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Article

The potential of computational modeling to predict disease course and treatment response in patients with relapsing multiple sclerosis

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Abstract: As of today, 20 disease modifying drugs (DMD) have been approved for the treatment of relapsing multiple sclerosis (MS) and, based on their efficacy, they can be grouped into moderate-efficacy DMDs and high-efficacy DMDs. The choice of the drug mostly relies on the judgement and experience of neurologists and the evaluation of therapeutic response can only be obtained by monitoring clinical and magnetic resonance imaging (MRI) status during follow up. In an era where therapies are focused on personalization, the aim of this study is to develop a modeling infrastructure to predict the evolution of relapsing MS and the response to treatments. We built a computational modeling infrastructure named UISS (Universal Immune System Simulator) able to simulate the main features and dynamics of the immune system activities. We extended UISS to simulate all the underlying MS pathogenesis and its interaction with the host immune system. This simulator is a multi-scale, multi-organ, agent based simulator with an attached module capable of simulating the dynamics of specific biological pathways at the molecular level. We simulated six MS patients with different relapsing-remitting courses. These patients were characterized on the basis of their age, sex, presence of oligoclonal bands, therapy and MRI lesion load at onset. The simulator framework is made freely available and can be used following the links provided in the availability section. Even though the model can be further personalized employing immunological parameters and genetic information, based on the available data we generated a few simulation scenarios for each patient, including those who matched the real clinical and MRI history. Moreover, for two patients, the simulator anticipated the timing of subsequent relapses, which really occurred, suggesting that UISS may have the potential to assist MS specialists in predicting the course of the disease and the response to treatment.

Keywords: Computational modeling; Agent Based Modeling; Systems Biology; Multiple Sclerosis; Immunity; Degenerative disease.

1. Introduction

Multiple sclerosis (MS) is a heterogeneous disease resulting from the interaction of environment, genetics, and dysregulated immune response. Substantial evidence indicates that MS is an autoimmune disorder in which immune response is directed to myelin components, but neurodegenerative processes also occur and contribute to the buildup of neurological damage. The leading hypothesis on MS pathogenesis suggests that auto-reactive T-lymphocytes, exhibiting the Th1 and Th17 phenotype, become activated in the periphery and gain access to the CNS through a

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disrupted blood-brain barrier (BBB) [1]. Once in the brain, auto-reactive immune cells trigger inflammation by recruiting B-lymphocytes, macrophages, and microglia. Tissue damage results from the release of inflammatory cytokines and other detrimental factors such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [2]. The mainstay of MS treatment is immunomodulating or immunosuppressive drugs and the therapeutic scenario has dramatically changed over the last two decades with the advent of several monoclonal antibodies and oral medications. About 13 drugs are now approved for the treatment of MS, but active substances are in fact 13 as interferon 1 beta, glatiramer acetate and dimethyl fumarate are marketed under several brand names. Over the last decade DMDs for MS have been classified by regulatory authorities as first-line, second-line and third-line therapies, mostly based on their safety profiles. However, according to their evidence of efficacy, drugs for MS are now grouped by clinicians into moderate-efficacy DMDs and high-efficacy DMDs [3]. As of today, the assessment of therapeutic response can only result from clinical and magnetic resonance imaging (MRI) data and it requires months of careful monitoring to be reliable. In an era where therapies are oriented to personalization, tools to predict the evolution of MS and the response to treatments at individual level are eagerly needed.

In recent years, the concept of early therapeutic intervention has become popular also in the field of MS. A substantial amount of data indicate that brain damage occurs from onset and proceeds relentlessly, even during clinically silent phases [4–7]. Furthermore, long-term studies show that patients who were treated earlier have better outcomes as far as relapse rate, disability accumulation and brain volume loss [8]. The ideal target of MS treatment is now the achievement of no evidence of disease activity (NEDA) which encompasses the absence of clinical relapses, disability progression and new MRI lesions [9]. The most common treatment strategy so far has been that of escalating from a moderate- to a high-efficacy drug if disease activity was still persistent or resurgent. However, for patients with highly active disease at presentation the use of high-efficacy therapies form onset is recommended to prevent accumulation of disability [10]. Furthermore, some of the high-efficacy DMDs such as alemtuzumab and cladribine have profound depleting effects on T and B lymphocytes and may lead to a reshaping of the antigen-specific repertoire, reproducing to a minor extent the effects of autologous hematopoietic stem cell transplantation. These treatments are referred to as immune reconstitution therapies and are the prototype agents of induction strategy for MS [11].

Whatever the initial therapeutic approach is, a close clinical and MRI monitoring is warranted during the first year of treatment, as the recurrence of disease activity should prompt a switch to more efficacious drugs. For this purpose, MRI results more sensitive than clinical parameters as new lesions tend to occur 5 to 10 times more frequently than relapses, however, performing MRI at short intervals is infeasible in daily practice. In addition, first line drugs, although comparable for efficacy, exhibit different mechanisms of action (MoA) and individual response may be heterogeneous. Thus far, there is no laboratory test that has been validated as a predictor of drug responsiveness or therapeutic failure in MS and therefore the search for early and reliable tools to guide the treatment is still ongoing. Finally, it is largely believed that a personalized approach would be the most efficacious in the treatment of multifactorial diseases such as MS, but this clinical strategy is still far to be put into practice due to the high number of variables that need to be considered [12].

In the last years, different models focused in describing MS dynamics in general and the relapsing-remitting multiple sclerosis (RRMS) type from a mechanistic point of view have been proposed. In this regard, an exhausting overview of computational modeling applied to MS has been published by Pappalardo et al. [13].

Vélez de Mendizábal et al. [14] presented a Stochastic Differential Equations (SDE) model able to qualitatively reproduce the typical oscillating behavior of RRMS. However, such model is quite simplistic, as it takes into account only a restricted number of entities involved into the developing of the disease. In [12] the authors present an agent based model developed with the NetLogo software. The model is based on the same hypotheses of the previous one, but with a more complete description of the involved entities, including some thoughts about the possible positive effects of vitamin D [15], and the role of the Blood Brain Barrier (BBB) [16]. However, both the two approaches do not take into account any specific treatment.

Differently, in[17–20] a computational methodology based on the Petri Net formalism has been proposed to qualitatively analyse the RRMS behaviour under the administration of daclizumab.

Recently, Kannan and collaborators [21] developed a computational model that reproduces the principal types of MS and accounts for several features of the disease progression. Kotelnikova and co-workers [22] presented a mathematical model that reproduced the different disease courses, despite the heterogeneity of genetic and environmental factors, supporting the hypothesis that a common pathogenesis is at the origin of different MS subtypes, and this applies also when genetic and environmental factors are heterogeneous. Although the process by which constitutional (genetics, ethnicity, gender) and environmental (viral, sun exposure, diet) elements would interact in MS pathogenesis is still far to be elucidated, data derived from the natural history of the disease and longitudinal MRI studies provide prognostic tools currently used in daily practice. It must be said that all the models described above limit their functions to a general qualitative description of the disease, and none of them is specifically tailored with patient data to provide personalized simulations under the administration of a specific treatment. The model we present here is the only one that, to our best knowledge, is able, at the present time, to deliver an accurate description of the disease dynamics, including the effects of the most common treatments. Moreover, our model is designed to use specific patient data in order to provide personalized simulations.

A computational model of MS dynamics, as the one we are going to describe, could overcome the difficulties in handling the large number of information required to generate a disease profile at individual level, helping to predict the course of the illness and to choose the best therapeutic option.

Based on these premises we envisaged to build a computational model for reproducing and predicting the immune system dynamics in MS patients and we tuned the model with clinical and MRI features as a preliminary attempt to personalize the framework. The next step would be to incorporate immunological features (cytokines level, lymphocytes sub-population counts, HLA profile) to refine the in silico model and hence increase its predictive power. The final aim would be the generation of a baseline profile for each patient that could be translated into a computational model to assist clinicians in predicting the evolution of the disease and the response to different drugs.

2. Materials and Methods

2.1. The computational model

MS is a neuroinflammatory disease with an extremely complex immunopathology that involves practically all the immune system machinery. Moreover, as in the case of other autoimmune diseases, the building of a computational framework should consider also the role of immunoregulatory mechanisms. Indeed, there is the necessity to have a methodology that describes in an integrated manner all the entities and their relationships acting in the biological scenario. Ontology is an approach that formally defines and assigns the types, properties, and interrelationships among the entities within a particular domain. Building an ontology specifically designed for MS aims to give a strong contribution to acquire a better understanding of the critical properties of the phenomenon i.e., the system entities, how they are organized, and their dynamics. Malhotra et al. [23] developed an exhaustive ontology from MS, using scientific literature and expert review under the Protégé OWL environment [24]. Protégé is a complete editor for developing ontologies and it is released under an open-source license. It also allows, through the use of a reasoner engine, to check the consistency of the ontology. We used Protégé to build a complete ontology that contains: i) the classes needed to describe both the immune system and specific disease related features (done also using the work done by Malhotra et al. [23]); ii) the object properties related to the interactions and localization; iii) entities (i.e., individuals) that are linked to the classes; iv) data properties that provide an extensive description of the interactions. Once the ontology description is completed, the next step is the choice of the best computational technique able to host all the components of interest.

Several immunological case studies [25] describe in an exhaustive way the use of unified modelling language (UML) in representing and capturing the dynamics of individual model entities

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in a system-level emergent properties. UML formalism could be a valuable complement in modeling complex systems, especially in immunology and, in a future perspective, it could be useful to provide a metamodel combining both language in the definition of a biological system. Both UML modelling and OWL ontology own strengths and weaknesses, similarities and differences. It is worth mentioning that ontology could be used to represent interactions in an effective way. An example of this use is shown in Figure 1. We are, however, going to better formalize this methodology.

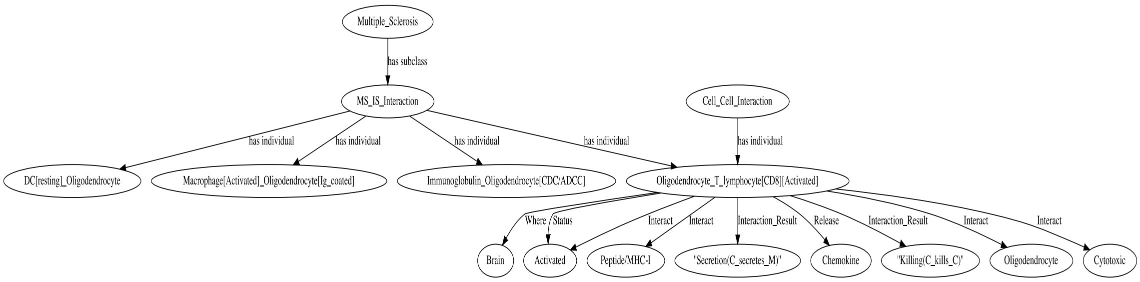

Figure 1. An overview of the ontology of a specific autoimmune interaction during the MS dynamics. A previously activated cytotoxic T lymphocyte encounter its target i.e., an oligodendrocyte, in the white matter portion of the brain. It recognizes, at the molecular level, the peptide/MHC-I complex that is exposed on the surface of the oligodendrocyte. The result of the interaction is the killing of the oligodendrocyte by the cytotoxic T lymphocyte and the local release of chemokine factors that will attract further activated effector immune cells.

We used an agent-based modeling (ABM) approach to build the simulation framework given its suitability to be used as a cellular and molecular simulation technique and taking into account our previous successful experiences in simulating the dynamics of immune system in several pathologies. In particular, we adapted and extended our immune system simulation framework [16,26–28], now named "Universal Immune Simulator Framework" (UISS), to model the dynamics of the immune system in MS along with the therapeutic effects of teriflunomide, fingolimod, IFN-β1a, ocrelizumab and natalizumab. The advantages of this modeling methodology are well known: entities as well as biological functions and interactions can be described very closely to the biological reality; at the same time, this approach allows a mathematician or a computer scientist to describe the scenario using a logic and rational framework. Finally, the ABM approach allows flexibility and further extension and refinement of the model without significant additional effort.

The ABM is built over five main concepts. Identification concept include all the entities with their functions and activities that play both a role in the immune system dynamics and in the evolution of MS. In particular, we considered all the relevant immune cells and molecules along with their specific properties.

The concept of localization is devoted to establish the position in which the entities are located especially during interactions. We considered two specific compartments, the brain (white matter) and the peripheral lymph nodes. Effector cells (B and T cells) are challenged with auto-antigens and consequently activated into lymph node compartment while the inflammatory and immune system activity against the target take place into the white matter compartment. The class named "treatments" includes all the details and the effects of the therapies we tested by the simulator. The biological scenario class identifies all the specific elements and interactions of MS we took into account. We describe in details this concept later on. To characterize all the cellular and molecular activities, functions and interactions, the computational framework was built as a polyclonal model, i.e. it is able to represent all the possible binding sites of B and T lymphocytes, Major Histocompatibility Complexes of both class I and II, antigens (MHC-I and MHC-II), epitopes and peptides. The technique used is based on a binary strings representation of the binding sites. Both molecular and cellular entities interact following procedures describing the recognition processes and the following events according to the immune system response. The model also implements processes encompassing phagocytosis, antigen presentation, cytokine and chemokines release, cell activation, cytotoxicity and immunoglobulins secretion.

In our simulation framework, interactions among entities take place when they are in a range in which they can "sense" each other. The term "sense" means that two entities can interact if they are located at the same site of the structure developed for the simulation i.e., in the same lattice point. We used a lattice that represents a specific volume of peripheral lymph nodes or blood. Entities interact following a specific stochastic rule that is a function of the Hamming distance between binary strings that, as told before, mimic the receptor binding. Presence of cytokines is also considered and they can act by favoring or inhibiting the probability of the interaction.

The model takes into account innate and adaptive immunity (both cellular and humoral) and immune memory.

2.2. Introdution to the simulation framework

To model MS dynamics, we used the UISS simulator framework. It is based on the agent based model paradigm that has been developed to represent the immune system response to general pathogens [12,29,30]. In computer science, agent based models represent a simulation technique in which entities, conversely to differential equations systems, are followed individually. In this way, complex emergent behaviors can arise and lead to the prediction of non-coded dynamics.

The entities, namely "agents", are usually placed on a simulation space (i.e., a lattice), and in each lattice point we can have many entities. Such agents can be heterogeneous in nature, can have internal properties (i.e. lifetime, internal state and energy), and can act (i.e. move, interact with other agents in their neighborhood, modify their internal state or die) individually or as a result with the interaction with other agents. ABM have several advantages. By definition, they can be stochastic and include both delays and a spatial description. Furthermore, they permit to better describe the biological aspects and behaviors of the involved entities. As a consequence of that, the accuracy in the description is usually limited by the biological knowledge rather than the modeling methodology. Nonlinear behaviors, as well as the capacity to add further complexity and biological knowledge, do not represent a problem in solving the model. Such methods are also intrinsically numerically stable as most of the involved variables are represented by integer numbers, so very few complex floating point operations are required.

UISS is entirely written in ANSI C-99 standard programming language, allowing to be architecture-independent. The modelling approach used by UISS is the same used by Celada and Seiden [31].

In UISS we considered both cellular and molecular entities. Usually, cellular entities are followed individually and are modeled as singular agents. Molecules are instead considered by their concentration per lattice-site. Cell agents have different properties, such as position, half-life, and an internal state from a certain set of suitable states. Their dynamics is realized by means of state changes. A state change takes place when a cell agent interacts with another agent, like a cell, a molecule or both of them. The most relevant cells belonging to the IS have been considered, including B lymphocytes, helper, cytotoxic and regulatory T lymphocytes and natural killer cells. Monocytes are represented as well and we take care of macrophages and dendritic cells. Some entities, like B and T cells, also have specific receptors for modeling specificity, as their real counterparts. For what concerns molecules, the model distinguishes between simple small molecules like interleukins or signaling molecules in general and more complex molecules like immunoglobulins and antigens, for which we need to represent the specificity.

At the same level of entities, immune system activities are represented. They include both interactions and functions. Functions refer to the main immune system tasks. In particular, UISS takes care of the diversity of specific elements, major histocompatibility classes restriction, clonal selection by antigen affinity, thymus education of T cells, antigen processing and presentation (both the cytosolic and endocytic pathways are implemented), cell–cell cooperation, homeostasis of cells created by the bone marrow, hypermutation of antibodies, cellular and humoral response and immune memory.

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In UISS, as in most ABM approaches, time is discrete. This means that all system activities are followed and measured using equally-spaced time intervals. At each time interval, all the immune system activities, such as the interaction and the diffusion processes, hold.

An interaction between two entities is a complex action which eventually ends with a state change of one or both entities. In order to interact, the entities must be "near enough". More specifically, physical proximity is modeled through the concept of lattice-site. All interactions among cells and molecules take place within a lattice-site in a single time step, so that there is no correlation between entities residing on different sites at a fixed time. In UISS, the simulation space can be either represented by a 2D $L \times L$ hexagonal lattice (6 neighbors) or by a 3D as a $L \times L \times L$ cubic lattice, with periodic boundary conditions or using rigid walls on the edges, according to the problem we are dealing with. All entities are allowed to move with uniform probability between neighboring lattices in the grid with equal diffusion coefficient (Brownian motion). This simulation space is used to represent, more from a biological point of view rather than a physical point of view, three anatomical compartments: the thymus, the bone marrow and a portion of a generic secondary organ.

Interactions can be seen as Bernoulli events, so each interaction has a given probability p to happen. Interactions can be classified as aspecific or specific interactions. Aspecific reactions are those that refer to the use of aspecific receptors. For example, if we take into account Toll-like receptors (TLRs), we know that they will recognize with low specificity Pathogen Associated Molecular Patterns (PAMPs) expressed by pathogens.

These will not explicitly be modeled in UISS, but instead a fixed probability p' will be used for all the interactions that involve the same couple TLR-PAMP. Specific reactions are those who involve cells coming from adaptive immunity and that are equipped with specific receptors. Specific interactions need a recognition phase between the two entities; In this case the probability p of interaction will depend upon the result of the recognition phase, in which the affinity between the involved receptors plays a major role.

UISS represents receptors and ligands as binary strings and uses a string-matching rule to model affinity. This clever idea introduced by Farmer and Packard [32] represents a simple way to mimic the typical molecular complementarity mechanism between receptors. While this may seem a rough approximation of the real biological event, millions of recognitions can be executed efficiently, allowing the study large-scale properties of the immune system. Furthermore, models based on this approach produced accurate results when benchmarked to experiment, suggesting that the abstraction captures important features of receptor/ligand binding and does not represent a limiting factor for the study of many biological scenarios [27,33]. The binding rule of the string-matching procedure mimics as well the complementarity mechanism between two receptors by using the Hamming distance. This distance measures the number of mis-matching bits between two strings.

As a consequence of that, repertoires are represented in the model as sets of strings, and the set of lymphocytes receptors is represented by bit-strings of length h that forms the so called "shape space". A clonal set of cells is characterized by the same clonotypic receptor, i.e. by the same bit-string of length l the potential repertoire of receptors scales as 2^l.

UISS can be then defined as a bit-string polyclonal lattice method. Bit-string refers to the fact that the molecules and the specificity among molecules is represented, and polyclonal identifies the capability to have more clones of different specificity of lymphocytes. Finally, lattice means that a discrete lattice is used to represent the space.

Hematopoiesis and thymus selection represent two of the most important processes that regulate the immune system functions. Hematopoiesis is a biological process that describes, for example, the formation of blood cells derived from haematopoietic stem cells. In UISS, such process is used to describe the production of B and T lymphocytes in the "bone marrow compartment". Moreover, a further selection of TH and cytotoxic T cells holds in the "thymus compartment" out by the thymus selection.

In order to keep the system in a metastable state in absence of perturbations (cell *homeostasis*), haematopoiesis is modelled as an Ornstein-Uhlenbeck mean reverting process that can be described by the following differential equation:

$$\frac{dx_t}{dt}(t) = \frac{ln2}{\tau_i}(x_i(0) - x_i(t)) + \frac{d\varepsilon}{dt}(t)$$

Where $\overline{x}$ is the typical mean number of entities, $x(t)$ the number of entities at time t, η is the speed of the process to revert back to the mean value, and $\sigma d\varepsilon(t)$ is a random stochastic noise. At each time step, the formula is applied to calculate the number of cells that should be added (or removed) from the simulation.

Note that in the presence of an immune response the number of lymphocytes is always greater than the initial value. This means that $dx_i/dt < 0$, so more cells will be removed than in the absence of an antigen. On the other hand, cell clones specific for the antigen that eventually will become memory will grow. The combination of these two actions causes the shift of the immune repertoire towards specificities that recognize the antigen.

Thymus selection is instead used to guarantee that in UISS a repertoire of MHC-restricted, self-tolerant T cells is available. The process uses a selective procedure to mimic the real biological events that happen during T cell maturation in the thymus. This procedure is composed by two sub sequential stochastic procedures: a first positive selection and a second negative selection. In the first phase, the T cells with a receptor that has low affinity to the MHC molecules taken alone (class I for TC and class II for TH) are removed as they are not useful.

Those cells that surpass the first phase, are exposed to the second selection phase, in which T cell receptors are compared with an MHC-self-peptide complex. If high affinity between the two strings is found, such T cells will be eliminated to avoid autoreactivity.

Of course, receptors, MHC molecules and (self) peptides are always represented as binary strings, and the affinity is always calculated as described before. The self antigen is defined by specifying in the input datafile of UISS, a subset of binary strings considered as self peptides in the set $\{0, 2^l - 1\}$, where *l* is the chosen binary-string length.

We note here that a T cell receptor will be positively selected only if it matches at least one MHC molecule with an affinity greater or equal to $l/2 - m_0$, where m_0 is the minimal number of mismatching bits.

Many other biological processes that are part of the IS machinery are implemented. These include Hayflick limit on cell duplication, management of immune memory, hyper-mutation of Antibodies, bystander effect, istotype switching, anergy, antigen digestion and presentation and B cell receptor mutation.

As already described, entities may have a number of internal states depending on their type. We recall here the most common internal states, sketching some typical scenarios that bring to their appearing.

- New born cells are introduced in the **Resting** state.
- A cell becomes **Active** when it is stimulated through an interaction with another entity. For example, TH cell activation occurs with the interaction with an antigen if TCR binds the antigen.
- **Anergic** state. In this state the cell does not interact. This state applies, for example, to B, TH and TC cells.
- Status **Intern** applies only to Antigen Presenting Cells (APC). When an APC (i.e., a M, DC or B cells) encounters the antigen, it may be directed recognized by membrane bound receptors, for example those on surface of a naive B cell. Unlike B cells, T cells do not recognize antigens directly. They "see" antigen as peptides only in association with the host surface MHC (Major Histocompatibility Complex) molecules. Since MHC molecules can only bind peptide molecules of 7-15 amino acids long, T cells only recognize their specific antigen in the form of short peptides. The antigen presenting cells such as dendritic cells and B-cells take up antigen and partially degrade it into peptides which then occupy the antigen-presenting groove in MHC class I and MHC class II molecules.
- When cells present peptides via MHC molecules their status changes to **PresI** (MHC class I) or **PresII** (MHC class II).

- Status **Duplicating** (applies to TH, TC and B cells) is achieved when a cell has been activated and stimulated to start the clonal division.
- **BoundToAb** status applies to specific cells (for example pathogen-infected cells). This state represents the fact that a cell has been recognized by an antibody. Cells in this status may die by action of Ab-complement or by means of natural killer cells.
- Status **Infected** applies to virus target cells. It identifies viral penetration via permeabilization of host cell membrane, when it begins local replication and spread. Each cell can be in different internal states and all cells are tracked individually throughout the course of an experimental run. This status can be **Actively** or **Silently** infected, according to the fact that the virus is replicating or not.

Interactions represent the most important source of entities state changing and drive the evolution of the system. We can have different types of interactions, that include "molecule-molecule" interactions, "cell-molecule" interactions, and "cell-cell" interactions, or combination of them. A list of some of the most important interactions (far from being complete) of UISS are finally sketched as follows.

- Epithelial cell-pathogen. If an epithelial cell encounters**,** for example, a specific virus or intracellular bacteria, the cell will be infected by the pathogen (status **Infected**), then the cell may also present its infected status **(PresI)** to the immune system cells**.** Note here that, instead of epithelial cells that are reported here as an example, we can have different cells that represent target of different pathogens.
- IG_Ag. If a soluble immunoglobulin (IG) encounters its specific antigen (Ag), the IG binds to Ag and forms an immunocomplex (that can be captured by a macrophage). The binding probability is, as already said, a function of the Hamming distance of the two entities.
- B_Antigen. If a naive B lymphocyte expresses at the cell surface a membrane IG which is specific for the native antigen (calculated according the Hamming distance between the two strings), the B lymphocyte internalizes the membrane IG and the bound Ag (state **Intern**), and then processes the IG-Ag complex into peptides which are then presented by MHC-II (status **PresII**) at the B lymphocyte surface. We recall here that the binding probability is a function of the Hamming distance of the B receptor and the peptide (specific interaction). The B cell is now an APC (specialized antigen presenting cell).
- M_Antigen. If a macrophage encounters a native antigen or an immunocomplex, the macrophage internalizes the antigen or the immunocomplex (state **Intern**), and then processes it into peptides which are then presented by MHC-II (status **PresII**) at the macrophage cell surface. The M is now an APC (specialized antigen presenting cell).
- DC_Antigen. If a naive dendritic cell encounters a native antigen or an immunocomplex, the dendritic cell internalizes the antigen or the immunocomplex (state **Intern**), and then it processes it into peptides which are then presented by MHC-II (status **PresII**) at the dendritic cell surface. The DC cell is now an APC (specialized antigen presenting cell).
- TH_B. If a resting T helper lymphocyte (TH) encounters B lymphocyte that is presenting a given peptide-MHC-II complex (status **PresII**), the TH cell becomes an activated T helper lymphocyte (status **Active**) that helps the B cell to differentiate into plasma cell or memory cell. At the molecular level, the interaction holds if the T cell receptor (TR) at the surface of the Th cells binds specifically peptide-MHC-II complex (specific interaction). Then active Th proliferates and secretes interleukin 2 (IL2). At the same time, B lymphocyte proliferates and differentiates into a plasma cell (that secretes IG) or into a memory cell (with IG at its surface).
- TH-M and TH-DC. As already shown in the TH_B interaction. If a resting TH encounters a Macrophage or Dendritic cell in the **PresII** state, TH becomes activated and secretes interleukins that activate other cells of the immune response (NK, mast cells, cytotoxic T lymphocytes and so on).
- TC – Epithelial Cell. If cytotoxic **resting** T lymphocyte (TC) encounters an epithelial cell that is **infected** (and thus also **and PresI** status**),** for example, by a virus or intracellular bacteria, the cytotoxic T cell becomes, in presence of IL2, an **active** cytotoxic T lymphocyte that kills

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the other cell. At the molecular level, the T cell receptor (TR) at the surface of a resting cytotoxic T lymphocyte binds specifically peptide-MHC-I complex at the surface of the cell. It must be noted that such interaction with TC cells may also arise for other infected cells such as, for example, M infected by tuberculosis.

- IG-Epithelial cell (IG-Bacteria, IG-virus). If an immunoglobulin IG encounters an Infected epithelial cell that is **presenting** the antigen at its cell surface, and the soluble IG recognizes specifically the antigen, the opsonized cell (cell with bound IG on its surface) may be killed by complement dependent cytotoxicity (CDC) or by antibody dependent cell cytotoxicity (ADCC) mechanisms. At the molecular level, the first interaction is the recognition by the IG of the antigen expressed at the surface of the bacteria. Similar specific interaction with IG may also arise in other scenario that include binding of IG to cancer cells, infected cells, viruses, bacteria and so on.

Before starting the simulation, an initialization phase is executed. In this phase, the lattice is populated with the required number of entities. Then, the simulation is carried out for a given number of time-steps. At each time-step, both the interaction-driven processes and the non-interaction driven processes (i.e., movement or internal processes) are executed. It must be noted that, ideally, all processes within the time step should happen synchronously. As this does not represent a problem for movement or for internal dynamics that can be easily reproduced as serialized processes, it may be a possible source of bias for the interaction dynamics.

A total massive parallel execution is very difficult to achieve in actual computers, especially when the number of entities is very high. To this end, in order to minimize any bias, for each lattice-site a different random interaction scheme is generated both considering a random order of the interaction rules, and a random order in the list of agents that may interact within the same rule. For a given rule that refers to two entity types A and B, every entity of type A will be compared with all the entities of type B within the same site until a successful interaction occurs. Then, the next entity of type A is examined and compared again with all the entities of type B. The next interaction rule from the random interaction scheme is executed as soon as all the entities have had their chance to interact.

In UISS a set of core features is used to reproduce the standard IS machinery and its response against a generic pathogen. This core, that is composed by entities, processes and interactions, does not usually change unless novelties in the biological knowledge are discovered and assessed.

In order to simulate new pathologies, the set of basic features is extended, from time to time, with a set of specific features necessary to reproduce the missing involved biological mechanisms. While such extensions may change from a pathology to another, the core is kept.

2.3. Extension of the simulation framework to include MS pathogenesis and related treatments

MS affects brain and spinal cord [2,34], but we restricted to the brain oligodendrocytes (mostly present in the white matter region) the main target to measure demyelinating hits over the course of the disease. In particular, myelin basic protein (MBP) represents the oligodendrocyte associated antigen that we used to simulate the self-antigen recognition by the immune competent cells. The complete list of entities specifically used and/or added for modeling MS is reported in Table 1.

Table 1. Cellular and molecular entities implemented in the simulation framework. Initial quantities and half-life values were retrieved from literature in the field [31]. UISS framework takes into account B cells, TH (CD4+ T cells), TC (CD8+ T cells) CDC (conventional dendritic cells), M (macrophages), P cells (plasma B cells), IC (immunocomplexes), ODC (oligodendrocytes), IFN-G (interferon gamma), IL-x (interleukins of type x), TGFB (transforming growth factor beta), MBP (myelin basic proteins), IgG (Immunoglobulins class G) and chemokines (as generic chemokines).

Entity	Initial quantity per µL (or cubic millimeter)	Half-life (days or hours)
B	260	3.3 days
TH	876	3.3 days
TC	434	3.3 days
CDC	351	3.3 days
M	351	3.3 days
P	0	3.3 days
IC	0	4.0 days
ODC	45000	4.0 days
IFN-G	0	1.6 days
IL-2	0	1.6 days
IL-4	0	1.6 days
IL-10	0	1.6 days
IL-12	0	1.6 days
IL-17	0	1.6 days
IL-23	0	1.6 days
TGFB	0	1.6 days
MBP (myelin basic protein)	0	3 days
IgG	0	23.0 days
Chemokine (generic)	0	3.0 hrs

The model takes into consideration a 2D domain physical space: although this limits the representation space, all the processes and interactions are simulated between two main compartments. The first one is represented by lymph nodes while the second one is represented by a slice of white matter populated by oligodendrocytes. This results in a satisfactory approximation and allows the simulator to run in smoothly fashion.

In order to set the simulation space we used the same approach followed in [35]. To simulate inflammation and the resulting chemo attraction dynamics, we also added to the simulation framework the chemotaxis feature. Chemotaxis is a biological phenomenon in which some cells, like immune system cells, direct their movements according to certain chemical signals. Chemotaxis is a very complex process not yet fully understood. It involves many factors such as short and long-range interactions. To our best knowledge, there is no model able to completely represent this phenomenon.

In a first attempt to mimic short range chemotaxis effects, higher probabilities of being chosen are given to sites containing chemokines. Let x0 be a lattice-site and xi, i = 1 . . . 6 be its neighborhood. For every xi, i = 1 . . . 6, the probability pi is defined as:

$$p_i = \begin{cases} \frac{C_i}{\sum_{j=0}^{6} C_j}, & \sum_{j=0}^{6} C_j > 0 \\ \frac{1}{7}, & \sum_{j=0}^{6} C_j = 0 \end{cases} \quad (1)$$

where Ci represents the quantity of chemokines in the lattice site xi. Once a new site xi has been chosen for movement, the entity will move towards xi with probability p calculated as the following:

$$p = 1 - \min\left(\left(\frac{\sum_i^n E_i}{M_{cells}} \cdot K\right), 1\right) \quad (2)$$

where $\sum_i^n E_i$ denotes the total number of entities Ei in the lattice-site x, K is a constant ∈ (0,1) and Mcells is the maximum number of entities a lattice site can usually contain. The use of K is justified by the fact that in non-safe conditions i.e., in presence of inflammatory processes, biologists observed the infiltration of immune system cells such as macrophages in site. Thus, using $K < 1$ it is possible to have some chance for immune system cells to move into sites that are already full. In absence of chemokines, cells move through random walk.

The simulator takes care of the main interactions that happen during an autoimmune response. Besides the interactions that we already have into the simulation framework, we added specific interactions that are needed to simulate the inflammatory and autoimmune response against oligodendrocytes. We included the following interactions.

Resting dendritic cell and oligodendrocyte interaction. If a dendritic cell (DC) encounters an oligodendrocyte by means of cross-presentation mechanism DC captures and cross-present antigens from oligodendrocyte as peptide/MHC-I complex. In this case, being an oligodendrocyte a self cell, B7 proteins is not activated on DC.

Antigen presenting in peptide/MHC-I complex dendritic cell with B7 proteins not activated and cytotoxic T cell interaction. If in a lymph node, a DC that is presenting on its surface peptide/MHC-I complex (APC) with B7 proteins not activated, encounters a resting cytotoxic T lymphocyte, in the context of defective peripheral immune tolerance, cytotoxic T cell (auto-reactive clone) becomes activated. This simulates what happens in individuals where genetic predisposition combined with environmental stresses can lead to a breakdown in peripheral tolerance leading to autoimmunity [36].

Oligodendrocyte with IgG interaction. If self-reactive IgG recognize antigen expressed on the membrane of the oligodendrocyte, their binding leads to opsonization. The opsonized cell (cell with bound IG on its surface) may be killed by complement dependent cytotoxicity (CDC) or by antibody dependent cell cytotoxicity (ADCC).

Activated Macrophage with IgG coated oligodendrocyte interaction. If a macrophage encounters an opsonized oligodendrocyte, the macrophage kills the oligodendrocyte and releases chemokine factors in site.

Activated cytotoxic T cell with oligodendrocyte interaction. Into the white matter, if a cytotoxic T lymphocyte (CD8) encounters an oligodendrocyte, CD8 T cell kills the oligodendrocyte. Oligodendrocyte killing promotes the local secretion of chemokine factors. At the molecular level, the CD8 receptors recognize the MBP/MHC-I complex shown at the oligodendrocyte surface.

All of these interactions were implemented into the ontology. Figure 1 describes one of the most common autoimmune response against oligodendrocyte i.e., a CD8 activated T cell that kills the oligodendrocyte.

Although the etiology of MS is still mysterious, environmental, genetic, infectious and immunological factors contribute to MS pathogenesis and may be also conceived as risk factors [1]. In our simulation framework, we considered all of them with one or more representatives in each class of risk factors. In particular, we included EBV infectious agent that is believed necessary to the genesis of MS [37]. We simulated the mechanism of molecular mimicry that involves reactivity of T and B cells with either peptides or antigenic determinants shared by infectious and self-antigens (MBP in this simulation, which is a potential autoantigen). Genetic predisposition was considered by simulating, into the binary string that implements Human Leukocyte Antigen (HLA) genetic composition, those polymorphisms that render patients more prone to activate autoimmune response against self-antigens expressed on the surface of Oligodendrocyte (ODC) (e.g. MBP). For the specific immune system class, we considered malfunction of regulatory mechanisms: this presently involves both central and peripheral immune tolerance malfunctioning. We simulated central malfunction through an impaired thymus selection process that produces a larger number of auto-reactive T cells. Peripheral tolerance malfunction was implemented through an increased probability for an auto-reactive clone (both T and B) to be activated even in the absence of B7 proteins expressed on the surface of APCs. This prevented both induced anergy of autoreactive clones and a sufficient release of anti-inflammatory cytokines like IL-10, IL-4 and TGFB. Environmental factors were represented by low vitamin D levels and cigarette smoking as both were demonstrated to play a detrimental role

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in immune system regulatory mechanism and in MS. This risk factor matrix allowed us to obtain a good framework able to simulate the baseline conditions for a virtual patient developing MS. Another goal of our work is to study the evolution of relapsing MS in patients under licensed treatment such as teriflunomide, fingolimod, IFN-β1a, ocrelizumab, or natalizumab. Consequently, we needed to implement their mechanism of action inside the simulation framework.

To include effects of IFN-β1a drug into the model, we searched for literature containing summary of biological activity and dynamics of IFN-β1a. Laboratory and clinical studies [38] have shown that IFN-β1a modulates inflammation underlying MS activity, acting on a variety of processes and molecular mediators within the immune system. In particular, we inserted: i) IFN-β1a effect on the cytokine production, favoring the switch from a Th1 (pro-inflammatory) to a Th2 phenotype (anti-inflammatory) [39]; ii) inhibition of T-cell activation and reduced expression of MHC-II molecules, which in turn reduces self-antigen presentation [40]; iii) enhancements of CD4+ regulatory T cells activity [41]. For the modeling purposes, we considered IFN-β1a at the dosage of 44 mcg three times a week.

Teriflunomide acts through a specific non-competitive reversible inhibition of the enzyme dihydroorotate dehydrogenase (DHODH), localized within mitochondria organelle and highly expressed in proliferating lymphocytes [42]. This drug inhibits cell growth and division of proliferating T and B cells. To build the model of this mechanism of action, we extracted the topography of all the entities and the relative biochemical reactions from KEGG (Kyoto Encyclopedia of Genes and Genomes) PATHWAY Database [43]. With all these data ready, we developed a model network made of 29 different species involved in 13 enzymatic reactions. The model was realized through COPASI (COmplex PAthway SImulator), a software able to simulate and examine the dynamics of biological pathways [44]. The model accepts as input the dosage of teriflunomide and provides, as output, the probability to reduce T and B cell proliferation to the main simulation framework. For the modeling purposes, we considered teriflunomide at the dosage of 14 mg once a day.

Fingolimod is an oral prodrug that acts by inhibiting lymphocyte egress form lymph nodes, especially interleukin-17-producing T cells [45]. We simulated the net effect of the fingolimod by preventing T cells migration into the compartment of white matter and by inhibiting IL-17 producing cells (Th-17). For the modeling purposes, we considered fingolimod at the dosage of 0.5 mg once a day.

Natalizumab is a humanized monoclonal antibody that selectively binds to the α4-subunit of α4β1- and α4β7-integrins expressed on the surface of human leukocytes [46]. We simplified the mechanism of action taking into account the final effect that can be reproduced as a reduction of leukocyte migration in the white matter compartment [47,48]. For the modeling purposes, we considered natalizumab at the dosage of 300 mg every four weeks. Ocrelizumab is a humanized monoclonal antibody directed against CD20 B cells [49] and to represent its activity we exploited the processes already included into the computational framework that were both antibody – cell interaction and natural killer – Ab-opsonized cell interaction. In other words, when ocrelizumab is injected, it binds to B cells, which become targets for natural killer cells. For the modeling purposes, we considered ocrelizumab at the dosage of 600 mg every six months.

2.3. Simulation of real patients

The main target of this study is to have a simulation framework able to predict the dynamics of relapsing-remitting MS patients when treated with specific drugs. In [50], the authors present a multivariable survival and generalized linear models to predict individual treatment response for MS. However, they use a statistical approach that is very useful to identify patterns in the prevalent multiple sclerosis population, whose predictive value exceeds that of the isolated individual variables. We use a different approach that is based on a mechanistic model. The aim is to have a detailed analysis of the immune system behavior that could predict the outcome of a treatment in a specific MS patient. We limited the analysis of drugs to IFN-β1a, fingolimod and teriflunomide (first line drugs) and natalizumab and ocrelizumab (second line drugs). The simulator offers various strategies to reproduce a real patient in silico, hence to personalize the simulation. The optimal case would be a patient at disease onset with specific analyses that can provide data about: i) cytokine levels i.e., IL-2, IL-10, IL-12, IL-17, IFN-G, TGFB; ii) HLA genotype; iii) MRI lesion load; iv) lymphocyte subpopulation data; v) oligoclonal bands status; vi) vitamin D levels; vii) history of infections; viii) gender; xix) ethnicity. To obtain all the above-mentioned data in real patients, we should conduct a prospective and systematic study, but, at this stage, this would not be feasible without preliminary evidence that our simulation framework could be useful for the purpose. After the approval of our local ethics committee, we selected six patients who gave their informed consent. For each subject, we obtained age at MS onset, baseline MRI lesion load, oligoclonal bands status and the administered treatment. These features are widely accepted as prognostic parameters of MS evolution [51]. For the modeling aims, we associated the lesion load at onset with the probability for an oligodendrocyte to die by apoptosis, hence releasing in situ autoantigens that can then be captured by APC (dendritic cells and macrophages). The greater the lesion load at onset, the higher is the probability for an oligodendrocyte to die by apoptosis. The presence of oligoclonal bands is a biomarker that is associated with a more aggressive prognosis of MS [52,53]. As the oligoclonal bands are made of immunoglobulins of class G and/or M, they can be translated, in the simulation model, into an augmented autoimmune response in patients suffering of MS. Then we associated the presence of oligoclonal bands with an impaired function of the regulatory system in the simulated patient. Finally, we considered the age as an indicator of a generic immune system reactivity. The lower is the age at the onset, the more active is the general immune response against auto-antigens. Table 2 shows a summary of the clinical data obtained for each of the selected individuals.

Table 2. Patients data summary. Table shows, for each patient, the clinical information that we translated into simulation terms in order to reproduce and predict the MS dynamics.

Patient ID	Age range	MS age onset	OB	Pregnancy	Lesional load (YYYY/MM–number)	Spinal lesions (YYYY/MM–number)	Treatment
1070	20-29	20	yes	no	2007/03–32007/06–5 2010/10–77 2010/12–82 2013/05–59 2015/06–41	2013/05–6 2015/06–7	2007/09: IFN-β1a 2013/05: Fingolimod 2015/12: Natalizumab
3736	30-39	30	yes	yes, from 2015/11 to 2016/08	2014/09–22 2015/01–28	N/D	2015/02-2015/12: Natalizumab 2016/09: Natalizumab
2789	30–39	33	yes	yes, from 2012/10 to 2013/07 and from 2015/11 to 2016/08	2013/11–9 2013/12–9 2014/09–9 2017/01–14	N/D	2017/02 2017/02: Teriflunomide
5793	20-29	26	yes	no	2015/08–20 2015/09–22 2016/01–28	N/D	2016/06: Natalizumab
2070	30-39	31	no	no	2014/10–23 2015/03–37 2016/01–33	N/D	2014/12: IFN-β1a
2961	30-39	29	no	no	2015/03–49 2016/03–43 2017/04–65	N/D	N/A

Another aspect that we took into account is the pregnancy condition of some female patients. Several findings report that pregnancy ameliorates MS and other cell-mediated autoimmune conditions like psoriasis and rheumatoid arthritis through a temporary status of enhanced immunomodulation [54,55]. In this case, during the pregnancy period, we simulated an increased activity of the central and peripheral mechanisms of immune tolerance, leading to a lesser activation of auto-reactive T and B clones directed against ODC.

3. Results

The computational framework is able to reproduce the biological variability of the immune system repertoire. Setting an initial parameter that identifies the random seed of the simulation, the binary strings that are related to MHC-I and II are randomly chosen. Consequently, the initial immune system repertoire is shaped randomly selecting lymphocytes receptors that undergo, in a second step, to thymus selection (for T lymphocytes) and bone marrow maturation (for B cells and the other immune system cells). In other words, varying the initial random seed, the simulator is able to reproduce different immune repertoires for an identified patient. This feature is really important for almost all the simulations that involve the immune system dynamics.

3.1 Prediction robustness

On the basis of Rotstein and Montalban's last evidences [56], we considered some of the major predictor factors established for a poor prognosis in multiple sclerosis. They dealt with demographic and environmental factors, clinical factors, MRI observations and biomarkers. We used some of them, i.e., age, vitamin D levels, smoking, number of lesions, lesions volume and IgG and IgM oligoclonal bands in CSF, as simulator input parameters in order to verify poor prognostic factor evidences. To achieve a proof of model correctness, robustness and with the aim to reproduce biological diversity, we generated a sample set of 100 virtual subjects-specific models, from which we extracted 60 relevant behaviors of MS patients, classified in different level of disease severity: high (20), light (20) and medium (20). The disease severity was measured in terms of loss of ODC.

In the following figures (5-7), we show both the mean behavior (green line) and the +/- SD (blue lines) of the ODC dynamics respectively for those patients that show a high, medium and low level of ODC loss. Each cluster of patients is characterized by different values of age, vitamin D, smoking, number of lesions, lesions volume and IgG and IgM oligoclonal bands in CSF in a way that represents the correspondent prognostic severity scores.

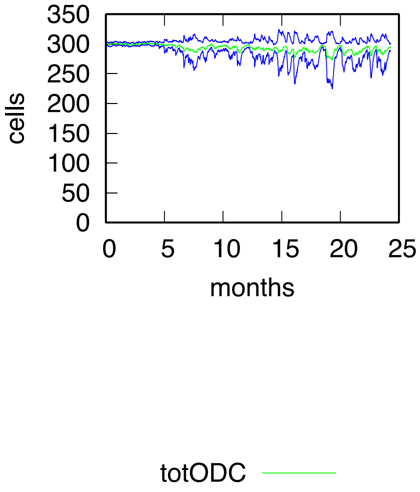

Figure 5. ODC dynamics revealed by 20 simulation cases of MS virtual patients. In this case, MS patients show a high loss of ODC levels.

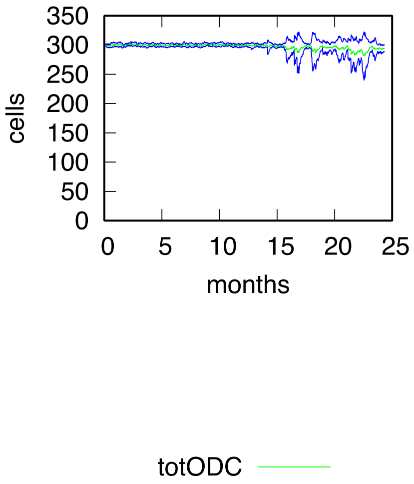

Figure 6. ODC dynamics revealed by 20 simulation cases of MS virtual patients. In this case, MS patients show a light loss of ODC levels.

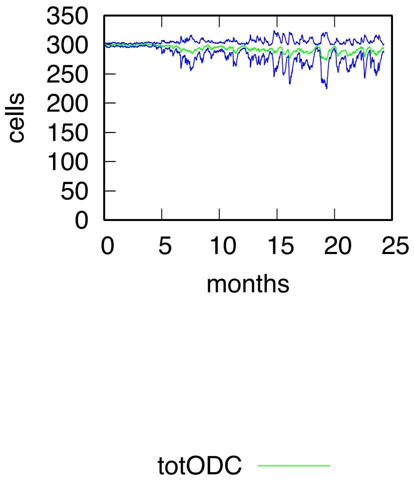

Figure 7. ODC dynamics revealed by 20 simulation cases of MS virtual patients. In this case, MS patients show a medium loss of ODC levels.

As one can see, results reflect the influence of the factors identified by Rotstein and Montalban in the prognosis of MS. Figure 5 shows a high number of relapses (measured as ODC loss): the simulated patients in this case are all affected by very low levels of vitamin D, high levels of lesions in terms of numerosity and volume, presence of oligoclonal bands, older age and a not negligible habit in smoking. Figure 6 shows a light number of relapses (measured as ODC loss): the simulated patients in this case are all affected by normal levels of vitamin D, light levels of lesions in terms of numerosity and volume, absence of oligoclonal bands, middle age and a negligible habit in smoking. Finally, figure 7 shows a medium number of relapses (measured as ODC loss): the simulated patients in this case are all affected by low levels of vitamin D, medium levels of lesions in terms of numerosity and volume, presence of oligoclonal bands, middle age and a not negligible habit in smoking.

3.2 Real patients predictions

We designed real patients simulations using the following procedure. For each of the six patients we set the simulator parameters based on the clinical, laboratory and MRI features of the patients i.e., age at onset, lesion load at onset and OB status. Given the wide variability of predictable MS

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dynamics stemming from only three baseline features, for each virtual patient we run the simulator using multiple different immune system repertoires randomly generated. We verified that 10 was the minimum number of simulations that allowed to obtain at least one model that could reasonably fit the available clinical data. These findings demonstrate that the simulation framework is potentially able to reproduce the real individual dynamics of MS. Figure 2 shows the simulations representing ODC dynamics that best fitted the clinical data obtained for each patient.

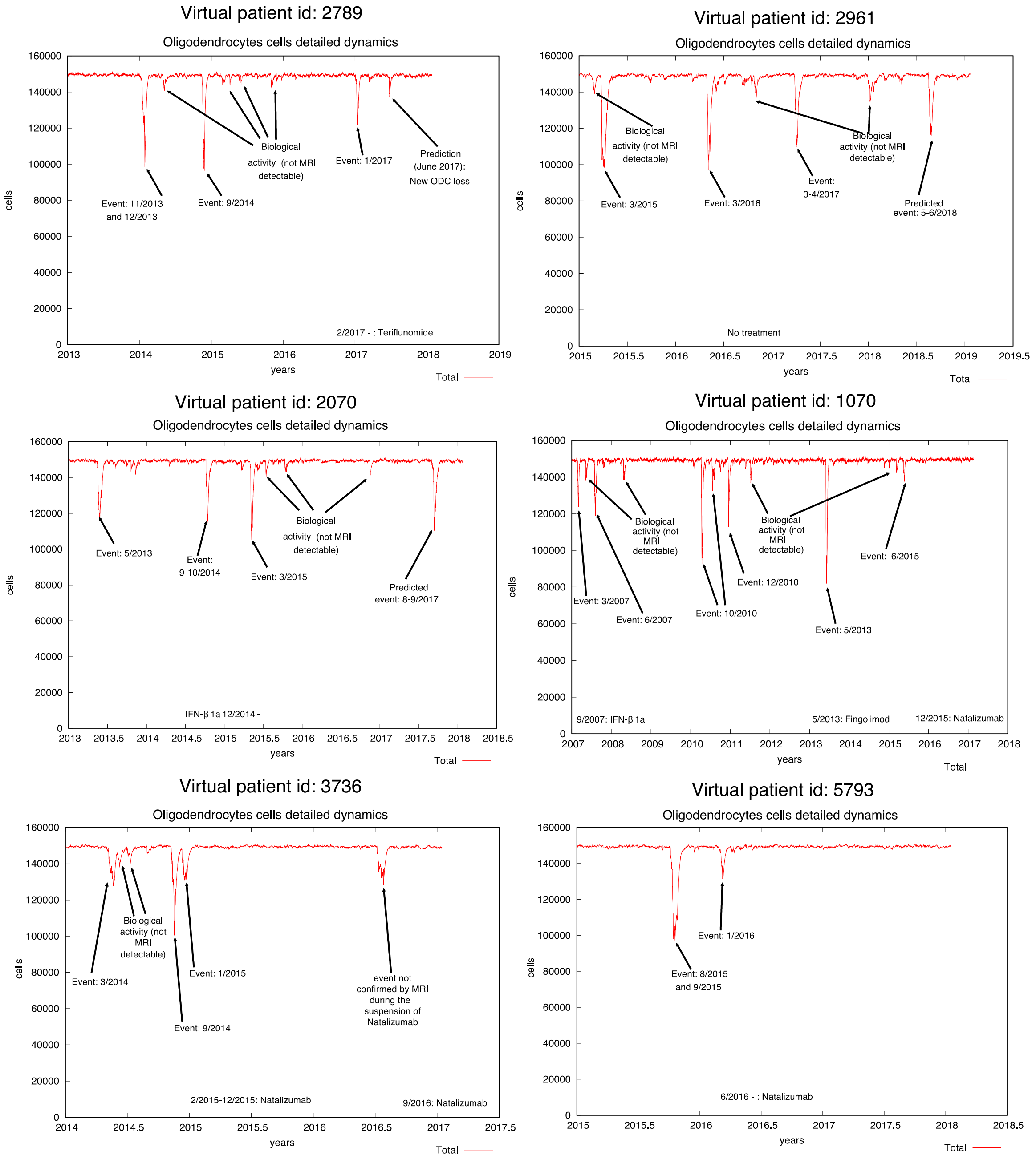

Figure 2. Simulated ODC dynamics for six patients. For each of the six patients, we depict the best simulation obtained, i.e.the one that parallels real patient data.

We simulated three cubic millimeters of white matter with ODC content explained before, i.e. $50x10^3$ ODC per cubic millimeter. We set as a MRI detectable event a lesion that involves at least $50x10^3$ ODC, approximating a uniform distribution of ODCs in the white matter tissue. If the ODC loss is greater or equal to $50x10^3$, this quantitively means that one or more lesions appeared and are MRI detectable. We were, at this stage, unable to have a better quantitative measure. We are working to have another quantitative method to put in relationship what the model measures in terms of ODC loss with real biological data. One of this may be represented by DNA methylation of the myelin

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oligodendrocyte glycoprotein gene in the blood [57]. Smaller ODC losses (undetectable by MRI) were classified as inflammatory biological activity and were considered useful for the analysis of treatment response. The data from Table 2 and Figure 2 shows that the predicted ODC dynamics is in good agreement with the MRI changes in lesion load. Predictions of potential adverse reactions have been obtained for each patients increasing the simulation time for one year. Furthermore, for the patient id 2789 and patient id 2070, the computational framework forecasted two events of ODC injury which were confirmed afterwards by clinical or MRI follow-up. This confirms that the simulation infrastructure was able also to correctly predict future relapse events due to a therapy that was potentially not effective for that patient.

In figures 3 and 4 we report further simulation plots to show both the immune system dynamics and oligodendrocytes dynamics while comparing an independent virtual MS patient with a healthy control case. The simulation time is 4.5 years. It is worth highlighting that in order to reproduce the typical dynamics in a relapsing-remitting MS virtual patient we set two specific parameters in the computational framework that control the regulatory (both central and peripheral) system.

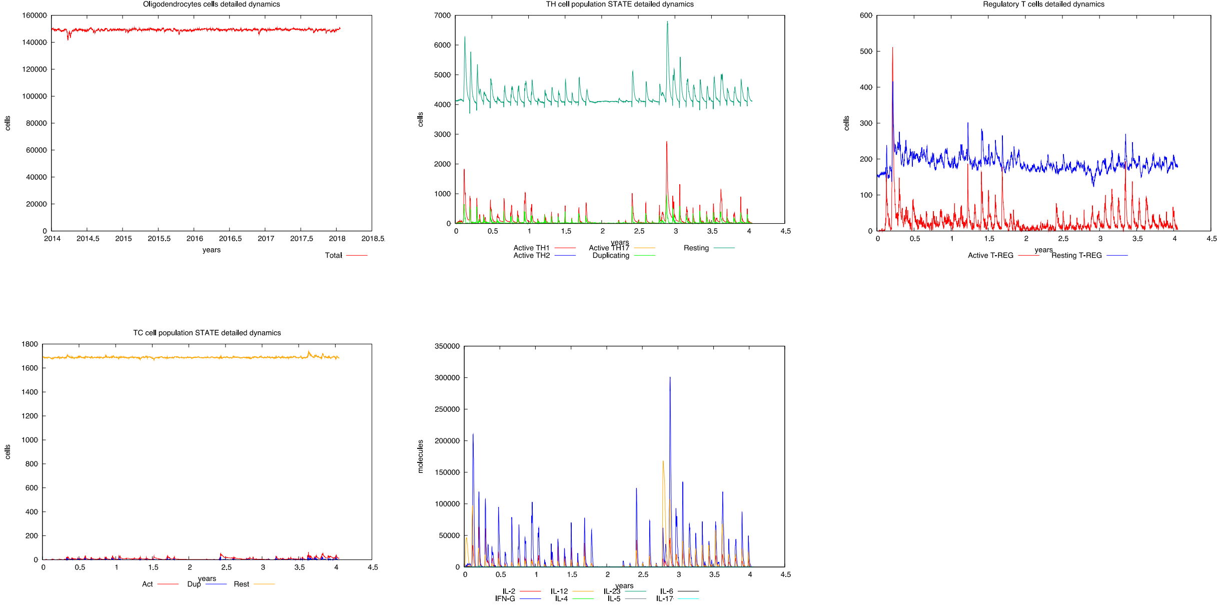

Figure 3. Simulated ODC, TH cell population, regulatory T cells, TC cell population and inflammatory and anti-inflammatory citokines dynamics for the virtual healthy control patient.

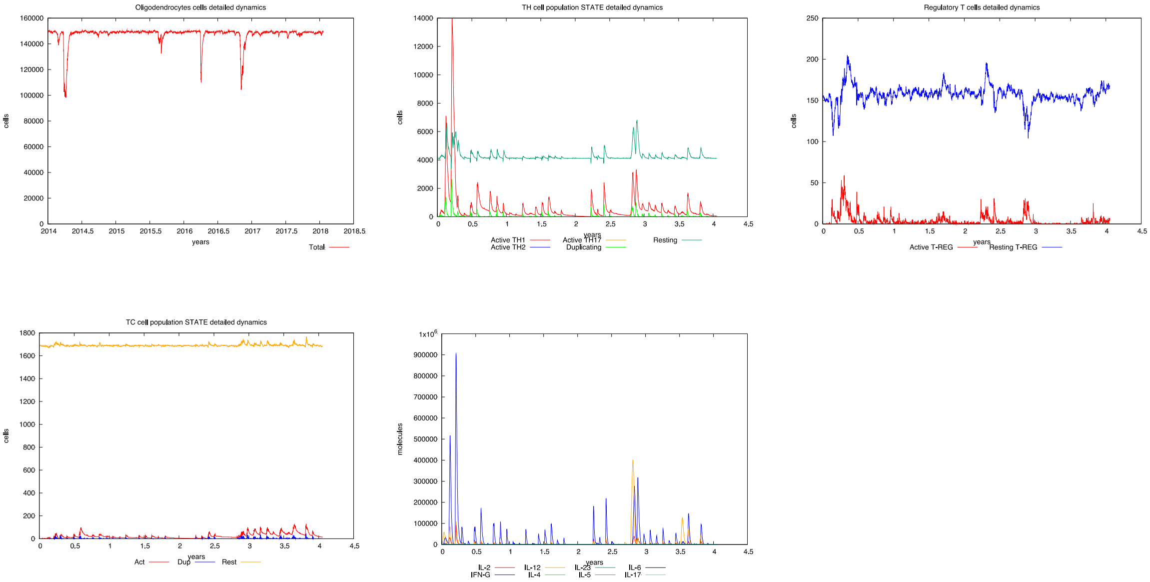

Figure 4. Simulated ODC, TH cell population, regulatory T cells, TC cell population and inflammatory and anti-inflammatory citokines dynamics for the MS virtual patient.

In each plot, it is depicted the dynamics over time of ODC cells, TH cell population state, regulatory T cells, T cell population and inflammatory and anti-inflammatory cytokines titers. ODC levels are almost flat in the healthy patient showing that no immune system activity is in progress; in the simulated MS patient three relapsing events occurred, showing ODC loss and then ODC recover. TH cell population levels show, in the MS patient, an increase of the primed helper T cells type 1 that, then, drive the consequent auto-reactive cytotoxic activity against ODC (leaded by CD8 T cells). Regulatory T cells activation is noticeably increased in the healthy control as it regulates and suppress auto-reactive immune response, while in MS simulated patient this activity is sensibly reduced. Inflammatory interferon gamma and interleukin 12 cytokines level are strongly increased in the MS patient, compared with the healthy one, showing an inflammatory condition that promotes consequent immune system activation. There are several models in the literature [12,14,16,21,22] that deal with MS modeling. However, they do not take into consideration specific immune system details and, more importantly, were not used in clinical settings to predict the outcome of MS therapies. Of course, our attempt is far from being complete but it is potentially ready to be verified in a systematic clinical study that we hope to set in due course.

The complete simulation framework is made freely available following the link https://combine.dmi.unict.it. Visitors have to sign up to launch the simulation framework. The interface to the simulator was developed using Flask micro server environment[1] and python programming language[2].

4. Conclusions

We presented a computational framework based on ABM paradigm that, to our best knowledge, is able to deliver an accurate description of the disease dynamics, including the effects of the most common treatments. It improves the current state of the art also making use of specific patient data in order to provide personalized simulations, mirroring the course of the relapsing-remitting phase of MS based on individual clinical, laboratory, and MRI features. Moreover, the simulator is able to predict the effects of the most used drugs against MS.

Currently, patients suffering from the first relapse of the disease (even with minor symptoms) are evaluated by a neurologist, who performs a brain MRI that confirms the presence of typical MS lesions. This diagnostic test hence represents the gold standard procedure used to diagnose MS and to track the disease's progression, also due to its non-invasiveness and sensitivity way of imaging the brain. Hence, the costs of the model do not impact at all during the diagnosis process as MRI scanning is mandatory diagnostic requirement; instead, the computational framework presented in this work could drastically reduce the costs deriving through a wrong therapy selection.

The final goal of our effort is to build a comprehensive profile, including also genetics, immunological, and environmental data so to reliably predict the real dynamics of MS at patient level and inform the therapeutic choice at early stages. The use of in silico models to simulate the clinical course of the disease and response to therapeutics has been recently acknowledged and recommended by regulatory agencies. Our model is a preliminary attempt to reproduce and predict the clinical and therapeutic dynamics at individual level and needs to be validated on a larger population and to be integrated with supplementary information to better shape patient profiles. Once refined, this simulator may reveal a helpful tool to assist MS specialists in predicting the course of disease at early stages and selecting the best treatment strategy, circumventing the need of a trial and error procedure.

Author Contributions: FP: drafted the manuscript, conceived and developed the model, analyzed data, performed numerical simulations, wrote the manuscript. GR: supervised the development of the model, analyzed data, wrote the manuscript. MP: developed the model, analyzed data, performed numerical simulations, wrote the manuscript. SM: gave useful insights and wrote the manuscript. DM: gave biomedical

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[1] http://flask.pocoo.org

[2] https://www.python.org

knowledge and data about MS, supervised the development of the model and drafted the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate: After the written approval of our local ethics committee (Comitato Etico dell'Azienda Ospedaliera "Garibaldi"), the six patients gave their informed consent to participate to this study.

Conflicts of Interest: The authors declare no conflict of interest.

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