LUND UNIVERSITY
Faculty of Science

Biophysical analysis of Immunotherapy and Cancer modeling

Simon Tropp

Thesis submitted for the degree of Master of Science
Project duration: 4 Months (30 ECTS)

Supervised by Tobias Ambjörnsson

Department of Astronomy and Theoretical Physics
Division of Computational Biology and Biological Physics
LU-TP 22-41
May 2022

Abstract

Accounting for 10 million deaths and 20 million new cases every year, cancer is one of the most common causes of death. The interest in trying to understand this lethal disease using mathematical models has increased steadily for many decades.

Methods for boosting the immune system to better fight cancer (immunotherapy) have also been long present. However, the full potential of immunotherapy methods is not yet understood due to the immune system's complexity. The immune system naturally fights cancer, but it is not able to keep up when tumors grow too quickly. This is where immunotherapy aims to enhance the immune system to avoid and reverse tumor escape. Existing immunotherapies only work for some cancers and patients, but a better understanding of the cancer-immune dynamics could lead to new and reliable cancer treatments.

Experimental images of tumor tissue, with single-cell resolution, are here shown in order to motivate the use of mathematical models for studies of cancer-immune dynamics. The behavior of individual immune cells is (through literature review) concluded to be governed by interactions with other immune cells. For this reason, the development of effective immunotherapies relies on the understanding of these cell-cell interactions, where spatial aspects are also believed to be of importance.

This review explores immunotherapy and mathematical cancer modeling by considering various cancer-immune interactions. The review aims to allow researchers from research fields outside of biology and medicine to find interest and potential in the study of immunotherapy.

Populärvetenskaplig beskrivning

Cancer är en av de vanligaste dödsorsakerna och står bakom 10 miljoner dödsfall varje år. De vanligaste och mest studerade behandlingarna av cancer inkluderar kemoterapi och cellgifter, men det finns även andra sätt att behandla cancer.

Ponera att kroppen själv kunde ta hand om cancer. Människans immunförsvar kan besegra både virus och bakterier, så intuitivt borde det inte vara mycket svårare för immunförsvaret att också eliminera cancerceller. Det hela är dock något mer komplicerat på cellnivå. Cancerceller är muterade celler som kan ändra både form och utseende, vilket i sin tur förvirrar immuncellerna. Tumörer (samlingar av cancerceller) kan även utveckla immunhämmande effekter, exempelvis så att immunceller blir tillsagda att det inte finns något problem att ta hand om. Cancerceller kan även "förklä sig" med särskilda proteiner (så kallade receptorer) som immuncellerna normalt associerar med friska celler.

I tidigt skede av tumörutveckling kan immunförsvaret hålla tumörer i vilande (icke-växande) tillstånd, men efter ett tag tenderar tumörer att växa för fort för att immunsvaret ska kunna hålla tillbaka dem. Det är i detta skede som tumörer utvecklar immunhämmande egenskaper. För att få immunförsvaret att åter få övertag i kampen med cancer introduceras *immunterapi*, som går ut på att förstärka immunförsvaret och hjälpa det överkomma de immunhämmande egenskaperna hos tumörer.

Fokus i denna uppsats är på matematisk modellering av tumörtillväxt och hur vårt immunförsvar interagerar med cancerceller. Genom att matematiskt definiera och utvärdera dessa interaktioner, kan bättre förståelse för viktiga parametrar ges. Effekter av rumslig fördelning av immunceller kan också undersökas och fastlås som väsentlig för att förutsäga utfall och beteenden av tumörer. Genom att modellera cancer-immun-dynamik och sätta i förhållande till observationer från faktisk tumörvävnad, kan matematisk modellering underbyggas och motiveras med dynamik från verkliga tumörer.

List of Abbreviations

APC Antigen-Presenting Cell.

CAR Chimeric Antigen Receptor.

CTL Cytotoxic T Lymphocyte.

DC Dendritic Cell.

IFN Interferon.

MHC Major Histocompatibility Complex.

NK cells Natural Killer cells.

ODE Ordinary Differential Equation.

PD-1 Programmed cell Death protein-1.

PDE Partial Differential Equation.

Th cell helper T cell.

TIL Tumor-Infiltrating Lymphocyte.

Treg regulatory T cell.

Contents

1 Introduction **4**
1.1 Cancer and Physics 4

2 An Overview of the System Components **5**
2.1 Cancer cells and Tumors 5
2.2 The Immune System 8

3 Immunotherapy **12**
3.1 Issues of Immunotherapy 16

4 Physics Methods of Immunotherapy **18**
4.1 Mathematical Models 18
4.1.1 Undisturbed Tumor Growth 20
4.1.2 Cancer–Immune Interactions 22
4.2 Spatial Distribution of Immune Cells 30
4.2.1 Physical Attributes 30
4.2.2 Mathematical Modeling 37
4.3 Physics Tools 40

5 Concluding Remarks **41**

Appendices **56**

A Complementary figures **56**

B Mathematical models **60**
B.1 Integro-differential model of antigen heterogeneity and evolution 60

C Data and complementary plots **62**

D Entropic aspects **68**

1 Introduction

Cancer is a worldwide disease that accounts for millions of deaths every year. As one of the most common causes of death, it is estimated that a total of 20 million people died due to cancer during the years 2020 and 2021. This implies that cancer is approximately the cause of every sixth death. Furthermore, it is estimated that the number of established cancer diagnoses during 2021 was about 20 million [1, 2]. These statistics are also expected to prevail into 2022 and continuously increase in the coming decades. However, it should still be mentioned that some types of cancers are observed to have a decreasing probability of causing death [2, 3].

The recent event of the worldwide coronavirus (SARS-CoV-2) 2019 pandemic, triggered an increase in the study of immunology [4, 5]. These intense studies of viruses and vaccines [6] might have contributed to an increased focus on the topic of *immunotherapy* (mobilization of the immune system to fight cancer cells) [7, 8]. It is believed that this increase is partly related to the disruption that the COVID-19 pandemic caused to cancer patients [9, 10]. However, the number of publications on the matter of mathematical modeling of cancer and tumors, have also been naturally increasing exponentially since the 1960s [11].

The complete potential and functionality of immunotherapy are not yet truly understood, but it seems to possess a great potential for successful cancer treatment. However, one should be aware that immunotherapy still has a long way to go before it can be established as a cure for cancer [12, 13]. On top of this, immunotherapy consists of several different concepts and methods, all gathered under the same name [14]. All of these methods will not be addressed in this thesis, instead the aim is to give an overview of the immunotherapy concept and its potential, mainly seen from a mathematical and physics point of view.

In the subsection below, the role of physics in cancer research is presented. Thereafter, an overview of the system components (cancer cells and the immune system) is given, followed by a detailed introduction to immunotherapy.

1.1 Cancer and Physics

Traditionally, cancer research is majorly performed by biologists, medical doctors, and biomedical engineers. However, the statistics presented above indicate that the research area requires more attention and new perspectives. This is where both physicists, mathematicians, and computer scientists can contribute with new ways of thinking and different techniques to potentially assist in new discoveries [13].

Arguably, physicists and engineers have historically played a big role (and still do) in the subject of cancer detection and cancer treatment, e.g. MRI (Magnetic Resonance Imaging), radiation therapy, and x-rays, have all been heavily studied and developed by physicists [15, 16]. Furthermore, lab-on-a-chip[1] systems and microchips (also heavily studied by biophysicists) are commonly used for 3-dimensional *in vitro* studies of microtumors [18, 19], and to monitor anti-cancer drugs and potential drug resistance [20, 21].

Mathematical modeling also plays a big role in cancer research as it helps to quantify tumor behavior. For example, it has been shown that the dynamics of tumors can

[1]Miniaturized biomedical laboratories, i.e. devices that integrate and automate laboratory functions onto "chips", see e.g. [17].

be described by Gompertzian[2] growth curves [22]. Therefrom, a phenomenological law (the Norton–Simon hypothesis) was derived, this law related the tumor growth characteristics to cytotoxic chemotherapy influence on tumor size. Specifically, it states a proportionality between the unperturbed tumor growth rate, at a specific tumor size, to the chemotherapy rate of regression in tumor volume at that size [23]. As a consequence, the international standard of *dose-dense* chemotherapy treatment was developed, which makes tumor destruction more likely in comparison to other methods [24, 25]. R. Simon and L. Norton also states in [23] that *"[...] it will remain imperative to use mathematical methods to guide clinical trial design."*. Their statement is substantiated by the many applications of mathematical modeling in a variety of cancer-related research and problems [13, 26, 27, 28].

Studies on immunotherapy have led to discoveries such that successful cancer recognition and infiltration by immune cells can be very effective [13]. For a set of different cancer types, it has even been shown that a high density of a specific kind of immune cell in the tumor yields a promising forecast [29, 30]. Related to this, it has been speculated that biologists would focus a lot on signaling pathways[3], while physicists find more interest in the effects of spatial organization on cell-cell interactions (introducing the concepts of maximum entropy and fractal dimensions, which might contribute to the development of more effective cancer treatments) [13].

The takeaway from this section is that collaboration between "traditional" cancer researchers and researchers from other fields (such as physics), might produce new and effective tools in the battle against cancer.

2 An Overview of the System Components

To fully understand immunotherapy, it is essential to have a good understanding of its system components. In an attempt to provide the reader with the necessary knowledge to understand and follow along with the models and methods presented in this thesis, this section contains an overview of both what cancer cells are and how they arise, as well as a detailed introduction to the immune system and its constituents. The readers which are already familiar with cancer and immunology may skip directly to Section 3.

2.1 Cancer cells and Tumors

Cancer arises when control systems in a cell are flawed or malfunctioning, specifically, the control systems that promote and protect against cell growth (where malfunction could cause irresponsible and uncontrolled proliferation[4]). Proliferation is otherwise a necessity for humans to go from being a fertilized egg to an adult with a cell count in the trillions, where the majority of the cell proliferation eventually stops (e.g. when organs reach their full size). Some proliferation, however, continues as skin cells and body cavities experience "erosion" and need to be replenished as time goes by. This life-long proliferation is essential, but if it is not properly controlled in a cell, this cell is on its way to becoming a cancer cell [31].

[2]A sigmoid-like function implying that the growth (of an arbitrary quantity) is the slowest at the beginning and end of time.

[3]Chemical reaction series where the proteins of a cell (together) govern cell functions like death and division.

[4]Proliferation of cells is the process in which they grow and divide into daughter cells.

The reason for occasional failure in the control systems is due to gene alterations in the proteins constituting the systems. These alterations may arise from mutations (occurs spontaneously when cellular DNA is faultily copied to daughter cells, or from byproducts of cellular metabolism; e.g. accelerated by fatty foods, smoking, or radiation/UV exposure). Mutations are otherwise a natural part of cellular life and are generally harmless, but sometimes a mutated gene may cause uncontrolled proliferation (a mutated gene with this ability is called an *oncogene*, and the normal or "healthy" version of this gene is called a *proto-oncogene*). In short, a normal gene of a cell might mutate and cause uncontrolled cell growth. The oncogene is also heritable during cell proliferation, which implies that uncontrolled cell growth may result in many unhealthy mutant clones. Other gene alterations, that may arise as a consequence, might grant the ability to invade nearby tissue or organs (metastasize) or to evade the immune system [26, 31].

A tumor is then a collection of many[5] such aggressive malfunctioning cells with genetic alterations, in other words, altered tissue due to evolutionary processes. Both evolution and natural selection are indeed affecting the cells in the tumor microenvironment, where survival is the ultimate goal for the tumor. Therefore, resistance, rapid proliferation, and evolvability[6] are all tumor desired cell properties, just as the aspirations of natural selection-based systems. This causes a natural increase of oncogenes in the tumor cells [26, 33, 34].

To go one step further, "signaling pathways" are what enable tumors to spread and grow. Specifically, when mutations cause these signaling pathways to be de- or over-activated, tumor growth may accelerate. Some of these pathways can also be coupled to the mechanobiology[7] of the tumors in their microenvironment. That is, proteins linking a cell to another can cause the cells to perceive how stiff their surroundings are, in turn leading to proliferating, shapeshifting, and traveling (via signaling pathways) cancer cells. As a consequence, cells within the tumor tend to increase the collagen fiber density, causing their microenvironment to be stiff and the tumor to become a lump. Individual cancer cells can also be remarkably stiff or soft, where the soft cancer cells easily deform and efficiently metastasize with high motility [13, 36].

Going back to the root of the problem, it would be ideal if malfunctioning control systems could be avoided. In fact, cells do have inbuilt safeguard systems for this exact reason, both mutation-preventing and mutation-fixing [31]. Specifically, cells have repair systems that quickly repair minor DNA damages (more than 20 000 mutation repairs per day in a single human cell) [37]. When the DNA damage is severe or when the repair system misses fixing a mutation, a mutation monitoring safeguard system is activated. This system may, for example, give the repair system more time to fix the mutation by preventing the damaged cell from proliferating, or when the damage is too extreme, it will cause the cell to undergo *apoptosis*[8] to eliminate the problem [31].

As a side note, this safeguard system contains the significant *p53-protein*, which modulates cell growth, and is therefore referred to as a tumor suppressor. The encoding genes of protein p53 (and others like it) are similarly referred to as tumor suppressor genes (or anti-oncogenes, cf. oncogene from previously). Research shows that most humans with

[5]Tumors of 1 cm^3 are usually considered to consist of 10^9 cells, but 10^8 could be more realistic [32].

[6]The capacity of a system to evolve adaptively. In context, the ability of a cell to generate variations in its heritable traits.

[7]A scientific field of study that focuses on physical forces and changes in the mechanical properties of cells and tissues (see more in [35]).

[8]Activation of an intracellular death program, also known as "Programmed cell death". In other words, the cell is instructed to kill itself [38].

tumors have mutations in these tumor suppressor genes, and mice designed to have mutated p53 genes were found to develop and perish from cancer in ages ranging from merely 10 weeks to seven months [31, 39].

To conclude, it is only when the normal cell simultaneously experiences mutations in its proto-oncogenes and tumor suppressor genes, that cancer can be developed. About 4-7 growth-promoting and safeguard-disabling mutations are thought to be necessary for the most common cancers to arise, which usually takes decades in humans. But there are also some common childhood cancers, for example, retinal cancer, which can arise from mutations in a single tumor suppressor gene (RB1) [31, 40].

Moreover, each type of cancer is generally assigned to one of two categories, namely, non-blood cell cancers (solid tumors) and blood cell cancers, see Figure 1. The solid tumors are further categorized considering the type of cell they arise from, for example, the most frequently discovered human tumors that metastasize in essential organs (e.g. lung and colon cancer) are called *carcinomas* and arise from epithelial cells[9]. On the other hand, blood cell cancers (e.g. leukemia) arise when blood stem cells' descendants do not mature to stop proliferating, in turn leading to more immature blood cells and anemia[10] or immunodeficiency [31, 41].

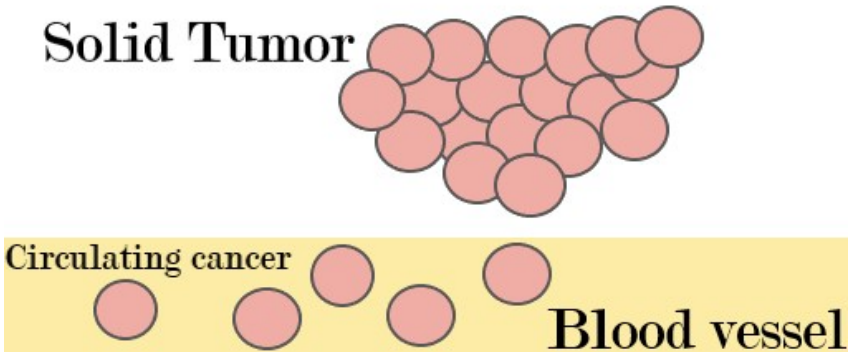


Figure 1: **Comparison of solid tumors and blood cell cancers.** The cancerous cells in the solid tumor together form a "stationary" complex, while blood cell cancer implies that the cancerous cells are circulating individually in the blood vessels.

Finally, cancer is said to be either spontaneous or virus-associated. Spontaneous cancers are what has been described above (i.e. spontaneous mutations), while virus-associated cancers are accelerated by a virus infection (which is the case for $\sim$20% of all human cancers). Specifically, there are viruses (e.g. hepatitis-B and papillomavirus) that can produce *oncoproteins* in the virus-infected cells to negatively affect their safeguard systems and p53 protein [42, 43]. Notably, virus-associated cancers still arise from spontaneous mutations, but the additional viral infection accelerates the production of cancerous cells by reducing the number of needed mutations [31].

[9] A cell that is present in body surfaces like skin, blood vessels, and the urinary tract.
[10] Too few healthy red blood cells in the body carry oxygen to body tissues.

2.2 The Immune System

The immune system is complex and involves many different components. These components are traditionally split into two groups, the *innate immune system* and the *adaptive immune system* [44]. But it is not as simple as saying that these two parts of the immune system have different tasks and functionalities, because it is their cooperation that results in our powerful defense against viruses and bacteria. Indeed, immunology is a subject that continuously evolves and contains many details (both small and big), where almost every rule has exceptions. The fact that the immune system involves plentiful components, all interacting with each other, complicates matters further. Specifically, it does not make sense to study one component at a time, since its actions will be dependent on the actions of other immune system components [31]. However, in this section, the bigger picture is considered, and the goal is to understand the essentials of the immune system.

The innate immune system is the first line of defense when invaders enter the body [45]. The name comes from the fact that this system seems to be a natural part of all animals, and has been so for over 500 million years (at least some of its components). One component of the innate immune system is the *macrophage* (Greek - "big eater") defender cell, which can detect bacterium using its receptors. More precisely, bacterium membranes consist of unfamiliar fats and carbohydrates which "signal" to the macrophages (and other members of the *phagocyte*-family) to activate and go dispose of the potentially dangerous microbe dressed with these molecules. The phagocytes' disposal process is called *phagocytosis* and starts at contact, where the macrophage (or other phagocytes) "eats" the bacterium by putting it in a vesicle (phagosome) and then taking it inside of itself. Next, the bacterium vesicle is combined with another vesicle (lysosome) containing bacteria-killing enzymes and chemicals. Furthermore, the macrophages do not only eat bacteria, the "big eaters" also dispose of general "garbage" in our bodies and can even eat cancer cells (see more in Section 3) [31, 44, 46].

Macrophages and all other blood cells in our bodies are produced from stem cells in the bone marrow. As these stem cells divide into two daughter cells, one of these will become a new stem cell (self-renewing), while the other is set off to grow into a mature blood cell. The daughter cell is given a set of choices[11] for what kind of blood cell it will mature into (see Figure A.1 in Appendix A), which includes macrophages and red blood cells etc. [47].

The stem cells destined to become macrophages are called *monocytes* once they have entered our bloodstream, the monocytes then head for the capillaries to exit the blood and enter our tissue, where they finally mature into macrophages. When the macrophages then battle invaders, they secrete *cytokines* (proteins) which act as a means of communication between immune cells. These messengers can then tell nearby monocytes and immune cells to enter the tissue and assist in the battle. Since the macrophages are able to quickly recognize a variety of common bacteria and invaders, the innate immune system finishes most battles in a few days [31]. In short, when the garbage collecting guards (macrophages) detect invaders, they start to take care of the issue themselves, while simultaneously sending messengers (cytokines) to recruit other cells, see Appendix A Figure A.2 for an illustrative depiction of the process.

Except for the macrophages, the innate immune system can also take care of bacteria

[11] It is not a literal choice of the cell, instead, it is carefully governed by our body to make sure that we always have enough of each kind.

with its Natural Killer cells (NK cells). The NK cell (a so-called *lymphocyte*) can not only kill bacteria but also virus-infected and cancerous cells (see more in Section 3) [48]. These cells are also produced in the bone marrow, and have cytokine-producing functions like the macrophages, causing them to be helpful when our body is under attack [49]. However, in absence of an attack, they live relatively short lives with a turnover[12] of about 2 weeks [50]. In comparison to other immune cells, this is a rapid turnover, which is present in both young and elderly people. However, they are also relatively fast at killing damaged, infected, and transformed cells [51].

When in action, the NK cells can secrete defense-engaging cytokines such as the gamma Interferon (IFN), IFN-γ. However, for the NK cells to be effective, they need to be activated (similar to the macrophages). Inactive NK cells can also kill and produce cytokines, but these processes are majorly boosted in active NK cells. The activation is accomplished through signals (specifically, the IFN-α and IFN-β cytokines), which are produced by other immune system cells when there is an attack. In contrary to the engulfing macrophages, the activated NK cells kill the "bad cells" using apoptosis, i.e. injecting them with "suicide"-causing enzymes (e.g. granzyme B) [48].

The NK cells also need to determine whether a cell is bad or not. This is made possible through *activating* and *inhibitory* surface receptors (see Figure 2). The activating receptors activate the killer instinct of the NK cells when the connection to the target cell is slowed down by unfamiliar molecules on the cell's surface (suggesting that the cell could be virus-infected or cancerous). On the other hand, the inhibitory receptors inhibit the killer instinct by recognizing the familiar (i.e. generally present on healthy cells) *class I MHC molecule*[13] [31, 52].

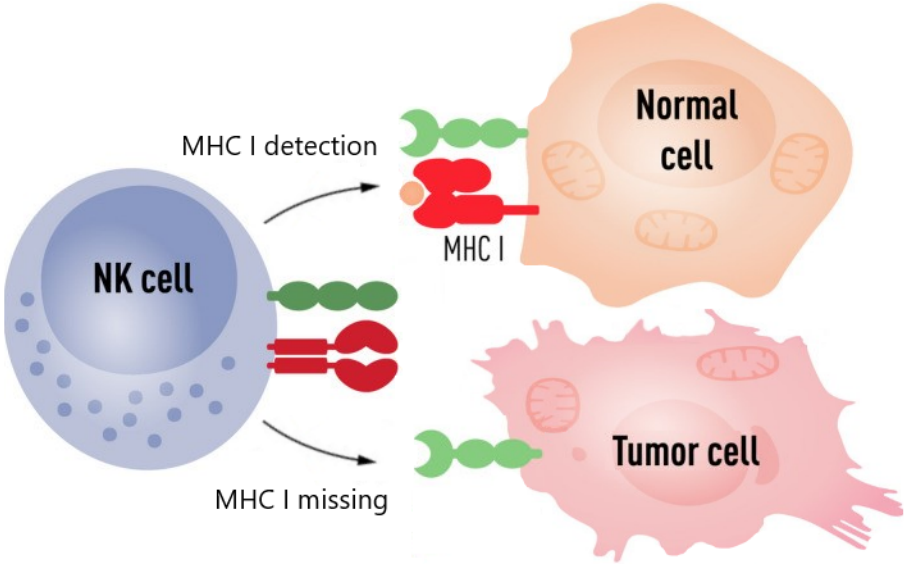


Figure 2: **Illustration of how NK cells differentiate between normal and cancerous cells.** The activating receptor (top on NK cell) successfully connects to most cells, while the inhibitory receptor (bottom on NK cell) usually does not find the MHC I molecule (bottom on normal cell) on tumor cells. For virus-infected cells, the receptors can be blocked by molecules, then the balance between the signals of these two receptors determines the action of the NK cell. Adapted from [52]CC BY.

[12]Continuous shedding and subsequent replacement with younger cells (two half-life cycles).

[13]Major Histocompatibility Complex (MHC) are proteins that "present" *antigens* to lymphocytes. Separated into two classes (I and II), class I functions as a surface-bound "display" for what is going on inside its cell.

Moving on to the **adaptive immune system**, its name comes from the fact that it is able to defend our bodies against most invaders. Specifically, if given time to prepare (e.g. through vaccines), the adaptive immune system can develop a defense against most foreign invaders. The immunity is provided by *antigens* inducing special proteins (*antibodies*) to circulate in our blood. These antibody molecules consist of different proteins and can bind to antigens. The production of antibodies takes place in a specific kind of white blood cells known as *B cells* (another lymphocyte, also originating from stem cells, see Appendix A Figure A.1), which can further mature into antibody-generating *plasma B cells* [45, 53]. Antibody molecules can also bind to cell receptors, and each kind of antibody only binds to one specific kind of antigen. Once the receptors of a B cell bind to their associated antigen, the B cell starts to proliferate to build up a reliable defense [54].

Notably, antibodies themselves do not kill, they only "tag" (*opsonize*) the intruders to let other cells know that it is an invader and should be eaten or killed. Specifically, antibodies can encourage phagocytosis of the invader by bringing it close to a phagocyte and forming a bridge (see Figure 3) [31].

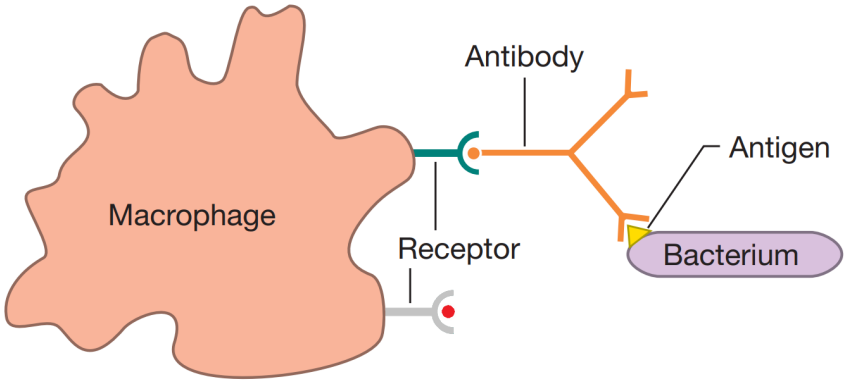


Figure 3: **Illustration of an antibody forming a bridge.** The antibody is bound to the antigen of an invader (bacterium) and brings it close to a phagocyte (macrophage). This process encourages the phagocyte to engulf the bacterium (which is expressing foreign molecules that are detected by other phagocyte receptors). Adapted with permission from [31].

Another important cell of the adaptive immune system is the lymphocyte known as the *T cell*. This cell also comes from the bone marrow (Appendix A Figure A.1) and looks almost identical to the B cell. However, different T cells can have different tasks, therefore, a T cell is generally classified as a killer T cell, helper T cell (Th cell), or regulatory T cell (Treg). The killer T cell is usually referred to by the name Cytotoxic T Lymphocyte (CTL) since it specializes in killing infected and cancerous cells by initiating their "suicide modes" (cytotoxic effect). On the other hand, Th cells do not kill directly, instead they secret cytokines (messengers, including IFN-γ from previously) to inform other cells of the immune system about an issue. Finally, Treg cells regulate the "intensity" of the immune system, to avoid the immune system running out of control and starting to attack healthy cells (i.e. autoimmune disease) [31].

For the T cells to know what to kill, they need to be presented to the antigens of the invaders (see Figure A.3 in Appendix A for the complete cycle of action of T cells killing cancer cells). The presentation is performed by the previously mentioned MHC proteins, where it is the T cell receptors' role to recognize the presented antigen. The class I MHC molecules know when there is an issue inside a cell and present antigens directly

to the CTLs (direct killing), while the class II MHC molecules know about extracellular issues and present antigens to the Th cells (indirect killing). The class I MHC molecules naturally exist on the surface of most cells, while class II MHC molecules can be produced by the macrophage, i.e. an Antigen-Presenting Cell (APC). Specifically, the macrophage "decorates" its surface with class II MHC molecules displaying protein fragments of the bacterium it just engulfed. The Th cells can then examine these class II MHC molecules with their receptors to identify the threat [31, 55].

Similar to the macrophages and NK cells, activation of the T cells and B cells is necessary for them to be effective with their immunosurveillance. For example, the Th cell (and other cells of the adaptive immune system) is activated when it is presented to its corresponding antigen by an APC and simultaneously recognizes a "control key"-protein on the APC's surface (see Figure 4). This two-key system is necessary to avoid accidental activation of the immune system. If the control protein was present, the Th cell starts to proliferate to build up a squadron of cells able to detect the specific antigen [55].

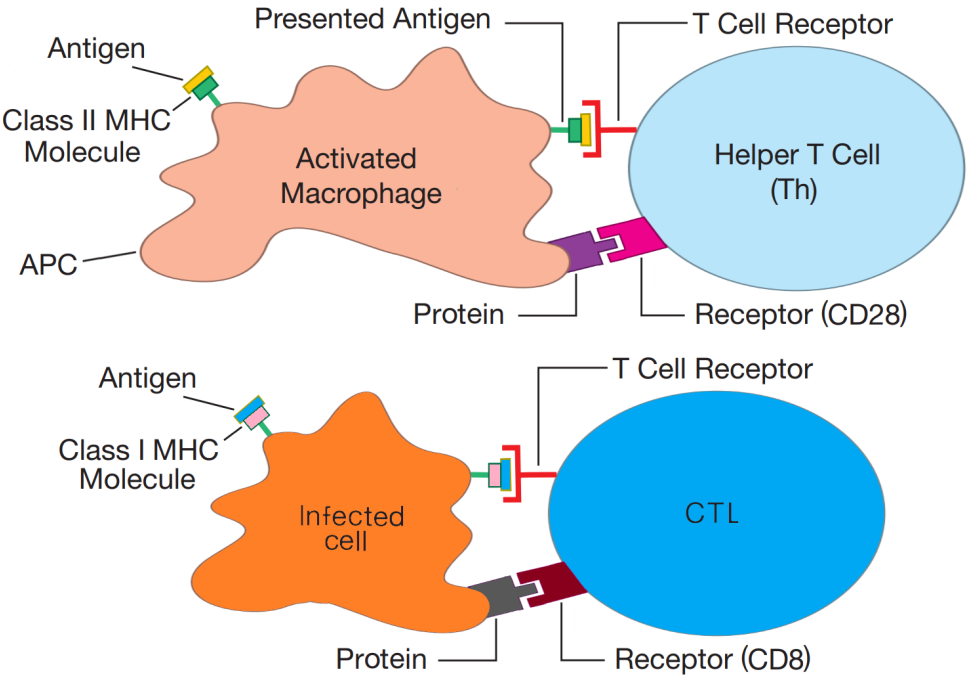


Figure 4: **T cell activation by MHC molecules.** (Top) An activated macrophage acts as an APC to activate a Th cell by presenting an antigen on its class II MHC molecule. The T cell receptors recognize the antigen and a specific protein on the APC surface (to ensure the Th cell that the APC can be "trusted"). (Bottom) An infected or cancerous cell is "examined" by a CTL using its T cell receptor and co-receptor, when CTLs recognize antigens in association with class I MHC molecules, they kill the opposing cell through granzyme secretion. Adapted with permission from [31].

Moreover, since both the T cells and APCs are widely spread out in our bodies, it seems unlikely that the APC which found the invader also will find a T cell with the correct T cell receptors to recognize the invader. However, our bodies contain special "meet-up spots" to make this process more likely. These meet-up spots are, specifically, our secondary lymphoid organs, e.g. *lymph nodes*, which are part of a lymphatic system (for more details on the lymphatic system, see [56], and the figure therein) [31, 56, 57].

Both B cells and T cells travel between nodes to "scout" for their corresponding

antigens. Since the lymph also tends to carry invaders and APCs to the lymph nodes, there is now a relatively small and confined place where the necessary communication for adaptive immune system activation can take place (thus increasing the likelihood for the interactions to happen) [57].

Furthermore, the most important APCs are the *dendritic cells* (see Appendix A Figure A.1). These function similarly to other APCs and will capture protein fragments from dying cells to present for T cells, that is, they can activate T cells to take care of tumors and ongoing infections. Specifically, dendritic cells collect MHC complexes from infected or cancerous cells to display on their surfaces [58].

To conclude this introduction to the immune system, the innate system is always in place and ready to deal with invaders, but when the "war" becomes too great, the adaptive system has to help get rid of the invaders. But it does take time for the specialized B cells and T cells to proliferate and build up their forces, therefore, the innate system has to do its best in the meantime. Furthermore, when the war is over, some B and T cells (specialized on the specific invader) will stick around as *memory cells*. These memory cells will simplify the process of a counterattack if reinfection occurs, allowing the immune system to act quickly (cf. vaccines) [53, 57, 59].

3 Immunotherapy

The full potential of the immune system is still beyond our understanding, but as the previous section emphasizes, it is powerful. Even though the immune system and immunotherapy are not fully understood, existing methods make use of the immune system to treat cancer. For example, checkpoint inhibitors (awarded a Nobel Prize in 2018) are one class of immunotherapy that focuses on how immunosuppressing proteins (immune checkpoints) can be blocked to "unleash" the immune system [14, 60].

When surface proteins of T cells bind to other cells, the immune checkpoints act to regulate the immune system and prevent overreaction. The surface proteins of the T cells (checkpoint proteins) are part of the "two-key" system previously explained in relation to Figure 4. Typically, cancer cells avoid T cells by bearing the right partner proteins. Therefore, when the T cell binds to the cancer cell, the "control-key" is detected and the T cell does not take any action. By using checkpoint inhibitor drugs, the checkpoint proteins can be blocked to avoid detection of the control-key, thus causing the T cells to attack cancer cells [61].

Consequently, checkpoint inhibitors increase the likelihood of cancer cell detection and elimination by the immune system [60]. Specifically, there is a checkpoint protein called Programmed cell Death protein-1 (PD-1), with corresponding partner protein (ligand) PD-L1. PD-1 is a surface protein of T cells, and PD-L1 exists on both normal and cancerous cells, the production of PD-L1 can even be major in some tumors. In the event where PD-1 of a T cell binds to PD-L1 of a cancer cell, the T cell is "told off" from killing the cancer cell. When immune checkpoint inhibitor drugs then block PD-1 and/or PD-L1 proteins, T cells will start killing the cancer cells, see Figure 5 [61].

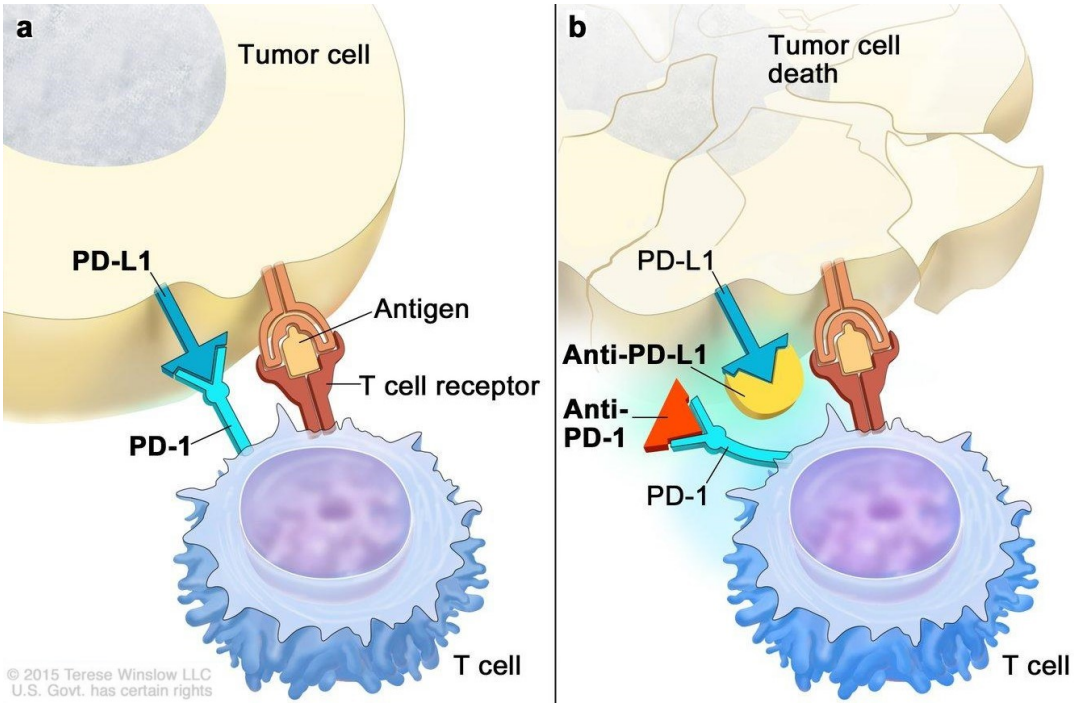


Figure 5: **Illustration of how checkpoint inhibitors work**, considering the proteins PD-L1 (on the tumor cell) and PD-1 (on the T cell). (a) When PD-1 binds to PD-L1, the T cell is kept inactive. (b) The binding of PD-L1 and PD-1 is prevented through blockage using immune checkpoint inhibitors anti-PD-L1 and anti-PD-1, causing the T cell to become active and kill the tumor cell. Adapted with permission from [61].

Some key surface proteins of cancer cells can even be disabled or locked through binding engineered antibodies to them, thus making it easier for cells of the immune system to kill cancer. The antibodies also opsonize the cancer cells to make them easier to detect by the immune system, they can even bring T cells close to cancer cells (see Figure 6). This specific kind of checkpoint inhibitor is known as antibody therapy [14, 60].

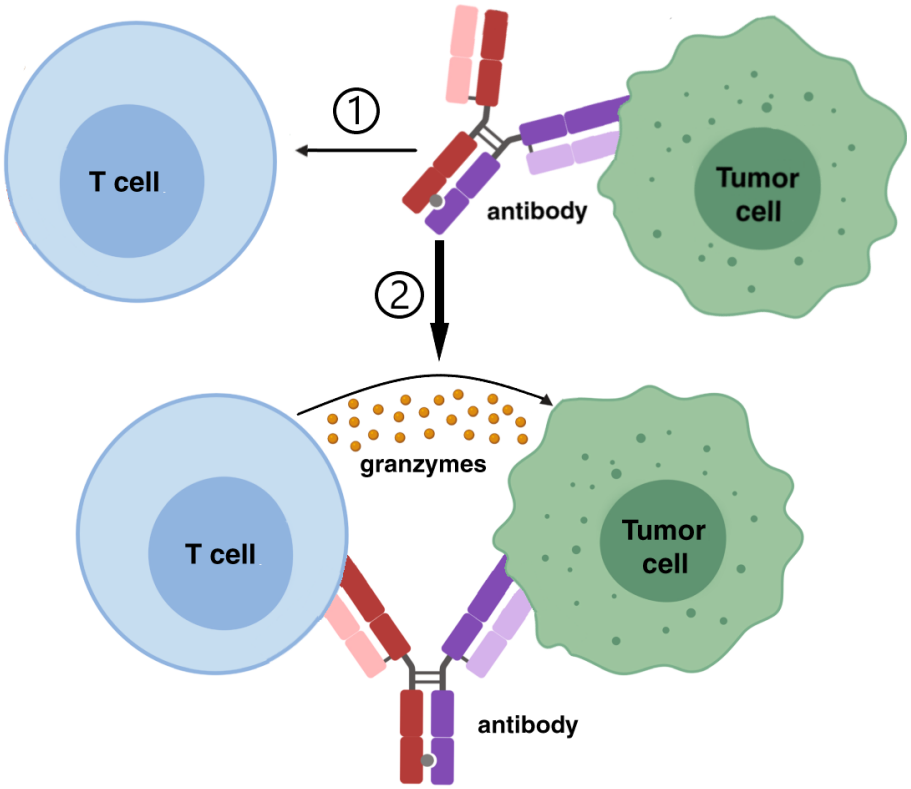


Figure 6: **Illustration of an antibody bringing a T cell close to a tumor cell**, such that the T cell can "inject" the tumor cell with granzymes (suicide-causing enzymes). Adapted from [62]CC BY.

It is also possible to engineer T cells to be super-activated and more prone to kill cancer cells, e.g., through Chimeric Antigen Receptor (CAR) T cell therapy [60]. This kind of therapy (T cell transfer therapy or adoptive cell therapy) involves the extraction of immune cells from the patient suffering from cancer, these cells will then proliferate in a lab, and later be re-injected into the patient. In CAR T cell therapy, the extracted T cells are modified to produce CAR proteins, which allows the T cells to attach to surface proteins on cancer cells (for details, see Appendix A Figure A.4). Alternatively, Tumor-Infiltrating Lymphocyte (TIL) therapy can be applied, which uses artificial selection to extract TILs[14] from a tumor sample. The TILs are then left to proliferate and build up in number before they are re-injected into the patient [63].

In principle, most T cells can recognize tumor proteins as foreign. However, tumors tend to disarm and deactivate T cells in their microenvironment by secreting chemical signals, causing T cells to only recognize and kill cancer cells under certain circumstances. Therefore, one branch of immunotherapy focuses on how to activate these inactive T cells. For example, this can be achieved by one type of dendritic cell that "decorates" itself with cancerous proteins from tumors. Through stimulating these dendritic cells, the effect of immunotherapy can be improved (specifically, the growth rate of some tumors in mice has been shown to decrease) [64, 65].

There is a specific type of dendritic cell that interacts with cancer-killing T cells, and another type that encourages T cells to activate in regressing tumors. The relevant dendritic cells are activated by the specific interferon known as type-I IFN. Therefore,

[14]Most tumors contain some tumor-infiltrating lymphocytes that can recognize the cancer cells.

stimulation of dendritic cells can be performed by inducing an increase of this cytokine [58, 65].

Unfortunately, interferon treatment of cancer can induce side effects, for example, flu symptoms and lowering of blood cell count [66]. Direct or targeted interferon delivery is therefore necessary, alternatively, a drug could potentially be used to have the cancer cells themselves produce IFN type-I. Notably, many tumor cells naturally secrete IFN type-I, but these are too few to cause the dendritic cells to activate. Furthermore, too many IFNs are generally toxic to cells, even slight alterations can cause dramatic immune responses [65].

In general, the precise cytokine dosage to achieve safe activation of T cells is crucial for immunotherapy to be harmless. Through genetically engineered immune system cells, the development of technologies with accurately controlled signaling is possible. By studying and applying different technologies, possible upper and lower boundaries of cytokine dosage could potentially be established for the safe activation of T cells (at least the degree of cytokine control could be evaluated). If these boundaries can be determined, mathematical simulations and models could possibly predict the necessary degree of T cell activation (cytokine dosage) to regulate the response of the immune system in a controlled manner. Noteworthy, it is important to carefully consider the balance between apoptosis-encouraging and apoptosis-inhibiting cytokines, but this is where the coexistence of computer models and experiments can help to formulate new relationships, molecular mechanisms, and establishment of optimal dosage [67].

It has been experimentally proved that cancer cells can be weakened (made less tolerant) to CTLs by direct injection of mature (antigen-loaded) dendritic cells into the tumor, or by endogenous dendritic cell depletion [68]. Tumor infiltration by T cells is also known to contribute to a good prognosis in multiple different cancer types (e.g. triple-negative breast cancer) [69]. Furthermore, in adoptive cell transfer therapy, the T cells need to be primed with their corresponding antigen to interact with the tumor (e.g. by stimulating endogenous dendritic cells). Alternatively, the T cells can be genetically modified to engage dendritic cells by secreting a specific cytokine (FLT3L). In fact, research proves that this modification increases the efficiency of the infused T cells [70, 71].

Methods such as vaccination with viral tumor antigens, stimulation of T cell receptors, and engaged dendritic cells, all increase the efficiency of infused T cells. By further adding checkpoint inhibitors to the above combination of methods, the inactivation of tumor-reactive T cells can be avoided, and the overall effect of adoptive cell therapy is further improved [70].

Notably, the adaptive immune system is not alone in the fight against cancer cells and tumors, the macrophages and NK cells of the innate immune system can also help [46, 52]. Macrophages physically ingest cells (phagocytosis) and are also able to devour cancer cells. However, the tumor microenvironment tends to suppress the macrophages' ability to fight the tumor. Especially, the cancer cells can express a specific receptor (CD47) to deactivate the macrophages, but this suppression of the macrophages can be avoided by using checkpoint inhibitors (cf. method for increasing cancer cell detection of T cells). Furthermore, the tumor can also take (indirect) advantage of the macrophages to metastasize throughout the body. The relation may be complex, but it is believed that macrophages' ability to fight cancer can be enhanced through immunotherapy [46].

The usage of NK cells in immunotherapy is similar to the T cell-based immunotherapies. Even though T cell-based therapies are currently the most researched and reliable, NK cell-based therapies show promising potential by having more manageable safety

profiles and being less transfer sensitive (relevant in adoptive therapy). The NK cells also have the advantage that they can recognize and kill target cells without the need for antigen presentation. Specifically, when the NK cell faces a tumor cell, the class I MHC molecule is likely to be absent (see Figure 7, cf. Figure 2). If the NK cell's inhibitory receptor is not stimulated, the NK cell will kill the opposing tumor cell and secrete cytokines to attract more immune cells [52, 72].

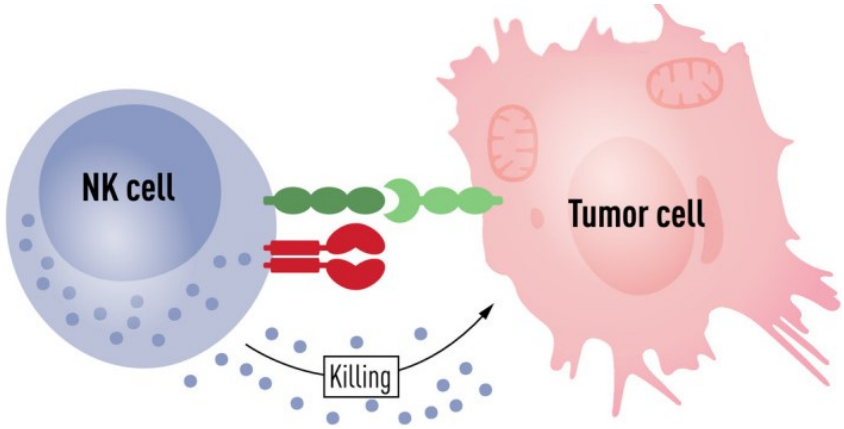


Figure 7: **Illustration of an NK cell killing a tumor cell** by inducing apoptosis (release of cytolytic granules, like granzyme B) due to the tumor cell's lack of class I MHC molecules on its surface. Adapted from [52]CC BY.

To concluded, some common immunotherapies are presented in Table 1 below.

Table 1: Excerpt of some immunotherapies, composed from [14].

Immunotherapy	**Definition**
Immune checkpoint inhibitors	Drug-induced blockage of immune checkpoints that down-regulate the immune system.
T-cell transfer therapy	Boosting of T cells' ability to detect and kill cancer cells.
Monoclonal antibodies	Laboratory created proteins that bind to cancer cells to mark (highlight) them.
Treatment vaccines	Teaching the immune system to recognize tumor-associated antigens to destroy cancer cells.
Immune system modulators	Boosting immune response against cancer by using agents (cytokines) that affect the immune system.

3.1 Issues of Immunotherapy

The above (and other) immunotherapies tend to solve separate cancer-related problems. There seems to be no single optimal immunotherapy, in fact, the individual aspect plays a major role. The concept of a (non-static) framework was recently proposed in the form of a "cancer immunogram", which aims to describe the effects of different immunotherapies on an individual basis (with a focus on biomarkers). This framework emphasizes that some patients may suffer from insufficiently foreign cancer, making it difficult to establish T cell response. Therefore, treatment with a combination of immunotherapies and biomarkers might be necessary [73].

Furthermore, cytokines play an essential role in the activation of the immune system to fight cancer, however, there are many different kinds of cytokines. Even though some cytokines have positive effects on immunotherapy, cytokines can in general either promote or suppress tumor progression. In fact, certain cytokines are necessary for the tumor-promoting activity of suppressor cells (immunosuppression). Some cytokines, secreted by tumor-infiltrating lymphocytes, even promote cancer metastasis through angiogenesis[15]. Therefore, the presence of cytokines can also be associated with tumor survival and malignancy [74].

Undeniably, a major problem with immunotherapy is that there exist immunosuppressive mechanisms present in tumor microenvironments. Such molecules (cytokines) can be produced by the cancer cells themselves, but also by Treg cells and macrophages. These cytokines negatively affect the different lymphocytes (like the NK cells) by suppressing their metabolism and helpful cytokine secretion. These unfavorable cytokines can even hinder dendritic cells from acting as APCs [72, 75].

Arguably, the factors that influence the immunological possibility to fight cancer can be generalized into seven different tumor features. (1) Tumors tend to sustain themselves with growth factors (generally these are also immunosuppressive). (2) Tumors tend to be unaffected by anti-growth signals, consequently, there will be local immunosuppression. (3) Through overexpression of cell-death inhibitors, tumors can avoid apoptosis. (4) Mutations in the p53-gen cause uncontrolled cell replication. (5) Continuous angiogenesis implies tumor production of factors that can inhibit activation of T cells and maturation of dendritic cells. (6) Metastasis can locally supply tumors with different troublesome immunological features. (7) Tumors can also evade detection by the immune system by continuous adaption and immunosuppressive networks in its microenvironment (to inactivate approaching cells of the immune system) [76].

To avoid some of these immunosuppressive factors, the method of immune checkpoint blockade is reasonable to apply. However, the application of this treatment only seems to be effective regarding certain types of cancer. Even when the initial response and prognosis appear to be good (e.g. for patients with melanoma), a tendency of developing resistance to the treatment is observed [77]. Extensive research on the specifics of these mechanisms will help to improve immunotherapy methods and contribute toward defeating the existing resistance. This can for example be studied by addressing the evolutionary dynamics of checkpoint blockade resistance, but there is still a lot of research needed [78].

The immune system can recognize and destroy cancer cells by having T cells attach to tumor-specific antigens (neoantigens), but tumors are still able to escape. For this purpose, normal cells can be engineered to express specific neoantigens, such that *in vivo* observations can determine which neoantigens provoke immune response. While tumor cells with immunogenic antigens are easily detected and likely to be destroyed, the opposite holds for nonimmunogenic antigens. Notably, even tumors with few immunogenic antigens tend to be killed, but when the amount is substantially small (every hundredth cell), the tumor survives since the T cells have "too little to work with". This infers that individuals with heterogeneous tumors (a large variety of clonal cells) generally benefit less from immunotherapy [79].

In the following section, the effects and efficiency of immunotherapy will be investigate considering mathematical and physical perspectives.

[15]A process leading to the development of new blood vessels from existing blood vessels.

4 Physics Methods of Immunotherapy

4.1 Mathematical Models

Mathematical and computational models allow for the investigation of immune system components in an integrated manner. The models can provide understanding of how immunological components interact with each other, and allow for investigation of the effect of specific conditions. Furthermore, models used in immunology are often "universal", for example, models constructed to describe infection-dynamics can be applicable when describing interactions of the immune-cancer system [80].

So-called phenomenological (quantitative) models, aim to extract patterns (such as correlation coefficients or regressions) from data [81]. Due to the development of large data processing using deep learning methods, complex phenomenological models can make predictions and find patterns without knowing any underlying system mechanisms [82]. Therefore, to understand the underlying system mechanisms, studies using mechanistic models are best suited. Such models can consider multiple different interacting components at once, however, this causes the models to often be simplified[16], but they still provide mechanistic insights. Contrary to phenomenological models, mechanistic models require system-specific knowledge and assumptions about the component interactions [80].

It is also important to notice that the many and diverse mathematical models that attempt to describe and quantify tumor systems, all suffer from reductionism. The two extremes would be exhaustive biology models (considering as many cell-cell signals as possible) and simple deterministic models (designed for deterministic and mathematical analyses on optimization and control) [83].

Specifically, different models and what they model can be split into four categories: (1) Simple growth models - A single population of cells. (2) Compartmental models - Coupling of cell populations, e.g. represented by a probabilistic or deterministic Ordinary Differential Equation (ODE) describing population size. (3) Variability - Heterogeneity represented by continuous cell-variables (space, age, size, etc.), each variable described by a Partial Differential Equation (PDE). (4) Agent-based models - Probabilistic or deterministic evolutionary rules for cell variables (e.g. spatial and age-related). Furthermore, these four models can also be combined. One example would be cell populations described by agent-based models, where the connection of the populations is described by signaling molecules, which in turn are described by spatially structured PDEs [83].

The compartmental models are usually considered the most common in immunological modeling, where cytokine concentrations and population size of a specific cell type are commonly modeled using ODEs [80]. However, just as with the different immunotherapies, there is no ideal mathematical model. Depending on the circumstances and the available parameter data, different models may be best suited. In the context of immunotherapy, the relation and interactions between tumors and the immune system are of most interest. For example, regarding checkpoint inhibitor therapies, the dynamic process describing the relation between tumor cells and the immune system can be modeled (known as immunoediting, which considers the three phases: elimination, equilibrium, and escape [84]). It would also be relevant to investigate and model the diffusion of drugs and nutrients in tumor spheroids [83].

Tumor spheroids correspond to one common way to represent solid tumors' structure,

[16]Yet, the physicist knows that simplified equations can often accurately describe system behaviors.

where the composition is often generalized into three different layers, see Figure 8.

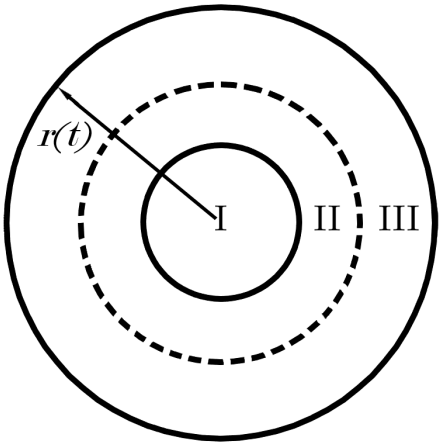


Figure 8: **Structural depiction of a solid tumor**. The composition of multi-cellular tumor spheroids with some radius $r(t)$ (only the outer [largest] radius is marked here since it is usually this radius that is considered when modeling solid tumors). The composition is generally idealized into three layers: Necrotic core (I), Quiescent (non-proliferating) cells (II), and Proliferating cells (III).

Notably, tumor progression eventually tends to result in metastasis. This invasive growth can be sporadic and less predictable than compact growth. Therefore, mathematical models generally regard tumor spheroids, which also represent the most promising (yet versatile) models [83, 85].

In the most basic and generic case, compartmental growth (of some population X, e.g. cancer cells) can be modeled by considering a growth rate (g) and a death rate (d) according to,

$$X(t + dt) = X(t) + [gX(t) - dX(t)]\mathrm{d}t. \tag{1}$$

The model is discrete and uses a time step of size $\mathrm{d}t$, where the population after each time step has increased or decreased with the difference between the growth and death rate of the population at the last time step.

To obtain a continuous model, Eq. (1) can be turned into an ODE by allowing the time step to be infinitesimal. The growth is then described by,

$$X'(t) = gX(t) - dX(t), \tag{2}$$

with the corresponding solution

$$X(t) = X(0)e^{(g-d)t}, \tag{3}$$

where it is noted that $g > d$ results in an exponential increase, and $g < d$ in an exponential decrease towards zero.

The ODE in Eq. (2) corresponds to simple population growth, specifically, the immunological and cellular contexts demand more variables to be realistically described by mathematical models. On small scales (e.g. in the context of extinction dynamics), biological systems are also governed by randomness, which can not be accounted for with ODEs due to their determinism. Therefore, stochastic models, although mathematically complicated to analyze, could also be considered. [80].

Moreover, most ODE models lack the spatial aspect, and can at best only approximate representations of space. Instead, models which include spatial coordinates are usually formulated in terms of PDEs. However, these are usually cumbersome in comparison to ODEs, and they still do not optimally account for immunological spatial features [80].

The fifth of the earlier presented model categories was agent-based models, these are well suited for including spatial representations. Contrary to the compartmental ODE models, these consider populations on an individual basis. Agent-based models are illustratively realistic and account for influenced, stochastic, or governed movement of each system component (such as the T cells), as well as their spatial interactions with other system components. The individual component behaviors in these models are commonly determined using parameters sampled from probability distributions to implement diversity. Notably, agent-based models are often difficult to analyze due to the large amount of system-defining parameters and quantitative information needed [80].

For a hands-on experience and an introduction to the use of mechanistic simulation models in immunology, the `R`-package DSAIRM (Dynamical Systems Approach to Immune Response Modeling) is available, see [86, 87].

4.1.1 Undisturbed Tumor Growth

Gompertzian growth curves are phenomenological models that describe undisturbed tumor growth. This growth model assumes that the proliferation rate decreases exponentially with time [22, 83]. The equations of the system can then be written as,

$$\begin{cases} V'(t) = P(t) \cdot V(t) \\ P'(t) = -\gamma P(t) \end{cases}, \tag{4}$$

where $V(t)$ is the time-dependent tumor volume, $P(t)$ is its proliferation rate, and γ is the rate at which the growth slows down. Solving for $V(t)$ gives,

$$V(t) = V_0 \exp\left(\frac{P_0}{\gamma}(1 - e^{-\gamma t})\right), \tag{5}$$

where V_0 is the initial volume, and P_0 is the initial proliferation rate. Considering the derivative of Eq. (5), Eqs. (4) can conveniently be written on a one-dimensional form,

$$V'(t) = P_0 V(t) - \gamma V(t) \ln V(t). \tag{6}$$

According to Eq. (6), the volumetric growth rate of tumors depends on one growth-promoting term (directly proportional to the tumor volume) and one death term (proportional to the tumor volume and its logarithm).

Alternatively, tumor growth is also sometimes [83] modeled by the Bertalanffy equation [88],

$$V'(t) = P_{\text{surf.}} \cdot V(t)^{2/3} - M \cdot V(t). \tag{7}$$

The ODE assumes that the total growth scales with the proliferation rate ($[P_{\text{surf.}}] = [\text{length}/t]$) and the tumor's surface area, with a decrease (due to cell death) proportional to its volume with some constant $[M] = 1/[t]$. In comparison to Figure 8, the Bertalanffy equation can be interpreted to take the different layers into account, where it is only the proliferating cells (surface layer) that cause the tumor to increase in its volume. The proliferation term ($P_{\text{surf.}}$) is thus dependent on the width of the proliferation layer (assumed to be constant, see more in relation to Eq. (9) on Page 22).

The analytical solution of the Bertalanffy equation is computationally heavy, however, its growth curve obtained through a numerical solution is given along with a Gompertzian and a logistic growth curve in Figure 9 below. Notably, the logistic growth curve is included due to its (to be seen in Section 4.1.2) frequent appearance in mathematical models.

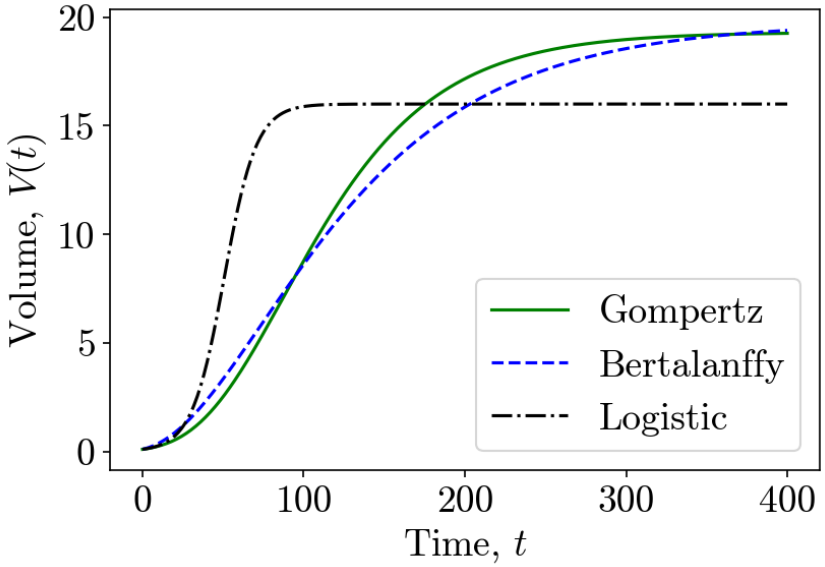


Figure 9: **Time-dependent tumor growth.** Note that the unit of $V(t)$ is considered arbitrary (could be cm^3, or the number of cells, etc.). The growth functions assume a proliferation rate of $P = 0.1/\text{day}$ (or, e.g., [cm/day] for the Bertalanffy equation) and an initial tumor volume of $V_0 = 0.1$. The growth is modeled by one Gompertzian curve (green line, corresponding to Eq. (5)), one Bertalanffy curve (blue dashed line, corresponding to a numerical solution of Eq. (7)), and also one logistic curve (black dot-dashed line corresponding to a numerical solution of $V'(t) = PV(t)(1 - V(t)/\kappa)$, which appears in models considering tumor carrying capacity, κ). Here, $\gamma = 0.019/\text{day}$, $M = 0.037/\text{day}$, and $\kappa = 16$ ($[\kappa] = [V(t)]$).

The features of the sigmoidal curves emphasize the tendency of tumors to initially have exponential growth, then enter a more linear phase, and finally a plateau phase (where saturation is reached).

It should also be noted that, in influenced tumor growth, a death term $-c(t)V(t)$ should be included in the growth models, where the function $c(t)$ represents the cytotoxic effect of drugs, treatment, or immune cells on the tumor [83]. Furthermore, if the natural proliferation rate can be influenced, $P = P(V, t)$ should be considered with some proportionality to an effective function $P_e(t)$. This proportionality is, for example, accounted for in the model presented in [83],

$$V'(t) = \left(\frac{P(V,t)}{1 + P_e(t)} - c(t) \right) V(t). \tag{8}$$

The growth models from Eqs. (6-8) do no not explicitly assume any structural geometry of the tumors (except for Eq. (7)), i.e. the volumetric variable can also be interpreted as a literal cell count (with parameter units adjusted accordingly). These kinds of growth models aim to supply a macroscopic and phenomenological growth quantification, without assumptions on the tumor structure. Thorough models should also consider tumor

heterogeneity and geometry, e.g. through spatially distributed models (however, heterogeneity is not necessarily directly correlated to space). Other parameters that might be more relevant than spatial aspects include the tumor cell ages (cell-division cycles and internal cell traits on the individual scale) and drug-induced resistance (adaptability of the tumor microenvironment) [83].

Contrary to the models presented above, growth models can also be constructed to explicitly consider the tumor geometry and structure. The most simple ones assume tumor spheroids growing in a sigmoidal manner (cf. Figure 8 and Figure 9). Models considering tumor spheroids commonly assume perfect symmetry, both geometrically (with some radius $r(t)$) and property-wise (e.g. proliferation, necrosis, and diffusion) [89]. Volumetric models are then rewritten in terms of the tumor radius by replacing $V(t)$ with $\frac{4}{3}\pi r(t)^3$. A well-studied model originated from observations of constant proliferation rate (P) in the proliferating layer of tumor spheroids (III in Figure 8) [90], and that this outer layer of the tumor has a constant width (w) [91]. The model was later written to have the tumor initially grow exponentially [92] (omitted here), and finally through the introduction of a general death term (proportional to the spheroid radius and a rate constant, γ) [89], the model reads,

$$r'(t) = Pr\left[\frac{w}{r} - \left(\frac{w}{r}\right)^2 + \frac{1}{3}\left(\frac{w}{r}\right)^3\right] - \gamma r. \tag{9}$$

Moreover, undisturbed tumor growth has also been described in spatial models using PDEs (see more in Section 4.2.2).

4.1.2 Cancer–Immune Interactions

The interactions between cancer cells and immune cells have already been explored and accounted for in Sections 2 and 3, but to summarize and recall these cancer-immune interactions, the diagram below in Figure 10 is considered. It should be noted that the mathematical interpretation of these cancer-immune interactions (mainly cancer cell death through cytotoxic effect, and immune cell inactivation) are commonly characterized using ODEs [83].

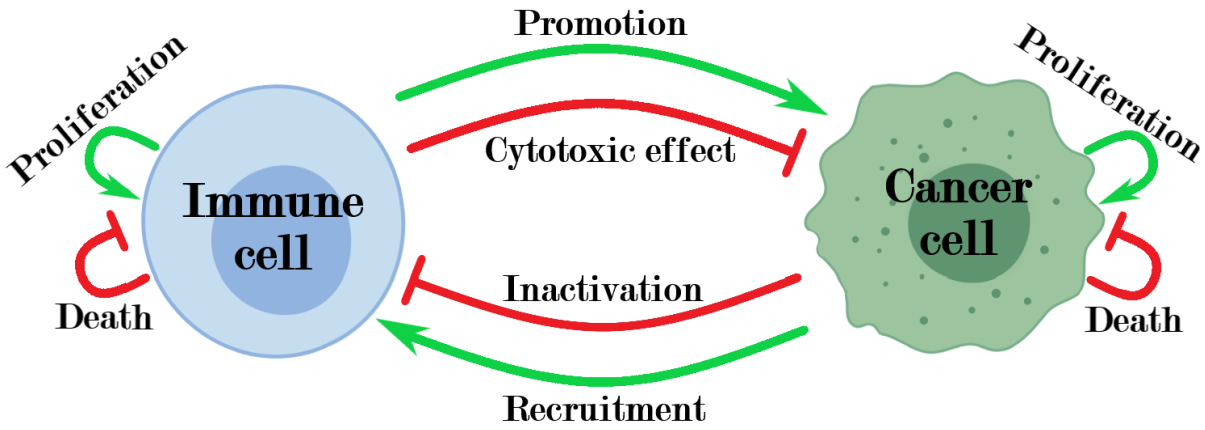


Figure 10: **Diagram depicting growth-associated cancer-immune interactions.** The green arrows represent growth stimulation, and the red bar lines represent growth inhibition. It should be noted that most models neglect the promoting effect that immune cells can have on cancer cells.

The dynamical process of the immune-cancer system is of great importance in the context of immunotherapy, and it is referred to as immunoediting. As briefly mentioned earlier, immunoediting considers three different phases: **Elimination** - through sufficient cytokine production, tumor antigen recognition by dendritic cells, or active CTLs and NK cells, tumor cells are eliminated by the immune system. **Equilibrium** - through immune cell evasion and inactivation, some tumor cells avoid elimination. **Escape** - escalated growth of the tumor cells that survived the elimination phase and evaded immune detection in the equilibrium phase (e.g. through immunosuppressive cytokine secretion or an apoptosis encouraging tumor microenvironment) [93]. The three phases are illustratively depicted below in Figure 11 (and an even more extensive illustrative poster of cancer immunoediting can be found in [93]).

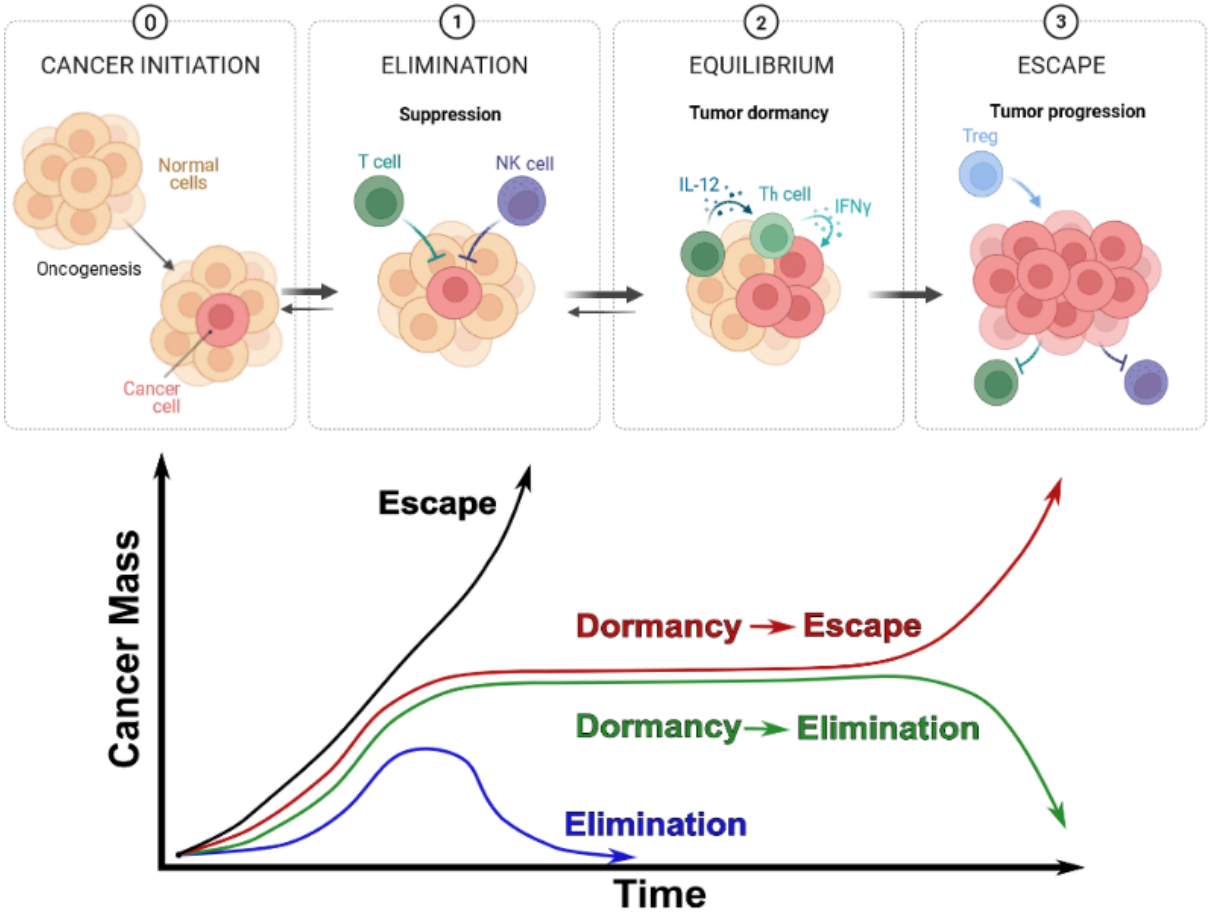


Figure 11: **Illustration of immunoediting and its three phases.** ⓪ The initial condition, where a normal cell has developed an oncogene and turns into a cancer cell (oncogenesis). ① The elimination phase, where immune cells such as T cells and NK cells suppress the cancer cell from proliferating. ② The equilibrium phase, where the tumor is dormant and anti-tumor cytokines (IL-12 and IFN-γ) are maintaining the cancer in its equilibrium state. ③ The escape phase, where the tumor will progress by evading the immune system and taking advantage of the Treg cells. The bottom panel shows the time-dependent growth profiles corresponding to the immunoediting process. Adapted from [94, 95]CC BY.

With the dynamical aspects in consideration, a simple and straightforward way to investigate cancer-immune interactions is to regard the Lotka-Volterra [96, 97] (predator-prey) model, with the number of cancer cells as the prey and the number of immune cells as the predator. Notably, this model neglects many of the essential interactions shown in Figure 10, however, it acts as a good starting point. The equations of such a system look like this,

$$\begin{aligned} C'(t) &= P_C C - \gamma C I \\ I'(t) &= P_I C I - \delta I, \end{aligned} \tag{10}$$

where P_C is the proliferation rate of the cancer cell population $C = C(t)$, γ is the death rate of cancer cells (proportional to both the cytotoxic immune cell population, $I = I(t)$, and the cancer cell population). Furthermore, the immune cell population is growing with some proliferation rate P_I (which is affected by the cancer cell population[17]), and δ is the natural death rate of the immune cells. Note that the tumor volume function $V(t)$, from the previous section, can correspond to the same thing as $C(t)$. The change of notation from this point onward is to remove the geometric analogy and, instead, consider the size of a cancer population as the number of cancer cells in the tumor. The interaction between cancer cells and immune cells according to the Lotka-Volterra model can be seen in the phase portrait below.

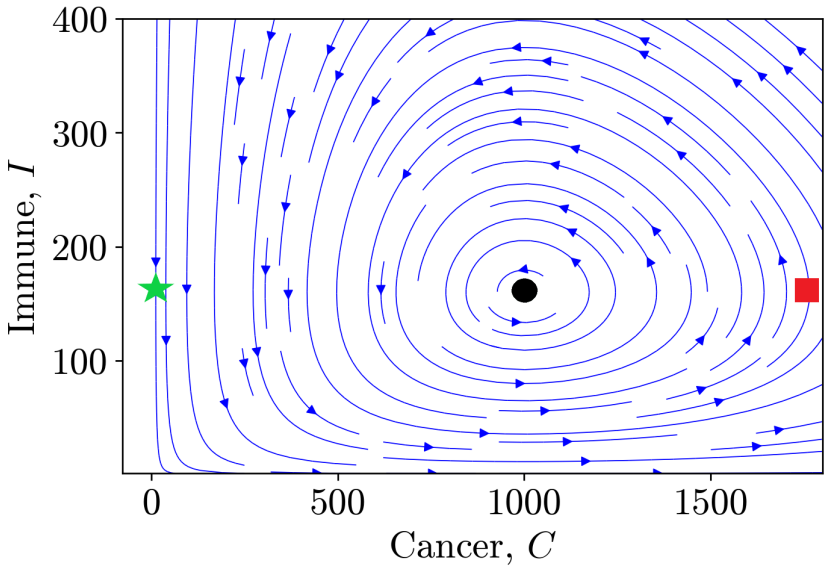


Figure 12: **Phase portrait of the predator-prey model** according to numerical solutions of Eqs. (10). The phase portrait represents how the system evolves, where the direction of time is indicated by the arrows. The green star indicates a state of possible tumor elimination, the black circle represents a stable state for a dormant tumor, and the red square indicates a state of possible tumor escape. The specific parameters used are $P_C = 0.08/\text{day}, \gamma = 0.0005\ \text{day}^{-1}\text{cells}^{-1}, P_I = 0.0002\ \text{day}^{-1}\ \text{cells}^{-1}$, and $\delta = 0.2/\text{day}$.

[17] Originating from the fact that the predators survive on nutrition from the prey, which is not the case for cancer and immune cells. However, as conveyed by the introductory section, if the immune system recognizes a great population of intruders (or cancer cells), an increase of immune cells can take place to deal with the issue. Yet, as also discussed, immune cells can have a hard time recognizing cancer cells, so the matter is complicated. But as to be seen, this proportionality remains even in the more realistic models.

A more physical extension of the predator-prey model was presented in 1973 by Bell [98],

$$\begin{aligned} C'(t) &= P_C C - \gamma \frac{CI}{1+C+I} \\ I'(t) &= P_I \frac{CI}{1+C+I}(1-\nu I) - \delta I, \end{aligned} \tag{11}$$

where ν is an antibody production inhibiting constant. If the antibody production is considered unlimited ($\nu = 0$) the only difference to the predator-prey model in Eqs. (10) is that Bell's model introduces finite limits to the population growth ($\frac{1}{1+C+I}$). Even though Bell's model has similarities to the predator-prey model, they exhibit dramatically different behaviors.

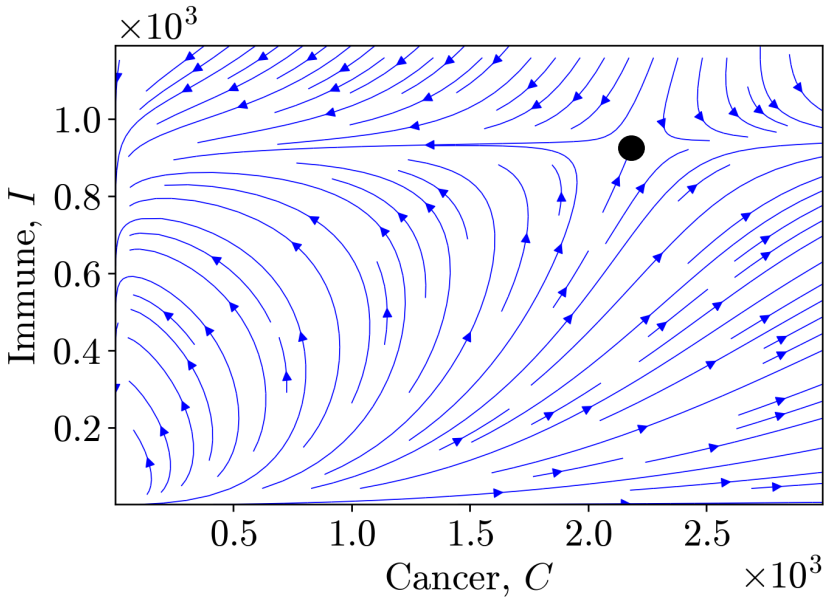


Figure 13: **Phase portrait of the Bell model** from Eqs. (11). The black circle marks an unstable state between tumor escape (to its right) and tumor elimination (to its left). Notably, this model is not meant to represent cancer-immune interactions, and even in the context of modeling viral bacterial infections (its intended use), Bell deems it difficult to select biologically realistic parameter values. However, estimations based on his discussion in [98] are used here and read as $P_C = 0.9/\text{day}, \gamma = 3/\text{day}$, $P_I = 2.4/\text{day}, \delta = 0.1/\text{day}$, and $\nu = 0.001/\text{cells}$.

Other appearances that the model by Bell can take are extensively accounted for in [98]. But one interesting remark is that uncontrolled proliferation of the antigen (here, cancer) will occur when $\gamma P_I - \gamma\delta - P_I P_C \leq 0$, otherwise (for $\gamma < P_I$) the populations will oscillate towards a dormant tumor or (for $\gamma > P_I$) experience extreme oscillations, i.e. malignant tumor escape or complete tumor elimination (as seen in Figure 13) [28, 98].

Moving on to more realistic models for cancer-immune interactions, a (today classic) model was presented in 1979 by Stepanova [99]. The model assumes T-cell proliferation is stimulated by the tumor cells' antigens, and that the tumor growth rate is described by unperturbed tumor growth ($F(C)$, in accordance with the models presented in Section 4.1.1) subtracted by some death term proportional to the number of immune cells

and cancer cells. The model reads,

$$\begin{aligned} C'(t) &= F(C) - \gamma CI \\ I'(t) &= P_I\,(1 - \beta C)\,CI - \delta I + \alpha, \end{aligned} \tag{12}$$

where the immune cell proliferation is now suppressed by an immunosuppressive coefficient, β, related to the cancer cell population. In opposite to the natural immune cell death (δI), a natural generation (i.e supply from the bone marrow) of immune cells is also accounted for.

For a sufficiently small tumor, the immune system can prevent its growth, but after a critical point, the tumor escapes. This can be interpreted as an unstable manifold and is best represented in a phase portrait.

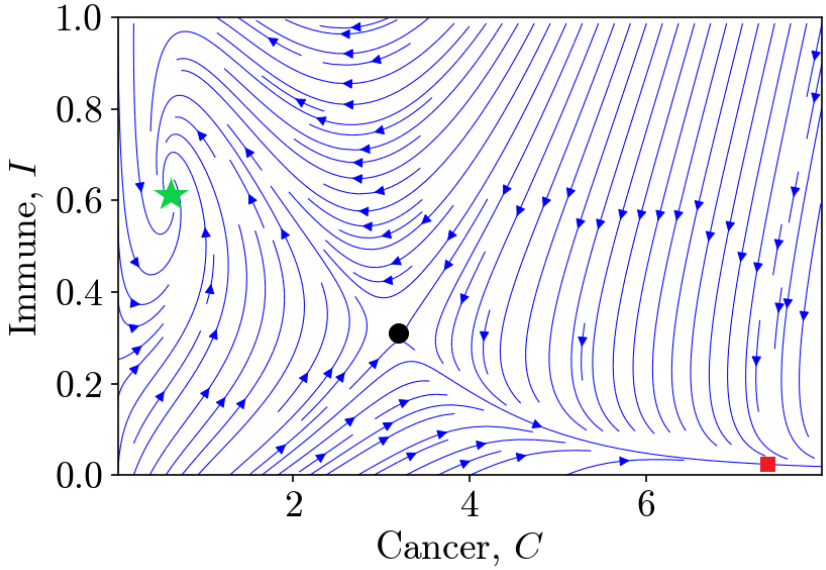


Figure 14: **Phase portrait of the Stepanova model** from Eqs. (12), considering tumor growth by the Bertalanffy equation from Eq. (7), i.e. $F(C) = P_C C^{2/3} - MC$. The parameterization has here been done in a normalized and unitless manner to better illustrate and the model behavior and disregard any volumetric assumptions, thus $P_C = 0.6, M = 0.098, \alpha = 0.1, \beta = 0.3, \gamma = 1, \delta = 0.4$, and $P_I = 0.5$ (inspired by parameters used in [83]). The green star represents a stable state for a nonmalignant (dormant) tumor, the black circle represents an unstable state, and the red square represents (malignant) tumor escape.

Another model introduces the necessity of the formation of cancer-immune cell complexes for any cell death to occur. The model, along with a kinetic scheme of cancer-immune interactions (see Figure 15), was proposed in 1994 by Kuznetsov et al. [100] and will be referred to as the Kuznetsov model from now on.

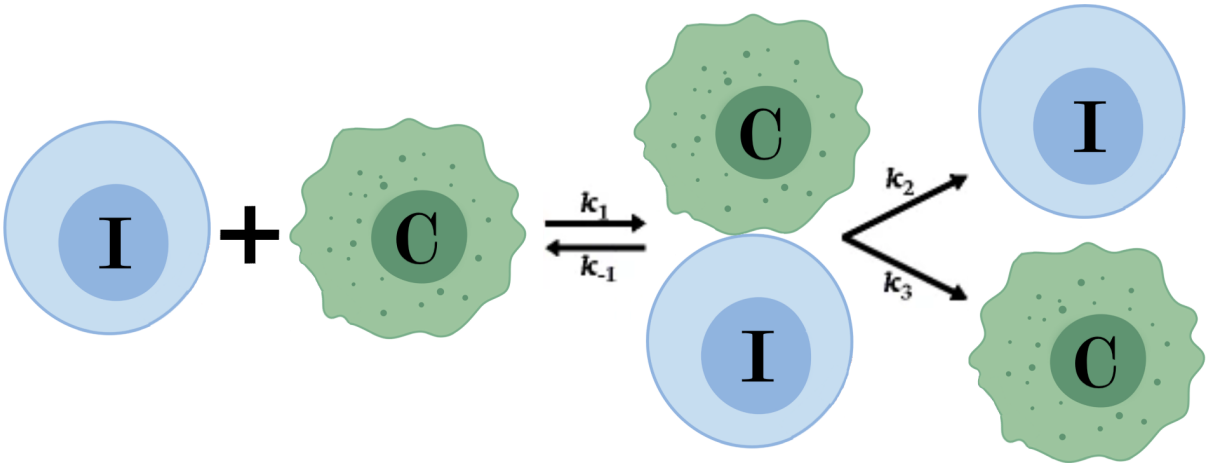


Figure 15: **A kinetic scheme of cancer-immune interactions** proposed in [100], where **I** denotes active CTLs and NK cells, and **C** represents cancer cells. At a rate of k_1, cancer and immune cells form complexes (which are also decomposed at a rate k_{-1}). The complexes also result in only immune cell survival with a rate of k_2, and only cancer cell survival (i.e. dead or inactivated immune cells) with a rate of k_3.

A simplified (2D) version of the Kuznetsov model can be seen below,

$$\begin{aligned} C'(t) &= P_C C\left(1-\frac{C}{\kappa}\right) - \gamma CI \\ I'(t) &= P_I \frac{CI}{g+C} - mCI - \delta I + \alpha, \end{aligned} \tag{13}$$

where, in relation to the k-rates from Figure 15, $\gamma = Kk_2$ and $m = Kk_3$, with $K = \frac{k_1}{k_2+k_3+k_{-1}}$ (considering β from Eqs. (12), $m = P_I\beta$). The cancer growth rate is here described by logistic growth (cf. $C'(t)$ from Eqs. (12) with $F(C) = PC(1-C/\kappa)$). The immune cell proliferation ($P_C CI$) is seen to be suppressed by the cancer population and some constant (g), while the total growth rate is (except for natural death and supply) influenced by cancer-induced immune-death ($-mCI$). Note that the complete Kuznetsov model also regards a third ODE, describing the formed complexes. However, the dimensional reduction is obtained by letting the complexes be approximately described by the product KCI (see details in [100]), which here allows for the depiction of the model in 2D phase portraits, see Figure 16.

A complete exposition of the parameters from Eqs. (13) can be seen below in Table 2 (along with their most realistic values according to [100, 101]).

Table 2: Description of the parameters in the Kuznetsov model from Eqs. (13).

Parameter	**Description**	**Estimated value**
P_C	Proliferation rate of cancer cells	0.18 day^{-1}
γ	Tumor cell death rate	1.101×10^{-7} day^{-1}cells^{-1}
κ	Tumor's maximum carrying capacity	5.0×10^8 cells
P_I	Proliferation rate of immune cells	0.1245 day^{-1}
δ	Natural death rate of immune cells	0.0412 day^{-1}
m	Immunosuppressive constant	3.422×10^{-10} day^{-1}cells^{-1}
g	Experimentally measured constant	2.019×10^7 cells
α	Natural generation of immune cells	1.3×10^4 cells day^{-1}

Notably, the Kuznetsov model takes density limitation into account by letting the cancer proliferation term depend on the maximum carrying capacity, κ, of the tumor (which is especially motivated considering tumor spheroids [102]). Moreover, the Kuznetsov model can not have any closed orbits or limit cycles, but depending on the used parameters, it can have zero, one, or three equilibrium points [28]. The Kuznetsov model also generally predicts a dormant tumor through its inward spiraling phase portrait, like the one seen in Figure 16.

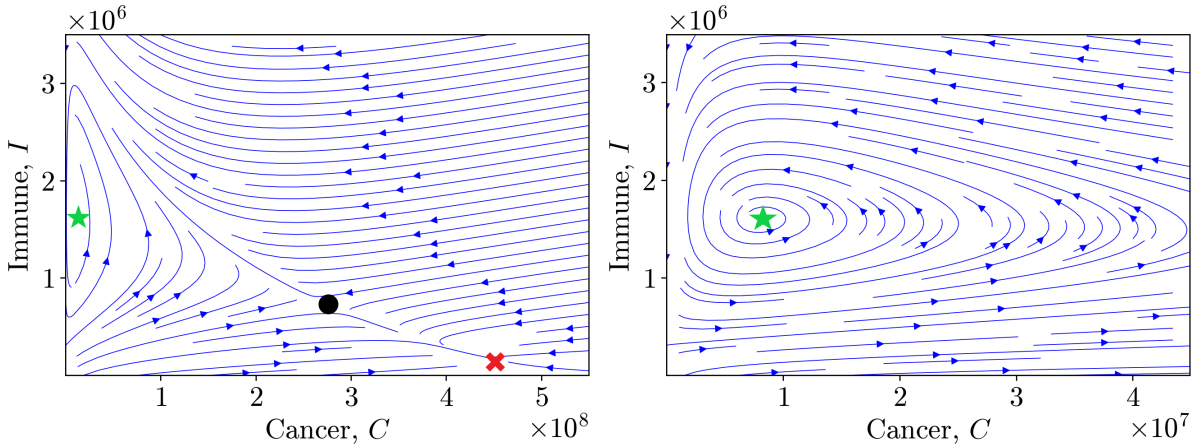


Figure 16: **Phase portraits of the Kuznetsov model** corresponding to Eqs. (13). The complete behavior of the model is seen in (a), while (b) shows the phase portrait for tumors of a magnitude lower (compared to (a)). The inward spiral results in a stable benign tumor (denoted by a green star), the black circle marks an unstable state, and the red ✖ indicates a stable malignant tumor. The used parameter values are the same as in Table 2.

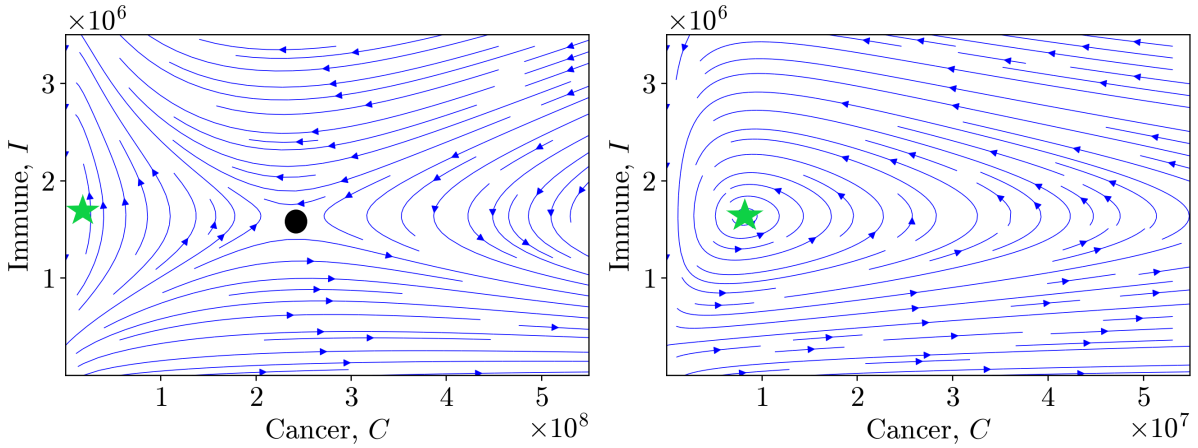
The phase portraits seen in Figure 16 highlight a phenomenon known as "sneaking through", as a tumor that appears to become dormant, escapes in the end. The phenomenon depends on the ratio between the induced inactivation (death) of immune cells and cancer cells, i.e. $\frac{k_3}{k_2}$ from Figure 15 (or $\frac{m}{\gamma} \approx 0.0034$, considering Table 2) [28]. For $k_3 \ll k_2$, the immune system is too effective, thus sneaking through is only possible when the ratio is above a certain value ($\frac{k_3}{k_2} \gtrsim 0.0029$, numerically obtained using $m = 2.89 \times 10^{-10}$ day^{-1}cells^{-1} and γ from Table 2). The existence of this threshold infers that immunosuppressive features are essential for tumor escape, which predicts the effectiveness of checkpoint inhibitor drugs (artificially decreasing k_3).

From here, it is natural to introduce a generalized model (stated [103], adapted in [28]),

$$\begin{aligned} C'(t) &= \overbrace{P_C f_1(C)}^{\text{Tumor growth}} C - \overbrace{\gamma f_2(C,I)}^{\text{Predation}} CI \\ I'(t) &= \underbrace{P_I f_3(C)}_{\text{Recruitment}} CI - \underbrace{\delta f_4(C)}_{\text{Inactivation}} I + \underbrace{\alpha f_5(C)}_{\text{Generation}} . \end{aligned} \tag{14}$$

This generalized model can be easily adopted to obtain the special cases from Eqs. (10)-(13) (and others, see e.g. Table 10.1 in Ref. [28]), for example, the Kuznetsov model from Eqs. (13) can be further adapted to large tumors by considering the Gompertzian

growth curve from Eq. (6) (instead of logistic growth). In this case, $f_1 = 1 - \frac{1}{\kappa P_C} \ln C$, while everything else is identical to Eqs. (13). The phase portraits now look like,



(a) (b)

Figure 17: **Phase portraits of the modified Kuznetsov model**, considering Gompertzian tumor growth. The behavior of the model is seen in (a), while (b) considers tumors of a magnitude smaller (notably identical to Figure 16 (b)). The black circle marks an unstable state, the green star marks tumor dormancy, and the used parameter values are the same as in Table 2 and Figure 16. Also, note that the phenomenon of "sneaking through" is still present (bottom left in (a) and bottom right in (b)).

The next logical step is to introduce a third ODE, for example, the process of how the cancer-killing immune cells (CTLs) are recruited, could be accounted for. One group of immune cells that encourage CTL proliferation (through cytokine production, e.g. IFN-γ) and are partly responsible for CTL recruitment, are the Th cells. A model which accounts for this cytokine production, by assuming that the proliferation rate of CTLs is proportional to the number of Th cells, was presented in 2018 by Dritschel et al. [104].

$$\begin{aligned} C'(t) &= \overbrace{P_C C\left(1-\frac{C}{\kappa}\right)}^{\text{Logistic growth}} - \overbrace{k_2 C I_T}^{\text{Predation}}, \\ I'_{Th}(t) &= \overbrace{\iota_{Th}}^{\text{Infiltration}} + 2P_{Th}\overbrace{\frac{\tilde{C} C I_{Th}}{\tilde{C}^2 + C^2}}^{\text{Proliferation}} - \overbrace{\delta_{Th} I_{Th}}^{\text{Natural death}}, \\ I'_T(t) &= \overbrace{\iota_T}^{\text{Infiltration}} + \overbrace{P_T I_T I_{Th}}^{\text{Proliferation}} - \overbrace{k_3 I_T C}^{\text{Inactivation}} - \overbrace{\delta_T I_T}^{\text{Natural death}}. \end{aligned} \tag{15}$$

The parameters are the same as introduced earlier (except for the immune cells' infiltration rate, ι), where the k-rates from Figure 15 are also considered. The subscripts denote the cell corresponding to the specific parameter (Th = T helper cells, T = CTLs, and C = cancer cells). The $\tilde{C}$-parameter corresponds to the number of cancer cells when the proliferation rate of Th cells is half-maximum. When $C = \tilde{C}$, the individual Th cell's maximum proliferation rate (P_{Th}) is obtained, and the proliferation term is seen to be similar to previous proliferation terms.

The Dritschel model has shown that the immunoediting process is dependent on the CTLs' and Th cells' infiltration rates, where both of these immune cells are crucial for

tumor elimination. It also indicates an increased effect of tumor growth suppression by combining immune system-boosting immunotherapies with the blocking of tumor-induced immunosuppressors [104].

More variables could also be accounted for by introducing more ODEs (see for example [105]), however, as explained, ODEs can only approximately represent spatial aspects. The next section, therefore, moves on from ODE-based models.

4.2 Spatial Distribution of Immune Cells

4.2.1 Physical Attributes

This section considers sets of data containing spatial information of cells from 2D-slices of tumor tissues. The data corresponds about 6500 tumor samples taken from a total of roughly 3500 different cancer patients, see explicit details in Appendix C. The data have generously been supplied by [106, 107].

An example of the spatial data collected from each tumor sample is seen in the figure below, which considers cell-coordinates in a sample taken from a breast cancer patient.

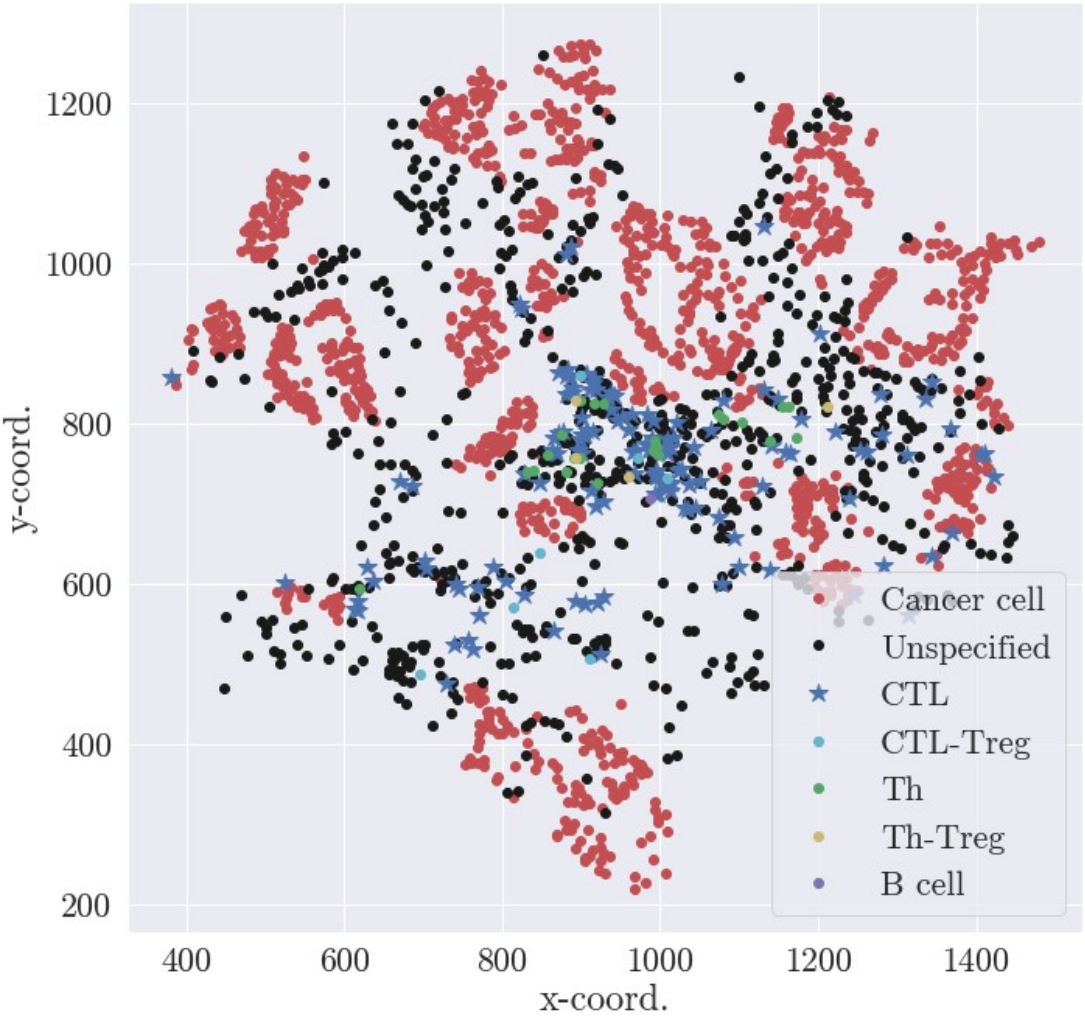


Figure 18: **Spatial distribution of tumor-infiltrating lymphocytes** and cancer cells in tumor tissue taken from a breast cancer patient. The killer T cells (CTLs) are separately marked with stars to further highlight them.

All mathematical models accounted for in the previous subsection assumed that immune cells (CTLs) kill cancer cells. By observing the spatial distributions of the CTLs and cancer cells depicted in Figure 18, it can be noted that the CTL-dense areas (e.g. around $(x, y) = (1000, 800)$ or $(800, 600)$) contain fewer cancerous cells than the areas with no or sparse CTLs. To explicitly investigate if the lack of cancer cells is generally related to the presence of CTLs, the number of CTLs and cancer cells in each tumor sample can be compared. Specifically, by counting the number of CTLs and cancer cells in Figure 18 and all other of the 6500 tumor samples, the scatter plot in Figure 19 is obtained[18].

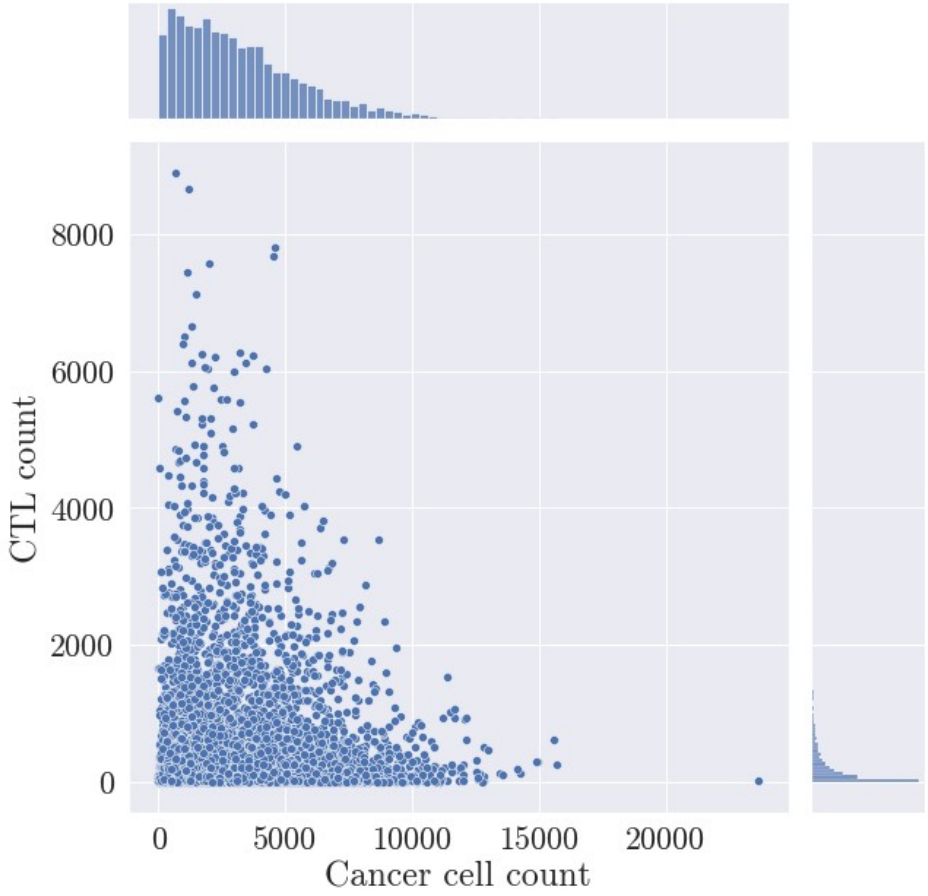


Figure 19: **Number of CTLs in relation to the number of cancer cells.** Each data point corresponds to a separate tumor sample from an unspecified cohort.

Notice in Figure 19 that the lower the CTL count, the larger the cancer cell count is able to get. However, from the the right-hand histogram, it is noted that the tumor samples are more likely to only have a few number of CTLs (samples with few CTLs are more common). By also considering the peak of the histogram on the top in Figure 19, the tumor samples appear to most likely contain about 2000 cancer cells, where the frequency of larger cancer cell counts seem to decrease with Gaussian-like behavior.

Notably, the data in Figure 19 corresponds to 12 different cancer types (cohorts). It has previously been mentioned that tumors can differ a lot in behavior depending on their

[18]For the relations between each of the other cells depicted in Figure 18 (B cells, Th cells, etc.), see Appendix C Figure C.1 and Figure C.2 (for logarithmic scale).

region of occupation, therefore, it could be more reasonable to study the CTL occurrence in the 12 cohorts separately. To investigate if this assumption is valid, the data from Figure 19 is separated into 12 different plots in Figure 20, where each panel considers a different cancer type (tumor cohort).

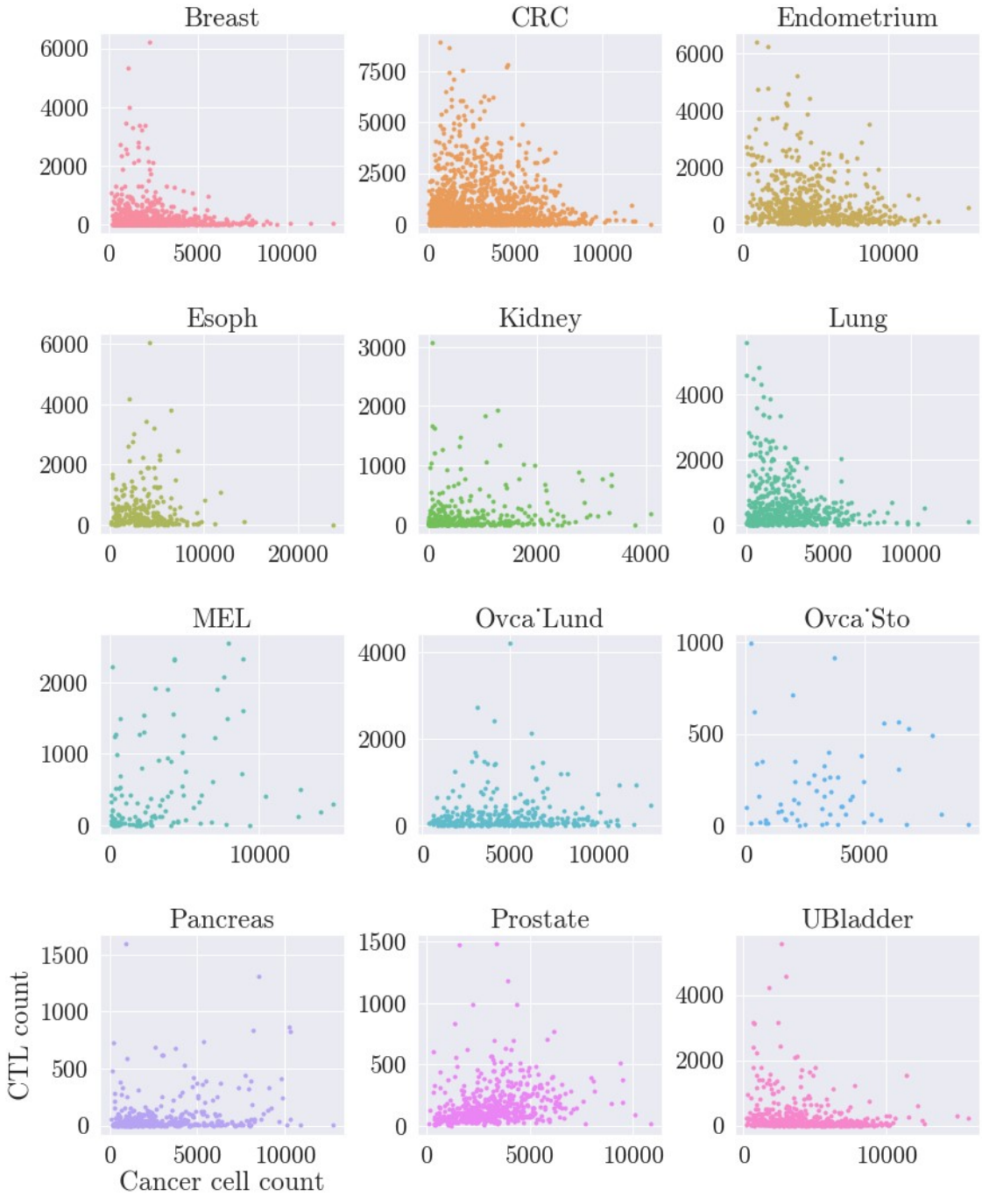


Figure 20: **CTL count in comparison to the number of cancer cells**, separated by type (cohort). Here, CRC is Colorectal cancer, Esoph is Esophageal, MEL is Melanoma, and Ovca'Lund and Ovca'Sto correspond to Ovarian cancer data from research teams from Lund University and the Karolinska Institute (Stockholm), respectively. Some cohorts (e.g. Ovca'Sto and MEL) are noted to consist of relatively few data points, therefore not allowing for any clear trends to be established.

Some of the cohorts in Figure 20 (Ovca'Lund and Prostate) show sporadic behavior where a high count of CTLs can also be found in tumor samples with large cancer cell counts. In particular, these cohorts show no clear relation between CTL count and cancer cell count. Other cohorts (Breast, CRC, Esoph, and Lung) correlate particularly well to the general behavior observed in Figure 19, implying the potentially important role of CTLs in benign tumors of these types. In fact, it has previously been shown that a high density of tumor-infiltrating lymphocytes (like the CTLs) is strongly correlated to a better prognosis for patients suffering from cancers known as 'HER2-positive breast cancer' and 'triple-negative breast cancer', where the latter one is known to have the highest concentration of infiltrating lymphocytes of all breast cancer types [108]. The trend seen for colorectal cancer (CRC) is also expected from previous research establishing that a high density of CTLs can decrease the rate of recurrence in colorectal carcinoma [29, 30].

Both Figure 19 and Figure 20 show that a high count of CTLs in most cancers limits the number of cancerous cells in a tumor. It is even considered that the role of CTL-density in tumors is well known to be important in cancer prognosis, and that focus should be put on other immune cells. But at the same time, it is deemed worth considering their spatial distributions and association with cancer recurrence [13].

Therefore, before moving on from the CTLs, some spatial distribution aspects of the well-known cancer-killing immune cells (given through empirical observations in the studied data) are considered. Specifically, in Figure 18 it was noted that there are CTL-dense areas, in particular, it is observed that the CTLs seem to appear in clusters. The general behavior of CTL-clustering can be investigated statistically by considering the relative frequency of specific cluster sizes, i.e. $f(I_{\text{cluster}}) = \frac{\text{Occurrence of cluster size}}{\text{Total number of clusters}}$, where $f(I_{\text{cluster}})$ is the relative frequency (or empirical probability) of CTL-clusters of size I_{cluster}. The process of cluster classification and the resulting cluster behavior is seen below in Figure 21.

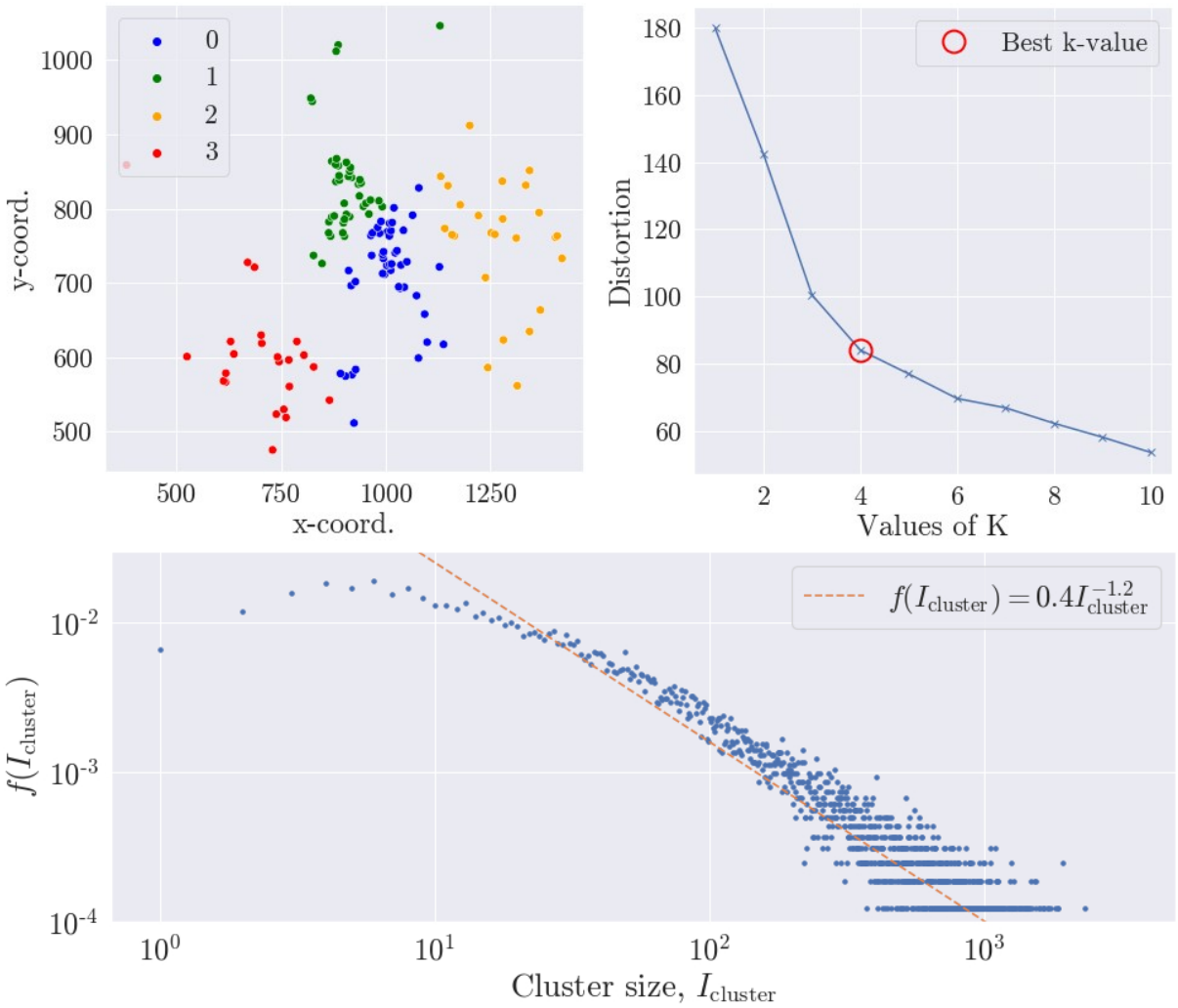


Figure 21: **Clustering of CTLs in tumors.** The top-left panel illustrates the four classified CTL-clusters of the single tumor sample depicted in Figure 18. The clustering process is performed using the k-means method, where the optimal number of clusters (k) per tumor sample is found using the "Elbow method" (illustrated in the top-right panel as finding the point of maximum curvature in a distortion plot). The `KneeLocator()`-function of the Python package `kneed` (which builds upon the 'kneedle' algorithm introduced in [109]) makes it possible to classify the CTL-clusters and their sizes in each of the 6500 tumor samples. The relative frequency (f) of all found cluster sizes can then be collectively plotted for all samples on log-log scale (the bottom panel).

The general clustering behavior (regarding cluster size) of CTLs in all of the considered tumor samples can be observed in Figure 21 to approximately follow a power law. The power-law[19] indicates the presence of scale-invariance in cluster formation of CTLs. Note that the used clustering algorithm needs to know in what range the optimal number of clusters (k) is expected to be, the maximum number of clusters per sample was here set to 20 (i.e. $k \in [1, 20]$, however, no tumor samples were found to be optimally described by more than 6 clusters).

Briefly moving on from the CTLs, it was in Sections 2.2 and 3 noted that also the NK cells and macrophages of the immune system can be major contributors to the killing of

[19] A tentative exploration of the power-law's implications can be found in Appendix D.

cancer cells. The data sets considered, also account for these innate immune cells and some of their variants. The spatial data is still in the form as described in Appendix C, however, now with the innate immune system cells instead of TILs. The innate immune cells accounted for in the data are put into relation with the number of cancerous cells (similarly to Figure 19) in the figure below.

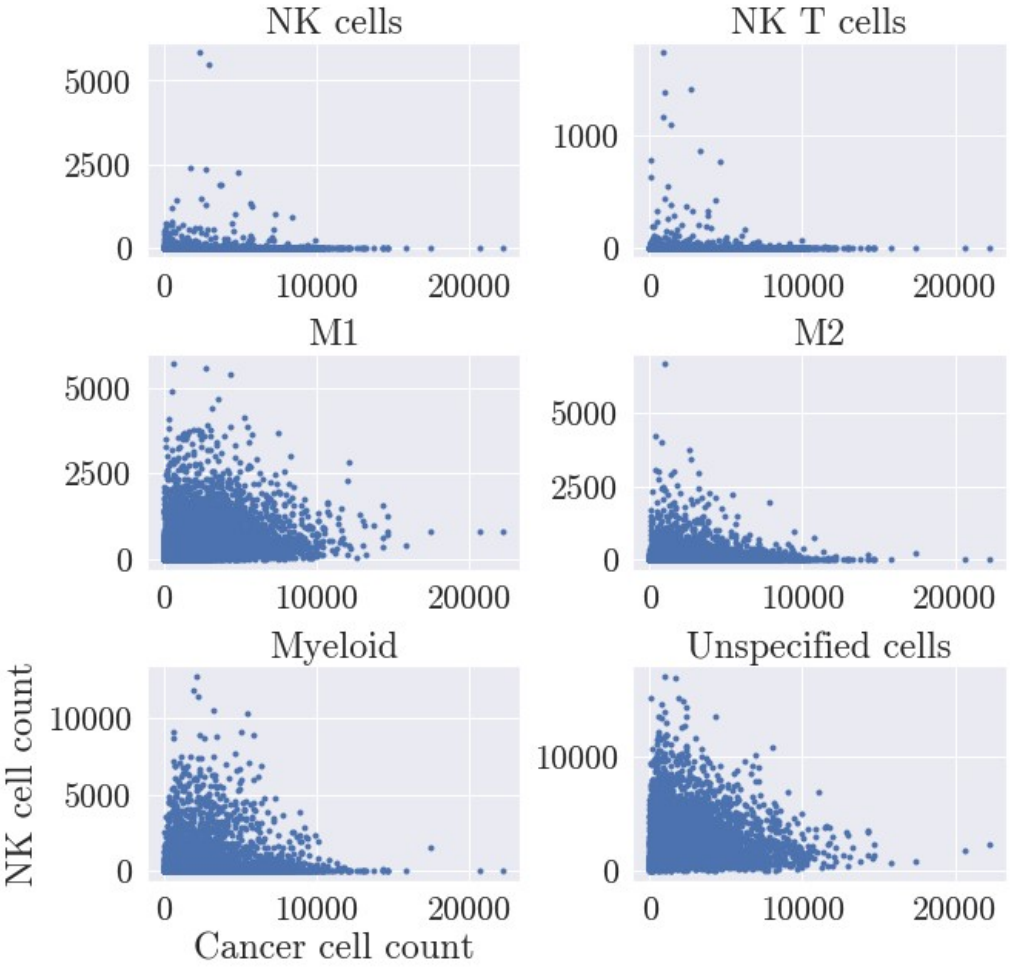


Figure 22: **Relation between the number of immune cells and cancer cells.** Here, M1 and M2 are different subgroups of macrophages, and more on the relation between Myeloid cells and NK cells can be found in [110]. Some distinguishing features are more evident when plotted on a logarithmic scale, see Appendix C Figure C.3. Note that the behavior of the "Unspecified cells"-panel can be influenced by many different cells, but also by the fact that the total number of cells in each tumor sample is finite (which holds in all cases).

Notice that the NK cells behave similarly to the CTLs in relation to the number of cancerous cells. However (especially evident from the logarithmic scale in Appendix C Figure C.3) the M1s' and M2s' relation to the number of cancer cells stand out. Both M1 and M2 are commonly referred to as tumor-associated macrophages, implying that they are common in tumor microenvironments. From previous research, it is also well-known that M1 macrophages are tumor resistant and have anti-tumor effects such as being able to distinguish between normal cells and tumor cells, while M2 macrophages promote tumor proliferation, invasion, metastasis, and angiogenesis [111, 112].

The M1s can have positive regulatory effects such as increasing the number of activated NK cells and promoting T cell proliferation [111]. On the other hand, the regulatory effects of M2s can directly inhibit the proliferation of CTLs by Treg recruitment [113]. Surgical removal of these tumor-associated macrophages has also proven to block Treg recruitment, thereby, inhibiting tumor growth [114]. The many immunosuppressive and tumor-supportive features of M2s can cause cascade reactions in tumor progression, rapidly increasing the process of forming malignant tumors. Specifically, the ratio between CTLs and M2s has been proven to predict the survival of cancer patients better than many other clinical parameters [107, 112].

Thus the consideration of NK cells and macrophages is also motivated for study in presence and distribution in the tumor microenvironments. A third group of immune cells that the provided data sets account for (which also was introduced in Section 2.2) are the APCs (antigen-presenting cells), these were deemed essential for the activation of other immune cells. A brief investigation of their relation to the number of cancerous cells is therefore also motivated. The APCs considered are Dendritic Cell (DC) variations, see Figure 23 below.

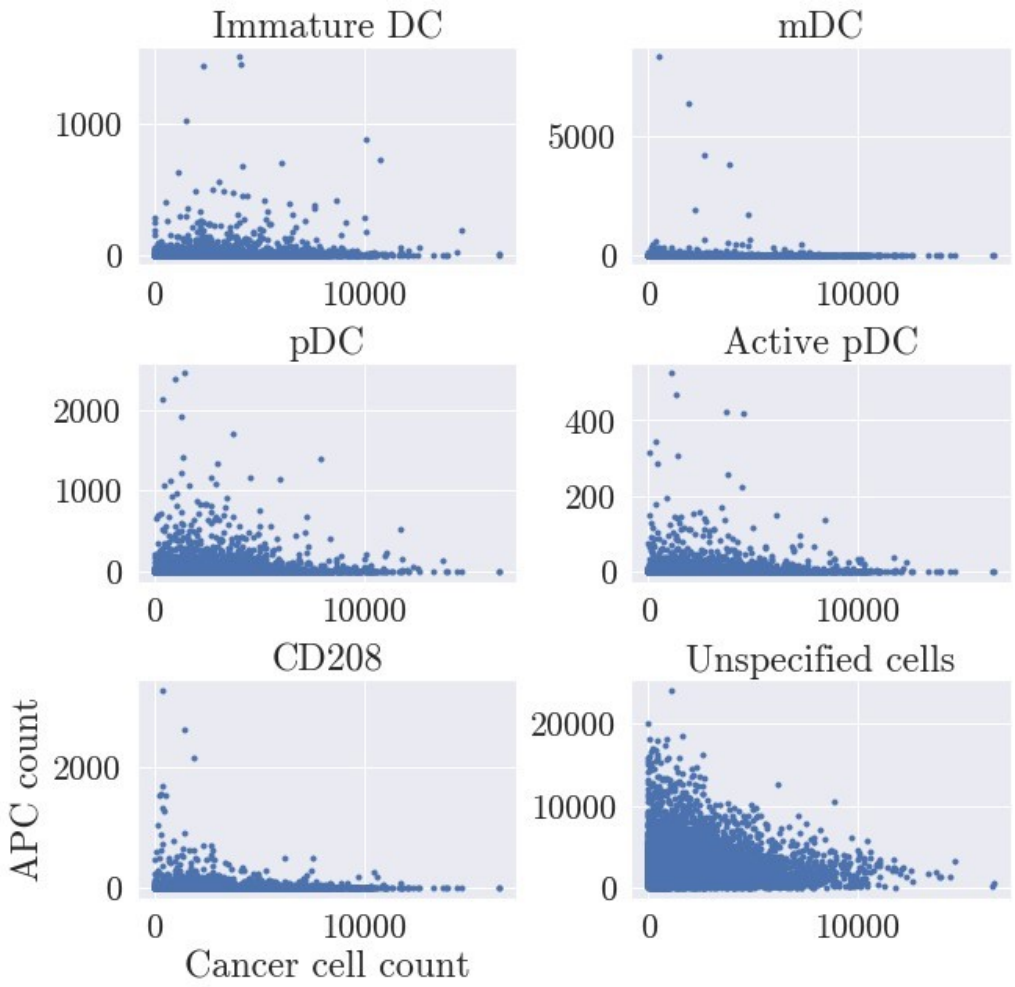


Figure 23: **APC count in relation to the number of cancer cells in its corresponding sample.** The specifics of the different kinds of APCs (DCs), can be explored further in [115, 116]. However, mDCs are myeloid DCs, pDCs are plasmacytoid DCs, and CD208 is also known as DC-LAMP. For logarithmic scale, see Appendix C Figure C.4.

The different APCs in the tumor samples are noted to differ in their relation to the cancer cell count. Notably, it has been shown that immature DC-conditioned Th cells are immunosuppressive, while mature DCs (like mDC and pDC) induces immunostimulatory Th cells [117]. These results are well reflected by the data from Figure 23. Furthermore, T cells related to two specific cytokines (IL-10 and TGF-β), are known to increase in number independent of whether the DCs are mature or immature, while their suppressive functions only are coupled to immature DCs (inhibition of the IL-10 and TGF-β related receptors on DCs have also proven to enhance activation of CTLs and to kill cancer cells) [117, 118].

The physical attributes and relations between the number of immune cells and cancer cells in tumor microenvironments, suggest that models of the interactions between immune cells and cancer cells are motivated by the dynamics of actual tumor microenvironments. The considered data also does not contain any interpretation or aspect of a time variable, the static "images" emphasize the need of models that incorporate spatial parameters. Building upon the (solely time-dependent) ODEs from Section 4.1.2, models that can make predictions for, e.g., $I(x, y, z, t)$ are in the next section considered using PDEs.

4.2.2 Mathematical Modeling

In comparison to the simplistic (undisturbed) tumor growth models from Eqs. (6-9), more comprehensive models can be introduced by accounting for spatial aspects using PDEs. One such model takes growth saturation into account by considering glucose (main nutrient) concentrations, G, and the volumetric fractions of tumor cells (C), healthy cells (H), and extracellular space (S). The model also applies Heaviside functions (Θ) mainly to simplify the influence of the death and proliferation rates on the glucose level, with the intention to ease its analytical study [119]. The model reads,

$$
\begin{aligned}
\frac{\partial C}{\partial t} &= \overbrace{PC \cdot \Theta(G - G_p) \cdot \Theta(S - S_{cr})}^{\text{Proliferation}} - \overbrace{MC \cdot \Theta(G_d - G)}^{\text{Death}} - \overbrace{\frac{1}{r^2}\frac{\partial \left(vCr^2\right)}{\partial r}}^{\text{Convection}} \\
\frac{\partial H}{\partial t} &= \overbrace{-\frac{1}{r^2}\frac{\partial \left(vHr^2\right)}{\partial r}}^{\text{Convection}}, \\
\frac{\partial G}{\partial t} &= \overbrace{N_\alpha H[1 - G]}^{\text{Inflow}} + \overbrace{\frac{D_G}{r^2}\frac{\partial^2 \left(Gr^2\right)}{\partial r^2}}^{\text{Convection}} - \overbrace{Q_p C \cdot \Theta\left(G - G_p\right) \cdot \Theta\left(S - S_{cr}\right)}^{\text{Consumption by proliferating cells}} \\
&\quad - \underbrace{Q_q C\left[\Theta\left(G_p - G\right) \cdot \Theta\left(S_{cr} - S\right) + \Theta\left(S - S_{cr}\right)\right] \cdot \Theta\left(G - G_d\right)}_{\text{Consumption by quiescent cells}}
\end{aligned}
\tag{16}
$$

with cell velocity $v = \frac{N_{\alpha,cr}}{\mu[S_0 - S_{cr}]}\frac{\partial S}{\partial r}$, where $S = 1 - (H + C)$. In contrast to the previous models, all volumetric fractions are here spatially dependent, i.e. $C = C(x, y, z, t)$, $H = H(x, y, z, t)$, and $G = G(x, y, z, t)$. The dynamics of the convective motions depend on gradients of stress that in turn depend on the death and proliferation of the cancer cells. Furthermore, tumor proliferation is possible for glucose concentrations (G) above the critical point G_p, and an extracellular space fraction (S) greater than the critical fraction S_{cr}. For glucose concentrations lower than $G_d < G_p$, the tumor cells are assumed to die due to malnutrition [83, 119]. See Table 3 for a complete description of the model parameters.

Table 3: Description of parameters from Eqs. (16), with values from [119].

Parameter	**Description**	**Estimated value**
P	Proliferation rate of tumor cells	$0.03\ \mathrm{h}^{-1}$
M	Death rate of tumor cells	$0.003\ \mathrm{h}^{-1}$
N_α	Nutrient generation (supply) level	$1.1 \times 10^{-3}\ \mathrm{s}^{-1}$
D_G	Glucose diffusion coefficient	$2.8 \times 10^{-6} \frac{\mathrm{cm}^2}{\mathrm{s}}$
Q_p	Proliferating cells' glucose consumption rate	$1.2 \times 10^{-16} \frac{\mathrm{mol}}{\mathrm{cells \cdot s}}$
Q_q	Quiescent cells glucose consumption rate	$3 \times 10^{-18} \frac{\mathrm{mol}}{\mathrm{cells \cdot s}}$
G_d	Critical glucose level for tumor cell survival	0.055 mM
G_p	——"—— for tumor cell proliferation	0.55 mM
r	Radial coordinate	-
S_0	Fraction of healthy cells' extracellular space	-
μ	Fluid viscosity	-

To analyze heterogeneous spatio-temporal dynamics of tumor cells and immune cells, tumor-infiltrating immune cells (e.g. T-cells) are considered in spatially-distributed models [120, 121]. If consideration is also taken to tumor antigen recognition, the mutation frequency in tumor antigens can be shown to play an important role in the effectiveness of the immune system [122]. On the topic of mutations in tumor antigens, a model accounting for antigen heterogeneity and evolution (i.e. mutation) has been presented in [123] (see Appendix B.1 for details).

A mathematical model describing spatio-temporal dynamics of tumor cells and immune cells in immunogenic solid tumors was presented in 2004 by Matzavinos et al. [121]. The model specifically considers the dynamics between a small non-necrotic multicellular tumor (without angiogenesis) and tumor-infiltrating cytotoxic lymphocytes. The dormancy phenomenon briefly accounted for in the previous section, is more extensively explored in this model, and the lymphocyte infiltration is accounted for using a set of different parameters.

Similar to the model by Kuznetsov from Eq. (13), immune cells and tumor cells are here assumed to form complexes, with the same rates as in Figure 15. The model consists of four nonlinear PDEs describing tumor-infiltrating CTLs (I), cancer cells (C), complexes (ζ), and chemokines[20] (χ), where all variables depend on spatial coordinates (x, y, z) and time (t). Recall,

$$I + C \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \zeta \begin{array}{l} \overset{k_2}{\longrightarrow} I \\ \underset{k_3}{\longrightarrow} C \end{array}$$

[20]Specific types of immune-activating cytokines.

The model is then written as,

$$
\begin{aligned}
\frac{\partial I}{\partial t} =& \overbrace{D_1\nabla^2 I}^{\text{Random motility}} - \overbrace{\mathcal{K}\nabla\cdot(I\nabla\chi)}^{\text{Chemotaxis}} + \overbrace{P_I\frac{CI}{g+C}}^{\text{Proliferation}} - \overbrace{\delta I}^{\text{Decay}} + \overbrace{\alpha\Theta(\mathbf{x})}^{\text{Generation}} \\
& - \overbrace{k_1 CI + (k_{-1}+k_2)\,\zeta}^{\text{Local kinetics}}, \\
\frac{\partial \chi}{\partial t} =& \overbrace{D_2\nabla^2\chi}^{\text{Diffusion}} + \overbrace{p\zeta}^{\text{Production}} - \overbrace{d\chi}^{\text{Decay}}, \\
\frac{\partial C}{\partial t} =& \overbrace{D_3\nabla^2 C}^{\text{Random motility}} + \overbrace{P_C C\left(1-\frac{C}{\kappa}\right)}^{\text{Logistic growth}} - \overbrace{k_1 CI + (k_{-1}+k_3)\zeta}^{\text{Local kinetics}}, \\
\frac{\partial \zeta}{\partial t} =& \overbrace{k_1 CI - (k_{-1}+k_2)\zeta}^{\text{Local kinetics}},
\end{aligned}
\tag{17}
$$

where ∇ is the differential operator of 3D-Euclidean space, i.e. $\hat{\mathbf{x}}\frac{\partial}{\partial x} + \hat{\mathbf{y}}\frac{\partial}{\partial y} + \hat{\mathbf{z}}\frac{\partial}{\partial z}$ in Cartesian coordinates. Many of the variables have been introduced earlier (including the Heaviside-function $\Theta(\mathbf{x})$, which here introduces a sub-region that initially consists solely of tumor cells, see details in [121]). All parameters are collectively accounted for in Table 4 below, along with their estimated values (adapted from [100, 101, 121]). Also, note that random motility (passive transport) and chemotaxis (active transport) represent two ways for the immune cells to infiltrate the tumor.

Table 4: Description of the parameters in the Matzavinos model from Eqs. (17).

Parameter	**Description**	**Estimated value**
P_C	Proliferation rate of cancer cells	0.18 day^{-1}
κ	Tumor's maximum carrying capacity	5.0×10^{8} cells $\cdot$ cm^{-1}
P_I	Proliferation rate of immune cells	0.1245 day^{-1}
δ	Natural death rate of immune cells	0.0412 day^{-1}
k_1	Complex formation rate	1.3×10^{-7} day^{-1}cells^{-1}cm
k_{-1}	Complex deformation rate	24.0 day^{-1}
k_2	Cancer death by immune cell	7.198 day^{-1}
k_3	Immune cell death by cancer	0.002 day^{-1}
g	Experimentally measured constant	2.02×10^{7} cells $\cdot$ cm^{-1}
α	Natural generation of immune cells	1.36×10^{4} day^{-1}cells $\cdot$ cm^{-1}
d	Decay rate of chemokines	1.155×10^{-2} day^{-1}
p	Production rate of chemokines	$[20, 3000]$ cell^{-1} min^{-1}
D_1	Random motility of immune cells	7.0×10^{-5} cm^2 day^{-1}
D_2	Diffusion coefficient of chemokine	$[10^{-4}, 10^{-2}]$ cm^2 day^{-1}
D_3	Random motility of cancer cells	$[0.06, 9]\times10^{-6}$ cm^2 day^{-1}
$\mathcal{K}$	Chemotaxis coefficient	1.728×10^{6} cm^2 day^{-1}M^{-1}

The Matzavinos model can be adopted for modeling of tumors exhibiting multi-layered cell growth or multi-cellular spheroid growth. The Matzavinos model has also demonstrated the existence of quasi-stationary (in time) and space-heterogeneous cell distributions, as well as stable limit cycles (verified with bifurcation analysis). These results

explain the complex heterogeneous spatio-temporal dynamics observed in the PDE system, and are believed to deepen the cancer dormancy insight, while also contributing to anti-cancer vaccine advancements [121]. For extensive simulations of time-relative spatial distributions of immune cells in tumor tissue, according to the Matzavinos model, see [121].

4.3 Physics Tools

This section briefly accounts for a few relevant tools that could be considered when researching physical aspects of immunotherapy.

AstroPath™ [data acquisition] is a platform used for the investigation of marker expressions on individual cells and their respective spatial coordinates. Multiplex immunofluorescent technologies are known for making visualization of molecular expression patterns possible, however, it is not suitable for large tumor areas. This is where AstroPath comes in, it draws from methods used in astronomy (which is well-known for large-scale imaging analyses) to perform multispectral imaging with a single-cell resolution of whole-tumor samples. AstroPath has since been used, among others, for multiplex immunofluorescent analyses to find prognostic predictors related to immunotherapy on melanoma cancers. Read more about the end-to-end workflow provided by AstroPath in [124]. Notably, this was the method used for compiling the cancer-patient data used in this thesis.

PhysiCell™ [simulation tool] is a framework built in `C++`, the simulator[21] is physics-based and intended to use for simulations and studies of large multi-cellular systems. Read more about the simulator in [125].

Moreover, an application powered by PhysiCell (using an Anaconda Jupyter-based GUI) has been presented in [126]. This application is used to study the effects of chemical communication (interactions) in dynamical multi-cellular systems. The model considers Invader cells (cf. cancer cells) whose survival and proliferation are dependent on some resource released by some Suppliers (cf. cancer-promoting cells). Scout cells (cf. APCs) are drawn towards these Invader cells and can secrete attack signals (cf. cytokines) when an Invader cell is encountered. The attack signals cause Attacker cells (cf. CTLs) to trace the attack signal back to its origin, when encountering an invader cell, the Attacker cells become activated and start to secrete a poison (cf. granzymes) to kill the Invader cells. The model is well applicable for modeling immunotherapy methods, however, it should be noted that it only considers chemical interactions (i.e. no contact-based interactions).

PyClone™ [mutation modeling] is a statistical modeling tool[22] for Python, created to study and identify clonal population structures. The model is used to analyze deeply sequenced mutations and can be described as a Bayesian clustering method. Its intended use is for the investigation of point-mutation occurrence in samples from heterogeneous cancers. For more details and its applications, see [127].

Immunotherapy with microchips [laboratory work] and the important roles of biophysics and lab-on-a-chip methods (considering single-cell analyses) are extensively motivated for cancer research [18]. One research team [19] focuses on the use of microchips to understand the mechanisms of cytotoxic killer cells (NK cells and CTLs). Through a physics-immunology platform, they study cytotoxic cells using microchips to gather

[21] Download at: `https://sourceforge.net/projects/physicell/` [Retrieved May 15, 2022].

[22] Reed more about the tool and how to install it at: `https://github.com/Roth-Lab/pyclone/` [Retrieved May 15, 2022].

a limited amount of tumor cells and cytotoxic cells within wells. The wells allow for individual cell monitoring at the same time as the complete cell population can be studied. Using these methods, they have observed trends of certain groups of immune cells being particularly effective in killing cancer cells.

By combining microchips and ultrasonic emitters, 3D microtumors can also be synthesized. Specifically, by matching the well-size with the frequency of the emitter, standing (sound) waves force the cells to cluster and form a 3D structure (microtumor) near the well center. These microtumors are then used for modeling solid tumor features in relation to the cancer-killing immune cells, which behave differently in 2D and 3D environments [19].

5 Concluding Remarks

The cells of the immune system are most often able to keep newly emerged tumors from escaping and becoming malignant. However, this phase of cancer elimination ends when the proliferation rate of the tumor eventually saturates the immune system's capacity. This is where the tumor escapes and starts to rapidly proliferate, with even more mutations as a consequence. It is in this escape phase that the tumor develops an immunosuppressive microenvironment, using features such as cytokine secretion and "recruitment" of immune cells (e.g. Tregs and macrophages) to encourage its growth further. But as attempted to expand upon and illustrate in this review, these changes do not have to be irreversible. Through manipulation, boosting, and/or inhibition of certain immunogenic features, immunotherapies can help reverse the process to re-enter the elimination phase and enhance the cancer-killing capabilities of the immune cells. Notably, there already exists immunotherapeutic treatments for cancer that are in use today, however, these only work for a limited amount of patients and cancer types. Consequently, more research is needed, and it is expected that a more comprehensive understanding of the events in between the elimination- and escape phase might lead to new findings and the development of immunotherapies that can avoid the transition to happen in the first place [128].

Some cells of the immune system (including CTLs, NK cells, M1 macrophages, and dendritic cells) naturally fight cancer, and the level of malignancy (density of cancerous cells) is in many tumors directly influenced by the presence of tumor-infiltrating lymphocytes, antigen-presenting cells, and natural killer cells in the tumor microenvironment. Other immune cells, like immature dendritic cells, are less helpful in moderating tumors and can influence Th cells to become immunosuppressive. The M2 macrophages also have directly immunosuppressive features, such as Treg recruitment that down-regulates the rate of proliferation for the CTLs.

The effect of cancer-fighting immune cells on tumor microenvironments can also vary significantly. The consideration of the number of CTLs in relation to the number of cancerous cells in a tumor microenvironment (Figure 19), shows that it is also common for tumors with low cancer cell counts to only have a few CTLs. Tumor-promoting immune cells can also be few in more malignant tumors (Figure 22 and Figure 23). Notably, the behavior and importance of particular immune cells vary depending on which organ the tumor is located in (Figure 20), e.g. prostate cancer only conveys a vague relation between the number of CTLs and cancer cells. In particular, tumors should not be studied in a general manner. Even though global features can be observed (Figures 19, 22, 23, and C.1), different cohorts need to be considered separately to model and predict tumor behaviors and outcomes in a realistic manner.

Immune cells also do not act individually, every cell's behavior is governed by other cells, e.g. the CTLs which need to be informed of an issue by Th cells, and by Tregs to be told at what rate they should proliferate and kill. In turn, Th cells and Tregs are dependent on the APCs finding and presenting antigens for them, in turn, dependent on cytokine secretion, etc. The spatial aspects have to be considered and, ideally, all involved immune cells and signaling cytokines should be accounted for to achieve accurate cancer prognostics.

Models considering spatial cell-distributions are able to approximately describe the dynamics of tumor microenvironments by considering chemokines (immune-activating cytokines) and outcome rates of cancer-immune interactions (k-rates and complexes, ζ), see Eq. (17). But in the process of developing effective immunotherapies, i.e. efficient ways to influence the natural behavior of the dynamics in the tumor environment, it is essential to account for other TILs than just the CTLs, as well as APCs and the members of the innate immune system (macrophages and NKs).

It is worth mentioning that a common and general issue with mathematical models in immunotherapy is that the actual application of treatments often results in unpredictable outcomes (e.g. harmful effects). Some well-motivated models have even been deemed too simplistic for being of practical use [83, 129]. The need for extensive models that incorporate multiple variables and interactions between cell populations is evident, but the high dimensionalities of the parameter spaces further complicate the parametrizations and introduce circumstantial parameters (estimated with large uncertainties within ranges of values, cf. Table 4).

The subject of immunotherapy is comprehensive, and far from everything has been accounted for in this review. Therefore, below follows some recommended further reading for the interested readers.

Further reading:

- Immunotherapy: The future of cancer treatment [130]
- Cancer immunotherapy: a brief review of the history, possibilities [...] [131]
- Cancer immunoediting and resistance to T cell-based immunotherapy [132]
- Nonequilibrium Physics in Biology [133] (in particular, Section IX)
- Dynamical properties of autoimmune disease models [...] [134]
- The immune contexture and Immunoscore in cancer prognosis [...] [135]
- The "Encyclopedia of Cancer Immune Microenvironment"[23] (cell densities)
- A review of mathematical and computational methods in cancer dynamics [136]
- More mathematical aspects in [105, 121]
- More on the physicists' role and importance in cancer research [13, 26]
- **Build your own model** - Simulation modelling for immunologists [80].

Key words that could ease the understanding of other literature:

- CD8+ = T killer cell (CTL)
- CD4+ = T helper cell (Th)
- CD3+ = T regulatory cell (Treg)
- TCR = T cell receptor
- TIL = Tumor-infiltrating lymphocyte
- TME = Tumor microenvironment
- Effector cells = Cytotoxic immune cells
- Immunoscore = Quantification of *in situ* immune infiltrations (for prognostics)

[23] An online database found at: `https://encima.one/` [Retrieved May 15, 2022].

Outlook

The fact that the immune system is a complex and efficient defense system suggests that it should also be able to fight cancer, and so it does, but the immune system tends to eventually be saturated, at least in humans. A scenario where the immune system rarely fails is in the bodies of bats, where their immune system can effectively protect them from developing cancer. In fact, bats are unaffected by many (typically deadly) viruses and therefore serve as asymptomatic reservoirs for these [137]. The seemingly robust immune system of bats suggests that there exist fundamental immune mechanisms that could lower the human cancer rate, or at least supply strategies for human cancer treatment. Specifically, the understanding of bats' tumor suppressor mechanisms might contribute to both cancer treatments and cancer prevention.

Some humans also seem to be less susceptible to viruses. The lack of knowledge of why has led to the concept of "immunological dark matter" (something "invisible" which is theorized to exist to explain the visible). The term seems to have arisen in relation to the discovery of some individuals being resistant or non-susceptible to SARS-CoV-2 [138]. However, no conclusions have yet been drawn about the nature of immunological dark matter, but it could be related to both geographical isolation and natural resistance [59]. Importantly, the name is just a placeholder for what is not yet fully understood, and there may not be any parallels to be drawn to immunotherapy, but it does emphasize how advanced and complex the immune system can be, and that more research is needed.

In regards to immunotherapeutic treatment utilization, the mathematical conclusion of dose-dense chemotherapy (drawn from the Gompertzian growth curve and the Norton-Simon hypothesis), might also play an important role in immunotherapy. However, the potential advantages of dose-dense immunotherapy still have to be further investigated.

In the area of cancer-immune modeling, the spatial aspect of cell distributions in tumor microenvironments has to be accounted for to a greater extent. Consideration also has to be taken to immune cells that directly, or indirectly, encourage immune cell and cancer cell proliferation and death. Analytical studies of such extensive models could then help investigate potential approximations and dimension reduction to determine key contributors and disregard redundant cell behaviors.

The spatial organization of immune and cancer cells is also expected to play an important role in attempts to disrupt tumors. In particular, entropic relations and cluster formations require further research. The presented power-law of relative cluster-size frequencies indicates the presence of scale-invariance, whose implications should be further studied. A tentative approach to investigate a potential relationship between the clustering of cytotoxic T lymphocytes and the malignancy of tumors is presented in Appendix D.

I hope that this review can stimulate further efforts in modeling of immunotherapy and cancer-immune interactions. In particular, I believe that more research about spatial aspects is needed, along with increased cooperation between different fields of study.

Acknowledgments

I give my sincere thank you to my supervisor for his helpful input and much-appreciated feedback, Tobias Ambjörnsson at the Division of Computational Biology and Biological Physics, Lund University. I also wish to express my gratitude towards Artur Mezheyeuski at the Department of Immunology, Genetics and Pathology, Uppsala University, for the granted access and usage of the cancer patient data.

References

[1] J Ferlay and M Ervik, et al. Global cancer observatory: Cancer today. https://gco.iarc.fr/today, 2020. Online; accessed February 7, 2022.

[2] World Health Organization (WHO). World cancer day: closing the care gap. https://www.who.int/news/item/03-02-2022-world-cancer-day-closing-the-care-gap, 2022. Online; accessed February 7, 2022.

[3] Rebecca L Siegel, Kimberly D Miller, Hannah E Fuchs, and Ahmedin Jemal. Cancer statistics, 2022. *CA: a cancer journal for clinicians*, 2022.

[4] Konstantin Chumakov, Michael S Avidan, Christine S Benn, Stefano M Bertozzi, Lawrence Blatt, Angela Y Chang, Dean T Jamison, Shabaana A Khader, Shyam Kottilil, Mihai G Netea, et al. Old vaccines for new infections: Exploiting innate immunity to control covid-19 and prevent future pandemics. *Proceedings of the National Academy of Sciences*, 118(21), 2021.

[5] Faith Osier, Jenny PY Ting, John Fraser, Bart N Lambrecht, Marta Romano, Ricardo T Gazzinelli, Karina R Bortoluci, Dario S Zamboni, Arne N Akbar, Jennie Evans, et al. The global response to the covid-19 pandemic: how have immunology societies contributed? *Nature Reviews Immunology*, 20(10):594–602, 2020.

[6] Nature News. Covid research: A year of scientific milestones. https://www.nature.com/articles/d41586-020-00502-w, May 2021. Online; accessed February 8, 2022.

[7] Frank L van de Veerdonk, Evangelos Giamarellos-Bourboulis, Peter Pickkers, Lennie Derde, Helen Leavis, Reinout van Crevel, Job J Engel, W Joost Wiersinga, Alexander PJ Vlaar, Manu Shankar-Hari, et al. A guide to immunotherapy for covid-19. *Nature Medicine*, pages 1–12, 2022.

[8] Douglas F Nixon, Daniela Marín-Hernández, and Nathaniel Hupert. Extreme immunotherapy: emergency immunology to defeat pandemics. *Molecular Medicine*, 27(1):1–6, 2021.

[9] Karim Hussien El-Shakankery, Joanna Kefas, and Rowan Miller. Covid-19, the future vaccine and what it means for cancer patients on immunotherapy. *Frontiers in Oncology*, page 3433, 2021.

[10] Ryan J Sullivan, Douglas B Johnson, Brian I Rini, Tomas G Neilan, Christine M Lovly, Javid J Moslehi, and Kerry L Reynolds. Covid-19 and immune checkpoint inhibitors: initial considerations. *Journal for immunotherapy of cancer*, 8(1), 2020.

[11] Renee Brady and Heiko Enderling. Mathematical models of cancer: when to predict novel therapies, and when not to. *Bulletin of mathematical biology*, 81(10):3722–3731, 2019.

[12] Johns Hopkins inHealth. Immunotherapy: Precision medicine in action. https://www.hopkinsmedicine.org/inhealth/about-us/immunotherapy-precision-medicine-action-policy-brief.html, 2016. Online; accessed February 8, 2022.

[13] C Yu Clare, Juliana C Wortman, Ting-Fang He, Shawn Solomon, Robert Z Zhang, Anthony Rosario, Roger Wang, Travis Y Tu, Daniel Schmolze, Yuan Yuan, et al. Physics approaches to the spatial distribution of immune cells in tumors. *Reports on progress in physics*, 84(2):022601, 2021.

[14] National Cancer Institute at the National Institutes of Health. Immunotherapy to treat cancer. `https://www.cancer.gov/about-cancer/treatment/types/immunotherapy`, 2019. Online; accessed February 8, 2022.

[15] Steve Webb. The contribution, history, impact and future of physics in medicine. *Acta Oncologica*, 48(2):169–177, 2009.

[16] Thomas Bortfeld and Robert Jeraj. The physical basis and future of radiation therapy. *The British journal of radiology*, 84(1002):485–498, 2011.

[17] Elveflow Team. Introduction to lab-on-a-chip 2020: review, history and future. *Elveflow*, 2021.

[18] Vasudha C Shukla, Tai-rong Kuang, Abirami Senthilvelan, Natalia Higuita-Castro, Silvia Duarte-Sanmiguel, Samir N Ghadiali, and Daniel Gallego-Perez. Lab-on-a-chip platforms for biophysical studies of cancer with single-cell resolution. *Trends in biotechnology*, 36(5):549–561, 2018.

[19] Tjärnlund Nils Johan and Arding (translator) Maxwell. Enhanced immunotherapy with microchips. `https://kaw.wallenberg.org/en/research/enhanced-immunotherapy-microchips`, 2019. Online; accessed February 9, 2022.

[20] Duc TT Phan, Xiaolin Wang, Brianna M Craver, Agua Sobrino, Da Zhao, Jerry C Chen, Lilian YN Lee, Steven C George, Abraham P Lee, and Christopher CW Hughes. A vascularized and perfused organ-on-a-chip platform for large-scale drug screening applications. *Lab on a Chip*, 17(3):511–520, 2017.

[21] Amy Wu, Kevin Loutherback, Guillaume Lambert, Luis Estévez-Salmerón, Thea D Tlsty, Robert H Austin, and James C Sturm. Cell motility and drug gradients in the emergence of resistance to chemotherapy. *Proceedings of the National Academy of Sciences*, 110(40):16103–16108, 2013.

[22] Larry Norton, Richard Simon, Harmar D Brereton, and Arthur E Bogden. Predicting the course of Gompertzian growth. *Nature*, 264(5586):542–545, 1976.

[23] Richard Simon and Larry Norton. The Norton–Simon hypothesis: designing more effective and less toxic chemotherapeutic regimens. *Nature Clinical Practice Oncology*, 3(8):406–407, 2006.

[24] Michael Untch, Jonas Bergh, Mark Citron, Clifford A Hudis, Jens Huober, Volker Möbus, and Günter Steger. Dose-dense therapy. *Breast Care*, 3(2):134, 2008.

[25] Marc L Citron. Dose-dense chemotherapy: principles, clinical results and future perspectives. *Breast Care*, 3(4):251–255, 2008.

[26] Franziska Michor, Jan Liphardt, Mauro Ferrari, and Jonathan Widom. What does physics have to do with cancer? *Nature Reviews Cancer*, 11(9):657–670, 2011.

[27] Dominik Wodarz and Natalia Komarova. *Dynamics of cancer: mathematical foundations of oncology*. World Scientific, 2014.

[28] Kathleen P Wilkie. A review of mathematical models of cancer–immune interactions in the context of tumor dormancy. *Systems biology of tumor dormancy*, pages 201–234, 2013.

[29] Bernhard Mlecnik, Marie Tosolini, Amos Kirilovsky, Anne Berger, Gabriela Bindea, Tchao Meatchi, Patrick Bruneval, Zlatko Trajanoski, Wolf-Herman Fridman, Franck Pagès, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *Journal of clinical oncology*, 29(6):610–618, 2011.

[30] Jérôme Galon, Anne Costes, Fatima Sanchez-Cabo, Amos Kirilovsky, Bernhard Mlecnik, Christine Lagorce-Pagès, Marie Tosolini, Matthieu Camus, Anne Berger, Philippe Wind, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*, 313(5795):1960–1964, 2006.

[31] Lauren M Sompayrac. *How the immune system works*. John Wiley & Sons, 2019.

[32] Ugo Del Monte. Does the cell number 10(9) still really fit one gram of tumor tissue? *Cell cycle*, 8(3):505–506, 2009.

[33] Gloria H Heppner and Fred R Miller. The cellular basis of tumor progression. *International review of cytology*, 177:1–56, 1997.

[34] Bernard Crespi and Kyle Summers. Evolutionary biology of cancer. *Trends in ecology & evolution*, 20(10):545–552, 2005.

[35] Chwee Teck Lim, Alexander Bershadsky, and Michael P Sheetz. Mechanobiology. *Journal of The Royal Society Interface*, 7(suppl_3):S291–S293, 2010.

[36] Darci T Butcher, Tamara Alliston, and Valerie M Weaver. A tense situation: forcing tumour progression. *Nature Reviews Cancer*, 9(2):108–122, 2009.

[37] Lawrence A Loeb. Human cancers express mutator phenotypes: origin, consequences and targeting. *Nature Reviews Cancer*, 11(6):450–457, 2011.

[38] Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. Programmed cell death (apoptosis). In *Molecular Biology of the Cell. 4th edition*. Garland Science, 2002.

[39] Lawrence A Donehower, Michele Harvey, Betty L Slagle, Mark J McArthur, Charles A Montgomery, Janet S Butel, and Allan Bradley. Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. *Nature*, 356(6366):215–221, 1992.

[40] Heidi Chial. Proto-oncogenes to oncogenes to cancer. *Nature education*, 1(1):33, 2008.

[41] National Cancer Institute SEER Training Modules. Cancer classification. `https://training.seer.cancer.gov/disease/categories/classification.html`, 2022. Online; accessed February 21, 2022.

[42] Martyn K White, Joseph S Pagano, and Kamel Khalili. Viruses and human cancers: a long road of discovery of molecular paradigms. *Clinical microbiology reviews*, 27(3):463–481, 2014.

[43] Cheryl Chan, Yu Wang, Pierce KH Chow, Alexander YF Chung, London LPJ Ooi, and Caroline G Lee. Altered binding site selection of p53 transcription cassettes by hepatitis b virus x protein. *Molecular and cellular biology*, 33(3):485–497, 2013.

[44] Ruslan Medzhitov and Charles Janeway Jr. Innate immunity. *New England Journal of Medicine*, 343(5):338–344, 2000.

[45] Charles A Janeway Jr, Paul Travers, Mark Walport, and Mark J Shlomchik. Principles of innate and adaptive immunity. In *Immunobiology: The Immune System in Health and Disease. 5th edition*. Garland Science, 2001.

[46] Cancer Research Institute. Immunotherapy fact of the day #8. `https://www.cancerresearch.org/en-us/join-the-cause/cancer-immunotherapy-month/30-facts/08`, 2018. Online; accessed February 22, 2022.

[47] Mayo Clinic. Stem cells: What they are and what they do. `https://www.mayoclinic.org/tests-procedures/bone-marrow-transplant/in-depth/stem-cells/art-20048117`, 2019. Online; accessed February 25, 2022.

[48] Eric Vivier, Elena Tomasello, Myriam Baratin, Thierry Walzer, and Sophie Ugolini. Functions of natural killer cells. *Nature immunology*, 9(5):503–510, 2008.

[49] Giorgio Trinchieri. Biology of natural killer cells. *Advances in immunology*, 47:187–376, 1989.

[50] Yan Zhang, Diana L Wallace, Catherine M De Lara, Hala Ghattas, Becca Asquith, Andrew Worth, George E Griffin, Graham P Taylor, David F Tough, Peter CL Beverley, et al. In vivo kinetics of human natural killer cells: the effects of ageing and acute and chronic viral infection. *Immunology*, 121(2):258–265, 2007.

[51] Charles T Lutz, Anush Karapetyan, Ahmad Al-Attar, Brent J Shelton, Kimberly J Holt, Jason H Tucker, and Steven R Presnell. Human NK cells proliferate and die in vivo more rapidly than T cells in healthy young and elderly adults. *The Journal of Immunology*, 186(8):4590–4598, 2011.

[52] Hans-Gustaf Ljunggren. Paths taken towards NK cell–mediated immunotherapy of human cancer—a personal reflection. *Scandinavian Journal of Immunology*, 93(1):e12993, 2021.

[53] Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. Chapter 24 The Adaptive Immune System. In *Molecular Biology of the Cell. 4th edition*. Garland Science, 2002.

[54] Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. B Cells and Antibodies. In *Molecular Biology of the Cell. 4th edition*. Garland Science, 2002.

[55] Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. Helper T Cells and Lymphocyte Activation. In *Molecular Biology of the Cell. 4th edition*. Garland Science, 2002.

[56] Healthdirect. Lymph nodes. `https://www.healthdirect.gov.au/lymph-nodes`, 2021. Online; accessed March 1, 2022.

[57] Lindsay B Nicholson. The immune system. *Essays in biochemistry*, 60(3):275–301, 2016.

[58] K Liu. Dendritic cells. *Encyclopedia of Cell Biology*, page 741, 2016.

[59] Thomas Parr, Anjali Bhat, Peter Zeidman, Aimee Goel, Alexander J Billig, Rosalyn Moran, and Karl J Friston. Dynamic causal modelling of immune heterogeneity. *Scientific Reports*, 11(1):1–17, 2021.

[60] Andrea Greif. 2018 Nobel Prize in Physiology or Medicine: Unleashing the Immune System. `https://www.lls.org/blog/2018-nobel-prize-physiology-or-medicine-unleashing-immune-system`, 2018. Online; accessed March 4, 2022.

[61] National Cancer Institute at the National Institutes of Health. Immune checkpoint inhibitors. `https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/checkpoint-inhibitors`, 2019. Online; accessed March 6, 2022.

[62] Ajit Singh, Sundee Dees, and Iqbal S Grewal. Overcoming the challenges associated with CD3+ T-cell redirection in cancer. *British Journal of Cancer*, 124(6):1037–1048, 2021.

[63] National Cancer Institute at the National Institutes of Health. T-cell transfer therapy. `https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/t-cell-transfer-therapy`, 2019. Online; accessed March 8, 2022.

[64] Ellen Duong, Tim B Fessenden, Emi Lutz, Teresa Dinter, Leon Yim, Sarah Blatt, Arjun Bhutkar, Karl Dane Wittrup, and Stefani Spranger. Type I interferon activates MHC class I-dressed CD11b+ conventional dendritic cells to promote protective anti-tumor CD8+ T cell immunity. *Immunity*, 2021.

[65] Massachusetts Institute of Technology. A stealthy way to combat tumors: Biologists show that helper immune cells disguised as cancer cells can help rejuvenate T cells that attack tumors. *ScienceDaily*. `www.sciencedaily.com/releases/2021/11/211119155623.htm`, 2021. Online; accessed March 9, 2022.

[66] American Society of Clinical Oncology. Interferon Therapy for Chronic Myeloid Leukemia. `https://www.cancer.org/cancer/chronic-myeloid-leukemia/treating/interferon-therapy.html#references`, 2018. Online; accessed May 5, 2022.

[67] Matteo Barberis, Tomáš Helikar, and Paul Verbruggen. Simulation of stimulation: cytokine dosage and cell cycle crosstalk driving timing-dependent t cell differentiation. *Frontiers in Physiology*, 9:879, 2018.

[68] Eileen M Higham, Ching-Hung Shen, K Dane Wittrup, and Jianzhu Chen. Cutting edge: delay and reversal of t cell tolerance by intratumoral injection of antigen-loaded dendritic cells in an autochthonous tumor model. *The Journal of Immunology*, 184(11):5954–5958, 2010.

[69] Xuefei Li, Tina Gruosso, Dongmei Zuo, Atilla Omeroglu, Sarkis Meterissian, Marie-Christine Guiot, Adam Salazar, Morag Park, and Herbert Levine. Infiltration of CD8+ T cells into tumor cell clusters in triple-negative breast cancer. *Proceedings of the National Academy of Sciences*, 116(9):3678–3687, 2019.

[70] Mie Linder Hübbe, Ditte Elisabeth Jæhger, Thomas Lars Andresen, and Mads Hald Andersen. Leveraging Endogenous Dendritic Cells to Enhance the Therapeutic Efficacy of Adoptive T-Cell Therapy and Checkpoint Blockade. *Frontiers in Immunology*, page 2349, 2020.

[71] Junyun Lai, Sherly Mardiana, Imran G House, Kevin Sek, Melissa A Henderson, Lauren Giuffrida, Amanda XY Chen, Kirsten L Todd, Emma V Petley, Jack D Chan, et al. Adoptive cellular therapy with T cells expressing the dendritic cell growth factor Flt3L drives epitope spreading and antitumor immunity. *Nature immunology*, 21(8):914–926, 2020.

[72] Jacob A Myers and Jeffrey S Miller. Exploring the NK cell platform for cancer immunotherapy. *Nature reviews Clinical oncology*, 18(2):85–100, 2021.

[73] Christian U Blank, John B Haanen, Antoni Ribas, and Ton N Schumacher. The "cancer immunogram". *Science*, 352(6286):658–660, 2016.

[74] Marcela Esquivel-Velázquez, Pedro Ostoa-Saloma, Margarita Isabel Palacios-Arreola, Karen E Nava-Castro, Julieta Ivonne Castro, and Jorge Morales-Montor. The role of cytokines in breast cancer development and progression. *Journal of Interferon & Cytokine Research*, 35(1):1–16, 2015.

[75] Martina Molgora, Domenico Supino, Domenico Mavilio, Angela Santoni, Lorenzo Moretta, Alberto Mantovani, and Cecilia Garlanda. The yin-yang of the interaction between myelomonocytic cells and NK cells. *Scandinavian journal of immunology*, 88(3):e12705, 2018.

[76] Laurence Zitvogel, Antoine Tesniere, and Guido Kroemer. Cancer despite immunosurveillance: immunoselection and immunosubversion. *Nature Reviews Immunology*, 6(10):715–727, 2006.

[77] Padmanee Sharma, Siwen Hu-Lieskovan, Jennifer A Wargo, and Antoni Ribas. Primary, adaptive, and acquired resistance to cancer immunotherapy. *Cell*, 168(4):707–723, 2017.

[78] David Liu, Jia-Ren Lin, Emily J Robitschek, Gyulnara G Kasumova, Alex Heyde, Alvin Shi, Adam Kraya, Gao Zhang, Tabea Moll, Dennie T Frederick, et al. Evolution of delayed resistance to immunotherapy in a melanoma responder. *Nature medicine*, 27(6):985–992, 2021.

[79] Matthew Tontonoz. The Immune System Can Fight Cancer. So Why Doesn't It? *Memorial Sloan Kettering Cancer Center*. https://www.mskcc.org/news/immune-system-can-fight-cancer-so-why-doesn-t-it, 2018. Online; accessed March 19, 2022.

[80] Andreas Handel, Nicole L La Gruta, and Paul G Thomas. Simulation modelling for immunologists. *Nature Reviews Immunology*, 20(3):186–195, 2020.

[81] Khuloud Jaqaman and Gaudenz Danuser. Linking data to models: data regression. *Nature Reviews Molecular Cell Biology*, 7(11):813–819, 2006.

[82] Travers Ching, Daniel S Himmelstein, Brett K Beaulieu-Jones, Alexandr A Kalinin, Brian T Do, Gregory P Way, Enrico Ferrero, Paul-Michael Agapow, Michael Zietz, Michael M Hoffman, et al. Opportunities and obstacles for deep learning in biology and medicine. *Journal of The Royal Society Interface*, 15(141):20170387, 2018.

[83] Maxim Kuznetsov, Jean Clairambault, and Vitaly Volpert. Improving cancer treatments via dynamical biophysical models. *Physics of Life Reviews*, 39:1–48, 2021.

[84] Gavin P Dunn, Lloyd J Old, and Robert D Schreiber. The three Es of cancer immunoediting. *Annu. Rev. Immunol.*, 22:329–360, 2004.

[85] Michele Zanoni, Michela Cortesi, Alice Zamagni, Chiara Arienti, Sara Pignatta, and Anna Tesei. Modeling neoplastic disease with spheroids and organoids. *Journal of hematology & oncology*, 13(1):1–15, 2020.

[86] Andreas Handel. A software package for immunologists to learn simulation modeling. *BMC immunology*, 21(1):1–7, 2020.

[87] Andreas Handel. Software DSAIRM: dynamical systems approach to immune response modeling. *GitHub*. https://ahgroup.github.io/DSAIRM/, 2019. Online; accessed April 10, 2022.

[88] Ludwig Von Bertalanffy. Quantitative laws in metabolism and growth. *The quarterly review of biology*, 32(3):217–231, 1957.

[89] M Marušić. Mathematical models of tumor growth. *Mathematical Communications*, 1(2):175–188, 1996.

[90] Alan D Conger and Marvin C Ziskin. Growth of mammalian multicellular tumor spheroids. *Cancer Research*, 43(2):556–560, 1983.

[91] J Landry, JP Freyer, and RM Sutherland. A model for the growth of multicellular spheroids. *Cell Proliferation*, 15(6):585–594, 1982.

[92] Tom E Wheldon. *Mathematical models in cancer research*. CRC Press, 1988.

[93] BioLegend Inc. Cancer Immunoediting. https://www.biolegend.com/en-us/cancer-immunoediting, 2014. Online; accessed April 15, 2022.

[94] BioRender. Cancer Immunoediting. https://app.biorender.com/biorender-templates/figures/5c65c7b6bce1963300935374/t-60808597be65c100ab26fabc-cancer-immunoediting, 2022. Online; accessed April 16, 2022.

[95] Kathleen P Wilkie, Philip Hahnfeldt, and Lynn Hlatky. Using ordinary differential equations to explore cancer-immune dynamics and tumor dormancy. *bioRxiv*, page 049874, 2016.

[96] Alfred J Lotka. Analytical note on certain rhythmic relations in organic systems. *Proceedings of the National Academy of Sciences*, 6(7):410–415, 1920.

[97] Royal Norton Chapman. *Animal ecology: with especial reference to insects*. Burgess-Brooke, Incorporated, 1926.

[98] George I Bell. Predator-prey equations simulating an immune response. *Mathematical Biosciences*, 16(3-4):291–314, 1973.

[99] NV Stepanova. Course of the immune reaction during the development of a malignant tumour. *Biophysics*, 24(5):917–923, 1979.

[100] Vladimir A Kuznetsov, Iliya A Makalkin, Mark A Taylor, and Alan S Perelson. Nonlinear dynamics of immunogenic tumors: parameter estimation and global bifurcation analysis. *Bulletin of mathematical biology*, 56(2):295–321, 1994.

[101] Mark Chaplain, Vladimir A Kuznetsov, ZH James, and LA Stepanova. Spatio-temporal dynamics of the immune system response to cancer. In *International Conference on Mathematical Models in Medical and Health Sciences*, 1997.

[102] RM Sutherland and RE Durand. Growth and cellular characteristics of multicell spheroids. *Spheroids in cancer research*, pages 24–49, 1984.

[103] Alberto d'Onofrio. A general framework for modeling tumor-immune system competition and immunotherapy: Mathematical analysis and biomedical inferences. *Physica D: Nonlinear Phenomena*, 208(3-4):220–235, 2005.

[104] Heidi Dritschel, Sarah L Waters, Andreas Roller, and Helen M Byrne. A mathematical model of cytotoxic and helper t cell interactions in a tumor microenvironment. *Letters in Biomathematics*, 5(S1), 2018.

[105] Grace E Mahlbacher, Kara C Reihmer, and Hermann B Frieboes. Mathematical modeling of tumor-immune cell interactions. *Journal of Theoretical Biology*, 469:47–60, 2019.

[106] Patrick Micke, Carina Strell, Johanna Mattsson, Alfonso Martín-Bernabé, Hans Brunnström, Jutta Huvila, Malin Sund, Fredrik Wärnberg, Fredrik Ponten, Bengt Glimelius, et al. The prognostic impact of the tumour stroma fraction: A machine learning-based analysis in 16 human solid tumour types. *EBioMedicine*, 65:103269, 2021.

[107] Artur Mezheyeuski, Max Backman, Johanna Mattsson, Alfonso Martín-Bernabé, Chatarina Larsson, Ina Hrynchyk, Klara Hammarström, Simon Ström, Joakim Ekström, Siarhei Mauchanski, et al. The ratio of cytotoxic lymphocytes to m2-like macrophages is prognostic in immunogenic tumors. *bioRxiv*, 2021.

[108] Peter Savas, Roberto Salgado, Carsten Denkert, Christos Sotiriou, Phillip K Darcy, Mark J Smyth, and Sherene Loi. Clinical relevance of host immunity in breast cancer: from tils to the clinic. *Nature reviews Clinical oncology*, 13(4):228–241, 2016.

[109] Ville Satopaa, Jeannie Albrecht, David Irwin, and Barath Raghavan. Finding a“ kneedle” in a haystack: Detecting knee points in system behavior. In *2011 31st international conference on distributed computing systems workshops*, pages 166–171. IEEE, 2011.

[110] Bartosz Grzywacz, Nandini Kataria, Niketa Kataria, Bruce R Blazar, Jeffrey S Miller, and Michael R Verneris. Natural killer–cell differentiation by myeloid progenitors. *Blood, The Journal of the American Society of Hematology*, 117(13):3548–3558, 2011.

[111] Yueyun Pan, Yinda Yu, Xiaojian Wang, and Ting Zhang. Tumor-associated macrophages in tumor immunity. *Frontiers in immunology*, page 3151, 2020.

[112] Jiuyang Liu, Xiafei Geng, Jinxuan Hou, and Gaosong Wu. New insights into m1/m2 macrophages: Key modulators in cancer progression. *Cancer Cell International*, 21(1):1–7, 2021.

[113] Tyler J Curiel, George Coukos, Linhua Zou, Xavier Alvarez, Pui Cheng, Peter Mottram, Melina Evdemon-Hogan, Jose R Conejo-Garcia, Lin Zhang, Matthew Burow, et al. Specific recruitment of regulatory t cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nature medicine*, 10(9):942–949, 2004.

[114] Jinlin Liu, Ning Zhang, Qun Li, Weiwei Zhang, Fang Ke, Qibin Leng, Hong Wang, Jinfei Chen, and Honglin Wang. Tumor-associated macrophages recruit ccr6+ regulatory t cells and promote the development of colorectal cancer via enhancing ccl20 production in mice. *PloS one*, 6(4):e19495, 2011.

[115] Kuntal Worah, Till SM Mathan, Thien Phong Vu Manh, Shivakumar Keerthikumar, Gerty Schreibelt, Jurjen Tel, Tjitske Duiveman-de Boer, Annette E Sköld, Annemiek B van Spriel, I Jolanda M de Vries, et al. Proteomics of human dendritic cell subsets reveals subset-specific surface markers and differential inflammasome function. *Cell reports*, 16(11):2953–2966, 2016.

[116] Kelli McKenna, Anne-Sophie Beignon, and Nina Bhardwaj. Plasmacytoid dendritic cells: linking innate and adaptive immunity. *Journal of virology*, 79(1):17–27, 2005.

[117] N Cools, VFI Van Tendeloo, ELJM Smits, M Lenjou, G Nijs, DR Van Bockstaele, ZN Berneman, and P Ponsaerts. Immunosuppression induced by immature dendritic cells is mediated by tgf-β/il-10 double-positive cd4+ regulatory t cells. *Journal of cellular and molecular medicine*, 12(2):690–700, 2008.

[118] Chutamas Thepmalee, Aussara Panya, Mutita Junking, Thaweesak Chieochansin, and Pa-thai Yenchitsomanus. Inhibition of il-10 and tgf-β receptors on dendritic cells enhances activation of effector t-cells to kill cholangiocarcinoma cells. *Human vaccines & immunotherapeutics*, 14(6):1423–1431, 2018.

[119] Maxim Kuznetsov. Combined influence of nutrient supply level and tissue mechanical properties on benign tumor growth as revealed by mathematical modeling. *Mathematics*, 9(18), 2021.

[120] Kevin Atsou, Fabienne Anjuère, Véronique M Braud, and Thierry Goudon. A size and space structured model describing interactions of tumor cells with immune cells reveals cancer persistent equilibrium states in tumorigenesis. *Journal of Theoretical Biology*, 490:110163, 2020.

[121] Anastasios Matzavinos, Mark AJ Chaplain, and Vladimir A Kuznetsov. Mathematical modelling of the spatio-temporal response of cytotoxic T-lymphocytes to a solid tumour. *Mathematical Medicine and Biology*, 21(1):1–34, 2004.

[122] Fiona R Macfarlane, Mark AJ Chaplain, and Tommaso Lorenzi. A stochastic individual-based model to explore the role of spatial interactions and antigen recognition in the immune response against solid tumours. *Journal of theoretical biology*, 480:43–55, 2019.

[123] Marcello Delitala and Tommaso Lorenzi. Recognition and learning in a mathematical model for immune response against cancer. *Discrete & Continuous Dynamical Systems-B*, 18(4):891, 2013.

[124] Sneha Berry, Nicolas A Giraldo, Benjamin F Green, Tricia R Cottrell, Julie E Stein, Elizabeth L Engle, Haiying Xu, Aleksandra Ogurtsova, Charles Roberts, Daphne Wang, et al. Analysis of multispectral imaging with the astropath platform informs efficacy of pd-1 blockade. *Science*, 372(6547):eaba2609, 2021.

[125] Ahmadreza Ghaffarizadeh, Randy Heiland, Samuel H Friedman, Shannon M Mumenthaler, and Paul Macklin. Physicell: An open source physics-based cell simulator for 3-d multicellular systems. *PLoS computational biology*, 14(2):e1005991, 2018.

[126] Paul Macklin and Randy Heiland. Physicell: invader-scout-attacker system, Feb 2019.

[127] Andrew Roth, Jaswinder Khattra, Damian Yap, Adrian Wan, Emma Laks, Justina Biele, Gavin Ha, Samuel Aparicio, Alexandre Bouchard-Côté, and Sohrab P Shah. Pyclone: statistical inference of clonal population structure in cancer. *Nature methods*, 11(4):396–398, 2014.

[128] Gabriella Beer. Science Surgery: 'Why doesn't the immune system attack cancer cells? *Cancer Research UK*. `https://news.cancerresearchuk.org/2019/02/28/science-surgery-why-doesnt-the-immune-system-attack-cancer-cells/`, 2019. Online; accessed March 19, 2022.

[129] Urszula Ledzewicz, Mohammad Naghnaeian, and Heinz Schättler. Optimal response to chemotherapy for a mathematical model of tumor–immune dynamics. *Journal of mathematical biology*, 64(3):557–577, 2012.

[130] Manisha Sahu and Hemakumari Suryawanshi. Immunotherapy: The future of cancer treatment. *Journal of Oral and Maxillofacial Pathology: JOMFP*, 25(2):371, 2021.

[131] Stanley J Oiseth and Mohamed S Aziz. Cancer immunotherapy: a brief review of the history, possibilities, and challenges ahead. *Journal of Cancer Metastasis and Treatment*, 3:250–261, 2017.

[132] Jake S O'Donnell, Michele WL Teng, and Mark J Smyth. Cancer immunoediting and resistance to t cell-based immunotherapy. *Nature reviews Clinical oncology*, 16(3):151–167, 2019.

[133] Xiaona Fang, Karsten Kruse, Ting Lu, and Jin Wang. Nonequilibrium physics in biology. *Reviews of Modern Physics*, 91(4):045004, 2019.

[134] Shingo Iwami, Yasuhiro Takeuchi, Yoshiharu Miura, Toru Sasaki, and Tsuyoshi Kajiwara. Dynamical properties of autoimmune disease models: tolerance, flare-up, dormancy. *Journal of theoretical biology*, 246(4):646–659, 2007.

[135] Daniela Bruni, Helen K Angell, and Jérôme Galon. The immune contexture and immunoscore in cancer prognosis and therapeutic efficacy. *Nature Reviews Cancer*, 20(11):662–680, 2020.

[136] Abicumaran Uthamacumaran and Hector Zenil. A review of mathematical and computational methods in cancer dynamics. *arXiv preprint arXiv:2201.02055*, 2022.

[137] Javier Koh, Yoko Itahana, Ian H Mendenhall, Dolyce Low, Eunice Xin Yi Soh, Alvin Kunyao Guo, Yok Teng Chionh, Lin-Fa Wang, and Koji Itahana. ABCB1 protects bat cells from DNA damage induced by genotoxic compounds. *Nature communications*, 10(1):1–14, 2019.

[138] Laura Spinney. COVID-19 expert Karl Friston:'Germany may have more immunological "dark matter"'. *The Observer*, 31(5):20, 2020.

[139] Daniel S Chen and Ira Mellman. Oncology meets immunology: the cancer-immunity cycle. *immunity*, 39(1):1–10, 2013.

[140] Artur Mezheyeuski. Personal communication. Unpublished. Dept. of Immunology, Genetics and Pathology, Uppsala University, Rudbeck Laboratory, 751 85 Uppsala, Sweden.

[141] Emery N Brown, Patrick L Purdon, Oluwaseun Akeju, K Solt, and RD Miller. Monitoring the state of the brain and central nervous system during general anesthesia and sedation. *Miller's Anesthesia*, 9:1279–1297, 2020.

[142] Michel Baranger. Chaos, complexity, and entropy. *New England Complex Systems Institute, Cambridge*, 2000.

[143] Frank L Lambert. Configurational entropy revisited. *Journal of Chemical Education*, 84(9):1548, 2007.

[144] Daniel F Styer. Insight into entropy. *American Journal of Physics*, 68(12):1090–1096, 2000.

[145] Alexander D Wissner-Gross and Cameron E Freer. Causal entropic forces. *Physical review letters*, 110(16):168702, 2013.

[146] Elias P Gyftopoulos. Entropies of statistical mechanics and disorder versus the entropy of thermodynamics and order. *J. Energy Resour. Technol.*, 123(2):110–118, 2001.

Appendices

A Complementary figures

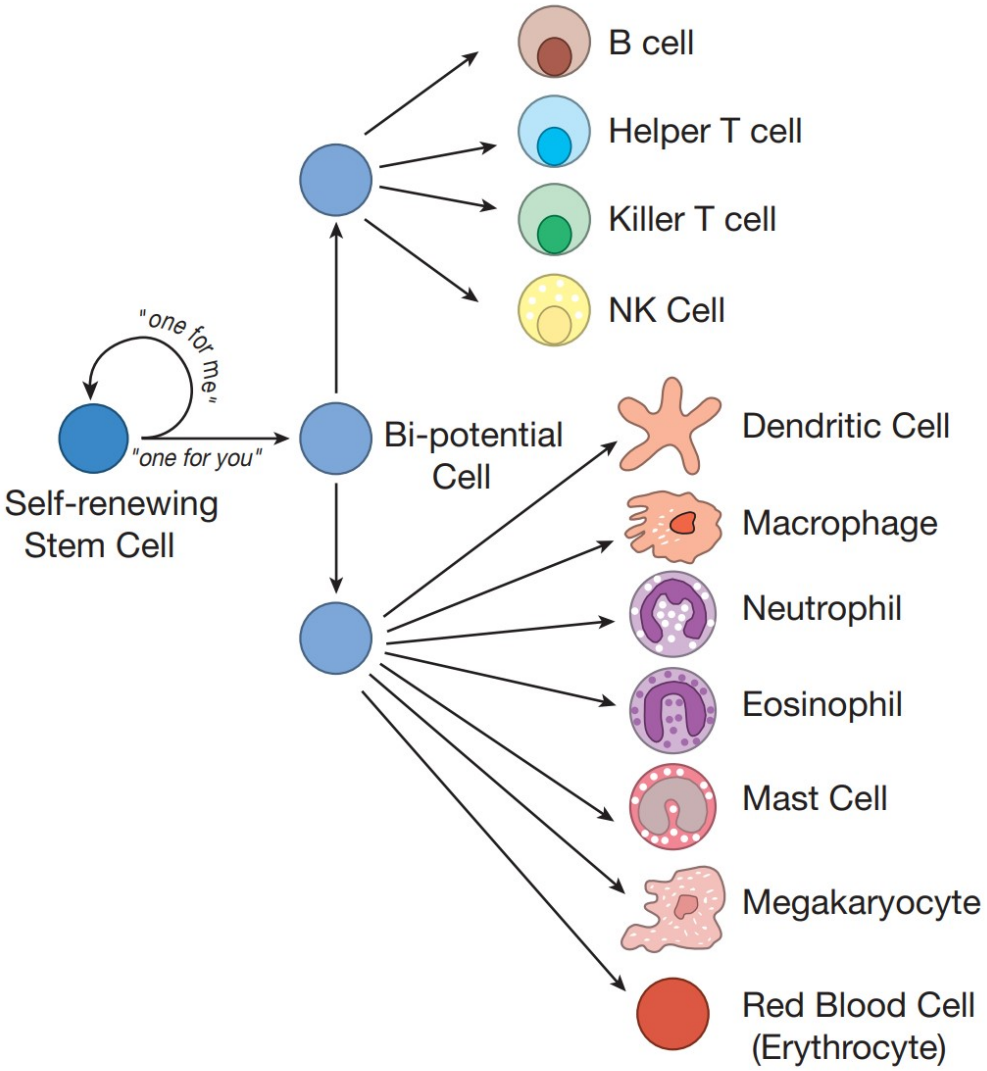


Figure A.1: **Illustration of the event where a stem cell splits into two daughter cells.** One daughter cell is destined to remain a stem cell, while the Bi-potential cell has 11 (many more in reality) options to "choose" between. Adopted with permission from [31].

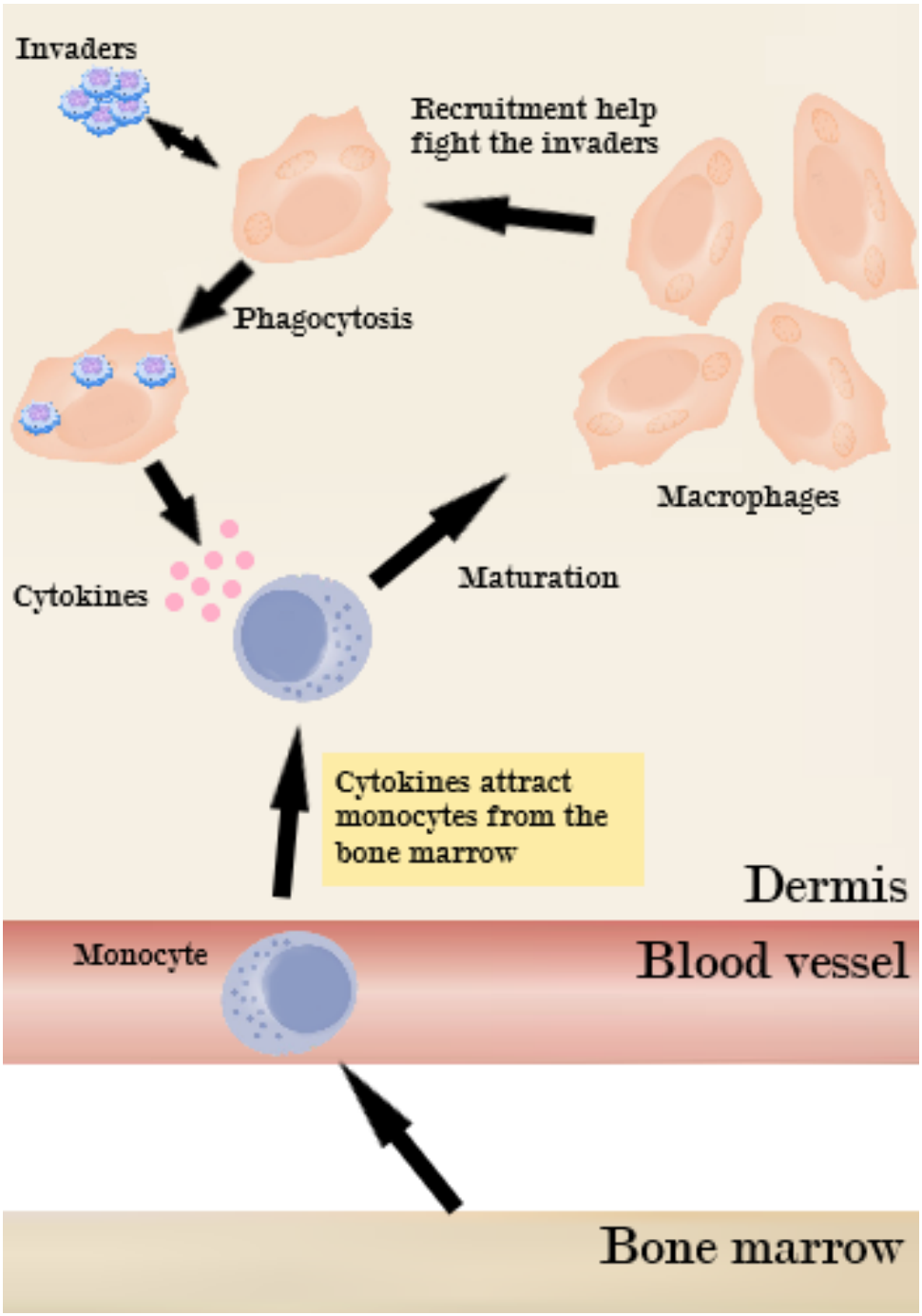


Figure A.2: **Illustration of the macrophage recruitment process.** The dermis layer is the second layer of the skin. The invader is engulfed and dissolved by a macrophage, which then secretes cytokines to recruit help (monocytes from a nearby blood vessel).

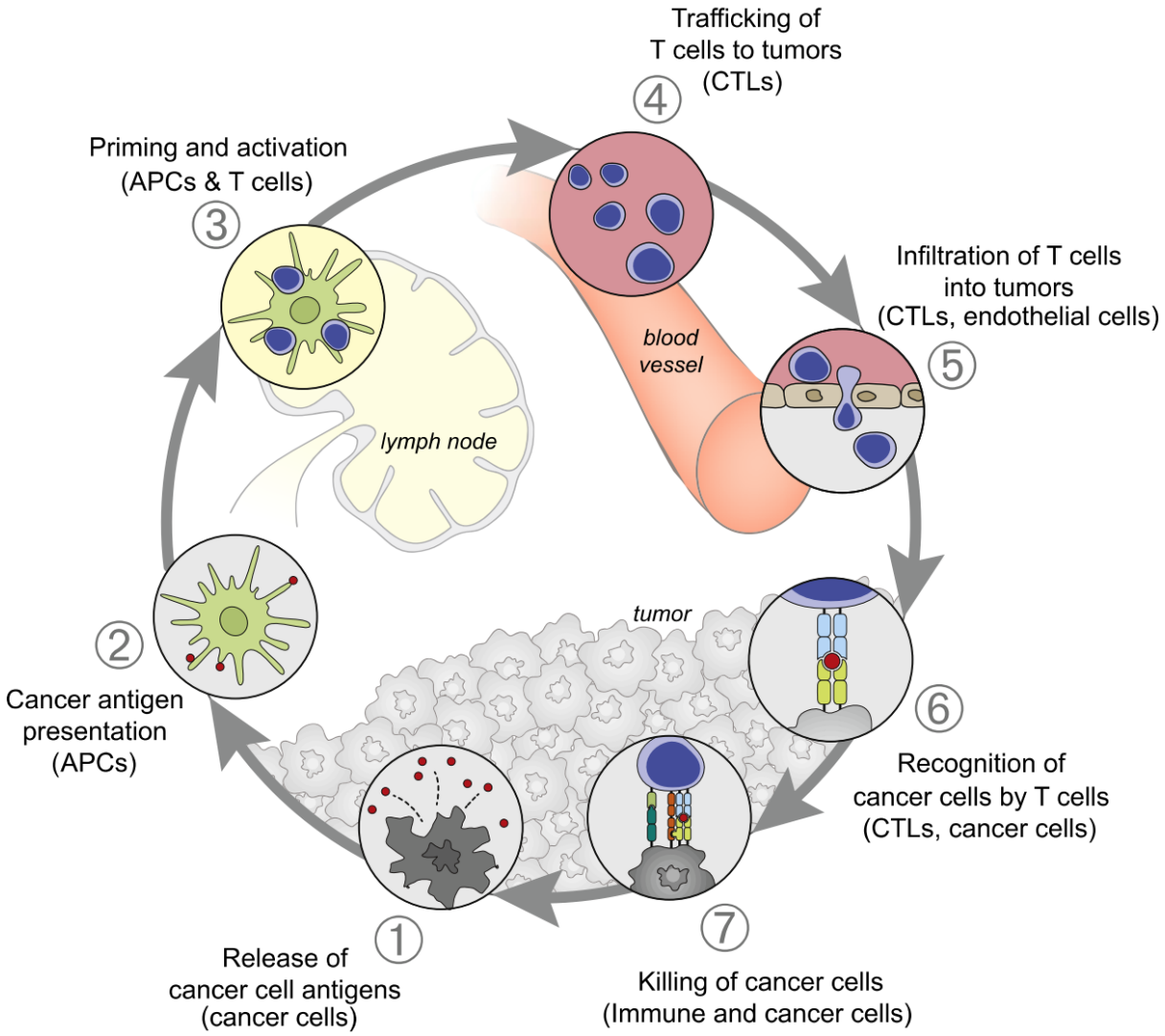


Figure A.3: **Illustration of the dynamical interactions between the immune system (T cells) and cancer cells.** The cycle of action shows the process of T cells being activated and transported to the tumor site. Each step implies the involved cells in parentheses, where APCs are antigen-presenting cells, and CTLs are cytotoxic T lymphocytes. Adapted with permission from [139].

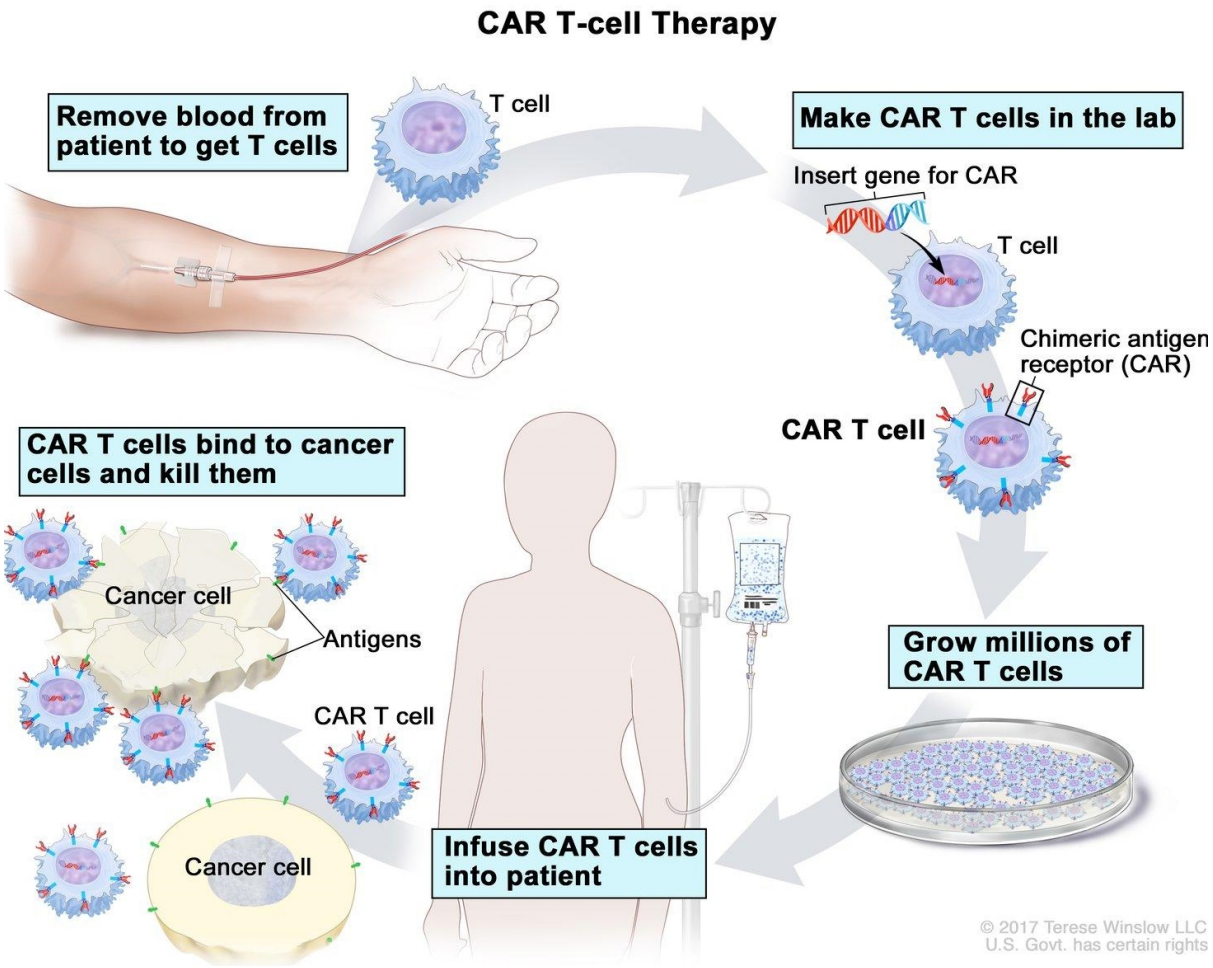


Figure A.4: **Illustration of the CAR T cell therapy.** The T cells are firstly extracted from a blood sample from the patient, such that they can be genetically engineered to express the Chimeric Antigen Receptor (CAR). Once some of these CAR T cells have been created, they are allowed to proliferate. Once a sizeable batch has been obtained (usually a few weeks), the CAR T cells are infused into the patient. Finally, the CAR T cells will eventually find the cancer cells and bind to them, consequently killing them. Adopted with permission from [63].

B Mathematical models

B.1 Integro-differential model of antigen heterogeneity and evolution

In [123], a model considering antigen heterogeneity and evolution (mutation) is introduced. In essence, the model considers five different cell populations (i), namely cancer cells ($i = 1$), APCs with no antigens on their surface ($i = 2$), APCs with a certain antigen ($i = 3$), naive T cells ($i = 4$), and activated T cells ($i = 5$). The model considers the phenomenons of cancer mutations that promote change of their expressed antigens, cancer cell proliferation and nutrient competition, cancer cell recognition (influenced by APCs), activation of naive T cells, proliferation of activated T cells, and immune induced cancer cell death.

The variable $u \in [0, 1]$ below, is a continuous structuring-variable used to identify the state of the specific cells. The biological interpretation of u for the different populations are (1) governs cancer cells' expression of antigens, (3) to identify APC exposed antigens, (4) cognate antigen of naive T cells, and (5) the antigens that activated T cells recognize and attack. Note that population 2 is an unstructured population (no u dependence).

The sizes of the five populations (1-5) are here, respectively, donated f_1, n_2, f_3, f_4, and f_5, where (at time t) n_2 is a real positive number, and $f_i : \mathbb{R}^+ \times u \in [0, 1] \to \mathbb{R}^+, \quad i \neq 2$. The model is presented below in Eqs. (18)-(22), with parameter explanation in Table 5.

$$\begin{aligned}
\frac{\partial}{\partial t} f_1(t,u) &= \int_U \mathcal{M}\left(u_*, u; \epsilon\right) f_1\left(t, u_*\right) du_* - f_1(t,u) \int_U \mathcal{M}\left(u, u_*; \epsilon\right) du_* + \\
&\quad + \kappa_1(u) f_1(t,u) - \mu_1(u) f_1(t,u) \int_U f_1\left(t, u^*\right) du^* + \\
&\quad - \mu^I f_1(t,u) \int_U e^{-\theta^I (u-u^*)^2 f_5(t,u^*) du^*} \\
&= \underbrace{\int_U \mathcal{M}\left(u_*, u; \epsilon\right) f_1\left(t, u_*\right) du_* - f_1(t,u)}_{\text{renewal and mutations}} + \underbrace{\kappa_1(u) f_1(t,u)}_{\text{proliferation}} \\
&\quad - \underbrace{\mu_1(u) f_1(t,u) \mathrm{n}_1(t)}_{\text{cell-cell competition}} - \underbrace{\mu^I f_1(t,u) \int_U e^{-\theta^I (u-u^*)^2} f_5\left(t, u^*\right) du^*}_{\text{cancer-immune competition}}
\end{aligned} \tag{18}$$

$$\begin{aligned}
\frac{d}{dt} n_2(t) &= -\gamma_2 n_2(t) \int_U f_1\left(t, u^*\right) du^* + \mu_2 \int_U \int_U f_3\left(t, u_*\right) f_3\left(t, u^*\right) du_* du^* \\
&= \underbrace{-\gamma_2 n_2(t) n_1(t) + \mu_3 \mathrm{n}_3^2(t)}_{\text{recognition, presentation and homeostatic regulation}}
\end{aligned} \tag{19}$$

$$\begin{aligned}
\frac{\partial}{\partial t} f_3(t,u) &= \gamma_2 n_2(t) f_1(t,u) - \mu_3 f_3(t,u) \int_U f_3\left(t, u^*\right) du^* \\
&= \underbrace{\gamma_2 n_2(t) f_1(t,u) - \mu_3 f_3(t,u) \mathrm{n}_3(t)}_{\text{recognition, presentation and homeostatic regulation}}
\end{aligned} \tag{20}$$

$$
\begin{aligned}
\frac{\partial}{\partial t} f_4(t,u) &= \kappa_4 f_4(t,u) - \mu_4 f_4(t,u) \int_U f_4\left(t,u^*\right) du^* + \\
&\quad + \int_U \int_U e^{-\theta^I (u_* - u^*)^2} \mathcal{A}^4\left(u,u_*,u^*\right) f_4\left(t,u_*\right) f_3\left(t,u^*\right) du_* du^* + \\
&\quad - f_4(t,u) \sum_j \int_U \int_U e^{-\theta^I (u - u^*)^2} \mathcal{A}^j\left(u_*,u,u^*\right) f_3\left(t,u^*\right) du_* du^* \\
&= \underbrace{\kappa_4 f_4(t,u) - \mu_4 f_4(t,u) \mathrm{n}_4(t)}_{\text{homeostatic regulation}} - \underbrace{\gamma_4 f_4(t,u) \int_U e^{-\theta^I (u - u^*)^2} f_3\left(t,u^*\right) du^*}_{\text{T-cell activation}}
\end{aligned}
\tag{21}
$$

$$
\begin{aligned}
\frac{\partial}{\partial t} f_5(t,u) &= \int_U \int_U e^{-\theta^I (u_* - u^*)^2} \mathcal{A}^5\left(u,u_*,u^*\right) f_3\left(t,u^*\right) f_4\left(t,u_*\right) du_* du^* + \\
&\quad + \kappa_5 f_5(t,u) - \mu_5 f_5(t,u) \int_U f_5\left(t,u^*\right) du^* \\
&= \underbrace{\gamma_4 f_3(t,u) \int_U e^{-\theta^I (u_* - u)^2} f_4\left(t,u_*\right) du_*}_{\text{T-cell activation}} + \underbrace{\kappa_5 f_5(t,u) - \mu_5 f_5(t,u) \mathrm{n}_5(t)}_{\text{clonal expansion}} .
\end{aligned}
\tag{22}
$$

Here, $U := [0,1]$, and $\mathrm{n}_i(t) = \int_U f_i(t,u)\,\mathrm{d}u$ is the number density of population $i \neq 2$ at time t, where $f_i(t,u)\,\mathrm{d}u$ represents the number of cells in population $i \neq 2$ belonging to the volume $\mathrm{d}u$ (normalized considering the number of cells at $t = 0$ in the system). Furthermore, t is normalized considering the average cancer cell's lifetime.

For more details and an explanation of the model's appearance, see [123].

Table 5: Model parameters corresponding to the model of Eqs. (18)-(22).

Biological Phenomena	**Parameters**
Mutations altering the antigenic expression	γ_1, ϵ
Cancer cell proliferation	κ^C
Competition among cancer cells	μ^C
Recognition and antigen presentation by APCs	γ_2
Activation of T-cells by APCs	γ_y, θ^I
Homeostatic regulation of APCs	μ_3
Homeostatic regulation of naive T-cells	κ_4, μ_4
Clonal expansion of activated T-cells	κ_5, μ_5
Immune destruction of cancer cells	μ^I, θ^I
Symmetric probability kernel for mutations	$\mathcal{M}$, see [123]
Probability density of cell transitions from state u_* to u (population 4 to 5)	$\mathcal{A}^i(u,u_*,u^*)$

C Data and complementary plots

The data consists of tumor tissue samples taken from 3359 different cancer patients, where two simultaneous tissue samples (from different tumor regions) were sometimes taken from the same patient, resulting in a total of 6380 tumor samples. The cell-coordinate data from each sample represents one 2D layer from the sample. Each sample is solely tumor tissue, but the insides of these tissues also consist of clusters of tumor cells 'sitting' in tumor-associated stroma. The individual cancerous cells were identified using a cytokeratin marker (which is expressed by cancer cells in epithelial cancers). For melanoma cancer, another marker was used, but for consistency reasons, it is also denoted as 'CK' in the data [140].

Notably, some samples are denser in cell counts than others, and some even have large regions consisting of voids (holes). Specifically, the density of cells varies depending on the type of tumor and stroma tissue considered (fat, muscle, organ parenchyma, etc.). Samples can also be damaged, e.g. contain large areas of defects or necroses, which have been manually removed during the data compilation, resulting in smaller sample sizes or internal 'holes'. But some tumors (adenocarcinomas) also contain growth patterns that themselves make glandular-like structures, resulting in 'holes' in the image. Furthermore, the samples have been physically removed from bigger tumor pieces using cylindrical 'punchers', causing the samples to be of different sizes [140].

In Table 6 on the next page, the data structure can be seen. Note that "1" marks the type of the cell, i.e. that some marker used to identify that specific kind of cell was detected in the cell on that specific location (rows with zeros in all cell categories, implies that the cell at that location is an "unspecified cell", meaning that it did not show any of the markers for the cells investigated).

Table 6: Structure of used data (here NK cells, with artificially constructed values).

cohort	Sample name tissue	Category	Cell X position	Cell Y position	NK	NKT	M1	Myeloid	M2	CK	ID_anonym
Breast	Breast_NK_5...	Stroma	7012.1	43005.6	1	0	0	0	0	0	p1...
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
Lung	Lung_NK_5...	Tumor	4012.1	23005.6	0	0	0	0	0	1	p2...
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮

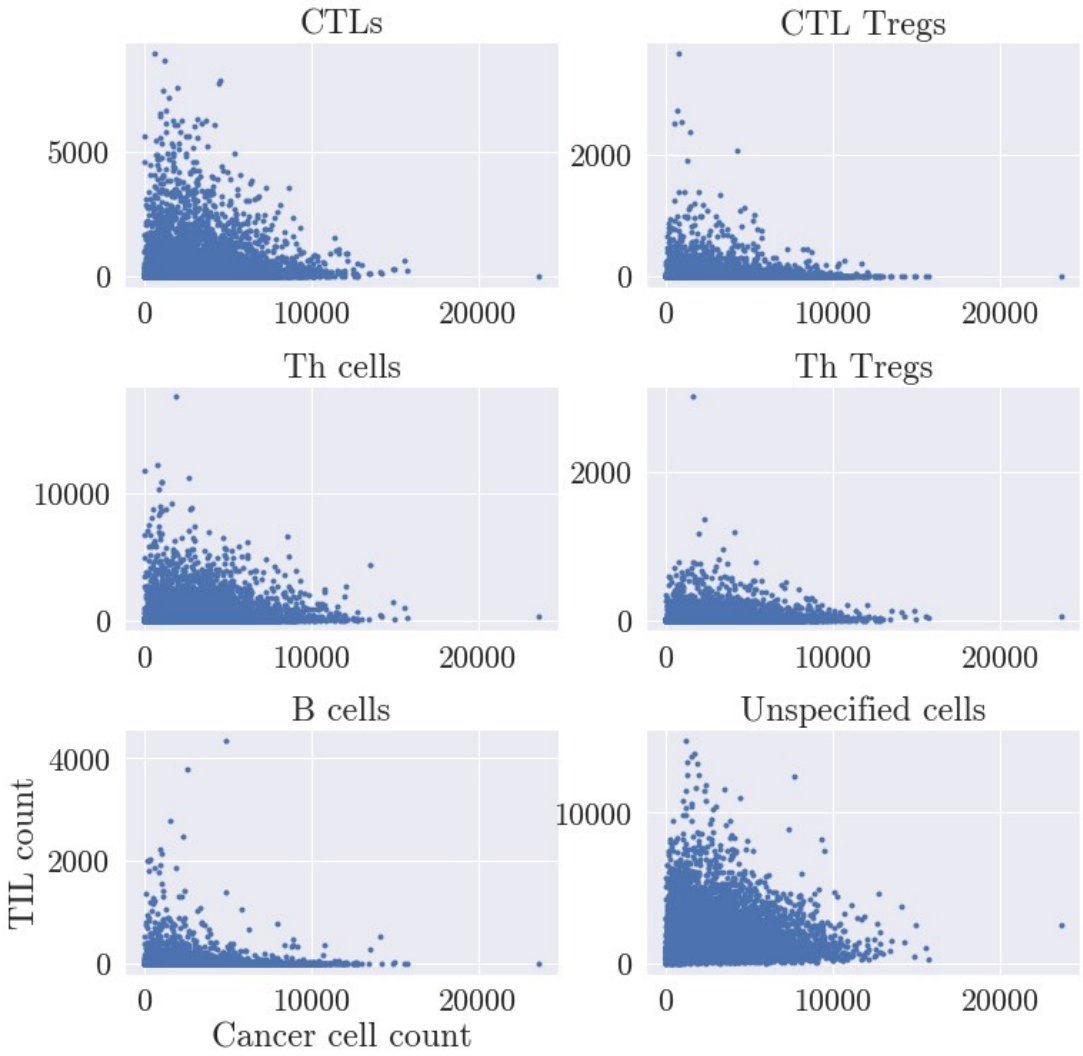


Figure C.1: **The number of each TIL-type in relation to the number of cancerous cells**. Each data point corresponds to a separate tumor sample. Directly there seem to be indications of a certain relation between the number of each TIL-type and the cancer cell count. For logarithmic scale, see Figure C.2.

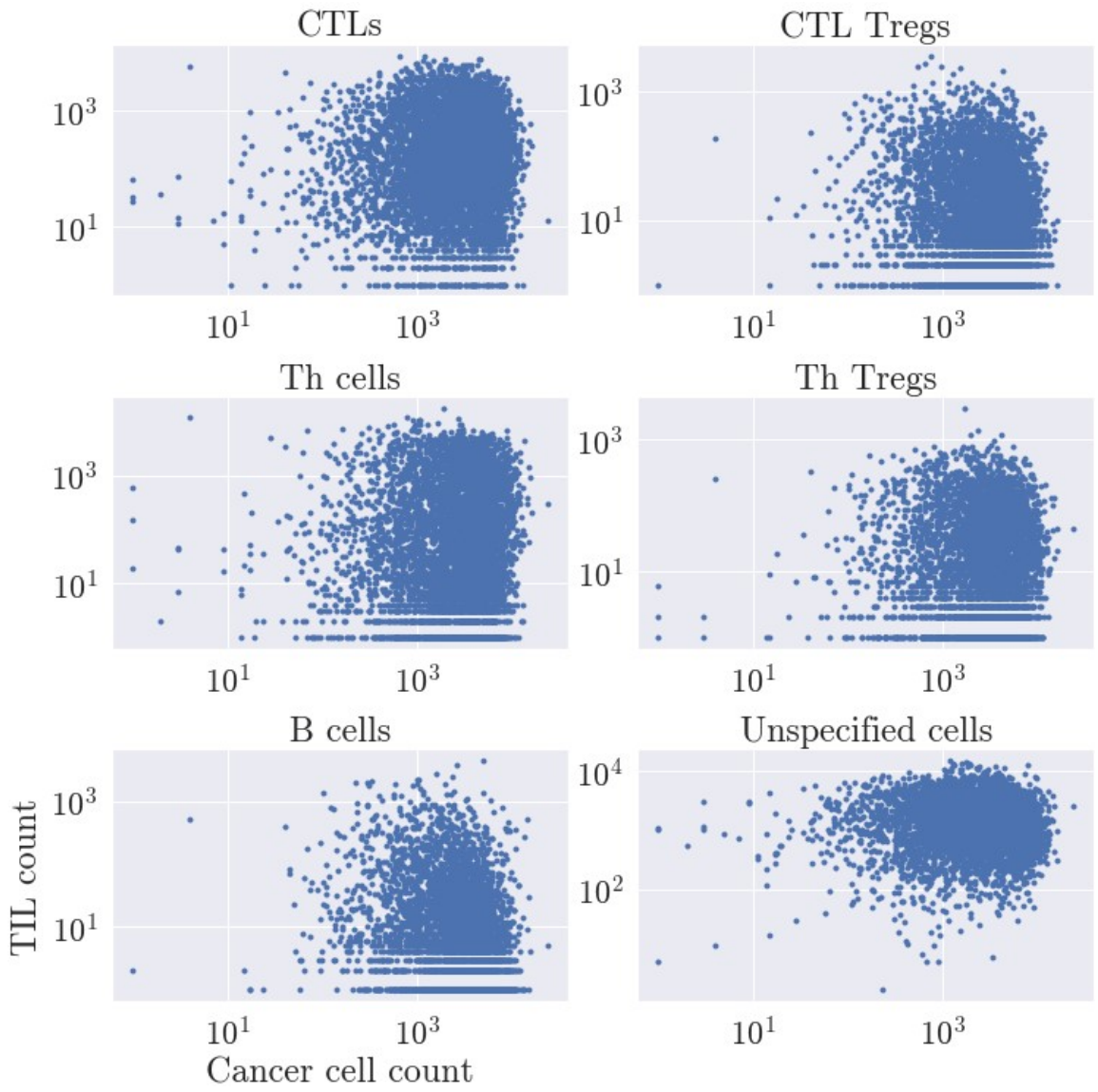


Figure C.2: **TILs plotted separately in relation to the number of cancer cells**, on a logarithmic scale. Corresponding to Figure C.1.

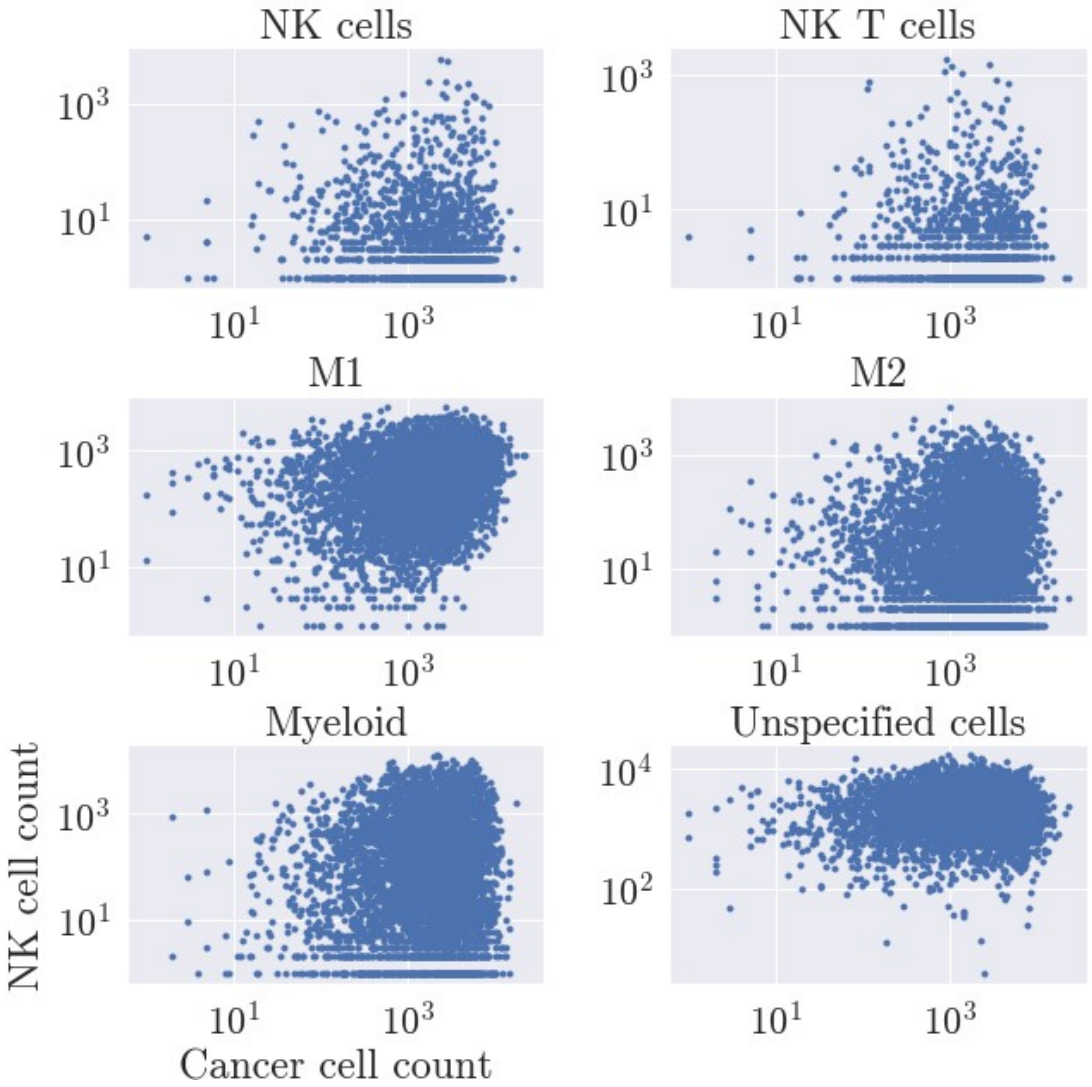


Figure C.3: **Relation between the number of NK cells and cancer cells on a logarithmic scale.** Corresponding to Figure 22.

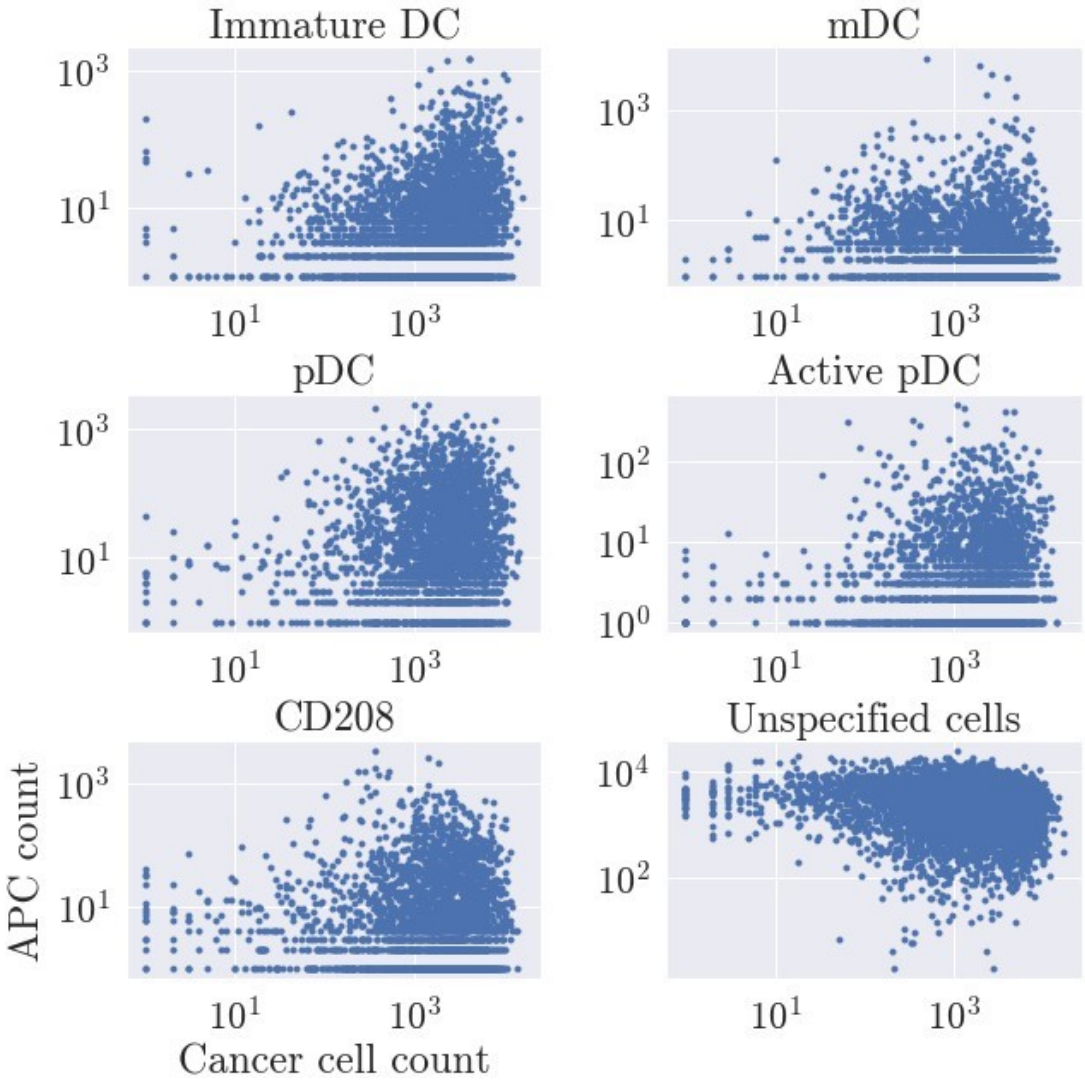


Figure C.4: **APC count in relation to the number of cancer cells on a logarithmic scale**. Corresponding to Figure 23.

D Entropic aspects

The concept of entropy has only been mentioned hastily, however, entropy is commonly explained as a measurement of the lack of consistency in a system [141]. Entropy is also known as an important quantity to many physicists, especially, entropy has proven to be useful when describing the behavior of chaotic processes [142]. Furthermore, entropy can be interpreted as a measure of possible ways for energy to be distributed in a system of molecules (through their available microstates[24], Ω). Macrostates then describe the global properties of a system, e.g. the sum of a sequence is a macroscopic property of the sequence [143].

The entropy in microcanonical ensembles is derived by counting the number of microstates corresponding to a specific macrostate ($S \propto \ln \Omega$), where the macrostate entropy is a measure of ways for a system to remain in the same macroscopic state while being microscopically different [144]. It is also known that nonequilibrium physical systems tend to maximize their entropy production at each instant, which in turn can be linked to their evolution towards entropically larger macrostates [145].

Explicitly, the microstates are here represented by formed clusters of immune cells (CTLs), thus the ensemble of all microstates is the set of all observed cluster formations. Therefore, the formally defined entropy,

$$S = -\sum_{i}^{M} p_i \log p_i, \tag{23}$$

where p_i is the probability for each of some M possible outcomes, has to be altered. In particular, the interpretation of M and p_i needs to be represented by other (cluster-related) metrics, which in this case are the number of clusters (N) and their respective sizes (I_{cluster}). The entropy related to the clustering of CTLs in each tumor is therefore given by,

$$S \approx -\sum_{n}^{N} I^{\phi}_{\text{cluster},n} \log \left(I^{\phi}_{\text{cluster},n} \right), \tag{24}$$

where $I_{\text{cluster},n}$ is the number of CTLs in the n^{th} cluster of in total N clusters. The exponent, ϕ, is included to introduce scale-invariance, such that consideration is taken to systems of unspecified size (i.e. arbitrarily large tumors).

The value of ϕ is obtained from a fitted power-law $f(I_{\text{cluster}}) = aI^{\phi}_{\text{cluster}}$, where the statistical interpretation of $f(I_{\text{cluster}})$ corresponds to the relative frequency (or empirical probability) of specific cluster sizes, i.e. $\frac{\text{Occurrence of cluster size}}{\text{Total number of clusters}}$. By then considering the generalized clustering behavior of CTLs in all tumors (using the adjusted entropy formula from Eq. (24)), the relation between entropy (of CTL-clustering) and the number of cancer cells can be quantified on the individual tumor scale.

By applying the value of $\phi = -1.2$ (found in Figure 21) in Eq. (24), the entropy related to the clustering of CTLs in each tumor sample can be investigated in relation to the number of cancerous cells in the same sample. The result is seen below,

[24] The microscopic configuration of a system, e.g. the position of atoms in gas or cells in tissue.

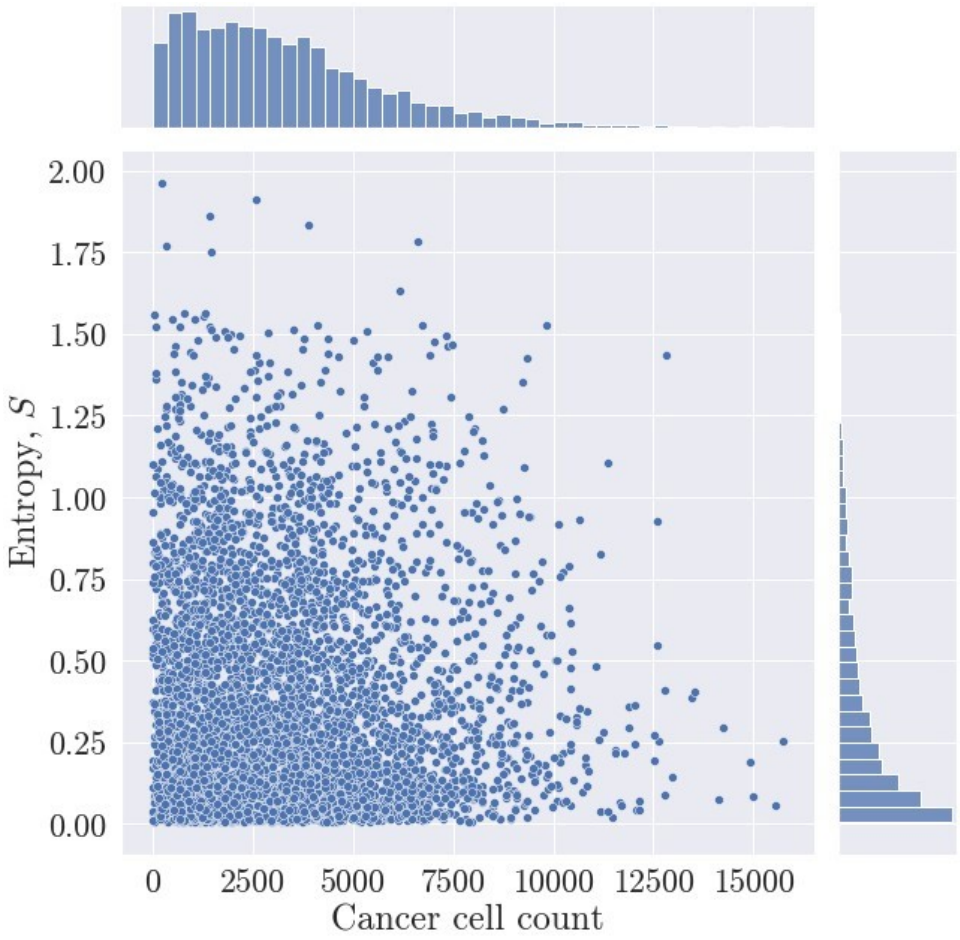


Figure D.1: **Clustering entropy of CTLs in relation to the number of cancerous cells.** Each data point represents a separate tumor sample.

Similarities to Figure 19 are seen, however, larger entropy is not necessarily solely explained by that a larger volume gives more microstates, therefore a more rigorous explanation is required [146].

The data investigated consists of no time aspect, and the role of entropy, entropy maximization, and system irreversibility should ideally be studied on an individual tumor basis with the time-dependent evolution of the cell structures in consideration. Therefore, this appendix only serves as a brief exploration of how the concept of entropy can be incorporated into the study of immunotherapy and expand the relevant "parameters" to investigate.