Contributions to Stochastic Modelling of the Immune System

Dissertation

zur

Erlangung des Doktorgrades (Dr. Rer. Nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Hannah Mayer

aus

Aachen

Bonn, Januar 2016

Angefertigt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn

1. Gutachter: Prof. Dr. Anton Bovier
2. Gutachter: Prof. Dr. Ellen Baake

Tag der Promotion: 8. April 2016
Erscheinungsjahr: 2016

Abstract

During the past century, mathematical modelling of biological processes rapidly developed into a field of high scientific interest. The diversity of mathematical approaches and biological applications is large. Some key objectives in this context are a better understanding of biological processes and, based on that, the prediction of processes which have not yet been investigated experimentally. The purpose of this thesis is to contribute to this area at the interface of stochastics and immunology.

The thesis consists of three parts. In the first part, we investigate the question how certain immune cells (so-called T-cells) recognise structures which are potentially harmful to an organism. This is studied by means of a stochastic model. Mathematically, this problem can be described as the task to distinguish particular signals from a noisy background. A signal is represented by a sum of real-valued random variables and we are interested in the probability of the event that this sum becomes extremely large. To analyse this probability we use techniques from the theory of large deviations. We prove that sharp estimates on the probabilities of large deviations hold, also in conditional setups. The estimates are interpreted in the biological context of T-cell activation.
In the second part, we analyse related problems in a setup which is independent of the biological application; as a consequence the results apply to a broader class of random variables. We establish strong large deviation results for certain conditional probability distributions. Moreover, we show that the behaviour of the random rate function can be characterised by an invariance principle.
In the third part, we introduce a stochastic, individual-based model from population dynamics that describes the evolution of cancer. This model offers the possibility to include the effects of particular immunotherapies. It allows to survey the development of heterogeneous tumour cell populations under the influence of certain immune cells and specific aspects of an inflammatory environment. The relevance of particular stochastic phenomena for this context is studied and illustrated by examples.

The models we investigate are examples of an effective interaction of mathematics and immunology. On the one hand, they show that biological concepts and questions can be stated more precisely with the help of mathematical models, and that the resulting models may be useful to predict the behaviour of a biological system. On the other hand, during the analysis of biological questions new mathematical problems and models arise, which are mathematically relevant, independent of the primary questions.

Zusammenfassung

Während des letzten Jahrhunderts hat sich die mathematische Modellierung biologischer Prozesse immer schneller zu einem wissenschaftlich hoch interessanten Gebiet entwickelt. Die Vielfalt mathematischer Ansätze und biologischer Anwendungen ist groß. Einige der wichtigsten Ziele in diesem Zusammenhang sind sowohl ein besseres Verständnis biologischer Prozesse als auch die darauf basierende Vorhersage von Vorgängen, die so noch nicht experimentell untersucht wurden. Das Ziel dieser Dissertation ist es, einen wissenschaftlichen Beitrag zu dieser Thematik an der Schnittstelle zwischen Stochastik und Immunologie zu leisten.

Die vorliegende Arbeit besteht aus drei Teilen. Im ersten Teil analysieren wir das Problem der Erkennung von für einen Organismus gefährlichen Strukturen durch bestimmte immunkompetente Zellen (sogenannte T-Zellen) und untersuchen es mithilfe eines stochastischen Modells. Mathematisch kann dieses Problem als die Aufgabe beschrieben werden, bestimmte Signale von einem verrauschten Hintergrund zu unterscheiden. Dabei wird ein Signal durch eine Summe von reellwertigen Zufallsvariablen repräsentiert und wir sind an der Wahrscheinlichkeit des Ereignisses interessiert, bei dem diese Summe besonders groß wird. Um diese Wahrscheinlichkeit zu analysieren, verwenden wir Techniken aus der Theorie der großen Abweichungen und beweisen, dass scharfe Abschätzungen für die Wahrscheinlichkeiten großer Abweichungen auch unter bestimmten bedingten Wahrscheinlichkeitsverteilungen gelten. Diese Abschätzungen werden im Kontext der Aktivierung von T-Zellen interpretiert.
Im zweiten Teil betrachten wir ähnliche mathematische Probleme, unabhängig von der biologischen Anwendung. Die mathematischen Resultate gelten dann für eine größere Klasse von Zufallsvariablen. Wir zeigen, dass scharfe Abschätzungen für die Wahrscheinlichkeiten großer Abweichungen unter bestimmten bedingten Wahrscheinlichkeitsverteilungen gelten. Darüber hinaus beweisen wir, dass die zufällige Ratenfunktion durch ein Invarianzprinzip charakterisiert werden kann.
Im dritten Teil führen wir ein Modell ein, das die Evolution von Krebserkrankungen beschreibt, wobei sowohl die spontane Entwicklung als auch diejenige unter bestimmten Immuntherapien betrachtet werden kann. Hierbei handelt es sich um ein stochastisches, individuen-basiertes Modell aus dem Bereich der Populationsdynamik. Es ermöglicht die Untersuchung der Entwicklung heterogener Tumorzell-Populationen unter dem Einfluss bestimmter immunkompetenter Zellen und einer inflammatorischen Umgebung. Die Relevanz gewisser stochastischer Phänomene für diesen Kontext wird untersucht und anhand von Beispielen verdeutlicht.

Die betrachteten Modelle sind Beispiele für eine erfolgreiche Interaktion von Mathematik und Immunologie. Sie zeigen einerseits, dass biologische Konzepte und Fragestellungen mithilfe von mathematischen Modellen präzisiert werden können und dass die resultierenden Modelle zur Vorhersage des Verhaltens eines biologischen Systems verwendet werden können. Andererseits entstehen aus der Analyse der biologischen Fragestellungen neue mathematische Probleme und Modelle, die unabhängig von der ursprünglichen Fragestellung mathematisch relevant sind.

Acknowledgements

I would like to take the opportunity to thank everybody who supported me during all the ups and downs of the time of my PhD studies.
First of all, I am glad to express my gratitude to my advisor Anton Bovier. He always trusted in me, and took the time and the patience to answer my sceptic questions. During the last seven years (that is the time when I first asked him for advice regarding my studies in an email), I not only benefited from his immense knowledge in mathematics and his perfect intuition about mathematical problems but also learned much about life, decision making (take the way of steepest descent) and, of course, how to eat with chopsticks. It was a pleasure to accompany him to many PhD defences and meetings. In addition, I would like to thank him very much for giving me the freedom to spend so much of my research time on immunology.
I would like to thank all former and present members of the stochastic groups for their support with smaller and bigger, mathematical or non-mathematical obstacles and for the pleasant atmosphere at the institute. This includes especially Mei-Ling, who puts a lot of effort in creating this atmosphere. In particular, I would like to thank the PhD students and PostDocs of the Bovier group: Martina, Kaveh, Carina, Lisa, Anna, Patrick, Rebecca and Adela as well as Sebastian (half member of this group), Loren, Nicola, Evangelia and Constanza. I will have fond memories on my time in the stochastic groups.
Sincere thanks go to Ellen Baake for the interest in my work as well as for accepting to be the co-examiner of this thesis. In some sense, the paper she has written with Frank den Hollander and Natali Zint brought me to the field of mathematical immunology. I am very grateful for that, for several discussions with Ellen and Frank and visits to their groups.
Furthermore, I would like to thank the other members of the committee Martin Rumpf and Waldemar Kolanus. I thank Martin Rumpf also for being my mentor during my PhD studies.
Within the ImmunoSensation cluster, I would like to thank not only my co-authors, Michael Hölzel, Meri Rogava and Thomas Tüting, but also Waldemar Kolanus, Joachim Schultze and several young investigators who all found time in their tight time schedules for discussions and explanations. Their interest in our work and the friendly atmosphere of these meetings encouraged me a lot.
I am grateful to Hugo van den Berg for his visit to Bonn and for sharing his experiences in mathematical modelling in immunology.
Special thanks go to Grant Lythe and Carmen Molina-París from Leeds, who always welcomed me very warmly at meetings of the QuanTi network. This way they made it possible for me to get to know different research and many researchers in the field of mathematical immunology.
The constant support of my whole family, especially from my husband Thomas, as well as from my close friends was very important for being able to complete the PhD studies. Thank you very much for all your patience, understanding and encouragement, in particular during the last months.
Funding from the Bonn International Graduate School of Mathematics, a graduate school of the Hausdorff Center for Mathematics, and the cluster of Excellence ImmunoSensation is gratefully acknowledged. I really appreciate the opportunities that were provided through this funding, as for example the impressive research atmosphere in Bonn as well as many visits to international conferences.

Contents

Abbreviations

ACT	Adoptive Cell Transfer Therapy
APC	Antigen Presenting Cell
APP	Antigen Presentation Profile
CD	Cluster of Differentiation
CLT	Central Limit Theorem
CTLA-4	Cytotoxic T Lymphocyte-Associated Antigen 4
DC	Dendritic Cell
gp100	Glycoprotein 100
i.i.d.	Independent, Identically Distributed
LDP	Large Deviation Principle
LLN	Law of Large Numbers
MHC	Major Histocompatibility Complex
PAMP	Pathogen-Associated Molecular Pattern
PD-1	Programmed Death-1
PRR	Pattern-Recognition Receptor
RWRE	Random Walk in Random Environment
TCR	T-Cell Receptor
TNF-α	Tumour Necrosis Factor-α

1. Introduction

The immune system of vertebrates is a very complex system containing many different components, which interact in a highly intertwined and sophisticated manner. A deep and complete understanding of these interactions is still missing in many aspects despite the advanced measuring techniques and the continuously increasing amount of data. This biological framework offers many challenging problems for mathematical modelling.

One aim of mathematical modelling is to combine theoretical and experimental observations and to enable a fruitful interaction between the involved disciplines. On the one hand, experimental observations can give rise to new theoretical concepts and stimulate the development of new mathematics. On the other hand, theory can be used to pinpoint even counter-intuitive interrelations, to guide experimental setups as well as to interpret and predict experimental results. These principles are well-established for the interaction of mathematics and physics, and receive growing attention in the field of biology [99]. Many articles and books are devoted to mathematical biology including applications in neuroscience, evolutionary theory, immunology and carcinogenesis, see e.g. [33, 99, 105, 106, 119].

In general, *mathematical models* describe concepts and interpretations of particular real situations, using the precise language of mathematics. Once the mathematical description is built up, the whole strength of mathematical theory, proven theorems and strategies to prove new results is applicable for the analysis of the problems of interest. In order to obtain meaningful results, a good balance between the level of detail, i.e. the closeness to real systems, and manageability, i.e. the feasibility from the mathematical point of view, is required, when choosing a model. Chapters 2 to 4 give examples where both is achieved: The models provide insights into the underlying biological systems and raise mathematical questions, which are interesting to study from the mathematical point of view, independent from the application.

For several reasons *stochastic* models are an appropriate choice in special immunological contexts. Many parameters are still unknown, and thus it is reasonable to describe the respective quantities by means of random variables. Moreover, in certain situations relevant cell populations are small and therefore random fluctuations play a crucial role. In other situations cells move around and meet each other randomly. As we will see in Chapter 2, also rare events, as for example encounters of particular rare cell types, are important in immune responses.

For many stochastic models the dependence on the detailed description of a system is relatively weak, and certain *universal* behaviour can be observed. This is comparable to classical limit theorems in probability theory, namely the law of large numbers and the central limit theorem: For a huge class of random variables or models the limiting behaviour is very similar, and can be described in a common framework.

This thesis consists of three parts: the first one, Chapter 2, investigates a stochastic model for the activation of certain immune cells, namely T-cells, and was published in the Journal of Mathematical Biology, [100]. From the mathematical point of view this requires the use of large deviation techniques. The generalisation of the mathematical results obtained in Chapter 2 constitutes Chapter 3 of this thesis. This chapter was published in the probability theory journal ALEA, [18]. Here, we establish a conditional strong large deviation result and a functional

central limit theorem for the random large deviation rate function. In the third part of this thesis, Chapter 4, we propose a stochastic, individual-based model to describe the evolution of cancer with and without treatment. Several examples illustrate certain stochastic phenomena arising in this setting. This chapter is based on the preprint [11].

The remainder of the introduction is organised as follows: Section 1.1 provides a brief overview of the immune system to a reader from a mathematical background. It introduces concepts and terminology used in this thesis. The content of this section is slightly more general than required to read Chapters 2 and 4. Section 1.2 on mathematical universalities arising in the context of this thesis complements the biological explanations. The purpose of this section is to elucidate the connections of the different chapters from the mathematical point of view and to sketch the position of the present thesis in mathematics. Finally, the main results of Chapters 2 to 4 are summarised in Section 1.3. All chapters are self-contained, i.e. each chapter can be read without reading other parts of this thesis.

1.1 Immunological context

The following subsections provide a brief introduction into the immune system and cancer immunotherapy, and mention some modelling approaches in immunology. The introduction of the basic immunological terms and concepts is based on [104].

1.1.1 Brief overview of the immune system

The major task of the immune system is to protect the body against foreign invaders such as bacteria, viruses, fungi or parasites. In some particular situations, such as cancer, also structures derived from the host itself may be harmful and have to be eliminated. At the same time it is important that the system is not overreactive, i.e. that allergies (potentially too strong immune responses to rather harmless structures) and auto-immune diseases are avoided. To fulfil this task a huge diversity of organs, cells and molecules interacts in a sophisticated manner, passing on information and controlling each other. In order to combat a pathogen reliably, the immune system recognises the presence of invaders, clears the infection effectively, regulates its own activity and forms a memory to better deal with future infections. Immunity can be split into two parts, so-called innate and adaptive immunity.

Innate immunity works in an unspecific way and provides direct protection against infections on the basis of recognition of certain patterns, which are common for pathogens. This part of the immune system is evolutionary older and can be found in all plants and animals [80]. In many organs, such as the skin or the intestinal tract, it forms a protective barrier against pathogens. A *pathogen* is a substance which can potentially cause a disease. Some infections can be cleared by the mechanisms of innate immunity alone, but most infections overcome the innate defence.

Adaptive immunity enables the individual to respond to a pathogen specifically, i.e. by recognition of specific characteristics of a given health threat. It is present only in vertebrates, and adapts and develops during the life-time of an individual (vertebrate). Furthermore, it is able to build up an immunological memory. This means that within a certain timeframe a second infection of the same or a very similar type can be controlled faster and more efficient due to the presence of free antibodies and specialised memory cells remaining from a first infection [14]. *Antibodies* are certain proteins, which bind to pathogens or parts of them and thereby allow for

the neutralisation of pathogens or infected cells. Any substance which is able to induce *anti*body *gen*eration is called *antigen*.

Innate and adaptive immunity are intertwined, and several steps of communication between these parts of immunity are required for an effective immune response.

T-cells are certain immune cells, belonging and contributing to adaptive immunity. They play a crucial role in the immune system. Furthermore, a focus of this thesis is on T-cells, too. Therefore, the following description of immunological processes is focussed on T-cells and closely related processes.

T-cells are a particular group of lymphocytes, special white blood cells. They can be classified in various subgroups according to their different functions, such as killing of infected cells and activation or regulation of other immune cells. All T-cells have in common that they can execute their function only upon interaction with other cells, namely *Antigen Presenting Cells* (APC). The notion of an APC is functional; it generally applies to cells displaying fragments of an antigen on their surface. Peptides on the surface of an APC are displayed by two classes of *Major-Histocompatibility-complexes* (MHC), MHC I and MHC II. They have a different structure and serve different purposes. Both types present peptides, which are degraded from proteins in the inner of an antigen presenting cell, in their peptide binding groove. The so-called peptide:MHC-complexes are displayed on the surface of an APC. *Professional* APCs express MHC I and MHC II molecules on their surface, whereas non-professional APCs not necessarily express MHC II. Mostly, the term professional APC refers to a specific set of immune cells, in particular dendritic cells, macrophages and B-cells. Let us describe these types of immune cells briefly.

Dendritic cells are located in most tissues. They internalise antigen and travel upon activation to the lymph nodes. There they meet and activate T-cells when indicated. This function is a crucial point in the communication of adaptive and innate immunity [13].

Macrophages, literally the "big eaters", play various important roles, of which some rely on their phagocytic activity in many tissues. Phagocytosis denotes the engulfment of extracellular material and can be compared to eating in higher order organisms. Apart from a scavenging function this provides direct control on pathogens as well as presentation of the collected material to other immune cells. T-cells which arrived in an infected tissue can be further activated by the resident macrophages. Recent research reveals that macrophages are a diverse set of cells with many differentiation states and functions [65, 137].

B-cells form another subset of lymphocytes, also bearing a unique, specific receptor on their cell surface. They are important in humoral immunity and are able to secrete their receptors as soluble antibodies.

Most immune cells carry a variety of different receptors on their surface, and some receptors are responsible for recognition of pathogens. In the context of innate immunity these are so-called pattern-recognition receptors (PRRs), which identify pathogens by means of characteristic patterns. Such Pathogen-Associated Molecular Patterns (PAMPs) stem from characteristics of certain groups of pathogens, as for example lipopolysaccharides which are present on particular bacteria. For instance, the activation of dendritic cells requires such recognition processes. It is a distinctive feature of B-cells and T-cells that they recognise antigen by *specific* chemical structures. Whereas B-cells and their secreted antibodies are able to interact with "original", unbound pathogens, T-cells can only detect antigens located on the surface of other cells.

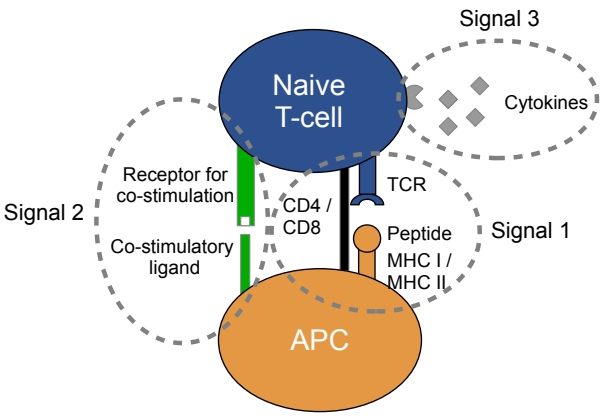


Figure 1.1: Interaction of T-cell and APC, adapted from Figure 8.19 in [104].

Let us have a closer look on recognition, signalling and activation on the example of T-cell activation since this is also subject to the modelling approach in Chapter 2.

T-cells are produced in the bone marrow and educated in an organ called thymus. Only T-cells which survive positive and negative selection are released from the thymus. Positive selection ensures that the T-cells can productively interact with APCs, and negative selection avoids (to some degree) auto-reactivity of T-cells. After their development in the thymus, T-cells enter the blood stream, migrate to lymphoid organs, search for targets and reenter the blood stream. When T-cells leave the thymus, they are so-called *naïve* T-cells which are not yet able to fulfil their function in the immune response. They have not encountered antigen so far. To obtain their effector type, they have to be activated by professional APCs. A T-cell interacts with an APC in a so-called immunological synapse, a bond between the cells. As long as this bond of cells takes place, different types of receptors and ligands on the cell surfaces interact and provide different signals to both cells.

Each T-cell bears many copies of one type of the so-called *T-cell receptor* (TCR), which determines the *clonotype* of the T-cell. The specificity of the T-cell relies on this receptor type. In addition, T-cells are equipped with *co-receptors*, either CD4 or CD8, which allow for the distinction between functionally different sets of effector T-cells: cytotoxic (mostly CD8) T-cells, which are able to kill other cells, and helper and regulatory (CD4) T-cells, which support and control immune responses in various ways. During an immunological synapse the TCRs interact with peptide:MHC-complexes. The co-receptors (CD4 or CD8) stabilise such bonds by connecting to invariant parts of the MHC molecules. CD4 receptors interact with MHC II molecules, and CD8 receptors engage with MHC I molecules [112]. The appearance of the peptides on MHC I and MHC II molecules is related to the origin of the peptides. More precisely, the presentation opportunities depend on whether the pathogen infected the presenting cell or whether and how the pathogen or fragments were engulfed from outside into the cell. This information is passed on to the T-cells by different ways of presentation, ensuring activation of suitable T-cells.

After successful interaction with a professional APC naïve T-cells proliferate and differentiate into effector cells. Most CD4 effector cells contribute to the activation of other immune cells and thus help to clear infections. Therefore, these CD4 T-cells are called *helper T-cells*. Naïve CD8 T-cells all differentiate into the same effector function, namely into *cytotoxic T-cells*. This

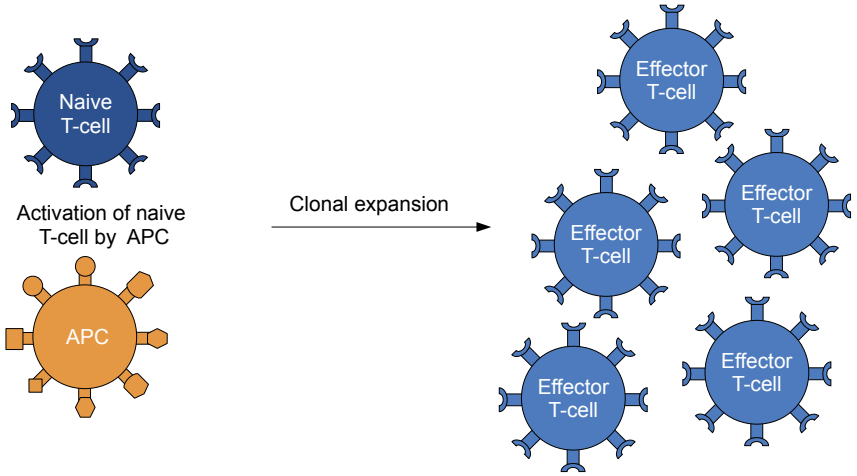


Figure 1.2: Naïve T-cell is activated by an APC, differentiates into effector T-cell and proliferates.

means that CD8 effector cells are able to kill infected cells. They do so with a high precision and neighbouring healthy cells are usually not damaged. Effector cells again interact with APCs but not necessarily with professional ones. Upon such interactions they can perform their effector function. For example, a cytotoxic T-cell can be stimulated by a cancer cell, which in this case functions as an APC. This results in killing of the cancer cell by the activated cytotoxic T-cell.

For its full activation a naïve T-cell requires three signals, see Figure 1.1. Signal 1 is mediated via the TCR and leads to initial activation. The co-stimulatory Signal 2 is required for survival and depends on stimulation of certain additional receptors on T-cells. Finally, Signal 3 guides differentiation into different effector cells. It is conveyed by cytokines, [40, 41]. *Cytokines* are small proteins which influence the behaviour of cells. They orchestrate immune responses in various ways, e.g. by activating or inhibiting cells or by guiding their movement through chemotaxis. Signals 2 and 3 in a sense "belong" to the APC and the environment, and represent the state of activation of the APC and innate immunity. Whether they are provided or missing, is independent of the specificity of the T-cell. Signal 1 regulates which T-cell clonotypes become activated by an activated APC that provides Signals 2 and 3. Only *activated* APCs can deliver co-stimulatory signals (Signal 2) to T-cells. Effector T-cells do not need co-stimulation to perform their task at the correct side, e.g. an infected cell.

Lymphocyte receptors are highly diverse, enabling T-cells and B-cells to recognise a huge diversity of pathogens by specific molecular structures. This is basically empowered by a genetic mechanism, by which the genes encoding for these receptors are rearranged in a random manner. The (safe) functionality of the cells is ensured by certain selection processes, as for example positive and negative selection of T-cells in the thymus. The effectivity of the repertoire results from clonal expansion of a rare suitable clone, i.e. an activated lymphocyte produces many copies of itself bearing the same receptor, compare Figure 1.2. This concept is sometimes called clonal selection theory and was introduced in [22]. It enables the immune system to create populations of the required clonotype which are large enough to cure an infection. After an immune response most T-cells die, but some *memory T-cells* remain. They are highly sensitive to antigen and can elicit a faster response to a second infection with the same or maybe a very similar pathogen [14, 82].

The actual signalling resulting from the interaction of receptors and their ligands and the events induced by this signal again constitute a complicated topic. The details are of interest also in ongoing biological research [1]. A lot of research is concerned with the signal transduction from the outside to the inside of a cell considering aspects such as phosphorylation and activation of transcription factors [113]. Whole signalling pathways can be tracked experimentally, but the complexity and the integration in the entire system are still not completely understood.

1.1.2 Immunotherapy of cancer

Cancer denotes a family of diseases characterised by increased cell division, avoidance of cell death, invasion into and distraction of surrounding tissues and organs as well as the ability to metastasize [84]. The way such diseases are treated has changed substantially in recent years. Although the disease is not curable in many cases, very often a status similar to that of a chronic disease can be achieved by treatment.

Classical therapeutic approaches are surgery, radiotherapy and chemotherapy. The treatment options depend strongly on the type, location and stage of the tumour. If a solid tumour is detected early enough, *surgery* can be curative. *Radiation* is applied locally, but damages also healthy cells in the close environment of the targeted tumour. For treatment of cancer at a metastatic stage a systemic treatment is required. *Chemotherapies* operate in such a way. The majority of chemotherapeutic approaches targets parts of the cell division programme and executes cytotoxic functions. Thus, healthy cells, in particular immune cells, are destroyed as well [84].

The lack of specificity in the above mentioned therapies as well as the high frequency of side effects and recurrences of tumours underline the necessity of the development of different treatment strategies. There exist already approaches in *targeted therapy* in clinical practice. This term refers mainly to the use of small molecules or particular antibodies that target specific structures of cancer cells. This way the destruction of other cells is avoided.

One set of therapeutic approaches, developed during the last decades, is so-called immunotherapy. *Immunotherapy* is a collective term for strategies which use the immune system to treat a disease, as for example cancer. In some cases such strategies target only immune cells, completely independent of the cancer cells. This is a strong change in treatment procedures [38].

Usually, the immune system is able to recognise and eliminate mutated endogenous cells, which might eventually give rise to cancer. But sometimes the abnormal cells manage to escape the immune system and to generate a tumour. The environment in an advanced tumour is often immunosuppressive, i.e. the immune system is silenced [94, 139, 140]. There are a lot of different strategies to unleash the immune system again. As described in [101] at least three key aspects in tumour immunology can be targeted: antigen presentation by dendritic cells, production of protective T-cell responses and the immunosuppressive environment of a tumour. For many immunotherapies T-cells are an essential point of action, see e.g. the overview given in [88]. The following list provides a few examples.

- Antibodies can be used as *checkpoint inhibitors*, i.e. to block T-cell receptors that mediate inhibitory signals. Programmed death-1 (PD-1) and cytotoxic T-lymphocyte associated antigen-4 (CTLA-4) are two receptors on T-cells, which are often stimulated by tumours and mediate cell death or anergy of T-cells. These receptors can be blocked by monoclonal antibodies in clinical practice already (Nivolumab targeting PD-1 and Ipilimumab targeting CTLA-4). Blocking these receptors keeps T-cells active.

- *Bispecific antibodies* are able to connect with tumour cells on the one end and T-cells on the other end of the antibody. On the side of the T-cell CD3, which is a compulsory part of the T-cell receptor, can be used as an anchor. The antibody not only binds to the TCR, but it also imitates the usual activation of the T-cell. Thus, the specificity of the T-cell is not relevant. For this approach it is necessary to identify a tumour antigen, which is not present on healthy cells, as a reliable binding site for the antibody.

- *Adoptive cell transfer therapy* (ACT) denotes the transfer of immune cells which are able to target cancerous cells into a patient. This strategy hinges on getting hands on specific T-cells [110]. For example, tumour-specific T-cells can be obtained from a patient, cultured and activated ex vivo, and then be re-transferred. A second way to obtain suitable T-cells is to engineer T-cells and their receptors genetically.

1.1.3 Mathematical modelling in immunology

The preceding description of the immune system raises a lot of questions on how the immune system *really* functions. These questions include but are not restricted to: How is it possible that the immune system is balanced between auto-immunity and effective defence? How are signals transduced from the outside into the inside of cells? How do T-cells find suitable APCs? How long does it take from the invasion of a pathogen to its detection by innate and adaptive immunity? How is an effective and safe T-cell repertoire maintained? How do cells communicate? How does directed cell migration work?

This list of questions could be extended, but it is already long enough to demonstrate that immunology poses a lot of challenging questions. Mathematical modelling can assist in answering them. A good overview on existing modelling approaches is provided in [103], a book containing a diverse mixture of articles about models of certain parts of the immune system. The need for a collaboration of immunology and mathematics is also pointed out in [23].

Many mathematical models in immunology are concerned with the activation of T-cells, see e.g. [52, 93, 95, 129, 130, 131, 138]. Another line of research studies T-cell development and the influence of selection processes on the actual T-cell repertoire [54, 89, 125]. The maintenance of an effective and diverse T-cell repertoire under homeostatic conditions is investigated with Markov process models in [115, 116, 117]. Brownian motion can be used to approach for example the movement of T-cells [49]. More generally, chemotaxis and movement of immune cells were described by different types of random walks [2, 69, 73, 134]. Reactions involved in immunity happen on different timescales. For example, the bonds between TCRs and p:MHC molecules are not as long-lived as bonds between T-cells and APCs [122]. Furthermore, many models for signalling are proposed [58].

1.2 Mathematical context

Stochastic objects can often be classified according to their limit behaviour. Properly rescaled, a system or particular characteristic quantities of it converge to the same limit for a set of systems. The limits can be used to describe a complex random object by a simpler object. Results concerning such kind of *universalities* play a crucial role in this thesis and are thus explained in the following. As a reference for the basic theorems and explanations introduced below see for

example [68]. In addition, we briefly introduce stochastic, individual-based models, which play an important role in Chapter 4.

1.2.1 Law of large numbers, central limit theorem and large deviation principle

Laws of large numbers (LLN) and central limit theorems (CLT) are classical results in probability theory. They are concerned with the asymptotic behaviour of rescaled sequences of random elements. While particular variants of them are taught in each introductory course for probability theory, they are in different contexts and for varying levels of complexity still subject of contemporary mathematical research.

Roughly speaking, an *LLN* states that under suitable conditions a properly rescaled sequence of random elements converges to a deterministic object in some sense. That means that LLNs are statements of the form

$$a_n^{-1} S_n \to C \quad \text{as} \quad n \to \infty, \tag{1.2.1}$$

where $(S_n)_{n\in\mathbb{N}}$ is a sequence of random elements, $(a_n)_{n\in\mathbb{N}}$ with $a_n \in \mathbb{R}$ is a scaling sequence with $a_n \to \infty$ as $n \to \infty$ and C denotes a deterministic limit. If this convergence holds in probability or almost surely, we speak about a weak LLN or a strong LLN, respectively. A *CLT* quantifies the random fluctuations of a particular order of magnitude around the limit obtained in the LLN. CLTs consider convergence results of the form

$$b_n^{-1}(S_n - a_n C) \to Z \quad \text{weakly}, \tag{1.2.2}$$

where $(b_n)_{n\in\mathbb{N}}$ with $b_n \in \mathbb{R}$ and $b_n \to \infty$ as $n \to \infty$ is an additional normalising sequence and Z is a random element.

The first published form of an LLN is due to Jacob Bernoulli and considers sums of independent, identical Bernoulli trials.

The typical modern formulation of the standard result about the behaviour of the empirical mean of a sequence of real-valued random variables is stated in the following Theorem 1.2.1.

Theorem 1.2.1 (LLN)**.** *Let $(X_i)_{i\in\mathbb{N}}$ be a sequence of independent, identically distributed random variables with $\mathbb{E}[|X_1|] < \infty$. Then,*

$$\lim_{n\to\infty} \frac{1}{n} \sum_{i=1}^{n} X_i = \mathbb{E}[X_1] \quad \textit{almost surely.} \tag{1.2.3}$$

A first version of the CLT was introduced by Abraham de Moivre and generalised by Pierre-Simon Laplace. The combination of their results, known as the De Moivre-Laplace Theorem, can be seen as a special case of the following formulation of the classical CLT. It states that the fluctuations of the empirical mean around its expectation converge to a Gaussian random variable.

Theorem 1.2.2 (CLT)**.** *Let $(X_i)_{i\in\mathbb{N}}$ be a sequence of independent, identically distributed random variables such that $\mathbb{E}[|X_1|] < \infty$ and $0 < \sigma^2 = \mathbb{V}[X_1] < \infty$. Then,*

$$\lim_{n\to\infty} \frac{1}{\sigma\sqrt{n}} \sum_{i=1}^{n} (X_i - \mathbb{E}[X_i]) = Z \quad \textit{in law}, \tag{1.2.4}$$

where Z is a Gaussian random variable with mean 0 and variance 1.

The sequences in the general formulations, (1.2.1) and (1.2.2), are in Theorems 1.2.1 and 1.2.2 given by $S_n = \sum_{i=1}^n X_i$, $a_n = n$ and $b_n = \sigma\sqrt{n}$. Both theorems characterise a universality in the limiting behaviour of partial sums of sequences of random variables, which is independent of the exact distribution of the components of the sum. This formally states that certain regularities can be observed in the occurrence of random events.

So-called *large deviation theory* is concerned with the analysis of the probabilities of deviations from the LLN beyond the CLT. The LLN stated above yields $S_n \sim \mu n$ and the CLT states that typical deviations are of order $\sqrt{n}$, i.e. the typical size of $S_n - \mu n$ is of order $\sqrt{n}$. In addition, one can ask for the probability of events where $S_n - \mu n$ is atypically large, for example of order n. This is done by large deviation theory. Probabilities of such events tend to 0 as n tends to infinity. In other words, large deviation theory deals with *rare* events.

Many results in this framework are formulated as large deviation principles (LDP). We present the concept of an *LDP* as well as some basic results following [45] and [46]. An LDP characterises the limiting behaviour of a sequence of probability measures, P_n, on a measurable space, $(\mathcal{X}, \mathcal{B})$, as n tends to infinity. More precisely, asymptotic exponential bounds on $P_n(B)$ for measurable sets $B \in \mathcal{B}$ are provided in terms of a rate function.

Definition 1.2.3. A function $I : \mathcal{X} \to [0, \infty]$ is called a rate function if $I \not\equiv \infty$, I is lower semi-continuous and has compact level sets.

Definition 1.2.4 (LDP)**.** A sequence of probability measures P_n on $(\mathcal{X}, \mathcal{B})$ is said to satisfy the large deviation principle (LDP) with rate function I and rate c_n if, for all $B \in \mathcal{B}$,

$$-\inf_{x \in B^\circ} I(x) \leq \liminf_{n\to\infty} c_n^{-1} \log P_n(B) \leq \limsup_{n\to\infty} c_n^{-1} \log P_n(B) \leq -\inf_{x \in \overline{B}} I(x), \tag{1.2.5}$$

where B° denotes the interior of a set B and $\overline{B}$ denotes its closure.

The basic result complementing Theorems 1.2.1 and 1.2.2 can be traced back to Cramér [39].

Theorem 1.2.5 (Cramér)**.** *Let $(X_i)_{i\in\mathbb{N}}$ be a sequence of independent, identically distributed random variables such that $\phi(\vartheta) = \mathbb{E}[\exp(\vartheta X_1)] < \infty$ for all $\vartheta \in \mathbb{R}$. Then, for all $a > \mathbb{E}[X_1]$,*

$$\lim_{n\to\infty} \frac{1}{n} \log \mathbb{P}(S_n \geq an) = -I(a), \tag{1.2.6}$$

where $I(z) = \sup_{\vartheta\in\mathbb{R}} (z\vartheta - \log \phi(\vartheta))$.

Here, the sequence P_n in the formulation of an LDP is induced by the sequence S_n, i.e. $P_n(B) = \mathbb{P}(S_n \in B)$ for measurable sets $B \in \mathcal{B}$. The sets B correspond to the intervals $[a, \infty)$ and the sequence c_n is given by $c_n = n$.

Opposed to the previous two theorems here the actual distribution of the random variables is more important. The *rate function*, $I(a)$, depends on the distribution via the logarithmic moment generating function and the limit is not determined by first and second moments alone. The moment generating function determines the law of a random variable completely, whereas first and second moments can be equal for large families of distributions.

LLN, CLT and LDP were subject of research during the last decades in various guises. The classical results and generalisations of them are not only used to establish other results, but it is also a generic research interest to obtain related statements in different contexts.

One line of generalisations, which developed mainly during the 1970's, establishes similar results, when the summands X_i take values in Banach spaces [75, 92]. This is a natural direction for generalisations since these spaces allow for a summation by their vector space structure and provide helpful convergence properties by completeness.

When the random elements X_i take values in certain function spaces, such results are usually referred to as *functional* or dynamic LLN, CLT or LDP. In connection with consistency of parameter estimates in statistics or economics also *uniform* LLNs are discussed [4, 81, 107]. When dependence of the random objects is introduced, one speaks about *ergodic theorems* in place of LLNs, but the terms CLT and LDP remain.

A more recent line of research arises in the setup of conditional probability distributions, as for example in the study of random walks in random environment or random conductance models. Such scenarios have been studied by many authors during the last years [3, 9, 15, 35, 47, 61, 62, 98].

The framework explained above considers countable sequences of random objects. There are also results for collections of random elements indexed by a continuous parameter, as for example in the context of perturbation theory [60].

1.2.2 Refined large deviation results

The results obtained by an LDP, such as estimates of the form

$$\lim_{n\to\infty} \frac{1}{n} \log \mathbb{P}\left(S_n \geq an\right) = -I(a), \tag{1.2.7}$$

are relatively imprecise. Approximations of the form (1.2.7) can be rephrased as

$$\mathbb{P}(S_n \geq an) = \exp[-n(I(a) + \mathrm{o}(1))] = \exp(-nI(a)) \exp(-n\mathrm{o}(1)), \quad n \to \infty. \tag{1.2.8}$$

The involved probabilities are approximated only up to a factor $\exp(-n\mathrm{o}(1))$, whose behaviour is unknown.

There are refinements of estimates of the form (1.2.7). The version for S_n a sum of independent, identically distributed (i.i.d.) random variables was analysed by Bahadur and Ranga Rao [12]. A further generalisation was proven by Chaganty and Sethuraman in [24] for sequences of real-valued random variables satisfying certain regularity conditions. The precise statement relies on the following two assumptions and can be formulated as Theorem 1.2.8. It will play a crucial role in Chapters 2 and 3.

Let us denote the logarithmic moment generating function of S_n by Ψ_n, i.e.

$$\Psi_n(\vartheta) \equiv \log \mathbb{E}[\exp(\vartheta S_n)], \quad \vartheta \in \mathbb{R}. \tag{1.2.9}$$

Assumption 1.2.6. There exist $\vartheta_* \in (0, \infty)$ and $\beta < \infty$ such that

$$|\Psi_n(\vartheta)| < \beta, \text{ for all } \vartheta \in \{\vartheta \in \mathbb{C} : |\vartheta| < \vartheta_*\} \tag{1.2.10}$$

for all $n \in \mathbb{N}$ large enough.

Assumption 1.2.7. $(a_n)_{n\in\mathbb{N}}$ is a bounded real-valued sequence such that the equation

$$a_n = \Psi_n'(\vartheta) \tag{1.2.11}$$

has a solution $\vartheta_n \in (0, \vartheta_{**})$ with $\vartheta_{**} \in (0, \vartheta_*)$ for all $n \in \mathbb{N}$ large enough.

We define $\sigma_n^2 \equiv \Psi_n''(\vartheta_n)$ with ϑ_n as in Assumption 1.2.7 and denote the moment generating function of S_n by Φ_n, i.e. $\Phi_n(\vartheta) \equiv \mathbb{E}[\exp(\vartheta S_n)]$. Furthermore, the analogue of the rate function $I(a)$ is defined as

$$I_n(a_n) \equiv a_n\vartheta_n - \Psi_n(\vartheta_n). \tag{1.2.12}$$

Theorem 1.2.8 (Theorem 3.3 in [24])**.** *Let S_n be a sequence of real-valued random variables. Let Ψ_n be their logarithmic moment generating function defined in* (1.2.9) *and assume that Assumptions 1.2.6 and 1.2.7 hold for Ψ_n. Assume furthermore that*

(i) $\lim_{n\to\infty} \vartheta_n\sqrt{n} = \infty$,

(ii) $\liminf_{n\to\infty} \sigma_n^2 > 0$, *and*

(iii) $\lim_{n\to\infty} \sqrt{n}\sup_{\delta_1\le|t|\le\delta_2\vartheta_n} \left|\frac{\Phi_n(\vartheta_n+it)}{\Phi_n(\vartheta_n)}\right| = 0 \quad \forall\, 0<\delta_1<\delta_2<\infty$,

are satisfied. Then

$$\mathbb{P}\left(S_n \ge na_n\right) = \frac{\exp(-nI_n(a_n))}{\vartheta_n\sigma_n\sqrt{2\pi n}}\left[1+o(1)\right],\ n\to\infty. \tag{1.2.13}$$

As many other proofs of large deviation results, the proof of this theorem involves an exponential change of measure. Such a change turns rare events into typical ones and thereby allows to use well-known results, such as CLTs, for the analysis of the behaviour of the random variables. The involved random variables are studied under the law $\widetilde{\mathbb{P}}$ defined by its distribution function $\widetilde{F_n}$. For F_n the distribution function of S_n and ϑ_n as defined in Equation (1.2.11) the tilted version of the distribution function is given by

$$\widetilde{F_n}(x) = \frac{1}{\mathbb{E}[\exp(\vartheta_n S_n)]}\int_{(-\infty,x]} \exp(\vartheta_n y)dF_n(y). \tag{1.2.14}$$

Theorem 1.2.8 is deduced from a local central limit theorem for $(n\sigma_n^2)^{-\frac{1}{2}}(S_n - na_n)$ under the *tilted* law $\widetilde{\mathbb{P}}$.

In principle, even finer estimates on the probabilities, as for example of Berry-Esseen type, can be obtained.

1.2.3 Conditional probability distributions

The description of real world phenomena by stochastic models often requires the incorporation of different sources of randomness. In such situations, one can study the behaviour of the objects conditioned on certain types or parts of the randomness. This is also the case for the model we investigate in Chapter 2.

Let us explain the idea on the example of random walks in random environment (RWRE). The motion of a particle can be described by means of a random walk, which is a classical object to study in probability theory. The particle moves according to certain rules on an underlying space, typically a sort of grids or graphs. In real situations, the medium in which the random walk evolves can be highly irregular. This is taken into account by representing the media as a random environment. For this purpose, a random variable is attached to each site of the underlying space. These random variables influence the transition probabilities or rates of a particle at this site. It is not clear in which cases and to which amount the long-term behaviour of the random walk

depends on the realisation of the environment. Thus, a rigorous study of such phenomena is required.

The system or results conditioned on the environment are often called *quenched*, whereas the corresponding objects where also the environment is averaged out are called *annealed*. These terms were shaped by statistical mechanics. It is important to note that probability measures conditioned on a source of randomness are a priori *random* measures. Additional technicalities arise, when dealing with these objects, and proofs become more involved.

1.2.4 A brief introduction to stochastic, individual-based models

Stochastic, individual-based models are a crucial mathematical ingredient in Chapter 4. Therefore, we describe this class of models briefly.

In such modelling approaches, interacting particle systems are used to describe the Darwinian evolution in a population with asexual reproduction, e.g. a population which consists of cells. The structure of *individual-based* models is defined on the level of its components. The macroscopic evolution is a result of the evolutionary events of the single particles. Each particle is equipped with a set of exponential waiting times, indicating together the time and type of the next evolution step for the whole population.

Here, we introduce a "basic" version of these models, following [25]. Each individual with trait $x \in \mathcal{X}$, where the trait space $\mathcal{X}$ is a compact subset of $\mathbb{R}^d$, can give birth to another individual or die. At a birth event, a mutant appears with a certain probability. More precisely, each individual is characterised by the following set of parameters:

- a natural birth rate, $b(x) \in \mathbb{R}_+$,
- a natural death rate, $d(x) \in \mathbb{R}_+$,
- a competition kernel, $c(x,y)K^{-1} \in \mathbb{R}_+$, indicating the competition felt by an individual of trait $x \in \mathcal{X}$ in presence of an individual of trait $y \in \mathcal{X}$,
- a probability, $u_K\mu(x)$, that a birth event induced by an individual of trait x is a mutation,
- a law, $M(x,dh)$, that describes the distribution of a mutant of type $x+h$
- scaling parameters, K and u_K, for the population size and the mutation probabilities.

The population at time t can be represented by a rescaled point measure

$$\nu_t^K = \frac{1}{K}\sum_{i=1}^{N_t} \delta_{x_i(t)}, \tag{1.2.15}$$

where $x_i(t)$ denotes the trait of the i-th individual at time t and N_t denotes the number of individuals at time t. To each individual of trait x three independent exponential clocks are attached with parameters:

- $(1-u_K\mu(x))b(x)$ for clonal reproduction
- $u_K\mu(x)b(x)$ for production of a mutant
- $d(x) + \int_{\mathcal{X}} c(x,y)\nu_t^K(dy)$ for logistic death due to age and competition.

Both types of reproduction clocks for an individual depend only on the trait of that individual. In contrast, the death clock depends (via the effect of competition) on the state of the whole population. When the clonal reproduction clock rings, another individual with trait x appears. A ring of the mutational reproduction clock indicates the appearance of a mutant of trait $y = x+h$, where $h \sim M(x, dh)$. Furthermore, when the death clock rings, an individual with trait x disappears.

The dynamics of the process can be summarised in terms of its generator. Let us define

$$\mathcal{M}^K \equiv \left\{ \frac{1}{K} \sum_{i=1}^{n} \delta_{x_i}, n \geq 0, x_i \in \mathcal{X} \right\} \tag{1.2.16}$$

as the set of finite rescaled counting measures on $\mathcal{X}$. The infinitesimal generator, $\mathcal{L}^K$, of the process of interest acts on bounded measurable functions ϕ from $\mathcal{M}^K$ into $\mathbb{R}$, for all $\eta \in \mathcal{M}^K$ by

$$\begin{aligned}\left(\mathcal{L}^K \phi\right)(\eta) = &\int_{\mathcal{X}} \left(\phi\left(\eta + \frac{\delta_x}{K}\right) - \phi(\eta)\right) b(x)(1 - \mu_K m(x)) K\eta(dx) \\ &+ \int_{\mathcal{X}} \int_{\mathbb{R}^d} \left(\phi\left(\eta + \frac{\delta_{x+h}}{K}\right) - \phi(\eta)\right) b(x)\mu_K m(x) M(x, dh) K\eta(dx) \\ &+ \int_{\mathcal{X}} \left(\phi\left(\eta - \frac{\delta_x}{K}\right) - \phi(\eta)\right) \left(d(x) + \int_{\mathcal{X}} c(x, y)\eta(dy)\right) K\eta(dx). \end{aligned} \tag{1.2.17}$$

This defines a continuous-time, measure-valued Markov process with density-dependence. Under regularity assumptions on the involved parameters, the existence and uniqueness in law of a process with generator $\mathcal{L}^K$ have been proven in [57].

This model and related ones as well as their asymptotic behaviour were studied rigorously for example in [10, 25, 26, 27, 28, 30, 31, 74, 102]. In these publications the long-term evolution of the system is investigated in the limits of large populations, rare mutations and small mutational effects in different combinations and on different timescales.

1.2.5 Law of large numbers, central limit theorem and large deviations in this thesis

Limit theorems appear in various manners in this thesis. One idea behind the studies in Chapter 2 is to unify the modelling framework and to show a higher robustness of previous results. We will see in Chapter 2 that rare events play a crucial role in the functioning of the immune response. Apart from proving large deviation results for conditional probability distributions it is a crucial part of Chapters 2 and 3 to derive a functional central limit theorem in order to describe the random rate function. The central limit theorem is functional in the threshold value a, which determines the size of the large deviation. These results are established by proving tightness and convergence in finite dimensional distributions. Several steps in the proof of the main results hinge on verifying uniform laws of large numbers, which are again functional in the parameter a.

The large population approximation in the context of stochastic, individual-based models in Chapter 4 can be seen as an LLN as well. It is derived from the LLN for density-dependent population processes of [55] and illustrated in Figure 1.3, which shows the trajectories of a stochastic system and of the solution of the corresponding deterministic limit.

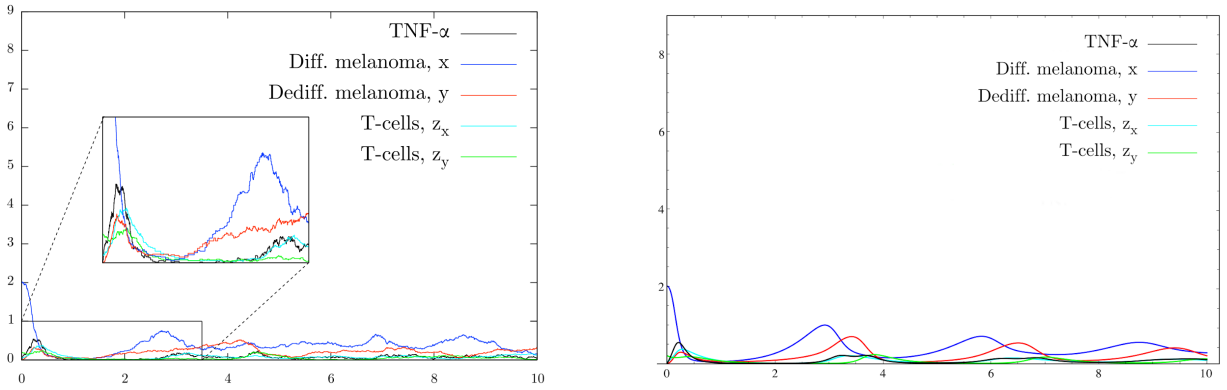


Figure 1.3: Each line describes the trajectory of one subpopulation of a population of interacting cancer and immune cells. On the left-hand side the trajectories of the stochastic process are shown, whereas on the right-hand side the trajectories of a solution of the deterministic system obtained in the limit are depicted. These pictures are taken from [11] and explained in more detail in Chapter 4.

1.3 Outline of the content of this thesis

The main part of the present thesis consists of three chapters. Each of these chapters is briefly summarised in one of the following subsections.

1.3.1 A mathematical justification for a specific recognition by T-cells

Chapter 2 of this thesis is concerned with a stochastic model for the activation of T-cells. The model describes the signal a T-cell receives via its T-cell receptors (TCRs). Our results can be seen as a mathematical justification for a *specific* recognition by T-cells. This chapter appeared as joint work with Anton Bovier in the Journal of Mathematical Biology, [100],

> H. Mayer and A. Bovier, *Stochastic modelling of T-cell activation*, Journal of Mathematical Biology, Volume 70, Number 1–2, pp. 99–132, 2015.

The model, which we consider, is a slight generalisation of the model proposed by van den Berg, Rand and Burroughs in [131] and further developed in [138]. In both publications particular distributions are used for the involved random variables. In contrast, we develop a *unified* framework, i.e. the results are more robust.

With regard to T-cell activation we are interested in the question how a reliable distinction between foreign and self structures is possible. More precisely, we ask: Is an immune response more likely in the presence of invaders? If yes, which T-cells become activated? Are those T-cells activated which are sensitive to a certain peptide species?

To answer these questions we analyse the probability of T-cell activation in three different scenarios, which are illustrated in Figure 1.4. Recall that a T-cell interacts with Antigen Presenting Cells (APC) and scans the sample of peptides presented on the APC with its receptors. A T-cell should become activated when foreign peptides are present on the APC. Let us describe the three cases that we study. The first one is concerned with the total probability that a T-cell with a randomly chosen clonotype is activated by a randomly chosen APC, i.e. by a randomly chosen Antigen Presentation Profile (APP), see Figure 1.4 (A). Case two investigates the probability

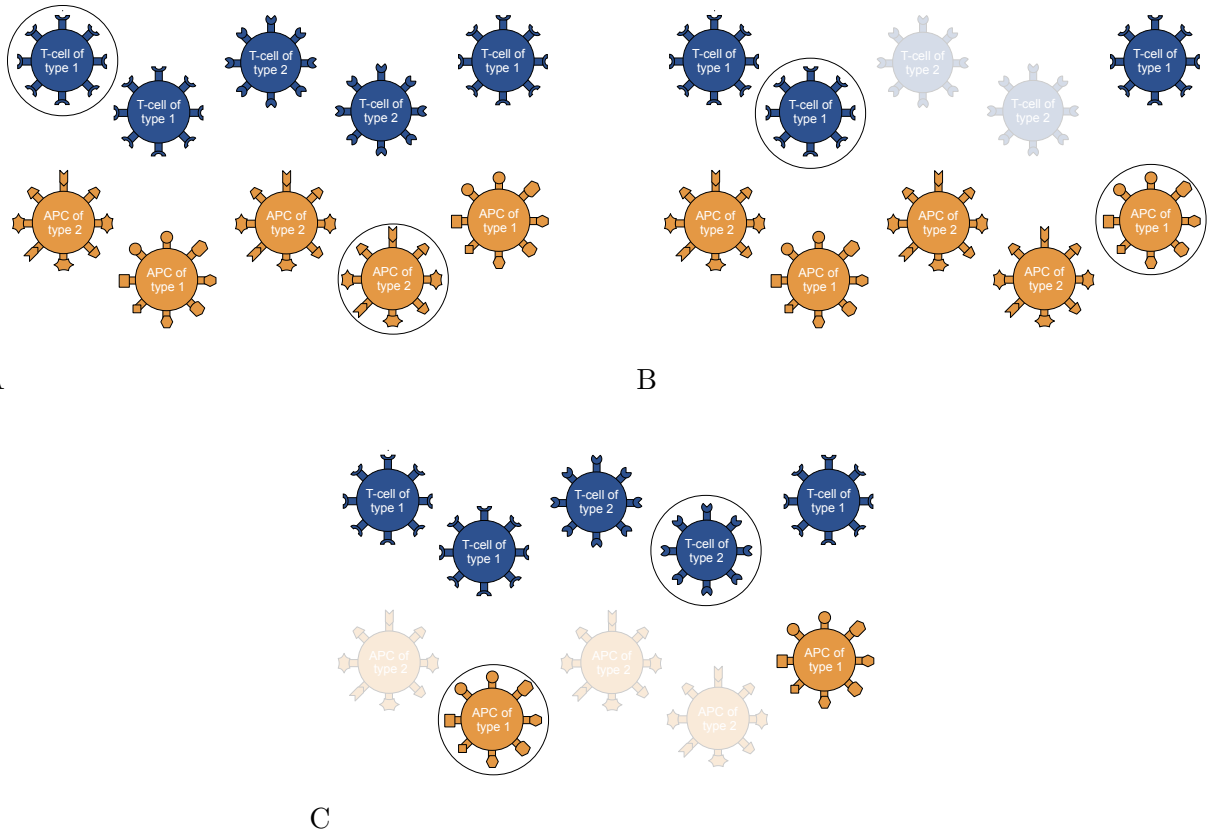


Figure 1.4: Illustration of different scenarios, for which the probability of T-cell activation is analysed. (A) The T-cell clonotype and the Antigen Presentation Profile (APP) are chosen at random. (B) The T-cell pool is restricted to one clonotype and the APP is chosen at random. (C) The T-cell clonotype is chosen at random and the pool of Antigen Presenting Cells is restricted to one APP.

that a T-cell of a fixed clonotype is activated by a randomly chosen APP, see Figure 1.4 (B). Finally, the third case examines the probability that a randomly chosen T-cell is activated by a fixed APP, Figure 1.4 (C). For all three cases the frequency of observed activations converges to a particular probability.

In mathematical terms, we study the probability that a large sum of random variables, $G_{i,n}(z_\mathrm{f})$, representing the stimulatory signal received by the T-cell, exceeds a certain threshold value, g_act,

$$\mathbb{P}\left(G_{i,n}(z_\mathrm{f}) \geq g_\mathrm{act}\right). \tag{1.3.1}$$

Here,

$$G_{i,n}(z_\mathrm{f}) \equiv q_n \sum_{j=1}^{n} Z_j W_{ij} + z_\mathrm{f} W_{if}, \quad \text{where} \tag{1.3.2}$$

- Z_j are i.i.d. random variables representing the number of copies of peptides of species j,
- W_{ij} are i.i.d. random variables representing the stimulation rate induced by a peptide of species j for a T-cell of clonotype i,

- z_{f} is a parameter denoting the number of foreign peptides,
- q_n is a parameter ensuring proportional displacement of self peptides and
- n denotes the number of different self peptide species present on an APC.

The sum in Equation (1.3.2) describes the signal induced by the self-background and the last term, $z_{\mathrm{f}}W_{if}$, the signal induced by the foreign peptide species.

Let us define $\mathcal{W}$ as the sigma-algebra generated by the stimulation rates, $(W_{ij})_{i,j\in\mathbb{N}}$, and $\mathcal{Z}$ as the one generated by the numbers of peptides, $(Z_j)_{j\in\mathbb{N}}$. Then, the three scenarios introduced above correspond to investigate

$$\mathbb{P}\left(G_{i,n}(z_{\mathrm{f}}) \geq g_{\mathrm{act}}\right), \quad \mathbb{P}\left(G_{i,n}(z_{\mathrm{f}}) \geq g_{\mathrm{act}}|\mathcal{W}\right) \quad \text{and} \quad \mathbb{P}\left(G_{i,n}(z_{\mathrm{f}}) \geq g_{\mathrm{act}}|\mathcal{Z}\right). \tag{1.3.3}$$

$\mathbb{P}\left(G_{i,n}(z_{\mathrm{f}}) \geq g_{\mathrm{act}}\right)$ refers to the case illustrated in Figure 1.4 (A), $\mathbb{P}\left(G_{i,n}(z_{\mathrm{f}}) \geq g_{\mathrm{act}}|\mathcal{W}\right)$ to the one in Figure 1.4 (B) and $\mathbb{P}\left(G_{i,n}(z_{\mathrm{f}}) \geq g_{\mathrm{act}}|\mathcal{Z}\right)$ to the one in Figure 1.4 (C).

By a law of large numbers, $n^{-1}G_{i,n}(z_{\mathrm{f}})$ converges almost surely to $\mathbb{E}[Z_1W_1]$ as n tends to infinity. From immunology we know that T-cell activation is a *rare* event since there are many encounters of T-cells and APCs all the time, but only very few of them lead to an immune response. Thus, the threshold value, g_{act}, has to be larger than $n\mathbb{E}[Z_1W_1]$ and large deviation techniques can be used to analyse the probabilities of interest. Large deviation results were also applied in previous work but not in the conditional (quenched) scenarios [125, 131, 138]. First steps into the direction of the analysis of these scenarios have been carried out in [121].

The investigation of the quenched cases adds value to the analysis of this type of models. As stated precisely in Theorems 2.3.3, 2.3.5 and 2.3.16, we can show that strong large deviation estimates hold in all three scenarios. Furthermore, the random rate function, appearing in the analysis of the quenched scenarios, can be characterised in terms of functional central limit theorems, see Theorems 2.3.7 and 2.3.17. With these tools it is possible to deduce that the probability of T-cell activation increases exponentially with the number of foreign peptides, z_{f}, for a fixed threshold value g_{act}/n when T-cell clonotype and APP are chosen at random or when an APP is fixed. In the case where the T-cell clonotype is fixed, the activation probabilities grow only then exponentially in z_{f} when the stimulation rate induced by the foreign peptide species, W_{if}, is large enough.

This implies that T-cell activation in this model is indeed more likely in the presence of foreign invaders. Moreover, it is gradually specific, i.e. only those T-cells are activated which are sensitive to the foreign peptide. There might be several peptides inducing a high stimulation rate for one given T-cell clonotype. That means that in this model crossreactivity plays also a role, fitting to reality [97, 136].

The results obtained in Chapter 2 are independent of the actual distribution of the random variables. This fact allows to use different distributions depending on the exact situation that should be modelled. Of course, the involved random variables have to satisfy some moment conditions. Mathematically, this can be seen as a universality in the behaviour of objects of the form (1.3.2).

1.3.2 Conditional large deviations for sums of weighted random variables

In Chapter 3 we consider large deviation results for partial sums, $S_n = \sum_{j=1}^{n} Z_jW_j$, conditioned on the sequence $(W_j)_{j\in\mathbb{N}}$, for sequences of real-valued, i.i.d. random variables, $(Z_j)_{j\in\mathbb{N}}$ and $(W_j)_{j\in\mathbb{N}}$.

We define $\mathcal{W}$ and $\mathcal{Z}$ as the sigma-algebras generated by $(W_j)_{j\in\mathbb{N}}$ and $(Z_j)_{j\in\mathbb{N}}$, respectively, and let $\mathcal{Z}$ and $\mathcal{W}$ be independent. For $a > \mathbb{E}[Z_1 W_1]$, we establish a sharp large deviation result for the conditional probability distribution, $\mathbb{P}(S_n \geq an|\mathcal{W})$, and an invariance principle for the random large deviation rate function. This chapter is joint work with Anton Bovier and was published in ALEA, [18],

> A. Bovier and H. Mayer, *A conditional strong large deviation result and a functional central limit theorem for the rate function*, ALEA Lat. Am. J. Probab. Math. Stat., Volume 12, Number 1, pp. 533–550, 2015.

Assuming that W_1 and Z_1 satisfy certain moment conditions and for a suitable interval J, we prove Theorem 3.1.6. Roughly speaking, it states that, as $n \to \infty$,

$$\mathbb{P}\left(\forall a \in J : \mathbb{P}(S_n \geq an|\mathcal{W}) = K_n^{\mathcal{W}}(a)\exp(-nI_n^{\mathcal{W}}(a))[1+\mathrm{o}(1)]\right) = 1. \tag{1.3.4}$$

Here, $K_n^{\mathcal{W}}(a)$ denotes a random prefactor and $I_n^{\mathcal{W}}(a)$ the random rate function. The result relies on a strong large deviation result due to Chaganty and Sethuraman, [24], restated in Theorem 1.2.8. Equation (1.3.4) means that the approximation provided by Theorem 1.2.8 holds almost surely, uniformly in $a \in J$. Subsection 3.3 of this thesis contains the proof of Theorem 3.1.6.

The rate function is given by

$$I_n^{\mathcal{W}}(a) = a\vartheta_n^{\mathcal{W}}(a) - \frac{1}{n}\sum_{j=1}^{n} f(W_j\vartheta_n^{\mathcal{W}}(a)), \tag{1.3.5}$$

where $f(\vartheta) \equiv \log\mathbb{E}[\exp(\vartheta Z_1)]$ and $\vartheta_n^{\mathcal{W}}(a)$, the tilting parameter, solves

$$a = \frac{d}{d\vartheta}\left(\frac{1}{n}\sum_{j=1}^{n} f(W_j\vartheta)\right). \tag{1.3.6}$$

Let us define $\vartheta(a)$ as the solution of

$$a = \frac{d}{d\vartheta}\mathbb{E}\left[f(W_1\vartheta)\right]. \tag{1.3.7}$$

We can split up the rate function into the sum of a deterministic function, $I(a)$, well-behaved random fluctuations, $\frac{1}{\sqrt{n}}X_n(\vartheta(a))$, and a small enough remainder term, $\frac{1}{n}r_n(a)$, where $r_n(a) \in \mathcal{O}(1)$, i.e.

$$I_n^{\mathcal{W}}(a) = I(a) + \frac{1}{\sqrt{n}}X_n(\vartheta(a)) + \frac{1}{n}r_n(a). \tag{1.3.8}$$

Note that an approximation of the rate function via a uniform law of large numbers without further characterisation of the remainder term, i.e. of the form

$$I_n^{\mathcal{W}}(a) = I(a) + R_n(a) \quad \text{with} \quad R_n \in \mathcal{O}\left(\frac{1}{\sqrt{n}}\right), \tag{1.3.9}$$

is not good enough, because the rate function is scaled by a factor n in the approximation of the probabilities, $\exp(-nI_n^{\mathcal{W}}(a))$. This means that also the error term is blown up by this factor and

we need a more precise control. To prove that Equation (1.3.8) holds in a suitable sense we prove that the fluctuation process, $(X_n(\vartheta(a)))_{a\in\bar{J}}$, converges in distribution, jointly with its derivatives, to a Gaussian process, provided the covariance structure is well-behaved. The description of $I_n^{\mathcal{W}}(a)$ by means of a functional central limit theorem is the subject of Theorem 3.1.9, which is proven in Subsection 3.4.

A typical condition required for large deviation results is that the moment generating function, $\mathbb{E}[\exp(\vartheta S_n)]$, exists on a certain interval. Here, we do not need this condition for the environment given by the W_j's and we require only the existence of the moment generating function of Z_1. The exact conditions imposed on W_1 depend on the distribution of Z_1, but are typically weaker than existence of exponential moments.

1.3.3 Modelling the evolution of cancer with and without treatment

In Chapter 4 a stochastic, individual-based model for the evolution of tumours with and without therapy is introduced and further examined by illustrative examples. This part of the thesis is based on joint work with Martina Baar, Anton Bovier and Loren Coquille from the mathematical department and Michael Hölzel, Meri Rogava and Thomas Tüting from the medical faculty in Bonn. The following preprint is available on the arXiv, [11],

> M. Baar, L. Coquille, H. Mayer, M. Hölzel, M. Rogava, T. Tüting and A. Bovier, *A stochastic individual-based model for immunotherapy of cancer*, arXiv:1505.00452, 2015.

Sections 4.1, 4.2 and 4.4 and Subsection 4.3.1 of Chapter 4 are rewritten with a different focus. The remainder of Section 4.3 contains only minor changes compared to the preprint, [11], in particular some pictures are not shown here. Section 4 of the preprint is not used in this thesis apart from small parts of Subsection 4.2 of the preprint. Except for the simulations shown in Section 4.4, the figures in Chapter 4 are taken from the preprint. Simulations were performed with a Gillespie-like algorithm implemented by Boris Prochnau.

A large part of this work is the actual choice of the model. The starting point from the *biological* point of view are the experimental findings by Landsberg et al. reported in [91]. It is shown there that melanoma cells escape therapy with cytotoxic T-cells by switching their phenotype. This so-called phenotypic plasticity is enhanced in the presence of particular cytokines secreted during inflammation. After an initial phase of remission very often a relapse appears.

In small populations random fluctuations can play a crucial role and modify the long-term evolution of a system. Since at least some subpopulations in a tumour are relatively small, in particular in a phase of remission, it is reasonable to use a probabilistic approach to model this setup. In order to build in the model the interactions of cancer and immune cell populations as well as the influence of the environment constituted by cytokines, we choose an individual-based model.

The starting point from the *mathematical* perspective are thus stochastic, individual-based models as introduced in Subsection 1.2.4. Recall that individuals in these models are characterised by a trait $x \in \mathcal{X}$. They can reproduce or die due to age or competition. At reproduction events a mutation occurs with probability $\mu(x)$. Note that in these models the population size, N_t, is not constant as it is for example in Wright-Fisher models. This is important for modelling the applications we have in mind, where a tumour shrinks and regrows in the context of therapy.

We extend the basic model in order to describe a therapeutic setup as explained in [91]. To do so we include a predator-prey relation between T-cells and cancer cells, switching of phenotypes of cancer cells and terms reflecting the production of a special cytokine and its influence on the cancer cells. Furthermore, we generalise the new framework in a way which allows to model also new therapy strategies, such as usage of several types of T-cells attacking different types of cancer cells. The observed phenotypic switch is relatively rapid, i.e. it takes place on a shorter timescale than genotypic mutations. A distinction of genotype and phenotype of cancer cells allows for assuming rare mutations and fast switches at the same time and for studying their interplay. This might also be a tool to better understand the impact of phenotypic and genotypic heterogeneity of tumours on therapy resistance.

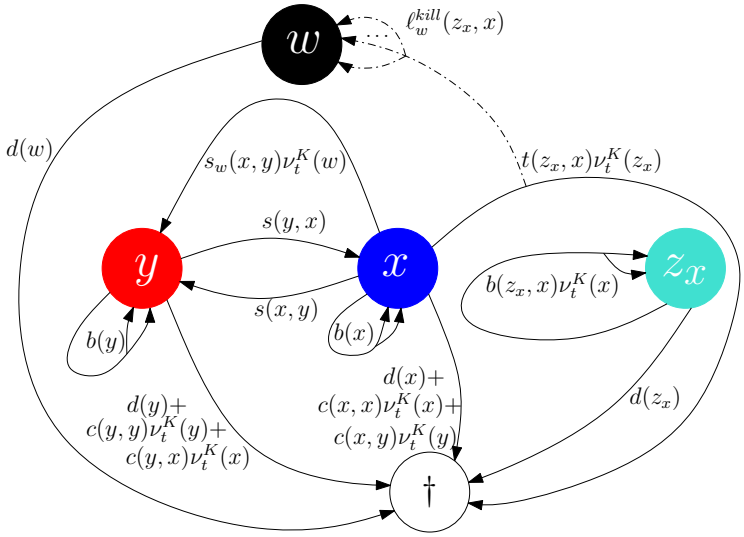


Figure 1.5: Dynamics of the process (without mutations) modelling the experiments described in [91]. x denotes differentiated melanoma cells, y dedifferentiated melanoma cells, z_x T-cells and w TNF-α. This picture is taken from [11].

The "small" model describing the experiments reported in [91] can graphically be represented as shown in Figure 1.5. Differentiated (original) melanoma cells are denoted by x and dedifferentiated melanoma cells appearing through switching by y. At time t each melanoma cell can divide at rate $b(x)$ or $b(y)$, switch at rate $s(x,y) + s_w(x,y)\nu_t^K(w)$ or $s(y,x)$, and die at rate $d(i) + c(i,i)\nu_t^K(i) + c(i,j)\nu_t^K(j)$ with $i,j \in \{x,y\}$ and $i \neq j$. At cell divisions the phenotype is maintained. The presence of T-cells, denoted by z_x, introduces an additional death rate $t(z_x,x)\nu_t^K(z_x)$ for differentiated melanoma cells, while the T-cells reproduce in presence of their target at rate $b(z_x,x)\nu_t^K(x)$. T-cells can also die or become exhausted and thus vanish at rate $d(z_x)$. When melanoma cells die from therapy, $\ell_w^{\text{kill}}(z_x,x)$ TNF-α molecules are secreted. Their presence enhances the switch towards dedifferentiated cells. TNF-α vanishes at rate $d(w)$. The evolution of the population is described by exponential waiting times with rates as explained above and indicated on the arrows in Figure 1.5. We do not consider mutations in this reduced setup since this was also not studied in [91].

In Subsection 4.3.1 we give examples describing the experiments of [91] qualitatively, whereas Subsection 4.3.3 aims at a quantitative description with physiologically reasonable parameters. Both examples are extended to a predictive setup, where a second type of T-cells targeting

dedifferentiated cancer cells with the switched phenotype is introduced. A possible influence of therapy on the incidence of mutations is presented by an example in Subsection 4.4.1.

2. Stochastic modelling of T-cell activation

Hannah Mayer and Anton Bovier

Abstract We investigate a specific part of the human immune system, namely the activation of T-cells, using stochastic tools, especially sharp large deviation results. T-cells have to distinguish reliably between foreign and self peptides which are both presented to them by antigen presenting cells. Our work is based on a model studied by [138] [J Math Bio 57(6):841-861]. We are able to dispense with some restrictive distribution assumptions that were used previously, i.e. we establish a higher robustness of the model. A central issue is the analysis of two new perspectives to the scenario (two different quenched systems) in detail. This means that we do not only analyse the total probability of a T-cell activation (the annealed case) but also consider the probability of an activation of one certain clonotype and the probability of a T-cell activation by a certain antigen presentation profile (the quenched cases). Finally, we see analytically that the probability of T-cell activation increases with the number of presented foreign peptides in all three cases.

2.1 Introduction and model setting

In the present paper we analyse a stochastic model for T-cell activation that was introduced by [131] and later developed and studied by [138]. While we allow for a slightly more general model setting, the main new contribution is the analysis of some different biological settings that correspond mathematically to various conditional probabilities. This is explained in detail below.

2.1.1 Biological perspective

Let us first of all say that the model we consider concerns only the mechanism of T-cell activation. There are many other processes involved in the immune response [104] that are not considered here at all. *T-cells* have the task to recognise foreign antigens against a noisy background of the body's own antigens. Any substance which is able to elicit an immune response is called *antigen* (from the term *anti*body *gen*erating). T-cells do not react with free antigens present in the body, but only with antigens presented by *antigen presenting cells* (APCs). APCs collect material in the body, internalize it and split it up in peptides which are afterwards presented on the surface of the APC.

In a so called *immunological synapse*, a bond between a T-cell and an APC, the T-cell scans the presented mixture of peptides using its receptors. There is only one receptor type on each T-cell and thus the T-cell is characterised by its T-cell receptor (TCR) clonotype. On the other hand there are many different peptide species on each APC. The task of a T-cell is to decide on the basis of signals received in an immunological synapse whether foreign antigens are present in the body, and to trigger an immune reaction when indicated (in reality, this involves a complex

interplay with other parts of the immune system which we do not deal with here). This task is made difficult by the following fact: according to [97] and [6], there exist about 10^{13} peptide species that should be recognised but only 10^7 different TCR clonotypes. This implies that a fully specific recognition, i.e. that each TCR clonotype recognises exactly one specific antigen, is impossible. Therefore, a certain degree of *cross-reactivity* has to be assumed. The presence of auto-immune diseases, allergies and heavy diseases caused e.g. by viruses and bacteria shows that the immune system faces a very difficult task.

2.1.2 The model

[131] and [138] presented a mathematical model that allows to interpret the functioning of T-cells as a statistical test problem. We briefly describe this model following [138].

Characterisation of the APC. An APC is characterised by the species of the presented peptides and the number of copies of each peptide species. Thus, each APC can be represented by a set of parameters z_j representing the numbers of peptides of species j, by the so-called Antigen Presentation Profile (APP). Peptide species are sometimes distinguished as *constituent* (i.e. being present in all cells) and *variable* (i.e. being present only in some cells), but this distinction plays no major rôle in the present paper (see, however [95]). The index j ranges over all peptide species present on this APC. We denote the number of foreign peptides present on the APC by z_f and allow, for simplicity, for only one species of foreign peptide on one APC. Note that the peptides on the APC are collected by a given APC and thus are a *random* sample from the total pool of peptides present in the body.

Characterisation of the T-cell. Each T-cell possesses one receptor type on its surface which determines the so-called TCR clonotype. The T-cell is characterised by the interaction of its receptors with the different peptide species. To each TCR clonotype i corresponds a set of association rates, a_{ij}, and dissociation rates, r_{ij}. Here i ranges over all TCR clonotypes and j over all peptide species. We use the following assumption in line with the approach of [138] and [131]. This scenario is discussed by [128] as the "MHC-limited case".

Assumption 2.1.1. There is an abundance of receptors on each T-cell in the region of interaction with the APC such that each released peptide is immediately bound by a receptor again. In this case association can be assumed as instantaneous and the association rates play no major rôle.

Remark 2.1.2. We see later that we have to work with random stimulation rates to investigate the event of T-cell activation. It will become clear that the qualitative behaviour of our results does not rely on the exact distribution of the stimulation rates. In particular, this distribution could depend on the association rates and thus Assumption 2.1.1 is not too restrictive.

Under Assumption 2.1.1, a T-cell is fully characterised by the set r_{ij}, where i refers to the particular TCR clonotype. We will see later that we are interested in the duration of peptide-receptor-complexes. The duration of a complex of type ij is denoted by t_{ij} and depends on the dissociation rates.

Activation criteria. The interaction of the cells produces a stimulating signal which the T-cell receives. This signal results in an activation of the T-cell, if certain criteria are met. The papers by [111, 124, 123] and [133] suggest the following assumption.

Assumption 2.1.3.

1. A T-cell receives a stimulus if a peptide-receptor-complex exists longer than a time t_*.
2. A T-cell sums up all the stimuli it gets, even those of different receptors.
3. A T-cell is activated if the sum of all stimuli exceeds a threshold value g_{act}.

Note that activation is induced by stimulation and thus the stimulation rates w_{ij} which result from a peptide-receptor-complex of type ij are important. The central quantity to analyse is then the *total stimulation rate* a T-cell receives. It is denoted by g_i and compounds all the parameters mentioned before.

The preliminary description demonstrates that we are concerned with a really high-dimensional problem and model. Merely the number of parameters for the dissociation rates in the model is at least of the order 10^{20} (10^7 TCR clonotypes times 10^{13} peptide species).

Stochastic model. The large number of parameters in this model makes a specification of all of them impossible. Therefore, [131] proposed a stochastic model.

A closer look at the system reveals that we must deal with different types and sources of randomness. This is crucial for the interpretation of the probabilities in specific setups. The dissociation rates are assumed to be random because they are unknown in detail. The APP is random because it represents a random sample of the peptides present in the body. In fact, this quantities are doubly stochastic because we have a random pool of peptides in the body and a random sample out of this pool is presented on the APC. For the sake of simplicity we do not model this double effect here. A further reason to assume randomness is that we consider a random encounter of two randomly meeting cells. Let us now specify the stochastic version of the model precisely.

Let $(\Omega, \mathcal{F}, \mathbb{P})$ be a probability space on which we define the following random variables. First, we characterise the APP.

(i) $n \equiv n_c + n_v + 1$ denotes the number of different peptide species on one APC, where n_c and n_v denote the number of constitutive and variable peptide species on each APC and one foreign peptide species is present.

(ii) N_c and N_v denote the number of constitutive and variable peptide species in the body.

(iii) The sample of constitutive and variable peptides is represented by positive random variables Z_j^c and Z_j^v, each of them representing the number of copies of a certain peptide species; they are independent and identically distributed (i.i.d.) in the class of constitutive and variable peptides, respectively.

(iv) Z_j^c and Z_j^v are bounded, and independent of each other.

(v) $\mathcal{Z}$ is the σ-algebra generated by Z_j^c with $j \in \{1, \dots, N_c\}$ and Z_j^v with $j \in \{1, \dots, N_v\}$.

(vi) We use a short-hand notation for the conditional distribution $\mathbb{P}^{\mathcal{Z}}(A) \equiv \mathbb{P}(A|\mathcal{Z})$ and denote the corresponding conditional expectation by $\mathbb{E}^{\mathcal{Z}}[\cdot]$.

It is reasonable to assume that Z_j^c and Z_j^v are bounded because the space on each APC is bounded.

Next, we come to the characterisation of the T-cell.

(i) The total number of TCR clonotypes is $N_1 \in \mathbb{N}$, the one of the peptide species is $N_2 \in \mathbb{N}$.

(ii) The index i denotes the TCR clonotype and j denotes the peptide species.

(iii) The dissociation rates of a complex of type ij are positive, i.i.d. random variables R_{ij} with distribution $\mathcal{P}$ and expectation value $\mathbb{E}[R_{ij}] = \overline{\tau}$, $\overline{\tau} \in \mathbb{R}_+$.

(iv) $\mathcal{R}$ is the σ-algebra generated by R_{ij} with $i \in \{1, \dots, N_1\}$ and $j \in \{1, \dots, N_2\}$.

(v) The times certain peptide-receptor-complex of type ij exist are positive i.i.d. random variables T_{ij} with conditional distribution $\mathbb{P}^{\mathcal{R}}(T_{ij} \in A) \equiv \mathbb{P}(T_{ij} \in A|\mathcal{R})$ and corresponding conditional expectation $\mathbb{E}^{\mathcal{R}}[T_{ij}] \equiv \mathbb{E}[T_{ij}|\mathcal{R}] = 1/R_{ij}$.

In this setting T_{ij} are random variables in a random environment, in other words they are also doubly stochastic. They display the individual duration of a concrete peptide-receptor-complex of type ij. Therefore, the expected duration of such a bond should be reciprocally proportional to the corresponding dissociation rate R_{ij}. According to (v) $\mathbb{E}[T_{ij}] = (\bar{\tau})^{-1}$. The joint distribution of all dissociation rates is given by the product measure $\mathcal{P}^{N_1 N_2}$. The presented sample is from the biological point of view independent of the dissociation rates and the duration of the peptide-receptor-bonds. Thus, $\mathcal{Z}$ and $\mathcal{R}$ are independent.

The parameters of the previous part can be considered as realisations of these random variables.

The stimulation rates. According to Assumption 2.1.3 a single bond between a receptor i and a presented peptide of species j in a synapse results in a stimulation signal if the binding time, T_{ij}, exceeds a certain threshold t_*. It is assumed that the relevant signal for the T-cell is the compound average number of stimuli in a synapse,

$$\frac{1}{t} \sum\nolimits_{k=1}^{N_j(t)} \mathbb{1}_{T_{ij}^k > t_*}, \tag{2.1.1}$$

where t indicates the time, for which this synapse lasts, $N_j(t)$ denotes the total number of bindings with a peptide of species j and T_{ij}^k are the respective binding times. Assuming that the number of bindings is very large, it is reasonable to assume that the relevant signal a T-cell is receiving from a given peptide species is given by

$$W_{ij} \equiv \lim_{t \uparrow \infty} \frac{1}{t} \sum\nolimits_{k=1}^{N_j(t)} \mathbb{1}_{T_{ij}^k > t_*} = \frac{\mathbb{P}^{\mathcal{R}}(T_{ij} > t_*)}{\mathbb{E}^{\mathcal{R}}[T_{ij}]} \quad \text{a.s.}, \tag{2.1.2}$$

where the last equality follows from elementary renewal theory. We will henceforth consider the random variables W_{ij} as the fundamental characteristics of a peptide-receptor interaction.

Remark 2.1.4. This approach might not be reasonable for certain T-cell types, e.g. for cytotoxic T-cells since these cells interact only for a very short time with the APC and an equilibrium can not be reached.

Notation. Let the variable z_{f} denote the number of presented foreign peptides of one particular species. The expected number of peptides present at one APC is given by $n_M \equiv n_c \mathbb{E}[Z_1^c] + n_v \mathbb{E}[Z_1^v]$.

The factor $q_n \equiv (n_M - z_\mathrm{f})n_M^{-1}$ used in the following ensures a proportional displacement of the presented self peptides by the foreign peptides[1].

We drop the index i in favour of a clear notation since it is fixed for one T-cell. The discussion above motivates [138] to define the *total stimulation rate* as follows.

Definition 2.1.5. With the notation introduced above, the total stimulation rate a T-cell receives is given by

$$G_n(z_\mathrm{f}) = q_n \left(\sum_{j=1}^{n_c} Z_j^c W_j + \sum_{j=n_c+1}^{n_c+n_v} Z_j^v W_j \right) + z_\mathrm{f} W_\mathrm{f}. \tag{2.1.3}$$

Note that W_f has the same distribution as the other random variables representing stimulation rates, and is independent of these random variables and $\mathcal{Z}$.

Interpretation of the probabilities.

The central quantity for our investigation is the probability of T-cell activation. If we consider just one certain encounter of an APC and a T-cell the "probability" of T-cell activation is either 0 or 1 since all parameters are fixed in that case. But one single event does not give much information on the actual situation because it is possible that this T-cell makes a mistake. So, we and in fact the immune system take another, namely a statistical, view to the scenario. We are concerned with investigating the following three different cases:

1.) The annealed case: Here we consider the overall probability that a randomly chosen T-cell is activated by a randomly chosen APC, $\mathbb{P}(G_n(z_\mathrm{f}) \geq g_\mathrm{act})$. This probability can be interpreted as the frequency with which activations occur. If m denotes the number of encounters of APCs and T-cells, then

$$\frac{1}{m}\#\ \{\text{activations of T-cells}\} \to \mathbb{P}(G_n(z_\mathrm{f}) \geq g_\mathrm{act}) \tag{2.1.4}$$

as m tends to infinity.

2.) The case quenched with respect to (w.r.t.) the TCR clonotype: In this case we investigate the conditional probability that a T-cell of a certain TCR clonotype is activated by a randomly chosen APC, $\mathbb{P}^{\mathcal{R}}(G_n(z_\mathrm{f}) \geq g_\mathrm{act})$. Here, different T-cells of one TCR clonotype are examining several APPs presented by different APCs and the results are averaged. The probability is to be interpreted as the frequency of activations of T-cells of a given clonotype in several synapses with different APCs presenting different APPs. That is, if $m_{\mathcal{R}}$ denotes the number of encounters of T-cells of clonotype i and different APCs, then

$$\frac{1}{m_{\mathcal{R}}}\#\{\text{activations of T-cells of clonotype } i\} \to \mathbb{P}^{\mathcal{R}}(G_n(z_\mathrm{f}) \geq g_\mathrm{act}) \tag{2.1.5}$$

as $m_{\mathcal{R}}$ tends to infinity. We shall see that these probabilities depend strongly on the sensitivity of the given TCR clonotype to the particular presented foreign peptide species.

[1] One may argue that scaling the random variables by a common factor is not the best choice to achieve a constant expectation. E.g., if the Z_j were assumed to be binomial random variables, it would be more reasonable to change the parameter in an appropriate way. However, this appears to have only little effect on the results and we keep following [138] at this point.

3.) The case quenched w.r.t. the APP: The conditional probability that a APC with a certain APP activates a randomly chosen T-cell, $\mathbb{P}^{\mathcal{Z}}(G_n(z_\mathrm{f}) \geq g_\mathrm{act})$, is analysed here. If $m_{\mathcal{Z}}$ denotes the number of encounters of T-cells of different clonotype with different APCs presenting the same APP, then

$$\frac{1}{m_{\mathcal{Z}}}\#\{\text{activations of T-cells by the given APP}\} \to \mathbb{P}^{\mathcal{Z}}(G_n(z_\mathrm{f}) \geq g_\mathrm{act}) \tag{2.1.6}$$

as $m_{\mathcal{Z}}$ tends to infinity. This is to be interpreted as the frequency of TCR clonotypes which are "sensitive" to the fixed APP under consideration. If one considers the idealistic situation of having one encounter for each TCR clonotype this implies that it is desired that

$$\mathbb{P}(G_n(0) \geq g_\mathrm{act}) \ll \frac{1}{N_1} \ll \mathbb{P}(G_n(z_f) \geq g_\mathrm{act}) \tag{2.1.7}$$

is fulfilled, where N_1 denotes the number of different TCR clonotypes. This is intuitively clear since no TCR clonotype should be activated by the self-background and at least one TCR clonotype should be activated if foreign peptides are present.

These three cases can be used to answer different biological questions. Depending on the particular question different distributions for the appearing random variables should be used. To calculate a probability which can be compared to biological parameters certain aspects have to be known or specified, for example the number of T-cells of the different clonotypes and the number of different APPs. Then questions, such as "How long does it take to elicit an immune response?", "How long does it take to encounter a suitable TCR clonotype?" can be asked. On the other hand, this also gives a feedback on the question whether the mathematical model is reasonable. The main question is which information on z_f is deducible from the behaviour of the T-cells. This information emerges only on the basis of several encounters and the resulting frequency of activations. It leads to the question whether there exists a threshold value g_act such that the presence of invaders is distinguishable from the self-background.

[138] considered only Case 1.). In our view the Cases 2.) and 3.) are even more relevant since they provide information on the activating APPs and the activated clonotypes.

Scaling and asymptotics. To obtain sensible analytic results one needs to consider certain numbers to be *large*. Clearly, the total number of peptide species on one APC, n, will be assumed the main large parameter. The number of foreign peptides has to be seen in comparison to this number, i.e. we understand that z_f *depends* on n, and one wants to know how large z_f has to be (as a function of n) for a reliable detection. The numbers n_c and n_v can in principle also be of different magnitude, e.g. one may think that $n_v \sim n$ and $n_c \sim n^\beta$, $\beta < 1$. For simplicity, we will consider here only the case $n_c \sim n_v \sim n$.

2.2 Results

2.2.1 Expectation value and variance of the total stimulation rate

We compute the expectation value and variance of the total stimulation rate as a function of the number of foreign peptides, z_f, in Cases 1.) , 2.), and 3.). It is easy to calculate these quantities and they allow a first insight into why and in which range a foreign self distinction may work.

We assume the existence of the involved first and second moments.

Case 1.) According to the following lemma an increasing number of foreign peptides does not change the expectation value but the variance of the total stimulation rate.

Lemma 2.2.1. *It holds that* $\mathbb{E}[G_n(0)] = \mathbb{E}[G_n(z_\mathrm{f})]$*, and that*

$$\mathbb{V}[G_n(z_\mathrm{f})] - \mathbb{V}[G_n(0)] = \left(\mathbb{V}[W_1] + \tfrac{\mathbb{V}[G_n(0)]}{n_M^2}\right) z_\mathrm{f} \left(z_\mathrm{f} - \tfrac{2n_M \mathbb{V}[G_n(0)]}{\mathbb{V}[W_1]n_M^2 + [G_n(0)]}\right). \tag{2.2.1}$$

We can see that the expectation value of $G_n(z_\mathrm{f})$ is independent of z_f. The variance depends quadratically on z_f, the difference of the variances as a function of z_f is a parabola with roots $2n_M\mathbb{V}[G_n(0)]/(\mathbb{V}[W_1]n_M^2 + \mathbb{V}[G_n(0)])$ and 0. The function decreases first but for z_f large enough it is monotonously increasing and positive. The qualitative behaviour shows up in this general setup, the exact shape depends only on the first and second moments of the involved random variables and not on their exact distribution. In the setting of an increasing variance and a constant expectation value the probability to exceed a threshold value larger than the expectation value increases. Thus, a T-cell activation may become more likely for a larger value of z_f. The increasing variance may allow to have the threshold value g_act on a level such that permanent reactions are avoided but an activation becomes more probable. We see here already that a detection can only be possible if z_f is large enough.

Case 2.) Because we consider conditional probabilities and expectations here, these quantities can coincide at most almost surely. We consider now the difference of the conditional expectations as a function of z_f, too. In this case the expectation value and the variance depend on z_f.

Lemma 2.2.2. *It holds that* $\mathbb{E}^\mathcal{R}[G_n(z_\mathrm{f})] - \mathbb{E}^\mathcal{R}[G_n(0)] = z_\mathrm{f}(W_\mathrm{f} - \mathbb{E}[W_1])$ *almost surely, and that*

$$\begin{aligned}\mathbb{V}^\mathcal{R}[G_n(z_\mathrm{f})] - \mathbb{V}^\mathcal{R}[G_n(0)] &= \left(\mathbb{V}^\mathcal{R}[W_1] + \frac{\mathbb{V}^\mathcal{R}[G_n(0)]}{n_M^2}\right) z_\mathrm{f} \left(z_\mathrm{f} - \frac{2n_M \mathbb{V}^\mathcal{R}[G_n(0)]}{\mathbb{V}^\mathcal{R}[W_1]n_M^2 + \mathbb{V}^\mathcal{R}[G_n(0)]}\right) \\ &= \frac{\mathbb{V}^\mathcal{R}[G_n(0)]}{n_M^2} z_\mathrm{f}\left(z_\mathrm{f} - 2n_M\right), \end{aligned} \tag{2.2.2}$$

where $\mathbb{V}^\mathcal{R}[X] := \mathbb{E}^\mathcal{R}[(X - \mathbb{E}^\mathcal{R}[X])^2]$.

Note that $\mathbb{V}^\mathcal{R}[W_1] = 0$ because W_1 is measurable w.r.t $\mathcal{R}$. $\mathbb{E}[W_1]$ is with positive probability not equal to W_f because we do not assume the stimulation rates to be distributed according to a Dirac measure. This is reasonable because we know from the biological background that the stimulation rates can vary. Hence, we have here an effect on the average stimulation rate a T-cell receives which depends on the stimulation rate associated to the foreign peptide. The conditional expectation of $G_n(z_\mathrm{f})$ increases with z_f if $W_\mathrm{f} > \mathbb{E}[W_1]$. The effect becomes enlarged for an increasing z_f. On the other hand, the variance is decreasing for an increasing value of z_f because z_f will never reach $2n_M$ from the biological point of view. Thus, the situation is different here. The parabolas describing the difference of the variances are random here.

Case 3.) Again, the conditional expectations can be at most almost surely equal. Here, the expectation value is almost surely independent of z_f but the variance depends again on z_f.

Lemma 2.2.3. *It holds that* $\mathbb{E}^{\mathcal{Z}}[G_n(z_{\mathrm{f}})] - \mathbb{E}^{\mathcal{Z}}[G_n(0)] = 0$ *almost surely, and that*

$$\mathbb{V}^{\mathcal{Z}}[G_n(z_{\mathrm{f}})] - \mathbb{V}^{\mathcal{Z}}[G_n(0)] = \left(\mathbb{V}^{\mathcal{Z}}[W_1] + \tfrac{\mathbb{V}^{\mathcal{Z}}[G_n(0)]}{n_M^2}\right) z_{\mathrm{f}} \left(z_{\mathrm{f}} - \frac{2n_M \mathbb{V}^{\mathcal{Z}}[G_n(0)]}{\mathbb{V}^{\mathcal{Z}}[W_1] n_M^2 + \mathbb{V}^{\mathcal{Z}}[G_n(0)]}\right), \quad (2.2.3)$$

where $\mathbb{V}^{\mathcal{Z}}[X] := \mathbb{E}^{\mathcal{Z}}[(X - \mathbb{E}^{\mathcal{Z}}[X])^2]$.

In this case the conditional expectation of the total stimulation rate is only almost surely independent of z_{f}. We also have random parabolas for the difference of the variances in this case. Note that this scenario is very similar to the one in the annealed case; there is an effect on the level of the variance but not on the level of the expectation.

In Figure 2.1 intervals are plotted for different values of z_{f}, such that the random variable $G_n(z_{\mathrm{f}})$ lies in these intervals with probability 0.99, under the tentative assumption that the standardised version of the total stimulation rate, $(G_n(z_{\mathrm{f}}) - \mathbb{E}[G_n(z_{\mathrm{f}})])(\sqrt{\mathbb{V}[G_n(z_{\mathrm{f}})]})^{-1}$, is standard normally distributed. In Case 1.) the intervals enlarge for z_{f} large enough because the variance of $G_n(z_{\mathrm{f}})$ increases. In Case 2.) the intervals shrink due to the decreasing variance but they move according to the value of the stimulation rate of the foreign peptide. In the presented case W_{f} is larger than its expectation $\mathbb{E}[W_{\mathrm{f}}]$. Thus, the intervals move to the right hand side. Case 3.) is very similar to Case 1.) except for a slight difference concerning the expectation value which is just almost surely independent of z_{f}, and thus slightly varying.

2.2.2 Large deviations

Encounters of T-cells and APCs happen permanently in the body, but only very few of them give rise to an immune reaction. Therefore, T-cell activation must be tuned (by suitable choice of the activation threshold g_{act}) such that activation and the corresponding immune response are rare events. This implies that to compute the activation probabilities, one needs to use large deviation techniques (see for example [46] or [45]). In particular, the computations of means and variances from the previous subsection 2.2.1 are insufficient.

We are concerned with a family of real-valued random variables $(S_n)_{n\in\mathbb{N}}$ and the probability that $n^{-1}S_n$ exceeds a threshold value a, $\mathbb{P}(S_n \geq na)$. A *large deviation* is a deviation from the expectation value of S_n of the order n. If the family of random variables under consideration satisfies a so called *large deviation principle* (LDP), the probability for a large deviation event decays exponentially in n with rate $I(a)$. The *rate function* $I(a)$ is obtained as the limit of the Fenchel-Legendre transformation of the logarithmic moment generating function of S_n, to wit $I(a) = \lim_{n\uparrow\infty} I_n(a)$, where

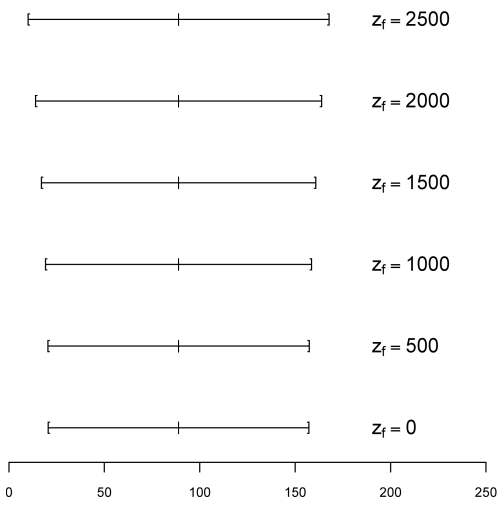
$$I_n(a) = \sup_{\vartheta}(a\vartheta - \Psi_n(\vartheta)) \equiv a\vartheta_n - \Psi_n(\vartheta_n), \quad (2.2.4)$$

and $\Psi_n(\vartheta) \equiv \frac{1}{n}\ln\mathbb{E}[\exp(\vartheta S_n)]$ and ϑ_n satisfies

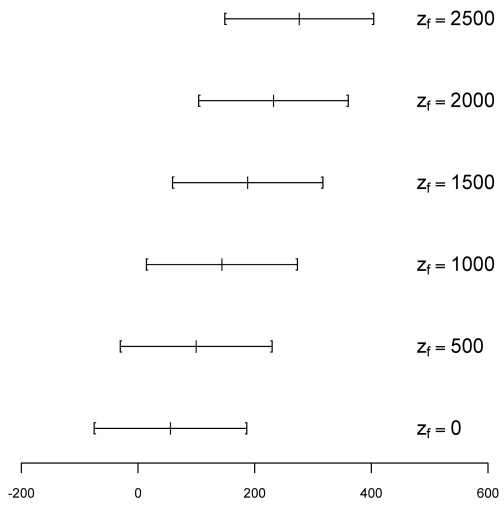
$$\Psi_n'(\vartheta_n) = a. \quad (2.2.5)$$

ϑ_n is known as the *tilting parameter* and is used to perform an exponential change of measure in many proofs of theorems in this field. The standard theorems of Cramér and Gärtner-Ellis then state

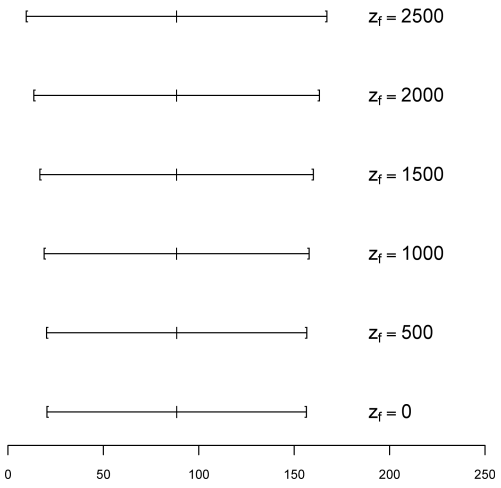
$$\mathbb{P}(S_n \geq an) = \exp(-nI(a)(1 + \mathrm{o}(1))). \quad (2.2.6)$$



(a) Case 1.)



(b) Case 2.)



(c) Case 3.)

Figure 2.1: Intervals containing $G_n(z_\mathrm{f})$ with probability and conditional probability 0.99.

As was pointed out by [138], this approximation is not sufficiently precise to calculate the actual probability because of the huge and poorly controlled multiplicative error term $\exp(n\mathrm{o}(1))$. Fortunately, there are stronger theorems available, known as *sharp large deviation results* or *exact asymptotics*. To use such results was already suggested by [131] and [126]. We obtain approximations of the form

$$\mathbb{P}(S_n \geq an) = \frac{\exp(-nI_n(a))}{\vartheta_n \sigma_n \sqrt{2\pi n}}(1 + \mathrm{o}(1)) \tag{2.2.7}$$

with the same notation as before and $\sigma_n^2 \equiv \Psi_n''(\vartheta_n)$. The standard theorem for S_n a sum of i.i.d. random variables is due to [12]. The generalization to independent, but not identically distributed random variables, which we need here, is based on results of [24]. We restate these

results in Section 2.3.

These techniques will allow us to achieve our goal, namely to check whether there is a threshold value g_{act} such that the probability of activation changes by orders of magnitude with z_{f}. We would like to know if the condition $\mathbb{P}(G_n(0) \geq g_{\mathrm{act}}) \ll \mathbb{P}(G_n(z_{\mathrm{f}}) \geq g_{\mathrm{act}})$ can be satisfied for physiologically reasonable values of z_{f}. Therefore, we look at the activation probabilities as a function of g_{act}. These functions are often called *activation curves*.

Application to the model of T-cell activation. We look at an artificial sequence of models which is characterised by an increasing number of peptide species, $n \equiv n_c + n_v + 1$. We assume that there exists $C \in (0, \infty)$ such that $\lim_{n\to\infty} n_c/n_v = C$. This implies that there exist $C_1, C_2 \in (0, \infty)$ such that $\lim_{n\to\infty} n_c/n = C_1$ and $\lim_{n\to\infty} n_v/n = C_2$. We use this condition to ensure some convergence properties, especially for the rate function. But this is also from the biological point of view a reasonable assumption because the ratio of the numbers of constitutive and variable peptide species is constant. The sequence of random variables S_n is given by $G_n(z_{\mathrm{f}})$ from Definition 2.1.5.

[138] used certain distributions for all appearing random variables to prove the applicability of Theorem 3.1.3 below. Afterwards, the approximations of the probabilities were calculated and compared to simulations. Thereby a separation of the activation curves for different values of z_{f} was obtained and a high coincidence of the approximation with the simulation was observed.

We prove the applicability of Theorem 3.1.3 in the Cases 1.), 2.), and 3.) in Section 2.3 under suitable conditions on the distributions and moment generating functions of the involved random variables. In this section we state just the approximations of the probabilities and the involved rate functions.

Remark 2.2.4. To obtain the following approximations of the probabilities we assume the existence of the moment generating and the conditional moment generating functions of $Z_1^c W_1$, $Z_1^v W_1$ and W_1. This condition is satisfied since we consider bounded random variables.

Case 1.) Let $M_c(\vartheta) \equiv \mathbb{E}[e^{\vartheta Z_1^c W_1}]$, $M_v(\vartheta) \equiv \mathbb{E}[e^{\vartheta Z_1^v W_1}]$ and $M(\vartheta) \equiv \mathbb{E}[e^{\vartheta W_1}]$ denote the moment generating functions of $Z_1^c W_1, Z_1^v W_1$ and W_1. Let $g_{\mathrm{act}}(n) \equiv an$ for $a > \mathbb{E}[G_n(z_{\mathrm{f}})]/n$. The activation probabilities can be approximated by

$$\begin{aligned}\mathbb{P}(G_n(z_{\mathrm{f}}) \geq g_{\mathrm{act}}(n)) =& \frac{\exp(-na\vartheta_n(a, z_{\mathrm{f}}) + n_c \ln M_c(q_n \vartheta_n(a, z_{\mathrm{f}})))}{\vartheta_n(a, z_{\mathrm{f}})\sigma_n\sqrt{2\pi n}} \\ & \times \exp(n_v \ln M_v(q_n\vartheta_n(a, z_{\mathrm{f}})) + \ln M(z_{\mathrm{f}}\vartheta_n(a, z_{\mathrm{f}})))(1 + \mathrm{o}(1)), \qquad (2.2.8)\end{aligned}$$

where $\vartheta_n(a, z_{\mathrm{f}})$ is chosen such that the argument of the exponential function attains its minimum. We write $\vartheta_n(a, z_{\mathrm{f}})$ and not just ϑ_n to visualise the dependence on a and z_{f}. We have proven this approximation in analogy to the proof of [138]. It only requires homogeneous distributions with certain properties in each block. The form of the dependence of the stimulation rates on the dissociation rates is not important. This can be interpreted as a tacit inclusion of competition of the peptides, association rates, loading fluctuations and similar aspects.

The interesting situation is when z_{f} becomes large, but remains small compared to n. In that case, the infimum will be attained for $\vartheta_n \sim a$, and since the law of W_1 is assumed to have bounded support, there will be a constant L such that $\frac{d}{d\vartheta} \ln M(z_{\mathrm{f}}\vartheta) \sim L z_{\mathrm{f}}$ for ϑz_{f} large enough. For $z_{\mathrm{f}} \gg \sqrt{n}$ we may apply this approximation since we know from the applicability of Theorem

3.1.3 that $\vartheta_n\sqrt{n} \to \infty$. A simple computation then shows that

$$\frac{\mathbb{P}(G_n(z_\mathrm{f}) \geq g_\mathrm{act}(n))}{\mathbb{P}(G_n(0) \geq g_\mathrm{act}(n))} \sim \exp\left(\vartheta_n(a,0) z_\mathrm{f}\left(L - a\frac{n}{n_M}\right)\right), \tag{2.2.9}$$

where $\vartheta_n(a,0)$ is the solution of Equation (2.2.5) for $z_\mathrm{f} = 0$. This implies that if a is chosen sufficiently small, the activation probability increases exponentially with z_f, as desired. To obtain Equation (2.2.9) we used two Taylor approximations: first we expanded $\vartheta_n(a, z_\mathrm{f})$ in $\vartheta_n(a,0)$ and after substituting this term in the rate function we expanded the resulting expression. This way it is possible to recover the probability of activation for the self-background and calculate the ratio of interest as stated by Equation (2.2.9).

Remark 2.2.5. The fact that if a is too big, the activation probability drops as z_f increases has a simple intuitive explanation: for very large a, the contribution of the foreign peptides is limited by the maximal value of W_f, whereas the reduction of the contribution of the other peptides makes it more unlikely to achieve an activation by a random fluctuation.

Case 2.) An upper index $\mathcal{R}$ on any previously defined object should signify the same object conditioned on the σ-algebra $\mathcal{R}$. We consider the conditional moment generating functions

$$M^{\mathcal{R}}_{\gamma,j}(\vartheta) = \mathbb{E}^{\mathcal{R}}[e^{\vartheta Z_j^\gamma W_j}] = \int \exp(\vartheta Z_j^\gamma W_j) dP_{Z_j^\gamma}, \gamma \in \{c, v\}, \tag{2.2.10}$$

where $P_{Z_j^\gamma}$ denotes the measure corresponding to $Z_j^\gamma, \gamma \in \{c, v\}$. Thus, the moment generating functions are random variables themselves. Because the numbers of copies, Z_j^c and Z_j^v, are independent of $\mathcal{R}$ and the stimulation rates are measurable w.r.t. $\mathcal{R}$, these moment generating functions are again i.i.d. random variables in each block. Due to the measurability of W_f w.r.t. $\mathcal{R}$ we have $M^{\mathcal{R}}(\vartheta) = \exp(\vartheta z_\mathrm{f} W_\mathrm{f})$. The resulting rate function, $I_n^{\mathcal{R}}(a, z_\mathrm{f})$, is also *random*. We need to apply a law of large numbers in the proof of the approximation of the probabilities in this case. Thus, $\ln(M^{\mathcal{R}}_{\gamma,j}(\vartheta)) \in L^1(\mathcal{P}^{N_1 N_2})$ for each $\vartheta \geq 0$ and $\gamma \in \{c, v\}$ is an important ingredient for the proof. Using this fact we can also establish convergence of the rate function according to a strong law of large numbers. But this convergence is not good enough for our purpose because the rate function and thus also the error term arising from the law of large numbers are scaled with a factor n in the large deviation approximation of the probabilities. Therefore, we have to prove a *functional central limit theorem* for a part of the rate function and we have to take into account the term which thus emerges. Thereby we obtain a process, Z_n, which converges weakly to a Gaussian process. Let

$$g_n(\vartheta) \equiv \frac{n_c}{n}\mathbb{E}\left[\ln \mathbb{E}^{\mathcal{R}}\left[e^{\vartheta Z_1^c W_1}\right]\right] + \frac{n_v}{n}\mathbb{E}\left[\ln \mathbb{E}^{\mathcal{R}}\left[e^{\vartheta Z_1^v W_1}\right]\right] \tag{2.2.11}$$

and $\vartheta_0^n(a, z_\mathrm{f})$ be defined as the solution of

$$a - \frac{z_\mathrm{f}}{n} W_\mathrm{f} = \frac{d}{d\vartheta} g_n(q_n \vartheta). \tag{2.2.12}$$

The (random) function $I_0^n(a, z_\mathrm{f}) \equiv a\vartheta_0^n(a, z_\mathrm{f}) - g_n(q_n \vartheta_0^n(a, z_\mathrm{f}))$ converges to a function $I_0(a)$. Let

$$Z_n(\vartheta) \equiv \frac{1}{\sqrt{n}}\left[\sum_{j=1}^{n_c}\left(\ln M^{\mathcal{R}}_{c,j}(\vartheta) - \mathbb{E}\left[\ln M^{\mathcal{R}}_{c,j}(\vartheta)\right]\right) + \sum_{j=n_c+1}^{n_c+n_v}\left(\ln M^{\mathcal{R}}_{v,j}(\vartheta) - \mathbb{E}\left[\ln M^{\mathcal{R}}_{v,j}(\vartheta)\right]\right)\right]. \tag{2.2.13}$$

With this notation the activation probabilities can be approximated almost surely according to

$$\begin{aligned}&\mathbb{P}^{\mathcal{R}}(G_n(z_{\mathrm{f}}) \geq g_{\mathrm{act}}(n))\\ =&\frac{\exp\left(-nI_0^n(a,z_{\mathrm{f}}) + \sqrt{n}Z_n(q_n\vartheta_0^n(a,z_{\mathrm{f}})) + z_{\mathrm{f}}W_{\mathrm{f}}\vartheta_0^n(a,z_{\mathrm{f}}) + nR_n\right)}{\sigma_n\vartheta_n\sqrt{2\pi n}}(1+\mathrm{o}(1)),\end{aligned} \tag{2.2.14}$$

where $R_n \in \mathcal{O}\left(\frac{1}{n}\right)$. In Section 2.3 we prove the joint weak convergence of the process $Z_n(q_n\vartheta_0^n(a,z_{\mathrm{f}}))$ and its derivatives which establishes that the expression for the probabilities is well-behaved.

As in Case 1.), the interesting situation is when z_{f} becomes large but z_{f}/n is small. A computation similar as in Case 1.) then shows that

$$\frac{\mathbb{P}^{\mathcal{R}}(G_n(z_{\mathrm{f}}) \geq g_{\mathrm{act}}(n))}{\mathbb{P}^{\mathcal{R}}(G_n(0) \geq g_{\mathrm{act}}(n))} \sim \exp\left(\vartheta_0^n(a,0)z_{\mathrm{f}}\left(W_{\mathrm{f}} - a\frac{n}{n_M}\right)\right). \tag{2.2.15}$$

This shows that for a given choice of a, the activation probability increases exponentially with z_{f} only if W_{f} is large enough (depending on the choice of a). That is, only the presence of foreign peptide species which interacts strongly with the particular TCR clonotype will lead to an increased activation frequency. This implies a certain degree of specificity. Although we have seen in Case 1.) that the overall probability of T-cell activation increases with z_{f}, this is not true for any TCR clonotype but just for those clonotypes which are equipped with a large enough value of W_{f}.

Note that the rate function in this case is *random*, that is the activation probabilities fluctuate from clonotype to clonotype by a factor of order $\exp(\sqrt{n}Z)$, where Z is random. This implies that, for the modulation of the activation probabilities due to foreign peptides to exceed these random fluctuations significantly, one should have that $z_{\mathrm{f}} \gg \sqrt{n}$. This appears to limit the sensitivity level for the recognition of foreign peptides.

Case 3.) An upper index $\mathcal{Z}$ denotes the objects conditioned on this σ- algebra. $\widetilde{\vartheta}^n(a,z_{\mathrm{f}})$ is the solution of

$$a = \frac{d}{d\vartheta}\widetilde{g}_n(q_n\vartheta) + \frac{d}{d\vartheta}\frac{1}{n}\ln\mathbb{E}\left[e^{\vartheta z_{\mathrm{f}}W_{\mathrm{f}}}\right], \tag{2.2.16}$$

where $\widetilde{g}_n(\vartheta) \equiv \frac{n_c}{n}\mathbb{E}\left[\ln\mathbb{E}^{\mathcal{Z}}\left[e^{\vartheta Z_1^c W_1}\right]\right] + \frac{n_v}{n}\mathbb{E}\left[\ln\mathbb{E}^{\mathcal{Z}}\left[e^{\vartheta Z_1^v W_1}\right]\right]$. $\widetilde{Z}_n(\vartheta)$ is defined by

$$\widetilde{Z}_n(\vartheta) \equiv \frac{1}{\sqrt{n}}\left[\sum_{j=1}^{n_c}\left(\ln M_{c,j}^{\mathcal{Z}}(\vartheta) - \mathbb{E}[\ln M_{c,j}^{\mathcal{Z}}(\vartheta)]\right) + \sum_{j=n_c+1}^{n_c+n_v}\left(\ln M_{v,j}^{\mathcal{Z}}(\vartheta) - \mathbb{E}[\ln M_{v,j}^{\mathcal{Z}}(\vartheta)]\right)\right] \tag{2.2.17}$$

and $\widetilde{Z}_n(q_n\widetilde{\vartheta}^n(a,z_{\mathrm{f}}))$ converges weakly to a Gaussian process. $\widetilde{I}^n(a,z_{\mathrm{f}}) \equiv a\widetilde{\vartheta}^n(a,z_{\mathrm{f}}) - \widetilde{g}_n(q_n\widetilde{\vartheta}^n(a,z_{\mathrm{f}}))$ converges to a function $\widetilde{I}(a)$.

The notations and the proof of the result are quite similar to Case 2.) but we can recognise some structural differences in the results here. We have already seen these differences between the two conditional scenarios in the analysis of the variances and the expectation values. Here, the probability of activation can be approximated by

$$\begin{aligned}&\mathbb{P}^{\mathcal{Z}}(G_n(z_{\mathrm{f}}) \geq g_{\mathrm{act}}(n))\\ =&\frac{\exp\left(-n\widetilde{I}^n(a,z_{\mathrm{f}}) + \sqrt{n}\widetilde{Z}_n(q_n\widetilde{\vartheta}^n(a,z_{\mathrm{f}})) + \ln\mathbb{E}[e^{\widetilde{\vartheta}^n(a,z_{\mathrm{f}})z_{\mathrm{f}}W_{\mathrm{f}}}] + nR_n\right)}{\sigma_n\vartheta_n\sqrt{2\pi n}}(1+\mathrm{o}(1)),\end{aligned} \tag{2.2.18}$$

where $R_n \in \mathcal{O}\left(\frac{1}{n}\right)$. In the rate function appears again a term which depends on the foreign peptide, namely $\ln \mathbb{E}[\exp\left(\widetilde{\vartheta}^n(a, z_\mathrm{f})z_\mathrm{f}W_\mathrm{f}\right)]$. But in contrast to Case 2.) this term is deterministic and the only randomness in the rate function lies in the fluctuation term which arises from conditioning. Thus, we are again dealing with a random rate function but this function does not vary from event to event by a term which is scaled with z_f.

The interesting situation is again when z_f becomes large, but remains small compared to n. As in Case 1.) there will be a constant L such that $\frac{d}{d\vartheta} \ln M(z_\mathrm{f}\vartheta) \sim Lz_\mathrm{f}$ for ϑz_f large enough. A simple computation then shows that

$$\frac{\mathbb{P}^{\mathcal{Z}}(G_n(z_\mathrm{f}) \geq g_{\mathrm{act}}(n))}{\mathbb{P}^{\mathcal{Z}}(G_n(0) \geq g_{\mathrm{act}}(n))} \sim \exp\left(\widetilde{\vartheta}^n(a,0)z_\mathrm{f}\left(L - a\frac{n}{n_M}\right)\right), \tag{2.2.19}$$

where $\widetilde{\vartheta}^n(a,0)$ is the solution of Equation (2.2.16) for $z_\mathrm{f} = 0$. As in Case 2.), there appears a fluctuation term $\sqrt{n}Z$. Therefore, we need again $z_\mathrm{f} \gg \sqrt{n}$ such that the impact of this fluctuation term is not too big. This order of z_f ensures again that we may use the approximation $\frac{d}{d\vartheta} \ln M(z_\mathrm{f}\vartheta) \sim Lz_\mathrm{f}$.

Remark 2.2.6. During an infection the body is flooded with the invader. Therefore, a significant ratio of the peptides presented on the APC belongs to the foreign invader and it is reasonable to consider the regime $z_\mathrm{f} \gg \sqrt{n}$. A clear indication that a sufficiently high presentation level of the targeted peptides is needed, has been established by [91] in the context of T-cell therapy of melanomas. Our work is based on the assumption $z_\mathrm{f} \ll n$ although parts of the results in Case 2.) are also valid for $z_\mathrm{f} \sim n$. If this is the regime of interest we suggest to use a convolution of the distribution of the foreign stimulation rate and the distribution of the part of G_n belonging to the self background since then the influence of the summand $z_\mathrm{f}W_\mathrm{f}$ is very large and this summand cannot be treated as the other summands. That is, one should consider

$$\begin{aligned}\mathbb{P}(G_n(z_\mathrm{f}) \geq an) &= \mathbb{P}(q_nG_n(0) \geq an - z_\mathrm{f}W_\mathrm{f}) \\ &= \int \mathbb{P}(n^{-1}G_n(0) \geq q_n^{-1}(a - n^{-1}z_\mathrm{f}W_\mathrm{f})|W_\mathrm{f})dP_{W_\mathrm{f}}\end{aligned} \tag{2.2.20}$$

and approximate the probability in the integral suitably, depending on the value of $q_n^{-1}(a - n^{-1}z_\mathrm{f}W_\mathrm{f})$.

2.3 Precise formulation of the results and proofs

To state the central large deviation result proven by [24] we introduce some notation. Let $\{S_n\}_{n\in\mathbb{N}}$ denote a sequence of real-valued random variables with moment generating functions $\Phi_n(\vartheta) \equiv \mathbb{E}[\exp(\vartheta S_n)], \vartheta \in \mathbb{R}$ and let Ψ_n be defined by $\Psi_n(\vartheta) \equiv \frac{1}{n}\ln\Phi_n(\vartheta)$.

Assumption 2.3.1. There exist $\vartheta^* \in (0,\infty)$ and $\beta < \infty$ such that

$$|\Psi_n(\vartheta)| < \beta, \text{for all } \vartheta \in B_{\vartheta_*} \equiv \{\vartheta \in \mathbb{C} : |\vartheta| < \vartheta_*\} \text{ and } n \in \mathbb{N}.$$

Notation. Let $(a_n)_{n\in\mathbb{N}}$ be a bounded real-valued sequence such that the equation

$$a_n = \Psi_n'(\vartheta) \tag{2.3.1}$$

has a solution $\vartheta_n \in (0, \vartheta_{**})$ with $\vartheta_{**} \in (0, \vartheta_*)$ for all $n \in \mathbb{N}$. $\sigma_n^2 \equiv \Psi_n''(\vartheta_n)$ is the variance of the tilted version of $n^{-1}S_n$ and $I_n(a_n) \equiv a_n\vartheta_n - \Psi_n(\vartheta_n)$ is the Fenchel-Legendre transform of Ψ_n. We will abusively refer to this as the *rate function*.

Theorem 2.3.2 ([24]). *If in the above setting*

(i) $\lim_{n\to\infty} \vartheta_n \sqrt{n} = \infty$

(ii) $\inf_{n\in\mathbb{N}} \sigma_n^2 > 0$ *and*

(iii) $\lim_{n\to\infty} \sqrt{n} \sup_{\delta_1 \le |t| \le \delta_2 \vartheta_n} \left| \frac{\Phi_n(\vartheta_n + it)}{\Phi_n(\vartheta_n)} \right| = 0 \quad \forall 0 < \delta_1 < \delta_2 < \infty,$

then

$$\mathbb{P}\left(S_n \ge n a_n\right) = \frac{e^{-nI_n(a_n)}}{\vartheta_n \sigma_n \sqrt{2\pi n}} \left(1 + o(1)\right), n \to \infty. \tag{2.3.2}$$

We give here the precise conditions that we impose on the distributions of the involved random variables to ensure the applicability of Theorem 3.1.3 with $S_n = G_n(z_\mathrm{f})$ in the three different cases.

Case 1.) This case has been considered in [138]. We state their result under slightly more general assumptions. Below the quantities Φ_n, Ψ_n and ϑ_n are defined as above with $S_n = G_n(z_\mathrm{f})$.

Theorem 2.3.3. *Let $(a_n)_{n\in\mathbb{N}}$ be defined by $a_n \equiv a$ and $g_{act}(n) = an$ such that $g_{act}(n) > \mathbb{E}[G_n(z_\mathrm{f})]$ and $a < \sup_{\vartheta\in\mathbb{R}} \frac{d}{d\vartheta}\Psi_n(\vartheta)$ for all $n \in \mathbb{N}$. Then Theorem 3.1.3 is applicable provided $z_\mathrm{f}/n \downarrow 0$, the distribution functions of $Z_1^c W_1$, $Z_1^v W_1$ and W_1 are neither lattice-valued nor concentrated on one point, and the corresponding moment generating functions $M_c(\vartheta), M_v(\vartheta)$ and $M(\vartheta)$ are finite for each $\vartheta \in \mathbb{R}$. The rate function is*

$$I_n(a, z_\mathrm{f}) = a\vartheta_n(a, z_\mathrm{f}) - \frac{n_c}{n} \ln M_c(q_n \vartheta_n(a, z_\mathrm{f})) - \frac{n_v}{n} \ln M_v(q_n \vartheta_n(a, z_\mathrm{f})) - \frac{1}{n} \ln M(z_\mathrm{f} \vartheta_n(a, z_\mathrm{f})). \tag{2.3.3}$$

Proof. The moment generating function of the random variable G_n is given by

$$\Phi_n(\vartheta) = M_c(q_n\vartheta)^{n_c} M_v(q_n\vartheta)^{n_v} M(z_\mathrm{f}\vartheta). \tag{2.3.4}$$

It reduces to $\Phi_n(\vartheta) = M_c(\vartheta)^{n_c} M_v(\vartheta)^{n_v}$ if $z_\mathrm{f} = 0$. Assumption 3.1.1 is satisfied because the following holds: For each $x \in \mathbb{R}_+$ and all $\vartheta < x$

$$\begin{aligned} \Psi_n(\vartheta) &\le \frac{n_c}{n} \ln M_c(q_n x) + \frac{n_v}{n} \ln M_v(q_n x) + \frac{1}{n} \ln M(z_\mathrm{f} x) \\ &\le \ln M_c(x) + \ln M_v(x) + \ln M(z_\mathrm{f} x) \equiv \beta(x) \end{aligned} \tag{2.3.5}$$

because $\Psi_n(\vartheta)$ is strictly increasing and $q_n, n_c/n$ and n_v/n are smaller than 1. $\vartheta_n(a, z_\mathrm{f})$ is defined as the (unique) solution of

$$a = \frac{n_c}{n} \left[\frac{d}{d\vartheta} \ln M_c(q_n\vartheta)\right] + \frac{n_v}{n} \left[\frac{d}{d\vartheta} \ln M_v(q_n\vartheta)\right] + \frac{1}{n} \left[\frac{d}{d\vartheta} \ln M(z_\mathrm{f}\vartheta)\right]. \tag{2.3.6}$$

This equation results from Equation (3.1.7) and the choice $a_n \equiv a$. The solution exists since the function $\frac{d}{d\vartheta}\Psi_n(\vartheta)$ runs from $\frac{1}{n}\mathbb{E}[G_n(z_\mathrm{f})] = \frac{d}{d\vartheta}\Psi_n(\vartheta)|_{\vartheta=0}$ to $\sup_{\vartheta\in\mathbb{R}} \frac{d}{d\vartheta}\Psi_n(\vartheta)$ and a lies in between these values. It is unique because $\frac{d}{d\vartheta}\Psi_n(\vartheta)$ is strictly increasing. Because $\frac{n_c}{n} \to C_1$, $\frac{n_v}{n} \to C_2$, $\frac{z_\mathrm{f}}{n} \to 0$ and $q_n \to 1$, Equation (2.3.6) converges. The limit equation is $a = C_1 \frac{d}{d\vartheta} \ln M_c(\vartheta) + C_2 \frac{d}{d\vartheta} \ln M_v(\vartheta)$. Thus, there exists $C \in (0, \infty)$ such that $\lim_{n\to\infty} \vartheta_n = C$. C is strictly positive

because $a > \frac{d}{d\vartheta}\Psi_n(\vartheta)|_{\vartheta=0}$. Consequently, Condition (i) of Theorem 3.1.3 is satisfied.
We define

$$\sigma_n^2 = \left(\frac{n_c}{n}\frac{d^2}{d\vartheta^2}\ln M_c(q_n\vartheta) + \frac{n_v}{n}\frac{d^2}{d\vartheta^2}\ln M_v(q_n\vartheta) + \frac{1}{n}\frac{d^2}{d\vartheta^2}\ln M(z_{\mathrm{f}}\vartheta)\right)\Bigg|_{\vartheta=\vartheta_n(a,z_{\mathrm{f}})}. \tag{2.3.7}$$

This equation converges as the previous one and the second derivatives of $\ln M_\gamma(q_n\vartheta)$, $\gamma \in \{c, v\}$ are positive due to the strict convexity of these functions. Thus, Condition (ii) of Theorem 3.1.3 is satisfied, too.
We define

$$\nu_\gamma^n(t) = \frac{M_\gamma(q_n(\vartheta_n(a,z_{\mathrm{f}}) + it))}{M_\gamma(q_n\vartheta_n(a,z_{\mathrm{f}}))}, \gamma \in \{c, v\} \quad \text{and} \quad \nu^n(t) = \frac{M(z_{\mathrm{f}}(\vartheta_n(a,z_{\mathrm{f}}) + it))}{M(z_{\mathrm{f}}\vartheta_n(a,z_{\mathrm{f}}))}. \tag{2.3.8}$$

These are the characteristic functions of the tilted random variables. The distribution functions corresponding to these characteristic functions are also neither lattice-valued nor concentrated on one point. Because $\vartheta_n(a, z_{\mathrm{f}}) \to C$ and $q_n \to 1$, there exist $\epsilon > 0$ and $n_0 < \infty$ for each $t \neq 0$ such that for all $n \geq n_0$

$$|\nu_\gamma^n(t)| \leq 1 - \epsilon, \gamma \in \{c, v\} \text{ and } |\nu^n(t)| \leq 1 - \epsilon.$$

We obtain

$$\begin{aligned}\left|\frac{\Phi_n(\vartheta_n(a,z_{\mathrm{f}}) + it)}{\Phi_n(\vartheta_n(a,z_{\mathrm{f}}))}\right| &= \left|\frac{M_c(q_n(\vartheta_n(a,z_{\mathrm{f}}) + it))^{n_c} M_v(q_n(\vartheta_n(a,z_{\mathrm{f}}) + it))^{n_v} M(z_{\mathrm{f}}(\vartheta_n(a,z_{\mathrm{f}}) + it))}{M_c(q_n\vartheta_n(a,z_{\mathrm{f}}))^{n_c} M_v(q_n\vartheta_n(a,z_{\mathrm{f}}))^{n_v} M(z_{\mathrm{f}}\vartheta_n(a,z_{\mathrm{f}}))}\right| \\ &= |(\nu_c^n(t))^{n_c}(\nu_v^n(t))^{n_v}\nu^n(t)| \\ &\leq (1-\epsilon)^n = \mathrm{o}\left(\frac{1}{\sqrt{n}}\right), \quad n \to \infty. \end{aligned} \tag{2.3.9}$$

It remains to consider the supremum over the values of t in Condition (iii). Since $\vartheta_n(a, z_{\mathrm{f}})$ converges, the supremum is taken over a compact set. Thus, this function attains a maximum on this interval and this can be bounded according to Equation (2.3.9). Therefore, Condition (iii) of Theorem 3.1.3 is satisfied and Theorem 3.1.3 is applicable. □

Remark 2.3.4. The case $z_{\mathrm{f}} \sim n$ requires a special treatment. This is, however, best relegated to the following Case 2.).

Case 2.) We denote by $\mathbb{E}_{\mathcal{P}^{N_1N_2}}[\cdot]$ the expectation w.r.t. the measure $\mathcal{P}^{N_1N_2}$ which is the joint distribution of all the dissociation rates. We would like to show that the conditions of Theorem 3.1.3 are almost surely satisfied. As already mentioned it is important that $\ln(M_{\gamma,j}^{\mathcal{R}}(\vartheta)) \in L^1(\mathcal{P}^{N_1N_2})$, where $\gamma \in \{c, v\}$. Under the assumption $M_\gamma(\vartheta) < \infty$ for each $\vartheta \in (0, \vartheta_{**})$ we have that $\mathbb{E}^{\mathcal{R}}[\exp(\vartheta Z_1^\gamma W_1)] \in L^1(\mathcal{P}^{N_1N_2})$. Combined with

$$0 \leq \ln(\mathbb{E}^{\mathcal{R}}[\exp(\vartheta Z_1^\gamma W_1)]) < \mathbb{E}^{\mathcal{R}}[\exp(\vartheta Z_1^\gamma W_1)], \text{ for } \vartheta \geq 0, \tag{2.3.10}$$

this yields

$$\ln(M_{\gamma,j}^{\mathcal{R}}(\vartheta)) = \ln(\mathbb{E}^{\mathcal{R}}[\exp(\vartheta Z_1^\gamma W_1)]) \in L^1(\mathcal{P}^{N_1N_2}), \tag{2.3.11}$$

where $\gamma \in \{c, v\}$. Below we denote by $\Psi_n^{\mathcal{R}}$, $\vartheta_n^{\mathcal{R}}(a, z_{\mathrm{f}}) \equiv \vartheta_n(a, z_{\mathrm{f}})$ the analogues of the quantities Ψ_n, $\vartheta_n(a, z_{\mathrm{f}})$ under the conditional expectations $\mathbb{E}^{\mathcal{R}}$. For notational simplicity we drop that superscript on $\vartheta_n(a, z_{\mathrm{f}})$, but it is important to keep in mind that this is now a random variable, too.

Theorem 2.3.5. *Let $(a_n)_{n\in\mathbb{N}}$ be defined by $a_n \equiv a$ and $g_{act}(n) = an$ such that $g_{act}(n) > \mathbb{E}^{\mathcal{R}}[G_n(z_\mathrm{f})]$ and $a < \sup_{\vartheta\in\mathbb{R}} \frac{d}{d\vartheta}\Psi_n^{\mathcal{R}}(\vartheta)$ for all $n \in \mathbb{N}$. Then Theorem 3.1.3 is almost surely applicable if the density of W_j is continuous and the moment generating functions $M_{c,j}^{\mathcal{R}}(\vartheta)$, $M_{v,j}^{\mathcal{R}}(\vartheta)$ and $M^{\mathcal{R}}(\vartheta)$ as well as $M_c(\vartheta)$, $M_v(\vartheta)$ and $M(\vartheta)$ are finite for each $\vartheta \in \mathbb{R}$. Then the rate function is*

$$I_n^{\mathcal{R}}(a, z_\mathrm{f}) = \left(a - \frac{z_\mathrm{f}}{n}W_\mathrm{f}\right)\vartheta_n(a, z_\mathrm{f}) - \frac{1}{n}\left(\sum_{j=1}^{n_c} \ln M_{c,j}^{\mathcal{R}}(q_n\vartheta_n(a, z_\mathrm{f})) + \sum_{j=n_c+1}^{n_c+n_v} \ln M_{v,j}^{\mathcal{R}}(q_n\vartheta_n(a, z_\mathrm{f}))\right). \tag{2.3.12}$$

Proof. Due to the monotonicity of the logarithmic moment generating function for each realisation of the stimulation rates and the boundedness of all involved random variables we can find again $\beta(x)$ such that $\Psi_n^{\mathcal{R}}(\vartheta) < \beta(x)$ for all $\vartheta < x$. Thus, Assumption 3.1.1 is satisfied. Recall that $\vartheta_n(a, z_\mathrm{f})$ is defined as the solution of the equations

$$a = \frac{1}{n}\left(\sum_{j=1}^{n_c} \frac{d}{d\vartheta} \ln M_c^{\mathcal{R}}(q_n\vartheta) + \sum_{j=n_c+1}^{n_c+n_v} \frac{d}{d\vartheta} \ln M_v^{\mathcal{R}}(q_n\vartheta)\right) + \frac{z_\mathrm{f}}{n}W_\mathrm{f}. \tag{2.3.13}$$

The solution $\vartheta_n(a, z_\mathrm{f})$ exists due to the choice of a and is unique due to the strict convexity of $\Psi_n^{\mathcal{R}}$.

For $\vartheta \geq 0$ we have, by the law of large numbers,

$$\begin{aligned}&\lim_{n\to\infty} \frac{1}{n}\left(\sum_{j=1}^{n_c} \ln M_{c,j}^{\mathcal{R}}(q_n\vartheta) + \sum_{j=n_c+1}^{n_c+n_v} \ln M_{v,j}^{\mathcal{R}}(q_n\vartheta)\right)\\ =&C_1\mathbb{E}_{\mathcal{P}^{N_1N_2}}\left[\ln M_{c,1}^{\mathcal{R}}(\vartheta)\right] + C_2\mathbb{E}_{\mathcal{P}^{N_1N_2}}\left[\ln M_{v,1}^{\mathcal{R}}(\vartheta)\right], \quad \mathcal{P}^{N_1N_2} - a.s.\end{aligned} \tag{2.3.14}$$

Since the derivatives of the summands satisfy the bounds

$$\begin{aligned}0 \leq \frac{d}{d\vartheta} \ln \mathbb{E}^{\mathcal{R}}[\exp(\vartheta q_n Z_1^\gamma W_1)] &= \frac{q_n W_1 \mathbb{E}^{\mathcal{R}}[Z_1^\gamma \exp(\vartheta q_n Z_1^\gamma W_1)]}{\mathbb{E}^{\mathcal{R}}[\exp(\vartheta q_n Z_1^\gamma W_1)]}\\ &\leq \frac{q_n W_1 Z_1^{\gamma,\max}\mathbb{E}^{\mathcal{R}}[\exp(\vartheta q_n Z_1^\gamma W_1)]}{\mathbb{E}^{\mathcal{R}}[\exp(\vartheta q_n Z_1^\gamma W_1)]} = q_n W_1 Z_1^{\gamma,\max},\end{aligned} \tag{2.3.15}$$

where $Z_1^{\gamma,\max}$ denotes the maximal value of Z_1^γ, $\gamma \in \{c, v\}$. Therefore they are integrable and hence the limit of the derivatives on the left-hand side of Equation (2.3.14) exists and is equal to the derivative of the right-hand side. If either $\lim_{n\to\infty} z_\mathrm{f}/n = 0$, or $\lim_{n\to\infty} z_\mathrm{f}/n = C > 0$, the equations determining $\vartheta_n(a, z_\mathrm{f})$ converge almost surely and therefore so does the solution $\vartheta_n(a, z_\mathrm{f})$. Thus, Condition (i) of Theorem 3.1.3 is again satisfied.

We have $(\frac{d^2}{d\vartheta^2}\Psi_n^{\mathcal{R}}(\vartheta))|_{\vartheta=\vartheta_n(a,z_\mathrm{f})} > 0$ for each n due to the strict convexity of $\Psi_n^{\mathcal{R}}$. So, it remains to check whether this holds true in the limit $n \to \infty$. $\lim_{n\to\infty} \frac{d^2}{d\vartheta^2}\Psi_n^{\mathcal{R}}(\vartheta)$ exists because the summands of the derivative are again bounded and therefore integrable, since

$$\begin{aligned}0 \leq \frac{d^2}{d\vartheta^2} \ln \mathbb{E}^{\mathcal{R}}[\exp(\vartheta Z_1^\gamma W_1)] &\leq \frac{q_n^2 W_1^2 \mathbb{E}^{\mathcal{R}}[(Z_1^\gamma)^2 e^{\vartheta q_n Z_1^\gamma W_1}]\mathbb{E}^{\mathcal{R}}[e^{\vartheta q_n Z_1^\gamma W_1}] - \mathbb{E}^{\mathcal{R}}[q_n W_1 Z_1^\gamma e^{\vartheta q_n Z_1^\gamma W_1}]^2}{\mathbb{E}^{\mathcal{R}}[e^{\vartheta q_n Z_1^\gamma W_1}]^2}\\ \leq \frac{q_n^2 W_1^2 (Z_1^{\gamma,\max})^2 \mathbb{E}^{\mathcal{R}}[e^{\vartheta q_n Z_1^\gamma W_1}]^2}{\mathbb{E}^{\mathcal{R}}[e^{\vartheta q_n Z_1^\gamma W_1}]^2} &= q_n^2 W_1^2 (Z_1^{\gamma,\max})^2.\end{aligned} \tag{2.3.16}$$

Thus, it is again allowed to interchange limit and derivative. Moreover, we have that

$$\frac{d^2}{d\vartheta^2}\left(C_1\mathbb{E}_{\mathcal{P}^{N_1N_2}}[\ln M_{c,1}^{\mathcal{R}}(\vartheta)]\right) = C_1\mathbb{E}_{\mathcal{P}^{N_1N_2}}\left[\frac{d^2}{d\vartheta^2}\ln M_{c,1}^{\mathcal{R}}(\vartheta)\right] > 0 \tag{2.3.17}$$

because $\ln M_{c,1}^{\mathcal{R}}(\vartheta)$ is strictly convex. Thus, this summand is positive and, analogously, so is the second one. Therefore, Condition (ii) of Theorem 3.1.3 is satisfied.

Next we check Condition (iii) on the characteristic function. We have to take into account that Z_j^c and Z_j^v should be lattice-valued random variables because they represent numbers of peptides. The characteristic function is given by

$$\left|\frac{\Phi_n^{\mathcal{R}}(\vartheta+it)}{\Phi_n^{\mathcal{R}}(\vartheta)}\right| = \left|\frac{\prod_{j=1}^{n_c} M_{c,j}^{\mathcal{R}}(q_n(\vartheta+it))\prod_{j=n_c+1}^{n_c+n_v} M_{v,j}^{\mathcal{R}}(q_n(\vartheta+it))M^{\mathcal{R}}(z_{\mathrm{f}}(\vartheta+it))}{\prod_{j=1}^{n_c} M_{c,j}^{\mathcal{R}}(q_n\vartheta)\prod_{j=n_c+1}^{n_c+n_v} M_{v,j}^{\mathcal{R}}(q_n\vartheta)M^{\mathcal{R}}(z_{\mathrm{f}}\vartheta)}\right|. \tag{2.3.18}$$

We can rewrite (2.3.18) as

$$\begin{aligned}
&\left(\exp\left(\tfrac{1}{n_c}\textstyle\sum_{j=1}^{n_c}\ln\left|\frac{M_{c,j}^{\mathcal{R}}(q_n(\vartheta+it))}{M_{c,j}^{\mathcal{R}}(q_n\vartheta)}\right|\right)\right)^{n_c}\left(\exp\left(\tfrac{1}{n_v}\textstyle\sum_{j=1}^{n_v}\ln\left|\frac{M_{v,j}^{\mathcal{R}}(q_n(\vartheta+it))}{M_{v,j}^{\mathcal{R}}(q_n\vartheta)}\right|\right)\right)^{n_v}\left|\frac{M^{\mathcal{R}}(z_{\mathrm{f}}(\vartheta+it))}{M^{\mathcal{R}}(z_{\mathrm{f}}\vartheta)}\right| \\
&= \exp\Bigg(n_c\left(\mathbb{E}_{\mathcal{P}^{N_1N_2}}\left[\ln\left|\frac{M_{c,1}^{\mathcal{R}}(q_n(\vartheta+it))}{M_{c,1}^{\mathcal{R}}(q_n\vartheta)}\right|\right]+\mathrm{o}(1)\right) \\
&\quad + n_v\left(\mathbb{E}_{\mathcal{P}^{N_1N_2}}\left[\ln\left|\frac{M_{v,1}^{\mathcal{R}}(q_n(\vartheta+it))}{M_{v,1}^{\mathcal{R}}(q_n\vartheta)}\right|\right]+\mathrm{o}(1)\right)+\ln\left|\frac{M^{\mathcal{R}}(z_{\mathrm{f}}(\vartheta+it))}{M^{\mathcal{R}}(z_{\mathrm{f}}\vartheta)}\right|\Bigg).
\end{aligned} \tag{2.3.19}$$

This expression can be bounded from above by

$$\begin{aligned}
&\exp\Bigg(n_c\left(\ln(1-\epsilon)\mathcal{P}^{N_1N_2}\left(\left|\frac{M_{c,1}^{\mathcal{R}}(q_n(\vartheta+it))}{M_{c,1}^{\mathcal{R}}(q_n\vartheta)}\right|\le 1-\epsilon\right)+\tilde{\epsilon}\right) \\
&\qquad + n_v\left(\ln(1-\epsilon)\mathcal{P}^{N_1N_2}\left(\left|\frac{M_{v,1}^{\mathcal{R}}(q_n(\vartheta+it))}{M_{v,1}^{\mathcal{R}}(q_n\vartheta)}\right|\le 1-\epsilon\right)+\tilde{\epsilon}\right)\Bigg).
\end{aligned} \tag{2.3.20}$$

For given $\epsilon > 0$, the probabilities

$$\mathcal{P}^{N_1N_2}\left(\left|\frac{M_{c,1}^{\mathcal{R}}(q_n(\vartheta+it))}{M_{c,1}^{\mathcal{R}}(q_n\vartheta)}\right|\le 1-\epsilon\right) \quad \text{and} \quad \mathcal{P}^{N_1N_2}\left(\left|\frac{M_{v,1}^{\mathcal{R}}(q_n(\vartheta+it))}{M_{v,1}^{\mathcal{R}}(q_n\vartheta)}\right|\le 1-\epsilon\right) \tag{2.3.21}$$

are strictly positive, uniformly in n for n large, due to the assumptions on the distribution of the stimulation rates. Therefore, there exists $\delta > 0$, such that for all n large enough, (2.3.20) is bounded from above by

$$\exp((n-1)(\tilde{\epsilon}-\delta)). \tag{2.3.22}$$

Since $\tilde{\epsilon}$ can be made arbitrarily small if n is large enough, $\delta - \tilde{\epsilon} > 0$ for such n, and so this expression tends to zero with n exponentially fast. We use the continuous density to control the supremum which appears in Condition (iii) of Theorem 3.1.3. It is again a crucial point that $\vartheta_n(a, z_{\mathrm{f}})$ converges such that the supremum is taken over a compact set and Condition (iii) is satisfied. □

Remark 2.3.6. Note that it suffices in order to check these conditions to assume $C_1 + C_2 > 0$. It is not necessary that both constants are strictly positive. It is also not necessary that the density of W_j is continuous but we did not want to state a technical condition.

Investigation of the rate function. We are concerned with the behaviour of the large deviation rate function and prove a functional central limit theorem with which we can characterise this. $g_n(q_n\vartheta)$ defined by Equation (2.2.11) converges to

$$C_1\mathbb{E}\left[\ln\mathbb{E}^{\mathcal{R}}\left[e^{\vartheta Z_1^c W_1}\right]\right]+C_2\mathbb{E}\left[\ln\mathbb{E}^{\mathcal{R}}\left[e^{\vartheta Z_1^v W_1}\right]\right]\equiv g(\vartheta). \tag{2.3.23}$$

In the rate function appears the process $Z_n(q_n\vartheta_0^n(a,z_\mathrm{f}))$ which is defined by Equation (2.2.13). The following theorem states our result. We use the short-hand notation $M_{c,1}^{\mathcal{R},a}\equiv\ln M_{c,1}^{\mathcal{R}}(\vartheta_0(a))$, where $\vartheta_0(a)$ denotes the limit of $\vartheta_0^n(a,z_\mathrm{f})$, the solution of Equation (2.2.12).

Theorem 2.3.7. *If there exists a constant C such that $g_n''(q_n\vartheta_0^n(a,z_\mathrm{f}))>C>0$, the rate function is given by*

$$I_n^{\mathcal{R}}(a,z_\mathrm{f})=I_0^n(a,z_\mathrm{f})-\frac{1}{\sqrt{n}}Z_n(q_n\vartheta_0^n(a,z_\mathrm{f}))-\frac{z_\mathrm{f}}{n}\vartheta_0^n(a,z_\mathrm{f})W_\mathrm{f}+R_n, \tag{2.3.24}$$

where $Z_n(q_n\vartheta_0^n(a,z_\mathrm{f}))$ converges weakly to the Gaussian process $Z_a+\overline{Z}_a$ and $R_n\in\mathcal{O}\left(\frac{1}{n}\right)$. Z_a and $\overline{Z}_a$ are both Gaussian processes with expectation functions $\mathbb{E}[Z_a]=0=\mathbb{E}[\overline{Z}_a]$ and covariance functions

$$\mathrm{Cov}(Z_a,Z_{a'})=C_1\left(\mathbb{E}\left[M_{c,1}^{\mathcal{R},a}M_{c,1}^{\mathcal{R},a'}\right]-\mathbb{E}\left[M_{c,1}^{\mathcal{R},a}\right]\mathbb{E}\left[M_{c,1}^{\mathcal{R},a'}\right]\right) \tag{2.3.25}$$

and

$$\mathrm{Cov}(\overline{Z}_a,\overline{Z}_{a'})=C_2\left(\mathbb{E}\left[M_{v,1}^{\mathcal{R},a}M_{v,1}^{\mathcal{R},a'}\right]-\mathbb{E}\left[M_{v,1}^{\mathcal{R},a}\right]\mathbb{E}\left[M_{v,1}^{\mathcal{R},a'}\right]\right). \tag{2.3.26}$$

Remark 2.3.8. The remainder term is given by

$$R_n=\frac{(Z_n'(q_n\vartheta_0^n(a,z_\mathrm{f})))^2}{2n(g_n''(q_n\vartheta_0^n(a,z_\mathrm{f}))+\frac{1}{\sqrt{n}}Z_n''(q_n\vartheta_0^n(a,z_\mathrm{f})))}+\mathrm{o}\left(\frac{1}{n}\right), \tag{2.3.27}$$

where the appearing process scaled with n converges weakly. Since we consider the regime $z_\mathrm{f}\gg\sqrt{n}$ the term $\frac{z_\mathrm{f}}{n}\vartheta_0^n(a,z_\mathrm{f})W_\mathrm{f}$ is of a higher order than the remainder.

As we already mentioned in Section 2.2 we need this approximation of the rate function on the level of the central limit theorem due to the scaling with the factor n in the expression for the probabilities. In order to prove this result we show weak convergence of the involved random processes and derive then an expression for the rate function. To establish the weak convergence of $Z_n(q_n\vartheta_0^n(a,z_\mathrm{f}))$, $Z_n'(q_n\vartheta_0^n(a,z_\mathrm{f}))$ and $Z_n''(q_n\vartheta_0^n(a,z_\mathrm{f}))$ as well as their joint weak convergence as processes on the Wiener Space with parameter a we show convergence of their finite dimensional distributions and tightness. To prove tightness we use the Kolmogorov-Chentsov criterion from [83]. Adapted to our notation we have to check the conditions

1. $Z_n(q_n\vartheta_0^n(a,z_\mathrm{f}))$ converges in finite dimensional distribution.
2. The family of initial distributions, $Z_n(q_n(\vartheta_0^n(\epsilon,z_\mathrm{f})))$, is tight.
3. There exists $C>0$ independent of a and n such that

$$\mathbb{E}\left[\left(Z_n(q_n\vartheta_0^n(a+h,z_\mathrm{f}))-Z_n(q_n\vartheta_0^n(a,z_\mathrm{f}))\right)^2\right]\leq C|h|^2. \tag{2.3.28}$$

Note that Condition (3) is fulfilled if

$$\mathbb{E}\left[\left(\frac{d}{da}Z_n(q_n\vartheta_0^n(a,z_{\mathrm{f}}))\right)^2\right]\leq C. \tag{2.3.29}$$

The same criteria with $Z_n(q_n\vartheta_0^n(a,z_{\mathrm{f}}))$ suitably replaced by the process under consideration can be used to prove the convergence of these processes.

We can handle the constitutive and the variable part separately. It suffices to check the conditions for the constitutive part because the sum in the variable part is built analogously. The following results are taken from [79]. We need this central limit theorem for triangular arrays to check Condition (1).

Definition 2.3.9. A *row-wise independent d-dimensional triangular array scheme* is a sequence (K^n) of elements of $\overline{\mathbb{N}}^*=\mathbb{N}\setminus\{0\}\cup\infty$ and a sequence of probability spaces $(\Omega^n,\mathcal{F}^n,P^n)$ each of one being equipped with an independent sequence $(\chi_k^n)_{1\leq k\leq K^n}$ of $\mathbb{R}^d$-valued random variables.

We restrict the scenario to row-wise independent schemes which satisfy

$$\sum_{1\leq k\leq K^n}\left|\mathbb{E}\left[h\left(\chi_k^n\right)\right]\right|<\infty \text{ and } \sum_{1\leq k\leq K^n}\mathbb{E}\left[|\chi_k^n|^2\wedge 1\right]<\infty \tag{2.3.30}$$

for each n, where h is a given truncation function. This condition does not depend on $h\in\mathcal{C}_t^d\equiv\{h:\mathbb{R}^d\to\mathbb{R}^d \text{ bounded, compact support, } h(x)=x \text{ in a neighbourhood of } 0\}$.

Definition 2.3.10. A row-wise independent array (χ_k^n) satisfies the *Lindeberg condition* if for all $\epsilon>0$ we have

$$\lim_{n\to\infty}\sum_{1\leq k\leq K^n}\mathbb{E}\left[|\chi_k^n|^2\mathbb{1}_{\{|\chi_k^n|>\epsilon\}}\right]=0. \tag{2.3.31}$$

Of course, this implies $\sum_k\mathbb{E}[|\chi_k^n|^2]<\infty$, provided Condition (2.3.30) is satisfied.

Theorem 2.3.11. *We suppose that the d-dimensional row-wise independent array satisfies Condition* (2.3.30) *and the Lindeberg condition, and let* $\xi^n=\sum_{1\leq k\leq K^n}\chi_k^n$. *Then*
a) If $\mathcal{L}(\xi^n)\to\mu$, *then* μ *is a Gaussian measure on* $\mathbb{R}^d$;
b) in order that $\mathcal{L}(\xi^n)\to\mathcal{N}(b,c)$, *the Gaussian measure with mean b and covariance matrix c, it is necessary and sufficient that the following two conditions hold:*
$[\beta]$ $\sum_{1\leq k\leq K^n}\mathbb{E}\left[\chi_k^n\right]\to b$
$[\gamma]$ $\sum_{1\leq k\leq K^n}\mathbb{E}\left[\chi_k^{n,j}\chi_k^{n,l}\right]\to c^{jl}$,
where $\chi_k^{n,l}$ *denotes the l-th component of* χ_k^n.

Using this theorem we can prove the following lemmata which we need to prove Theorem 2.3.7.

Lemma 2.3.12. $Z_n(q_n\vartheta_0^n(a,z_{\mathrm{f}}))$ *as a process on the Wiener Space with parameter a converges weakly to a Gaussian process if there exists a constant C such that* $g_n''(q_n\vartheta_0^n(a,z_{\mathrm{f}}))>C>0$.

In order to simplify the notation we define

$$Y_{a,j}^n\equiv\ln\mathbb{E}^{\mathcal{R}}\left[e^{q_n\vartheta_0^n(a,z_{\mathrm{f}})Z_j^cW_j}\right]-\mathbb{E}\left[\ln\mathbb{E}^{\mathcal{R}}\left[e^{q_n\vartheta_0^n(a,z_{\mathrm{f}})Z_j^cW_j}\right]\right]. \tag{2.3.32}$$

The constitutive part of the process $Z_n(q_n\vartheta_0^n(a,z_\mathrm{f}))$ is given by $Z_{n,c}(q_n\vartheta_0^n(a,z_\mathrm{f})) \equiv \frac{1}{\sqrt{n}}\sum_{j=1}^{n_c} Y_{a,j}^n$. To prove this lemma we have to check Conditions 1., 2., and 3. First we investigate the finite dimensional distributions of $Z_{n,c}$ in the following Lemma 2.3.13. Therefore, let $0 < a_1 < \cdots < a_m < \infty$, $a_i \in \mathbb{R}, m \in \mathbb{N}$. We are interested in the limiting behaviour of $\xi^n \equiv \sum_{1\le j\le K^n} \chi_j^n$ with $\chi_j^n \equiv \frac{1}{\sqrt{n}}(Y_{a_1,j}^n, \ldots, Y_{a_m,j}^n)$ and $j \in \{1,\ldots,n_c\}$.

Lemma 2.3.13. *Under the assumptions of Lemma 2.3.12, $\xi^n \equiv \sum_{j=1}^{n_c}\chi_j^n$ converges weakly to a Gaussian vector with expectation* 0 *and covariance matrix defined by*

$$C^{jl} = C_1\left(\mathbb{E}\left[M_{c,1}^{\mathcal{R},a_j}M_{c,1}^{\mathcal{R},a_l}\right] - \mathbb{E}\left[M_{c,1}^{\mathcal{R},a_j}\right]\mathbb{E}\left[M_{c,1}^{\mathcal{R},a_l}\right]\right). \tag{2.3.33}$$

Proof. We show that Theorem 2.3.11 is applicable in this case. We have

$$|\chi_j^n|^2 = \sum_{i=1}^m\left(\frac{1}{\sqrt{n}}\ln M_{c,j}^{\mathcal{R}}(q_n\vartheta_0^n(a_i,z_\mathrm{f})) - \mathbb{E}\left[\frac{1}{\sqrt{n}}\ln M_{c,j}^{\mathcal{R}}(q_n\vartheta_0^n(a_i,z_\mathrm{f}))\right]\right)^2 \le \frac{4m}{n}K^2 \tag{2.3.34}$$

where K is the global constant bounding each $M_{c,j}^{\mathcal{R}}$ for $\vartheta \in (0,\vartheta_{**})$, independent of j. We have to check that the Lindeberg condition (2.3.31) is satisfied. Since $|\chi_j^n| \le 2\sqrt{\frac{m}{n}}K$ there exists for each $\epsilon > 0$ $n_0 \in \mathbb{N}$ such that $|\chi_j^n| < \epsilon$ for all $n \ge n_0$. Therefore, each summand is 0 for $n \ge n_0$ and thus also the sum and the limit vanish. Part 1 of Condition (2.3.30) is satisfied because we consider centered random variables. Part 2 holds true due to

$$\sum_{j=1}^{n_c}\mathbb{E}\left[|\chi_j^n|^2\right] \le \max\sum_{j=1}^{n_c}|\chi_j^n|^2 \le n_c\frac{4mK^2}{n} \le 4mK^2 < \infty \tag{2.3.35}$$

according to (2.3.34). There can only appear finitely many summands which are equal to 1. Condition $[\beta]$ of Theorem 2.3.11 is satisfied because each χ_j^n has expectation 0 due to the construction. Condition $[\gamma]$ is satisfied since

$$\begin{aligned}\sum_{j=1}^{n_c}\mathbb{E}\left[\chi_j^{n,k}\chi_j^{n,l}\right] =& \frac{n_c}{n}\Bigg(\mathbb{E}\left[\ln M_{c,1}^{\mathcal{R}}(q_n\vartheta_0^n(a_k,z_\mathrm{f}))\ln M_{c,1}^{\mathcal{R}}(q_n\vartheta_0^n(a_l,z_\mathrm{f}))\right]\\ &- \mathbb{E}\left[\ln M_{c,1}^{\mathcal{R}}(q_n\vartheta_0^n(a_k,z_\mathrm{f}))\right]\mathbb{E}\left[\ln M_{c,1}^{\mathcal{R}}(q_n\vartheta_0^n(a_l,z_\mathrm{f}))\right]\Bigg).\end{aligned} \tag{2.3.36}$$

Letting now n tend to infinity we obtain

$$C_1\left(\mathbb{E}\left[M_{c,1}^{\mathcal{R},a_j}M_{c,1}^{\mathcal{R},a_l}\right] - \mathbb{E}\left[M_{c,1}^{\mathcal{R},a_j}\right]\mathbb{E}\left[M_{c,1}^{\mathcal{R},a_l}\right]\right). \tag{2.3.37}$$

Limit and integral are interchangeable because dominated convergence is applicable due to the boundedness of the logarithmic moment generating functions for $\vartheta \in (0,\vartheta_{**})$. □

To complete the proof of Lemma 2.3.12 we need to prove tightness. To do so, we use, as usual, the Kolmogorov-Chentsov criterion [83] and check the Conditions 2 and (3.4.7).

Proof of Lemma 2.3.12. The family of initial distributions is given by the random variables evaluated in $\vartheta_0^n(\epsilon,z_\mathrm{f})$ for an $\epsilon > 0$ because $a > n^{-1}\mathbb{E}^{\mathcal{R}}[G_n(z_\mathrm{f})] > 0$. This family is seen to be tight

using Chebychev's inequality:

$$\begin{aligned}&\mathbb{P}\left(\frac{1}{\sqrt{n}}\sum_{j=1}^{n_c}\left(\ln M^{\mathcal{R}}_{c,j}(q_n\vartheta^n_0(\epsilon,z_{\mathrm{f}}))-\mathbb{E}\left[\ln M^{\mathcal{R}}_{c,j}(q_n\vartheta^n_0(\epsilon,z_{\mathrm{f}}))\right]\right)\geq K\right)\\ \leq&\frac{\frac{1}{n}\sum_{j=1}^{n_c}\mathbb{V}[\ln M^{\mathcal{R}}_{c,j}(q_n\vartheta^n_0(\epsilon,z_{\mathrm{f}}))]}{K^2}=\frac{n_c}{n}\frac{\mathbb{V}[\ln M^{\mathcal{R}}_{c,1}(q_n\vartheta^n_0(\epsilon,z_{\mathrm{f}}))]}{K^2}.\end{aligned}\tag{2.3.38}$$

With $\frac{n_c}{n}\to C_1$ and $q_n\to 1$ exist $\delta,\bar{\delta}$ and n_0 such that $\frac{n_c}{n}\leq C_1+\delta$ and $q_n\vartheta^n_0(\epsilon,z_{\mathrm{f}})\leq\vartheta^n_0(\epsilon,z_{\mathrm{f}})+\bar{\delta}$ for all $n\geq n_0$. Thus,

$$\frac{n_c}{n}\mathbb{V}[\ln M^{\mathcal{R}}_{c,j}(q_n\vartheta^n_0(\epsilon,z_{\mathrm{f}}))]\leq(C_1+\delta)\mathbb{V}[\ln M^{\mathcal{R}}_{c,1}(\vartheta^n_0(\epsilon,z_{\mathrm{f}})+\bar{\delta}))]\tag{2.3.39}$$

for all $n\geq n_0$ and we obtain

$$\begin{aligned}&\mathbb{P}\left(\frac{1}{\sqrt{n}}\sum_{j=1}^{n_c}(\ln M^{\mathcal{R}}_{c,j}(q_n\vartheta^n_0(\epsilon,z_{\mathrm{f}}))-\mathbb{E}[\ln M^{\mathcal{R}}_{c,j}(q_n\vartheta^n_0(\epsilon,z_{\mathrm{f}}))])\geq K\right)\\ \leq&K^{-2}\max\left\{\max_{i\in 1,\dots,n_0-1}\tfrac{n_c(i)}{i}\mathbb{V}\left[Y^i_{\epsilon,j}\right],(C_1+\delta)\mathbb{V}\left[\ln M^{\mathcal{R}}_{c,1}(\vartheta^n_0(\epsilon,z_{\mathrm{f}})+\bar{\delta})\right]\right\}.\end{aligned}\tag{2.3.40}$$

For each $\widetilde{\epsilon}$ we can choose K large enough such that 2.3.40 $<\widetilde{\epsilon}$ and thus we have proven tightness of the initial distributions.

It remains to check Condition 3.4.7. We have

$$\begin{aligned}&\mathbb{E}\left[\left(\frac{d}{da}Z_{n,c}(q_n\vartheta^n_0(a,z_{\mathrm{f}}))\right)^2\right]=\mathbb{E}\left[\left(\frac{1}{\sqrt{n}}\sum_{j=1}^{n_c}\frac{d}{da}Y^n_{a,j}\right)^2\right]\\ =&\frac{1}{n}\left(\mathbb{E}\left[\sum_{j=1}^{n_c}\left(\frac{d}{da}Y^n_{a,j}\right)^2\right]+\sum_{j=1}^{n_c}\sum_{i=1,i\neq j}^{n_c}\underbrace{\mathbb{E}\left[\tfrac{d}{da}Y^n_{a,j}\tfrac{d}{da}Y^n_{a,i}\right]}_{=0}\right)=\frac{n_c}{n}\mathbb{E}\left[\left(\frac{d}{da}Y^n_{a,j}\right)^2\right]\end{aligned}\tag{2.3.41}$$

because $Y^n_{a,j}$ and thus $\frac{d}{da}Y^n_{a,j}$ are centered i.i.d. random variables. They are independent for different j because $\vartheta^n_0(a,z_{\mathrm{f}})$ depends only on the random variable W_{f} by definition. Thus, it is enough to show that $\mathbb{E}\left[(\frac{d}{da}Y^n_{a,j})^2\right]$ is bounded. Since $\mathbb{E}[X]$ and thus $X-\mathbb{E}[X]$ are bounded if the random variable X is bounded it suffices in our scenario to show boundedness of the uncentered random variable. Let $X^n_{a,j}\equiv\vartheta^n_0(a,z_{\mathrm{f}})Z^c_jW_j$. Then

$$\begin{aligned}0\leq&\frac{d}{da}\left(\ln\mathbb{E}^{\mathcal{R}}\left[e^{q_nX^n_{a,j}}\right]\right)=\frac{\mathbb{E}^{\mathcal{R}}\left[q_n\frac{d}{da}X^n_{a,j}e^{q_nX^n_{a,j}}\right]}{\mathbb{E}^{\mathcal{R}}\left[e^{q_nX^n_{a,j}}\right]}\\ \leq&q_n\max_a\left(\frac{d}{da}X^n_{a,j}\right)\frac{\mathbb{E}^{\mathcal{R}}\left[e^{q_nX^n_{a,j}}\right]}{\mathbb{E}^{\mathcal{R}}\left[e^{q_nX^n_{a,j}}\right]}\leq q_nZ^{c,\max}_1W^{\max}_1\max_a\frac{d}{da}\left(\vartheta^n_0(a,z_{\mathrm{f}})\right).\end{aligned}\tag{2.3.42}$$

We know by an application of the implicit function theorem that

$$\frac{d}{da}\vartheta^n_0(a,z_{\mathrm{f}})=\left(g''_n(q_n\vartheta^n_0(a,z_{\mathrm{f}}))\right)^{-1}.\tag{2.3.43}$$

Thus, we get tightness if $g''_n(q_n\vartheta^n_0(a,z_{\mathrm{f}}))>C>0$. □

With analogous calculations we get the convergence of the derivatives of $Z_n(q_n\vartheta_0^n(a,z_\mathrm{f}))$.

Lemma 2.3.14. *The processes $Z_n'(q_n\vartheta_0^n(a,z_\mathrm{f}))$ and $Z_n''(q_n\vartheta_0^n(a,z_\mathrm{f}))$ as processes on the Wiener Space with parameter a converge weakly if there exists $C>0$ such that $g_n''(q_n\vartheta_0^n(a,z_\mathrm{f}))>C>0$.*

Proof. In analogy to the previous proof we define

$$\left(Y_{a,j}^n\right)' = \frac{\mathbb{E}^{\mathcal{R}}\left[q_n Z_j^c W_j e^{q_n X_{a,j}^n}\right]}{\mathbb{E}^{\mathcal{R}}\left[e^{q_n X_{a,j}^n}\right]} - \mathbb{E}\left[\frac{\mathbb{E}^{\mathcal{R}}\left[q_n Z_j^c W_j e^{q_n X_{a,j}^n}\right]}{\mathbb{E}^{\mathcal{R}}\left[e^{q_n X_{a,j}^n}\right]}\right] \tag{2.3.44}$$

and

$$\begin{aligned}\left(Y_{a,j}^n\right)'' =& \frac{\mathbb{E}^{\mathcal{R}}\left[(q_n Z_j^c W_j)^2 e^{q_n X_{a,j}^n}\right]}{\mathbb{E}^{\mathcal{R}}\left[e^{q_n X_{a,j}^n}\right]} - \left(\frac{\mathbb{E}^{\mathcal{R}}\left[q_n Z_j^c W_j e^{q_n X_{a,j}^n}\right]}{\mathbb{E}^{\mathcal{R}}\left[e^{q_n X_{a,j}^n}\right]}\right)^2 \\ &- \mathbb{E}\left[\frac{\mathbb{E}^{\mathcal{R}}\left[(q_n Z_j^c W_j)^2 e^{q_n X_{a,j}^n}\right]}{\mathbb{E}^{\mathcal{R}}\left[e^{q_n X_{a,j}^n}\right]} - \left(\frac{\mathbb{E}^{\mathcal{R}}\left[q_n Z_j^c W_j e^{q_n X_{a,j}^n}\right]}{\mathbb{E}^{\mathcal{R}}\left[e^{q_n X_{a,j}^n}\right]}\right)^2\right].\end{aligned} \tag{2.3.45}$$

The constitutive parts of the processes under consideration are given by

$$Z_{n,c}'(q_n\vartheta_0^n(a,z_\mathrm{f})) = \frac{1}{\sqrt{n}}\sum_{j=1}^{n_c}\left(Y_{a,j}^n\right)' \quad \text{and} \quad Z_{n,c}''(q_n\vartheta_0^n(a,z_\mathrm{f})) = \frac{1}{\sqrt{n}}\sum_{j=1}^{n_c}\left(Y_{a,j}^n\right)''. \tag{2.3.46}$$

With the notation $(\chi_j^n)' = \frac{1}{\sqrt{n}}((Y_{a_1,j}^n)',\dots,(Y_{a_m,j}^n)')$ and $(\chi_j^n)'' = \frac{1}{\sqrt{n}}((Y_{a_1,j}^n)'',\dots,(Y_{a_m,j}^n)'')$ we obtain $|(\chi_j^n)'|^2 \le \frac{4m}{n}(Z_1^{c,\max}W_1^{\max})^2$ and $|(\chi_j^n)''|^2 \le \frac{4m}{n}(Z_1^{c,\max}W_1^{\max})^4$. Hence, the convergence of the finite dimensional distributions of $Z_{n,c}'$ and $Z_{n,c}''$ follows with the same argument as before.

Concerning the tightness of the initial distributions we get the two following bounds using again Chebychev's inequality:

$$\begin{aligned}&\mathbb{P}\left(\frac{1}{\sqrt{n}}\sum_{j=1}^{n_c}(Y_{\epsilon,j}^n)' \ge K\right) \\ \le& K^{-2}\max\left\{\max_{i\in 1,\dots,n_0-1}\frac{n_c(i)}{i}\mathbb{V}\left[(Y_{\epsilon,j}^i)'\right], (C_1+\delta)\mathbb{V}\left[\frac{\mathbb{E}^{\mathcal{R}}[Z_1^c W_1 e^{(\vartheta_0^n(\epsilon,z_\mathrm{f})+\bar\delta)Z_1^c W_1}]}{\mathbb{E}^{\mathcal{R}}[e^{(\vartheta_0(\epsilon,z_\mathrm{f})+\bar\delta)Z_1^c W_1}]}\right]\right\}\end{aligned} \tag{2.3.47}$$

and

$$\begin{aligned}\mathbb{P}\left(\frac{1}{\sqrt{n}}\sum_{j=1}^{n_c}(Y_{\epsilon,j}^n)'' \ge K\right) \le K^{-2}\max\Bigg\{&\max_{i\in 1,\dots,n_0-1}\frac{n_c(i)}{i}\mathbb{V}[(Y_{\epsilon,j}^i)''], \\ &(C_1+\delta)\mathbb{V}\left[\frac{\mathbb{E}^{\mathcal{R}}[(Z_1^c W_1)^2 e^{(\vartheta_0^n(\epsilon,z_\mathrm{f})+\bar\delta)Z_1^c W_1}]}{\mathbb{E}^{\mathcal{R}}[e^{(\vartheta_0^n(\epsilon,z_\mathrm{f})+\bar\delta)Z_1^c W_1}]} - \left(\frac{\mathbb{E}^{\mathcal{R}}[Z_1^c W_1 e^{(\vartheta_0^n(\epsilon,z_\mathrm{f})+\bar\delta)Z_1^c W_1}]}{\mathbb{E}^{\mathcal{R}}[e^{(\vartheta_0^n(\epsilon,z_\mathrm{f})+\bar\delta)Z_1^c W_1}]}\right)^2\right]\Bigg\}.\end{aligned} \tag{2.3.48}$$

Using again Condition 3.4.7 it is enough to bound

$$\frac{d}{da}\frac{\mathbb{E}^{\mathcal{R}}[q_n Z_j^c W_j e^{q_n X_{a,j}^n}]}{\mathbb{E}^{\mathcal{R}}[e^{q_n X_{a,j}^n}]} \tag{2.3.49}$$

and

$$\frac{d}{da}\left(\frac{\mathbb{E}^{\mathcal{R}}[(q_nZ_j^cW_j)^2e^{q_nX_{a,j}^n}]}{\mathbb{E}^{\mathcal{R}}[e^{q_nX_{a,j}^n}]}-\left(\frac{\mathbb{E}^{\mathcal{R}}[q_nZ_j^cW_je^{q_nX_{a,j}^n}]}{\mathbb{E}^{\mathcal{R}}[e^{q_nX_{a,j}^n}]}\right)^2\right). \tag{2.3.50}$$

These derivatives are given by

$$\frac{\mathbb{E}^{\mathcal{R}}[(\vartheta_0^n)'(a,z_{\mathrm{f}})(q_nZ_j^cW_j)^2e^{q_nX_{a,j}^n}]}{\mathbb{E}^{\mathcal{R}}[e^{q_nX_{a,j}^n}]}-\frac{\mathbb{E}^{\mathcal{R}}[(\vartheta_0^n)'(a,z_{\mathrm{f}})q_nZ_j^cW_je^{q_nX_{a,j}^n}]\mathbb{E}^{\mathcal{R}}[q_nZ_j^cW_je^{q_nX_{a,j}^n}]}{(\mathbb{E}^{\mathcal{R}}[e^{q_nX_{a,j}^n}])^2} \tag{2.3.51}$$

and

$$\begin{aligned}&\frac{\mathbb{E}^{\mathcal{R}}[(\vartheta_0^n)'(a,z_{\mathrm{f}})(q_nZ_j^cW_j)^3e^{q_nX_{a,j}^n}]}{\mathbb{E}^{\mathcal{R}}[e^{q_nX_{a,j}^n}]}-\frac{\mathbb{E}^{\mathcal{R}}[(q_nZ_j^cW_j)^2e^{q_nX_{a,j}^n}]\mathbb{E}^{\mathcal{R}}[(\vartheta_0^n)'(a,z_{\mathrm{f}})q_nZ_j^cW_je^{q_nX_{a,j}^n}]}{(\mathbb{E}^{\mathcal{R}}[e^{q_nX_{a,j}^n}])^2}\\&-2\frac{\mathbb{E}^{\mathcal{R}}[(q_nZ_j^cW_j)^2(\vartheta_0^n)'(a,z_{\mathrm{f}})e^{q_nX_{a,j}^n}]\mathbb{E}^{\mathcal{R}}[q_nZ_j^cW_je^{q_nX_{a,j}^n}]}{(\mathbb{E}^{\mathcal{R}}[e^{q_nX_{a,j}^n}])^2}\\&+2\frac{\mathbb{E}^{\mathcal{R}}[(\vartheta_0^n)'(a,z_{\mathrm{f}})q_nZ_j^cW_je^{q_nX_{a,j}^n}](\mathbb{E}^{\mathcal{R}}[q_nZ_j^cW_je^{q_nX_{a,j}^n}])^2}{(\mathbb{E}^{\mathcal{R}}[e^{q_nX_{a,j}^n}])^3}.\end{aligned} \tag{2.3.52}$$

We are able to bound the first derivative according to

$$-\max_a(\vartheta_0^n)'(a,z_{\mathrm{f}})(Z_j^{c,\max}W_j^{\max})^2\leq(2.3.51)\leq\max_a(\vartheta_0^n)'(a,z_{\mathrm{f}})(Z_j^{c,\max}W_j^{\max})^2. \tag{2.3.53}$$

Thus, we get the same criterion as in Lemma 2.3.12 for boundedness and thus tightness here. We can bound each summand in (2.3.52) very similar to the previous cases and end up with the bound $4\max_a(\vartheta_0^n)'(a,z_{\mathrm{f}})(Z_j^{c,\max}W_j^{\max})^3$. Thus, we again arrive at the same criterion to get tightness. □

Lemma 2.3.15. *$X_a^n=(Z_n(q_n\vartheta_0^n(a,z_{\mathrm{f}})),Z_n'(q_n\vartheta_0^n(a,z_{\mathrm{f}})),Z_n''(q_n\vartheta_0^n(a,z_{\mathrm{f}})))$ converges weakly if there exists $C>0$ such that $g_n''(q_n\vartheta_0^n(a,z_{\mathrm{f}}))>C>0$.*

Proof. The structure of the proof is the same. First, we consider the finite dimensional distributions. Let $Y_{a,j}^n,(Y_{a,j}^n)'$ and $(Y_{a,j}^n)''$ be defined as above. We investigate now

$$\chi_j^n\equiv\tfrac{1}{\sqrt{n}}(Y_{a_1,j}^n,(Y_{a_1,j}^n)',(Y_{a_1,j}^n)'',\dots,Y_{a_l,j}^n,(Y_{a_l,j}^n)',(Y_{a_l,j}^n)''). \tag{2.3.54}$$

These vectors are again independent for different j. The boundedness of $|\chi_j^n|^2$ follows directly by the boundedness in the previous cases. Again, $|\chi_j^n|$ tends to 0 such that the Lindeberg condition is satisfied. Part 1 of Condition 2.3.30 holds because we consider centered random variables. Part 2 holds because we can bound $|\chi_j^n|^2$. It can be shown that the initial distributions are tight using again Chebychev's inequality. To prove tightness we have to show that

$$\begin{aligned}&\mathbb{E}\left[|X_{a+h}^n-X_a^n|^2\right]\\=&\mathbb{E}\Big[(Z_n(q_n\vartheta_0^n(a+h))-Z_n(q_n\vartheta_0^n(a,z_{\mathrm{f}})))^2+(Z_n'(q_n\vartheta_0^n(a+h))-Z_n'(q_n\vartheta_0^n(a,z_{\mathrm{f}})))^2\\&+(Z_n''(q_n\vartheta_0^n(a+h))-Z_n''(q_n\vartheta_0^n(a,z_{\mathrm{f}})))^2\Big]\leq C|h|^2\end{aligned} \tag{2.3.55}$$

This holds true because we have already seen that each summand can be bounded by the right-hand side for a certain C. Thus, we have just to sum up the different constants. □

Finally, we come to the proof of the result about the rate function.

Proof of Theorem 2.3.17. We look at the $\vartheta_n(a, z_\mathrm{f})$ determining equation

$$a - \frac{z_\mathrm{f}}{n} W_\mathrm{f} = q_n g_n'(q_n \vartheta) + \frac{1}{\sqrt{n}} q_n Z_n'(q_n \vartheta) \tag{2.3.56}$$

and write the solution of this equation in the form $\vartheta_0^n(a, z_\mathrm{f}) + \delta^n(a, z_\mathrm{f})$, where $\vartheta_0^n(a, z_\mathrm{f})$ is defined as the solution of

$$a - \frac{z_\mathrm{f}}{n} W_\mathrm{f} = q_n g_n'(q_n \vartheta). \tag{2.3.57}$$

$\delta^n(a, z_\mathrm{f})$ denotes the stochastic perturbation of this equation caused by the process Z_n. By definition of $\vartheta_0^n(a, z_\mathrm{f})$, we have $q_n g_n'(q_n \vartheta_0^n(a, z_\mathrm{f})) = a - z_\mathrm{f}/n W_\mathrm{f}$. We can derive an expression for $\delta^n(a, z_\mathrm{f})$ using a first order Taylor expansion. To keep the notation short we drop the arguments and write just ϑ_0^n and δ^n. We obtain

$$\begin{aligned}
& a - \frac{z_\mathrm{f}}{n} W_\mathrm{f} = q_n \left[g_n'(q_n(\vartheta_0^n + \delta^n)) + \frac{1}{\sqrt{n}} Z_n'(q_n(\vartheta_0^n + \delta^n)) \right] \\
\Leftrightarrow \quad & a - \frac{z_\mathrm{f}}{n} W_\mathrm{f} = q_n \left[g_n'(q_n \vartheta_0^n) + q_n \delta^n g_n''(q_n \vartheta_0^n) + \frac{1}{\sqrt{n}} Z_n'(q_n \vartheta_0^n) + q_n \delta^n \frac{1}{\sqrt{n}} Z_n''(q_n \vartheta_0^n) + \mathrm{o}(\delta^n) \right] \\
\Leftrightarrow \quad & 0 = q_n^2 \delta^n \left(g_n''(q_n \vartheta_0^n) + \frac{1}{\sqrt{n}} Z_n''(q_n \vartheta_0^n) \right) + \frac{q_n}{\sqrt{n}} Z_n'(q_n \vartheta_0^n) + \mathrm{o}(\delta^n) \\
\Leftrightarrow \quad & \delta^n = \frac{-\frac{1}{\sqrt{n}} Z_n'(q_n \vartheta_0^n) + \mathrm{o}(\delta^n)}{q_n(g_n''(q_n \vartheta_0^n) + \frac{1}{\sqrt{n}} Z_n''(q_n \vartheta_0^n))} = \frac{-\frac{1}{\sqrt{n}} Z_n'(q_n \vartheta_0^n)}{q_n(g_n''(q_n \vartheta_0^n) + \frac{1}{\sqrt{n}} Z_n''(q_n \vartheta_0^n))} + \mathrm{o}(\delta^n).
\end{aligned} \tag{2.3.58}$$

The rate function can be rewritten as

$$\begin{aligned}
I_n^{\mathcal{R}}(a, z_\mathrm{f}) &= a \vartheta_n(a, z_\mathrm{f}) - \Psi_n^{\mathcal{R}}(\vartheta_n(a, z_\mathrm{f})) \\
&= \left(a - \frac{z_\mathrm{f}}{n} W_\mathrm{f} \right) (\vartheta_0^n + \delta^n) - g_n(q_n(\vartheta_0^n + \delta^n)) - \frac{1}{\sqrt{n}} Z_n(q_n(\vartheta_0^n + \delta^n)).
\end{aligned} \tag{2.3.59}$$

A second order Taylor expansion and reordering of the involved terms yields

$$\begin{aligned}
I_n^{\mathcal{R}}(a, z_\mathrm{f}) = & \underbrace{a \vartheta_0^n - g_n(q_n \vartheta_0^n)}_{=: I_0^n(a, z_\mathrm{f})} - \frac{z_\mathrm{f}}{n} W_\mathrm{f} \vartheta_0^n - \frac{1}{\sqrt{n}} Z_n(q_n \vartheta_0^n) + \underbrace{\left(\left(a - \frac{z_\mathrm{f}}{n} W_\mathrm{f} \right) - q_n g_n'(q_n \vartheta_0^n) \right)}_{=0} \delta^n \\
& - \frac{1}{\sqrt{n}} q_n \delta^n Z_n'(q_n \vartheta_0^n) - \frac{1}{2} (q_n \delta^n)^2 \left(g_n''(q_n \vartheta_0^n) + \frac{1}{\sqrt{n}} Z_n''(q_n \vartheta_0^n) \right) + \mathrm{o}((q_n \delta^n)^2)
\end{aligned} \tag{2.3.60}$$

The stochastic process $Z_n(q_n \vartheta_0^n(a, z_\mathrm{f}))$ converges weakly to the mentioned Gaussian process according to Lemma 2.3.12. g_n'' is of the order $\mathcal{O}(1)$ according to

$$\begin{aligned}
g_n''(q_n \vartheta) \to & C_1 \mathbb{E} \left[\frac{\mathbb{E}^{\mathcal{R}}[(Z_1^c W_1)^2 e^{\vartheta Z_1^c W_1})] \mathbb{E}^{\mathcal{R}}[e^{\vartheta Z_1^c W_1}] - \mathbb{E}^{\mathcal{R}}[Z_1^c W_1 e^{\vartheta Z_1^c W_1}]^2}{(\mathbb{E}^{\mathcal{R}}[e^{\vartheta Z_1^c W_1}])^2} \right] \\
& + C_2 \mathbb{E} \left[\frac{\mathbb{E}^{\mathcal{R}}[(Z_1^v W_1)^2 e^{\vartheta Z_1^v W_1}] \mathbb{E}^{\mathcal{R}}[e^{\vartheta Z_1^v W_1}] - \mathbb{E}^{\mathcal{R}}[Z_1^v W_1 e^{\vartheta Z_1^v W_1}]^2}{(\mathbb{E}^{\mathcal{R}}[e^{\vartheta Z_1^v W_1}])^2} \right]
\end{aligned} \tag{2.3.61}$$

Together with the joint weak convergence of the processes $Z_n'(q_n\vartheta_0^n(a,z_\mathrm{f}))$ and $Z_n''(q_n\vartheta_0^n(a,z_\mathrm{f}))$ this yields $\delta^n \in \mathcal{O}(1/\sqrt{n})$. Furthermore, this implies $f \in \mathrm{o}(1/\sqrt{n})$ for each $f \in \mathrm{o}(\delta^n)$ and $f \in \mathrm{o}(1/n)$ for each $f \in \mathrm{o}((\delta^n)^2)$. We plug in Equation (3.4.16) the expression for δ^n and obtain

$$\begin{aligned}
I_n^{\mathcal{R}}(a,z_\mathrm{f}) = I_0^n(a,z_\mathrm{f}) &- \frac{z_\mathrm{f}}{n}W_\mathrm{f}\vartheta_0^n - \frac{1}{\sqrt{n}}Z_n(q_n\vartheta_0^n) \\
&- \frac{q_n^2}{2}\left(\frac{-(\frac{1}{\sqrt{n}}Z_n'(q_n\vartheta_0^n))}{q_n(g_n''(q_n\vartheta_0^n)+\frac{1}{\sqrt{n}}Z_n''(q_n\vartheta_0^n))}+\mathrm{o}(\delta^n)\right)^2\left(g_n''(q_n\vartheta_0^n)+\frac{1}{\sqrt{n}}Z_n''(q_n\vartheta_0^n)\right) \\
&- \frac{q_n}{\sqrt{n}}Z_n'(q_n\vartheta_0^n)\left(\frac{-\frac{1}{\sqrt{n}}Z_n'(q_n\vartheta_0^n)}{q_n(g_n''(q_n\vartheta_0^n)+\frac{1}{\sqrt{n}}Z_n''(q_n\vartheta_0^n))}+\mathrm{o}(\delta^n)\right)+\mathrm{o}((\delta^n)^2) \\
= I_0^n(a,z_\mathrm{f}) &- \frac{z_\mathrm{f}}{n}W_\mathrm{f}\vartheta_0^n - \frac{1}{\sqrt{n}}Z_n(q_n\vartheta_0^n) - \frac{q_n^2}{2}\left(g_n''(q_n\vartheta_0^n)+\frac{1}{\sqrt{n}}Z_n''(q_n\vartheta_0^n)\right) \\
&\times\left[\frac{\frac{1}{n}(Z_n'(q_n\vartheta_0^n))^2}{q_n^2\left(g_n''(q_n\vartheta_0^n)+\frac{1}{\sqrt{n}}Z_n''(q_n\vartheta_0^n)\right)^2}-\frac{\frac{1}{\sqrt{n}}Z_n'(q_n\vartheta_0^n)\mathrm{o}(\delta^n)}{q_n\left(g_n''(q_n\vartheta_0^n)+\frac{1}{\sqrt{n}}Z_n''(q_n\vartheta_0^n)\right)}+\mathrm{o}((\delta^n)^2)\right] \\
&+ \frac{\frac{1}{n}(Z_n'(q_n\vartheta_0^n))^2}{g_n''(q_n\vartheta_0^n)+\frac{1}{\sqrt{n}}Z_n''(q_n\vartheta_0^n)}+\frac{1}{\sqrt{n}}Z_n'(q_n\vartheta_0^n)\mathrm{o}(\delta^n)+\mathrm{o}((\delta^n)^2). \qquad (2.3.62)
\end{aligned}$$

According to the observations concerning δ^n this equals

$$I_0^n(a,z_\mathrm{f}) - \frac{z_\mathrm{f}}{n}W_\mathrm{f}\vartheta_0^n - \frac{1}{\sqrt{n}}Z_n(q_n\vartheta_0^n) + \frac{\frac{1}{2n}(Z_n'(q_n\vartheta_0^n))^2}{g_n''(q_n\vartheta_0^n)+\frac{1}{\sqrt{n}}Z_n''(q_n\vartheta_0^n)} + \mathrm{o}\left(\frac{1}{n}\right), \qquad (2.3.63)$$

where $\frac{(Z_n'(q_n\vartheta_0^n))^2}{g_n''(q_n\vartheta_0^n)+\frac{1}{\sqrt{n}}Z_n''(q_n\vartheta_0^n)}$ converges weakly due to continuous mapping and the joint weak convergence of $Z_n'(q_n\vartheta_0^n)$ and $Z_n''(q_n\vartheta_0^n)$. This completes the proof of the theorem. □

Case 3.) Conditioning on $\mathcal{Z}$ produces again i.i.d. random variables because Z_j are measurable w.r.t. $\mathcal{Z}$ and the stimulation rates are independent of this σ-algebra.

Theorem 2.3.16. *Let $(a_n)_{n\in\mathbb{N}}$ be defined by $a_n \equiv a$ and $g_{act}(n) = an$ such that $g_{act}(n) > \mathbb{E}^{\mathcal{Z}}(G_n(z_\mathrm{f}))$ and $a < \sup_{\vartheta\in\mathbb{R}}\frac{d}{d\vartheta}\Psi_n^{\mathcal{Z}}(\vartheta)$ for all $n \in \mathbb{N}$. Then Theorem 3.1.3 is almost surely applicable provided $z_\mathrm{f}/n \downarrow 0$, the distribution functions of the stimulation rates are neither lattice-valued nor concentrated on one point and the moment generating functions $M_{c,j}^{\mathcal{Z}}(\vartheta)$, $M_{v,j}^{\mathcal{Z}}(\vartheta)$ and $M^{\mathcal{Z}}(\vartheta)$ as well as $M_c(\vartheta)$, $M_v(\vartheta)$ and $M(\vartheta)$ are finite for each $\vartheta \in \mathbb{R}$. The rate function is*

$$\begin{aligned}
I_n^{\mathcal{Z}}(a,z_\mathrm{f}) =& a\vartheta_n(a,z_\mathrm{f}) - \frac{1}{n}\left(\sum_{j=1}^{n_c}\ln M_{c,j}^{\mathcal{Z}}(q_n\vartheta_n(a,z_\mathrm{f}))\right. \\
&\left.+\sum_{j=n_c+1}^{n_c+n_v}\ln M_{v,j}^{\mathcal{Z}}(q_n\vartheta_n(a,z_\mathrm{f}))+\ln M(z_\mathrm{f}\vartheta_n(a,z_\mathrm{f}))\right). \qquad (2.3.64)
\end{aligned}$$

Proof. The proof of this theorem goes along the same lines as the analogous result in Case 2.) and will be skipped. □

Investigation of the rate function. In this case, the properties of the large deviation rate function can again be described by a functional central limit theorem. Using the notation from Section 2.2 we obtain the following result

Theorem 2.3.17. *If there exists a constant C such that $\widetilde{g}_n''(q_n\widetilde{\vartheta}^n(a,z_\mathrm{f})) > C > 0$, the rate function takes the form*

$$I_n^{\mathcal{Z}}(a,z_\mathrm{f}) = \widetilde{I}^n(a,z_\mathrm{f}) - \frac{1}{\sqrt{n}}\widetilde{Z}_n(q_n\widetilde{\vartheta}^n(a,z_\mathrm{f})) - \frac{1}{n}\ln M(z_\mathrm{f}\widetilde{\vartheta}^n(a,z_\mathrm{f})) + R_n, \tag{2.3.65}$$

where $R_n \in \mathcal{O}\left(\frac{1}{n}\right)$. $\widetilde{Z}_n(q_n\widetilde{\vartheta}^n(a,z_\mathrm{f}))$ converges weakly to the Gaussian process $Z_a + \overline{Z}_a$. Z_a and $\overline{Z}_a$ are both Gaussian processes with expectation functions $\mathbb{E}[Z_a] = 0 = \mathbb{E}[\overline{Z}_a]$ and covariance functions

$$\begin{aligned}\mathrm{Cov}(Z_a, Z_{a'}) = C\Big(&\mathbb{E}[\ln(\mathbb{E}^{\mathcal{Z}}[e^{\widetilde{\vartheta}_0(a)Z_1^c W_1}])\ln(\mathbb{E}^{\mathcal{Z}}[e^{\widetilde{\vartheta}_0(a')Z_1^c W_1}])] \\ &-\mathbb{E}[\ln(\mathbb{E}^{\mathcal{Z}}[e^{\widetilde{\vartheta}_0(a)Z_1^c W_1}])]\mathbb{E}[\ln(\mathbb{E}^{\mathcal{Z}}[e^{\widetilde{\vartheta}_0(a')Z_1^c W_1}])]\Big)\end{aligned} \tag{2.3.66}$$

and

$$\begin{aligned}\mathrm{Cov}(\overline{Z}_a, \overline{Z}_{a'}) = \widetilde{C}\Big(&\mathbb{E}[\ln(\mathbb{E}^{\mathcal{Z}}[e^{\widetilde{\vartheta}_0(a)Z_1^v W_1}])\ln(\mathbb{E}^{\mathcal{Z}}[e^{\widetilde{\vartheta}_0(a')Z_1^v W_1}])] \\ &-\mathbb{E}[\ln(\mathbb{E}^{\mathcal{Z}}[e^{\widetilde{\vartheta}_0(a)Z_1^v W_1}])]\mathbb{E}[\ln(\mathbb{E}^{\mathcal{Z}}[e^{\widetilde{\vartheta}_0(a')Z_1^v W_1}])]\Big).\end{aligned} \tag{2.3.67}$$

The approach to prove this result is the same as in Case 2.) and we will not present the details.

2.4 Conclusion and Outlook

The main new aspects of the present work are the investigation of the conditional scenarios and the establishment of a higher robustness of the model using classes of distributions instead of concrete distributions.

The first point allows a more precise understanding and interpretation of the activation mechanism. The presented results show that the parameter a can be chosen such that the activation probabilities increase exponentially with z_f for the regime $\sqrt{n} \ll z_\mathrm{f} \ll n$ in Cases 1.) and 3.). For Case 2.) this result depends on the actual value of the stimulation rate of the foreign peptide, W_f. This value has to be large enough in order that the probability of activation increases exponentially with z_f. In biological terms this means that the overall frequency of T-cell activation increases as desired. This growth is caused by those TCR clonotypes which interact strongly with the given foreign peptide species. This interpretation also suits to the histograms of stimulation rates and the explanation of these histograms by [95].

The generalised distribution assumptions allow the choice of different distributions depending on the mechanisms that should be included into the model. For example MHC-loading fluctuations, influence of different affinity of the different peptide species to the same receptor and maybe co-stimulation could be considered (see e.g. [128, 127]). For the relevance of co-stimulation in the immune response see, e.g. the recent review by [32].

One aim of future work is to investigate the mechanism of *negative selection*. Thereby T-cells which interact too strongly with the body's own structures are deleted. Negative selection was already investigated in a different model setting [131, 125] and also [138] included negative selection into numerical simulations. In this case the situation becomes mathematically more involved, since the selection causes dependencies in between the stimulation rates.

Acknowledgement. We are very grateful to Ellen Baake and Frank den Hollander for numerous discussions and valuable input. We thank an anonymous referee for helpful comments. A.B. is partially supported through the German Research Foundation in the Priority Programme 1590 "Probabilistic Structures in Evolution" and the Hausdorff Center for Mathematics (HCM). He is member of the Cluster of Excellence "ImmunoSensation" at Bonn University. H.M. is supported by the German Research Foundation in the Cluster of Excellence "ImmunoSensation" and the Bonn International Graduate School in Mathematics (BIGS).

3. A conditional strong large deviation result and a functional central limit theorem for the rate function

Anton Bovier and Hannah Mayer

Abstract We study the large deviation behaviour of $S_n = \sum_{j=1}^n W_j Z_j$, where $(W_j)_{j\in\mathbb{N}}$ and $(Z_j)_{j\in\mathbb{N}}$ are sequences of real-valued, independent and identically distributed random variables satisfying certain moment conditions, independent of each other. More precisely, we prove a conditional strong large deviation result and describe the fluctuations of the random rate function through a functional central limit theorem.

3.1 Introduction and Results

Let $(Z_j)_{j\in\mathbb{N}}$ be independent, identically distributed (i.i.d.) random variables and let $(W_j)_{j\in\mathbb{N}}$ be i.i.d. random variables as well. Define the σ-fields $\mathcal{Z} \equiv \sigma(Z_j, j \in \mathbb{N})$ and $\mathcal{W} \equiv \sigma(W_j, j \in \mathbb{N})$ and let $\mathcal{Z}$ and $\mathcal{W}$ be independent. Furthermore, define

$$S_n \equiv \sum_{j=1}^{n} Z_j W_j. \tag{3.1.1}$$

In this paper we derive *strong (local) large deviation* estimates on S_n *conditioned* on the σ-field $\mathcal{W}$. The random variables W_j can be interpreted as a random environment weighting the summands of S_n. Conditioning on $\mathcal{W}$ can thus be understood as fixing the environment. [34] investigates conditional large deviation estimates of such sums in the more general setup of i.i.d. random fields of random variables taking values in a Polish Space. His results concern, however, only the standard *rough* large deviation estimates. Local limit theorems have been obtained in the case $S_n \in \mathbb{R}$ (see e.g. [12, 24]) and for the case $S_n \in \mathbb{R}^d$ (see [78]), but these have, to our knowledge, not been applied to conditional laws of sums of the form (3.1.1).

Our result consists of two parts. The first part is an almost sure local limit theorem for the conditional tail probabilities $\mathbb{P}(S_n \geq an|\mathcal{W})$, $a \in \mathbb{R}$. The second part is a functional central limit theorem for the *random* rate function.

3.1.1 Strong large deviations

For a general review of *large deviation theory* see for example [46] or [45]. A *large deviation principle* for a family of real-valued random variables S_n roughly says that, for $a > \mathbb{E}\left[\frac{1}{n}S_n\right]$,

$$\mathbb{P}(S_n \geq an) = \exp\left[-nI(a)(1 + \mathrm{o}(1))\right]. \tag{3.1.2}$$

The Gärtner-Ellis theorem asserts that the *rate function*, $I(a)$, is obtained as the limit of the Fenchel-Legendre transformation of the logarithmic moment generating function of S_n, to wit

$I(a) = \lim_{n\to\infty} I_n(a)$, where $I_n(a)$ is defined by

$$I_n(a) \equiv \sup_{\vartheta}(a\vartheta - \Psi_n(\vartheta)) = a\vartheta_n - \Psi_n(\vartheta_n), \tag{3.1.3}$$

where $\Psi_n(\vartheta) \equiv \frac{1}{n}\log\mathbb{E}[\exp(\vartheta S_n)]$ and ϑ_n satisfies $\Psi_n'(\vartheta_n) = a$. Furthermore, define $\Phi_n(\vartheta) \equiv \mathbb{E}[\exp(\vartheta S_n)]$.

Strong large deviations estimates refine this *exponential asymptotics*. They provide estimates of the form

$$\mathbb{P}(S_n \geq an) = \frac{\exp(-nI_n(a))}{\vartheta_n\sigma_n\sqrt{2\pi n}}[1 + \mathrm{o}(1)], \tag{3.1.4}$$

where $\sigma_n^2 \equiv \Psi_n''(\vartheta_n)$ denotes the variance of $\frac{1}{\sqrt{n}}S_n$ under the *tilted* law $\widetilde{\mathbb{P}}$ that has density

$$\frac{d\widetilde{\mathbb{P}}}{d\mathbb{P}} = \frac{\mathrm{e}^{\vartheta_n S_n}}{\mathbb{E}\left[\mathrm{e}^{\vartheta_n S_n}\right]}. \tag{3.1.5}$$

The standard theorem for S_n a sum of i.i.d. random variables is due to [12]. The generalisation, which we summarise by Theorem 3.1.3, is a result of [24]. We abusively refer to $I_n(a)$ as the *rate function*. The following theorem is based on 2 assumptions.

Assumption 3.1.1. There exist $\vartheta_* \in (0,\infty)$ and $\beta < \infty$ such that

$$|\Psi_n(\vartheta)| < \beta, \text{ for all } \vartheta \in \{\vartheta \in \mathbb{C} : |\vartheta| < \vartheta_*\} \tag{3.1.6}$$

for all $n \in \mathbb{N}$ large enough.

Assumption 3.1.2. $(a_n)_{n\in\mathbb{N}}$ is a bounded real-valued sequence such that the equation

$$a_n = \Psi_n'(\vartheta) \tag{3.1.7}$$

has a solution $\vartheta_n \in (0, \vartheta_{**})$ with $\vartheta_{**} \in (0, \vartheta_*)$ for all $n \in \mathbb{N}$ large enough.

Theorem 3.1.3 (Theorem 3.3 in [24])**.** *Let S_n be a sequence of real-valued random variables defined on a probability space $(\Omega, \mathcal{F}, \mathbb{P})$. Let Ψ_n be their logarithmic moment generating function defined above and assume that Assumptions 3.1.1 and 3.1.2 hold for Ψ_n. Assume furthermore that*

(i) $\lim_{n\to\infty}\vartheta_n\sqrt{n} = \infty$,

(ii) $\liminf_{n\to\infty}\sigma_n^2 > 0$, *and*

(iii) $\lim_{n\to\infty}\sqrt{n}\sup_{\delta_1\leq|t|\leq\delta_2\vartheta_n}\left|\frac{\Phi_n(\vartheta_n+it)}{\Phi_n(\vartheta_n)}\right| = 0 \quad \forall\, 0 < \delta_1 < \delta_2 < \infty$,

are satisfied. Then

$$\mathbb{P}\left(S_n \geq na_n\right) = \frac{\exp(-nI_n(a_n))}{\vartheta_n\sigma_n\sqrt{2\pi n}}\left[1 + o(1)\right], \; n \to \infty. \tag{3.1.8}$$

This result is deduced from a local central limit theorem for $\frac{S_n - na_n}{\sqrt{n\sigma_n^2}}$ under the tilted law $\widetilde{\mathbb{P}}$ defined in (3.1.5).

Remark 3.1.4. There are estimates for $\mathbb{P}(S_n \in n\Gamma)$, where $S_n \in \mathbb{R}^d$ and $\Gamma \subset \mathbb{R}^d$, see [78]. Then the leading order prefactor depends on d and the geometry of the set Γ.

3.1.2 Application to the conditional scenario

Throughout the following we write $I_n^{\mathcal{W}}(a)$, $\vartheta_n^{\mathcal{W}}(a)$, $\Phi_n^{\mathcal{W}}(\vartheta)$, $\Psi_n^{\mathcal{W}}(\vartheta)$ and $\mathbb{E}^{\mathcal{W}}[\cdot]$ for the random analogues of the quantities defined in the previous section, e.g. $\Phi_n^{\mathcal{W}}(\vartheta) \equiv \mathbb{E}[\exp(\vartheta S_n)|\mathcal{W}]$.

Remark 3.1.5. One could also condition on a different σ-field $\mathcal{Y}$ as in the application to financial mathematics and an immunological model described in Section 3.2. In the proofs we just need the fact that $\mathcal{W} \subset \mathcal{Y}$ and $\mathcal{Z}$ is independent of $\mathcal{Y}$.

Theorem 3.1.6. *Let S_n be defined in (3.1.1). Assume that the random variables W_1 and Z_1 satisfy the following conditions:*

(i) Z_1 is not concentrated on one point.

(a) If Z_1 is lattice valued, W_1 has an absolutely continuous part and there exists an interval $[c,d]$ such that the density of W_1 on $[c,d]$ is bounded from below by $p > 0$.

(b) If Z_1 has a density, $\mathbb{P}(|W_1| > 0) > 0$.

(ii) The moment generating function of Z_1, $M(\vartheta) \equiv \mathbb{E}[\exp(\vartheta Z_1)]$, is finite for all $\vartheta \in \mathbb{R}$.

(iii) For $f(\vartheta) \equiv \log M(\vartheta)$, both $\mathbb{E}[f(\vartheta W_1)]$ and $\mathbb{E}[W_1 f'(\vartheta W_1)]$ are finite for all $\vartheta \in \mathbb{R}$.

(iv) There exists a function $F : \mathbb{R} \to \mathbb{R}$ such that $\mathbb{E}[F(W_1)]$ is finite and $W_1^2 f''(\vartheta W_1) \leq F(W_1)$ for all $\vartheta \in \mathbb{R}$.

Let $\vartheta_* \in \mathbb{R}_+$ be arbitrary but fixed. Let $J \equiv (\mathbb{E}[W_1]\mathbb{E}[Z_1], \mathbb{E}[W_1 f'(\vartheta_* W_1)])$ and let $a \in J$. Then

$$\mathbb{P}\left(\forall a \in J : \mathbb{P}(S_n \geq an|\mathcal{W}) = \frac{\exp(-nI_n^{\mathcal{W}}(a))}{\sqrt{2\pi n}\vartheta_n^{\mathcal{W}}(a)\sigma_n^{\mathcal{W}}(a)}(1 + o(1))\right) = 1, \tag{3.1.9}$$

where

$$I_n^{\mathcal{W}}(a) = a\vartheta_n^{\mathcal{W}}(a) - \frac{1}{n}\sum_{j=1}^{n} f\left(W_j \vartheta_n^{\mathcal{W}}(a)\right) \tag{3.1.10}$$

and $\vartheta_n^{\mathcal{W}}(a)$ solves $a = \frac{d}{d\vartheta}(\frac{1}{n}\sum_{j=1}^{n} f(W_j\vartheta))$.

This theorem is proven in Section 3.3.

Remark 3.1.7. The precise requirements on the distribution of W_1 depend on the distribution of Z_1. In particular, Condition (iii) does not in general require the moment generating function of W_1 to be finite for all $\vartheta \in \mathbb{R}$. Condition (iv) looks technical. It is used to establish Condition (ii) of Theorem 3.1.3 for all a at the same time. For most applications, it is not very restrictive, see Section 3.1.4 for examples.

3.1.3 Functional central limit theorem for the random rate function

Note that the rate function $I_n^{\mathcal{W}}(a)$ is *random*. Even if we may expect that $I_n^{\mathcal{W}}(a)$ converges to a deterministic function $I(a)$, almost surely, due to the fact that it is multiplied by n in the exponent in Equation (3.1.9), its fluctuations are relevant. To control them, we prove a functional central limit theorem. We introduce the following notation.

$$g(\vartheta) \equiv \mathbb{E}[f(W_1\vartheta)] \quad \text{and} \quad X_n(\vartheta) \equiv \frac{1}{\sqrt{n}}\sum_{j=1}^{n}\left(f(W_j\vartheta) - \mathbb{E}[f(W_j\vartheta)]\right). \tag{3.1.11}$$

Moreover, define $\vartheta(a)$ as the solution of the equation $a = g'(\vartheta)$.

In addition to the assumptions made in Theorem 3.1.6, we need the following assumption on the covariance structure of the summands appearing in the definition of $X_n(\vartheta)$ and their derivatives.

Assumption 3.1.8. There exists $C < \infty$, such that, for all $a, a' \in \bar{J}$, where $\bar{J}$ is the closure of the interval J,

$$\begin{aligned}
&\mathrm{Cov}\left(f(\vartheta(a)W_j), f(\vartheta(a')W_j)\right), \quad \mathrm{Cov}\left(W_j f'(\vartheta(a)W_j), W_j f'(\vartheta(a')W_j)\right), \\
&\mathrm{Cov}\left(W_j^2 f''(\vartheta(a)W_j), W_j^2 f''(\vartheta(a')W_j)\right), \quad \mathrm{Cov}\left(f(\vartheta(a)W_j), W_j f'(\vartheta(a')W_j)\right), \\
&\mathrm{Cov}\left(W_j f'(\vartheta(a)W_j), W_j^2 f''(\vartheta(a')W_j)\right), \quad \mathrm{Cov}\left(f(\vartheta(a)W_j), W_j^2 f''(\vartheta(a')W_j)\right) \text{ and} \\
&\mathbb{V}\left[W_j^3 f'''(\vartheta(a)W_j)\right]
\end{aligned}$$

are all smaller than C.

Theorem 3.1.9. *If $g''(\vartheta(a)) > c$ for some $c > 0$ and Assumption 3.1.8 is satisfied, then the rate function satisfies*

$$I_n^{\mathcal{W}}(a) = I(a) + n^{-1/2} X_n(\vartheta(a)) + n^{-1} r_n(a), \tag{3.1.12}$$

where

$$I(a) \equiv a\vartheta(a) - g(\vartheta(a)), \tag{3.1.13}$$

$$\left(X_n(\vartheta(a))\right)_{a\in\bar{J}} \xrightarrow{\mathcal{D}} \left(X_a\right)_{a\in\bar{J}}, \quad \text{as } n \to \infty, \tag{3.1.14}$$

where X is the Gaussian process with mean zero and covariance

$$\mathrm{Cov}(X_a, X_{a'}) = \mathbb{E}[f(W_1\vartheta(a))f(W_1\vartheta(a'))] - \mathbb{E}[f(W_1\vartheta(a))]\mathbb{E}[f(W_1\vartheta(a'))], \tag{3.1.15}$$

and

$$r_n(a) = \frac{(X_n'(\vartheta(a)))^2}{2\left[g''(\vartheta(a)) + \frac{1}{\sqrt{n}} X_n''(\vartheta(a))\right]} + o(1), \tag{3.1.16}$$

uniformly in $a \in \bar{J}$.

To prove Theorem 3.1.9 we show actually more, namely that the process

$$\left(X_n(\vartheta(a)), X_n'(\vartheta(a)), X_n''(\vartheta(a))\right)_{a\in\bar{J}} \xrightarrow{\mathcal{D}} \left(X_a, X_a', X_a''\right)_{a\in\bar{J}}, \tag{3.1.17}$$

(see Lemma 3.4.1 below). The proof of the theorem is given in Section 3.4.

3.1.4 Examples

In the following we list some examples in which the conditions of the preceding theorems are satisfied.

1. Let Z_1 be a Gaussian random variable with mean zero and variance σ^2. In this case,

$$f(\vartheta) = \log(\mathbb{E}[\exp(\vartheta Z_1)]) = \frac{1}{2}\sigma^2\vartheta^2, \quad f'(\vartheta) = \sigma^2\vartheta \tag{3.1.18}$$

$$f''(\vartheta) = \sigma^2, \quad \text{and} \quad f'''(\vartheta) = 0 \tag{3.1.19}$$

This implies that W_1 must have finite fourth moments to satisfy Assumption 3.1.8. Under this requirement Conditions (iii) and (iv) of Theorem 3.1.6 are met. According to Condition (ib) of Theorem 3.1.6, W_1 may not be concentrated at 0. Moreover,

$$g''(\vartheta) = \sigma^2 > c \tag{3.1.20}$$

independent of the distribution of W_1.

2. Let Z_1 be a binomially distributed random variable, $Z_1 \sim B(m, p)$. Thus

$$f(\vartheta) = m\log(1 - p + p\mathrm{e}^{\vartheta}) \tag{3.1.21}$$

$$f'(\vartheta) = m\frac{p\mathrm{e}^{\vartheta}}{1 - p + p\mathrm{e}^{\vartheta}} \leq m \tag{3.1.22}$$

$$f''(\vartheta) = m(p - p^2)\frac{\mathrm{e}^{\vartheta}}{(1 - p + p\mathrm{e}^{\vartheta})^2} \leq f''\left(\log\left(\frac{3p-1}{p}\right)\right) \tag{3.1.23}$$

$$f'''(\vartheta) = m(p - p^2)\mathrm{e}^{\vartheta}\frac{1 - 3p + p\mathrm{e}^{\vartheta}}{(1 - p + p\mathrm{e}^{\vartheta})^3} \in C_0. \tag{3.1.24}$$

Then W_1 has to satisfy (ia) of Theorem 3.1.6 and must have finite sixth moments. One can show that $f'(\vartheta)$, $f''(\vartheta)$ and $f'''(\vartheta)$ are bounded, $\mathbb{E}[f(\vartheta W_1)]$ and the moments depending on $f(\vartheta W_1)$ in Assumption 3.1.8 are finite. Furthermore, the assumption $0 < \mathbb{E}[W_1^2] < \infty$ implies that $g(\vartheta(a)) > c$ as required in Theorem 3.1.9.

Remark 3.1.10. In both cases it is not necessary that the moment generating function of W_1 exists.

3.1.5 Related results

After posting our manuscript on arXiv, Ioannis Kontoyiannis informed us about the papers [43] and [44], where some similar results on conditional large deviations are obtained. They concern sums of the form

$$\rho_n \equiv \frac{1}{n}\sum_{j=1}^{n}\rho(W_j, Z_j), \tag{3.1.25}$$

where $\mathbf{W} = (W_j)_{j\in\mathbb{N}}$ and $\mathbf{Z} = (Z_j)_{j\in\mathbb{N}}$ are two stationary processes with W_j and Z_j taking values in Polish spaces A_W and A_Z, respectively, and $\rho : A_W \times A_Z \to [0, \infty)$ is some measurable function. Their main motivation is to estimate the frequency with which subsequences of length n in the process Z occur that are "close" to W. To do this, they estimate conditional probabilities of the form

$$\mathbb{P}\left(\rho_n \leq D|\mathcal{W}\right), \tag{3.1.26}$$

obtaining, under suitable assumptions, refined large deviation estimates of the form

$$\frac{1}{n}\log\mathbb{P}\left(\rho_n \leq D|\mathcal{W}\right) = R_n(D) + \frac{1}{\sqrt{n}}\Lambda_n(D) + o(1/\sqrt{n}), \tag{3.1.27}$$

almost surely, where they show that $R_n(D)$ converges a.s. while $\Lambda_n(D)$ converges in distribution to a Gaussian random variable.

3.2 Applications

3.2.1 Stochastic model of T-cell activation

The immune system defends the body against dangerous intrusion, e.g. bacteria, viruses and cancer cells. The interaction of so-called T-cells and antigen presenting cells plays an important rôle in performing this task. Van den Berg, Rand and Burroughs developed a stochastic model of T-cell activation in [131] which was further investigated in [138] and [100]. Let us briefly explain this model.

The antigen presenting cells display on their surface a mixture of peptides present in the body. During a bond between a T-cell and a presenting cell the T-cell scans the presented mixture of peptides. The T-cell is stimulated during this process, and if the sum of all stimuli exceeds a threshold value, the cell becomes activated and triggers an immune response. The signal received by the T-cell is represented by

$$S_n \equiv \sum_{j=1}^{n} Z_j W_j + z_{\mathsf{f}} W_{\mathsf{f}}, \tag{3.2.1}$$

where W_j represents the stimulation rate elicited by a peptide of type j and Z_j represents the random number of presented peptides of type j. The sum describes the signal due to self peptides, $z_{\mathsf{f}} W_{\mathsf{f}}$ is the signal due to one foreign peptide type. From the biological point of view, T-cell activations are rare events and thus large deviation theory is called for to investigate $\mathbb{P}(S_n \geq na|\mathcal{Y})$, where $\mathcal{Y}$ is a σ-field such that W_j are measurable with respect to $\mathcal{Y}$ and Z_j are independent of $\mathcal{Y}$. For two examples of distributions discussed in [138], Theorems 3.1.6 and 3.1.9 can be applied. In both examples, the random variables Z_j are binomially distributed, and thus their moment generating function exists everywhere. W_j is defined by $W_j \equiv \frac{1}{\tau_j}\exp(-\frac{1}{\tau_j})$, where τ_j are exponentially distributed or logarithmic normally distributed, i.e. W_j are bounded and the required moments exist. Furthermore, W_1 has a density and Condition (ia) of Theorem 3.1.6 is met. Using Theorems 3.1.6 and 3.1.9, one can prove that the probability of T-cell activation for a given type of T-cell grows exponentially with the number of presented foreign peptides, z_{f}, if the corresponding stimulation rate W_{f} is sufficiently large. It is then argued that a suitable activation threshold can be set that allows significantly differentiate between the presence or absence of foreign peptides. For more details see [100].

3.2.2 Large portfolio losses

Dembo, Deuschel, and Duffie investigate in [42] the probability of large financial losses on a bank portfolio or the total claims against an insurer conditioned on a macro environment. The random variable S_n represents the total loss on a portfolio consisting of many positions, W_j is a $\{0,1\}$-valued random variable and indicates if position j experiences a loss, whereas the random variable Z_j is for example exponentially distributed and represents the amount of loss. They consider the probability conditioned on a common macro environment $\mathcal{Y}$ and assume that $Z_1, W_1, \ldots, Z_n, W_n$ are conditionally independent. Furthermore, they work in the slightly generalised setup of finitely

many blocks of different distributions. That is

$$S_n \equiv \sum_{\alpha=1}^{K} \sum_{j=1}^{Q_\alpha} Z_{\alpha,j} W_{\alpha,j}, \tag{3.2.2}$$

where $Z_{\alpha,j} \stackrel{\mathcal{D}}{=} Z_\alpha$ and $W_{\alpha,j} \stackrel{\mathcal{D}}{=} W_\alpha$ for each $\alpha \in \{1, \ldots, K\}$ and $\sum_{\alpha=1}^{K} Q_\alpha = n$. Moreover, the conditional probability of losses for each position is calculated and the influence of the length of the time interval, in which the loss occurs, is investigated. For more details see [42]. This analysis was generalised later in a paper by [114].

Remark 3.2.1. In general, the exponential distribution for Z_1 causes problems because the moment generating function does not exist everywhere. Evaluating at ϑW_j thus might yield to an infinite term depending on the range of W_j. In this application there is no problem because W_j is $\{0,1\}$-valued.

3.3 Proof of Theorem 3.1.6

Proof of Theorem 3.1.6. We prove Theorem 3.1.6 by showing that the conditional law of S_n given $\mathcal{W}$ satisfies the assumptions of Theorem 3.1.3 uniformly in $a \in J$, almost surely.

Assumption 3.1.1 is satisfied due to Conditions (ii) and (iii) of Theorem 3.1.6: For each $n \in \mathbb{N}$ and each realisation of $(W_j)_{j\in\mathbb{N}}$ $\Psi_n^{\mathcal{W}}(\vartheta)$ is a convex function. Furthermore,

$$\Psi_n^{\mathcal{W}}(\vartheta) \leq \max\{\Psi_n^{\mathcal{W}}(\vartheta_*), \Psi_n^{\mathcal{W}}(-\vartheta_*)\} \tag{3.3.1}$$

and

$$\lim_{n\to\infty} \max\{\Psi_n^{\mathcal{W}}(\vartheta_*), \Psi_n^{\mathcal{W}}(-\vartheta_*)\} = \max\{\mathbb{E}\left[f(W_1\vartheta_*)\right], \mathbb{E}\left[f(-W_1\vartheta_*))\right]\}, \quad \text{a.s.} \tag{3.3.2}$$

This implies that Assumption 3.1.1 is satisfied. To prove that Assumption 3.1.2 holds, note that, by the law of large numbers,

$$\lim_{n\to\infty} \frac{d}{d\vartheta}\Psi_n^{\mathcal{W}}(0) = \lim_{n\to\infty} \frac{1}{n}\mathbb{E}^{\mathcal{W}}[S_n] = \mathbb{E}[W_1]\mathbb{E}[Z_1], \quad \text{a.s.} \tag{3.3.3}$$

Next, by convexity, and again the law of large numbers

$$\liminf_{n\to\infty} \sup_{\vartheta\in[0,\vartheta_*]} \frac{d}{d\vartheta}\Psi_n^{\mathcal{W}}(\vartheta) = \liminf_{n\to\infty} \frac{d}{d\vartheta}\Psi_n^{\mathcal{W}}(\vartheta_*) = \mathbb{E}[W_1 f'(\vartheta_* W_1)], \quad \text{a.s.} \tag{3.3.4}$$

Recall that $\vartheta_n^{\mathcal{W}}(a)$ is defined as the solution of

$$a = \frac{1}{n}\sum_{j=1}^{n} \frac{d}{d\vartheta} \log M(W_j\vartheta) = \frac{1}{n}\sum_{j=1}^{n} W_j f'(\vartheta W_j). \tag{3.3.5}$$

For n large enough, the solution $\vartheta_n^{\mathcal{W}}(a)$ exists for $a \in J$ and is unique since the logarithmic moment generating function $\Psi_n^{\mathcal{W}}$ is strictly convex. Again by monotonicity of $\frac{d}{d\vartheta}\Psi_n^{\mathcal{W}}(\vartheta)$ in ϑ, and because of (3.3.3) and (3.3.4), for $a \in J$, $\vartheta_n^{\mathcal{W}}(a) \in (0, \vartheta_*)$, almost surely, for n large enough. Thus Assumption 3.1.2 is satisfied.

In order to establish Condition (i) of Theorem 3.1.3 we prove the following

Lemma 3.3.1. $\mathbb{P}(\forall a \in J : \lim_{n\to\infty} \vartheta_n^{\mathcal{W}}(a) = \vartheta(a)) = 1$.

Proof. First, using that $g'(\vartheta)$ is continuous and monotone increasing

$$\begin{aligned}
&\mathbb{P}\left(\forall a \in J : \lim_{n\to\infty} |\vartheta_n^{\mathcal{W}}(a) - \vartheta(a)| = 0\right) \\
&= \mathbb{P}\Bigg(\forall a \in J : \lim_{n\to\infty} \Big|g'(\vartheta_n^{\mathcal{W}}(a)) - \frac{1}{n}\sum_{j=1}^{n} W_j f'(\vartheta_n^{\mathcal{W}}(a)W_j) \\
&\qquad\qquad -g'(\vartheta(a)) + \frac{1}{n}\sum_{j=1}^{n} W_j f'(\vartheta_n^{\mathcal{W}}(a)W_j)\Big| = 0\Bigg) \\
&= \mathbb{P}\left(\forall a \in J : \lim_{n\to\infty} \Big|g'(\vartheta_n^{\mathcal{W}}(a)) - \frac{1}{n}\sum_{j=1}^{n} W_j f'(\vartheta_n^{\mathcal{W}}(a)W_j)\Big| = 0\right),
\end{aligned} \tag{3.3.6}$$

where we used that, by definition of $\vartheta(a)$ and $\vartheta_n(a)$,

$$\frac{1}{n}\sum_{j=1}^{n} W_j f'(\vartheta_n^{\mathcal{W}}(a)W_j) = g'(\vartheta(a)) = a. \tag{3.3.7}$$

Since we have seen that for $a \in J$, $\vartheta(a) \in [0, \vartheta_*]$ and, for n large enough, $\vartheta_n^{\mathcal{W}}(a) \in [0, \vartheta_*]$, the last line in (3.3.6) is bounded from below by

$$\mathbb{P}\left(\sup_{\vartheta\in[0,\vartheta_*]} \lim_{n\to\infty} \left|\frac{1}{n}\sum_{j=1}^{n} W_j f'(\vartheta W_j) - \mathbb{E}[W_1 f'(\vartheta W_1)]\right| = 0\right). \tag{3.3.8}$$

Denote the open ball of radius δ around ϑ by $B_\delta(\vartheta) \equiv \{\bar{\vartheta} \in \mathbb{R} : |\vartheta - \bar{\vartheta}| < \delta\}$. The following facts are true:

1. By Condition (iii) of Theorem 3.1.6 $W_1 f'(\vartheta W_1)$ is integrable, for each $\vartheta \in [0, \vartheta_*]$.
2. $W_1(\omega) f'(\vartheta W_1(\omega))$ is a continuous function of ϑ, $\forall \omega \in \Omega$.
3. $W_1 f'(\vartheta W_1)$ is monotone increasing in ϑ since $\frac{d}{d\vartheta}(W_1 f'(\vartheta W_1)) > 0$.
4. (1), (2), and (3) imply, by dominated convergence, that, for all $\vartheta \in [0, \vartheta_*]$,

$$\lim_{\delta\downarrow 0} \mathbb{E}\left[\sup_{\bar{\vartheta}\in B_\delta(\vartheta)} W_1 f'(\bar{\vartheta} W_1) - \inf_{\bar{\vartheta}\in B_\delta(\vartheta)} W_1 f'(\bar{\vartheta} W_1)\right] = 0. \tag{3.3.9}$$

Note that (4) implies that, for all $\vartheta \in [0, \vartheta_*]$ and for all $\epsilon > 0$, there exists a $\delta = \delta(\epsilon, \vartheta)$, such that

$$\left|\mathbb{E}\left[\sup_{\bar{\vartheta}\in B_{\delta(\epsilon,\vartheta)}(\vartheta)} W_1 f'(\bar{\vartheta} W_1) - \inf_{\bar{\vartheta}\in B_{\delta(\epsilon,\vartheta)}(\vartheta)} W_1 f'(\bar{\vartheta} W_1)\right]\right| < \epsilon. \tag{3.3.10}$$

The collection $\{B_{\delta(\epsilon,\vartheta)}(\vartheta)\}_{\vartheta\in[0,\vartheta_*]}$ is an open cover of $[0,\vartheta_*]$, and since $[0,\vartheta_*]$ is compact we can choose a finite subcover, $\{B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)\}_{1\le k\le K}$. Therefore

$$\begin{aligned}
&\sup_{\vartheta\in[0,\vartheta_*]}\left\{\left|\frac{1}{n}\sum_{j=1}^{n}W_jf'(\vartheta W_j)-\mathbb{E}[W_1f'(\vartheta W_1)]\right|\right\} \qquad (3.3.11)\\
&=\max_{1\le k\le K}\sup_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}\left\{\left|\frac{1}{n}\sum_{j=1}^{n}W_jf'(\vartheta W_j)-\mathbb{E}[W_1f'(\vartheta W_1)]\right|\right\}\\
&=\max_{1\le k\le K}\max\left\{\left|\sup_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}\left(\frac{1}{n}\sum_{j=1}^{n}W_jf'(\vartheta W_j)-\mathbb{E}[W_1f'(\vartheta W_1)]\right)\right|,\right.\\
&\qquad\left.\left|\inf_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}\left(\frac{1}{n}\sum_{j=1}^{n}W_jf'(\vartheta W_j)-\mathbb{E}[W_1f'(\vartheta W_1)]\right)\right|\right\}.
\end{aligned}$$

It suffices to show that for all $1\le k\le K$ and n large enough almost surely

$$\begin{aligned}
-\epsilon \quad &< \inf_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}\left(\frac{1}{n}\sum_{j=1}^{n}W_jf'(\vartheta W_j)-\mathbb{E}[W_1f'(\vartheta W_1)]\right)\\
&\le \sup_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}\left(\frac{1}{n}\sum_{j=1}^{n}W_jf'(\vartheta W_j)-\mathbb{E}[W_1f'(\vartheta W_1)]\right)<\epsilon. \qquad (3.3.12)
\end{aligned}$$

Note that

$$\begin{aligned}
&\sup_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}\left(\frac{1}{n}\sum_{j=1}^{n}W_jf'(\vartheta W_j)-\mathbb{E}[W_1f'(\vartheta W_1)]\right) \qquad (3.3.13)\\
&\le\frac{1}{n}\sum_{j=1}^{n}\sup_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}W_jf'(\vartheta W_j)-\inf_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}\mathbb{E}[W_1f'(\vartheta W_1)]
\end{aligned}$$

Since by convexity of f

$$W_jf'(-\vartheta_*W_j)\le\sup_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}W_jf'(\vartheta W_j)\le W_jf'(\vartheta_*W_j) \qquad (3.3.14)$$

and since these bounds are integrable by Condition (iii) of Theorem 3.1.6 also the supremum itself is integrable. Thus, the strong law of large numbers applies and (3.3.13) converges almost surely to

$$\mathbb{E}\left[\sup_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}W_1f'(\vartheta W_1)\right]-\inf_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}\mathbb{E}[W_1f'(\vartheta W_1)], \qquad (3.3.15)$$

which in turn, due to (3.3.10), is bounded from above by

$$\mathbb{E}\left[\sup_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}W_1f'(\vartheta W_1)-\inf_{\vartheta\in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)}W_1f'(\vartheta W_1)\right]<\epsilon. \qquad (3.3.16)$$

With a similar argument it can be shown that for all $1 \leq k \leq K$ and n large enough almost surely

$$\inf_{\vartheta \in B_{\delta(\epsilon,\vartheta_k)}(\vartheta_k)} \left(\frac{1}{n} \sum_{j=1}^{n} W_j f'(\vartheta W_j) - \mathbb{E}[W_1 f'(\vartheta W_1)] \right) > -\epsilon. \tag{3.3.17}$$

Thus, $\vartheta_n^{\mathcal{W}}(a)$ converges almost surely to $\vartheta(a)$. □

But for $a \in J$, we know that $\vartheta(a) > 0$, and since $\vartheta_n^{\mathcal{W}}(a)$ converges to $\vartheta(a)$, a.s., a fortiori, Condition (i) of Theorem 3.1.3 is satisfied, a.s.

Next we show that Condition (ii) of Theorem 3.1.3 is also satisfied, almost surely. To see this, write

$$\begin{aligned}
&\left(\frac{d^2}{d\vartheta^2} \Psi_n^{\mathcal{W}}(\vartheta) \right) \Big|_{\vartheta = \vartheta_n^{\mathcal{W}}(a)} \\
&= \frac{1}{n} \sum_{j=1}^{n} \frac{\mathbb{E}[W_j^2 Z_j^2 e^{\vartheta_n^{\mathcal{W}}(a) W_j Z_j} | \mathcal{W}] \mathbb{E}[e^{\vartheta_n^{\mathcal{W}}(a) W_j Z_j} | \mathcal{W}] - \left(\mathbb{E}[W_j Z_j e^{\vartheta_n^{\mathcal{W}}(a) W_j Z_j} | \mathcal{W}] \right)^2}{(\mathbb{E}[e^{\vartheta_n^{\mathcal{W}}(a) W_j Z_j} | \mathcal{W}])^2} \\
&= \frac{1}{n} \sum_{j=1}^{n} \mathbb{V}^{\vartheta_n^{\mathcal{W}}(a)}[W_j Z_1 | \mathcal{W}].
\end{aligned} \tag{3.3.18}$$

The conditional variance $\mathbb{V}^{\vartheta_n^{\mathcal{W}}(a)}[W_j Z_j | \mathcal{W}]$ is clearly positive with positive probability, since we assumed the distribution of Z_1 to be non-degenerate and W_j is non-zero with positive probability. We need to show that also the infimum over $n \in \mathbb{N}$ is strictly positive. Note that

$$\Psi''(\vartheta(a)) = \mathbb{E}[\mathbb{V}^{\vartheta(a)}[W_1 Z_1 | \mathcal{W}]] > 0. \tag{3.3.19}$$

We need the following lemma.

Lemma 3.3.2. $\mathbb{P}\left(\forall a \in J : \lim_{n \to \ă\infty} \Psi_n''(\vartheta_n^{\mathcal{W}}(a)) = \Psi''(\vartheta(a)) \right) = 1.$

Proof. Since trivially

$$\begin{aligned}
&|\Psi_n''(\vartheta_n^{\mathcal{W}}(a)) - \Psi''(\vartheta(a))| \\
&\leq |\Psi_n''(\vartheta_n^{\mathcal{W}}(a)) - \Psi''(\vartheta_n^{\mathcal{W}}(a))| + |\Psi''(\vartheta_n^{\mathcal{W}}(a)) - \Psi''(\vartheta(a))|,
\end{aligned} \tag{3.3.20}$$

Lemma 3.3.2 follows if both

$$\mathbb{P}\left(\forall a \in J : \lim_{n \to \infty} |\Psi_n''(\vartheta_n^{\mathcal{W}}(a)) - \Psi''(\vartheta_n^{\mathcal{W}}(a))| = 0 \right) = 1 \tag{3.3.21}$$

and

$$\mathbb{P}\left(\forall a \in J : \lim_{n \to \infty} |\Psi''(\vartheta_n^{\mathcal{W}}(a)) - \Psi''(\vartheta(a))| = 0 \right) = 1. \tag{3.3.22}$$

Now, $\Psi''(\vartheta)$ is a continuous function of ϑ and uniformly continuous on the compact interval $[0, \vartheta_*]$. This implies that

$$\forall \epsilon > 0 \, \exists \delta = \delta(\epsilon) : \forall \vartheta, \vartheta' : |\vartheta - \vartheta'| < \delta \quad |\Psi''(\vartheta) - \Psi''(\vartheta')| < \epsilon. \tag{3.3.23}$$

From the uniform almost sure convergence of $\vartheta_n^{\mathcal{W}}(a)$ to $\vartheta(a)$, it follows that

$$\forall \delta > 0 \, \exists N = N(\omega, \delta) : |\vartheta_n^{\mathcal{W}}(a) - \vartheta(a)| < \delta, \tag{3.3.24}$$

which in turn implies that

$$\forall n \geq N : |\Psi''(\vartheta_n^{\mathcal{W}}(a)) - \Psi''(\vartheta(a))| < \epsilon. \tag{3.3.25}$$

Therefore, Equation (3.3.22) holds. The proof of (3.3.21) is very similar to that of Lemma 3.3.1. The difference is that we cannot use monotonicity to obtain a majorant for $W_1^2 f''(\vartheta W_1)$, but instead use Condition (iv) of Theorem 3.1.6. Again, as in (3.3.8),

$$\begin{aligned}&\mathbb{P}\left(\forall a \in J : \lim_{n\to\infty} |\Psi_n''(\vartheta_n^{\mathcal{W}}(a)) - \Psi''(\vartheta_n^{\mathcal{W}}(a))| = 0\right)\\ &\geq \mathbb{P}\left(\sup_{\vartheta\in[0,\vartheta_*]} \lim_{n\to\infty} |\Psi_n''(\vartheta) - \Psi''(\vartheta)| = 0\right).\end{aligned} \tag{3.3.26}$$

Moreover, the following facts are true:

1. By Condition (iv) of Theorem 3.1.6 and the convexity of f, $0 \leq W_1^2 f''(\vartheta W_1) \leq F(W_1)$ and $\mathbb{E}[F(W_1)] < \infty$.
2. $W_1^2(\omega) f''(\vartheta W_1(\omega))$ is a continuous function of ϑ $\forall \omega \in \Omega$.
3. From (1) and (2) it follows by dominated convergence that for all $\vartheta \in [0, \vartheta_*]$ that

$$\lim_{\delta\downarrow 0} \mathbb{E}\left[\sup_{\bar\vartheta\in B_\delta(\vartheta)} W_1^2 f''(\bar\vartheta W_1) - \inf_{\bar\vartheta\in B_\delta(\vartheta)} W_1^2 f''(\bar\vartheta W_1)\right] = 0. \tag{3.3.27}$$

The proof of Lemma 3.3.2 proceeds from here exactly as the proof of Lemma 3.3.1, just replacing f' by f'' and W_1 by W_1^2. □

Condition (ii) of Theorem 3.1.3 now follows immediately.
Next we show that Condition (iii) is satisfied. We want to show that $\forall 0 < \delta_1 < \delta_2 < \infty$

$$\mathbb{P}\left(\forall a \in J : \lim_{n\to\infty} \sqrt{n} \sup_{\delta_1\leq|t|\leq\delta_2\vartheta_n^{\mathcal{W}}(a)} \left|\frac{\Phi_n^{\mathcal{W}}(\vartheta_n^{\mathcal{W}}(a) + it)}{\Phi_n^{\mathcal{W}}(\vartheta_n^{\mathcal{W}}(a))}\right| = 0\right) = 1. \tag{3.3.28}$$

As above we bound the probability in (3.3.28) from below by

$$\mathbb{P}\left(\lim_{n\to\infty} \sqrt{n} \sup_{\vartheta\in[0,\vartheta_*]} \sup_{\delta_1\leq|t|\leq\delta_2\vartheta} \left|\frac{\Phi_n^{\mathcal{W}}(\vartheta + it)}{\Phi_n^{\mathcal{W}}(\vartheta)}\right| = 0\right). \tag{3.3.29}$$

Therefore, (3.3.28) follows from the first Borel-Cantelli lemma if, for each $\delta > 0$,

$$\sum_{n=1}^{\infty} \mathbb{P}\left(\sqrt{n} \sup_{\vartheta\in[0,\vartheta_*]} \sup_{\delta_1\leq|t|\leq\delta_2\vartheta} \left|\frac{\Phi_n^{\mathcal{W}}(\vartheta + it)}{\Phi_n^{\mathcal{W}}(\vartheta)}\right| > \delta\right) < \infty. \tag{3.3.30}$$

Note that

$$\left|\frac{\Phi_n^{\mathcal{W}}(\vartheta + it)}{\Phi_n^{\mathcal{W}}(\vartheta)}\right| = \prod_{j=1}^{n} \left|\frac{M(W_j(\vartheta + it))}{M(W_j\vartheta)}\right| \tag{3.3.31}$$

is a product of functions with absolute value less or equal to 1. Each factor is the characteristic function of a tilted Z_j. According to a result of [56] there are 3 classes of characteristic functions.

Lemma 3.3.3 (Lemma 4 in Chapter XV in [56]). *Let ϕ be the characteristic function of a probability distribution function F. Then one of the following must hold:*

1. *$|\phi(\zeta)| < 1$ for all $\zeta \neq 0$.*
2. *$|\phi(\lambda)| = 1$ and $|\phi(\zeta)| < 1$ for $0 < \zeta < \lambda$. In this case ϕ has period λ and there exists a real number b such that $F(x + b)$ is arithmetic with span $h = 2\pi/\lambda$.*
3. *$|\phi(\zeta)| = 1$ for all ζ. In this case $\phi(\zeta) = \mathrm{e}^{\mathrm{i}b\zeta}$ and F is concentrated at the point b.*

Case (3) is excluded by assumption. Under Condition (ia) of Theorem 3.1.6 we are in Case (1). In this case it is rather easy to verify Equation (3.3.28). Namely, observe that there exists $0 < \rho < 1$, such that for all $\vartheta \in [0, \vartheta_*]$, for all $\delta_1 \leq t \leq \delta_2 \vartheta_*$, whenever $K^{-1} \leq |W_j| \leq K$, for some $0 < K < \infty$,

$$\left| \frac{M(W_j(\vartheta + it))}{M(W_j\vartheta)} \right| < 1 - \rho. \tag{3.3.32}$$

This implies that, for ϑ as specified,

$$\left| \frac{M(W_j(\vartheta + it))}{M(W_j\vartheta)} \right| \leq (1 - \rho)^{\mathbb{1}_{\{\frac{1}{K} \leq |W_j| \leq K\}}}. \tag{3.3.33}$$

Therefore,

$$\begin{aligned} &\mathbb{P}\left(\sqrt{n} \sup_{\vartheta \in [0,\vartheta_*]} \sup_{\delta_1 \leq |t| \leq \delta_2 \vartheta} \prod_{j=1}^{n} \left| \frac{M(W_j(\vartheta + it))}{M(W_j\vartheta)} \right| > \delta \right) \\ &\leq \mathbb{P}\left(\sqrt{n}(1-\rho)^{\sum_{j=1}^{n} \mathbb{1}_{\{\frac{1}{K} \leq |W_j| \leq K\}}} > \delta \right), \end{aligned} \tag{3.3.34}$$

where K is chosen such that $\mathbb{P}\left(\frac{1}{K} \leq |W_j| \leq K\right) > 0$. With $c_n \equiv \frac{\log \delta - \frac{1}{2}\log n}{\log(1-\rho)}$, the probability in the second line of (3.3.34) is equal to

$$\begin{aligned} &\mathbb{P}\left(\sum_{j=1}^{n} \mathbb{1}_{\{\frac{1}{K} \leq |W_j| \leq K\}} < c_n \right) \\ &\leq \sum_{k=1}^{\lceil c_n \rceil} \binom{n}{k} \mathbb{P}\left(\frac{1}{K} \leq |W_j| \leq K\right)^k \left[1 - \mathbb{P}\left(\frac{1}{K} \leq |W_j| \leq K\right)\right]^{n-k} \\ &\leq \lceil c_n \rceil \binom{n}{\lceil c_n \rceil} \left[1 - \mathbb{P}\left(\frac{1}{K} \leq |W_j| \leq K\right)\right]^{n - \lfloor c_n \rfloor}. \end{aligned} \tag{3.3.35}$$

Since $\binom{n}{\lceil c_n \rceil} \sim n^{C \log n}$ for a constant C, this is summable in n and (3.3.30) holds.

Case (2) of lattice-valued random variables Z_j, which corresponds to Condition (ib) of Theorem 3.1.6, is more subtle. Each of the factors in the product in (3.3.31) is a periodic function, which is equal to 1 if and only if $W_j t \in \{k\lambda, k \in \mathbb{Z}\}$, where λ is the period of this function. This implies that each factor is smaller than 1 if $W_j \notin \{k\lambda/t, k \in \mathbb{Z}\}$. The points of this set do not depend on ϑ and have the smallest distance to each other if t is maximal, i.e. $t = \delta_2 \vartheta_*$. Each

factor is *strictly* smaller than 1 if tW_j does not lie in a finite interval around one of these points. We choose these intervals as follows. Let

$$\widetilde{\delta} \equiv \min\left\{\frac{\lambda}{8\delta_2\vartheta_*}, \frac{d-c}{4}\right\} \tag{3.3.36}$$

and define the intervals

$$I(k,t,\widetilde{\delta}) \equiv \left[\frac{k\lambda}{t} - \widetilde{\delta}, \frac{k\lambda}{t} + \widetilde{\delta}\right]. \tag{3.3.37}$$

These disjoint and consecutive intervals are separated by a distance at least $6\widetilde{\delta}$ from each other. Then, for all $\vartheta \in [0,\vartheta_*]$ there exists $0 < \rho(\vartheta) < 1$, independent of t, such that

$$\left|\frac{M(W_j(\vartheta+it))}{M(W_j\vartheta)}\right| \leq (1-\rho(\vartheta))^{\mathbb{1}_{\{|W_j|\notin\cup_{k\in\mathbb{Z}} I(k,t,\widetilde{\delta})\}}}. \tag{3.3.38}$$

Furthermore, $\left|\frac{M(\theta+it)}{M(\theta)}\right|$ is continuous in θ, and thus its supremum over compact intervals is attained. Thus, for any $C > 0$ there exists $\bar{\rho} = \bar{\rho}(C,\vartheta_*) > 0$ such that, for all $\vartheta \in [0,\vartheta_*]$,

$$\left|\frac{M(W_j(\vartheta+it))}{M(W_j\vartheta)}\right| \leq (1-\bar{\rho})^{\mathbb{1}_{\{W_j\in[-C,C]\setminus\cup_{k\in\mathbb{Z}} I(k,t,\widetilde{\delta})\}}}. \tag{3.3.39}$$

We choose C such that the interval $[c,d]$ from Hypothesis (ia) is contained in $[-C,C]$. Then we get with Equations (3.3.38) and (3.3.39) that

$$\begin{aligned}
&\mathbb{P}\left(\sqrt{n}\sup_{\vartheta\in[0,\vartheta_*]}\sup_{\delta_1\leq|t|\leq\delta_2\vartheta}\prod_{j=1}^{n}\left|\frac{M(W_j(\vartheta+it))}{M(W_j\vartheta)}\right| > \delta\right)\\
&\leq \mathbb{P}\left(\sqrt{n}\sup_{\vartheta\in[0,\vartheta_*]}\sup_{\delta_1\leq|t|\leq\delta_2\vartheta_*}\prod_{j=1}^{n}(1-\bar{\rho})^{\mathbb{1}_{\{W_j\in[-C,C]\setminus\cup_{k\in\mathbb{Z}} I(k,t,\widetilde{\delta})\}}} > \delta\right)\\
&= \mathbb{P}\left(\sqrt{n}\,(1-\bar{\rho})^{\inf_{\delta_1\leq|t|\leq\delta_2\vartheta_*}\sum_{j=1}^{n}\mathbb{1}_{\{W_j\in[-C,C]\setminus\cup_{k\in\mathbb{Z}} I(k,t,\widetilde{\delta})\}}} > \delta\right).
\end{aligned} \tag{3.3.40}$$

With $c_n \equiv \frac{\log\delta - \frac{1}{2}\log n}{\log(1-\bar{\rho})}$ Equation (3.3.40) can be rewritten as

$$\begin{aligned}
&\mathbb{P}\Big(\inf_{\delta_1\leq|t|\leq\delta_2\vartheta_*}\sum_{j=1}^{n}\mathbb{1}_{\{W_j\in[-C,C]\setminus\cup_{k\in\mathbb{Z}} I(k,t,\widetilde{\delta})\}} < c_n\Big)\\
&\leq \mathbb{P}\Big(\inf_{\delta_1\leq|t|\leq\delta_2\vartheta_*}\sum_{j=1}^{n}\mathbb{1}_{\{W_j\in([-C,C]\cap[c,d])\setminus\cup_{k\in\mathbb{Z}} I(k,t,\widetilde{\delta})\}} < c_n\Big).
\end{aligned} \tag{3.3.41}$$

(3.3.41) is summable over n since the number of W_j contained in the "good" sets is of order n, i.e. $\#\{j : W_j \in [c,d]\setminus\cup_{k\in\mathbb{Z}} I(k,t,\widetilde{\delta})\} = \mathcal{O}(n)$. Define

$$K(t) = \#\{k : I(k,t,\widetilde{\delta}) \cap [c,d] \neq \emptyset\}, \tag{3.3.42}$$

and let $k_1,\dots k_{K(t)}$ enumerate the intervals contained in $[c,d]$. Let $m_1(t),\dots,m_{K(t)}(t)$ be chosen such that $W_{m_i(t)} \in I(k_i,t,\widetilde{\delta})$. Note that $m_i(t)$ are random. The probability in the last line of (3.3.41) is bounded from above by

$$\mathbb{P}\left(\inf_{\delta_1\leq|t|\leq\delta_2\vartheta_*}\sum_{j=1}^{n}\mathbb{1}_{\{W_j\in[c,d],|W_j-W_{m_1(t)}|>2\widetilde{\delta},\dots,|W_j-W_{m_{K(t)}(t)}|>2\widetilde{\delta}\}} \leq c_n\right). \tag{3.3.43}$$

Since there are only finitely many intervals of length $2\widetilde{\delta}$ with distance $6\widetilde{\delta}$ to each other in $[c,d]$, there exists $K<\infty$ such that $\sup_{t\in[\delta_1,\delta_2\vartheta_*]}K(t)<K$. Thus, the probability in (3.3.43) is not larger than

$$\begin{aligned}
&\mathbb{P}\left(\exists_{m_1,\dots,m_K\in\{1,\dots,n\}}:\sum_{j=1}^{n}\mathbb{1}_{\left\{W_j\in[c,d],|W_j-W_{m_1}|>2\widetilde{\delta},\dots,|W_j-W_{m_K}|>2\widetilde{\delta}\right\}}\leq c_n\right)\\
&\leq\sum_{m_1,\dots,m_K=1}^{n}\mathbb{P}\left(\sum_{j=1}^{n}\mathbb{1}_{\left\{W_j\in[c,d],|W_j-W_{m_1}|>2\widetilde{\delta},\dots,|W_j-W_{m_K}|>2\widetilde{\delta}\right\}}\leq c_n\right)\\
&\leq n^K\mathbb{P}\left(\sum_{j=1}^{n}\mathbb{1}_{\left\{W_j\in[c,d],|W_j-W_{m_1}|>2\widetilde{\delta},\dots,|W_j-W_{m_K}|>2\widetilde{\delta}\right\}}\leq c_n\right).
\end{aligned}\tag{3.3.44}$$

The indicator function vanishes whenever $j=m_i$ with $i\in\{1,\dots,K\}$. Thus,

$$\begin{aligned}
&\mathbb{P}\left(\sum_{j=1}^{n}\mathbb{1}_{\left\{W_j\in[c,d],|W_j-W_{m_1}|>2\widetilde{\delta},\dots,|W_j-W_{m_K}|>2\widetilde{\delta}\right\}}\leq c_n\right)\\
&=\mathbb{P}\left(\sum_{j\notin\{m_1,\dots,m_K\}}^{n}\mathbb{1}_{\left\{W_j\in[c,d],|W_j-W_{m_1}|>2\widetilde{\delta},\dots,|W_j-W_{m_K}|>2\widetilde{\delta}\right\}}\leq c_n\right)\\
&=\mathbb{P}\left(\sum_{j=K}^{n}\mathbb{1}_{\left\{W_j\in[c,d],|W_j-W_1|>2\widetilde{\delta},\dots,|W_j-W_K|>2\widetilde{\delta}\right\}}\leq c_n\right)
\end{aligned}\tag{3.3.45}$$

due to the i.i.d. assumption. (3.3.45) is equal to

$$\sum_{l=0}^{\lceil c_n\rceil}\binom{n-K}{l}\mathbb{P}(A)^l\left(1-\mathbb{P}(A)\right)^{n-K-l}\leq\lceil c_n\rceil\binom{n-K}{\lceil c_n\rceil}(1-\mathbb{P}(A))^{n-K-\lceil c_n\rceil}.\tag{3.3.46}$$

Here A is the event

$$A=\left\{W\in[c,d],|W-W_1|>2\widetilde{\delta},\dots,|W-W_K|>2\widetilde{\delta}\right\},\tag{3.3.47}$$

where W is an independent copy of W_1. We show that $\mathbb{P}(A)$ is strictly positive.

$$\begin{aligned}
\mathbb{P}(A)&=\int_{[c,d]}\mathbb{P}\left(|W-W_1|>2\widetilde{\delta},\dots,|W-W_K|>2\widetilde{\delta}\,\middle|\,W\right)dP_W\\
&\geq\int_{[c,d]}\mathbb{P}\left(W_i\in[W-2\widetilde{\delta},W+2\widetilde{\delta}]^c\cap[c,d],\forall i\in\{1,\dots,K\}\right)dP_W,
\end{aligned}\tag{3.3.48}$$

where P_W denotes the distribution of W. Since the random variables $W_1,\dots,W_K,W$ are independent of each other, this is equal to

$$\int_{[c,d]}\mathbb{P}\left(W_1\in[W-2\widetilde{\delta},W+2\widetilde{\delta}]^c\cap[c,d]|W\right)^K dP_W,\tag{3.3.49}$$

and due to the lower bound on the density of P_W postulated in Hypothesis (ia), this in turn is bounded from below by

$$(p(d-c-4\widetilde{\delta}))^K\int_{[c,d]}dP_W\geq(d-c)p^{K+1}(d-c-4\widetilde{\delta})^K\equiv\widetilde{p}\in(0,1].\tag{3.3.50}$$

Combining Equations (3.3.46) and (3.3.50) we obtain

$$(3.3.44) \leq n^K \lceil c_n \rceil \binom{n}{\lceil c_n \rceil} \widetilde{p}^{n-K-\lceil c_n \rceil} \tag{3.3.51}$$

which is summable over n, as desired. Thus all hypotheses of Theorem 3.1.3 are satisfied with probability one, uniformly in $a \in J$, and so the conclusion of Theorem 3.1.6 follows. □

3.4 Proof of Theorem 3.1.9

In order to prove Theorem 3.1.9 we need the joint weak convergence of the process X_n, defined in (3.1.11) and its derivatives, as stated in Lemma 3.4.1. Define on the closure, $\bar{J}$ of the interval J (recall the definition of J in Theorem 3.1.6), the processes $(\widehat{X}_a^n)_{a\in\bar{J}}$, $n \in \mathbb{N}$, via

$$\widehat{X}_a^n \equiv (X_n(\vartheta(a)), X_n'(\vartheta(a)), X_n''(\vartheta(a))). \tag{3.4.1}$$

Lemma 3.4.1. *The family of processes $(\widehat{X}_a^n)_{a\in\bar{J}}$ defined on $\Big(C(\bar{J},\mathbb{R}^3), \mathcal{B}(C(\bar{J},\mathbb{R}^3)\Big)$, converges weakly, as $n \to \infty$, to a process $(\widehat{X}_a)_{a\in\bar{J}}$ on the same space, if there exists $c > 0$, such that, for all $a \in \bar{J}$, $g''(\vartheta(a)) > c$, and if Assumption 3.1.8 is satisfied.*

Proof. As usual, we prove convergence of the finite dimensional distributions and tightness.

More precisely, we have to check that:

1. $(\widehat{X}_a^n)_{a\in\bar{J}}$ converges in finite dimensional distribution.
2. The family of initial distributions, i.e. the distributions of $\widehat{X}_b^n$, where $b \equiv \mathbb{E}[Z_1 W_1]$, is tight.
3. There exists $C > 0$ independent of a and n such that

$$\mathbb{E}\left[\|\widehat{X}_{a+h}^n - \widehat{X}_a^n\|^2\right] \leq C|h|^2, \tag{3.4.2}$$

which is a Kolmogorov-Chentsov criterion for tightness, see [83, Corollary 14.9].

First, we consider the finite dimensional distributions. Let

$$\begin{aligned} Y_{a,j} &\equiv f(\vartheta(a)W_j) - \mathbb{E}\left[f(\vartheta(a)W_j)\right] \\ Y'_{a,j} &\equiv W_j f'(\vartheta(a)W_j) - \mathbb{E}[W_j f'(\vartheta(a)W_j)] \quad \text{and} \\ Y''_{a,j} &\equiv W_j^2 f''(\vartheta(a)W_j) - \mathbb{E}[W_j^2 f''(\vartheta(a)W_j)]. \end{aligned} \tag{3.4.3}$$

Moreover, let $\ell \in \mathbb{N}$, $a_1 < a_2 < \cdots < a_\ell \in \bar{J}$ and

$$\chi_j \equiv \left(Y_{a_1,j}, Y'_{a_1,j}, Y''_{a_1,j}, \ldots, Y_{a_\ell,j}, Y'_{a_\ell,j}, Y''_{a_\ell,j}\right) \in \mathbb{R}^{3\ell}. \tag{3.4.4}$$

These vectors are independent for different j and the components $(\chi_j)_k, 1 \leq k \leq 3\ell$, have covariances $\mathrm{Cov}((\chi_j)_k, (\chi_j)_m) = \mathcal{C}_{km} < C$ for all $k, m \in \{1, \ldots, 3\ell\}$, according to Assumption 3.1.8. Therefore, $1/\sqrt{n}\sum_{j=1}^n \chi_j$ converges, as $n \to \infty$, to the 3ℓ-dimensional Gaussian vector with mean zero and covariance matrix $\mathcal{C}$ by the central limit theorem. This proves convergence of the finite dimensional distributions of $(\widehat{X}_a^n)_{a\in\bar{J}}$.

The family of initial distributions is given by the random variables evaluated at $\vartheta(b)$. This family is seen to be tight using Chebychev's inequality

$$\begin{aligned}\mathbb{P}\left(\|X_b^n\|_2 > C\right) &\leq \frac{\mathbb{V}\left[\sqrt{X_n(\vartheta(b))^2 + (X_n'(\vartheta(b)))^2 + (X_n''(\vartheta(b)))^2}\right]}{C^2} \\ &\leq \frac{\mathbb{E}\left[X_n(\vartheta(b))^2 + (X_n'(\vartheta(b)))^2 + (X_n''(\vartheta(b)))^2\right]}{C^2}. \end{aligned} \tag{3.4.5}$$

which is finite by Assumption 3.1.8. For each ϵ we can choose C large enough such that (3.4.5) $< \epsilon$. It remains to check Condition (3). Since

$$\begin{aligned}\mathbb{E}\left[\|\widehat{X}_{a+h}^n - \widehat{X}_a^n\|^2\right] &= \mathbb{E}\left[(X_n(\vartheta(a+h)) - X_n(\vartheta(a)))^2\right] \\ &\quad + \mathbb{E}\left[[X_n'(\vartheta(a+h)) - X_n'(\vartheta(a)))^2\right] \\ &\quad + \mathbb{E}\left[(X_n''(\vartheta(a+h)) - X_n''(\vartheta(a)))^2\right], \end{aligned} \tag{3.4.6}$$

we need to show that each of the three terms on the right-hand side is of order h^2. Note that $\mathbb{E}\left[[X_n(\vartheta(a+h)) - X_n(\vartheta(a))]^2\right] \leq C|h|^2$ if

$$\mathbb{E}\left[\left(\tfrac{d}{da} X_n(\vartheta(a))\right)^2\right] \leq C. \tag{3.4.7}$$

Since $X_n(\vartheta(a)) = \frac{1}{\sqrt{n}} \sum_{j=1}^n Y_{a,j}$,

$$\mathbb{E}\left[\left(\tfrac{d}{da} X_n(\vartheta(a))\right)^2\right] = \frac{1}{n} \sum_{j=1}^n \mathbb{E}\left[\left(\tfrac{d}{da} Y_{a,j}\right)^2\right]. \tag{3.4.8}$$

Each summand can be controlled by

$$\begin{aligned}&\mathbb{E}\left[\left(\tfrac{d}{da} Y_{a,j}\right)^2\right] = \mathbb{E}\left[\left(\tfrac{d}{da} f(\vartheta(a) W_j)\right)^2\right] - \left(\mathbb{E}\left[\tfrac{d}{da} f(\vartheta(a) W_j)\right]\right)^2 \\ &= \left(\tfrac{d}{da}\vartheta(a)\right)^2 \left(\mathbb{E}\left[W_j^2 f'(\vartheta(a) W_j)^2\right] - \left(\mathbb{E}\left[W_j f'(\vartheta(a) W_j)\right]\right)^2\right) \\ &= \left(\tfrac{d}{da}\vartheta(a)\right)^2 \mathbb{V}\left[W_j f'(\vartheta(a) W_j)\right]. \end{aligned} \tag{3.4.9}$$

By the implicit function theorem,

$$\frac{d}{da}\vartheta(a) = \left(g''(\vartheta(a))\right)^{-1}. \tag{3.4.10}$$

Thus, Equation (3.4.7) holds since $g''(\vartheta(a)) > c$ by assumption and $\mathbb{V}\left[W_j f'(\vartheta(a) W_j)\right]$ is bounded by Assumption 3.1.8. The bounds for the remaining terms follow in the same way by controlling the derivatives of $X_n'(\vartheta(a))$ and $X_n''(\vartheta(a))$. We obtain

$$\begin{aligned}\mathbb{E}\left[\left(\tfrac{d}{da} Y_{a,j}'\right)^2\right] &= \mathbb{E}\left[\left(\tfrac{d}{da} W_j f'(\vartheta(a) W_j)\right)\right] - \left(\mathbb{E}\left[\tfrac{d}{da} W_j f'(\vartheta(a) W_j)\right]\right)^2 \\ &= \left(\tfrac{d}{da}\vartheta(a)\right)^2 \mathbb{V}\left[W_j^2 f''(\vartheta(a) W_j)\right] \end{aligned} \tag{3.4.11}$$

and

$$\begin{aligned}\mathbb{E}\left[\left(\tfrac{d}{da}Y''_{a,j}\right)^2\right] &= \mathbb{E}\left[\left(\tfrac{d}{da}W_j^2 f''(\vartheta(a)W_j)\right)\right] - \left(\mathbb{E}\left[\tfrac{d}{da}W_j^2 f''(\vartheta(a)W_j)\right]\right)^2 \\ &= \left(\tfrac{d}{da}\vartheta(a)\right)^2 \mathbb{V}\left[W_j^3 f'''(\vartheta(a)W_j)\right]. \end{aligned} \tag{3.4.12}$$

In both formulae the right hand sides are bounded due to Assumption 3.1.8. This proves the lemma. □

Proof of Theorem 3.1.9. Recall that $\vartheta_n^{\mathcal{W}}(a)$ is determined as the solution of the equation

$$a = g'(\vartheta) + \frac{1}{\sqrt{n}}X'_n(\vartheta). \tag{3.4.13}$$

Write $\vartheta_n^{\mathcal{W}}(a) \equiv \vartheta(a) + \delta^n(a)$, where $\vartheta(a)$ is defined as the solution of

$$a = g'(\vartheta). \tag{3.4.14}$$

Note that $\vartheta(a)$ is deterministic while $\delta^n(a)$ is random and $\mathcal{W}$-measurable . The rate function can be rewritten as

$$I_n^{\mathcal{W}}(a) = a(\vartheta(a) + \delta^n(a)) - g(\vartheta(a) + \delta^n(a)) - \frac{1}{\sqrt{n}}X_n(\vartheta(a) + \delta^n(a)). \tag{3.4.15}$$

A second order Taylor expansion and reordering of the terms yields

$$\begin{aligned} I_n^{\mathcal{W}}(a) = \quad & \underbrace{a\vartheta(a) - g(\vartheta(a))}_{\equiv I(a)} - \frac{1}{\sqrt{n}}X_n(\vartheta(a)) \\ & + \underbrace{(a - g'(\vartheta(a)))}_{=0}\delta^n(a) - \frac{1}{\sqrt{n}}\delta^n(a)X'_n(\vartheta(a)) \\ & - \frac{1}{2}(\delta^n(a))^2\left(g''(\vartheta(a)) + \frac{1}{\sqrt{n}}X''_n(\vartheta(a))\right) + \mathrm{o}((\delta^n(a))^2). \end{aligned} \tag{3.4.16}$$

Note that the leading terms on the right-hand side involve the three components of the processes $\widehat{X}^n$ whose convergence we have just proven. We obtain the following equation for $\delta^n(a)$ using a first order Taylor expansion.

$$\begin{aligned} a &= g'(\vartheta(a) + \delta^n(a)) + \frac{1}{\sqrt{n}}X'_n(\vartheta(a) + \delta^n(a)) \qquad (3.4.17)\\ &= g'(\vartheta(a)) + \frac{1}{\sqrt{n}}X'_n(\vartheta(a)) + \delta^n(a)\left(g''(\vartheta(a)) + \frac{1}{\sqrt{n}}X''_n(\vartheta(a))\right) + \mathrm{o}(\delta^n(a)), \end{aligned}$$

which implies

$$\delta^n(a) = \frac{-\frac{1}{\sqrt{n}}X'_n(\vartheta(a))}{g''(\vartheta(a)) + \frac{1}{\sqrt{n}}X''_n(\vartheta(a))} + \mathrm{o}(\delta^n(a)). \tag{3.4.18}$$

Lemma 3.4.1 combined with $g''(\vartheta(a)) = \mathcal{O}(1)$ yields $\delta^n(a) = \mathcal{O}(1/\sqrt{n})$. We insert the expression for $\delta^n(a)$ into Equation (3.4.16) to obtain

$$\begin{aligned}
&I_n^{\mathcal{W}}(a) \\
&= I(a) - \frac{1}{\sqrt{n}} X_n(\vartheta(a)) - \frac{1}{2}\left(g''(\vartheta(a)) + \frac{1}{\sqrt{n}} X_n''(\vartheta(a))\right) \\
&\times \left[\frac{{}^1\!/_n (X_n'(\vartheta(a)))^2}{\left(g''(\vartheta(a)) + \frac{1}{\sqrt{n}} X_n''(\vartheta(a))\right)^2} - \frac{\frac{1}{\sqrt{n}} X_n'(\vartheta(a)) \mathrm{o}(\delta^n)}{\left(g''(\vartheta(a)) + \frac{1}{\sqrt{n}} X_n''(\vartheta(a))\right)} + \mathrm{o}((\delta^n)^2)\right] \\
&+ \frac{\frac{1}{n}(X_n'(\vartheta(a)))^2}{g''(\vartheta(a)) + \frac{1}{\sqrt{n}} X_n''(\vartheta(a))} + \frac{1}{\sqrt{n}} X_n'(\vartheta(a)) \mathrm{o}(\delta^n) + \mathrm{o}((\delta^n)^2). \qquad (3.4.19)
\end{aligned}$$

Combining this with the bound (3.4.18), it follows that

$$I_n^{\mathcal{W}}(a) = I(a) - \frac{1}{\sqrt{n}} X_n(\vartheta(a)) + \frac{1}{n} r_n(a), \qquad (3.4.20)$$

where

$$r_n(a) \equiv \frac{\frac{1}{2}(X_n'(\vartheta(a)))^2}{g''(\vartheta(a)) + \frac{1}{\sqrt{n}} X_n''(\vartheta(a))} + \mathrm{o}(1). \qquad (3.4.21)$$

$r_n(a)$ converges weakly due to the continuous mapping theorem and the joint weak convergence of $X_n'(\vartheta(a))$ and $X_n''(\vartheta(a))$. This completes the proof of the theorem. □

Acknowledgement. We are very grateful to Frank den Hollander for numerous discussions and valuable input. A.B. is partially supported through the German Research Foundation in the Priority Programme 1590 "Probabilistic Structures in Evolution", the Hausdorff Center for Mathematics (HCM), and the Cooperative Research Centre 1060 "The Mathematics of Emergent Effects". He is member of the Cluster of Excellence "ImmunoSensation" at Bonn University. H.M. is supported by the German Research Foundation in the Bonn International Graduate School in Mathematics (BIGS) and the Cluster of Excellence "ImmunoSensation".

4. A stochastic approach to develop effective immunotherapy strategies

4.1 Introduction

In this chapter we discuss a stochastic, individual-based model for the evolution of tumours. The model allows to study the evolution of pure tumours as well as the co-evolution of a tumour and a therapeutic environment. It is explained on the example of an immunotherapy for metastatic melanoma, namely the adoptive cell transfer therapy (ACT) of particular T-cells. The transferred T-cells are capable of killing melanoma cells expressing a certain surface marker. It has been shown experimentally that melanoma cells react to the therapy-induced inflammation by phenotypic plasticity, i.e. by switching their phenotype. The relevant surface marker is downregulated in the switched melanoma cells. Thus, these melanoma cells are not recognised by the T-cells [91].

The proposed model is able to describe the phenomena observed in the experiments. We apply it to simulate different therapy protocols, as for example different dosages or protocols involving several types of T-cells targeting different types of cancer cells. Phenotypic and genotypic heterogeneity of cancer cells within a single tumour enables therapy resistance in many cases [76, 77, 96]. Therefore, it is important to understand the interplay of heterogeneity and treatment. The model which we explain in the following serves this purpose.

In this model from population dynamics the population of cancer cells grows logistically and interacts with therapeutic agents in a predator-prey relation. We include two sources of changes of traits of cancerous cells: rare genetic mutations and fast phenotypic alterations, which we call *switches*. In the context of therapy the switch is environment-dependent, i.e. it is influenced by the molecules present in the tumour. The interplay of slow changes via mutations and fast changes via switches is worth studying with or without the context of cancer. First steps in the analysis of this setup are described in [11]. Here we focus on the biological applications and the phenomena relevant for treatment strategies, as for example the role of heterogeneity for resistance and the appearance of a relapse. We demonstrate that the occurrence of different types of relapses under the same treatment protocol can be explained by random fluctuations, which alter the long-term behaviour of the system of a tumour under treatment. For instance, when subpopulations become extinct, the system is attracted to an equilibrium that is different from the attractive equilibrium in a scenario when the subpopulation survived. This is illustrated by examples and numerical simulations in Section 4.3. In addition, we explain in Subsection 4.4.1 that cancer evolution can be accelerated by an increased mutation rate during therapy in certain setups. Furthermore, some phenomena can be observed that would maybe not have been anticipated without the model. These findings can be understood very well once observed, see for example the paragraph on the influence of the initial intensity of treatment in Subsection 4.3.4.

Let us now explain the biological and mathematical frameworks in more details.

4.1.1 Cancer evolution and therapy resistance

Cancer is still a leading cause of death throughout the world. In 2012, eight million people died due to cancer and 14 million new incidences were recorded according to the WHO [135]. Although many therapeutic approaches are successful in the beginning, very often a relapse appears after some time [76]. In order to improve treatment strategies and to achieve durable therapy responses, it is necessary to understand the mechanisms of resistance and the evolution of cancer with and without treatment.

Cancer denotes a family of diseases characterised by increased cell division, avoidance of cell death, invasion into and distraction of surrounding tissues and organs as well as the ability to metastasize [84]. In a healthy organism, cells divide and die in a controlled and balanced way. The majority of cancerous diseases arises from genetic mutations. The disease develops in a multistep process, in which several evolutional steps equip cancer cells with different properties [50, 84, 108]. This process is called *tumerogenesis*. Initiation of cancer is often caused by mutations in so-called *oncogenes* or *tumour suppressor genes*. Such genes encode for example for proliferative signalling, growth suppressors or programmed cell death, and influence the cell cycle in various ways [71, 84]. Nearly all cancers develop from a single cell [84]. The descendants of this cell accumulate various mutations. Some of them cause an evolutionary growth advantage compared to the surrounding cells. Such mutants are selected by Darwinian evolution.

This brief description shows already that the concept of a tumour as only an accumulation of cells is too simplistic and not appropriate. Instead tumours should be understood as complex tissues. They consist of many different cell types, which interact in a complex and structured way. This enables the tumour to take over the control and to evade a lot of control and security mechanisms of healthy organisms, e.g. control by the immune system. Apart from risk factors, such as environmental exposure, or inherited genetic variation, the simple intrinsic property of cell division rate is a crucial determinant for the probability that a certain tissue develops cancer in an individuals life time, see [118].

As pointed out in several reviews, the heterogeneous structure of tumours can be seen as a driving cause for the failure of treatment, see [21, 64, 77, 96] and references therein. The heterogeneity is not restricted to genotypic diversity, but includes also phenotypic changes which are not induced by genotypic changes [21, 77, 96]. Sometimes resistance is acquired very fast and the corresponding changes can be reversible. These facts indicate the crucial role of phenotypic heterogeneity [77]. Genotypic and phenotypic adaptations differ with respect to stability and heritability. Heritability of mutations is (relatively) stable and advantageous mutations can fixate. The question whether an alteration has to be heritable and thus selectable in order to be relevant is currently debated [21, 96]. Therapy induces a rapid, strong and not necessarily permanent change of the environment. This can be seen as a strong selective pressure acting in a short time frame so that changes do not necessarily need to be inherited to provide a selective advantage according to [59]. The presence of different cell types enables the tumour to exploit niches formed by therapy [66, 96]. In addition, the microenvironment strongly influences which cell types are fit at a given time [36]. According to [77] the tumour resides in a "metastable order", which can be perturbed or destroyed by the new environment created by therapy. The important role of the simultaneous, spontaneous phenotypic plasticity of tumour and immune cells is emphasized in [77].

Despite the development of new therapeutic approaches for the treatment of metastatic cancers durable responses to therapy are still hard to achieve. Resistance as well as recurrence are

issues for most targeted therapy strategies, [64, 76], and thus it is essential to understand the underlying mechanisms rigorously. Immunotherapeutic approaches, i.e. therapeutic strategies which activate the immune system to target cancer, are promising [38, 101, 109, 120]. However, they are also subject to the problem of resistance.

Melanoma under adoptive cell transfer therapy with cytotoxic T-cells: Therapeutic approach and resistance mechanism

Landsberg et al. investigate the system of melanomas under adoptive cell transfer therapy with cytotoxic T-cells in mice [91]. Before treatment a melanoma consists mainly of differentiated cells, but rare dedifferentiated cells may be present. Melanoma cells coexist in a dynamic equilibrium and switch phenotypes in both directions. As a therapeutic agent cytotoxic T-cells are transferred into mice. These T-cells are able to recognise a certain melanocyte-specific surface marker, glycoprotein 100 (gp100). They are capable of killing differentiated melanoma cells, i.e. cells bearing these markers. This therapeutic strategy induces an inflammation, during which (apart from other cytokines) the pro-inflammatory cytokine Tumour Necrosis Factor-α (TNF-α) is produced. It was shown in in vitro experiments, where cell division was inhibited, that the switch is reversible and that it occurs without cell division. The switch towards dedifferentiated cells is enhanced in the presence of TNF-α. In dedifferentiated melanoma cells the surface marker gp100, required for recognition, is down-regulated. Thus, dedifferentiated melanoma cells cannot be controlled by these T-cells. The inflammation-induced *phenotypic plasticity* serves as a resistance mechanism in this system. A relapse occurs after some time in almost every case. Phenotypic plasticity was not only observed in mouse models but also for human cell lines, showing its clinical relevance.

In addition to the effect of phenotypic plasticity, T-cells can become exhausted after some time, i.e. they lose their functionality and are not able to kill melanoma cells anymore. The state of exhaustion can in principle be reversed by re-activation. In the experiments re-activation of the T-cells could only delay the appearance of the relapse. According to careful control experiments conducted by Landsberg et al. the influence of other immune cells and cytokines in the tumour microenvironment can be neglected in the context of the situation described above [91].

4.1.2 Mathematical modelling of cancer evolution

Cancer research is an active and fast developing field, as for example indicated by the update of the publication about the "hallmarks of cancer" [71] after roughly ten years [72]. Mathematical models may help to structure the huge amount of data and to keep track of the relevant mechanisms. Moreover, they may assist in sharpening biological questions and in planning experiments. There are many mathematical modelling approaches in the context of cancer evolution, in particular in connection with immunotherapy of cancer, see for example [53, 64, 90, 108]. In general, efficient mathematical modelling of the interaction between cancer and immune cells provides a better understanding of the mechanisms behind therapy failure and may ultimately help to develop more efficient treatment protocols. So far, many deterministic and some stochastic approaches as for example branching process models, [5, 20, 50, 51], or rate models, [70], were used to study the development and treatment of cancer. To our knowledge, branching process models are mainly used to describe the early onset of cancer and do not include the interaction with therapy. Evolutionary theory can be seen as a unifying framework for understanding cancerous diseases and resistance at various stages [64, 67, 132].

Here we choose a *probabilistic* model since random effects play a crucial role and deterministic models, such as [53], cannot describe these effects. This is partially based on the fact that the number of cells and molecules in the entire tumour is relatively small, especially during remission. The model, we present in the following, comes from population dynamics and is a stochastic, *individual-based* model with density-dependence. In individual-based models the structure of the model is defined on the level of the components (called individuals, agents or particles) of the system. The model at hand is an extension of the individual-based stochastic models for adaptive dynamics introduced by Metz et al. [102] and developed and analysed by many authors in recent years, see e.g. [10, 16, 17, 19, 25, 26, 27, 29, 37, 48].

We aim to understand the co-evolution of tumour and immune cell populations and therefore include additional mechanisms, describing their interactions, into the "basic" model that was described for example in [25] or [31]. In the basic model a population grows logistically and at birth events mutations may occur. Mutant populations can invade a population and either replace the resident population or coexist with some resident traits. The models describe the Darwinian evolution of a population with asexual reproduction, mediated by heredity, mutation and selection. Most of the research in this area is concerned with the asymptotic behaviour of the models. Typically, various combinations of the limits of large populations, rare mutations and small mutational effects are considered on different timescales.

In our model, we allow for two varieties of changes in cancer cells: fast (possibly reversible) switches, which affect only the phenotype of cells, and slow genetic mutations. The switching behaviour is influenced by an environment, which in this description is constituted by cytokines. Cytokines are molecules working as chemical messengers in the immune system. The description of the evolution of these particles is also part of the stochastic model and is not described by separate, deterministic equations as in [29]. The relation between therapeutic agents, here T-cells, and cancer cells is modelled as a predator-prey term. In [37] the long-term evolution of predator-prey systems is studied. In their model general escape strategies of prey are accounted for by changing relevant parameters. We model the escape mechanism, namely switching, directly. Moreover, in our model competition cannot only increase the death rate of the individuals, but also affect their reproduction behaviour and decrease their cell division rate.

4.2 The model

We consider three classes of particles. In each class certain evolutionary events take place. More precisely, we distinguish cancer cells, T-cells (certain immune cells) and cytokines (chemical messengers of the immune system).

Cancer cells are characterised by a genotype and a phenotype. $(g, p) \in \mathcal{G} \times \mathcal{P}$ denotes the trait of a cancer cell with genotype g and phenotype p. They can divide, die (due to age, competition or therapy) or switch their phenotype. If they die from therapy, cytokines are produced at the same time. Furthermore, mutations (i.e. a change of the genotype) may appear at cell divisions.

T-cells can divide or die. Their trait is denoted by $z \in \mathcal{Z}$. At distinguished T-cell reproduction events cytokines are released.

Cytokines can only disappear, but cannot reproduce themselves. Their trait is denoted by $w \in \mathcal{W}$. Their presence influences the switching behaviour of cancer cells.

The whole trait space, $\mathcal{X}$, is a finite set of the form

$$\begin{aligned}\mathcal{X} &= \mathcal{G} \times \mathcal{P} \cup \mathcal{Z} \cup \mathcal{W} \\ &= \left\{g_1, \ldots, g_{|\mathcal{G}|}\right\} \times \left\{p_1, \ldots, p_{|\mathcal{P}|}\right\} \cup \left\{z_1, \ldots, z_{|\mathcal{Z}|}\right\} \cup \left\{w_1, \ldots, w_{|\mathcal{W}|}\right\}. \end{aligned} \tag{4.2.1}$$

Let us define

$$\mathcal{M}^K \equiv \left\{ \frac{1}{K} \sum_{i=1}^{n} \delta_{x_i}, n \geq 0, x_i \in \mathcal{X} \right\} \tag{4.2.2}$$

as the set of finite, rescaled counting measures on $\mathcal{X}$. K is a parameter scaling the population size, which is sometimes called carrying capacity. The state of the population at time t can be represented by the measure

$$\nu_t^K = \frac{1}{K} \sum_{i=1}^{N_t} \delta_{x_i(t)} \quad \in \mathcal{M}^K, \tag{4.2.3}$$

where N_t denotes the number of individuals at time t and $x_i(t) \in \mathcal{X}$ denotes the trait of the i-th individual at time t.

All evolutionary events happen at exponential waiting times depending on parameters and on the state of the population (density-dependence) as explained in Subsections 4.2.1 to 4.2.3. The sequence of these waiting times can be used to construct a continuous-time, measure-valued Markov process, which describes the co-evolution of the whole system.

For the sake of clarity we explain the generator and the dynamics of the process in three parts.

4.2.1 Natural growth

This part describes the natural growth behaviour of all individuals. Each cancer cell of type (g, p) divides at a **natural birth rate** $b(p)$. At each cell division a mutation can appear with probability $\mu(p)$. Whenever a mutation occurs, the trait of the mutant is distributed according to a **mutation kernel** $m((g,p),(g',p'))$, where in this setup $m((g,p),(g',p'))$ can be seen as a transition probability. In particular, $m((g,p),(g,p)) = 0$ and $\sum_{(g',p') \in \mathcal{G} \times \mathcal{P}} m((g,p),(g',p')) = 1$. T-cells divide at a natural birth rate $b(z)$, whereas cytokines do not reproduce since they are molecules. We do not allow for mutations of T-cells here to keep the approach simple and since this is not necessary for the applications we want to look at. All cells and cytokines die and vanish at **natural death rates** $d(p), d(z)$ and $d(w)$, respectively.

Furthermore, competition for resources can have two effects: On the one hand, cell division is lowered, modelled via a **competition kernel** $K^{-1}c_b(p,p')$. We call this effect birth-reducing competition in the following. If this effect sets the birth rate already to a level zero, then the kernel acts as an additional death rate. On the other hand, the death rate of individuals is increased, modelled via a competition kernel $K^{-1}c(p,p')$. Both kernels signify the influence of individuals of phenotype p' on individuals of phenotype p. Note that we could include competition between T-cells and cancer cells or among T-cells, but for the sake of simplicity we consider competition only among cancer cells.

The infinitesimal generator of this part of the process, $\mathcal{L}_G^K$, acts on bounded measurable functions ϕ from $\mathcal{M}^K$ into $\mathbb{R}$, for all $\eta \in \mathcal{M}^K$ by

$$
\begin{aligned}
\left(\mathcal{L}_G^K\phi\right)(\eta) = & \sum_{(g,p)\in\mathcal{G}\times\mathcal{P}} \left(\phi\left(\eta+\tfrac{\delta_{(g,p)}}{K}\right)-\phi(\eta)\right) \\
& \quad \times (1-\mu(p))\left\lfloor b(p) - \sum_{(\tilde{g},\tilde{p})\in\mathcal{G}\times\mathcal{P}} c_b(p,\tilde{p})\eta(\tilde{g},\tilde{p})\right\rfloor_+ K\eta(g,p) \\
& + \sum_{(g,p)\in\mathcal{G}\times\mathcal{P}} \left(\phi\left(\eta-\tfrac{\delta_{(g,p)}}{K}\right)-\phi(\eta)\right) \\
& \quad \times \left(d(p) + \sum_{(\tilde{g},\tilde{p})\in\mathcal{G}\times\mathcal{P}} c(p,\tilde{p})\eta(\tilde{g},\tilde{p}) + \left\lfloor b(p) - \sum_{(\tilde{g},\tilde{p})\in\mathcal{G}\times\mathcal{P}} c_b(p,\tilde{p})\eta(\tilde{g},\tilde{p})\right\rfloor_-\right) K\eta(g,p) \\
& + \sum_{z\in\mathcal{Z}} \left(\phi\left(\eta+\tfrac{\delta_z}{K}\right)-\phi(\eta)\right) b(z) K\eta(z) + \sum_{z\in\mathcal{Z}} \left(\phi\left(\eta-\tfrac{\delta_z}{K}\right)-\phi(\eta)\right) d(z) K\eta(z) \\
& + \sum_{w\in\mathcal{W}} \left(\phi\left(\eta-\tfrac{\delta_w}{K}\right)-\phi(\eta)\right) d(w) K\eta(w) \\
& + \sum_{(\tilde{g},\tilde{p})\in\mathcal{G}\times\mathcal{P}} \sum_{(g,p)\in\mathcal{G}\times\mathcal{P}} \left(\phi\left(\eta+\tfrac{\delta_{(\tilde{g},\tilde{p})}}{K}\right)-\phi(\eta)\right) \\
& \quad \times \mu(p) m((g,p),(\tilde{g},\tilde{p}))\left\lfloor b(p) - \sum_{(g',p')\in\mathcal{G}\times\mathcal{P}} c_b(p,p')\eta(g',p')\right\rfloor_+ K\eta(g,p). \qquad (4.2.4)
\end{aligned}
$$

4.2.2 Therapy

Let us now describe all effects related to therapy. In the presence of their target of phenotype p T-cells of type z divide according to an additional **reproduction kernel** $K^{-1}b(z,p)$. At each such activity-related reproduction event a number of $\ell_w^{\mathrm{prod}}(z,p)$ cytokines of type w are released. Cancer cells with phenotype p die in the presence of T-cells of type z according to a **therapy kernel** $K^{-1}t(z,p)$. At such activity-related death events a number of $\ell_w^{\mathrm{kill}}(z,p)$ cytokines of type w are produced. The relation of T-cells and cancer cells can be seen as a predator-prey system. The generator of this part of the process, $\mathcal{L}_T^K$, acts via

$$
\begin{aligned}
\left(\mathcal{L}_T^K\phi\right)(\eta) = & \sum_{(g,p)\in\mathcal{G}\times\mathcal{P}} \sum_{z\in\mathcal{Z}} \left(\phi\left(\eta-\tfrac{\delta_{(g,p)}}{K}+\sum_{w\in\mathcal{W}}\ell_w^{\mathrm{kill}}(z,p)\tfrac{\delta_w}{K}\right)-\phi(\eta)\right) t(z,p)\eta(z)K\eta(g,p) \qquad (4.2.5) \\
& + \sum_{z\in\mathcal{Z}} \sum_{(g,p)\in\mathcal{G}\times\mathcal{P}} \left(\phi\left(\eta+\tfrac{\delta_z}{K}+\sum_{w\in\mathcal{W}}\ell_w^{\mathrm{prod}}(z,p)\tfrac{\delta_w}{K}\right)-\phi(\eta)\right)\left(b(z,p)\eta(g,p)\right)K\eta(z)
\end{aligned}
$$

4.2.3 Switching

Each cancer cell can switch its phenotype p into phenotype p' without changing its genotype g according to a **switch kernel** $K^{-1}s^g(p,p')$. In the presence of a cytokine of type w there is an additional **messenger-induced switch kernel** $K^{-1}s_w^g(p,p')$. The generator of this part of the

process, $\mathcal{L}_S^K$, acts via

$$\left(\mathcal{L}_S^K\phi\right)(\eta) = \sum_{(g,p)\in\mathcal{G}\times\mathcal{P}}\sum_{\tilde{p}\in\mathcal{P}}\left(\phi\left(\eta+\frac{\delta_{(g,\tilde{p})}}{K}-\frac{\delta_{(g,p)}}{K}\right)-\phi(\eta)\right) \times\left(s^g(p,\tilde{p})+\sum_{w\in\mathcal{W}}s_w^g(p,\tilde{p})\eta(w)\right)K\eta(g,p). \tag{4.2.6}$$

The measure-valued process $(\nu_t^K)_{t\geq 0}$ is a Markov process, whose law is characterised by its infinitesimal generator $\mathcal{L}^K$, which captures the dynamics described above and is defined by

$$\mathcal{L}^K = \mathcal{L}_G^K + \mathcal{L}_T^K + \mathcal{L}_S^K. \tag{4.2.7}$$

Remark 4.2.1.

1. All "visible" properties of the cancer cells such as birth or death rates depend only on the phenotype. The genotype defines the set of possible phenotypes and the dynamic equilibria via the associated switch kernels. The switch kernels specify which phenotypes are expressed by a given genotype in which proportions in a (dynamic) environment.
2. Switching, T-cell reproduction and killing of melanoma cells are multiple events in this description: at one time the state of several populations is changed. For switching one cancer cell disappears and another one appears, for T-cell reproduction a T-cell and cytokines appear, and for killing a cancer cell disappears and cytokines appear.
3. Such models can also be constructed on a continuous trait space. In order to avoid technicalities we restrict ourselves to the finite trait space, which is sufficient for the applications in this thesis.

4.2.4 Large population approximation

The preceding explanation defines a sequence of continuous-time, measure-valued Markov processes, which describe the co-evolution of tumour and immune cells as well as the influence of the inflammatory environment. We are interested in the asymptotic behaviour of the system in the limit of large populations, i.e. we let the carrying capacity K tend to infinity. In this limit, the sequence of rescaled processes, $((\nu_t^K)_{t\geq 0})_K$, converges almost surely to the solution of a system of differential equations, as stated in Corollary 4.2.3. This result can be seen as a law of large numbers. It is a consequence of a law of large numbers for a collection of density-dependent population processes with finitely many traits due to Ethier and Kurtz, [55]. Let us now introduce some notation in order to restate this result.

Let $E \subset \mathbb{R}^d$ and $E_K = E \cap \{K^{-1}u : u \in \mathbb{Z}^d\}$. We consider a collection of non-negative functions $\beta_l, l \in \mathbb{Z}^d$ on E, such that for $x \in E_K$ and $\beta_l(x) > 0$ also $x + K^{-1}l \in E_K$. Let $((X_K(t))_{t\geq 0})_K$ be a sequence of Markov jump processes with state space E_K and transition rates $q_{x,y}^K$ from x to y given by

$$q_{x,y}^K \equiv K\beta_{K(y-x)}(x) \quad \text{for} \quad x, y \in E_K. \tag{4.2.8}$$

We define $F(x) \equiv \sum_l l\beta_l(x)$ and denote by $(Y_l)_{l\in\mathbb{Z}^d}$ a family of standard Poisson processes and by $\widetilde{Y}_l(u) \equiv Y_l(u) - u$ the centred versions of these processes. When $X_K(0)$ denotes the initial condition, X_K satisfies

$$X_K(t) = X_K(0) + \sum_l \frac{l}{K}\widetilde{Y}_l\left(K\int_0^t \beta_l(X_K(s))\mathrm{d}s\right) + \int_0^t F(X_K(s))\mathrm{d}s \tag{4.2.9}$$

as long as only finitely many jumps occurred.

Theorem 4.2.2 (Theorem 2.1 in Chapter 11 in [55]). *Suppose that for each compact $S \subset E$,*

$$\sum_l |l| \sup_{x\in S} \beta_l(x) < \infty \tag{4.2.10}$$

and that there exists $M_S > 0$ such that

$$|F(x) - F(y)| \le M_S|x-y|, \qquad x, y \in S. \tag{4.2.11}$$

Suppose that X_K satisfies Equation (4.2.9), $\lim_{K\to\infty} X_K(0) = x_0$, and X satisfies

$$X(t) = x_0 + \int_0^t F(X(s))\mathrm{d}s, \quad t \ge 0. \tag{4.2.12}$$

Then, for every $T \ge 0$,

$$\lim_{K\to\infty} \sup_{t\in[0,T]} |X_K(t) - X(t)| = 0 \quad a.s. \tag{4.2.13}$$

Since the trait space $\mathcal{X}$ is finite, the population in our model can be represented as a vector $V_K(t) := (\nu_t^K(x))_{x\in\mathcal{X}}$ of dimension $d = |\mathcal{G}| \cdot |\mathcal{P}| + |\mathcal{Z}| + |\mathcal{W}|$. We present the population as a measure in the definition of the model (see Equation (4.2.3)), since this is more convenient for continuous trait spaces.

Corollary 4.2.3. *Suppose that the initial conditions converge almost surely to a deterministic limit, i.e. $\lim_{K\to\infty} V_K(0) = v(0)$. Then, for each $T \in \mathbb{R}_+$ the sequence of rescaled processes $(V_K(t))_{0\le t\le T}$ converges almost surely as $K \to \infty$ to the d-dimensional deterministic process which is the unique solution to the following dynamical system:*

$$\begin{aligned}
\frac{\mathrm{d}\mathfrak{n}_{(g,p)}}{\mathrm{d}t} &= \mathfrak{n}_{(g,p)} \Bigg((1-\mu(p)) \Bigg\lfloor b(p) - \sum_{(\tilde g,\tilde p)\in\mathcal{G}\times\mathcal{P}} c_b(p,\tilde p)\mathfrak{n}_{(\tilde g,\tilde p)} \Bigg\rfloor_+ \\
&\qquad - \Bigg\lfloor b(p) - \sum_{(\tilde g,\tilde p)\in\mathcal{G}\times\mathcal{P}} c_b(p,\tilde p)\mathfrak{n}_{(\tilde g,\tilde p)} \Bigg\rfloor_- - d(p) - \sum_{(\tilde g,\tilde p)\in\mathcal{G}\times\mathcal{P}} c(p,\tilde p)\mathfrak{n}_{(\tilde g,\tilde p)} \\
&\qquad - \sum_{z\in\mathcal{Z}} t(z,p)\mathfrak{n}_z - \sum_{\tilde p\in\mathcal{P}} \Bigg(s^g(p,\tilde p) + \sum_{w\in\mathcal{W}} s_w^g(p,\tilde p)\mathfrak{n}_w \Bigg) \Bigg) \\
&\quad + \sum_{(\tilde g,\tilde p)\in\mathcal{G}\times\mathcal{P}} \mathfrak{n}_{(\tilde g,\tilde p)} \Bigg(\mu(\tilde p)\, m((\tilde g,\tilde p),(g,p)) \Bigg\lfloor b(\tilde p) - \sum_{(g',p')\in\mathcal{G}\times\mathcal{P}} c_b(\tilde p,p')\mathfrak{n}_{(g',p')} \Bigg\rfloor_+ \Bigg) \\
&\quad + \sum_{\tilde p\in\mathcal{P}} \mathfrak{n}_{(g,\tilde p)} \Bigg(s^g(\tilde p,p) + \sum_{w\in\mathcal{W}} s_w^g(\tilde p,p)\mathfrak{n}_w \Bigg), \qquad (g,p) \in \mathcal{G}\times\mathcal{P} \\
\frac{\mathrm{d}\mathfrak{n}_z}{\mathrm{d}t} &= \mathfrak{n}_z \Bigg(b(z) - d(z) + \sum_{(g,p)\in\mathcal{G}\times\mathcal{P}} b(z,p)\mathfrak{n}_{(g,p)} \Bigg), \qquad z \in \mathcal{Z} \\
\frac{\mathrm{d}\mathfrak{n}_w}{\mathrm{d}t} &= -\mathfrak{n}_w d(w) + \sum_{(g,p)\in\mathcal{G}\times\mathcal{P}} \mathfrak{n}_{(g,p)} \sum_{z\in\mathcal{Z}} \Big(\ell_w^{\mathrm{kill}}(z,p)\, t(z,p) + \ell_w^{\mathrm{prod}}(z,p)\, b(z,p) \Big)\mathfrak{n}_z, \qquad w \in \mathcal{W}.
\end{aligned} \tag{4.2.14}$$

More precisely, $\mathbb{P}\left(\lim_{K\to\infty} \sup_{0\le t\le T} |V_K(t) - \mathfrak{n}(t)| = 0\right) = 1$, where $\mathfrak{n}(t)$ denotes the solution to Equations (4.2.14) with initial condition $v(0)$.

Proof. The process $V_K(t)$ can be constructed explicitly from a finite collection of Poisson processes. To each possible event corresponds a standard Poisson Process Y_l. The index $l \in \mathbb{Z}^d$ encodes the possible transitions between (non-rescaled) population states and $\beta_l : \mathbb{R}^d \to \mathbb{R}_+$ yields the corresponding transition rates $q^K_{x,y} = K\beta_l(x)$ from x to y, where $l = K(x-y)$. For a fixed order of the d elements of $\mathcal{X}$, say $x_1, \ldots, x_d$, we denote for each $x \in \mathcal{X}$ by e_x the unit vector with a 1 at position k, when $x = x_k$. In a similar way we denote by v_x the k-th entry of a vector $v \in \mathbb{R}^d$, when $x = x_k$. With a slight abuse of notation, the different possible events, their corresponding l and β_l for a *rescaled* population in state $v \in \mathbb{R}^d$ are

1. Birth of an individual of type x (clonal or mutational):

$$l = e_x, \tag{4.2.15}$$

with

$$\begin{aligned}\beta_l(v) &= (1-\mu(p))\left[b(p) - \sum_{(\tilde{g},\tilde{p})\in\mathcal{G}\times\mathcal{P}} c_b(p,\tilde{p})v_{(\tilde{g},\tilde{p})}\right]_+ v_{(g,p)} \\ &\quad + \sum_{(\tilde{g},\tilde{p})\in\mathcal{G}\times\mathcal{P}} \left(\mu(\tilde{p})\, m((\tilde{g},\tilde{p}),(g,p)) \left[b(\tilde{p}) - \sum_{(g',p')\in\mathcal{G}\times\mathcal{P}} c_b(\tilde{p},p')v_{(g',p')}\right]_+\right) v_{(\tilde{g},\tilde{p})},\end{aligned} \tag{4.2.16}$$

when $x = (g,p)$ and

$$\beta_l(v) = b(z)v_z, \tag{4.2.17}$$

when $x = z$.

2. Production of a T-cell of type z in presence of a cancer cell with phenotype p combined with cytokine production:

$$l = e_z + \sum_{w\in\mathcal{W}} \ell^{\text{prod}}_w(z,p)e_w, \tag{4.2.18}$$

with

$$\beta_l(v) = \left(\sum_{g\in\mathcal{G}} b(z,p)v_{(g,p)}\right) v_z. \tag{4.2.19}$$

3. Death of a particle of type x (natural or due to competition):

$$l = -e_x \tag{4.2.20}$$

with

$$\beta_l(v) = \left(d(p) + \sum_{(\tilde{g},\tilde{p})\in\mathcal{G}\times\mathcal{P}} c(p,\tilde{p})v_{(\tilde{g},\tilde{p})} + \left[b(p) - \sum_{(\tilde{g},\tilde{p})\in\mathcal{G}\times\mathcal{P}} c_b(p,\tilde{p})v_{(\tilde{g},\tilde{p})}\right]_-\right) v_{(g,p)} \tag{4.2.21}$$

when $x = (g,p)$ and

$$\beta_l(v) = d(x)v_x, \tag{4.2.22}$$

when $x \in \mathcal{Z} \cup \mathcal{W}$.

4. Therapy-induced death of a cancer cell of type (g,p) in presence of a T-cell of type z combined with cytokine production:

$$l = -e_{(g,p)} + \sum_{w \in \mathcal{W}} \ell_w^{\text{kill}}(z,p) e_w, \tag{4.2.23}$$

with

$$\beta_l(v) = t(z,p) v_z v_{(g,p)}. \tag{4.2.24}$$

5. Switch from type (g,p) to type $(g,\tilde{p})$,

$$l = -e_{(g,p)} + e_{(g,\tilde{p})} \tag{4.2.25}$$

with

$$\beta_l(v) = \left(s^g(p,\tilde{p}) + \sum_{w \in \mathcal{W}} s_w^g(p,\tilde{p}) v_w \right) v_{(g,p)}. \tag{4.2.26}$$

For transitions not mentioned above $\beta_l(v) = 0$. Furthermore, $V_K(t)$ satisfies

$$V_K(t) = V_K(0) + \sum_l l K^{-1} \widetilde{Y}_l \left(K \int_0^t \beta_l \left(V_K(s) \right) \mathrm{d}s \right) + \int_0^t F\left(V_K(s) \right) \mathrm{d}s, \tag{4.2.27}$$

where $\widetilde{Y}_l(u) = Y_l(u) - u$ is the Poisson process centred at its expectation and $F(v) \equiv \sum_l l \beta_l(v)$ for $v \in \mathbb{R}^d$. Since only finitely many transitions are possible and all event rates are finite and continuous in v we have for each compact set S,

$$\sum_l |l| \sup_{v \in S} \beta_l(v) < \infty. \tag{4.2.28}$$

Moreover, $F(v)$ is Lipschitz continuous on compact subsets of $\mathbb{R}_+^d$ and $\mathfrak{n}(t)$ satisfies for every $t \geq 0$

$$\mathfrak{n}(t) = v(0) + \int_0^t F(\mathfrak{n}(s)) \mathrm{d}s. \tag{4.2.29}$$

Thus, the claimed result follows from Theorem 4.2.2. □

The meaning of this convergence result is illustrated in Figure 4.1. The trajectories of the stochastic process, as shown on the left-hand side of Figure 4.1, converge to the trajectories of the solution of the deterministic system, as shown on the right-hand side. Each line represents the rescaled population size of one subpopulation. The underlying example is explained in more detail in Subsection 4.3.2, where also the parameters used for the generation of the pictures are specified.

4.2.5 Simulations

The examples in the following Sections 4.3 and 4.4 rely on simulations. Simulations were run with a computer programme implemented by Boris Prochnau. The programme is based on a Gillespie-like algorithm, [63], where all events for the stochastic process are simulated, i.e. the simulation is exact and does not use approximative results.

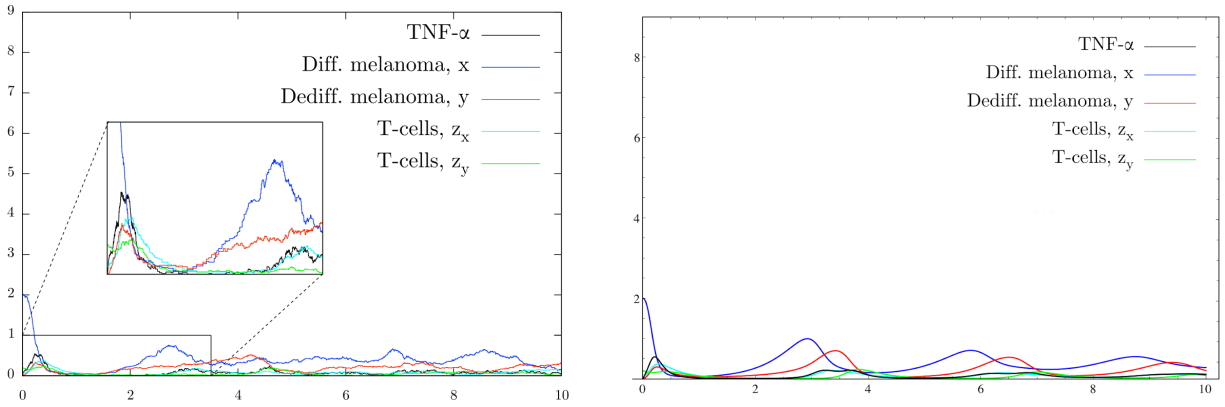


Figure 4.1: Each line describes the trajectory of one subpopulation of a population of interacting cancer and immune cells. On the left-hand side the trajectories of the stochastic process are shown, whereas on the right-hand side the trajectories of a solution of the deterministic system obtained in the limit are depicted.

Note that for $X_1, \ldots, X_k$ independent, exponentially distributed random variables with parameters $\lambda_1, \ldots, \lambda_k$

$$\min\{X_1, \ldots, X_k\} \sim \text{Exp}(\lambda_1 + \cdots + \lambda_k). \tag{4.2.30}$$

Due to this property it is possible to use following simplification in order to obtain the sequence of evolutionary events for models as described above: Sample one exponential random variable indicating the time for the next evolution step and decide which event happened afterwards instead of sampling exponential random variables for all possible events in order to obtain their minimum and the corresponding event.

The steps performed in the algorithm can be summarised as follows.

1. Initialize: Read the initial data and parameters.

2. Calculate and save for each trait, which is present in the population at this step, the event rates for each type of event and the total event rate for this trait.

3. Calculate the total event rate for the whole system, r_{tot}, and sample the time for the next evolution step from an exponentially distributed random variable with parameter r_{tot}.

4. Choose which event happens:

   - Sample from a uniform distribution on the interval $[0, r_{tot}]$, *which* trait changes. To each trait corresponds a part of the interval $[0, r_{tot}]$ of length r_x, where r_x denotes the total event rate of trait $x \in \mathcal{X}$. When the sampled number falls into the interval corresponding to trait x, this trait is changed.
   - Sample from a uniform distribution on the interval $[0, r_x]$, *how* this trait is changed, i.e. which event happens. To each event for the chosen trait corresponds a part of the interval $[0, r_x]$ of length $r_{eventtype}$, where $r_{eventtype}$ denotes the corresponding event rate. When the sampled number falls into the interval corresponding to a certain event type, execute this event.

5. Execute the chosen event. When the event is a birth event for a cancer cell, decide whether a mutation appears. If a mutation appears, sample the trait of the mutant. When the event is a switch, sample the phenotype, to which the individual switches. As long as the maximal number of iterations is not reached, go back to Step 2.

4.3 Influence of random fluctuations on the appearance of a relapse

A crucial difference between the stochastic systems we consider here and their deterministic counterparts is the possibility of extinction of (sub)populations in the stochastic system. Already the extinction of one subpopulation can alter the long-term behaviour of the whole system. For example, the populations can be attracted to different equilibria, depending on the set of surviving traits.

In the context of cancer and treatment, this can be seen as an explanation for different types of relapses observed under the same treatment protocol. This is explained in more detail in the following subsections.

4.3.1 Therapy with T-cells of one specificity

The aim of the following example is to model the experiments of [91] *qualitatively*. That means that we want to construct an example which includes the treatment of melanoma with cytotoxic T-cells and the escape from therapy by phenotypic plasticity. Thus, the trait space consists of four traits: differentiated melanoma cells with trait x, dedifferentiated melanoma cells with trait y, T-cells with trait z_x and cytokines (TNF-α) with trait w.

Both melanoma populations grow logistically and switch in both directions. When T-cells are introduced into the system, they reproduce in presence of their target, while differentiated melanoma cells die at an additional rate in the presence of these T-cells. TNF-α is secreted, when melanoma cells are killed. T-cells can also die or become exhausted and thus vanish at a certain rate that takes both effects into account. The presence of TNF-α introduces an additional unidirectional switch from differentiated to dedifferentiated melanoma cells. TNF-α vanishes at a certain rate. The deterministic limit of a stochastic model of the form introduced in the previous sections, which incorporates the effects just mentioned, is given by

$$\begin{aligned}
\dot{\mathfrak{n}}_x &= \mathfrak{n}_x\big(b(x) - d(x) - c(x,x)\mathfrak{n}_x - c(x,y)\mathfrak{n}_y - s(x,y) - s_w(x,y)\mathfrak{n}_w - t(z_x,x)\mathfrak{n}_{z_x}\big) + s(y,x)\mathfrak{n}_y \\
\dot{\mathfrak{n}}_y &= \mathfrak{n}_y\big(b(y) - d(y) - c(y,y)\mathfrak{n}_y - c(y,x)\mathfrak{n}_x - s(y,x)\big) + s(x,y)\mathfrak{n}_x + s_w(x,y)\mathfrak{n}_w\mathfrak{n}_x \\
\dot{\mathfrak{n}}_{z_x} &= \mathfrak{n}_{z_x}\big(b(z_x,x)\mathfrak{n}_x - d(z_x)\big) \\
\dot{\mathfrak{n}}_w &= -\mathfrak{n}_w d(w) + \big(\ell_w^{\mathrm{kill}}(z_x,x)\, t(z_x,x) + \ell_w^{\mathrm{prod}}(z_x,x)\, b(z_x,x)\big)\mathfrak{n}_x\mathfrak{n}_{z_x}.
\end{aligned} \tag{4.3.1}$$

Due to the switch terms a rigorous analysis of the system, even a calculation of the fixed points, is difficult. Therefore, we analyse the system for the following set of parameters and

initial values numerically. Parameters and initial conditions are chosen as

$$\begin{array}{llll} b(x)=3 & b(y)=3 & b(z_x,x)=8 & \ell_w^{\text{kill}}(z_x,x)=1 \\ d(x)=1 & d(y)=1 & t(z_x,x)=28 & \ell_w^{\text{prod}}(z_x,x)=0 \\ c(x,x)=0.3 & c(y,x)=0 & d(z_x)=3 & d(w)=15 \\ c(x,y)=0 & c(y,y)=0.3 & & s_w(x,y)=4 \\ s(x,y)=0.1 & s(y,x)=1 & & \end{array} \tag{4.3.2}$$

and

$$(\mathfrak{n}_x,\mathfrak{n}_y,\mathfrak{n}_{z_x},\mathfrak{n}_w)(0)=(2,0,0,0.05,0). \tag{4.3.3}$$

The system has three fixed points, but only one of them is stable. We denote the stable fixed point by P_{xyz_xw}. All populations are present at a non-zero level at this fixed point. For initial conditions as in the example, the deterministic system is attracted to this fixed point. In addition to P_{xyz_xw} there are two more fixed points: First, P_{0000}, where all populations are absent. This fixed point is unstable. Second, P_{xy00}, where both melanoma populations are present, but T-cells and TNF-α are absent. This fixed point is unstable in the four-dimensional space but stable in the invariant subspace where $\{\mathfrak{n}_x=0\}$.

This fact is very important when the stochastic system is considered. As mentioned above the *deterministic* system is attracted to P_{xyz_xw} for initial conditions as indicated in (4.3.3) or similar ones. The T-cell population, $\mathfrak{n}_{z_x}$, increases in presence of its target, while the differentiated melanoma population, $\mathfrak{n}_x$, shrinks in the presence of the T-cells. TNF-α is secreted and its presence intensifies switching form differentiated to dedifferentiated melanoma cells. Thus, the population of dedifferentiated melanoma cells, $\mathfrak{n}_y$, grows. After a short phase of oscillation the system levels off around P_{xyz_xw}.

Let us now study the *stochastic* system. One possible behaviour of the stochastic system is that it approaches P_{xyz_xw}, too. A typical trajectory, i.e. one close to the one of the deterministic system, passes through a phase of remission. In this phase the T-cell population drops to a low level and becomes extinct with positive probability. Once this subpopulation died out, also the TNF-α population becomes extinct. Both populations cannot reappear and the remaining melanoma cells equilibrate around P_{xy00}, the fixed point which is stable in the respective invariant subspace. The case where the T-cell population survives is qualitatively shown in Fig. 4.2 C, and the one where they become extinct is shown in Fig. 4.2 D. The pictures include a second T-cell population, which becomes extinct quite fast and has only very little impact. This is explained in more detail in the following Subsection 4.3.2.

Note that depending on the choice of parameters (in particular switching, therapy or cross-competition), a variety of different behaviour is possible.

To analyse the extinction times and probabilities in the phenomenon described above is both interesting and challenging. From the medical point of view, a better understanding of the circumstances for extinction of the therapeutic agents is desirable. From the mathematical point of view, it is important to note that the system in this example is not at equilibrium at the time of extinction. The probability of extinction in the phase of remission depends strongly on K. The extinction probability can only be high in a short phase as long as the value of the minimum, $\mathfrak{n}_{z_x}^{\min}$, is small enough compared to K. Thus, for very large K this effect probably disappears and a population can only become extinct after a long enough time.

4.3.2 Therapy with T-cells of two specificities

A therapy can only be called successful if the whole tumour is eradicated or kept small for a long time. A natural idea is thus to inject two types of T-cells in future therapies as suggested in [91]. To model this scenario, we add T-cells attacking the dedifferentiated cells as new actors to the setting described above. We denote them by z_y. The system contains one more predator-prey term between y and z_y:

$$\begin{aligned}
\dot{\mathfrak{n}}_x &= \mathfrak{n}_x\Big(b(x) - d(x) - c(x,x)\mathfrak{n}_x - c(x,y)\mathfrak{n}_y - s_w(x,y)\mathfrak{n}_w - s(x,y) - t(z_x,x)\mathfrak{n}_{z_x}\Big) + s(y,x)\mathfrak{n}_y \\
\dot{\mathfrak{n}}_y &= \mathfrak{n}_y\Big(b(y) - d(y) - c(y,y)\mathfrak{n}_y - c(y,x)\mathfrak{n}_x - s(y,x) - t(z_y,y)\mathfrak{n}_{z_y}\Big) + \big(s_w(x,y)\mathfrak{n}_w + s(x,y)\big)\mathfrak{n}_x \\
\dot{\mathfrak{n}}_{z_x} &= \mathfrak{n}_{z_x}(b(z_x,x)\mathfrak{n}_x - d(z_x)) \\
\dot{\mathfrak{n}}_{z_y} &= \mathfrak{n}_{z_y}(b(z_y,y)\mathfrak{n}_y - d(z_y)) \\
\dot{\mathfrak{n}}_w &= -\mathfrak{n}_w d(w) + (\ell_w^{\mathrm{kill}}(z_x,x)\, t(z_x,x) + \ell_w^{\mathrm{prod}}(z_x,x)\, b(z_x,x))\mathfrak{n}_x\mathfrak{n}_{z_x} \\
&\qquad + (\ell_w^{\mathrm{kill}}(z_y,y)\, t(z_y,y) + \ell_w^{\mathrm{prod}}(z_y,y)\, b(z_y,y))\mathfrak{n}_y\mathfrak{n}_{z_y}
\end{aligned} \tag{4.3.4}$$

In addition to parameters (4.3.2), we use the following ones:

$$\begin{array}{lll} t(z_y,y) = 28 & \ell_w^{\mathrm{kill}}(z_y,y) = 1 & d(z_y) = 3 \\ b(z_y,y) = 14 & \ell_w^{\mathrm{prod}}(z_y,y) = 0 & \end{array} \tag{4.3.5}$$

and initial conditions:

$$(\mathfrak{n}_x, \mathfrak{n}_y, \mathfrak{n}_{z_x}, \mathfrak{n}_{z_y}, \mathfrak{n}_w)(0) = (2, 0, 0, 0.05, 0.2, 0). \tag{4.3.6}$$

The introduction of z_y adds two new fixed points: $P_{xyz_xz_yw}$ is the new stable fixed point with all non-zero populations, and P_{xy0z_yw} corresponds to the absence of the T-cell population of type z_x. The invariant subspaces are now $\{\mathfrak{n}_{z_x} = 0\}$, in which P_{xy0z_yw} is stable, $\{\mathfrak{n}_{z_y} = 0\}$, in which P_{xyz_x0w} is stable and $\{\mathfrak{n}_{z_x} = 0\} \cap \{\mathfrak{n}_{z_y} = 0\}$, in which P_{xy000} is stable. Note that P_{xyz_x0w}, corresponding to P_{xyz_xw} from the last subsection, is unstable in the enlarged space.

With the same initial conditions as before and $\mathfrak{n}_{z_y}(0)$ small but positive, the *deterministic* system is attracted to the stable fixed point $P_{xyz_xz_yw}$: the T-cell population, $\mathfrak{n}_{z_x}$, increases in presence of its target x, TNF-α is secreted, and the differentiated melanoma population shrinks due to killing and switching, the population of dedifferentiated melanoma grows, but is regulated and kept at a low level by the T-cells of type z_y. Similarly, $\mathfrak{n}_x$ is regulated by $\mathfrak{n}_{z_x}$.

We choose the parameters such that the minima of the two types of T-cells during remission are low, so that they have a large enough probability to die out in the stochastic system. Since at the beginning of therapy no or only very few dedifferentiated melanoma cells are present, the population of T-cells of type z_y starts growing only later. In order to avoid their early extinction a higher initial amount of these T-cells can be injected. There are now five main different scenarios in the stochastic system (see Figure 4.2). Either the T-cells of type z_x (B), or the T-cells of type z_y (C), or both of them die out (D). Also all populations can survive for some time fluctuating around their joint equilibrium (A). The fifth scenario is a cure, i.e. the extinction of the entire tumour due to the simultaneous attack of the two different T-cell types (F). T-cells and TNF-α vanish since they are not produced any more in the absence of their target. Of course, transitions between the different scenarios are also possible, e.g. the system could pass from Case (A) to (B)

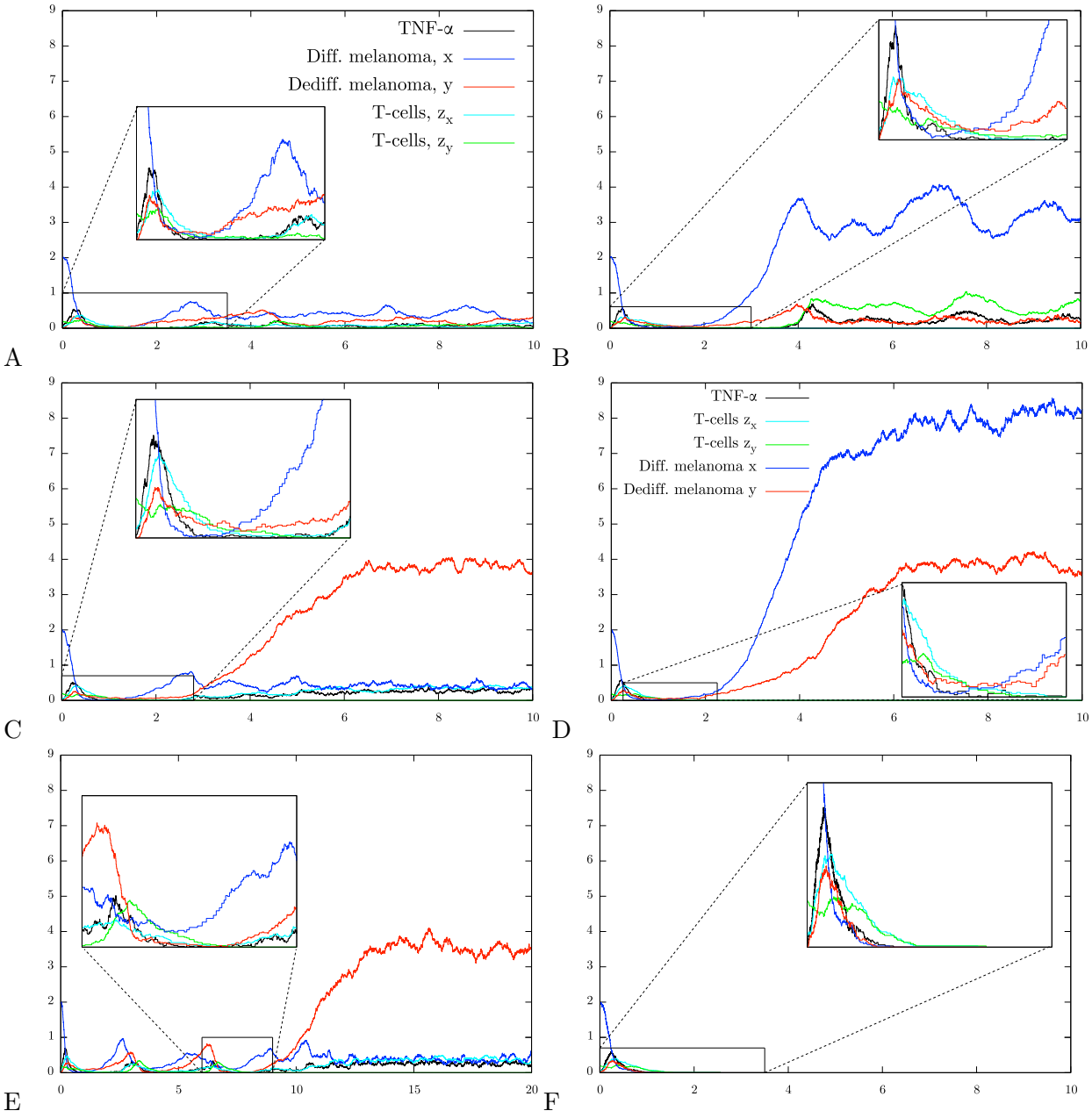


Figure 4.2: Simulations of the stochastic evolution of melanoma under T-cell therapy for parameters (4.3.3) and (4.3.5). The graphs show the number of individuals divided by 200 versus time. Possible scenarios for therapy with T-cells of two specificities: (A) T-cells z_x and z_y survive and the system stays close to $P_{xyz_xz_yw}$, (B) T-cells z_x die out and the system is attracted to P_{xy0z_yw}, (C) T-cells z_y die out and the system is attracted to P_{xyz_x0w}, (D) Both T-cell types z_x and z_y die out and the system is attracted to P_{xy000}. (E) Transition between cases (A) and (C). (F) the tumour is eradicated (corresponding to P_{00000}).

or (C) and then to (D), see Figure 4.2 (E). Furthermore, note that setting the switch from x to y to zero introduces an additional scenario: it is then possible that a relapse appears, which

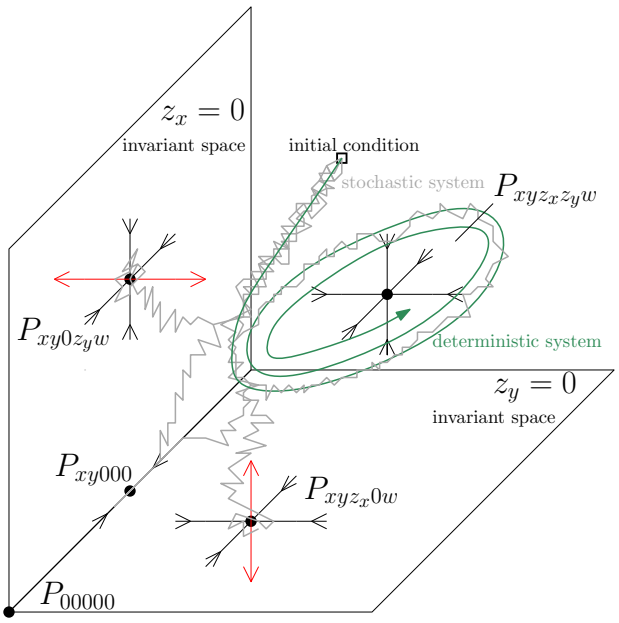


Figure 4.3: Sketch of the invariant subspaces, stability of the fixed points, and schematic representation of the dynamics of the deterministic and the stochastic processes.

consists only of differentiated melanoma cells.

Starting from our choice of initial conditions, the deterministic system converges to $P_{xyz_xz_y}$, but the stochastic system can hit one of the invariant hyperplanes due to fluctuations, and is driven to different possible fixed points, see Figure 4.3. The transitions between the different scenarios can be seen as a metastability phenomenon.

4.3.3 Reproduction of experimental observations and predictions

Comparison of experimental observations and simulations

The parameters of Subsections 4.3.1 and 4.3.2 are chosen ad hoc to highlight the influence of randomness and the possible behaviour of the system. Let us now show that our models are capable to reproduce the experimental data of Landsberg et al. [91] *quantitatively*. The choice of parameters is explained below (Subsection 4.3.4).

Figure 4.4 (A) shows the experimental data of [91], whereas Figure 4.4 (B) shows the results of our simulations. Each curve describes the evolution of the diameter of the tumour over time. In the stochastic system two situations can occur: first, the relapse consists mainly of differentiated melanoma cells and the tumour reaches its original size again after 90 days. This is the case if the T-cells die out. Second, the relapse consists mainly of dedifferentiated cells and the tumour reaches its original size again after roughly 190 days. This is the case if the T-cells survive the phase of remission, become active again and kill differentiated cancer cells. In the simulations the therapy with one type of T-cells pushes the tumour down to a microscopic level for 50 to 60 days, as in the experimental data. The curves marked ACT in the experimental data in Figure 4.4 (A) are matched by simulation data when the T-cells die out (Differentiated relapse in Figure 4.4 (B)). In the experiments there might be T-cells, which lose their function, e.g. due to exhaustion, and cannot kill the differentiated melanoma cells. This effect is to be seen as

included in the death rate of T-cells in the model. They can be re-stimulated and become active again, which is marked as ACT+Re in Figure 4.4 (A). Although our model does not include re-stimulation, the case of surviving T-cells in the simulations (Dediff. relapse in Figure 4.4(B)) can qualitatively be interpreted as the case of ACT+Re. Note that the scales of the axes are the same in both figures and that the experimental findings are met very well by the simulations. The simulated curves under treatment start at the beginning of the treatment and not at day zero. The detailed pictures showing the evolution of melanoma and T-cell populations during the therapy are given in Figure 4.5.

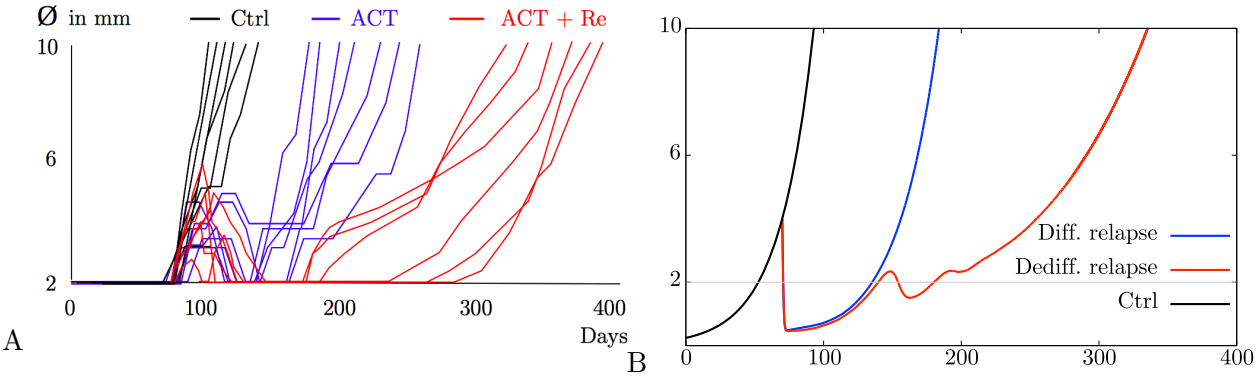


Figure 4.4: Comparison of experimental data obtained by Landsberg et al. with simulations for biologically reasonable parameters. The graphs show the diameter of the tumour measured in millimeters versus time in days after tumour initiation: (A) experimental data, (B) simulated data ($K = 10^5$ and $\mathfrak{n}_{z_x}(0) = 0.02$).

4.3.4 Predictions about success of therapy with two T-cell types and the influence of the initial dose

As there is no data for the case of two T-cells, numerical simulations of such a therapy strategy should be seen as predictions. For the new T-cell population (of type z_y) we choose the same parameters as for the first population (of type z_x), just the target is different. The therapy seems to be very promising: almost all simulations show a cure for these parameters, only very few times a relapse occurs. Nevertheless, the behaviour of the system (e.g. the probability to end up in the different scenarios) depends strongly on the choice of certain parameters, as pointed out in Subsections 4.3.1 and 4.3.2. In order to give a reliable prediction we need data to obtain safer estimates for the most important parameters, which seem to be the switching and therapy rates as well as initial values.

The initial values play an important role for the success of a therapy. In the case of therapy with T-cells of one specificity, increasing the initial amount of T-cells has the following effect: the melanoma cells are killed faster, the population of differentiated melanoma cells reaches a lower minimum and as a consequence the T-cells pass through a lower and broader minimum. The probability that the T-cells die out increases, and a differentiated relapse is more likely than in the case of a smaller initial T-cell population. Moreover, the broadening of the minima causes a "delay" and both kind of relapses (consisting mainly of differentiated or dedifferentiated cells) appear later. But since the extinction of T-cells is more likely, the tumour may reach its original size earlier, see Figure 4.6. For an initial value ten times as large as in Figure 4.4 (B) the

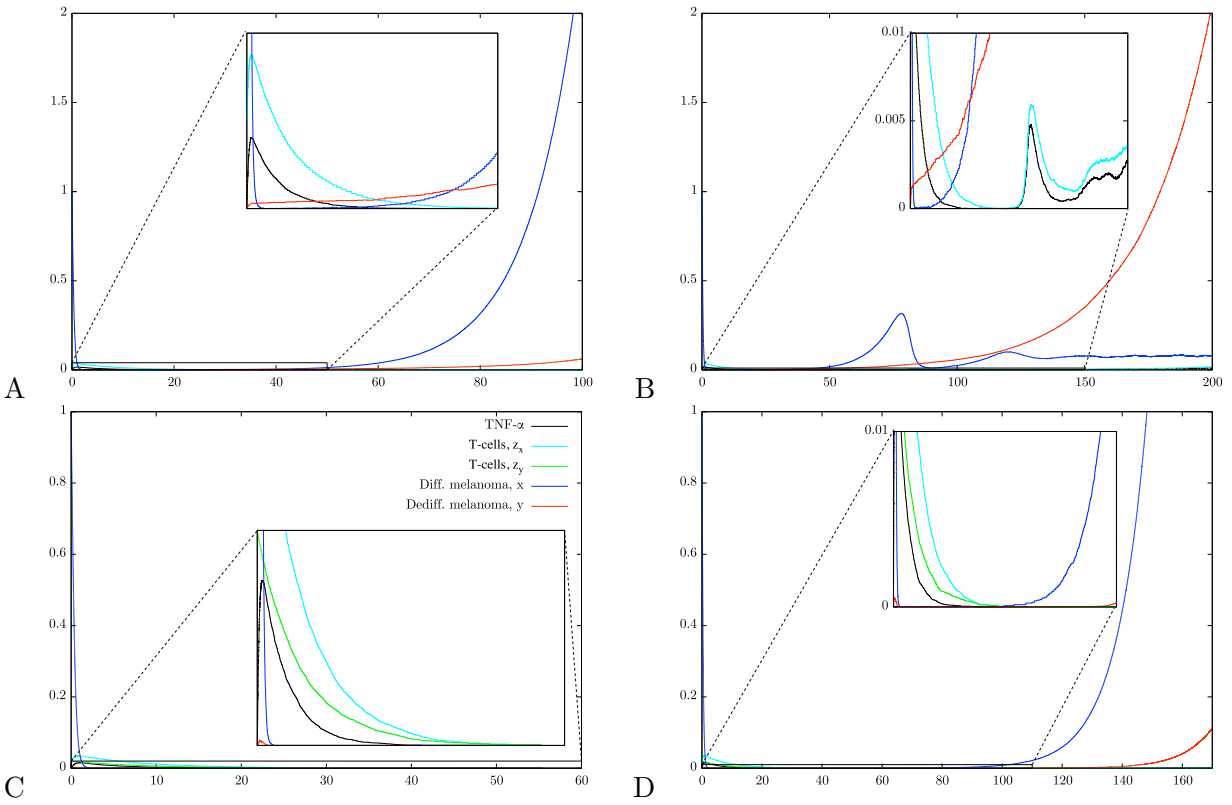


Figure 4.5: Simulations for biological parameters. Therapy with one T-cell type: (A) differentiated relapse (T-cells z_x die out), (B) dedifferentiated relapse (T-cells z_x survive), Therapy with two T-cell types: (C) cure, (D) differentiated relapse (both T-cell types die out).

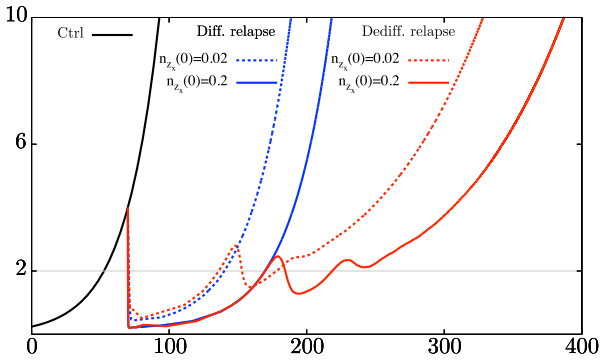


Figure 4.6: Simulations for different initial doses of T-cells: $\mathfrak{n}_{z_x}(0) = 0.2$ and $\mathfrak{n}_{z_x}(0) = 0.02$.

probability of an eradication of the tumour is still very small. If the number of T-cells initially is half the number of tumour cells, the probability of a favourable outcome is much higher. But such a high amount of T-cells is unrealistic.

Physiologically reasonable parameters

We explain here how we choose the biological parameters. Some parameters can be estimated from the experimental data. Recall that the subject of [91] is to investigate the behaviour of melanoma under T-cell therapy in mice. Without therapy the tumour undergoes only natural birth, death and switch events.

- *Choice of birth and death rates:* We assume that the number of cells in the tumour is described by
$$N_t \approx N_0 \exp(rt), \tag{4.3.7}$$
where N_t denotes the number of cells at time t, N_0 the initial population size and r the overall growth rate. Note that the estimate of the growth rate is independent of the initial value. Figure 4.4 (A) shows that the tumour needs roughly 50 days (without therapy) to grow from 2 mm diameter to 10 mm diameter. Since the structure of a melanoma is 3-dimensional, this corresponds roughly to $N_{50} = 125N_0$, which implies $r = 0.1$. Unfortunately, no data that allow to estimate the ratio of birth and death events are provided. As long as mutations are not considered this should not have a big impact and we choose $b = 0.12$ and $d = 0.02$ for the differentiated as well as the dedifferentiated cells. Landsberg et al. observed that the growth kinetics appear to be the same for both cell types, see Supplementary Figure 11 in [91].

- *Choice of the competition:* We assume that the competition has a very little effect here because the tumour grows exponentially in the observed time frame and does not come close to its equilibrium. We choose the competition between melanoma cells of the same type as $c(x,x) = c(y,y) = 0.00005$ and between different types of melanoma cells as $c(x,y) = c(y,x) = 0.00002$. The values are not set to 0 since the melanoma can grow only up to a finite size.

- *Choice of the switch parameters:* We can now estimate the switching parameters by using the data of Supplementary Figure 9e in [91]. In this experiment where cell division is inhibited, we can set $b = 0$. Furthermore, the amount of TNF-α is constant and we set here $\mathfrak{n}_w = 2$. Thus, the dynamics of the melanoma populations is described by
$$\begin{aligned} \dot{\mathfrak{n}}_x &= \mathfrak{n}_x\big(- d(x) - c(x,x)\mathfrak{n}_x - c(x,y)\mathfrak{n}_y - 2s_w(x,y) - s(x,y)\big) + s(y,x)\mathfrak{n}_y \\ \dot{\mathfrak{n}}_y &= \mathfrak{n}_y\big(- d(y) - c(y,y)\mathfrak{n}_y - c(y,x)\mathfrak{n}_x - s(y,x)\big) + \big(2s_w(x,y) + s(x,y)\big)\,\mathfrak{n}_x \end{aligned} \tag{4.3.8}$$
At the beginning of their observations the switch is very slow and speeds up after the first 24 hours. We assume that there is a delay until the reaction really starts and thus we choose the proportions at day 1 ($\mathfrak{n}_x = 0.81$ and $\mathfrak{n}_y = 0.19$) as initial data and choose switching parameters such that roughly the concentrations at day 2 ($\mathfrak{n}_x = 0.45$ and $\mathfrak{n}_y = 0.54$) and 3 ($\mathfrak{n}_x = 0.24$ and $\mathfrak{n}_y = 0.72$) are reached as shown in Figure 4.7. Thereby we obtain $s(x,y) = 0.0008, s(y,x) = 0.065$ and $s_w(x,y) = 0.33$. Note that the experiments we refer to provide only in vitro data and it is not clear if the in vivo situation is similar.

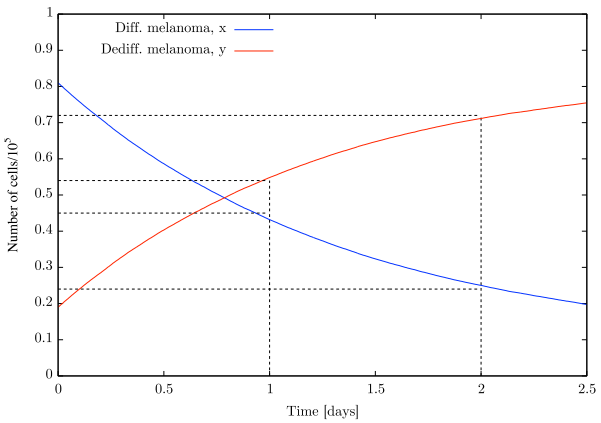


Figure 4.7: Switch in the in vitro experiments for inhibited cell division and constant concentration of TNF-α. Dashed lines indicate experimental data.

- *Choice of parameters concerning T-cells:* It remains to characterise the T-cells. Their natural birth rate is set to 0 since they are transferred by adoptive cell transfer and not produced by the mice themselves and do not proliferate in absence of targets. We assume that they have a relatively high birth rate depending on the amount of cancer cells present, $b(z_x, x) = b(z_y, y) = 2$ and produce one TNF-α molecule when they divide, $\ell_w^{\text{prod}}(z_x, x) = \ell_w^{\text{prod}}(z_y, y) = 1$. Furthermore, we assume that 4.5 cancer cells can be killed per hour (including indirect mechanisms), $t(z_x, x) = t(z_y, y) = 108$. The rate of death for the T-cell population is chosen as $d(z_x) = d(z_y) = 0.12$. These parameters are chosen such that the qualitative behaviour of the tumour was recovered. We choose the same parameters for the second T-cell type as for the first one because there are no data concerning the second T-cell type.

- *Choice of starting values and the scale K:* We set $K = 10^5$, the initial value for the differentiated melanoma cell population to 1 and to 0 for the population of dedifferentiated melanoma cells. The ratio of differentiated and dedifferentiated cells is not known for small tumours, which do not result from cell transfer of cells of in vitro cell lines. The initial value of the T-cell population is set to 0.02. We assume that the T-cells appear directly in the tumour, i.e. the migration phase into the tumour is not modelled.

To sum up, biological rates (per day) and initial conditions (in 100 000 cells) are:

$$
\begin{array}{llll}
b(x) = 0.12 & b(y) = 0.12 & b(z_x, x) = 2 & \ell_w^{\text{prod}}(z_x, x) = 1 \\
d(x) = 0.02 & d(y) = 0.02 & t(z_x, x) = 108 & \ell_w^{\text{kill}}(z_x, x) = 0 \\
c(x, x) = 5 \cdot 10^{-5} & c(x, y) = 2 \cdot 10^{-5} & d(z_x) = 0.12 & d(w) = 0.2 \\
c(y, x) = 2 \cdot 10^{-5} & c(y, y) = 5 \cdot 10^{-5} & & s_w(x, y) = 0.33 \\
s(x, y) = 0.0008 & s(y, x) = 0.065 & & \\
\mathfrak{n}_x(0) = 1 & \mathfrak{n}_y(0) = 0 & \mathfrak{n}_{z_x}(0) = 0.02 & K = 10^5
\end{array}
\tag{4.3.9}
$$

The additional parameters in the case where a second T-cell is used are:

$$\begin{array}{lll} t(z_y, y) = 108 & \ell_w^{\text{prod}}(z_y, y) = 1 & d(z_y) = 0.12 \\ b(z_y, y) = 2 & \ell_w^{\text{kill}}(z_y, y) = 0 & \mathfrak{n}_{z_y}(0) = 0.02 \end{array} \tag{4.3.10}$$

4.4 Mutations

This section studies the appearance of rare mutations in large populations.

One part of [11] is concerned with the interaction of rare mutations and fast switches in a setup without therapy. It is pointed out by examples that the initial growth rate of a mutant of type (g, p) cannot be used as a fitness concept in this setting. Instead it is proposed to use multi-type branching processes to describe a mutant population of genotype g including all its associated phenotypes, when the mutant arrives in a resident population at equilibrium. Moreover, it is sketched how a trait substitution sequence or even a polymorphic evolution sequence on the genotypic space can be obtained in future work. These processes are rigorously studied in [25] and [31] in a framework where only rare mutations but no switches are allowed. The behaviour of multi-type branching processes is for example investigated in [7, 8, 85, 86, 87].

4.4.1 Interplay of mutation and therapy

When competition for resources, as for example nutrients or oxygen, lowers the cell division rate of cells, more cell divisions and thus mutations may appear in populations at a size smaller than the corresponding equilibrium of this monomorphic population. This phenomenon is particularly interesting in the context of therapy, when tumours shrink under treatment. For such conditions earlier mutations may appear during therapy and the evolution of cancer may be accelerated.

The simplest setup to study this effect in our model consists of a monomorphic population of cancer cells of type (g, p), which can mutate to one fitter type (g', p'). For the sake of simplicity we exclude switching and consider only birth-reducing competition (the competition kernel increasing the death rate is set to 0). To include the effect of therapy, we consider one type of T-cells, z, targeting cancer cells of type (g, p) but not of type (g', p'), and neglect the role of cytokines in line with excluding switches. We are interested in the case of rare mutations in large populations, i.e. when mutation probabilities μ^K tend to zero as K tends to infinity. More precisely, we assume that $\mu^K \ll 1/(K \log K)$. This condition ensures that ecological and evolutionary time-scales are separated, i.e. that the system has time to equilibrate before a next mutant appears.

The limiting deterministic system that describes the interactions of the populations of type $(g, p), (g', p')$ and z has the following form

$$\begin{aligned} \dot{\mathfrak{n}}_{(g,p)} &= \mathfrak{n}_{(g,p)} \Big(b(p) - d(p) - c_b(p,p)\mathfrak{n}_{(g,p)} - c_b(p,p')\mathfrak{n}_{(g',p')} - t(z,p)\mathfrak{n}_z \Big) \\ \dot{\mathfrak{n}}_{(g',p')} &= \mathfrak{n}_{(g',p')} \Big(b(p') - d(p') - c_b(p',p')\mathfrak{n}_{(g',p')} - c_b(p',p)\mathfrak{n}_{(g,p)} \Big) \\ \dot{\mathfrak{n}}_z &= \mathfrak{n}_z (b(z,p)\mathfrak{n}_{(g,p)} - d(z)). \end{aligned} \tag{4.4.1}$$

The mutation term does not appear in the deterministic system and the difference between birth-reducing competition and usual competition is thus not visible. The effects we are looking for are intrinsically stochastic and happen on time-scales diverging with K.

We define $r(p) \equiv b(p) - d(p)$, and assume that $r(p) \neq 0$ and that $\frac{d(z)}{b(z,p)} \neq \frac{r(p)}{c_b(p,p)}$. The system (4.4.1) *without* the mutant population of type (g', p') has at least three equilibria,

1.
$$\bar{\mathfrak{n}}^1 = (0,0), \tag{4.4.2}$$
where the cancer and the T-cell population are absent,

2.
$$\bar{\mathfrak{n}}^2 = \left(\frac{r(p)}{c_b(p,p)}, 0\right), \tag{4.4.3}$$
where the cancer population reaches an equilibrium mediated via competition and the T-cell population is absent and

3.
$$\bar{\mathfrak{n}}^3 = \left(\frac{d(z)}{b(z,p)}, \frac{r(p) - c_b(p,p)\frac{d(z)}{b(z,p)}}{t(z,p)}\right), \tag{4.4.4}$$
where both populations are present.

The stability of these fixed points can be analysed by the eigenvalues of the Jacobian of the system (4.4.1) without the equation describing the mutant population. This Jacobi matrix is given by
$$\begin{pmatrix} r(p) - 2c_b(p,p)\mathfrak{n}_{(g,p)} - t(z,p)\mathfrak{n}_z & -t(z,p)\mathfrak{n}_{(g,p)} \\ b(z,p)\mathfrak{n}_z & b(z,p)\mathfrak{n}_{(g,p)} - d(z) \end{pmatrix}. \tag{4.4.5}$$
Evaluating the matrix at the equilibria, we obtain the following set of eigenvalues and criteria for stability:

1. The eigenvalues corresponding to $\bar{\mathfrak{n}}^1$ are $\lambda_1^1 = r(p)$ and $\lambda_2^1 = -d(z)$. Thus, when $r(p) > 0$ this fixed point is unstable.

2. The eigenvalues corresponding to $\bar{\mathfrak{n}}^2$ are $\lambda_1^2 = -r(p)$ and $\lambda_2^2 = b(z,p)\frac{r(p)}{c_b(p,p)} - d(z)$. Thus, when $r(p) > 0$ and $\frac{d(z)}{b(z,p)} > \frac{r(p)}{c_b(p,p)}$ this fixed point is stable.

3. The eigenvalues corresponding to $\bar{\mathfrak{n}}^3$ are
$$\lambda_{1,2}^3 = -\frac{c_b(p,p)d(z)}{2b(z,p)} \pm \sqrt{\frac{(c_b(p,p)d(z))^2}{4b(z,p)^2} - \frac{d(z)}{b(z,p)}\left(b(z,p)r(p) - c_b(p,p)d(z)\right)}. \tag{4.4.6}$$
Thus, when
$$\frac{d(z)}{b(z,p)}(b(z,p)r(p) - c_b(p,p)d(z)) > 0 \Leftrightarrow \frac{r(p)}{c_b(p,p)} > \frac{d(z)}{b(z,p)}, \tag{4.4.7}$$
this fixed point is stable.

Let us now consider the behaviour of the stochastic system. The total mutation rate of the population of type (g,p) at time t is given by
$$\mathfrak{m}(\nu_t^K(g,p)) \equiv \mu^K(p)\left\lfloor b(p) - c_b(p,p)\nu_t^K(g,p)\right\rfloor_+ \nu_t^K(g,p)K. \tag{4.4.8}$$
The function $\mathfrak{m}(\nu_t^K(g,p))$ is strictly positive as long as $\nu_t^K(g,p) \in (0, b(p)/c_b(p,p))$; it is a parabola opened downwards and attaining its maximum at $b(p)/(2c_b(p,p))$.

Let us consider a tumour population of type (g,p) at an equilibrium without or before therapy, i.e.

$$\nu_t^K(g,p) = \bar{\mathfrak{n}}^2_{(g,p)} = \frac{r(p)}{c_b(p,p)}. \tag{4.4.9}$$

Note that for $d(p) < b(p)/2$ it holds that $\bar{\mathfrak{n}}^2_{(g,p)} > b(p)/(2c_b(p,p))$. Thus, for such parameters the total mutation rate at this equilibrium is not maximal and smaller populations,

$$\nu_t^K(g,p) \in \left(\frac{d(p)}{c_b(p,p)}, \frac{r(p)}{c_b(p,p)}\right), \tag{4.4.10}$$

have a higher total mutation rate.

The time until a (not necessarily successful) mutation occurs is exponentially distributed with approximate parameter equal to

$$\mu^K(p)K \cdot \left(b(p) - c_b(p,p)\bar{\mathfrak{n}}^2_{(g,p)}\right)\bar{\mathfrak{n}}^2_{(g,p)} = \mu^K(p)K \cdot d(p)\frac{r(p)}{c_b(p,p)}. \tag{4.4.11}$$

For a therapy, where $\bar{\mathfrak{n}}^3$ is stable and the tumour remains at a smaller equilibrium created by the constant presence of therapeutic agents, the effect of birth-reducing competition becomes apparent. The waiting time for a mutation for such an equilibrium is exponentially distributed with approximate parameter

$$\mu^K(p)K \cdot \left(b(p) - c_b(p,p)\bar{\mathfrak{n}}^3_{(g,p)}\right)\bar{\mathfrak{n}}^3_{(g,p)} = \mu^K(p)K \cdot \left(b(p) - c_b(p,p)\frac{d(z)}{b(z,p)}\right)\frac{d(z)}{b(z,p)}. \tag{4.4.12}$$

Thus, the expected waiting time for a mutation is smaller during treatment if

$$\bar{\mathfrak{n}}^3_{(g,p)} \in \left(\frac{d(p)}{c_b(p,p)}, \frac{r(p)}{c_b(p,p)}\right). \tag{4.4.13}$$

This is illustrated in Figure 4.8, which was generated with parameters and initial values as indicated in (4.4.14).

In this example, treatment leads to earlier mutations and thereby accelerates the evolution towards more aggressive tumour variants. The long-term evolution of the system is crucially influenced.

Remark 4.4.1. Note that in a situation where competition increases death rates, a mutation is more unlikely during therapy since less mutations happen in smaller populations.

Depending on the choice of parameters, the appearance of a mutant in a population at an equilibrium of the form $\bar{\mathfrak{n}}^3$ can induce several different scenarios. By varying the cross-competition between the resident and the mutant population in the above example we observe the following three scenarios: first, the mutant invades, but the resident and the T-cell population survive at a different equilibrium, see Figure 4.9 (A). Second, the mutant invades, the resident population drops to a lower level due to competition and the T-cell population becomes extinct, when there are not enough target cells, see Figure 4.9 (B). Third, the mutant invades, replaces the resident type and the T-cell population becomes extinct, see Figure 4.8 (B).

Treatment strategies have to be adapted to these different situations. If the mutant is resistant to the former therapy (as assumed in our choice of parameters) a different treatment approach is

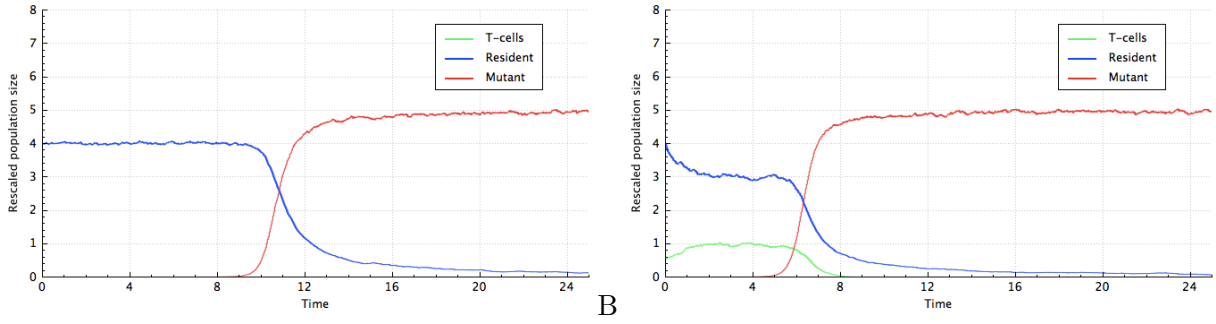


Figure 4.8: Simulations of mutation events under the influence of birth-reducing competition.
(A) For a population at high equilibrium without therapy and with few cell divisions.
(B) For a population at low equilibrium under therapy with many cell divisions.

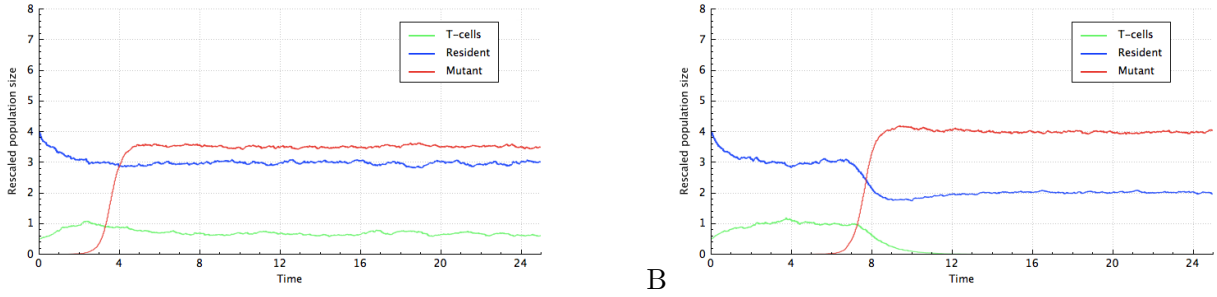


Figure 4.9: Simulations of mutation events in a cancer population under treatment with different values of the birth-reducing competition felt by the resident type from the mutant type.
(A) For a low value, here $c_b(p, p') = 0.1$, the resident and the T-cell population survive.
(B) For an intermediate value, here $c_b(p, p) = 0.5$, the resident survives but the T-cell population becomes extinct.

necessary. When the resident and the mutant population are present, a combination therapeutic approach might be indicated.
The simulations are obtained with the following parameters:

$$\begin{array}{llll} b(p) = 5 & b(p') = 6 & b(z,p) = 1 & m((g,p),(g',p')) = 1 \\ d(p) = 1 & d(p') = 1 & t(z,p) = 1 & \mu^K(p) = 3 \cdot 10^{-5} \\ c_b(p,p) = 1 & c_b(p',p) = 0.5 & d(z) = 3 & K = 10^3 \\ c_b(p,p') = 0.8 & c_b(p',p') = 1 & & \\ \mathfrak{n}_{(g,p)}(0) = 4 & \mathfrak{n}_{(g',p')}(0) = 0 & \mathfrak{n}_z(0) = 0 \text{ or } 0.5 & \end{array} \tag{4.4.14}$$

The values for $c_b(p, p')$ were adapted to obtain Figure 4.9 as indicated in the caption of that figure.

4.5 Discussion, outlook and open questions

Phenotypic and genotypic heterogeneity within single tumours are crucial for therapy resistance. We proposed a stochastic model from population dynamics, which includes both, phenotypic and genotypic alterations. It describes the experimental findings reported in [91] and can be used to

simulate and analyse new treatment protocols. Realistic predictions require reliable parameter estimates. To obtain such estimates further experimental data are necessary.

Furthermore, in future work our model can help to analyse the different impacts of phenotypic and genotypic changes. Such changes occur on different timescales and vary with respect to stability and heritability. Questions regarding these differences are also addressed experimentally, see [66].

In order to better approximate the reality, the model can be extended by including other cell populations, e.g. macrophages, different T-cell subpopulations, the effect of exhaustion or general networks of cytokine-immune-cell interactions.

Mutations are of particular interest in the context of cancer evolution. Not only from the biological point of view but also mathematically, it is interesting and challenging to consider different types of mutations. For example, mutations may happen on so-called care taker genes, which are relevant for DNA repair mechanisms, [84]. Such mutations can induce a lot of follow-up mutations since errors at DNA replication are not corrected as reliable as before. Mathematically spoken, such mutations change mutation probabilities themselves. Proliferation rates are important for the development of mutations and thus cancer, too. This was illustrated by the example on the accelerated evolution of cancer under treatment in a context, where competition lowers cell division rates.

Another challenging line of generalisation is to introduce a spatial structure into the model, taking into account that a tumour is a three-dimensional tissue with different areas. These areas show for example different types of cancer cells or variable levels of oxygen and acidosis. As a consequence therapeutic agents can target and infiltrate the tumour only inhomogeneously. A better understanding of this structure can help to find effective treatment strategies.

Bibliography

[1] O. Acuto, V. Di Bartolo, and F. Michel. Tailoring T-cell receptor signals by proximal negative feedback mechanisms. *Nat. Rev. Immunol.*, 8(9):699–712, 2008.

[2] W. Alt. Biased random walk models for chemotaxis and related diffusion approximations. *J. Math. Biol.*, 9(2):147–177, 1980.

[3] S. Andres, M. T. Barlow, J.-D. Deuschel, and B. M. Hambly. Invariance principle for the random conductance model. *Probab. Theory Related Fields*, 156(3-4):535–580, 2013.

[4] D. W. K. Andrews. Generic uniform convergence. *Econometric Theory*, 8(2):241–257, 1992.

[5] T. Antal and P. Krapivsky. Exact solution of a two-type branching process: models of tumor progression. *J. Stat. Mech.*, 2011(08):P08018, 2011.

[6] T. Arstila, A. Casrouge, V. Baron, J. Even, J. Kanellopoulos, and P. Kourilsky. A Direct Estimate of the Human $\alpha\beta$ T Cell Receptor Diversity. *Science*, 286(5441):958–961, 1999.

[7] K. B. Athreya. Some results on multitype continuous time Markov branching processes. *Ann. Math. Statist.*, 39:347–357, 1968.

[8] K. B. Athreya and P. E. Ney. *Branching processes*. Dover Publications, Inc., Mineola, NY, 2004. Reprint of the 1972 original [Springer, New York; MR0373040].

[9] L. Avena, F. den Hollander, and F. Redig. Law of large numbers for a class of random walks in dynamic random environments. *Electron. J. Probab.*, 16:no. 21, 587–617, 2011.

[10] M. Baar, A. Bovier, and N. Champagnat. From stochastic, individual-based models to the canonical equation of adaptive dynamics - in one step. *arXiv:1505.02421*, 2015.

[11] M. Baar, L. Coquille, H. Mayer, M. Hölzel, M. Rogava, T. Tüting, and A. Bovier. A stochastic individual-based model for immunotherapy of cancer. *arXiv:1505.00452*, 2015.

[12] R. R. Bahadur and R. Ranga Rao. On deviations of the sample mean. *Ann. Math. Statist.*, 31:1015–1027, 1960.

[13] J. Banchereau and R. M. Steinman. Dendritic cells and the control of immunity. *Nature*, 392(6673):245–252, 1998.

[14] M. J. Bevan and A. W. Goldrath. T-cell memory: You must remember this... *Current Biology*, 10(9):R338–R340, 2000.

[15] M. Biskup and T. M. Prescott. Functional CLT for random walk among bounded random conductances. *Electron. J. Probab.*, 12(49):1323–1348, 2007.

[16] B. Bolker and S. W. Pacala. Using Moment Equations to Understand Stochastically Driven Spatial Pattern Formation in Ecological Systems. *Theor. Popul. Biol.*, 52(3):179 – 197, 1997.

[17] B. M. Bolker and S. W. Pacala. Spatial moment equations for plant competition: understanding spatial strategies and the advantages of short dispersal. *Am. Nat.*, 153(6):575–602, 1999.

[18] A. Bovier and H. Mayer. A conditional strong large deviation result and a functional central limit theorem for the rate function. *ALEA Lat. Am. J. Probab. Math. Stat.*, 12(1):533–550, 2015.

[19] A. Bovier and S.-D. Wang. Trait Substitution Trees on Two Time Scales Analysis. *Markov Process. Related Fields*, 19(4):607–642, 2013.

[20] I. Bozic, T. Antal, H. Ohtsuki, H. Carter, D. Kim, S. Chen, R. Karchin, K. W. Kinzler, B. Vogelstein, and M. A. Nowak. Accumulation of driver and passenger mutations during tumor progression. *Proc. Natl. Acad. Sci. U.S.A.*, 107(43):18545–18550, 2010.

[21] A. Brock, H. Chang, and S. Huang. Non-genetic heterogeneity a mutation-independent driving force for the somatic evolution of tumours. *Nat. Rev. Gen.*, 10(5):336–342, 2009.

[22] F. M. Burnet. A modification of Jerne's theory of antibody production using the concept of clonal selection. *CA: a cancer journal for clinicians*, 26(2):119–121, 1976.

[23] R. E. Callard and A. J. Yates. Immunology and mathematics: crossing the divide. *Immunology*, 115(1):21–33, 2005.

[24] N. R. Chaganty and J. Sethuraman. Strong large deviation and local limit theorems. *Ann. Probab.*, 21(3):1671–1690, 1993.

[25] N. Champagnat. A microscopic interpretation for adaptive dynamics trait substitution sequence models. *Stochastic Process. Appl.*, 116(8):1127–1160, 2006.

[26] N. Champagnat, R. Ferrière, and G. Ben Arous. The Canonical Equation of Adaptive Dynamics: A Mathematical View. *Selection*, 2:73–83, 2001.

[27] N. Champagnat, R. Ferrière, and S. Méléard. From individual stochastic processes to macroscopic models in adaptive evolution. *Stochastic Models*, 24(suppl. 1):2–44, 2008.

[28] N. Champagnat and P.-E. Jabin. The evolutionary limit for models of populations interacting competitively via several resources. *J. Differential Equations*, 251(1):176–195, 2011.

[29] N. Champagnat, P.-E. Jabin, and S. Méléard. Adaptation in a stochastic multi-resources chemostat model. *J. Math. Pures Appl.*, 101(6):755–788, 2014.

[30] N. Champagnat and S. Méléard. Invasion and adaptive evolution for individual-based spatially structured populations. *J. Math. Biol.*, 55(2):147–188, 2007.

[31] N. Champagnat and S. Méléard. Polymorphic evolution sequence and evolutionary branching. *Probab. Theory Related Fields*, 151(1-2):45–94, 2011.

[32] L. Chen and D. B. Flies. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat. Rev. Immunol.*, 2013.

[33] J. E. Cohen. Mathematics is biology's next microscope, only better; biology is mathematics' next physics, only better. *PLoS Biol.*, 2(12):e439, 2004.

[34] F. Comets. Large deviation estimates for a conditional probability distribution. Applications to random interaction Gibbs measures. *Probab. Theory Related Fields*, 80(3):407–432, 1989.

[35] F. Comets, N. Gantert, and O. Zeitouni. Quenched, annealed and functional large deviations for one-dimensional random walk in random environment. *Probab. Theory Related Fields*, 118(1):65–114, 2000.

[36] A. L. Correia and M. J. Bissell. The tumor microenvironment is a dominant force in multidrug resistance. *Drug Resist. Updat.*, 15(1):39–49, 2012.

[37] M. Costa, C. Hauzy, N. Loeuille, and S. Méléard. Stochastic eco-evolutionary model of a prey-predator community. *J. Math. Biol. (online first)*, 2015.

[38] J. Couzin-Frankel. Cancer immunotherapy. *Science*, 342(6165):1432–1433, 2013.

[39] H. Cramér. Sur un nouveau théoreme-limite de la théorie des probabilités. *Actualités scientifiques et industrielles*, 736(5-23):115, 1938.

[40] J. M. Curtsinger, C. M. Johnson, and M. F. Mescher. CD8 T cell clonal expansion and development of effector function require prolonged exposure to antigen, costimulation, and signal 3 cytokine. *The Journal of Immunology*, 171(10):5165–5171, 2003.

[41] J. M. Curtsinger, C. S. Schmidt, A. Mondino, D. C. Lins, R. M. Kedl, M. K. Jenkins, and M. F. Mescher. Inflammatory cytokines provide a third signal for activation of naive CD4+ and CD8+ T cells. *The Journal of Immunology*, 162(6):3256–3262, 1999.

[42] A. Dembo, J.-D. Deuschel, and D. Duffie. Large portfolio losses. *Finance Stoch.*, 8(1):3–16, 2004.

[43] A. Dembo and I. Kontoyiannis. The asymptotics of waiting times between stationary processes, allowing distortion. *Ann. Appl. Probab.*, 9(2):413–429, 1999.

[44] A. Dembo and I. Kontoyiannis. Source coding, large deviations, and approximate pattern matching. *IEEE Trans. Inform. Theory*, 48(6):1590–1615, 2002.

[45] A. Dembo and O. Zeitouni. *Large deviations techniques and applications*, volume 38 of *Stochastic Modelling and Applied Probability*. Springer-Verlag, Berlin, 2010. Corrected reprint of the second (1998) edition.

[46] F. den Hollander. *Large deviations*, volume 14 of *Fields Institute Monographs*. American Mathematical Society, Providence, RI, 2000.

[47] F. den Hollander, R. dos Santos, and V. Sidoravicius. Law of large numbers for non-elliptic random walks in dynamic random environments. *Stochastic Process. Appl.*, 123(1):156–190, 2013.

[48] U. Dieckmann and R. Law. Moment approximations of individual-based models. In U. Dieckmann, R. Law, and J. A. J. Metz, editors, *The geometry of ecological interactions: simplifying spatial complexity*, pages 252–270. Cambridge University Press, 2000.

[49] G. M. Donovan and G. Lythe. T-cell movement on the reticular network. *J. Theoret. Biol.*, 295:59–67, 2012.

[50] R. Durrett. Cancer modeling: a personal perspective. *Not. Am. Math. Soc.*, 60(3):304–309, 2013.

[51] R. Durrett, D. Schmidt, and J. Schweinsberg. A waiting time problem arising from the study of multi-stage carcinogenesis. *Ann. Appl. Probab.*, 19(2):676–718, 2009.

[52] O. Dushek, R. Das, and D. Coombs. A role for rebinding in rapid and reliable T cell responses to antigen. *PLoS Comput. Biol.*, 5(11):e1000578, 12, 2009.

[53] R. Eftimie, J. Bramson, and D. Earn. Interactions Between the Immune System and Cancer: A Brief Review of Non-spatial Mathematical Models. *Bull. Math. Biol.*, 73(1):2–32, 2011.

[54] C. Ernst and E. Baake. Rare event simulation in immune biology: Models of negative selection in T-cell maturation. *arXiv:1407.5503*, 2014. Proceedings of the 10th International Workshop on Rare Event Simulation, RESIM 2014, 27-29 Aug 2014, Amsterdam, The Netherlands, p. 43.

[55] S. N. Ethier and T. G. Kurtz. *Markov processes.* Wiley Series in Probability and Mathematical Statistics: Probability and Mathematical Statistics. John Wiley & Sons, Inc., New York, 1986.

[56] W. Feller. *An introduction to probability theory and its applications. Vol. II.* Second edition. John Wiley & Sons Inc., New York, 1971.

[57] N. Fournier and S. Méléard. A microscopic probabilistic description of a locally regulated population and macroscopic approximations. *Ann. Appl. Probab.*, 14(4):1880–1919, 2004.

[58] P. François, G. Voisinne, E. D. Siggia, G. Altan-Bonnet, and M. Vergassola. Phenotypic model for early T-cell activation displaying sensitivity, specificity, and antagonism. *Proc. Natl. Acad. Sci. U.S.A.*, 110(10):E888–E897, 2013.

[59] S. A. Frank and M. R. Rosner. Nonheritable Cellular Variability Accelerates the Evolutionary Processes of Cancer. *PLoS Biol.*, 10(4):e1001296, 2012.

[60] M. I. Freidlin and A. D. Wentzell. *Random perturbations of dynamical systems*, volume 260 of *Grundlehren der Mathematischen Wissenschaften [Fundamental Principles of Mathematical Sciences]*. Springer, Heidelberg, third edition, 2012. Translated from the 1979 Russian original by Joseph Szücs.

[61] C. Gallesco, N. Gantert, S. Popov, and M. Vachkovskaia. A conditional quenched CLT for random walks among random conductances on $\mathbb{Z}^d$. *Markov Process. Related Fields*, 20(2):287–328, 2014.

[62] N. Gantert, S. S. Kim, and K. Ramanan. Cramér's theorem is atypical. *arXiv:1508.04402*, 2015.

[63] D. T. Gillespie. A general method for numerically simulating the stochastic time evolution of coupled chemical reactions. *J. Comp. Phys.*, 22(4):403–434, 1976.

[64] R. J. Gillies, D. Verduzco, and R. A. Gatenby. Evolutionary dynamics of carcinogenesis and why targeted therapy does not work. *Nat. Rev. Cancer*, 12(7):487–493, 2012.

[65] F. Ginhoux, J. L. Schultze, P. J. Murray, J. Ochando, and S. K. Biswas. New insights into the multidimensional concept of macrophage ontogeny, activation and function. *Nat. Immunol.*, 17(1):34–40, 2016.

[66] N. E. Glodde. *c-MET inhibition improves T-cell immunotherapy of mouse melanomas but cannot prevent outgrowth of genetic antigen loss variants*. PhD thesis, University of Bonn, 2016.

[67] M. Greaves and C. C. Maley. Clonal evolution in cancer. *Nat. Rev.*, 481(7381):306–313, 2012.

[68] G. R. Grimmett and D. R. Stirzaker. *Probability and random processes*. Oxford University Press, New York, third edition, 2001.

[69] N. Gritti, M. Caccia, L. Sironi, M. Collini, L. D'Alfonso, F. Granucci, I. Zanoni, and G. Chirico. Modeling Leukocyte-Leukocyte Non-Contact Interactions in a Lymph Node. *PLoS One*, 2013.

[70] P. B. Gupta, C. M. Fillmore, G. Jiang, S. D. Shapira, K. Tao, C. Kuperwasser, and E. S. Lander. Stochastic State Transitions Give Rise to Phenotypic Equilibrium in Populations of Cancer Cells. *Cell*, 146(4):633–644, 2011.

[71] D. Hanahan and R. A. Weinberg. The hallmarks of cancer. *Cell*, 100(1):57–70, 2000.

[72] D. Hanahan and R. A. Weinberg. Hallmarks of cancer: the next generation. *Cell*, 144(5):646–674, 2011.

[73] T. H. Harris, E. J. Banigan, D. A. Christian, C. Konradt, E. D. T. Wojno, K. Norose, E. H. Wilson, B. John, W. Weninger, A. D. Luster, et al. Generalized Lévy walks and the role of chemokines in migration of effector CD8+ T cells. *Nature*, 486(7404):545–548, 2012.

[74] J. Hofbauer and K. Sigmund. Adaptive dynamics and evolutionary stability. *Appl. Math. Lett.*, 3(4):75–79, 1990.

[75] J. Hoffmann-Jørgensen and G. Pisier. The law of large numbers and the central limit theorem in Banach spaces. *Ann. Probability*, 4(4):587–599, 1976.

[76] C. Holohan, S. Van Schaeybroeck, D. B. Longley, and P. G. Johnston. Cancer drug resistance: an evolving paradigm. *Nat. Rev. Cancer*, 13(10):714–726, 2013.

[77] M. Hölzel, A. Bovier, and T. Tüting. Plasticity of tumour and immune cells: a source of heterogeneity and a cause for therapy resistance? *Nat. Rev. Cancer*, 13(5):365–376, 2013.

[78] M. Iltis. Sharp asymptotics of large deviations in $\mathbf{R}^d$. *J. Theoret. Probab.*, 8(3):501–522, 1995.

[79] J. Jacod and A. N. Shiryaev. *Limit theorems for stochastic processes*, volume 288 of *Grundlehren der Mathematischen Wissenschaften [Fundamental Principles of Mathematical Sciences]*. Springer-Verlag, Berlin, 1987.

[80] C. A. Janeway Jr and R. Medzhitov. Innate immune recognition. *Annu. Rev. Immunol.*, 20(1):197–216, 2002.

[81] R. I. Jennrich. Asymptotic properties of non-linear least squares estimators. *Ann. Math. Statist.*, 40:633–643, 1969.

[82] S. M. Kaech, E. J. Wherry, and R. Ahmed. Effector and memory T-cell differentiation: implications for vaccine development. *Nat. Rev. Immunol.*, 2(4):251–262, 2002.

[83] O. Kallenberg. *Foundations of modern probability*. Probability and its Applications (New York). Springer-Verlag, New York, second edition, 2002.

[84] D. Kasper, A. Fauci, S. Hauser, D. Longo, J. Jameson, and J. Loscalzo. *Harrison's Principles of Internal Medicine 19/E (Vol. 1 & Vol. 2)*. McGraw Hill Professional, 2015.

[85] H. Kesten and B. P. Stigum. Additional limit theorems for indecomposable multidimensional Galton-Watson processes. *Ann. Math. Statist.*, 37:1463–1481, 1966.

[86] H. Kesten and B. P. Stigum. A limit theorem for multidimensional Galton-Watson processes. *Ann. Math. Statist.*, 37:1211–1223, 1966.

[87] H. Kesten and B. P. Stigum. Limit theorems for decomposable multi-dimensional Galton-Watson processes. *J. Math. Anal. Appl.*, 17:309–338, 1967.

[88] S. Kobold, P. Duewell, M. Schnurr, M. Subklewe, S. Rothenfusser, and S. Endres. Immunotherapy in tumor-activated T cells as a new treatment modality. *Dtsch. Arztebl. Int.*, 112:809–815, 2015.

[89] A. Košmrlj, A. K. Chakraborty, M. Kardar, and E. I. Shakhnovich. Thymic selection of T-cell receptors as an extreme value problem. *Phys. Rev. Lett.*, 103(6):068103, 2009.

[90] V. A. Kuznetsov, I. A. Makalkin, M. A. Taylor, and A. S. Perelson. Nonlinear dynamics of immunogenic tumors: Parameter estimation and global bifurcation analysis. *Bull. Math. Biol.*, 56(2):295–321, 1994.

[91] J. Landsberg, J. Kohlmeyer, M. Renn, T. Bald, M. Rogava, M. Cron, M. Fatho, V. Lennerz, T. Wölfel, M. Hölzel, and T. Tüting. Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation. *Nature*, 490(7420):412–416, 10 2012.

[92] M. Ledoux and M. Talagrand. *Probability in Banach spaces*. Classics in Mathematics. Springer-Verlag, Berlin, 2011. Isoperimetry and processes, Reprint of the 1991 edition.

[93] M. Lever, P. K. Maini, P. A. van der Merwe, and O. Dushek. Phenotypic models of T cell activation. *Nat. Rev. Immunol.*, 14(9):619–629, 2014.

[94] D. Lindau, P. Gielen, M. Kroesen, P. Wesseling, and G. J. Adema. The immunosuppressive tumour network: myeloid-derived suppressor cells, regulatory T cells and natural killer T cells. *Immunology*, 138(2):105–115, 2013.

[95] F. Lipsmeier and E. Baake. Rare event simulation for T-cell activation. *J. Stat. Phys.*, 134(3):537–566, 2009.

[96] A. Marusyk, V. Almendro, and K. Polyak. Intra-tumour heterogeneity: a looking glass for cancer? *Nat. Rev. Cancer*, 12(5):323–334, 2012.

[97] D. Mason. A very high level of crossreactivity is an essential feature of the T-cell receptor. *Immunol. Today*, 19(9):395 – 404, 1998.

[98] P. Mathieu. Quenched invariance principles for random walks with random conductances. *J. Stat. Phys.*, 130(5):1025–1046, 2008.

[99] R. M. May. Uses and abuses of mathematics in biology. *Science*, 303(5659):790–793, 2004.

[100] H. Mayer and A. Bovier. Stochastic modelling of T-cell activation. *J. Math. Biol.*, 70(1-2):99–132, 2015.

[101] I. Mellman, G. Coukos, and G. Dranoff. Cancer immunotherapy comes of age. *Nature*, 480(7378):480–489, 2011.

[102] J. A. J. Metz, S. A. H. Geritz, G. Meszéna, F. J. A. Jacobs, and J. S. van Heerwaarden. Adaptive dynamics, a geometrical study of the consequences of nearly faithful reproduction. In *Stochastic and spatial structures of dynamical systems (Amsterdam, 1995)*, Koninklijke Nederlandse Akademie van Wetenschappen Eerste Reeks, 45, pages 183–231. North-Holland, Amsterdam, 1996.

[103] C. Molina-París and G. Lythe. *Mathematical models and immune cell biology.* Springer Science & Business Media, New York, 2011.

[104] K. Murphy, P. Travers, and M. Walport. *Janeway's immunobiology.* Taylor & Francis, 7th edition, 2008.

[105] J. D. Murray. *Mathematical Biology I: An Introduction*, volume 17 of *Interdisciplinary Applied Mathematics.* Springer, New York, NY, USA, 2002.

[106] J. D. Murray. *Mathematical Biology II: Spatial Models and Biomedical Applications*, volume 18 of *Interdisciplinary Applied Mathematics.* Springer, New York, NY, USA, 2003.

[107] W. K. Newey. Uniform convergence in probability and stochastic equicontinuity. *Econometrica*, 59(4):1161–1167, 1991.

[108] P. C. Nowell. The clonal evolution of tumor cell populations. *Science*, 194(4260):23–28, 1976.

[109] S. A. Rosenberg. Cancer immunotherapy comes of age. *Nat. Clin. Pract. Oncol.*, 2(3):115, 2005.

[110] S. A. Rosenberg and N. P. Restifo. Adoptive cell transfer as personalized immunotherapy for human cancer. *Science*, 348(6230):62–68, 2015.

[111] E. Rothenberg. How T Cells Count. *Science (New York, NY)*, 273(5271):78, 1996.

[112] M. G. Rudolph, R. L. Stanfield, and I. A. Wilson. How TCRs bind MHCs, peptides, and coreceptors. *Annu. Rev. Immunol.*, 24:419–466, 2006.

[113] J. E. Smith-Garvin, G. A. Koretzky, and M. S. Jordan. T cell activation. *Annu. Rev. Immunol.*, 27:591, 2009.

[114] K. Spiliopoulos and R. B. Sowers. Recovery rates in investment-grade pools of credit assets: a large deviations analysis. *Stochastic Process. Appl.*, 121(12):2861–2898, 2011.

[115] E. R. Stirk, G. Lythe, H. A. van den Berg, G. A. D. Hurst, and C. Molina-París. The limiting conditional probability distribution in a stochastic model of T cell repertoire maintenance. *Math. Biosci.*, 224(2):74–86, 2010.

[116] E. R. Stirk, G. Lythe, H. A. van den Berg, and C. Molina-París. Stochastic competitive exclusion in the maintenance of the naïve T cell repertoire. *J. Theoret. Biol.*, 265(3):396–410, 2010.

[117] E. R. Stirk, C. Molina-París, and H. A. van den Berg. Stochastic niche structure and diversity maintenance in the T cell repertoire. *J. Theoret. Biol.*, 255(2):237–249, 2008.

[118] C. Tomasetti and B. Vogelstein. Variation in cancer risk among tissues can be explained by the number of stem cell divisions. *Science*, 347(6217):78–81, 2015.

[119] C. J. Tomlin and J. D. Axelrod. Biology by numbers: mathematical modelling in developmental biology. *Nat. Rev. Gen.*, 8(5):331–340, 2007.

[120] S. L. Topalian, G. J. Weiner, and D. M. Pardoll. Cancer immunotherapy comes of age. *J. Clin. Oncol.*, 29(36):4828–4836, 2011.

[121] H. Uebis. *Stochastische Modellierung der Fremdkörpererkennung in T-Zellen.* Diploma thesis, Rheinische Friedrich-Wilhelms-Universität, 2011.

[122] S. Valitutti, D. Coombs, and L. Dupré. The space and time frames of T cell activation at the immunological synapse. *FEBS letters*, 584(24):4851–4857, 2010.

[123] S. Valitutti and A. Lanzavecchia. Serial triggering of TCRs: a basis for the sensitivity and specificity of antigen recognition. *Immunol. Today*, 18(6):299–304, 1997.

[124] S. Valitutti, S. Müller, M. Cella, E. Padovan, and A. Lanzavecchia. Serial triggering of many T-cell receptors by a few peptide MHC complexes. *Nature*, 375(6527):148–151, 1995.

[125] H. van den Berg and C. Molina-París. Thymic Presentation of Autoantigens and the Efficiency of Negative Selection. *J. Theor. Med.*, 5(1):1–22, 2003.

[126] H. van den Berg and D. Rand. Foreignness as a matter of degree: the relative immunogenicity of peptide/MHC ligands. *J. Theor. Biol.*, 231(4):535–548, 2004.

[127] H. van den Berg and A. K. Sewell. Dynamic Tuning of T Cell Receptor Specificity by Co-Receptors and Costimulation. In C. Molina-París and G. Lythe, editors, *Mathematical Models and Immune Cell Biology*, pages 47–73. Springer, 2011.

[128] H. A. van den Berg, N. J. Burroughs, and D. A. Rand. Quantifying the Strength of Ligand Antagonism in TCR Triggering. *Bull. Math. Biol.*, 64(4):781–808, 2002.

[129] H. A. van Den Berg, C. Molina-Paris, and A. K. Sewell. Specific T-cell activation in an unspecific T-cell repertoire. *Science progress*, 94(3):245–264, 2011.

[130] H. A. van den Berg and D. A. Rand. Antigen presentation on MHC molecules as a diversity filter that enhances immune efficacy. *J. Theor. Biol.*, 224(2):249–267, 2003.

[131] H. A. van den Berg, D. A. Rand, and N. J. Burroughs. A Reliable and Safe T Cell Repertoire based on Low-affinity T Cell Receptors. *J. Theor. Biol.*, 209(4):465 – 486, 2001.

[132] T. L. Vincent and R. A. Gatenby. Modeling cancer as an evolutionary game. *Int. Game Theory Rev.*, 7(3):331–346, 2005.

[133] A. Viola and A. Lanzavecchia. T Cell Activation Determined by T Cell Receptor Number and Tunable Thresholds. *Science (New York, NY)*, 273(5271):104, 1996.

[134] W. Weninger, M. Biro, and R. Jain. Leukocyte migration in the interstitial space of non-lymphoid organs. *Nat. Rev. Immunol.*, 14(4):232–246, 2014.

[135] WHO. WHO Fact Sheet No 297, Cancer. `http://www.who.int/mediacentre/factsheets/fs297/en/`. Accessed: 10th January 2016.

[136] K. W. Wucherpfennig, P. M. Allen, F. Celada, I. R. Cohen, R. De Boer, K. C. Garcia, B. Goldstein, R. Greenspan, D. Hafler, P. Hodgkin, et al. Polyspecificity of T cell and B cell receptor recognition. *Semin. Immunol.*, 19(4):216–224, 2007.

[137] J. Xue, S. V. Schmidt, J. Sander, A. Draffehn, W. Krebs, I. Quester, D. De Nardo, T. D. Gohel, M. Emde, L. Schmidleithner, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity*, 40(2):274–288, 2014.

[138] N. Zint, E. Baake, and F. den Hollander. How T-cells use large deviations to recognize foreign antigens. *J. Math. Biol.*, 57(6):841–861, 2008.

[139] W. Zou. Immunosuppressive networks in the tumour environment and their therapeutic relevance. *Nat. Rev. Cancer*, 5(4):263–274, 2005.

[140] W. Zou. Regulatory T cells, tumour immunity and immunotherapy. *Nat. Rev. Immunol.*, 6(4):295–307, 2006.