These variables are used to determine the cell state and keep track of the molecular quantities inside the cell. The cell internal variables are: VRNA (viral RNA), VDNA (viral DNA in both cytoplasm and nucleus), PROVIRUS (integrated cDNA), MRNA
(mRNA transcripts), and VP (translated viral protein). At the start of
the simulations all variables are set to 0 and if a cell gets infected the
number of viral RNAs infecting it is assigned to VRNA. The value of
each internal variable changes based on its current value and its rate of
change. Figure 3 shows the structure of a single cell. Squares are the
internal variables and arrows are their rates of change. An incoming
arrow shows an increase in the variable value, while an outgoing
arrow shows a decrease.

Figure 3. Cell structure in the rate-based approach. Squares are the cell
internal variables and arrows are their rates of change. An incoming arrow
shows an increase in the variable value, while an outgoing arrow shows a
decrease.

Different steps in viral replication occur with different rates. These
rates are inferred from literature and listed in Table 1. The rates of
major steps in HIV replication are: Reverse transcription, nuclear
transport, integration, activation, mRNA transcription, exporting to
cytoplasm and translation rates. Each rate is rescaled to the time step of the simulation and is used as a probability for stochastic occurrence of events. As soon as the cell is infected ($T_{EI}$) the reverse transcription process occurs with the average rate of $k_{rt}$ nucleotides per minute where the length of HIV RNA component is 9479 nucleotides [19]. The reverse transcription process mainly consists of 3 steps: First synthesis of the first-strand DNA from the viral RNA, next synthesis of the second-strand DNA and finally the full-length double-strand DNA formation which leads to viral RNA degradation. We have considered these 3 steps of reverse transcription by creation of the first and the second-strand DNAs in our model which together form up a full-length viral DNA. Nuclear transfer rate, $k_{transfer}$, is the rate by which the viral DNA produced by reverse transcription transfers to the nucleus. The viral DNA in the nucleus is integrated into the host genome, degraded or circulated. DNA circularization is neglected in our model because it is very limited [28]. The viral DNA in the nucleus is integrated into the host cell DNA with rate $k_{integration}$ and the cell becomes latently infected ($T_{LI}$). A latently infected cell may become activated with an activation rate of $k_{activation} = 3 \times 10^{-3}$ per day. Activation of the cell is important for a latently infected cell to produce infected mRNA molecules by transcription and marks the passage from early stages of infection to the late stages.

In the previous model of HIV intracellular replication by Reddy et al. [8] all cells were considered to be in an activated state at the time of infection, whereas in our model we make a distinction between activated ($T_{AI}$) and inactivated ($T_{LI}$) cells. At every time step we check for the cell activation. As soon as the cell is activated the transcription process is initiated, otherwise no transcription of viral genes occurs in the infected cell. mRNA transcription is obtained by cellular factors and cell activation and there are three major classes of transcripts: unspliced, singly spliced, and multi-spliced [20]. In our simulation, we are not modeling the splicing event of mRNA transcripts and only consider the full-length mRNAs. Splicing plays regulatory roles and is required for efficient mRNA transcription. It basically adds a delay to the process of transcription, which is balanced in our simulation by the
time delay added for stochastic cell activation. The cell produces mRNA transcripts at every time step with an average transcription rate of $k_{\text{transcription}}$. The viral mRNA transcripts are exported to the cell cytoplasm by the export rate $k_{\text{export}}$. In the cytoplasm the mRNA transcripts are translated to viral proteins with translation rate $k_{\text{translation}}$. The list of rates and parameters used in this approach is summarized in Table 1.

Table 1. Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse transcription rate</td>
<td>165 nucleotides per minute</td>
<td>[21]</td>
</tr>
<tr>
<td>Activation rate</td>
<td>$3 \times 10^{-3}$ per day</td>
<td>[22]</td>
</tr>
<tr>
<td>Integration rate</td>
<td>4.5 copies per hour</td>
<td>[8]</td>
</tr>
<tr>
<td>Transcription rate</td>
<td>1000 copies per hour</td>
<td>[8]</td>
</tr>
<tr>
<td>Translation rate</td>
<td>262 proteins per hour</td>
<td>[8]</td>
</tr>
<tr>
<td>Export rate to the cytoplasm</td>
<td>2.6 copies per hour</td>
<td>[8]</td>
</tr>
<tr>
<td>Transfer rate to the nucleus</td>
<td>0.012 copies per hour</td>
<td>[10]</td>
</tr>
<tr>
<td>Half life of mRNA</td>
<td>0.2 per hour</td>
<td>[8]</td>
</tr>
<tr>
<td>Elimination rate of proteins</td>
<td>$1.4 \times 10^{-5}$ proteins per second</td>
<td>[23]</td>
</tr>
<tr>
<td>Death rate of uninfected T cell ($\mu_{UD}$)</td>
<td>0.03 per day</td>
<td>[18]</td>
</tr>
<tr>
<td>Death rate of infected T cell ($\mu_{ID}$)</td>
<td>0.5 per day</td>
<td>[18]</td>
</tr>
</tbody>
</table>

The modeling flowchart of the virus intracellular replication cycle (in the rate-based approach) is presented in Figure 4. At the start of the simulation cell gets infected randomly based on MOI. The function Replication is executed at each iteration in the simulation. Details of
the replication function are shown in the flowchart with a remark that the function checks the occurrence of an event at each step by taking a random number between 0 and 1. The event occurs if the random number is within the range of the event rate, otherwise it goes to the next step.

The advantage of this approach in comparison to ODE models is first the stochasticity of the model which appears at every step of virus replication. Also cells in the model are considered as individual entities and by looking into these individual cells one can obtain a better understanding of the dynamics of virus replication process at a single cell level.

Figure 4. Flowchart of the rate-based model showing the simulation of HIV intracellular replication.

2.3.2 Stochastic diffusion-based approach

In the diffusion-based approach we are simulating the HIV intracellular replication based on the movement of particles inside the cell. The cell is an individual entity in the model and includes the cellular and viral compartments, which have a significant role in the viral replication cycle. The cellular compartments are: the cell DNA,
nucleus, cytoplasm (cell area) and tRNA (transfer RNA), while the viral compartments are viral RNA, viral DNA, viral mRNA and viral proteins. We have defined a 2-dimentional grid of 100×100 for placing the cell. The position of the cell is assumed to be constant during the simulation run. The compartments inside the cell are modeled as agents with some properties and behaviors such as position, size and movement. The agents in the model are as follows:

- **Cellular nucleus**: The cell nucleus has a size and a fixed position in the center of the cell.
- **Cellular DNA**: The cell DNA has a size and a fixed position in the center of the cellular nucleus.
- **Cellular tRNA**: The cell tRNA is randomly positioned inside the cell cytoplasm. It binds to the primary binding site (PBS) of the viral RNA and act as a primer for initiation of reverse transcription process.
- **Viral RNA**: HIV contains 2 viral RNAs which are released inside the cell cytoplasm after infection. Viral RNA randomly moves in the cytoplasm area of the cell.
- **Viral DNA**: Viral DNA is the product of reverse transcription process. Viral DNA randomly moves inside the cell area (both the cytoplasm and the nucleus). The viral DNAs inside the nucleus are identified as viral cDNA.
- **Integrated DNA**: The viral DNA which is transferred to the nucleus and is integrated into the host cell DNA.
- **Viral mRNA**: Viral mRNA is produced in the nucleus and can be exported to the cytoplasm. mRNAs randomly move in both nucleus and cytoplasm areas of the cell.
- **Viral protein**: Viral proteins are produced in the cytoplasm by translation of viral mRNAs.

At each time step the moving agents can move one grid point, either left, right, up or down, within the cell area or remain in the same grid position (Finite size effects are not studied). The movement of viral particles inside the cell is known to be based on both diffusion and
moving along microtubules [24]. Movement along microtubules leads to a faster transfer of particle toward the cell nucleus. In this simulation, we are modeling the movement of particles only based on the diffusion and the diffusion coefficient of particles is tuned to compensate this assumption in the model. The tuned value for the diffusion coefficient is 0.010415 $\mu m^2/sec$, which is approximately 50 times bigger than the largest diffusion coefficient known for HIV particles inside the human 293T cells (50-220 $nm^2/sec$) [29]. The advantage of this approach is that we take into account the spatial effects of the cell which cannot be represented in the rate-based model. We chose the cell radius equal to 40 grid points (gp). The average T cell radius is between 4 to 4.5 $\mu$m [25]. Considering the cell size 4$\mu$m each grid point would be 0.1$\mu$m. In T lymphocytes the diameter of the cell is approximately twice that of its nucleus. Hence, we used the value of 2 for the cell to nucleus size ratio [25, 26], so the nucleus radius would be 20 gp. If the length of T cell DNA is 0.6$\mu$m, given that each grid point is 0.1 $\mu$m, the DNA length would be 6 gp. The amount of tRNA in the cell is a high value (198000 per cell [25]) however, not all the tRNAs are available to the virus, as a primer for reverse transcription, and a large proportion is used by the cell machinery. Hence, the initial value of the tRNA is used as a free parameter in the model. At the start of simulation, the cell randomly gets infected based on cell infection model (Section 2.1). Then the cell and agents are assigned to the simulation grid. At each time step every moving agent performs a random movement over the grid area and checks for collision. Events in the model occur upon collision between agents and collisions will be processed by the agent-collision rules listed in Table 2.
Table 2. Agent Collision Rules

<table>
<thead>
<tr>
<th>IF COLLISION</th>
<th>THEN (event)</th>
<th>Rules</th>
</tr>
</thead>
<tbody>
<tr>
<td>(agent1-agent2)</td>
<td>Rules</td>
<td></td>
</tr>
<tr>
<td>vRNA-tRNA</td>
<td>Reverse transcription</td>
<td>Produce vDNA, and vRNA degradation</td>
</tr>
<tr>
<td>vDNA-cell nucleus</td>
<td>Nucleus transfer</td>
<td>vDNA transfers from the cytoplasm to the nucleus (cDNA)</td>
</tr>
<tr>
<td>cDNA – cell DNA</td>
<td>Integration</td>
<td>Produce integrated provirus and reduce cDNA</td>
</tr>
<tr>
<td>mRNA – cell nucleus</td>
<td>Export to cytoplasm</td>
<td>mRNA export from the nucleus to the cytoplasm</td>
</tr>
</tbody>
</table>

Figure 5. (a) Simulation visualization of early stages of the simulationviral RNAs (red) are assigned to the cell cytoplasm environment. The purple circles are the cellular tRNA available in the cell (b) Simulation visualization of late stages of simulation: viral mRNA (small green) mainly in the cell nucleus, and translated viral proteins (big green) in the cell cytoplasm. The purple circles are the cellular tRNA available in the cell.
Cell activation, transcription and translation occurs with the same rates and conditions explained in the rate-based approach. We have considered these rates constant over time. Figure 5 a shows a snapshot of the model visualization at the early stages of the simulation. The visualized entities are: the cell, the nucleus and the cellular DNA which are all positioned on the center of the grid, while viral RNAs (red) and cellular tRNAs (purple) are randomly positioned in the cell cytoplasmic area. Figure 5 b shows a snapshot of the model at the late stages of the simulation where viral mRNA (small green) is transcribed from the cell DNA inside the nucleus and viral proteins (big green) are translated in the cell cytoplasm.

The model is implemented in Java programming language for both approaches. We have used MASON (A Multi-Agent Simulation library core in Java [27]) libraries and 2D visualization tools in our simulation. The result data of each cell at each run are recorded in a Hash Table at certain time points. These times can be changed at start of the simulation according to the interest of the experiment. The data saved in the history files are used later for analyzing the results in MATLAB.

2.4 Simulation results

Although we modeled the intracellular processes at a single cell level, we are analyzing the results over a population of cells. This is closer to what happens in in-vivo and in-vitro experiments, where measurements are an average over all the cells in a volume of blood or on a petri dish. In order to compare our model results with the experimental data we run the simulation for several populations of cells and average over these populations. At each run we compute the first replication cycle up to 72 hours post-infection, corresponding to 3 days, which is approximately 12 hours more than the average life-span of an infected cell. In what follows we will discuss the simulation results of both approaches described in Section 3.1.

The initial number of cells in the simulation is set to $10^3$ which is the average number of cells in a ml of blood [1]. Integration of viral DNA into the host cell DNA is a key step in the virus replication cycle and if
it doesn’t occur, the virus is incapable of fully replicating and producing new virions. In experiments conducted by Scott et al. [28] detection of integrated DNA was accomplished using fluorescence-monitored PCR. They used the MOI = 0.4 in their experiments and limited their measurements to a single cycle of HIV replication. We used the same MOI value in our simulation which indicates that there is on average 0.4 viruses per cell. The simulation results of integrated cDNA are shown in Figure 6. The graph in 6 a is a result of the rate-based approach, while 6 b refers to the diffusion-based approach. The blue (solid lines) line is the average amount of integrated cDNA per cell over time and the red (dashed lines) lines are the standard deviation of the output. Error bars show the experimental data that are measured in 12, 24, 48, and 72 hours post-infection [28]. The concentration of the integrated cDNA first rises, reaches a plateau and then slowly decreases. The decrease in the number of integrated cDNA reflects the death of infected cells. The integrated cDNA is also detected earlier in the simulation results comparing to the experimental data. This difference is explained by the limited sensitivity in detection and accurate quantification of integrated cDNA in PCR experiments [32]. The simulation results of integrated cDNA are consistent with the experimental data in [28]. However, there is an over estimation in the first 36 hours of the diffusion-based approach, which is due to the assumptions made on the diffusion of particles inside the cell. The particles inside an infected cell are in different sizes and have different diffusion coefficients, but we have assumed the same diffusion coefficient for all moving particles. Although this assumption influences the final biological results of the diffusion-based approach, we are fixing it by improving the missing data values. Nonetheless, the modeling approach would still be the same.
Figure 6. Simulation results of integrated cDNA per cell at MOI = 0.4 (average over 10 simulation runs of 1000 cells); (a) Rate-based approach; (b) Diffusion-based approach.

Viral mRNA is the product of transcription process. Once the cell is activated, the transcription process starts. We have measured the
average copies of mRNA transcripts per cell produced over 1000 cells at different MOIs and compared it with experimental data on genomic HIV-1 mRNA molecules. In experiments conducted by Barbosa et al. [10], mRNA of two groups of patients with different viral loads was measured. The patients in the first group had a high viral load and patients in the second group had a low viral load. Accordingly, we choose a range for MOI from 0.01 to 10 and run the simulation for certain values in this range. Then we plot the average amount of mRNA transcripts produced after 72 hours in each run versus the value of MOI in that run (Figure 7 a). The y-axis is the amount of mRNA transcripts produced per cell (72 hours post-infection) and x-axis is the MOI range. Figure 4 a is the results of the rate-based approach and each data point is an average over 1000 cells. The red bars show the range of mRNA produced per cell in the two groups of patients with high (solid bar) and low (dashed bar) viral loads. From these data points we observed that the simulation results of viral mRNA produced per cell is within the same range ($1.0 \times 10^2$-$1.0 \times 10^2$ genomes per cell) as the data collected from patients in experiment [10]. Figure 7 b illustrates the trend of viral mRNA production over time for certain MOI values in the rate-based approach. We can see that as the value of MOI increases, the cell activation and transcription processes start earlier and more mRNA transcripts are produced.
2.5 Conclusions

We presented a model of HIV-1 intracellular replication, where infected cells undergo a single cycle of virus replication. The cell is an individual entity and cell infection is modelled by a stochastic process. The model keeps track of the main viral proteins and genetic materials inside the cell during the virus replication. Using this model we analyzed each step of the viral replication cycle from reverse transcription to translation. Two simulation approaches were used for implementing the model: rate-based approach and diffusion-based approach. In the rate-based approach the intracellular dynamics of the cell is based on the rates that are inferred from literature, while in the diffusion-based approach the dynamics is based on the modelling intracellular compartments as diffusing agents. Therefore, in the diffusion-based approach the spatial information of the cell is taken into account. The simulation results on the amount of cDNA integrated into the host DNA and the number of mRNA transcripts produced per cell were consistent with the experimental data.
Both simulation approaches are stochastic and by looking at individual cells we get a more realistic description of the dynamics inside the cell. This is a more precise description of the in-vivo and in-vitro experiments compared to the large-scale deterministic models in the literature. For future directions the model can be adapted or extended in both single-cell level and population level. The diffusion-based approach can be extended at the single cell level to focus more on the behavior of the cell under viral replication. A more detailed description of the replication processes such as the movement of particles along the microtubules or the regulatory effects of mRNA splicing will allow a better understanding of the intracellular mechanisms. At a population level the rate-based approach can be extended to simulate re-infection and interaction between cells such as cell-cell transmission of the virus. So far, our model focuses on the first round of replication, while it can be enhanced to have a second, third or multiple rounds of replication. Both approaches in modeling HIV intracellular replication as a whole can be used for investigating the effect of various HIV inhibitors or new drug agents on the replication process. Effect of various drugs can be modeled either in the form of rates or real agents. This will help to identify the efficiency of drugs acting at different stages of virus replication.

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3. A Simulation Framework to Investigate in vitro Viral Infection Dynamics

3.1 Introduction

In the past ten years there has been a growing interest in modeling viral infections to study the characteristics of their dynamics. Early mathematical models were typically based on ordinary differential equations (ODEs) and focused on extracting key parameters of the infections dynamics [1, 2]. Later on, interest moved to investigating how spatial relations affected the system dynamics, thus moving to cellular automata (CA) models [3,4,5]. More recently the attention has shifted focus to viral respiratory diseases due to the increasing danger of pandemics such as the “Spanish flu” that caused over 50 million deaths worldwide in 1918 or the more recent H1N1 swine flu. The risk of new pandemics has a high impact on society and new research projects are being funded to investigate the different scales of threat, from the epidemiological spread of a virus in a population to its early phases of the infection in a cell culture. Among the papers published on this topic, in particular on the influenza virus [20,21,22], there are Bocharov’s ODE model [6] and Beauchemin’s CA model [3,4,7]. Both models described the dynamics of the influenza infection in the upper epithelial areas of the lungs and were validated by clinical data. Although they take into account the effect of the immune system on the dynamics of the disease, describing the infection until its final outcome (more than a week after the first infection), none of them analyzed which mechanisms where critical in the dynamics of the first and second round of infection. To understand the key mechanisms of viral respiratory diseases, we focus instead on early phases of viral infection by using in vitro experiments observing lung cells up to 24 hours post infection (PI). The in vitro experiments provide measurements of virus titer, spatial characteristics of cell growth and, through green fluorescent protein (GFP) imaging, of the infection spread.

Our computational model focuses on simulating the early stages of a
viral infection in a population of cells plated on a culture well. The choice of a CA model was natural since the in vitro infections being studied use host lung cancer cell lines that form a fixed mono-layer in which spatially dependent aspects of infection may be present [8,9]. We developed this computational model using the Multi-Agent System Visualization (MASyV) platform [3]. In contrast to previous models, we explicitly focus on the dynamics of virus spread on a population of cells, supported by experimental data from an in vitro model system. We also explicitly model the infectious viral particles as discrete entities, whereas in previous models the infection of cells followed simple CA rules depending on the states of neighboring cells. These viral particles are released by infected cells according to a specific function based on time post infection, and move over the well with a random walk algorithm. This representation allows us to model the mechanisms of virus spread in an environment where the virus is not confined and can also infect cells not adjacent to the infected ones.

In Section 2 we describe the model design and its main features. We also describe the SARS infection experimental data used to parameterize the model. In Section 3 we present a sensitivity analysis that identifies the critical mechanisms characterizing the early phases of the infection. We also show that the model can explain the experimentally observed virus titer data and allows a deeper understanding of the infection dynamics in the in vitro experiments.

3.2 Materials and Methods

3.2.1 Simulation Environment

The computational model is built using Beauchemin’s MASyV platform. The software consists of a server providing I/O and supervisory services to the various client modules where the simulation is actually coded. Our choice to use MASyV was partially driven by flexible and powerful graphical visualization routines that facilitate comparison to images provided by the experimental collaborators. MASyV has a C-based API and is open source allowing
finalized custom models to be easily shared. We discuss novel contributions and differences from the previous modules. The original module details are presented in Beauchemin et al. [3].

Our model reproduces a viral infection on a population of cells plated on a culture well. In our client we consider, as the target of the viral infection, Calu-3 cells that are a human airway epithelial cell line derived from human lung cancer. We model these host cells using a 130x130-site CA model where each site represents either one calu-3 cell or an empty space. At the beginning of the simulation each lattice site is initialized and labelled with “uninfected” or “empty” states as described below in Section 2.2. Uninfected cells are initially stochastically infected with virus through a first round of infection at the beginning of the simulation, described in Section 2.3, and once infected progress through the following states:

- Containing: initial infection state representing viral entry and hijacking of host cell mechanisms necessary for viral replication.
- Expressing: cell is actively producing and assembling virus capsids and genomes internally, but has not begun releasing virion.
- Infectious: Assembled virion is being released from the host cell according to the release function (Section 2.4)
By examining the experimental viral titer data shown in Figure 1 we derived temporal delay of the state transition between Containing and Infectious. The ~ log₁₀ 3 viral titer measurements at time points 0, 4 and 7 hours post infection are residual virus left over from the initial infection after washing. We set the delay for release of new infectious viral particles to 7 hours to represent the jump in observed virus titer between 7 and 12 hours. Cells release virus particles according to a viral release function, described below. Virus particles are entities that diffuse from one lattice point to another according to a random walk algorithm (Section 2.4). These free-floating virus particles stochastically infect uninfected cells in a second round of infection. The CA lattice is therefore like a tissue of immobile cells with infectious virions moving over it. We represent only infectious viral particles to
relate their count in the model with the viral titer measured in terms of Plaque Forming Unit per mL (PFU/mL). A PFU is a measure of the number of viral particles capable of forming plaques per unit of volume, thus only infectious particles are counted in the viral titer. The CA lattice is updated synchronously and the boundary conditions for both the epithelial and viral particles are periodic, i.e. epithelial cells and viral particles can grow or move outside the boundary of the virtual well. We use a Honeycomb neighborhood: each lattice site is considered adjacent to six sites and cells can replicate only if a neighboring site is empty or if it contains a dead cell. Only uninfected cells are allowed to replicate. When cells on the lattice are initialized all uninfected cells are stochastically assigned a TIME_TO_DEATH value between 1 and an arbitrary CELL_LIFESPAN variable. CELL_LIFESPAN was fitted in order to have natural cell death for 5% of the cells within the simulated 24 hours. CELL_LIFESPAN is not affected by infection as in vitro experimental observations showed no increase in cell death due to infection within 24 hours.

3.2.2 Cell placement

In the laboratory before an in vitro infection is performed, $10^6$ cells are first plated in each of the 6 culture wells present in a dish, and given time to settle down on the well surface to create a cell mono-layer, before the virus is applied to the cell culture. In the plating process, a suspension of cells is plated onto the surface of the well that adhere to the plastic in small clumps or as single cells forming "islands" that are homogeneously distributed throughout the well. Cells located on the inside of these islands (surrounded by other cells) do not replicate due to contact inhibition, whereas cells on the outer edges of each island replicate until they are completely surrounded by other cells. As a result each island continues to grow until there is no adjacent empty space on the well surface.
To represent this behavior we used a set of images (Figure 2) that shows how cells are distributed on the culture well just before the infection is performed. The number of cells forming each isolated island was counted for each image obtaining a distribution of island sizes with an average of 116 cells per island and a standard deviation of 74 cells per island. This data was used to generate randomly positioned clusters of cells on the grid, each with a size extracted from the measured distribution. Islands are placed iteratively until the simulated confluency matches the experimentally measured confluency.

Figure 3 shows lattice initialization using different CONFLUENCY parameter values. A value of .45 was derived based on calculations from well surface area, the total number of cells and the size of a single cell (~20 µm² see ATCC catalogue). This value was used for simulations performed in the results section.
A cell line doubling time is defined as the time required for the population of cells to double in number. We used a doubling time of 48 hours for Calu-3 cells (Ardizzoni Lab, personal communication). Cell density directly affects local cell growth since cells completely surrounded by other cells are not able to replicate. For this reason in our model the initial spatial distribution of uninfected cells affects the overall cell growth on the virtual culture well. In order to obtain a correct cell growth in our model we used a parameter called DIVISION_TIME that measures the time necessary to a uninfected cell to duplicate. We adjusted DIVISION_TIME to match the simulated doubling time with the experimentally-derived one. This was accomplished by analyzing cell growth of uninfected cells over 48 hours as a function of time for different values of the parameter. This led us to fix the value of DIVISION_TIME to 10 hours.

3.2.3 First round of infection

Cells in the culture well are initially infected using a Multiplicity of Infection (MOI) of 2 (see section 2.7.4). The MOI is calculated as the ratio of infectious viral particles to the number of Calu-3 target cells.
By definition the proportion of infected cells is given by the Poisson distribution that describes the infection process [12]. Using the experimental MOI would theoretically give us the exact percentage of infected cells at the beginning of the experiment but even though the number of particles can be measured with good accuracy not all the viral particles used in this first infection process are infective and the estimate is derived using VeroE6 cells. This leads to the definition of an effective MOI, that is the MOI given by the number of particles able to truly infect the Calu-3 host cells. Initially viral particles infect a proportion of the plated Calu-3 cells before being washed away. We use the resulting proportion of infected cells estimated through the standard MOI definition as a starting point of our simulation.

After cells are initialized on the lattice, they are assigned an infected state according to Equation 2, which describes the probability of at least one viral particle entering a cell. In the equations below, $n$ represents the number of virus particles and MOI is the multiplicity of infection used in the experiment. Equation 2, the probability of a cell being infected by 1 or more virus particles, is derived from Equation 1, the probability of being infected by a single virus particle, commonly used to describe viral infection as a Poisson process [13]. Although the expected MOI is experimentally known, as mentioned in the “Experimental Data” section it is often over-estimated. We add the free parameter INFECTIOUSNESS for two purposes: 1) to scale MOI over-estimation and 2) predict the number of initially infected cells when this data is not available. In the sensitivity analysis we discuss the importance of this parameter.

\[
P(n) = \frac{MOI^n \times e^{-MOI}}{n!} 
\]

(1)

\[
P(n > 0) = 1 - P(n = 0) = 1 - e^{-INFECTIOUSNESS \times MOI}
\]

(2)
3.2.4 Viral release and diffusion

After cells transition to an infectious state virus particles are released with a probability described by a sigmoidal function shown in Figure 4. The probability that one or more infectious viral particles are released in each time step is given by $V(t)$ (Equation 3). In this equation $t$ represents time since the cell was infected and parameters $A$, $B$, and $C$ are derived by fitting an experimentally-derived viral release curve produced by Hui et al. [11]. Since we had no direct measure of the amount of virions per cell released by SARS infected cells we rescaled the sigmoidal function with the free parameter VIRALRELEASE. When $V(t) > 1$ an infectious cell will release one or more virions with a probability of 1. Decimal digits less than one are treated as a probability for an additional virus particle to be released.

Figure 4. Viral Release Function: After cells transition to an infectious state virus particles are released with a probability described by this sigmoidal function. Its equation is shown in Eq. 5 and rescaled by the VIRAL_RELEASE parameter.
\[ V(t) = VIR\_RELEASE \times \frac{C}{B + e^{-A \tau}} \]  

(3)

Infectious virions diffuse in the virtual well according to a simple random walk: for each virion in the virtual well at each time step of the simulation we perform a number of Random Walk steps given by the free parameter NUM_DIFF_STEPS. Each viral particle has an equal chance to move to one of the six neighboring lattice sites and at each time step performs NUM_DIFF_STEPS movements on the lattice. The need of using a free parameter for the diffusion of virions was given by the lack of data regarding the virion diffusion coefficient on the well with specific conditions of the media used to cover the cell culture. Viral titer measures only infectious particles present in the media using Plaque Forming Units (PFU) per ml. For this reason we model only infectious particles [13]. In addition it is more computationally efficient to release and diffuse only the infectious viral particles.

3.2.5 Second round of infection

Once released, virus particles may infect cells with an uninfected state that are located at the same lattice site. We assume each viral particle independently infects a nearby cell with a probability following the binomial distribution \( P(n) \) (Equation 4) For each lattice site we compute the probability \( P(n) \) that an uninfected cell gets infected by \( n \) viral particles given \( N \) total local virus particles as follows:

\[ P(n) = \binom{N}{n} p_{BP}^n (1 - p_{BP})^{N-n} \]  

(4)

where \( p_{BP} \) represents the probability of a virus-receptor binding event leading to a cell’s infection by a single viral particle during a time step. We refer to the free parameter \( p_{BP} \) as BINDING_PROB below. Once a cell is successfully infected, \( n \) viral particles are removed from the virtual media located at the lattice site of the infected cell.
3.2.6 Experimental data

3.2.6.1 Viral Stocks

For these experiments we used a virus derived from our severe acute respiratory syndrome coronavirus (SARS-CoV) wild type infectious clone in which we engineered the green fluorescent protein (GFP) in place of open reading frame 7a/b. SARS-CoV GFP stock titers were calculated using standard plaque tittering methods. Briefly, confluent monolayers of VeroE6 cells in 6 well plates were infected with serial 1:10 dilutions (usually 10e-1 to 10e-6) of stock virus for 1 hour at 37°C. The monolayers were covered with a solution of 0.8% low melting point agarose (Seachem), 1X minimal essential media high glucose (MEM, Invitrogen), 10% fetal clone II (Hyclone), and 1X antibiotic/antimycotic (Invitrogen), which solidifies trapping the virus to allow cell to cell viral spread but not release of virions into the media. Plates were incubated at 37°C for 36 hours, stained with neutral red for 2 to 5 hours, the stain removed and the plaques (holes in the monolayer generated when viruses kill the host cells) are visualized and counted on a light box. Stock titers were calculated as plaque forming units (pfu) per mL.

3.2.6.2 Viral infection
Calu-3 cells were plated at a density of 1x10⁶ per well in 6 well plates in MEM containing 10% fetal clone II and 1X antibiotic/antimycotic. Cells were incubated at 37°C at 5% CO2 levels for 2 days prior to infection with a media change 24 hours post plating. SARS-CoV GFP stocks (7.5x10⁷) were used to infect each well at a multiplicity of infection of 2 or the addition of 2 infectious virions per cell in each well. At each time point, 100uL of media was harvested from each well to titer via plaque assay. (Number of cells per well assumed to be 2x10⁶ at 2 days post plating.)

3.2.6.3 Microscopy
Images shown in Figure 2 were taken using phase contrast microscopy at standard exposure times and at 10X magnification. At the indicated
times post infection, images of the infected and mock-infected Calu-3 cells were taken in living cells real time using a fluorescent light to excite GFP [23]. At each time point, three to five fields of SARS-GFP infected cells were assessed using ImageJ (http://rsbweb.nih.gov/ij/). In ImageJ, we gated the light signal to generate spots in each cell and used the spot counting algorithm determine the total number of cells per field. GFP positive cells were then counted and averaged.

3.2.7 Quantifying model fit to experimental data

For each candidate parameter set θ a simulation fitness (Eq. 5) was calculated based on a comparison of two experimental measurements: virus titer and proportion infected cells. Both components of simulation fitness, F(θ), are normalized by a maximum error (ME) term to balance their contribution.

\[
F(\theta) = \frac{F_{\text{titer}}}{M_{\text{titer}}} + \frac{F_{\text{GFP}}}{M_{\text{GFP}}}
\]  

(5)

\[
F_{\text{titer}} = \sum (\log_{10}(V_{\text{Exp}}(t)) - \log_{10}(\alpha \times V_{\text{Sim}}(t)))^2
\]  

(6)

\[
F_{\text{GFP}} = |I_{\text{Exp}} - I_{\text{Sim}}|
\]  

(7)

Randomized weight bootstrapping (1000 iterations) was used to determine MEtiter and the derivation of MEGFP is described below. Equation 6 shows Ftiter is the sum of squares difference between experimentally derived virus titer, VTexp, (Figure 1) and scaled virus titer produced by the simulation, VTSim. Only the last three time points (7, 12, 24 hours) are compared because earlier time point titer values were skewed by residual virus. Simulation output, VTSim, is scaled by α to account for differences in the number of simulated cells versus the number of in vitro cells. Using a lattice size of 130x130 an average of 7,636 total starting simulated cells were produced. The virus titer was scaled by a factor of 10^6/7,636 because the experimental virus titer was produced from a population of 10^6 in vitro cells.
As described in the Experimental Data Section 2.7, in vitro GFP measurements were used to quantify the proportion of infected cells at 12 hours post infection. This additional information was used to constrain our model’s parameter space such as the number of infected cells. The FGFP portion of simulation fitness (Equation 7) is the absolute value of the difference between simulated proportion of infected cells $I_{\text{sim}}$ to experimentally measured proportion infected, $I_{\text{exp}}$. Since $I_{\text{exp}}$ was measured to be .11 (on average 11 of 100 cells are infected) at 12 hours, a maximum error, $ME_{\text{GFP}}$, of .89 was used to normalize FGFP between zero and one.

3.3 Results

3.3.1 Sensitivity analysis of free parameters

To assess the importance of model free parameters to simulation outcome, a Latin Hypercube sampling (LHS) sensitivity analysis was performed [14,15]. This two-tiered sampling method was implemented by dividing each free parameter’s range into 1000 intervals. Intervals for each parameter were shuffled without replacement and then randomly sampled using a uniform distribution. All possible probability parameter values were considered for INFECTIOUSNESS AND BINDING_PROB between 0 and 1. Maximum values for NUM_DIFF_STEPS and VIR_RELEASE were arbitrarily chosen (12 and 30 respectively). A Spearman rank correlation was then used to measure statistical dependence, Rho, between free parameter and single run simulation fitness.

The following are free parameters (described above) contained in our model and assessed using LHS:

- **INFECTIOUSNESS**: scales number of infectious viral particles for the initial infection
- **BINDING_PROB**: Probability with which a single virion infects an uninfected cell in a single time step
- **NUM_DIFF_STEPS**: Number of steps for the diffusion of virions at each iteration (random walk)
• **VIR_RELEASE**: scales the number of virus particles released from each infected cell

![Graphs showing sensitivity analysis results](image)

Figure 5. Sensitivity Analysis Results: Using Latin Hypercube sampling, we examine the relationship between free parameters and simulation fitness. A significant correlation was identified for INFECTIOUSNESS (A) and VIR_RELEASE (D). Spearman correlations and p-values derived using the AS 89 method are shown. Blue lines indicate Loess regression fitted curve. Armand will update these graphs to specify Rho instead of cor, infectiousness instead of viral_fitness and remove p-value estimates.
Figure 5 shows that INFECTIOUSNESS and VIR_RELEASE were significantly correlated with the simulation fitness while BINDING_PROB and NUM_DIFF_STEPS are not. We see that lower values of INFECTIOUSNESS and VIR_RELEASE result in improved fitnesses whereas larger values result in dramatically poor fitness.

Figure 6. Time-Dependent Parameter Contributions: Dynamics of free parameter correlations with virus titer and proportion of infected cells over time are shown above. (A) INFECTIOUSNESS and VIR_RELEASE parameters are shown to be significantly correlated with virus titer. (B) indicates that INFECTIOUSNESS has a strong correlation with the number of infected cells, but eventually becomes insignificant. Spearman correlations are shown and a grey box indicates a lower quality correlation threshold of |.2|.

We also examined how free parameters affect simulation output with time. Using LHS sampling, we tested relations between free parameters and two simulation outputs: virus titer and % of infected cells. Figure 6A shows that INFECTIOUSNESS and VIR_RELEASE are significant over 24 hours of simulated infection and that their correlations start to diverge at ~12 hours. Again, BINDING_PROB and NUM_DIFF_STEPS do not show a significant correlation with virus titer. However, parameters necessary for the second round of infection do impact the proportion of infected cells shown in Figure 6B.
where we see an increase in BINDING_PROB and VIR_RELEASE correlation between 7 and 12 hours, corresponding to a decrease in the INFECTIOUSNESS correlation. This decrease can be attributed to the second round of infection in which a greater population of cells has been infected. The simultaneous increase in correlation of BINDING_PROB and VIR_RELEASE indicates that additional rounds of infection are playing a significant role in viral spread.

3.4 Discussion

Although simplistic compared to in vivo model systems, the interpretation of in vitro experiments is still confounded by biological complexity and disparate data types. Explanatory models are critical for understanding and hypothesis generation. This agent-based modeling framework may be used to investigate first and second round of infection mechanisms using free parameters able to be tuned to allow the model to incorporate disparate types of experimental data. We also take into account spatial aspects of infection, including biases in culture well cell growth and diffusion of infectious virions. Virus titer data and GFP infectivity data from a SARS infection of Calu-3 cells is used as an example to illustrate the model’s capacity to interpret experimentally derived data. LHS sensitivity analysis indicates that a small population of cells is initially infected and that additional rounds of infection are responsible for virus titer measurements.

A significant relationship between INFECTIOUSNESS and both the simulation fitness and simulation outputs (virus titer and proportion infected cells) indicates the importance of this parameter on the resulting infection dynamics. This result also demonstrates the importance of the accurate cell and infectious virus particle counts for the MOI calculation. Finally our model highlights the importance of intracellular processes leading to virus release. One possible future step is to include additional detail regarding intracellular processes of virus replication and move to a multi-scale spatio-temporal model.
Future work is planned to incorporate microarray data and make predictions regarding host response and examine connections between infection status and host response. Simulated annealing has been used to identify free parameters that fit the described model to virus titer data and may be used to predict the number of infected cells or other un-measured data types to support experimental modeling efforts. These off-line predictions could then be used to interpolate a single-cell function representing host response post-infection. We also plan to train the model with data from multiple virus strains to investigate how virus population and host response dynamics differ. Finally, we also plan to investigate the effects of initial spatial distribution of infected cells on viral pathogenesis for multiple virus strains.

References


4. HIV reservoirs and immune surveillance evasion cause the failure of structured treatment interruptions: a computational study

4.1 Introduction

The increase in life expectancy of HIV positive individuals raised both costs and side effects of combination Anti-Retroviral Therapy (cART), stimulating research into Structured Treatment Interruptions (STI). Latest clinical trials on STI [1,2] indicated they are less effective than continuous treatment. We show that the STI tested so far in clinical trials would fail regardless of the emergence of drug resistance and that this failure is caused by HIV reservoirs and immune evasion.

For many HIV positive individuals treatment interruptions are unavoidable. Although clinical studies proved that increased risks are associated to cART interruptions, patient-initiated unstructured treatment interruptions are still quite common in the clinical practice [3]. A recent systematic review [4] of cohort studies and clinical trials indicates a proportion of unstructured treatment interruptions ranging from 5.8% to 83.1% with a median of 23.1%. The mean duration of cART interruptions ranges from 11.5 days to 18 months with a median of 150 days. The main reported reasons for treatment interruptions are laboratory toxicity and clinical side effects. Less frequent arguments are patient compliance, treatment fatigue, intercurrent illness and other reasons.

The problem of unstructured interruptions is getting worse because of the increasing duration of treatment. Recent studies suggest an earlier use of cART as a way to fight effectively the HIV epidemics [5,6]. Current cART guidelines defer the treatment to the time when CD4+ counts drop below 350 (European guidelines - EACS) or 500 cells per microliter (US guidelines - DHHS and IAS-USA) whereas recent studies indicate that an early start of cART (CD4+ counts >500 cells per microliter) could significantly improve survival [5,7-9]. Regardless of
the success of anti-retroviral therapies, HIV’s ability to mutate and evade both antiviral treatments and vaccines shifted the attention from curing affected individuals to fighting the epidemics. Some predictive models investigate the effects of different strategies on the HIV epidemics [6,10]. Recent clinical investigations [9] indicate that a more intensive and earlier use of cART is effective in reducing the spread of the virus. Indeed, another study highlighted the effectiveness of preventive use of cART in reducing the chance of being infected in case of sexual contact with a seropositive individual [11]. Strategies aimed at reducing the spread of infection not only extend the duration of cART for an infected individual but also increase the number of individuals simultaneously under cART, raising the already high global cost of cART treatments.

STI aimed at discontinuing the therapy according to a schedule so as to minimize the side effects without losing substantial protection. In a large, randomized clinical trial [1,2] STI were associated with an increased risk of death and opportunistic diseases connected to treatment interruptions. Earlier studies [12,13] indicated positive results for STI, in some cases associated to supporting drugs [14]. Subsequently, many clinical studies on different STI schedules resulted in generally neutral or negative outcomes [15-17], although the reasons are still not fully understood. Results from the Staccato randomized trial indicated substantial drug savings and did not result in increased drug resistance in the STI arm, while treatment related adverse events were more frequent with continuous treatment [18].

Hereafter we resort to a computational model to gain a better understanding of the reasons of STI failure. A number of mathematical models describe the HIV infection dynamics and the related immune response. Some of them take into account the use of cART [19-21]. Most mathematical models use a continuous description of time, allowing the use of optimal control techniques to search for the best time to deliver the therapy [22-24]. Recently a mathematical model searched for the underlying reasons of STI failure [25]. That model confirms that viral mutation and the emergence of drug resistant
strains may be accelerated by STI, although only activated CD4+ cells and macrophages were considered as possible hosts for the virus. The study of HIV through cellular automata and agent-based models is also common [26-31] due to the discrete nature of the biological entities involved in the phenomena. In this work we use a well-established and validated agent-based model (ABM) of HIV infection [32-36]. See Supporting Information S1 for a brief description of the model used in this work.

Our aim is to gain a better understanding of the reasons behind STI failure: are they inherent to the HIV dynamics or consequential of other mechanisms like the emergence of drug resistance? By excluding to model the resistance to the drugs we restrict the possible causes of STI failure to mechanisms such as virus reservoirs in macrophages and resting/memory CD4+ T lymphocytes. In addition, in this simplified formulation, we search for an optimal STI and compare it to the optimal one found in a previous work [32]. A similar attempt to optimize clinical treatments using in silico modeling has been applied to cancer-preventing vaccinations [37]. In that case the predictions of the computational model were validated through specific in vivo experiments on mice. That paper proved that an integrated in vivo-in silico approach is able to improve mathematical and biological models for cancer immunoprevention.

In the present work we investigate treatment interruptions with two in silico simulations: in the first simulation we test three STI used in clinical trials and compare their efficacy to that of the continuous (i.e., uninterrupted) treatment, while in the second simulation we search for an optimal STI scheduling and compare it with random treatment and continuous treatment. By ‘optimal schedule’ we mean the one that less impairs the ability to mount an immune response while keeping the amount of drugs used to a minimum. We use the Simulated Annealing (SA) technique to search for the optimal STI schedule. We simulate the disease progression for a group of 250 virtual (i.e., in silico) HIV positive patients. For each group of virtual patients we compare the effects of different treatment strategies on the HIV infection over a

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therapeutic period of 48 weeks, three years after seroconversion. We finally evaluate the efficacy of each STI schedule by challenging the immune system of the virtual HIV patients with a simulated opportunistic bacterial infection at the end of the treatment.

4.2 Results

4.2.1 Optimization results

To quantitatively evaluate the effect of a STI schedule of cART we define a fitness function that measures the health of the virtual patient’s immune system and the amount of drugs received over the 48 weeks period. The fitness function is described in the Materials and Methods section. We performed 50 SA optimizations by using different initial conditions. These optimizations resulted in 50 optimized treatment schedules with an average fitness score of 2.325 and a standard deviation of 0.012. The optimal treatment schedules have 29 weeks of therapy on average and a standard deviation of 3 weeks. The optimized schedules are shown in a histogram (Figure 1) in which each bin represents the frequency of a given week of therapy in the optimized schedules.

Figure 1. Optimized HAART Histogram: the histogram shows 50 optimized therapeutic schedules. Each bin represents the frequency of a given week of therapy in the optimized schedules.
The histogram shows a peak every 3 weeks spaced out by 2 weeks in which the drug administration has a lower but still significant frequency. Due to the requirement of providing a schedule as regular as possible, we define as “optimal” a schedule having 2 weeks of therapy followed by an interruption of 1 week for 32 weeks after the initial two months. The optimization shows a different pattern for the initial two months of treatment (Figure 2). The optimal schedule has 29 weeks of therapy and is shown in Figure 2. According to our simulations the last 4 weeks of the therapeutic period have limited importance. In other words, the effect of cART at later times is somehow less important, supporting the current opinion that it is more rewarding to support the immune system with anti-retroviral treatment at earlier times.

Figure 2. At time 0 a virtual patient is infected with HIV. The therapeutic period of 48 weeks starts after 3 years of untreated infection (day 1096). The optimal therapy is shown in the figure. At the end of the 48 weeks of therapy we inject a fixed amount of bacteria in the virtual patient, challenging his immune system. The patient dies if the antigens exceed a threshold of $4 \times 10^6$ bacteria/ml. An efficient therapy is capable of restoring the patient’s immune system enough to contain the bacterial infection.

The aim of the treatment was to suppress the HIV viral load and induce immune restoration, resulting in an immune system capable of
dealing with opportunistic infections. To assess the efficacy of the optimal therapy indicated by the SA algorithm, we ran simulations of an opportunistic infection starting immediately after the therapeutic period. Bacteria were injected in the virtual patients as soon as the 48 weeks of therapy were over. The immune system reaction against those bacteria depended on its efficiency at injection time. We compared the result of the schedule optimized by the SA algorithm with three different simulated control groups (Figure 3).

![Opportunistic Infection – Survival Curves](image)

Figure 3. Survival curves: percentage of survivors over time in a population of 250 HIV+ virtual patients. 30 days after bacterial challenge survival rates are the following: continuous therapy 91.53%, optimal therapy 90%, random therapy 81.85% and void therapy 45.38%. A reduction of 40% of the drug intake corresponds to less than 5% reduction in the survival rate.

In the first group ("Continuous Therapy") the drugs were administered every day for a therapeutic period of 48 weeks as prescribed by the cART guidelines; in the second group ("Random Therapy") the total amount of drug was equal to that of the optimal therapy but administration within the therapeutic period was performed randomly; finally, in the last group, there was no therapy ("Void Therapy"). To measure the effectiveness of the optimized STI we monitored the survival curve in a population of 250 virtual HIV+
patients infected by a bacterium 4 years after their initial HIV infection. For the same 250 patients we compared the effects of continuous therapy, void therapy, optimal therapy and random therapy.

Our results show that the optimal therapy provided a survival rate comparable to the continuous therapy using 40% less drug. Survival rate for untreated patients was 45.4%. The optimal therapy performed significantly better than the void one (survival rate 90% versus 45.4%). The optimal therapy also performed better than a random therapy with the same amount of drugs (a survival rate of 90% vs. 81.9%). The latter result indicates that the schedule identified by the SA algorithm is optimized for both the amount of medicine and the administration schedule, supporting the importance of finding a specific scheduling to reduce therapeutic failures.

4.2.2 Comparison with clinical trials

Even though different structured treatment schedules have been tested in clinical trials, a comparison with multiple trials is often difficult due to the heterogeneity of their experimental conditions. The clinical trials that tested the STI simulated in our work have different durations and different criteria for the enrollment of patients. Such diversity makes it difficult to compare their results with a single optimized schedule. In terms of initial conditions and treatment duration our simulations can be related to the clinical trial by Dybul et al.[16]. As in Dybul’s clinical trial, our simulations have a treatment period of 48 weeks, enroll patients with similar CD4$^+$ cells counts (CD4$^+$ cells $>300$ cells/mm$^3$) and test the STI schedule with 8 weeks of treatment and 4 weeks of interruption. The main difference is that in our simulations we use patients that have been never exposed to HAART and thus with a higher viral load whereas the clinical trial enrolled patients receiving a 3-drug HAART regimen with HIV RNA levels <500 copies/mL plasma for $>6$ months and <50 copies/mL at screening.
Figure 4. Model validation: CD4⁺ cell counts /mm³ at three different time points (Baseline, Week 40 and Week 48). In the upper panel we compare the median CD4⁺ cell counts of 250 virtual patients with the data of real patients from the continuous treatment arm of Dybul’s clinical trial. In the lower panel we compare the median CD4⁺ cell counts of 250 virtual patients with the data of real patients from the STI arm of Dybul’s clinical trial.
The parameters that control the pharmacodynamics of HIV in our model have been tuned by using clinical information from about twenty-two patients selected at the Clinical Department of the National Institute of Infectious Diseases “L. Spallanzani” in Rome [38]. No special tuning has been performed for the model parameters of the three STI schedules tested in the present work. For this reason we can validate our model results by comparing the simulations for both continuous treatment and the structured treatment interruption with data from Dybul’s clinical trial. Those data consists of the median and range of CD4+ cell counts at baseline, at week 40 and at week 48 of the treatment period observed in 52 patients randomized in the continuous and STI arm of the clinical trial. In Figure (4) we compare the simulations results with the values observed in the clinical trial. Simulated CD4+ cell counts are within the ranges observed in the clinical trial.

In Table 1 we show the variation of immunological parameters for all the schedules tested in our virtual cohort. In the table we report the median value and the range observed in the virtual cohort at week 40 and week 48 of the treatment period.

Results of the STI strategies tested in clinical trials are shown in Figure 5. As observed in the clinical trials, all the STI strategies are associated with an increased number of deaths compared to the continuous treatment. The ratio of deaths associated to the “Week On / Week Off” strategy over that of continuous therapy is 1.95 at the end of the 30 days after opportunistic bacterial infection. The same ratio is 3.52 for the “4 Weeks On / 4 Weeks Off” strategy and 2.05 for the “ 8 Weeks On / 4 Weeks Off”. We observe a ratio of deaths of 1.19 for the Optimal therapy and 2.14 for the Random therapy.
Table 1. Variations of immunological and virological parameters: In the upper part of the table we report the data from Dybul's clinical trial [16]. In the lower part of the table we show the results of our simulations. For each treatment schedule tested in our simulations we report the median value for the most important immunological and virological parameters. In

<table>
<thead>
<tr>
<th>CD4+ T cell count cells/mm3</th>
<th>WEEK1 median (range)</th>
<th>Week40 median (range)</th>
<th>Week 48: median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous Treatment</td>
<td>562 (460-1060)</td>
<td>653 (547-884)</td>
<td>675 (559-883)</td>
</tr>
<tr>
<td>8 Weeks On/4 Weeks Off Treatment</td>
<td>562 (460-1060)</td>
<td>607 (468-863)</td>
<td>615 (477-864)</td>
</tr>
<tr>
<td>Optimal Treatment</td>
<td>562 (460-1060)</td>
<td>613 (487-884)</td>
<td>620 (490-882)</td>
</tr>
<tr>
<td>Void Treatment</td>
<td>561 (459-1069)</td>
<td>492 (383-993)</td>
<td>482 (378-950)</td>
</tr>
<tr>
<td>Random Treatment</td>
<td>561 (459-1069)</td>
<td>589 (488– 883)</td>
<td>599 (487-882)</td>
</tr>
<tr>
<td>4 Weeks On/4 Weeks Off Treatment</td>
<td>562 (460-1060)</td>
<td>575 (473- 883)</td>
<td>583 (470-882)</td>
</tr>
<tr>
<td>Week On / Week Off Treatment</td>
<td>562 (460-1060)</td>
<td>579 (441-884)</td>
<td>587 (450-882)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VIRAL LOAD Log_{10} (virions/ml)</th>
<th>WEEK1 median (range)</th>
<th>Week40 median (range)</th>
<th>Week 48: median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous Treatment</td>
<td>3.59 (ND - 4.51)</td>
<td>ND (ND - 3.40)</td>
<td>ND (ND - 3.41)</td>
</tr>
<tr>
<td>8 Weeks On/4 Weeks Off Treatment</td>
<td>3.59 (ND - 4.51)</td>
<td>1.85 (ND - 3.47)</td>
<td>3.72 (ND - 4.99)</td>
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<td>Optimal Treatment</td>
<td>3.59 (ND - 4.51)</td>
<td>ND (ND - 4.50)</td>
<td>ND (ND - 5.16)</td>
</tr>
<tr>
<td>Void Treatment</td>
<td>4.32 (ND - 5.18)</td>
<td>4.28 (ND - 5.14)</td>
<td>4.26 (ND - 5.06)</td>
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<td>Random Treatment</td>
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<td>3.18 (ND - 4.71)</td>
<td>3.35 (ND - 4.64)</td>
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<tr>
<td>4 Weeks On/4 Weeks Off Treatment</td>
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<td>3.33 (ND - 4.38)</td>
<td>4.01 (ND - 5.12)</td>
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<tr>
<td>Week On / Week Off Treatment</td>
<td>3.59 (ND - 4.51)</td>
<td>1.85 (ND - 4.38)</td>
<td>ND (ND - 4.36)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Provirus virus/mm3</th>
<th>WEEK1 median (range)</th>
<th>Week40 median (range)</th>
<th>Week 48: median (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous Treatment</td>
<td>817 (4 – 1699)</td>
<td>193 (1 - 385)</td>
<td>155 (1 – 352)</td>
</tr>
<tr>
<td>8 Weeks On/4 Weeks Off Treatment</td>
<td>817 (4 – 1699)</td>
<td>341 (1-1140)</td>
<td>282 (1 – 1151)</td>
</tr>
<tr>
<td>Optimal Treatment</td>
<td>817 (4 – 1699)</td>
<td>236 (1-1146)</td>
<td>196 (1 – 1624)</td>
</tr>
<tr>
<td>Void Treatment</td>
<td>817 (4 – 1699)</td>
<td>529 (446 - 1516)</td>
<td>725 (1 – 1494)</td>
</tr>
<tr>
<td>Random Treatment</td>
<td>817 (4 – 1699)</td>
<td>1313 (2 – 4155)</td>
<td>274 (1 – 1224)</td>
</tr>
<tr>
<td>4 Weeks On/4 Weeks Off Treatment</td>
<td>817 (4 – 1699)</td>
<td>474 (1-1482)</td>
<td>409 (1 – 1528)</td>
</tr>
<tr>
<td>Week On / Week Off Treatment</td>
<td>817 (4 – 1699)</td>
<td>266 (1 – 1007)</td>
<td>212 (1 – 883)</td>
</tr>
</tbody>
</table>
parentheses we report the minimum and maximum values observed in the population of 250 virtual patients. For the viral load measurement ND indicates level of infectious virions below the detection level (<50 virions/ml).

![Opportunistic Infection – Survival Curves](image)

Figure 5. STI Survival curves for different STI strategies: percentage of survivors over time in a population of 250 HIV+ virtual patients. 30 days after bacterial challenge, survival rates are the following: Week On/Week Off STI 83.60%, 8 Weeks On/4 Weeks Off 82.66% and 4 Weeks On/4 Weeks Off 70.28%. Survival rates for continuous therapy and void therapy are 91.53% and 45.38% respectively.

The effects of the different STI on virological and immunological parameters are shown in Figure (6). The parameters that show the strongest correlation with the survival of the virtual patients in the long term are CD4⁺ cell count and provirus levels. The levels of provirus seem to be responsible for the failure of STI treatment as much as the CD4⁺ cells count. The “4 Weeks On / 4 Weeks Off” schedule has the worst survival because of the high level of provirus even though it shows a CD4⁺ cells count comparable to that of the “Week On / Week Off” STI. The difference in the ratio of deaths associated to “8 Weeks On / 4 Weeks Off” and the optimal STI (2.05 versus 1.19) should be due to their difference in the level of provirus,
since both schedules show comparable CD4+ cell counts. These results lead us to point out the importance of HIV reservoirs as one of the main causes of STI failure. As expected, a treatment interruption longer than 10 days leads to a viral rebound and a decrease in CD4+ cell count. We observe that the viral rebound is proportional to the duration of the interruption. Treatment interruptions of one or two weeks have a smaller viral rebound whereas for longer interruptions the viral load reaches levels comparable to the ones before treatment (data not shown).

For both optimal and continuous treatment the outcome of the opportunistic infection is correlated more to the level of provirus than to the level of CD4+ lymphocytes. For all other schedules it seems that both CD4+ and provirus are relevant in determining fatal outcomes of the bacterial infection. In Figure 7 we show the CD4+ cell counts versus provirus level of the 250 virtual patients for each of the treatment schedules tested in the present work. For each STI schedule we separate the 250 virtual patients in two groups (survivors and casualties) depending on the outcome of the opportunistic infection. We perform a Kolmogorov-Smirnov two-sample test to compare the distributions of the CD4+ cell counts at the end of the treatment period in both groups. The null hypothesis is that the CD4+ cell counts in the group of survivors and the one in the group of casualties are from the same distribution. For all the treatment schedules tested, except the optimal and continuous treatment, the null hypothesis is rejected with p-values between 1.1*10^{-5} and 1.6*10^{-2}. Surprisingly for the optimal and continuous treatment the CD4+ cell count are not related to the outcome of the opportunistic infection (p-value 0.29 and 5.1*10^{-2}). We perform the same test to compare the distributions of provirus in survivors and casualties. In this case the null hypothesis is rejected for all the schedules with p-values between 9.1*10^{-12} and 1.5*10^{-2}.
Figure 6. Simulation results. Median CD4⁺ cells count (upper panel) and provirus levels (lower panel) of the 250 virtual patients during the 48 weeks treatment period. In each panel we show the different STI tested in this study, the optimal therapy and the continuous therapy. The levels of provirus seem to be responsible for the failure of STI treatment as much as the CD4⁺ cells count. The “4 Weeks On / 4 Weeks Off” schedule has the worst survival curve because of the high level of provirus even though it has a CD4⁺ cells count comparable to that of the “Week On / Week Off” STI.
Figure 7. Survivors and Casualties. In each panel we show the CD4\(^+\) cell counts versus provirus of the 250 virtual patients at the end of the treatment period. The black markers indicate the patients that survived the opportunistic bacterial infection, whereas the red markers indicate the patients that died within 30 days of the bacterial infection.

4.3 Discussion

The three STI strategies (“Week On/Week Off”, “4 Weeks On/4 Weeks Off” and “8 Weeks On/4 Weeks Off”) have lower performances compared to continuous treatment regardless of the emergence of drug resistance since the ratio of death associated to a clinically tested
STI strategy over that of continuous treatment is between 1.95 and 3.52. Since in the model we don’t allow HIV to develop resistance to cART drugs, the failure of STI seems to be inherent to the HIV infection dynamics, rather than caused by the emergence of drug resistant strains. We conclude that the amount of HIV reservoirs both in macrophages and latently infected resting/memory CD4+ T lymphocytes are sufficient to cause a failure of the STI schedules tested so far in clinical trials regardless of drug resistance. This observation may have relevant implications for the design of future treatment strategies. Indeed, efforts directed towards the reduction of the size of HIV reservoirs in humans, might facilitate the adoption of subsequent strategies aimed at reducing the antiretroviral treatment exposure, such as STI or treatments with less drugs [39].

We also show that, without the emergence of drug resistance, it is possible to find an optimized STI whose efficacy is close to that of a continuous treatment with a reduction of 40% in drug administration. In addition by showing the failure of the “8 Weeks On / 4 Weeks Off” STI we show that adherence to the optimal treatment schedule is more important than the amount of drug taken over the treatment period. The efficacy of random therapies with the same amount of drug of the optimal treatment is sensibly lower than that of optimal treatment. Results indicate a ratio of death of 2.14 for the random treatment instead of 1.19 observed for the optimal. We conclude that the SA optimization was successful in identifying an optimal schedule of drug administration for the set of conditions studied in this work.

The result obtained in a previous work [32] on a shorter therapeutic period (6 months) and optimized by using a genetic algorithm indicated an optimized therapy with a week-on week-off pattern after a period of about 2 months of continuous therapy. The initial period of continuous therapy is not necessary according to our study, possibly because the immune system is not compromised enough to require an initial strong recovery phase. In the previous study the percentage of survivors for full and optimal therapy was comparable (34.1% and 30.5% respectively) whereas random and void therapy had lower
performances (21.9% and 20.2%). The differences in the optimized treatments indicate that the best therapeutic strategy changes with the progressive damage that HIV infection inflicts on the immune system. Since at 3 years post infection the immune system is usually not severely compromised, in the present study the percentage of survivors is higher than the one observed in the previous study in which the therapy started almost 8 years after HIV infection. An interesting difference is the effect of random therapy. In our study we notice that a random therapy has less negative effects if the patient receives early (CD4+ cell counts > 500 cell/mm³) cART. If treatment starts later random therapy has virtually no effect, as shown in [32]. This result indicates that unstructured interruptions have a less negative effect on the short term when the immune system is still healthy but further investigation is required to evaluate their effects in the long term.

The increased risk of opportunistic infections associated to provider-directed structured therapeutic interruptions led to abandoning this strategy in favor of the safety of a continuous treatment. Yet continuous treatment is not always possible as suggested by the proportion of patient-initiated unstructured interruptions. Since new studies suggesting the beneficial effects of early treatment may increase the duration of cART in a patient’s life, a further growth of the already common unstructured interruptions can be foreseen. Moreover, so far, no clinical study has compared the risk of opportunistic infections associated with unstructured interruptions to the risk associated with STI. Finally, the sustainability of life-long antiretroviral therapy at global level, both from an economic and toxicity points of view, still represents a major challenge. For these reasons, exploring the mechanisms behind the failure of structured therapeutic interruptions and the possible optimal STI is still an open topic.

The ethical problems associated to further studies of STI in light of the previous failures make the use of modeling techniques appealing. The possibility of simulating STI in silico to predict the success or failure of a given STI strategy is a powerful tool that can support the design of
clinical studies without having impact on human beings. The optimization of treatment interruptions is another clear example of the usefulness of modeling approaches to foster the understanding of complex problems.

For the HIV infection the lack of fully predictive animal models [40] makes it difficult to accurately validate the prediction of computational models. It is very difficult to address the ethical implications of testing computational predictions on humans, given the potential loss of human lives that could be caused by a wrong prediction. Yet, clinical trials are still needed and any tool that could be used to assist those trials should be considered.

Regardless of the difficulties in validating the predictions of our model, our results can be used to orientate qualitatively the design of clinical trials. In silico simulations could be used to predict if a STI schedule might fail because of the size of the HIV reservoir regardless of the emergence of drug resistance. In this way clinical trials could be directed toward schedules with a higher chance of success.

4.4 Materials and Methods

4.4.1 In silico approach

In this paper we adopt a two phases approach similar to the one used in Castiglione et al. 2007 [32] to study the existence of an optimal treatment that minimizes the drug administration without compromising the immune system response to HIV. Firstly we search for the optimal STI schedule ("optimal therapy") by using the simulated annealing optimization algorithm. In this phase, looking at the conditions of the immune system at the end of the therapeutic period, we evaluate the potentially administered therapeutic schedule by means of computer simulations. The SA algorithm is applied to search for the optimal schedule of cART interruptions that maximizes both the immune system recovery and viral control and, at the same time, minimizes the amount of drugs used in a therapeutic period.
Secondly, once we have found the optimal schedule, we test it by challenging the immune system with an opportunistic infection simulated by introducing a bacterium in a group of 250 virtual patients. Therefore, we compare the survival curves of the different control groups with the survival curve of the optimal therapy. In the first control group patients have a continuous course of therapy (i.e., uninterrupted) for the whole period (“continuous therapy”). Patients of the second control group receive the same amount of medicine as in the optimal therapy, but the drugs are randomly administered in time (“random therapy”). The last control group consists of untreated patients (“void therapy”), that is, patients that do not receive therapy at all. Performing this test in silico allows us to use the same virtual patients for each group, whereas in clinical trials the control groups consist of different individuals. Such choice enables us to directly relate the survival of rates of each group to the effect of the different therapies.

In addition to the search for an optimal STI we also study the effects of STI strategies tested in clinical trials by comparing their effect on the HIV dynamics of the virtual patients over a period of 48 weeks of treatment. As control groups for this experiment we use groups of 250 virtual patients under continuous treatment and void treatment. Since the model does not include drug resistance, the outcomes of the STI strategies can be traced back to the dynamics of the immune response to the HIV virus and in particular to the action of antibody producing B lymphocytes and to the CD4+ and CD8+ T cells.

4.4.2 Quantitative evaluation of STI

The simulation starts at time zero with an injection of HIV viral particles and the HIV infection progresses untreated for three years. At that point the disease is already in its chronic phase and we start a therapeutic period of 48 weeks. During the therapeutic period we administer the cART according to a given schedule represented by TherStr, a 48-bit-long string, where a 0 stands for a week without therapy (i.e., an interruption) and a 1 stands for a full week of therapy.
(Figure 8). We assume the efficacy of the therapy to drop to zero during an interruption so that the HIV life cycle may progress unhindered through all its stages during the interruptions. Most cART regimens administer a daily cocktail of at least three drugs, commonly two RTIs and one PI. Although we could optimize the therapy on a daily basis, we decided to constrain the minimum administration/interruption period to one week. Note that this is not a fundamental limitation in our approach, but rather a practical one since no patient would be able to follow a one-year-long therapeutic schedule changing on a daily basis. In addition this choice allows a direct comparison with a previous study based on a genetic algorithm. Another constraint to the simulated cART dictated by medical practice is that RTI and PI drugs are always administered at the same time. At the end of the 48 weeks we measure the efficacy of the therapy by assigning a score given by our fitness function. At the beginning of the therapeutic period, the average CD4\(^+\) count measured in the virtual patients is about 500 cells per microliter.

![HAART schedule as a 48-bit string](image)

**Figure 8.** HAART schedule is represented as a 48-bit string. In the string, the \(j\)th bit set to 1 means that during the \(j\)th week the HAART is administered to the patient.

To quantitatively evaluate the effect of a STI schedule of cART we define a fitness function \(F_i\) for the \(i\)th virtual patient. The fitness function measures the health of the virtual patient’s immune system as...
the outcome of a simulation and the amount of drugs received over the 48 weeks period. Since the whole immune/HIV dynamics depends on the therapy administered, the fitness score is a function of TherStr. $F_i$ is the sum of three terms $H_i$, $D_i$, and $Z_i$.

- $H_i$ takes into account the fitness of HIV which is computed as the average of the sum of both virus contained in infected cells and free viral particles divided by the viral set point here defined as the sum of free virus and virus in infected cells at time the treatment starts $t_s$;
- $D_i$ is a measure of the fitness of the immune system and is given by the average of the ratio between the CD4$^+$ count of the healthy individual (i.e., CD4$^+$ count at time = 0, just before the infection) and the CD4$^+$ count during the treatment period;
- $Z_i$ measures the amount of drugs used as the ratio between the number of weeks of active therapy and the 48 weeks of treatment.

In order to partially account for inter-patient variability, the optimization algorithm tries to minimize $F_i$, the average fitness score of eight virtual patients.

$$F = \sum_{i=1}^{8} F_i = \sum_{i=1}^{8} H_i + D_i + Z_i$$  \hspace{1cm} (1)

where

$$H_i = \frac{1}{(t_e - t_s)} \sum_{t=t_s}^{t_e} \frac{H_i(t, \text{TherStr})}{H_i(t_s)}$$  \hspace{1cm} (2)

$$D_i = \frac{1}{(t_e - t_s)} \sum_{t=t_s}^{t_e} \frac{D_i(t = 0)}{D_i(t, \text{TherStr})}$$  \hspace{1cm} (3)

$$Z_i = \frac{z_i(\text{TherStr})}{(t_e - t_s)}$$  \hspace{1cm} (4)
In the formulas above $t_s$ indicates the time the therapy starts (three years post infection); $t_e$ the time the therapy ends (i.e., 48 weeks after $t_s$); $H_i(t, \text{TherStr})$ is the sum of free virions and proviral HIV in infected cells; $D_i(t, \text{TherStr})$ is the T helper cell count in the simulated space and $z_i(t, \text{TherStr})$ is a function such that $z_i(t)=1$ if the therapy is active at time $t$, zero otherwise. Minimizing the fitness function implies decreasing the viral load and the amount of drugs used, whereas increasing the CD4$^+$ cell count over a period of 48 weeks. C-ImmSim uses a time-step corresponding to 8 hours of real life.

4.4.3 Simulated annealing optimization

To find the optimal schedule of the cART during the 48 weeks therapeutic period we use a Simulated Annealing algorithm [41]. To optimize the STI we perform in silico experiments on a test group of 8 virtual patients. The size of the test group is purposefully chosen equal to the number of cores available in each node of the computer cluster used to perform the optimization. This choice allows us to simulate in parallel the HIV infection on 8 virtual patients, by performing the computation on a single node.

We adapted the classic simulated annealing algorithm to take as input a therapeutic schedule in the form of a 48 bits string where each bit represents a week of therapy. We define an algorithm to update the therapeutic schedule that modifies the previous schedule by adding, removing or shifting 1 week of therapy (1 bit). By using this algorithm we assume that two consecutive configurations in the Markov chain have comparable fitness scores (i.e., the energy of two consecutive configurations is comparable), which is a requirement for a correct annealing optimization.

The simulated annealing is characterized by a set of parameters described in Table 2. For the annealing we resort to a geometric cooling schedule [42]. We tested also logarithmic and adaptive cooling schedules. The logarithmic cooling was soon discarded due to the long
computational times required and the adaptive did not result in a better convergence.

A similar optimization problem has been studied in the past by following an approach based on a genetic algorithm (GA) [32]. The GA study looked for the optimal therapy over a period of six months starting after 7.5 years post infection. One of the main differences between the present work and the previous one [32] is that we reduce the time between seroconversion and treatment from 7.5 to 3 years. This reduction allows considering the positive effect of early cART, but increases the difficulty of the optimization because the effect of cART on CD4 recovery is dampened by the healthier immune system at the time of treatment initiation. Another important difference is that we attempt to optimize the schedule over a longer period (close to 1 year) instead of just 24 weeks. Extending the therapeutic period to one year increases the complexity of the optimization problem. In the previous study the state space had $2^{24}$ possible configurations. In the present work we face a state space of $2^{48}$ configurations. Doubling the therapeutic period allows to test if the optimal STI can yield results similar to those observed in the shorter STI previously studied [32]. Clinical studies on STI indicated a reduction of the benefits over longer time periods [15,16,43]. By using an extended period of STI we have the chance to verify if we observe a similar behavior in the model.

In each iteration of the optimization algorithm we evaluated the fitness function as the average among $N=8$ virtual patients. The evaluation of the fitness of a given STI schedule required the simulation of 4 years of HIV infection dynamics in each individual patient. Most of the computational time is due to the C-ImmSim simulations, whereas the annealing algorithm has a relatively small impact on the computation time. The longest annealing optimizations use MAXITER equal to 27000 and take about 15 days to complete (Figure 9) using a L5520 eight-core processor having a clock rate equal to 2.26 GHz.
We explore stochastically the configuration space of $2^{48}$ different therapeutic schedules. We stop the optimization when the standard deviation of the fitness score for the last homogeneous Markov chains (i.e., parameter MARKOVCHAIN in the Table 2) is lower than an arbitrary threshold set equal to $10^{-4}$. Each instance of the optimization algorithm results in an optimized schedule and a corresponding fitness score. We find several local optimal therapies with comparable fitness scores. As usual with most stochastic algorithms we do not have any warranty that a better solution could not be found by continuing the search.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>INIT$_T$</td>
<td>0.2-0.045</td>
<td>Initial Temperature</td>
<td>We tested several values of the initial temperature. In all cases INIT$_T$ is high enough to accept most the moves in the state space at the beginning of the algorithm. The use of a high INIT$_T$ ensures the system is not trapped in a capture basin.</td>
</tr>
<tr>
<td>MAXITER</td>
<td>9000-27000</td>
<td>Maximum number of iterations of the Metropolis algorithm</td>
<td>It is the maximum number of times C-ImmSim is launched. It is also one of the criteria that cause the termination of the SA.</td>
</tr>
<tr>
<td>MARKOVLENTH</td>
<td>5-10</td>
<td>Length of the homogeneous Markov chain at a given temperature</td>
<td>The last configuration in the Markov chain should represent the configuration at thermal equilibrium for the given temperature.</td>
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<tr>
<td>MARKOVCHAINS</td>
<td>10-20</td>
<td>Number of Markov chains used to calculate the standard deviation of the fitness score</td>
<td></td>
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<tr>
<td>THRESHOLD</td>
<td>10$^{-4}$</td>
<td>Value of the standard deviation below which the SA is stopped</td>
<td>It’s one of the stop criteria. The SA ends when the standard deviation of the fitness is lower than THRESHOLD.</td>
</tr>
<tr>
<td>NRUNS</td>
<td>8</td>
<td>It is the number of virtual patients over which the fitness score is averaged.</td>
<td>Corresponds to the number of cores used for the parallelization of the SA algorithm</td>
</tr>
<tr>
<td>C</td>
<td>0.996-0.999</td>
<td>Parameter characterizing the geometric cooling schedule for the SA</td>
<td>Geometric Cooling: $T_{k+1} = CT_k$ where $T_{k+1}$ is the temperature of the next Markov chain and $T_k$ is the temperature of the current one</td>
</tr>
</tbody>
</table>

Table 2: Parameters of the simulated annealing algorithm
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5. *In silico* studies reveal two phases of sustained response of a patient’s immune system to treatment during primary HIV infection

5.1 Introduction

The question when to initiate combination antiretroviral therapy (cART) is still a core controversy within the medical community [1-3]. The controversy revolves around two main issues: the first is fighting the HIV pandemic at global level and the second is choosing the treatment strategy that increases the life expectancy and the quality of life of individual patients. Regarding the first issue the general opinion has shifted toward the Test & Treat strategy that recommends continuous treatment as soon as they are identified [4]. The rationale behind this choice is that suppressing HIV viral load lowers the risk of new infections, possibly reducing the size of the epidemic [5-7]. Despite the most recent US guidelines recommend adopting the Test & Treat strategy many researchers are strongly advocating further evaluation of the consequences of such strategy and its real chances of defeating the pandemics [8-10]. However, identifying the best treatment strategy for an individual patient is still an unsolved problem and is the topic of this paper.

Since 1998 the National Institute of Health has updated guidelines in which, over the years, the best time to start cART for an individual patient has slowly shifted toward earlier treatment [4]. Recently, several studies reported benefits of temporary cART during the primary infection including lowering of the viral setpoint [11, 12] and limitation of viral reservoirs [13], which re-opened the discussion of starting treatment early. On the other hand, various studies have not observed changes in the viral setpoint [14, 15] or have reported a worsening in the disease progression [16, 17]. Because of such conflicting results the optimal conditions to start cART are still controversial.
The currently available clinical data is insufficient to identify the most opportune time for the patient to start treatment. Although new clinical trials are being performed, the potential risk to patients’ health slows down the clinical investigations. Our aim is to predict quantitatively the sustained response of the immune system to HIV infection with respect to treatment, during the first 4 years post infection. To this end we define a Sustained Response Score (SRS) for the adaptive immune response to a perturbation at a given time post-infection, under the assumption that cART perturbs the progression of the untreated HIV infection in the infected patients. We validate our predictions numerically and by use of available clinical data from the Primo-SHM study [18]. Obtaining statistical significance requires a large dataset on the concentration of T helper lymphocytes and viral particles over time. Data from hospitals and clinical trials is too sparse, providing only up to a few data points per patient. Therefore we generate datasets by simulating the HIV infection in 500 virtual patients for a period of 6 years post infection in time steps of 8 hours. We perform the simulations using an established agent-based model that has been validated using clinical data [19-23].

5.1.1 Model

We model the adaptive immune response to HIV infection in each virtual patient by simulating individual immune cells, viral particles, and cytokines, which interact in a volume of four microliters of a lymph node (Figure 1a). For this purpose we use C-ImmSim, a validated agent-based model of HIV infection [19-23]. The free parameters of this model have been tuned and validated against clinical data over the past 10 years. The first reason for choosing C-ImmSim is that it can be used to interpolate existing clinical data. The second reason is that it simulates the underlying virology of the adaptive immune response more closely than mathematical models [24-27]. The study of HIV through cellular automata and agent-based models is quite common [19-23,28-30] due to the discrete nature of the biological entities involved as well as the complexity of their collective dynamics. The main advantage of C-ImmSim over mathematical
models for HIV infection lies in its ability to simulate different populations of each cell type, distinguished by their specificity to the HIV virus. We assume that mechanisms like cross-reactivity, affinity maturation and the complex interplay of the HIV virus with the different subsets of immune cells play a fundamental role in the HIV dynamics. C-ImmSim has been used to investigate the differences in early versus deferred treatment [21] and phenomena associated with the HIV infection [19,20,22,30].

Figure 1. a) The in silico experiments are performed in a 3-dimensional ellipsoidal lattice. The lattice represents a lymph node with a volume of 4 microliters. Each lattice point represents a microscopic volume in which stochastic interactions occur between the modeled entities. Some of the entities in the model are shown in the sketch: CD4+ T-lymphocytes, B-lymphocytes, antibodies (Ab), macrophages (MA), dendritic cells (DC) and interferon γ; b) The interactions between the main cellular compartments in the adaptive immune response. This network describes the adaptive immune response to HIV, that in our model is simulated by a three dimensional agent-based model of a lymph node. The virus can be in two forms: free virus (infectious virions) and viral genome copies contained in both silently and actively infected cells (provirus). The complexity of this network does not lie in its topological complexity but is hidden in the complex spatio-temporal interactions (arrows) between the nodes.

The network of interactions between the main cellular compartments involved in the adaptive immune response and the virus is
summarized in Figure 1b. The complexity of this network lies in the edges (the interactions) and not in the connectivity of the nodes.

5.1.2 Virtual clinical data

We vary three parameters to generate 500 different virtual patients: the first two parameters represent the two major histocompatibility complexes (MHC-I and MHC-II) of the virtual patients’ immune cells. The third parameter distributes up to 4096 different MHC-I and MHC-II cell receptors over the population of cells for the specific patient. The virtual clinical data used for this analysis consists of cell counts per microliter for each of the main cellular entities involved in the adaptive immune response: T helper lymphocytes (CD4+ cells), cytotoxic lymphocytes, macrophages, B cells and dendritic cells. In addition, the data includes viral particle counts per milliliter (viral load) and the amount of virus inside the infected cells per microliter (provirus). The model stores both cellular and viral data for every 8 hours of simulated time from a single lymph node of 4 microliters for each of the 500 different virtual patients. A minor limitation of the model is that it simulates the immune response to the HIV virus only in a lymph node and not in other types of lymphatic tissues that have different cellular densities. This limitation has been addressed during the validation of the model, when we used clinical data on viral load, CD4+ cell counts and time to AIDS. In this sense our model of the HIV response in a lymph node replicates the dynamics of the HIV infection in an infected patient.

5.1.3 Sustained response score

Let \( X \) and \( Y \) be two stochastic dynamical processes for a given initial state, i.e., each element \( X(t) \) is a random variable of the state of process \( X \) at time \( t \). We define the point-to-point sustained response score \( S_{X \rightarrow Y}(t, t + \Delta) \) of the state of a stochastic process \( Y(t + \Delta) \) on the
previous state of another stochastic process $X(t)$ by their mutual information [31], defined as

$$S_{X\rightarrow Y}(t,t+\Delta) = I\left(X(t), Y(t+\Delta)\right) = \sum_{x \in X(t)} \sum_{y \in Y(t+\Delta)} p(x,y) \log \left( \frac{p(x,y)}{p(x)p(y)} \right).$$

(1)

Where, $p(x,y)$ is defined as the joint probability that $X(t)=x$ and $Y(t+\Delta)=y$; $p(x)$ and $p(y)$ are the corresponding marginal probabilities.

For combinations of the stochastic processes of the provirus and CD4$^+$ cells Equation 1.1 describes a causality relation because there is no third process that influences both provirus and CD4$^+$ cells simultaneously. This would create a non-zero mutual information due to correlation [32,33]. The only exception here is in the macrophages processes, but their dynamics is considerably noisier than that of CD4$^+$ cells and provirus. The main contribution of the macrophages is to act as reservoirs but such characteristic is already contained in the provirus process.

We use this point-to-point sustained response score $S_{X\rightarrow Y}(t,t+\Delta)$ to define a point-wise sustained response score $S_{X\rightarrow Y}(t)$ as the characteristic time at which $S_{X\rightarrow Y}(t,t+\Delta)$ decays to a constant as function of $\Delta$. Intuitively, this is how long a state of process $X$ at time $t$ has an effect on the subsequent behavior of process $Y$. We observe that $S_{X\rightarrow Y}(t,t+\Delta)$ typically first grows to a peak and then decays roughly exponentially, as function of $\Delta$ (see Methods). Therefore, in words, we define the sustained response score $S_{X\rightarrow Y}(t)$ as the time of the peak $\mu$ plus the halftime of the decay $\tau$, and multiply this quantity by the height of the peak $\iota$ in order to correct for the magnitude of the impact. SRS is a measure of the response of the immune system of a patient to a temporary treatment. A high SRS at time $t$ means the
beneficial effect of treatment started at time \( t \) will last longer. In formula,

\[
S_{X\rightarrow Y}(t) = t \cdot (\mu + \tau), \text{where} \\
t = S_{X\rightarrow Y}(t, t + \mu), \\
\mu = \arg \max_\Delta S_{X\rightarrow Y}(t, t + \Delta), \\
\tau = \left\{ \Delta > \mu : S_{X\rightarrow Y}(t, t + \Delta) = \frac{1}{2} \cdot \frac{1}{t} \right\}.
\]  

5.2 Results

We focus our analysis on the three most important indicators of the HIV infection: CD4\(^+\) cells (T helper lymphocytes), Provirus (the amount of virus hidden in infected cells) and viral load (\( \log_{10} \) viral RNA copies/ml). The key elements to evaluate the progression of HIV in a patient are its CD4\(^+\) cell count and the amount of viral genome copies in the infected cells (provirus) whose role in the survivability of virtual HIV infected patients has been already shown [30]. The role of provirus in the HIV infection has been recently reassessed experimentally, indicating that the size of replication-competent latent HIV reservoirs can be up to 60-fold greater than previously estimated [34]. Since early treatment has been reported to affect the size of HIV reservoirs [13] and the viral set point [11, 12], both directly related to the provirus, the SRS of the provirus with respect to other indicators (Figure 2c and 2d) is a relevant quantity for the most opportune timing of a treatment.

Our results confirm that the immune system has the longest sustained response to treatment during the acute phase of the HIV infection, as indicated by a very high SRS during the first two months after infection. In addition to this phase the results indicate a secondary phase during which the SRS plateaus are still at a relatively high value. The duration of this phase is roughly ten months. In Figure 2 we show the SRS of all the possible pairs with provirus and CD4\(^+\) cells counts. Each point of the SRS curve is the product of the mutual information at the peak (green marker in Figure 2e) and the sum of the
delay in the mutual information peak plus the information dissipation time. In Figure 2e each graph shows the change in mutual information in the CD4⁺ cells over time generated by a perturbation of the provirus level at the initial time point (respectively at about 2, 6 and 22 months post infection as indicated by the red markers in Figure 2d).

Figure 2: Panels a,b,c,d: Sustained Response Score over time of all the possible pairs with CD4⁺ cells counts and provirus. SRS is proportional to the duration of the response of the immune system to a temporary treatment. A high SRS at time t means the beneficial effect of treatment started at time t will last longer. Each panel shows the SRS of the second entity to changes in the first entity. For example panel d shows the effect on the CD4⁺ cell count due to a change in the provirus. Panels a and b show a plateau in the SRS of CD4⁺ cells.
in the first 12 months post infection. The SRS for the plateau is 5 times higher than the SRS relative to the chronic phase of the infection. Panels c and d show as expected a peak in the SRS of the provirus during the acute phase of the HIV infection. After this peak we observe a plateau (until 10 months post infection) with a SRS about 5 times higher that the SRS of the chronic phase on the infection. The plateaus in the four panels show the increased beneficial effects of cART during the first 10 months post infection. The red markers in panel d represent the SRS calculated from the Mutual information graphs shown in panel e. Panel e (lower set of 3 graphs): Mutual information for provirus at time t versus CD4$^+$ cells over time. Mutual information is a measure of the correlation of the CD4$^+$ cell count over time to changes in the Provirus at various times (90 days, 180 days and 500 days respectively). From a medical perspective it measures the impact on the CD4$^+$ cells recovery if the size of HIV reservoir is affected by treatment with cART at a given time. The curve fitting was performed over all the data points after the peak of mutual information. We used an exponential curve fitting to calculate the information dissipation time as the time at which 50% of the information over the baseline was lost.

5.2.1 CD4$^+$ cells Sustained Response Score

The sustained response score for CD4$^+$ cells in the first 10 – 12 months post infection, shown in Figure 2a and 2b, is significantly higher than the baseline, where the baseline is defined as the SRS at the chronic phase of the infection. For the CD4$^+$ cell count we find that there is only one phase of sustained response due to treatment that lasts up to 12 months post infection. Interestingly during the acute phase the SRS of CD4$^+$ cells is low. The phase of high SRS could mark the period during which a fluctuation in the total amount of T lymphocytes might have a long lasting effect on the disease progression.

5.2.2 Provirus Sustained Response Score

The sustained response score of the provirus is characterized by two phases: an initial intense but short phase and a longer but milder secondary phase. The initial phase of sustained response closely follows the acute phase of the HIV infection and has a SRS about 5 times higher than the baseline score pertaining the chronic phase of
the infection. This first phase lasts only a few weeks but starting cART during this phase, as expected, ensures the long-term benefits documented in literature [21, 35]. The secondary phase starts at about 3 months post infection and lasts about 6 months. During this secondary phase of sustained response, although clearly lower than during the acute phase of the infection, the HIV infection still has a SRS significantly higher than the baseline level for the next four years of infection. This higher score implies that a perturbation in the system, such as temporary cART, will have a longer lasting effect than a similar treatment applied anytime after this period. In other words, reducing the growth of provirus (a measure of HIV reservoirs) during this secondary phase of sustained response has a long lasting effect both on the size of HIV reservoirs (Figure 2 panel c) and on the CD4+ cells counts (Figure 2 panel d) at later times.

5.2.3 Validation

Our interpretation of the SRS is validated numerically and by a clinical study published in 2012 by the PRIMO-SHM study group [18]. The clinical study assessed the benefits of temporary cART during primary HIV infection. It shows that a period of 24 weeks of cART started within 6 months post infection lowers the viral set point and defers the restart of cART during chronic HIV infection. This is consistent with our prediction that a secondary phase of strong sustained response lasts up to about 10 months post infection.

In Figure 3 we compare the simulation results for 500 virtual patients with the clinical data from PRIMO-SHM study. In absolute terms the simulation results are shifted compared to the clinical data. A possible explanation is that in PRIMO-SHM all the enrolled patients had a symptomatic acute infection, which could indicate a bias to fast progressors. In our simulations we excluded such fast progressors. This explanation is supported by the lower CD4+ cell counts observed in the clinical trial in comparison to the simulated ones. A more aggressive infection might also explain the stronger effect of
temporary treatment on the viral set point observed in the PRIMO-SHM trial.

Figure 3: Validation. Comparison of the in silico experiments (continuous lines) with clinical data of the PRIMO-SHM study group (circle and square markers) on viral load and CD4⁺ cell count after randomization/treatment interruption in the no treatment and treatment arms. Although in both panels the clinical data appears to be rescaled by a constant factor, the dynamics is qualitatively comparable. In the inset of panel a) we show the prediction of the effect of temporary cART on the size of HIV reservoirs after treatment interruption.

Qualitatively in both in silico experiments and clinical data the temporary treatment has a long lasting effect on both CD4⁺ cell counts and viral load. In our experiments the viral load converges to the untreated level after roughly one year, faster than observed in the Primo-SHM study. Conversely the CD4⁺ cell counts in our experiments show the same dynamics of the clinical study.

5.3 Discussion

The PRIMO-SHM clinical study shows that starting a temporary cART within 6 months post infection lowers the viral set point. However, it is unknown to what extent this effect is due to patients in the acute phase. It is known that temporary cART administered during the
acute phase has a life long effect, but it is unclear how this effectiveness decreases over time. We predict a phase of long-lasting sustained response during the acute phase followed by a plateau of roughly ten months during which the sustained response of the immune system is still more intense than the one relative to the chronic phase. Our study provides an explanation to the temporary beneficial effect of cART observed in the PRIMO-SHM study.

It is well known that cART is associated to an increase in the CD4+ cells count at any time during the HIV infection. Less is known about the effects of cART on HIV reservoirs at different stages of the infection. In our analysis the size of HIV reservoirs evaluated by the provirus is reduced by the use of temporary cART for a period of about 1 year after treatment interruption. The correlation between the survivability of patients and the size of the population of infected cells has been observed in in silico experiments [30], highlighting the importance of controlling HIV reservoirs to reduce the number of deaths in the long term. The temporary reduction of the size of HIV reservoirs confirms the previously observed lack of long-term effects of treatment started after the acute infection. The inability of the immune system to identify and eliminate silently infected cells of the HIV reservoirs is a possible explanation of the slow decrease of Provirus under cART.

5.4 Methods

5.4.1 Estimating mutual information from discrete data
The mutual information $I(X_t, Y_u)$ between the random variables $X_t$ and $Y_u$ is estimated by constructing an equidistant contingency table [33] of the two corresponding vectors $x_t^1, \ldots, x_t^{500}$ and $y_u^1, \ldots, y_u^{500}$, which are the 500 cell counts of all virtual patients at time $t$. To construct this table we divide the range of values of each vector into $h$ bins of equal size. Here we use a variable $h$ proportional to the resolution at which we discretize the data. We set the resolution parameter at 0.05. This allows us to use more bins when the variance
in the data becomes bigger. Two observed pairs of cell counts are considered equal if they fall into the same bin.

In general, mutual information measures both causation and correlation simultaneously. However, here we use mutual information as a measure of causation due to the particular underlying dynamics of the HIV immune response, shown in Figure 1b. For the pairs of cell types we analyze, there is only a directed path between the cell types; there is no third cell type that has a directed path to both analyzed cell types.

5.4.2 Estimating the time-delay $\tau$ of the peak mutual information
We calculate the mutual information using data from 500 virtual patients with different immune systems. The heterogeneity of the virtual patients is necessary to model the observed variability in the immune response to HIV and the stochastic properties of the model introduce noise in the data. In order to identify the presence of a peak in the mutual information averaged over the whole set of virtual patients we need to filter such noise. To do so we use a Gaussian kernel with a fixed bandwidth for a Nadaraya-Watson kernel regression [36]. After smoothing the average mutual information we observe two main types of curve: in the first type the mutual information has a peak at time 0 and decreases exponentially toward an asymptotic value. This means that the amount of information the first entity possesses about the second entity is highest at time 0 and decreases afterward. In the second type we observe a delay in the peak in the mutual information. This can be interpreted as the first entity having more information about the status of the second entity that is a number of time steps in the future.

5.4.3 Estimating the information dissipation time $d_i$
We calculate the information dissipation time for each mutual information curve by fitting an exponential function to the mutual information curve. For the curves with a time-delay in the peak of mutual information we fit only the data from the peak onward. The
information dissipation time \( d \), is defined as the number of days between the time of the peak of mutual information and time at which 50% of the intensity is lost.

References


Supporting Information

In Figure S1 we show the three indicators that drive the SRS of all the possible pairs with CD4+ cells counts and provirus. In each panel the first graph displays the delay in the peak of mutual information. This delay indicates the time it takes to a perturbation in a variable to have its maximum impact on the other variable. Such delay is present only in the pairs with CD4+ as the susceptible variable and disappears after the acute phase. The second graph shows the information dissipation time. Its calculation is discussed in the methods section. For all the pairs the information dissipation time is between three and seven times higher than the baseline in the first ten months post infection. This result clearly shows how a perturbation in the system during the first nine months affects the system for a much longer period than any other time in the first four years of the infection. The intensity of the mutual information peak over time is shown in the third graph of each panel. We observe a peak in the intensity corresponding to the acute phase of the HIV infection only in the pair with provirus as the susceptible variable. As expected the main peak is close to the acute phase, since the first month is crucial to the dynamics of the provirus as it aggressively infects healthy CD4+ cells. The unexpected result is the presence of a second period of about 24 months in which the mutual information peak starts to slowly lose intensity only after 12 months post infection.
Figure S1: The three variables used to compute the SRS. Each panel a-d shows the value over time of the three variables for one of the possible pairs of CD4$^+$ cells counts and provirus. In each panel the first graph displays the delay in the peak of mutual information. This delay indicates the time it takes to a perturbation in a variable to have its maximum correlation on the other variable. The second graph shows the information dissipation time discussed in the methods section. The intensity of the mutual information peak over time is shown in the third graph of each panel.
6. Summary and Conclusions

Infectious diseases are a threat for our modern society due to the increased population density on Earth, so being able to understand and predict the dynamics of infectious diseases is extremely important. Viral infections are by their nature complex adaptive multiscale phenomena. Their microscopic dynamics within cells leads to the emergence of macroscopic effects within individuals and populations. For this reason viral infections should be studied at different spatio-temporal scales and, eventually, as a single multiscale hierarchical model.

One of the grand challenges of computational science is to develop such a multiscale model able to replicate the biological mechanisms from the molecular to epidemiological levels. However, our understanding of the biological and computational complexity is still far from complete.

In this thesis we have investigated the complexity of the interplay between viruses, immune system and drugs at several spatio-temporal scales (from molecular and cellular scales up to the scale of virtual patients). Our contribution has both computational and biological aspects. In the computational domain we have developed new models at intracellular and cellular levels and introduced the use of mutual information to the analysis of an established agent-based model. We have shown that ABM can be used to model viral infections at each spatio-temporal scale and that it is a suitable modeling framework to create a multiscale model to simulate viral infection and the immune response to them. In the biological domain we improved the understanding of the dynamics of HIV infection under cART, providing an insight into the causes of the failure of structured treatment interruptions and quantified the duration of a period of sustained immune response to temporary cART during primary infection.
For the computational domain our first contribution is the development of a stochastic model of HIV-1 intracellular replication. We simulate the HIV life cycle within a single cell, from the capsid release into the cytoplasm until the production of new viral genome, keeping track of the main viral proteins and genetic materials inside the cell. We use two different simulation approaches to implement the model and compare them on the basis of the amount of integrated cDNA and the amount of transcribed viral mRNA after one round of viral replication. We identify the need for the diffusion-based approach of additional experimental data on the movement of HIV proteins within the cytoplasm.

Our second contribution, this time at intercellular scale, is a framework able to support in vitro experiments by simulating both cellular growth and viral replication in a monolayer of cells. This framework is conceived to allow the future implementation of the lower spatio-temporal scale dynamics instrumental to an accurate simulation of the new viral particles released from infected cells. We also evaluate the critical role played by some parameters and using our model we concretely helped the experimental biologists to identify an issue in their cell line that explained the discrepancy between our simulations and the experimental results of the infection.

The last significant contribution in the computational domain is to have considered for the first time the flow of mutual information in the complex network formed by the immune cells and the virus at the scale of individual patients.

The significant contributions in the biological domain focus on the new insight on the dynamics of HIV infection at the scale of individual patients. Our new approach that measures the mutual information between cells types during untreated HIV infection allows us to interpret the results observed in the PRIMO-SHM clinical trial. The PRIMO-SHM clinical study observed that starting a temporary cART within 6 months post infection lowers for a long period of time the viral set point, delaying the need to restart the cART treatment. Our
study explains and quantifies the temporary beneficial effect of cART observed in the PRIMO-SHM study. We observe a known, short phase of long-lasting sustained response during the acute phase followed by a yet unknown plateau of roughly ten months that was never observed before during which the sustained response of the immune system is still more intense than the one relative to the chronic phase.

We show that the role of provirus in the dynamics of HIV infection was so far underestimated. We predict that the amount of HIV reservoirs both in macrophages and latently infected resting/memory CD4+ T lymphocytes are sufficient to cause a failure of the STI schedules tested so far in clinical trials regardless of drug resistance. This observation may have relevant implications for the design of future treatment strategies. We also predict that the temporary reduction of the size of HIV reservoirs is the cause of the temporary beneficial effects observed in the Primo-SHM clinical trial, confirming the key role of provirus in the HIV dynamics. It is worth noticing that a recent study published in Cell highlights the previously neglected role of provirus as the possible reason for our inability to cure HIV.

Lastly, we also show that, without the emergence of drug resistance, it is possible to find an optimized STI whose efficacy is close to that of a continuous treatment with a reduction of 40% in drug administration. In addition by showing the failure of the “8 Weeks On / 4 Weeks Off” STI we show that adherence to the optimal treatment schedule is more important than the amount of drug taken over the treatment period.
7. Discussion

In addition to the modeling challenges that we discussed in this thesis, there are technical limitations that also need to be overcome to succeed in an exhaustive replication of biological systems in silico. Achieving this grand challenge requires computing tools powerful enough to handle a multiscale model that simulates all or even parts of the spatio-temporal scales investigated so far. The discovery of new computing paradigms promises a realistic solution to the technical aspects of such multiscale models. New computing paradigms like general-purpose computing on graphics processing units (GPGPU) and cloud computing have provided solutions to maximize the utilization of currently available computing power. For example, GPGPU is extremely effective to perform fine-grained parallel computations such as simulating the individual molecular interactions (microscale) within a multiscale model of a virtual human. On the other hand cloud computing offers the infrastructure as a service (IaaS) model that provides resources on demand and scalability that would be essential to large-scale hierarchical computations. Simulating the immune response from molecular to cellular level within a single lymph node requires intensive computation, especially when an infection is taking place. A larger multiscale model that simulates all the lymph nodes in the human body interconnected by lymphatic vessels would greatly benefit from cloud computing since the simulation would not know in advance when and in which lymph node the infection will trigger the peak of computation. Thus using IaaS model would minimize the cost of running such a computationally intensive simulation, guarantee effective resource consumption and eventually make this multiscale simulation feasible.
Samenvatting

Infectieziektes zijn een bedreiging voor onze moderne maatschappij door de toegenomen populatiedichtheid in de wereld, dus het is zeer belangrijk om in staat te zijn om infectieverspreidingen beter te begrijpen en te voorspellen. Virale infecties zijn van nature complexe en adaptieve processen op meerdere tijds- en ruimteschalen (multischaal). De microscopische processen binnen en tussen cellen leiden tot emergente, macroscopische effecten in personen en populaties. Om deze reden is het nodig om virale infecties te bestuderen op meerdere spatio-temporele schalen, en uiteindelijk in één integraal, multi-schaal perspectief.

Eén van de grote uitdagingen voor de computationale wetenschappen is het ontwikkelen van een dergelijk multi-schaal model welke biologische mechanismen kan reproduceren van het moleculaire niveau tot het epidemiologische niveau. Echter, ons begrip van de biologische en computationale complexiteit van dit probleem is nog verre van compleet.

In deze Dissertatie hebben we de complexiteit onderzocht van de interactie tussen virussen, het immuunsysteem, en medicijnen op meerdere spatio-temporele schalen (van het moleculaire en cellulaire niveau tot op het niveau van virtuele patiënten). Onze bijdrage heeft zowel biologische als computationale aspecten. In het computationale domein hebben we nieuwe modellen ontwikkeld op het intracellulaire en intercellulaire niveau en hebben we het gebruik van informatietheorie geïntroduceerd in de analyse van een bestaand 'agent-based' computermodel (ABM). We hebben aangetoond dat het ABM kan worden gebruikt om virale infecties te modelleren op iedere spatio-temporele schaal en dat het een adequaat kader biedt om een multi-schaal model te creëren om virale infecties en de respons van het immuunsysteem te simuleren. In het biologische domein verbeteren we het begrip van de dynamiek van HIV-infectie onder cART-therapie, hetgeen inzicht biedt in de oorzaken van het falen van gestructureerde onderbrekingen van therapie, als ook de tijdsduur kwantificeert van de gecontinueerde immuunrespons na een tijdelijke cART-therapie gedurende de primaire infectieperiode.
In het computationele domein is onze eerste bijdrage de ontwikkeling van een stochastisch model van HIV-1 intracellulaire replicatie. We simuleren de cycles van HIV in één enkele cel, van de HIV-capside in het cytoplasma tot de productie van het nieuwe virale genoom, waarbij de belangrijke eiwitten en genetische materialen binnen de cel worden gevolgd. We gebruiken twee verschillende simulatiemethodes om het model te implementeren en vergelijken ze op basis van de hoeveelheid geïntegreerde cDNA en getranscribeerd virale mRNA na één ronde van virale replicatie. We identificeren de noodzaak voor de diffusie-gebaseerde methode van additionele gegevens van experimenten over de mobiliteit van HIV-eiwitten in het cytoplasma.

Onze tweede bijdrage, dit keer op de intercellulaire schaal, is een modelkader dat in staat is om in vitro experimenten te ondersteunen door het simuleren van celgroei en virale replicatie in één enkele laag van cellen. Dit kader is bedoeld om toekomstige implementatie van dynamica op de lagere spatio-temporele schalen te ondersteunen, om zo een accurate simulatie mogelijk te maken van de nieuwe virusdeeltjes afkomstig uit geïnfecteerde cellen. We evaluëren tevens de kritische rol van enkele parameters en gebruiken ons model op een concrete manier om experimentele biologen te helpen om probleem te identificeren in hun experiment, hetgeen een verklaring leverde voor de discrepantie tussen de voorspellingen van het model en de experimentele observaties.

Onze laatste contributie in het computationele domein is het gebruik van de stroming van ‘mutual information’ (een centrale maat uit de informatie-theorie) in het complexe netwerk van interacties tussen cellen van het immuunsysteem en het virus op de schaal van individuele patiënten.

Onze bijdragen in het bioogische domein richten zich op nieuwe inzichten in de dynamiek van de HIV-infectie op de schaal van individuele patiënten. Onze nieuwe methode meet de ‘mutual information’ tussen verschillende typen cellen in een onbehandelde HIV-infectie, hetgeen ons in staat stelt om de observaties van de Primo-SHM klinische testen te interpreteren. In de Primo-SHM klinische testen observeerde men dat het starten van een tijdelijke
cART binnen 6 maanden post-infectie leidde tot een langdurige verlaging van de virale setpoint wat de noodzaak om cART te herhalen uitstelt. Onze studie verklaart en kwantificeert het tijdelijke positieve effect van cART geobserveerd in de Primo-SHM studie. We observeren een reeds bekende, korte fase waarin het starten van een tijdelijke cART een langdurig effect heeft (acute fase), maar we observeren daarnaast nog een tot nu toe onbekende fase van ongeveer tien maanden waarin het starten van cART nog steeds een significant langdurigere invloed heeft op de virale setpoint vergeleken met de chronische fase.

We tonen aan dat de rol van provirus dusver onderschat is. We voorspellen dat de hoeveelheid HIV-reservoirs in macrofagen en latent geïnfecteerde CD4+ T lymfocyten voldoende zijn om te verklaren waarom experimenten met gestructureerde onderbrekingen van therapie mislukt zijn. Deze observatie kan significante gevolgen hebben voor het ontwerp van toekomstige therapieschema’s.

Verder voorspellen we dat de tijdelijke verlaging van de grootte van HIV-reservoirs de oorzaak is van het tijdelijke positieve effect geobserveerd in de Primo-SHM studie, wat de centrale rol van provirus bevestigt in het verloop van de HIV-infectie. Van belang hierbij is een recente studie in het vakblad Cell waarin de voorheen onderschatte rol van provirus in verband wordt gebracht met het tot nu toe mislukken van de HIV-epidemie een half toe te roepen.

Als laatst tonen we aan dat, indien er geen resistentie tegen medicijnen optreedt, het mogelijk is om een geoptimaliseerd schema te vinden van therapie-onderbrekingen waarbij de effectiviteit van therapie nagenoeg gelijk blijft aan die van de continue therapie, met een verlaging van 40% van de benodigde hoeveelheid medicijnen. Aan de hand van het falen van het “8 weken therapie, 4 weken rust”-schema tonen we aan dat het nauwgezet gehoorzamen van het optimale schema belangrijker is dan de hoeveelheid medicijnen die ingenomen worden.
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List of Publications

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