In order to provide a proper theoretical framework for targeting disease-associated mast cells, **chapter 1** describes basic mechanisms of both innate and adaptive immunity. Diverse roles played by cellular immunity in response to bacterial and viral infections are summarized, and the clinical relevance of immune therapy is highlighted. Paradoxical roles of mast cells are described, and strategies for modulating functional roles of mast cells are addressed. Systems Biology approaches towards the elucidation of the complexity of cancer-related inflammation and targeting strategies based on such approaches, are sketched. The insights provided in this chapter illustrate that cancer is a systems biology disease in which immune cells play bi-functional roles within the tumor microenvironment. Although it is widely accepted that the immune response inhibits tumorigenesis, immune evasion by tumor cells and negative performance of immune cells, can be beneficial for tumor growth by supporting tumor inflammation.

In **chapter 2**, we first summarize mechanisms by which antigens elicit inflammatory effects, and outline the application of peptidic drugs and the combination of cellular modulation with implantation of healthy fibroblasts. We draw a formalized network diagram of many of the processes involved with the aim of integrating immunobiology with experimental data in a formal, consistent manner. Here a bacterial antigen is the invigorator of the inflammatory network, with cross-reactive antigen (CRA) as the factor activating B cells. Once activated, B cells normally secrete the complete form of antibodies which has two immunoglobulin (Ig) heavy chains and two Ig light chains covalently bound to each other by cystine bridges. However, the activated B-cell also secretes excessive amounts of so-called free light chains of these Igs (FLCs). These mostly antigen specific molecules are used as biomarkers of autoimmune diseases such as asthma and rheumatoid arthritis. The FLCs bind to mast cells and make these activatable by antigens. Activated mast cells attack the invading bacteria but also damage the fibroblasts around them by secreting TNF-α. In innate immunity, the inflammatory network that is triggered by the bacterial antigen mounts an immediate response in the sense of an acute inflammation, which is driven by mast cells and should be detrimental to the invading bacterial pathogen. The same network can however also engage in chronic inflammation, which is a pathological condition. Because of the complexity of the network with a plethora of positive and negative feedback loops, it is difficult to understand (i) what may cause it to switch from acute to chronic inflammation and (ii) if and (iii) how it could be switched back.

We propose that *in silico* simulation of this inflammatory network might help to understand system behaviors of inflammation and to generate some answers to these questions. We thereto provide the network diagram that we had already made, with rate and balance equations and integrate these as functions of time and parameter values which we estimated on the basis of the scientific literature and physical chemical considerations. The *in silico* simulation showed that excessive antigen influx into the network may turn acute inflammation into chronic inflammation, that this mechanism was depending on the ratio between healthy and dying fibroblasts, and that the fibroblasts are crucial in wound healing. This model provides a potent example of how systems immunology may help to explore cellular immunity in an integrative way. With the models we present, it is not only possible to illustrate the known biology, but also to extrapolate behaviors to therapeutically targeted
environments such as that of chronic inflammation treated with anti TNF-α. It might be attractive for the pharmaceutical industry to develop new generations of drugs based on such, more integrative, approaches. Furthermore, chapter 2 highlights the potential of individualized Medicine based on the quantity of adaptive immune cells such as B-cells: the number of B-cells impacts the antigen threshold between acute and chronic inflammation.

Chapter 3 describes a possible pro-cancer function of mast cells. It reports on mouse and human studies highlighting activation of mast cells, as evidenced by tryptase secretion, in various cancer contexts. Increased numbers of mast cells (as evidenced by toluidine blue staining) as well as tryptase expression (functional staining) are associated with the pathophysiology of tumor progression, including human skin, lung, colon, and pancreas tumors and murine melanoma. In mouse samples, mast cells are shown to be degranulated. When considering the pro-tumorigenic effect of mast cell degranulation, it is important that mast cell granules possess a high content of bioactive substances some of which could contribute to tumor growth, such as tryptase, TNF-α, VEGF, and histamine. Mast cells may not only be a diagnostic marker for cancer but also constitute a therapeutic target in cancer therapy. The release of mediators by mast cells in response to tumor cell growth may be a doubly edged sword. On the one hand, mast cells secrete cytokines, such as IL-4, that kill tumor cells, but on the other hand mast cells secrete TNF-α, which may promote tumor inflammation by killing healthy tissue cells and creating space and resource access for tumor cells.

Again a balance between processes with positive and negative regulatory loops seems to determine whether mast cell activation promotes or retards tumorigenesis. We therefore set out to extend the systems-biology approach of innate immunity of chapter 2 towards addressing the role of innate immunity in tumorigenesis. Chapter 3 thereby links the innate sensing of tumor cells by the mast cell with its activation that may well promote tumor pathogenesis. Conventional and cellular targeting strategies of mast cells are highlighted and implantation of fibroblasts is simulated by using COPASI-software to make our cancer model predictive. Therapeutically, considering anti-FLC drugs in the cancer model suggested in an in silico treatment aimed at achieving tumor stabilization and re-growth of fibroblasts. In supplemental material to this chapter (presented as Chapter 7), we present experimental data that suggest that Ig free-light chains (FLCs) are indeed tumor growth modulators in cancer. Both human and mouse studies suggest that targeting FLC has potential for inhibiting tumor inflammation by its interference with the activation of mast cells. Taken together, experimental, modeling and literature studies now suggest that mast cells are involved in tumor inflammation and progression.

In chapter 4 we deployed analytical methods to inspect the fundamental characteristics and actionability of our model: stability analysis and sensitivity analysis were implemented in conjunctions with empirical analyses in silico. The stability of the model’s fixed points were assessed by linear stability analysis based on the eigenvalues of the Jacobian matrix. This information is crucial for stability of steady state under perturbations by the system’s inherent dynamics. Chapter 4 also describes the robustness and tolerance of our model when it comes to significant changes in concentrations of reactants and parameters. It addresses
the local robustness of steady states and whether any bi-stability is maintained. Sensitivity analysis is used to quantify which species and parameters have the largest impact on the model’s behavior, both for acute and for chronic inflammation. A major outcome of this chapter is that in the absence of fibroblast ingrowth, our model is not really bistable: the transition from acute to chronic inflammation is then irreversible. Treatment with anti-FLC peptide only transiently eliminates the model’s chronic inflammation. Fibroblast implantation or the stimulation of stem cells that generate fibroblasts, is essential for the acute to chronic transition to become reversible and for the systems to become bistable in the sense that also the acute state reached after the chronic state, becomes stable.

In chapter 5 we outline the various functional roles of mast cells in the immune system and highlight their diagnostic potential in cancer. We also discuss the potential of considering mast cells as drug target in cancer therapy. We give pathology evidence demonstrating mast cell degranulation as well as illustrating targeting strategies such as anti-IgE therapy. We describe the Stem Cell Factor (SCF)-mediated signaling pathway which is responsible for the development of mast cells, and discuss whether the targeting of signaling molecules involved in this development by kinase inhibitors blocking mast cell activation, might offer therapeutic potential.

In chapter 6 we discuss how Systems Biology may be used to analyze the link between chronic inflammation and tumor growth. Given the tremendous complexity of cancer, are we ready to study the inflammatory nature of cancer? Are we sufficiently equipped, both in terms of the appropriate quantitative experiments and in terms of multi-scale modelling, to use modeling approaches to predict and to monitor diseases as complex as cancer? Before 2000, it would have been impossible to answer in the affirmative. Systems analysis was invigorated however by the completion of the human genome sequence in 2001. Together with the advent of protein interaction maps and their online application by 2003, new technologies such as mass spectrometry proteomics and metabolomics have launched a next phase of systems approaches towards complex organisms like the human. Initially systems biology focused on intracellular networks, shying away from multicellular networks such as the immune cell environment and the tumor milieu. Ambitiously, we have here looked at multicellular immunity when developing a semi-artificial inflammatory network for computer simulation. There is much to be learned from immunobiology which is vital for curing many human diseases definitively. And there may even be more to be learned when this is put into the quantitative network perspective of a new Systems Immunology. As challenging as it may be, such learning should lead to more rational and science-based therapies of the many multifactorial diseases mankind still suffers from, at last.

In supplementary chapter 7 we summarize examples of other work that has been done by us in immunology and drug targeting. We discuss the model validation based on these experimental studies and the extent to which the model can be used to predict disease development in silico.