Mathematical model of detrimental inflammation in the late stage of colorectal cancer

VICTORIA WOLD

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SUPERVISORS

Christian Kersten (Sørlandet hospital HF)

Yuriy Rogovchenko (UiA)

CO-SUPERVISOR

Svitlana Rogovchenko (UiA)

University of Agder, 2019
Faculty of Engineering and Science
Department of Mathematical Sciences

Sammendrag

Tykktarmskreft er den nest mest vanlige kreftformen blant kvinner, og den tredje mest vanlige blant menn, med 1.8 millioner nye tilfeller og 861.000 dødsfall i 2018 ifølge Health Organization GLOBOCAN. Matematisk modellering av tykktarmskreft kan kaste et bedre lys på dynamikken til denne dødelige sykdommen, og foreslå ulike strategier for behandling. Vi studerer interaksjonen mellom viktige biomarkører foreslått av en konseptuell matematisk modell av skadelig inflammasjon i senstadiet av tykktarmskreft. Vi ønsker å bruke matematiske modeller for å forbedre vår forståelse av kompliserte prosesser i immunsystemet som påvirker kreftcellenes dynamikk. Vår konseptuelle modell omfatter 22 forskjellige likninger laget basert på diagrammet som beskriver interaksjonen mellom cellene vi anslår å være de mest signifikante for skadelig inflammasjon i dette stadiet av kreften. Cellene kommuniserer ved hjelp av et antall cytokiner som anses å spille den mest signifikante rollen i systemet. Basert på tidligere forsknings resultater fra andre, retter vi vårt fokus på inflammasjons cytokinet IL-6. Denne kan, på grunn av dens sterke korrelasjon med CRP, være en viktig faktor for pasientens medisinske forløp som påvirker forventet levealder. Vi har enda ikke testet denne konseptuelle modellen på grunn av manglende klinisk data. Kalibrering av parametere, analytiske og numeriske analyser er overlatt til vårt fremtidige arbeid med prosjektet.

Abstract

This thesis combines mathematical modeling and biology in cellular dynamics. The work employs mathematical methods to explore biological processes. By designing a model consisting of ordinary differential equations we try to help improve the understanding of the complex interactions between the immune system and cancer cells. The model focuses on a particular type of immune response, namely the detrimental inflammation in the advanced stage of colorectal cancer.

Acknowledgements

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From the deep of my heart, thank you all!

Contents

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Glossary

This glossary has been made for the reader to understand biological terms in the thesis. It provides medical terms. Some of the glossary has been inspired from Bourfia Y. [1], but most from Dr. Kersten`s advice.

Adaptive immune cells	Manifest antigen-specific receptors expressed on the surface of T and B-lymphocytes.
Angiogenesis	Evolution of new blood vessels. This contributes to the spread and growth of cancer. Angiogenesis is also involved in healing wounds and repair blood flow to tissue after injury.
Antigen	Structures bound by antibodies or cell surface, B or T cells antigen receptors.
Apoptosis	Cell death
Bystander cells	Are here defined as benign non-cancerous cells in the tumor microenvironment. They can consist of the extracellular matrix, several types of cells, like endothelia cells, macrophages, lymphocytes, fibroblasts and smooth muscle cells.
B cell	Important cell of the adaptive immune system. A lymphocyte developed in the bone marrow of an organism which produces antibodies and is co-managing, together with T cells, the adaptive immune system`s functions.
Cancer	A group of diseases involving abnormal cell growth with the potential to invade or, spread to other parts of an organism.
Carcinogenesis	Formation of cancer where normal cells are transformed into cancer cells.
CD8+ T cell	Cytotoxic T cell, which is a subtype of T lymphocytes that kills cancer cells -, or cells that are otherwise infected or damaged.
Cell crosstalk	The cell crosstalk that is describing the transitions between the cells where the cytokines bring a message of how to act or might be produced by the cells themselves.
Chemokines	Family of small cytokines, signaling proteins secreted by cells.
Cytotoxicity	Quality of being toxic to cells, for example immune cells

Cytokines	Small proteins that are important in cell signaling. They are produced by a broad range of cells, including immune cells like macrophages, neutrophils, B- and T lymphocytes.
Differentiation	Cellular differentiation is a process where a cell matures from stem cells and precursors to mature cells.
Growth factor	Substance capable of stimulating cellular growth, proliferating, healing and cellular differentiation, like cytokines.
Immune response	The body's mechanism of safeguarding the body's functioning by detecting and eradicating dead own cells and foreign substances or microbes.
Immunotherapy	Treatment of disease by modulating the functioning of the immune system.
Innate immune cells	Cells, which recognize molecular patterns assert on the surface of invading microbes and membrane bound receptors in order to screen and eradicate foreign substances, microbes and dead cells. They also present these particles to more specialized cells of the adaptive immune system.
Lymphocyte	Subtype of a white blood cell in the immune system. It includes natural killer cells, T cells, B cells
Macrophages	Type of white blood cell of the innate immune system that engulfs and digests cellular debris, foreign substances, microbe's cancer cells and anything else that interrupts the body cells on its surface.
Natural killer cell	Type of cytotoxic lymphocyte critical to innate immune system. They provide rapid responses to infection and respond to tumor formation.
Neutrophils	The most abundant type of granulocytes and the most frequent type of white blood cells. Form an essential part of the innate immune system.
Phenotype	Composite of the organism's observable characteristics or traits. Results from in two basic factors: organism`s genetic code, or factors that involve influence of environment.
Proliferate	To grow or multiply by rapidly producing new tissue, parts, cells or offspring.
T cell	Important cell of the adaptive immune system, a lymphocyte that participate in cellular immunity, including to-cell

	communication. The major T cell categories are T-helper (CD4) and T- cytotoxic T cell (CD8).
TGF-beta	Transforming growth factors beta; a growth factor synthesized by skeletal cells; found in most species.
Th1	T helper cell type 1 derived from CD4 + T cells. Differentiated after exposure to cytokines like IL-12, TNF-alpha, and INF-alpha.
Th2	T helper cell type 2 derived from CD4 + T cells. Differentiated after exposure to IL-4, IL-6, IL-10 and IL-13.
Treg cells	T regular cells are an immunosuppressive subtype of CD4 + T which regulate immune tolerance. They play an important role in the suppression of the anti-tumor immune response.
Tumor	Uncontrolled growth of tissue in which cell multiplication is progressive. Malignant tumors are also called neoplasms, which means that they are composed of a new and actively growing tissue.

Abbreviations

In this section we collected abbreviations that are most frequently used in the text.

CAF(s), cancer-associated fibroblast(s);

DC(s), dendritic cell(s);

EMC, extracellular matrix;

EGF, epidermal growth factor;

G-CSF, granulocyte colony stimulating factor (CSF3);

GM-CSF, granulocyte-macrophage colony stimulating factor (CSF2);

IFN, interferon;

IL, interleukin;

M1, type 1 macrophage;

M2, type 2 macrophage;

M.CSF, macrophage colony stimulating factor (CSF1);

MDSC(s), myeloid derived suppressor cell(s);

MSI, microsatellite instability

N1, type 1 neutrophil;

N2, type 2 neutrophil;

NK(s), natural killer (cells);

PD-L1, programmed death receptor 1 receptor);

TGF, transforming growth factor;

Th0, immature T helper cell precursor;

Th1, type 1 T-helper cell;

Th2, type 2 T-helper cell;

Th17, type 17 T-helper cell;

TME, tumor microenvironment;

TNF, tumor necrosis factor;

Treg(s), regulatory T (cells);

TAM(s), tumor-associated macrophage(s);

TAN(s), tumor-associated neutrophil(s)

Introduction

Colorectal cancer (CRC) is the leading type of cancer in Norway [2]. In this thesis, we focus on final stage of this disease, when the cancer has metastasized, detrimental inflammation which is accelerating the development of the tumor and the patient has a short life expectancy. Most often CRC does not cause symptoms until it reaches an advanced stage and has metastasized [Dr. C. Kersten personal communication]. Frequently, the immune system is impaired at this stage of disease and often leads to detrimental inflammation, fueling the tumor growth rather than inhibiting it. From time of diagnosis, an otherwise healthy patient receiving optimal treatment has a median survival of about 25 months.

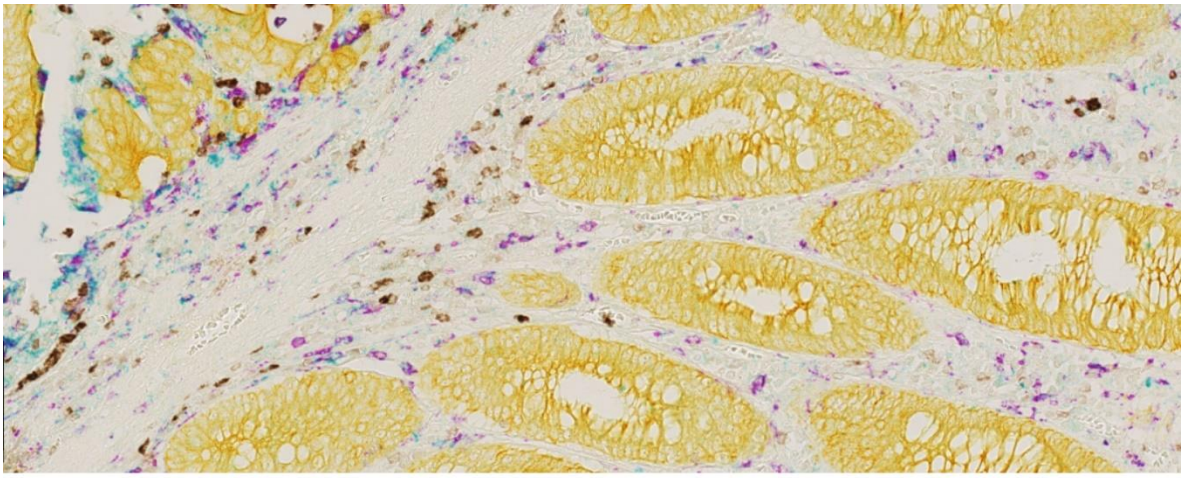


Figure 1. A human colorectal cancer specimen, illustrating the malignant tumor and its bystander cells in the microenvironment. It contains cancer cells (yellow) and different types of bystander cells: Adaptive/lymphoid profile: CD8 (brown), CD4 (purple), B cells (silver). Extracted with courtesy from Köstner: [1]

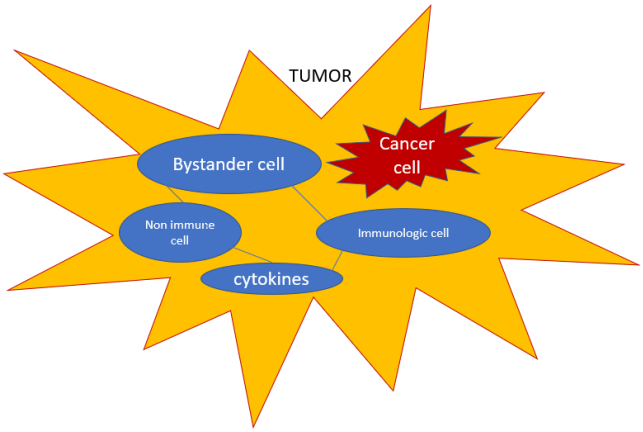
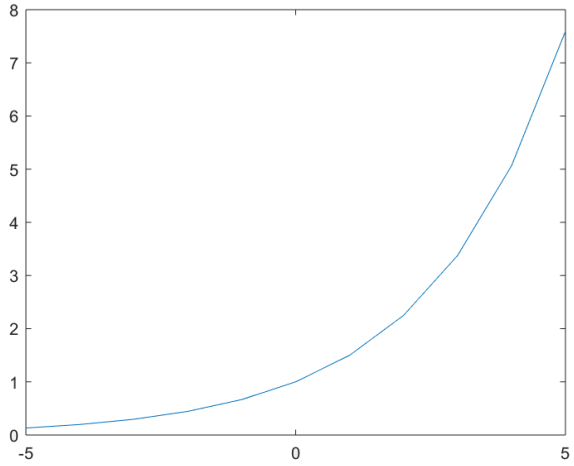


Figure 2. Simplification of a malignant tumor and its bystander cells in the microenvironment. It contains cancer cells and bystander cells, which in turn are comprised of non-immune cells and immune cells. These cells either interact directly or via cytokines.

When deciding what shape of equation for cancer cells we wanted to use in the model, we had to consider different potential shapes of the functional growth. Exponential functions can describe fast growing cancers like acute, untreated leukemia, and are in the assumed shape of:

$$f(x) = ab^x \quad ,$$

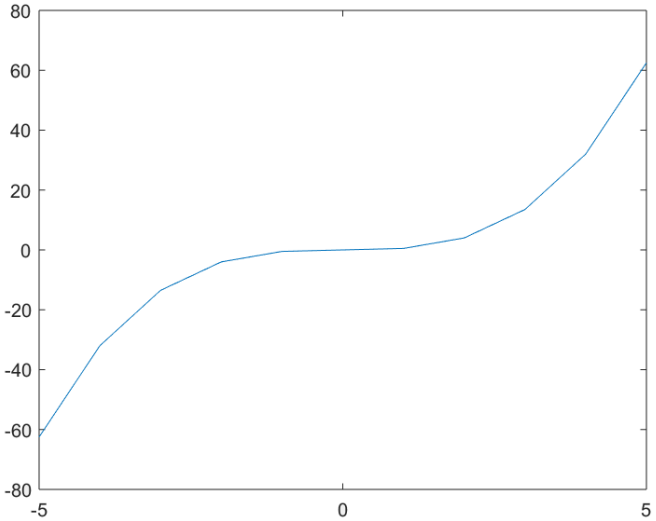


$$a = 1, \qquad b = 1.5, \qquad -5 \geq x \leq 5$$

Figure 3. Exponential curve simulated in MATLAB

Power functions can describe slow growing cancers like neuroendocrine pancreatic tumors, and are in the assumed shape of:

$$f(y) = cy^a \ ,$$



$$a \geq 3, c = 0.5, -5 \geq y \leq 5$$

Figure 4. Power curve produced in MATLAB

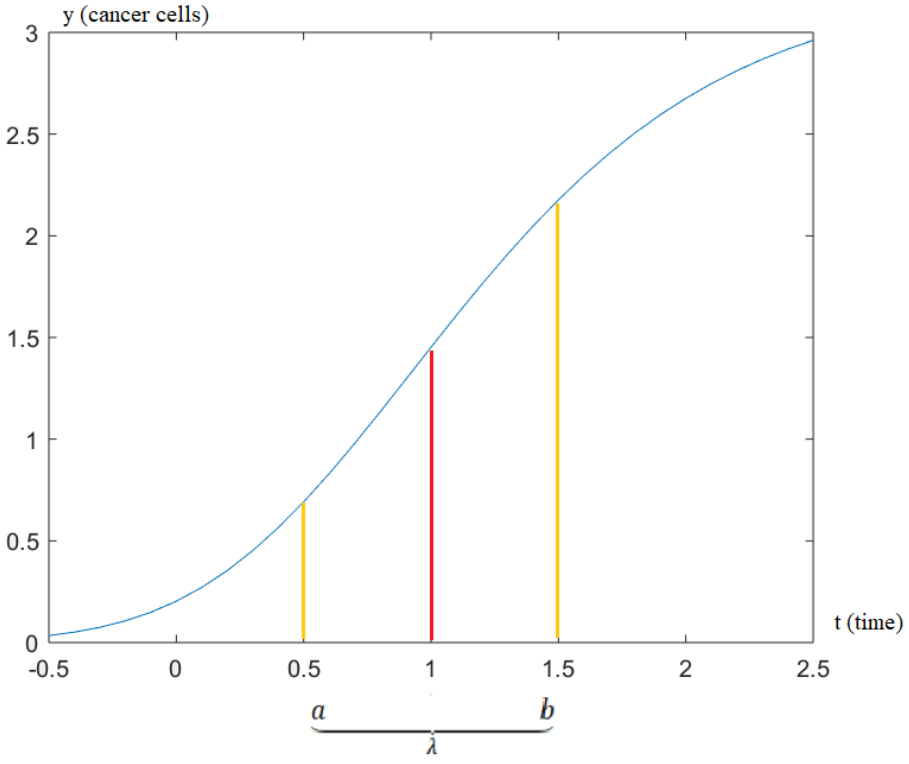
An application of logistic curve is used in medicine, where the logistic differential equation is used to model the growth of tumors. The logistic function that we are using in this paper is describing colorectal cancer, and has the assumed shape taken from the generalized logistic curve [3] with carrying capacity. Denoting with $C(t)$ as the density of cancer cells at time t, and its dynamics governed by:

$$C'(t) = k(\left(1 - \frac{C}{C_0}\right)^{v})C,$$

which is of the type: $C' = F(C)C, \quad F'(C) \leq 0$

where $F(C)$ is the proliferation rate of the cancer cells, and has the solution on the Richards differential equation:

$$C(t) = A + \frac{k - A}{(C + e^{-B(t-M)})^{1/v}},$$



$$A = 1, M = 0, k = 3.25, C = 1, B = 1.5, v = 0.25$$

Figure.5 Logistic curve produced in MATLAB

Figure 5. shows the growth of cancer exemplified by a logarithmic slope. The interval between a and b, defines λ (the slope), representing the growth rate of cancer in the late stage of CRC, influenced by different factors such as detrimental inflammation. The steepness of the slope can be influenced by therapy, and other favorable or non-favorable parameters. Exponential growth is desired to be avoided, as it represents accelerated tumor growth. We divide the curve into three parts. The first part represents the early stages of CRC development, i.e. stage I and II. The second part stage III and IV (our part of interest a-b), with the endpoint where the slope changes (b). It gives us a logarithmic scale where we for example have $a = (0.5, 0.7), b = (1.5, 2.18)$ as our points of interests, $k = 3{,}25$ is the carrying capacity and $B = 1.5$ is the growth rate. The third part representing the detrimental inflammation in final stage of CRC when $t > 1.5$. The part after (b) is assumed to be the growth in the terminal phase of a patient's life.

The motivation for this thesis is based on clinical experiences and research "*To Reduce CRP and Target Inflammation on Colorectal Cancer, written by Dr. Christian Kersten (2019)" [4].* Data from the scientific foundation, has motivated and inspired us to aim for the creation of a mathematical model describing detrimental inflammation in late stage colorectal cancer. We will use this model in order to explore the importance of IL-6 in this type of cancer. Published clinical data, as far as they are available, will be used to extrapolate the clinical significance of IL-6, using CRP as a surrogate marker with a proven statistical correlation in this clinical setting. CRP is a common marker used in the clinics to measure inflammation.

Mathematical modeling of the dynamics in colorectal cancer can not only provide mechanical insight but may in the future lead to personalized treatment that can be applied to identify which drugs are most efficient for the patient. There are several examples of successful use of applied mathematics in hospitals. Both Sørlandet Hospital HF and The Norwegian Radium Hospital have used this method for some years with success. At the University of Agder (UiA), Per Henrik Hogstad - associate professor in mathematics, physics and computer science has developed a mathematical visualization and simulation program named SimReal [5]. Parallel to this he has, together with some of his students, collaborated with The Norwegian Radium Hospital and SINTEF, combining mathematics, ICT and medicine. Later SimReal will be combined with these external projects. In one of the medical projects a prototype application to control a linear accelerator (ray machine) connected to a fictive patient database was created to give an automatic treatment for each individual patient [6]. The prototype was tested for months and further developed at different Norwegian radiotherapy centers. Other medicine projects were connected to the programming of mathematical transformations (using Wavelets) of medical image information. These mathematical transformations were used to find small tumors at an early stage in mammography images (The Norwegian Radium Hospital) and noise reduction of vein edges in ultrasound (SINTEF) [7]. A medicine project has started, but not yet finished, at Sørlandet Sykehus, detecting (using Wavelets) the thickness of bone structure, which helps doctors to detect osteoporosis.

At Oslo University Hospital, a multidisciplinary group of mathematicians, biologist and oncologists, led by Vessela N. Kristensen [8] is focusing on gene expression profiles in breast cancer. The mathematicians are led by professor Arnoldo Frigessi from the Oslo Center for

Biostatistics and Epidemiology (OCBE) and BigInsight. The Oslo Centre for Biostatistics and Epidemiology (OCBE) is a joint center integrating the activities of the Department of Biostatistics, UiO and the Section of Biostatistics, Epidemiology and Health Economics, OUS [9]. BigInsight is a center for research-based innovation, started in April 2015 as one of the third generation Norwegian Centres for Research-based Innovation. BigInsight produces innovative solutions for key data-driven challenges facing our consortium of private, public and research partners, by developing original statistical and machine learning methodologies. Fulfilling the promise of the big data revolution, they invent analytical tools to extract knowledge from complex data and deliver BIG INSIGHT [10].

Biological simplification of late stage colorectal cancer biology with focus on detrimental inflammation

The following diagram is very important for our project. It provides a simplified illustration of the interplay between tumor cells, innate (i.e. macrophages M1 and M2, Neutrophils N1 and N2) and adaptive immune cells (i.e. Tregs, CD8 + T cells, Th1 and Th2 cells) in the microenvironment of late stage colorectal cancer. The cell interplay is mediated by soluble molecules, like cytokines (i.e. IL-1, IL-4, IL-6, IL-10, IL-12, IL-13, TNF-alpha or TGF-Beta) or by the direct cell-to cell contact, called cell crosstalk. The red factors are promoting the tumor growth and green factors are contributing to the elimination of cancer cells.

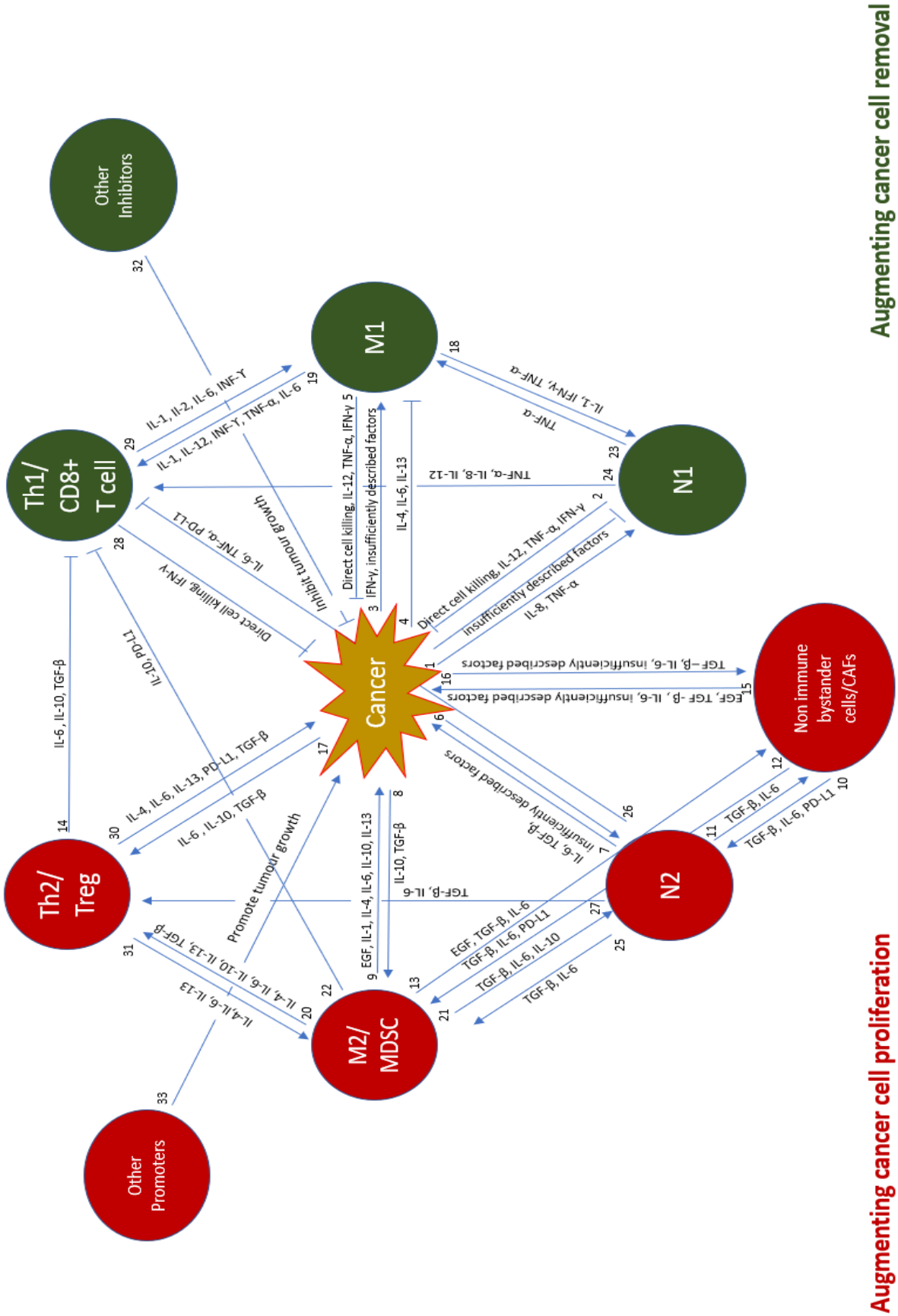


Figure 6. The Tumor Microenvironment and the assumed most important cancer cell extrinsic factors in the context of detrimental inflammation in late stage CRC.

The information used to support our diagram in Figure 6 is collected in the following table where we have illustrated each of the 33 interactions between the cells with the list of cytokines involved and the references to the corresponding literature. For those interactions where we do not have any relevant references we are guided by the educated guess and clinical and research experience of Dr. Kersten.

Table 1. Interaction network in detrimental inflammation of end stage colorectal cancer

#	Description	References
1	**Cancer >> pro-N1: Cancer >> anti-N1:** IL-1, IFN-γ, TNF-α	[11]
2	**N1 >> anti-Tumor:** Direct cell killing, IL-12, TNF-α, IFN-γ	[12]
3	**Cancer >> pro-M1:** IFN-γ	[13]
4	**Cancer >> anti-M1:** IL-4, IL-6, IL-13	Educated guess and experience
5	**M1 >> anti-Tumor:** Direct cell killing, IL-12, TNF-α, IFN-γ, NOS	[14]
6	**Cancer >> pro-N2:**	[15]
7	**N2 >> pro-Tumor:** IL-6, TNF-α, PD-L1,	[11, 13, 16]
8	**Cancer >> pro-M2:** EGF, IL-1, IL-4, IL-6, IL-10, IL-13	[14, 17]
9	**M2 >> pro-Tumor:** EGF, IL-4, IL-6, IL-10, IL-13	[14, 17]
10	**CAFs >> pro-N2**	[18, 19]
11	**N2 >> pro-CAFs:** TGF-β, IL-6	Intelligent guess and experience
12	**CAFs >> pro-M2**	[20]
13	**M2 >> pro-CAFs:** EGF, TGF-β, IL-6	Educated guess and experience

14	**TH2/Treg >> anti-TH1/CD8:** IL-6, IL-10, TGF-β	Educated guess and experience
15	**CAFs >> Tumor:**	[20], [21]
16	**Cancer >> pro CAFs:**	[20] [21]
17	**Cancer pro-TH2/Treg:** IL-6, IL-10, TGF-β	Educated guess and experience
18	**M1 >> N1**	[22]
19	**M1>>TH1/CD8:** IL-1, IL-12, INF-ϒ, TNF-α, IL-6	Educated guess and experience
20	**M2/MDSC pro- TH2/Treg:** IL-4, IL-6, IL-10, IL-13, TGF-β	[17]
21	**M2/MDSC >> pro- N2:** TGF-β, IL-6, IL-10	[23]
22	**M2 >> anti-TH1/CD8:** IL-10, PD-L1	Educated guess and experience
23	**N1 >> M1:** TNF-α,	[22]
24	**N1 >> CD8:** TNF-α, IL-8, IL-12,	[24]
25	**N2 >> pro- M2/MDSC:** TGF-β, IL-6,	[22], [23]
26	**N2 >> anti-TH1/CD8:** IL-6, TNF-α, PD-L1	Educated guess and experience
27	**N2 >> TH2/Treg:** TGF-β, IL-6	Educated guess and experience
28	**TH1/CD8 >>anti-tumor:** Direct cell killing, IFN-γ	Educated guess and experience
29	**TH1/CD8 >> M1:** IL-1, Il-2, IL-6, INF-ϒ	Educated guess and experience
30	**TH2/Treg >> pro-tumor:** IL-4, IL-6, IL-13, PD-L1, TGF-β	Educated guess and experience
31	**TH2/Treg >> pro-M2/MDSC:** IL-4, IL-6, IL-13,	[17]
32	**Other Tumor Inhibitors**	Educated guess and experience

33	**Other Tumor Promoters**	Educated guess and experience

Motivation

The motivation for this thesis is based on clinical experience of Dr. Christian Kersten including that reported in the paper "*To Reduce CRP and Target Inflammation on Colorectal Cancer"* [4]. He kindly allowed us to also use parts of this unpublished material in this thesis. For more than a century, there has been a discussion about the effect of beneficial and detrimental immune responses in cancer. For colorectal cancer the beneficial prognostic importance of cytotoxic T (CD8) cells has been robustly demonstrated by Jerome Galon [25]. Still, therapeutic effects from immunotherapeutic drugs like PDL inhibitors are very poor and largely comprised to a small subgroup of CRC patients with high mutational load, i.e. MSI-high patients.

The existence of activated, but tumor promoting immune responses with subsets of activated neutrophils, macrophages and regulatory T cells (Tregs) could explain the lack of therapeutic effect on checkpoint inhibitors. Inflammation has emerged as a major factor promoting cancer development and progression, and detrimental chronic immune responses are well documented in many cancers i.e. hepato-cellular carcinoma. IL-6 appears to be one of the key cytokines, promoting both effects on tumor proliferation and a detrimental inflammatory response.

Systemic C-reactive protein (CRP) levels reflect a systemic inflammatory response (SIR) and may be an easy assessable surrogate marker for IL-6 activation, since IL-6 is a potent inducer of CRP. Around 30% of IV stage CRP patients (mCRC setting), have a so-called SIR, a CRP level above 30 mg/L as seen in the paper of Thomsen, Kersten et.al [26]. In work by co-workers of C. Kersten, increasing CRP values are strongly associated with impaired prognosis in every molecular subtype of mutation Thomsen, Kersten et.al [26]. There have been documented strong prognostic value of elevated CRP levels in all stages of CRC. Analyzing multiple markers of SIR in metastatic (stage IV) CRC patients treated within a large phase III trial. Confirmed the prognostic significance of a SIR.

The markers of SIR were at least equal to that of prognostic significance of CRP. Patient groups with CRP levels of $\leq$10, 11-30, 31-60, and > 60 mg/L, experienced a median overall survival of 24.3, 20.6,17.1 and 12.3 months, respectively. The study showed a CRP and BRAF mutation status with an additive prognostic significance where a high CRP (>60) conferred to a median survival of only 3.8 months whereas survival was 33.8 month in the group with low CRP ($\leq$ 10). The most important discovery was that CRP values were highly correlated (correlation of 0.661) with IL-6 levels, and thus must reflect plasma levels of IL-6.

These data from the foundation and initiated the aim of creating a mathematical model describing detrimental inflammation in late stage colorectal cancer. We will used model in order to explore the importance of IL-6. The published clinical data will be used to extrapolate the clinical significance of IL6, using CRP as a surrogate marker with a proven statistical correlation in this particular clinical setting.

Cancer as a complex multifaceted disease

This section is based on the work of Friedman [27], and R. J. Seager [28]. Cancer has emerged as the most common cause of death in western world (Friedman, 2012), [27]. It can be divided into over two hundred types. They have their own specific characteristics but share some common features. Cancer is defined by gene mutations that lead to local proliferation of damaged cells and at a later stage metastasis, when cancer cells spread to other parts of the human body. The metastasized cancer cells can lead to death, when they interfere with other parts of the normal body functions.

A tumor is a mass of abnormal cells which grow in an uncontrolled fashion. The tumor is said to be benign if the mass remains clustered together and confined to the cavity. If the tumor is braking out of the cavity, and proliferating into the extracellular matrix, the tumor has become malignant. A process called metastasis defines invasion of malignant cancer cells to lymphatic vessels or the blood stream, subsequent transportation to other areas of the body, where they create a secondary tumor. Chronic inflammation is a common feature of many cancers [28]. It is manifested as an immune suppression of beneficial anti-tumor responses but represents at the same time a self-sustaining tumor promoting continued inflammation. Inflammation is mediated by direct cell-to-cell contracts within the tumor microenvironment (TME), as well as circulating chemokines, growth factors and cytokines.

Hallmarks of cancer

Seminal work has proposed ten hallmarks of cancer to provide a framework for the understanding of the multistep development of human malignant tumors by Hannah & Weinberg [29].

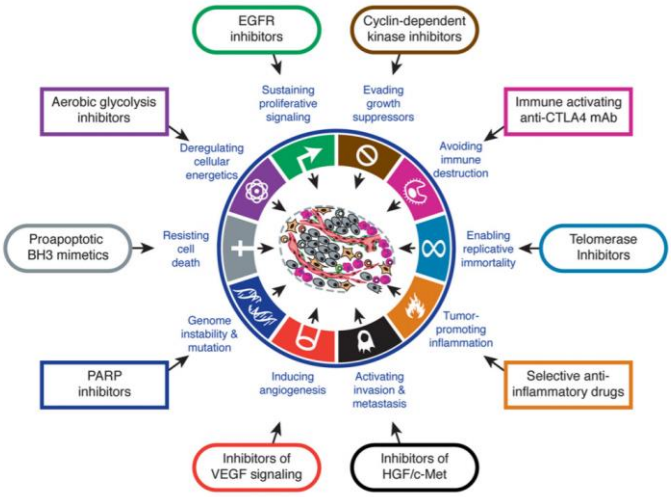


Figure 7. Therapeutic targeting of the hallmarks of cancer. Extracted with publisher`s permission from Hanahan & Weinberg [7]

Hallmark nr. 1: *Tumor promoting inflammation*. Contributing to multiple hallmarks including, growth factors, survival factors, extracellular factors, angiogenesis, invasion and metastasis.

Hallmark nr. 2: *Evading growth suppressors.* This feature describes the negative regulation of cell proliferation, partly through tumor suppressor genes (i.e. TGF-Beta). By inactivating these suppressor genes, tumor cells can continuously grow and divide.

Hallmark nr. 3: *Avoiding immune destruction* happens when large solid tumors circumvent detection and evade destruction or actively suppress an immune response.

Hallmark nr. 4: *Enabling replicative immortality* occurs, when i.e. the tumor cells use an enzyme called telomerase which adds telomeres onto the end of the chromosomes in DNA, and then the tumor cells can divide endlessly.

Hallmark nr. 5: *Activating invasion & metastasis* describes the ability of cancer cells spreading to distant organs, where they form new tumors, *called* metastases.

Hallmarks nr. 6: *Inducing Angiogenesis* describes the formation of new blood vessels in order to supply nutrients to the tumor.

Hallmark nr.7: *Genome instability & mutation* is happening when the tumor cell DNA changes continuously, thereby acquiring new malignant traits, a process that leads to tumor evolution.

Hallmark nr. 8: *Resisting programmed cell death*. Benign, but aging cells use to die in a controlled manner, called apoptosis. This process is tuned by a fine balance between pro-apoptotic and anti-apoptotic proteins and is disabled in cancer cells.

Hallmark nr. 9: *Deregulating cellular energetics* occurs when the tumor cells convert glucose into lactate in the absence of oxygen. This process is facilitated by the biosynthesis of macromolecules (amino acid precursors) for formation of daughter cells.

Hallmark nr. 10: *Sustaining proliferative signaling* by the production of own growth factors.

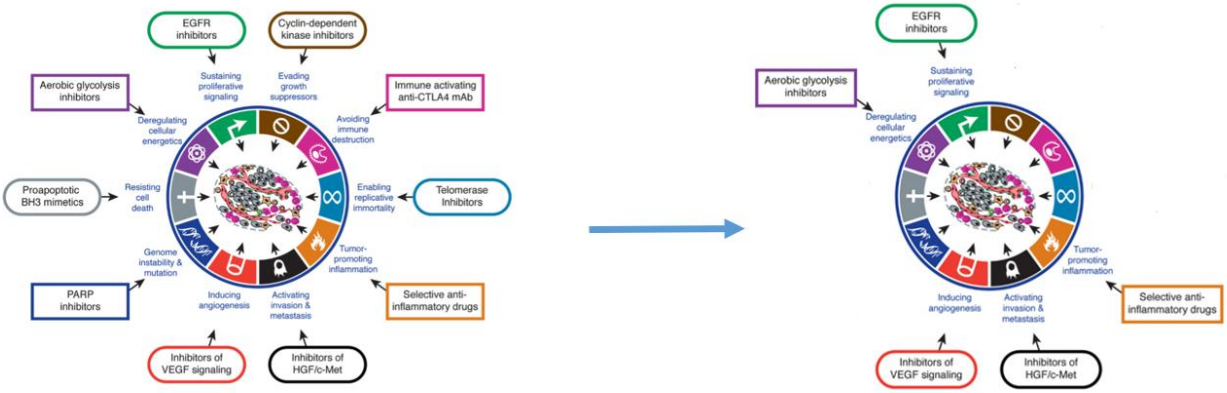


Figure 8. Therapeutic targeting of the hallmarks of cancer. Here we present the five hallmarks that are considered to be of particular relevance at the late stage of CRC with detrimental inflammation. Extracted with publisher`s permission from Hanahan & Weinberg [8].

To comprehensively understand malignant tumor behavior, all these hallmarks need to be considered. However, mathematically modulating all factors, which might be of importance in cancer appears to be beyond the scope of this thesis. For the late stage colorectal cancer with detrimental inflammation, five of these hallmarks are of particular relevance. These five hallmarks are highlighted in figure 8 and are taken into consideration while constructing a mathematical model with IL-6 at its core and CRP as a documented surrogate marker.

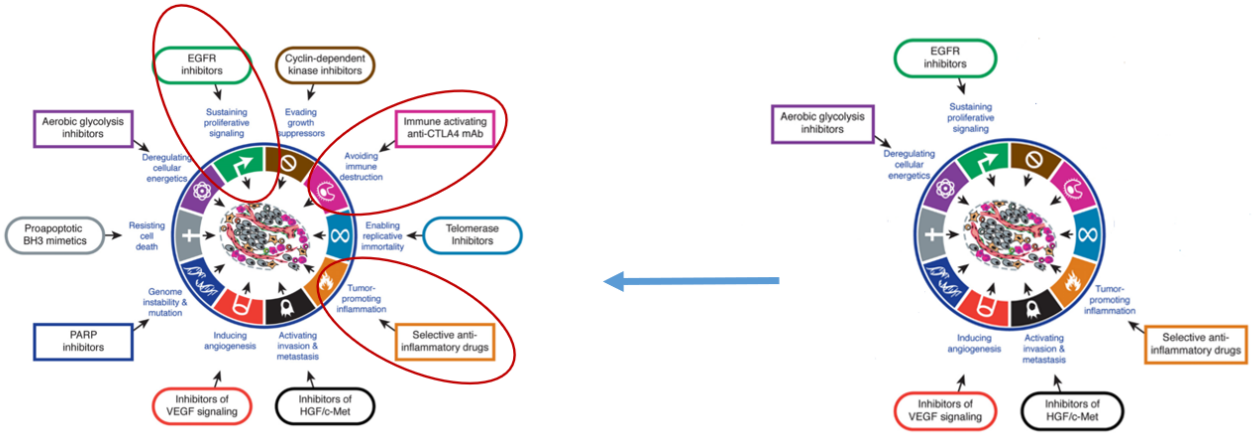


Figure 9. Therapeutic targeting of the hallmarks of cancer. Here we present the three highlighted hallmarks that will be discussed; avoiding immune destruction, tumor-promoting inflammation, and proliferation. Extracted with publisher`s permission from Hanahan & Weinberg [9].

At this early stage of research within mathematical modelling in metastatic colorectal cancer and within the scope of this thesis, we chose to further simplify the mathematical model. Since we want to modulate the situation of detrimental inflammation in late stage colorectal cancer, we choose to focus on the three presumably most relevant hallmarks of cancer: avoiding immune destruction, tumor -promoting inflammation and proliferation.

Colorectal cancer

Colorectal cancer (CRC) is the third leading cause of cancer worldwide [30] This type of cancer often starts as growth of colonic epithelium, leading to a polyp in the lumen of the colon. [31] The classical model of CRC oncogenesis is a polyp-to-cancer sequence progression. The established malignant tumor can progress from a localized (stage I and II) to advanced/late stage disease (stage III and IV). Stage I is defined as cancer growth through the mucosa and invasion of the muscular layer of the colon or rectum. It has not yet spread into the nearby tissue. At Stage II, the cancer has grown through the wall of the colon or rectum but has not spread to nearby tissue or lymph nodes. In stage III, the cancer has spread to local lymph nodes, but not to distant parts of the body. At stage IV, the cancer has spread to extended

parts of the body, such as the lungs or liver. In this thesis we are focusing on the later stages of CRC, when detrimental inflammation has been shown to be at its highest.

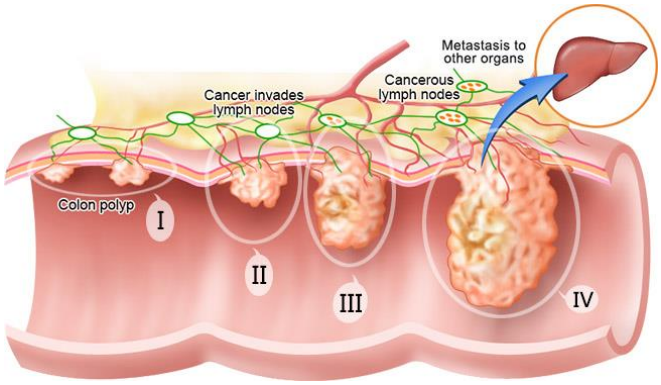


Figure 10. The stages of the colorectal cancer. Retrieved from: [10] on 13 of August 2019.

Immune system and its cells and cytokines

Innate and adaptive immune system

The immune system is often classified into the innate (first- line) and the adaptive (specialized second- line) response. Cells of the innate immune system employ proteins that attach molecules on the surface of invading microbes [32]. The innate response combines proteins and small molecules that are either present in biological fluids or discharge from cells as they are stimulated. Examples of this are cytokines, that determine the function of other cells, chemokines that captivate inflammatory leucocytes, and enzymes that commit to tissue inflammation.

The adaptive immune system manifest mainly for its target antigens, unlike the innate mechanisms of host defense. Antigen-specific receptors are expressed on the surface of T and B-lymphocytes. In the adaptive immune response, cells of monocytes/macrophages are important when phagocyting the antigens, converting them into forms that can activate T cell responses. The innate response of immune system represents the first line of host defense. The adaptive response becomes prominent after some days, through antigen- specific B and T cells that undergo expansion. The innate and adaptive immune systems co-operate closely but are often described as separate arms of host response.

The double-edged sword of the immune system in cancer

The concept of the *"double-edged sword of the immune system in cancer"* takes the longstanding notion of the existence of *"beneficial and detrimental immune responses in cancer"* into account. In a simplified view, inflammation can be divided into two alternative routes; beneficial (anti-tumor) cell orientation like M1, N1, Th1 cells, and their respectively cytokines, or detrimental (pro-tumor) cell orientation like M2, N2, and Th2 cells. Both types of orientation used their distinct portfolio of cytokines.

Early in the tumorigenesis, macrophages attracted to the TME can adapt a M1 phenotype and support anti-tumor immune responses. There will be a release of large amounts of IL-1, IL-6, IL-12, TNF-alpha, and INF-gamma leading to Th1 lymphocytes. As the tumor progresses, frequently more detrimental inflammation is observed. Here, the macrophages in the TME can change towards the tumor promoting M2 phenotype (TAMs). This type is induced by exposure to IL-4, IL-13, IL-10 and TGF-Beta, among others [33].

Tumor associated macrophages (TAMs) support metastasis, tumor growth and survival. They are attracted to the cytokines of myeloid suppressor cells (MDSC) from the bone marrow, corresponding cytokines (M-CSF/CSF) that can be produced by cancer cells, cancer associated fibroblast (CAFs), or other tumor-associated cells. An important pro-inflammatory factor that is upregulated by TAMs is IL-6. It protects the cell from cell death by inhibiting apoptosis. TAMs play an important role in tumor progression and metastasis and have a prominent role in detrimental inflammation. In aggressive cancers with poor prognosis, there is frequently an overexpression of key instigators of M2 differentiation, as well as high densities of TAMs in the tumor. It is also assumed that there will also be lower densities of M1 macrophages present in this stage of cancer.

Much research has been performed about the role of TAMs in cancer, but much less about the role of tumor associated neutrophils (TANs). TANs are also assumed to have tumor suppressing (N1) and tumor promoting (N2) phenotypes. Due to the cells being “short-lived” compared to other cells, the role of these innate immune cells in cancer is not well established. One argument for their importance is the role of N2 neutrophils promoted by the angiogenic chemokines in the TME. Neutrophils are primarily involved in fighting off microbial infections and healing wounds. TANs also play an important role in detrimental inflammation and are constantly recruiting to the TME. N2 neutrophils also secrete high numbers of cytokines that suppress immune responses like for instance IL-10. We can therefore assume that neutrophil N2 phenotype also add to tumor suppression, while N1 phenotype add to the tumor inhibition in detrimental inflammation of late stage cancer.

T lymphocytes oversee the adaptive immune response and are a class within the immune cells. T lymphocytes can be divided into Helper T cells (CD4+) and cytotoxic T cells (CD8+) [4]. T-regular cells (Tregs) are a particular type of CD4+ T helper cells, that mostly assert an immunosuppressive function leading to immune tolerance. In certain cancers, including CRC, T regs are found in high numbers in the microenvironment. They are believed to play an important role in the suppression of anti-tumor immune response. Cancer cells can have the ability to evolve in several ways avoiding- and suppressing immune response from the T cells. They can recruit immunosuppressive T regular cells, together with MDSCs, and change the ability to produce apoptosis for activated T cells, as well as developing different signaling processes that inhibit T cell movement.

Natural killer cells (NK) develop in the bone marrow [32] from bone marrow stroma cells stimulated by the cytokine IL-2. They represent a small fraction of lymphoid cells and peripheral blood cells in lymphoid tissues. NK cells have no antigen-specific receptors and are

important in host defense. Due to the complexity, NK cells are left out of the current model, but would need to be revisited when calibrating the current model in the future.

MDSCs are found elevated inn all patients with inflammation and tumor-directed suppression of immune response [33]. They are known to inhibit proliferation of CD4 + and CD8 + T cells, block activation of NK cells, and polarize T helper Th1 to Th2 (tumor promoting phenotype). From monocyte progenitors, IL-6 may have a role in the differentiation and proliferation of MDSCs. In the balance of M1/M2, MDCSs also plays a key role, and helps the proliferation of Tregs.

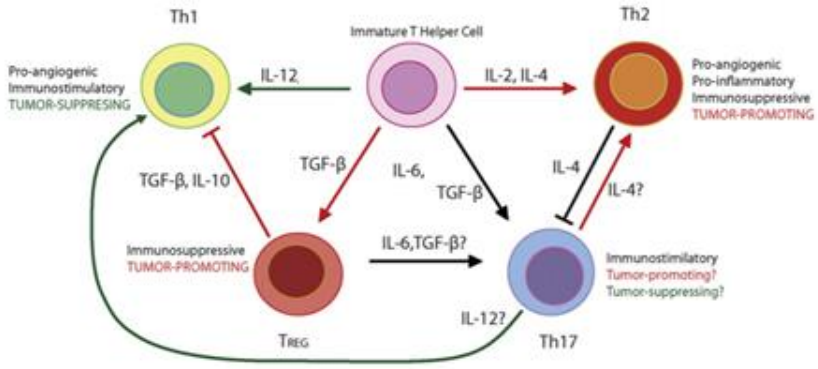


Figure 11. T helper cell differentiation. Modified to fit our situation. Extracted from open access in [11].

The fig 12 sketches T helper cell differentiation. Th1 and Th2 cells are both derived from CD4 + T helper cells. Exposed to IL-12, TNF-alpha and INF-alpha, the cells differentiate into Th1 cells. Or conversely, Th2 cells from differentiation exposed to IL-4 and IL-13. These cytokines also connect the cytotoxic capacity of M1 macrophages and M2. Beneficial inflammation is inducing Th1 cytokine response, meanwhile Th2 cells are more expressed by high levels of IL-4, IL-6, IL-10 and IL-13 associated with immune responses to detrimental inflammation. Both Th1 and Th2 produce profiles that are hostile to one another.

B cells are responsible for the release of antibodies associated with the immune response. Activated B cells have been shown to have an important role in autoimmune diseases. The role of B lymphocytes in cancer progression is unknown. In our setting B cells are most likely less important to bring into the model, but this may be refined and the future and is currently under investigation by PhD-student of Dr. C. Kersten and others.

Programmed death-ligand 1 (PD-L1) is expressed on the cancer cell membrane and other cells. It can induce T cell apoptosis. It is bound by its ligand programmed cell-death protein 1 (PD-1), that works as an immune checkpoint receptor. Binding to this receptor can inactivate T cell immune response in cancer [55]. Blocking this immune checkpoint receptor is employed therapeutically and often referred to as immunotherapy. By this the potential of a pre-existing

immune response against cancer is unleashed and can lead to long-lasting anti-tumor responses, leading sometimes even to cure. Discovery of this mechanism was awarded with the Nobel prize in medicine in 2018.

CD8 + T cells are a decisive factor in cancer inhibition. They kill tumor cells directly and recruit other immune cells [34]. CD8+ T cells are the most important cells to remove tumor cells through specifically recognizing tumor antigens. To limit tissue damage, il-10- which is an immunosuppressive cytokine is produced by effector CD8 + T cells that play a regulatory role.

Non-immune bystander cells

Non- immune bystander cells contain all the benign non-cancerous, non-immune cells in the TME. Their proportion varies and can make up to 90% i.e. in pancreatic cancer and Hodgkin's Lymphoma, but are here, at the late stage of colorectal cancer, assumed to comprise a minor part, i.e. - 10 % of the tumor proportion [educated guess and [15], [35]. These bystander cells are mainly comprised of cancer associated fibroblasts (CAFs), that produce soluble molecules like IL-6, TGF-beta or EGF. It can also consist of the extracellular matrix, several types of cells, like endothelia cells, macrophages, lymphocytes, and smooth muscle cells.

Late stage CRC with detrimental inflammation

Detrimental inflammation (possibly induced by autoimmune, bacterial, viral, parasite, toxic and/or intrinsic stimuli) can cause normal cells to develop mutations and resistance to apoptosis. "(C. Kersten, personal communication, May 2018.)" Both beneficial -and detrimental Inflammation can recruit leukocytes such as neutrophils and macrophages to the tumor microenvironment. Macrophages release cytokines and growth factors which may attack the tumor or may aid in nourishing the tumor.

Recent research confirms that detrimental inflammation in the colon may lead to oncogenesis. Inflammatory bowel diseases, like ulcerative colitis and Crohn 's disease, are in the group of inflammatory conditions. They fall into the class of autoimmune diseases, in which the body's own immune system attacks parts of the digestive system. Detrimental inflammation frequently results in the activation of both innate and adaptive immune systems with inherent chronic feedback loops that partly consist of pro-inflammatory cytokines and lead to the perpetuation of the inflammation.

During detrimental inflammation, endothelial cells trigger the recruitment of monocytes from the blood into the inflamed area. Upon entering the colon, monocytes differentiate into macrophages, which secrete tumor necrosis factor alpha (TNF-a), IL-6, CRP, and other soluble factors. CRC and subsets of fibroblast secrete proinflammatory cytokines, transforming growth factor beta (TGF-b) and IL-6. As stated above, *"Inflammation can be divided into two alternative routes; beneficial (anti-inflammatory) markers like M1, N1, Th1, and their respectively cytokines, detrimental (pro-inflammatory) markers like M2, N2, Th2, with cytokines".* The detrimental inflammation affects the tumor microenvironment, proliferation, survival, migration, angiogenesis, as well as suppressing beneficial immune responses.

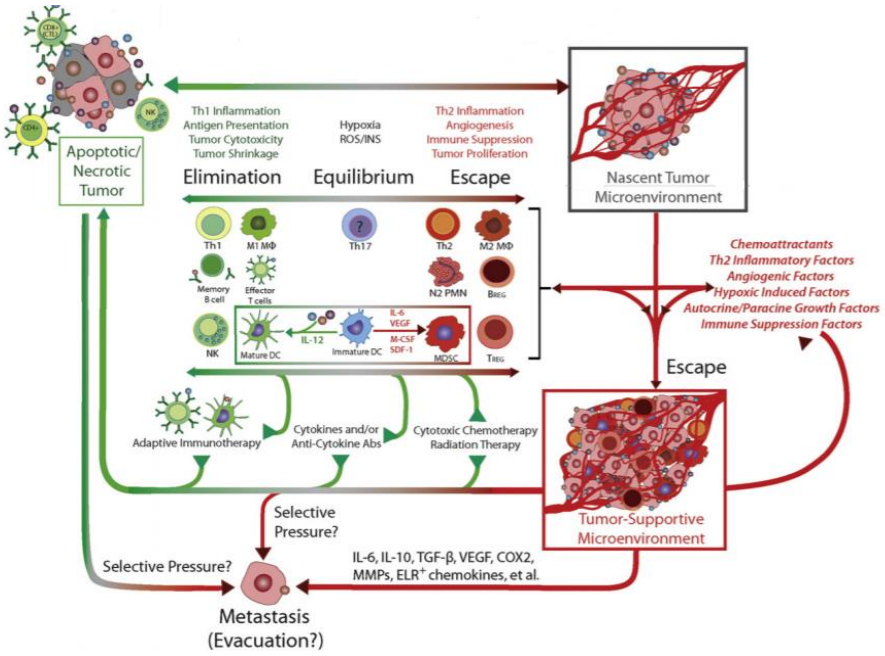


Figure 12. A model of immunoediting in tumor progression. Extracted from an open access paper [12].

C-reactive protein and IL-6

Interleukin-6 is an acute inflammatory cytokine, and there is also evidence that IL-6 may serve as a promoting factor in CRC at the detrimental inflammatory stage. There are reports on poor prognosis in CRC with high levels of IL-6 as seen in Thomsen et al. [26]. It exerts the effect on many cells and is the most important inducer of C-reactive protein (CRP). CRP is a sensitive marker of systemic inflammation produced by hepatocytes under transcriptional controlled by IL-6 in response to infection, advanced cancer and detrimental inflammatory conditions. IL-6 is the most important inducer of CRP and a positive association between IL-6 and CRP has been described in patients with late stage colorectal cancer [26]. Studies have shown higher level of CRP in preoperative blood from patients with CRC than healthy controls, see, for example, [26]. It has been suggested that high levels of CRP may result from the disease progression and can be used as an indicator of poor outcomes.

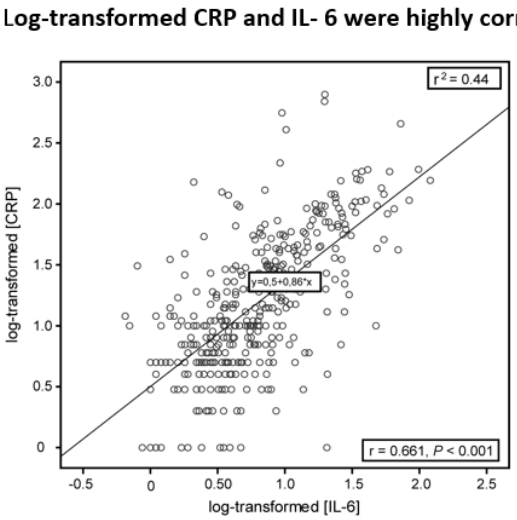


Figure 13. Correlation between IL-6 and CRP. Extracted from: [13].

To relate IL-6 and CRP, we use published clinical data that have demonstrated the correlation between CRP and IL-6 as seen in Thomsen et al [26]. We also use its corresponding data from the randomized phase III NORDIC-VII study, to see that this correlation data between the factor are based on patient study. It shows that r^2= 0,44, meaning that 44% of the total variation of CRP can be explained or deduced by IL-6. Most clinical data are available for CRP values, which are measured regularly – at very low costs and we therefore use in the current model CRP as an surrogate marker for IL-6.

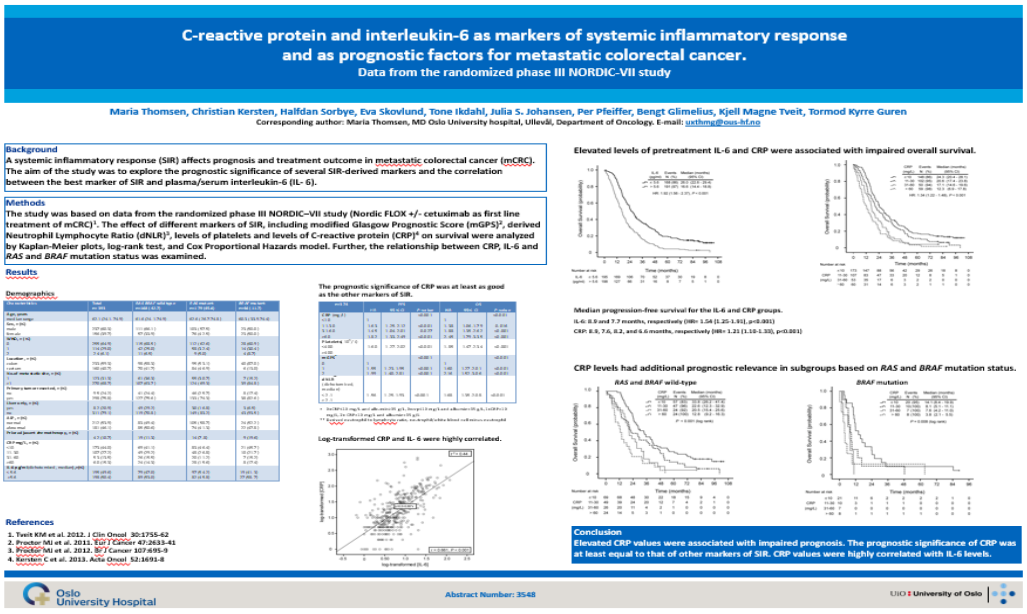


Figure 14. C-reactive protein and interleukin-6 as markers of systemic inflammatory response and as prognostic factors for metastatic colorectal cancer. Extracted from: [14]

Different types of mathematical models

Theoretical models have been used to explain experimental observations and generate new hypotheses regarding immunological phenomena [35]. For instance, theoretical models have been used to describe T cell receptor signaling- and activation, to model B and T cell turnover, and immune response during specific infections and their therapies. Coombs at el 2011 [36], proposed a mechanistic hypothesis to highlight the complex spatial and non-spatial receptor dynamics involved in T cell activation and receptor signaling. However, due the lack of data, these models cannot be confidently parametrized for now.

Mathematical models based on ordinary differential equations (ODEs) are most frequently exploited to date. They describe interactions among large numbers of cells/molecules, that can be calibrated against available data, and require a shorter simulation time. ODEs can be investigated using a variety of mathematical techniques, including stability and bifurcation results, existence of bounded solutions, uncertainty and sensitivity analysis, optimal control methods. The use of ODEs often leads to biologically realistic solutions. These types of models have been used, for example, to develop various immune simulator platforms.

Stochastic differential equations (SDEs) are derived from ODEs by considering random factors, for instance when the reaction rate between the components of the system is probabilistic. Computationally these models are more complex than ODEs. The SDEs are also more difficult to parametrize. They are less often used for mathematical modelling in medicine compared to ODEs.

Partial differential equations (PEDs) are also more complex than ODEs and are used more in the context of modelling spatial and age-related aspects of biomedical processes. These models cannot be calibrated very easily and require a longer simulation time compared to ODEs. PDEs can be investigated in more detail when powerful analytical techniques are available.

Hybrid models are the most complex models and combine different types of equations. When simulating a model, one must simulate different components separately (i.e., ODEs, PDEs, and using specific methods for each component). That's why they are computationally demanding. They are also the most difficult to calibrate, since each component must be calibrated separately using specific methods.

Rational models are based on observation, theory, and assumptions about the behavior of physical components of a system. Empirical models reflect the main features of available data. Applications of models are important in medicine and health care including prediction, planning, and evaluation of preventive and control measures, measurement of health level, assessment of risk of illness and patient diagnosis. Our conceptual model is combining mathematical techniques of rational modeling and empirical experience of a medical doctor and related data reported in medical literature.

Descriptive models are useful in community diagnosis and epidemiologic research, while predictive or simulating models may describe mechanisms of a phenomenon and predict the

future course of a condition. Odenbaugh [37] provides the list of pragmatic uses for a mathematical model in biology: "(1) Models are used to explore possibilities. (2) Models give scientists simplified means by which they can investigate more complex systems. (3) Models provide scientists with conceptual frameworks. (4) Models can be used to generate accurate predictions. (5) Models can be used to generate explanations".

Brady and Enderling [38] explained the six steps in the design of mathematical models of cancer. Our presentation of the material in this section follows what was suggested in this article.

1. Identify a putative biomarker

Mathematical modelling simulates the dynamics and the mechanisms of tumor. Biomarkers of tumor need to be identified both for simulating cancer and for clinical therapy. Biomarkers could be obtained by calculating the tumor volume from medical images or number of cancer cells in a patient's blood for liquid tumors. One can use surrogate markers such as prostate-specific antigen in liquid biopsy or even measurements from experiments on mice for solid cancers. Mathematical modelling simulates the dynamics of tumor and their underlying mechanisms, while statistical models may correlate random variables (pre -treatments tumor size with treatment outcome).

In our model we selected IL-6/CRP as an important biomarker for several reasons. First it is easy and inexpensive to obtain CRP values for patients on a regular basis. Using CRP as a marker of SITR and surrogate marker of IL-6 levels, the physician can evaluate the efficiency of the treatment and adjust it accordingly. Second, for our future studies we selected to use anti-IL-6 as a factor in the anti-inflammatory treatment. There is ongoing research on the use of anti-inflammatory drugs based on the anti-IL-6 antibody -Tocilizumab, which is approved for the treatment of rheumatoid arthritis.

2. Development of a mechanistic model

Dynamic models such as mechanistic differential equations, cellular automata or agent-based models describe the change of putative biomarkers over time. Ordinary differential equations (ODEs) are often enough if only temporal data are available. If temporal dynamics is insufficient to explain the observed biomarkers dynamics, partial differential equations (PEDs) should be used if spatiotemporal data are available. Number of model's variables and parameters should be determined with outmost care and limited based on the available data. If longitudinal data are limited (often in clinical studies), non-mechanistic or statistical models may be considered. Such models, however, may have limited ability to predict novel treatment protocols.

Our conceptual mechanistic model has in the beginning many variables and parameters covering the most general situation. It describes the combination of three hallmarks of cancer which were not modeled together until now. After the initial analysis the number of variables is slightly reduced.

3. Calibrate model with existing data

While a wide range of machine learning and analytical methods are already established to identify simpler models, major advancements in the field are still needed to calibrate mathematical models more precisely. It is important to remember that a mathematical model has to match and anticipate data from known analysis in order to simulate novel treatment protocols. Ideally, one should acquire model parameters from data whose validity has been confirmed by clinical experiments and/or research. When handling data in an attempt to resolve patient-specific treatment protocols, it is crucial to identify which parameters influence the dynamics of the disease for individuals and which parameters can be homogeneous across a patient population. It is important to keep at least a minimal number of patient-specific parameters in order to achieve a personalized treatment protocol-prediction.

To our knowledge, the model presented here, provides the most complete description of the colorectal cancer in the final stage in the presence of detrimental inflammation. It contains many parameters which characterize the interaction between the cells. We use cytokines to model the cell crosstalk and this requires large sets of parameters many of which are not yet reported in the literature and should be calibrated using experimental data and educated assumptions.

4. Validate model with untrained data

When calibrated with data, a mathematical model must be validated against independent data. Brady and Enderling suggest that a model's ability to fit untrained data should be evaluated via methods such as R^2 analysis, and the parameters should be held constant. Data should be randomly stratified into model calibration and validation if independent training and testing data sets are not available. To learn and test parameters, leaving-one-out studies may be used for smaller data sets. When applying this an internal control is given and prevent overfitting to trained data.

For our model we will be trying to obtain the joint contribution of cytokines to the processes governed by differential equations. The sources for cytokine productions may be different and produced cytokines can influence different cells. Since it is unlikely, we will be able to get information about each of these contributions we will attempt to evaluate the cumulative effect of a given cytokine.

5. Evaluate predictive performance for known treatment

A model is not predictive even though it has the ability to fit data. The model must be able to predict responses to treatment protocols for available data, before it is used to predict novel treatments. Brady and Enderling mentioned, random stratification of a single data set into a model training, validation, and prediction performance evaluation may be required and acceptable, but an independent prediction evaluation is the standard. The purpose of the model is to decide, how to evaluate model prediction performance. If a model aims to predict binary events (survival, resistance, responses) area under the operating characteristic curve may be applied. A conventional notion of acceptable cut-offs for predictive performance is yet to be established in the field of mathematical oncology. These models may be used to

stimulate and predict untested treatments, if and only if the predictive performance for known treatment responses and outcomes is sufficiently high.

For our model, it is not only important to relate the information about CRP/IL-6 levels to patient`s conditions but also to predict possible effect of anti-inflammatory drug treatment. Our goal would be to make this correlation between the CRP values and model`s predictions efficient and easily manageable with the clinical data from real patients. In [41] Friedman et al. mention the use of the model to explore efficacy of a drug; anti-IL-6 (tocilizumab) which is also useful in our case. This drug has already been shown good results of systemic juvenile arteritis Kersten`s article [4]. In future studies we aim to look in more detail at the way they implemented the treatment by anti-IL-6.

6. Simulate and predict untested treatments

Brady and Enderling assert that it is important to limit the exploration space to treatments that can be derived from calibrated and validated models, when used to simulate alternative treatments. Treatments that the model was not trained to predict should not be stimulated. Predictions into untested doses should be met with caution and limited confidence, as training data contain only single-dose levels and the biological dose responses curves are unknown. It is straightforward to simulate arbitrary treatments protocols, biologically important bounds (such as drug agent half-life or toxicity) and clinical feasibility (like radiation therapies can rarely be delivered more than twice a day or on the weekend due to logistical constrains) must be honored. Once can only draw conclusions about the evaluated regimes when comparing predicted responses to innovate treatments with data. Rigorous optimal control should be approached, and an exhaustive analysis should be provided to claim, “optimal therapy”.

When we model the effect of an anti-inflammatory drug on the patient`s condition we will be mathematically following the solution curve for the cancer cells density until the inflection point where the rate growth becomes steeper and additional drugs should be admitted.

This thesis brings us to the stage 2. We have focused our attention on modeling CRC at detrimental inflammation stage, using IL-6/CRP as one of the important characteristics of the patient`s conditions. The next step in our work is to calibrate the model using the existing data, which, due to complexity of a model and lack of experimental data, is a difficult task.

Our model

In this thesis, we use ordinary differential equations (ODEs) for modeling CRC. We build our model using ideas exploited by Friedman and collaborators to model pancreatic cancer [40] and chronic pancreatitis [41]. Based on the clinical data from oncologist (C. Kersten) indicating certain similarity between CRC and pancreatic cancer with respect to detrimental inflammation, we modify Friedman`s models including new factors particularly important for CRC. Our model incorporates three important hallmarks of cancer [29] proliferation, avoiding immune destruction and tumor-promoting inflammation.

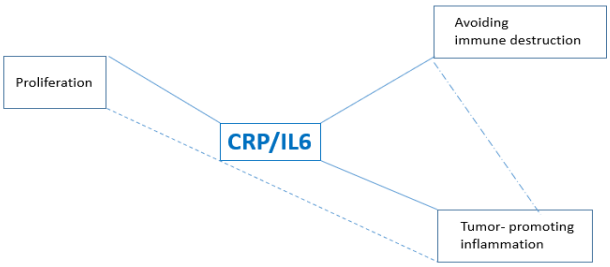


Figure 15. Three important hallmarks, by Dr. C. Kersten, 2019 (Personal communication, May 2019)

Our model features interaction between the most important cells like cancer cells, macrophages, neutrophils, T helper cells, non-immune bystander cells/CAFs, and cytokines like IL-6/CRP, TGF-beta, IL-10, TNF-alpha, EGF, PD-L1 serve as the means of the cells crosstalk. Our model is built to reflect the interaction between the main factors of CRC described in the diagram, see Figure 6.

Based on the research on IL-6 as an important biomarker in CRC treatment reported recently in the literature of Dr. C. Kersten`s article [4] we use IL-6 as an important variable to describe the interactions between cells at the late stage of CRC. We also want to use the model to explore the impact of treatment with anti-IL-6 drugs in CRC in the future. The model is intended to be used to find the survival rate of patients with CRC.

Mathematical modelling of the late stage of the colorectal cancer with detrimental inflammation

Tumor proliferation is increased or decreased by a myriad of cancer cell intrinsic and extrinsic factors. We are taking cancer cell extinctic factors of the microenvironment into account, that is, augmenting cancer cell proliferation (red group of parameters) and cancer cell removal (green group of parameters), as shown in figure 6. We translate the schematic simplification of tumor growth into a mathematical model as described in figure 6. We have two groups of cytokines and cells which will either promote tumor growth or contribute to the elimination of the tumor.

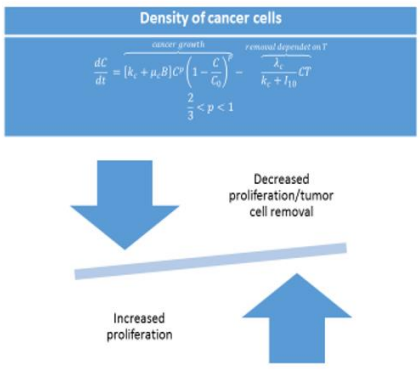


Figure 16. Density of cancer cells, by Dr. C. Kersten, 2019 (Personal communication, May 2019)

Assumptions

In this thesis we use many assumptions. Due to the lack of clinical data about the late stage of colorectal cancer, most of our parameters will be either unknown at the moment or taken from other articles. Calibration of the parameters is the next step for the future work. We also acknowledge that the model we designed may have missing parts because we cannot account for all factors that influence the tumour growth and restrict our selection following the guidance from the medical doctor who leads clinical research. Therefore, we established an additional promoter and inhibitor parts which include possible new factors which will be considered in the later work.

Clinical

The model applies to otherwise healthy and fit human beings without comorbidities, receiving optimal medical treatment under full compliance. Our assumption based on Dr. Kersten`s clinical experience is that a healthy person that tolerates optimal therapy has a median survival of 25 months, at diagnosis of stage IV colorectal cancer. We will use this information in developing a model of two sets of parameters (tumor promoting - and tumor elimination factors) that increase or decrease this survival rate. Our optimal goal is to estimate life expectancy of the patients based on this model.

Biomedical grounding

In theory and probably also in reality, one could envision all types of feedback loops between all cells and factors involved. The model presented here starts with the assumption that it includes most important interactions. Further feedback-loops could be envisioned and may be incorporated in the future, if emerging evidence suggests calibrating the model in this regard. Some factors and cells are acknowledged as important in the context of detrimental inflammation in late stage colorectal cancer but are at this stage of development and in the scope of this thesis not incorporated in the model. Further evidence in the future and/or calibrating work by the author or others, may hopefully lead to a refined calibration of this model and include these or other factors: NK cells, Th17 cells, VEGF, Toll-like receptors, chemokines and others. The interactions between TH1/TH2, N1/2 and M1/2 are not binary but are characterized by plasticity, which allows transition from one group of cells to another under the influence of cytokines and other factors. It is gaining increasing appreciation, but the details and quantities of pro-tumoral inflammation are hitherto insufficiently characterized. This applies especially for the neutrophilic granulocytes. The model presented here aims at understanding of this concept but acknowledges that the details of the N1/2 and M1/2 concepts must be calibrated in the future with emerging data. Intracellular cancer cell signaling and/or (epi)genetic mutations are not included in this model.

Theoretical foundations for the growth modeling

In this chapter we will present some general ideas for modelling cancer cells. We will recall some techniques that are generally used in the courses of differential equations, see for example “A short course in ordinary differential equations” by Kong [42] and [43]. An

important class of first order differential equations involves the independent variable that doesn't appear explicitly in the right hand-side and has the form:

$$\frac{dX_n}{dt} = f(X_n).$$

This equation can be discussed in the context of growth or decline of the population of a given species, that is an important issue in fields of medicine, ecology and economics. The important thing is to analyze the stability of the solutions. This we will discuss later in this section. Let X_n be the population of the given species at time t. The simplest hypothesis concerning the variating of population is that the rate of change is proportional to the current value of X_n; that is,

$$\frac{dX_n}{dt} = rX_n,$$

where the constant to proportionality r is called the rate of growth or decline, depending on whether its positive or negative. If we let $r > 0$, the population is growing. Solving the equation with the initial condition

$$X_n(0) = X_{n_0},$$

We obtain

$$X_n = X_{n_0}e^{rt}.$$

This mathematical model with $r > 0$ predicts that the population will grow exponentially for all time for several values of X_{n_0}. This exponential growth has been observed to be reasonably accurate for many populations, for limited periods of time for example growth of bacteria. However, it is clear that such ideal conditions cannot continue indefinitely, by limitations of space, food supply, or other resources that will reduce the growth rate and bring an end to uninhibited exponential growth. This type of cell growths is observed in the initial stages of cancer but in our case (final stage) this type of growth cannot be used. To take account for the fact that the growth rate depends on the population, we replaced the previous constant r by a function $h(X_n)$ and thereby obtained the modified equation

$$\frac{dX_n}{dt} = h(X_n)X_n.$$

We now want to choose $h(X_n)$ so that when y is small, $h(X_n)$ decreases as X_n grows larger, and $h(X_n) < 0$ when X_n is sufficiently large. The simplest function that has these properties is $h(X_n) = r - aX_n$, where a is also a positive constant. Using this function in the one above, we obtain

$$\frac{dX_n}{dt} = (r - aX_n)X_n.$$

This equation is known as Verhulst equation or the logistic equation. It is often convenient to write in the equivalent form:

$$\frac{dX_n}{dt} = r\left(1 - \frac{X_n}{X_{0n}}\right)X_n,$$

Where $X_{0n} = r/a$. The constant r is called the intrinsic growth rate – that is, the growth rate in the absence of any limiting factors. For the constant solutions of this equation the derivative $\frac{dX_n}{dt} = 0$ for all t, so any constant solution must satisfy the algebraic equation:

$$r\left(1 - \frac{X_n}{K_{0n}}\right)X_n = 0.$$

Thus, the constant solutions are $X_n = 0$ & $X_n = X_{0n}$. These solutions are called the equilibrium solutions of the original equation because they correspond to no change or variation in the value of y as t increases. In the same way, any equilibrium solutions of more general equations can be found by locating the roots of $f(X_n) = 0$. The zeros of $f(X_n)$ are also called critical or equilibrium points.

In the figure, the growth rate, dX_n/dt, is plotted with the cell growth density X_n. This is called a phase-plot and is often due to population dynamics. If $0 < X_n < X_{0n}$, then $dX_n/dt > 0$ and thus, the solution grows (point in the graph moves to the right). If $X_n < 0$ or $X_n > X_{0n}$ ($X_n < 0$ no biological sense), the cell density declines (point in the graph moves to the left). The arrows show that the equilibrium $X_n = 0$ is unstable, wheras the equilibrium $X_n = X_{0n}$ is stable. From the biological point of view, this means that after small deviation of growth of cancer cells from $X_n = 0$, the growth never returns to the stable equilibrium. Instead, growth of cancer cells increases until they reach the stable equilibrium $X_n = X_{0n}$. After any deviation from $X_n = X_{0n}$ the growth returns to this stable equilibrium.

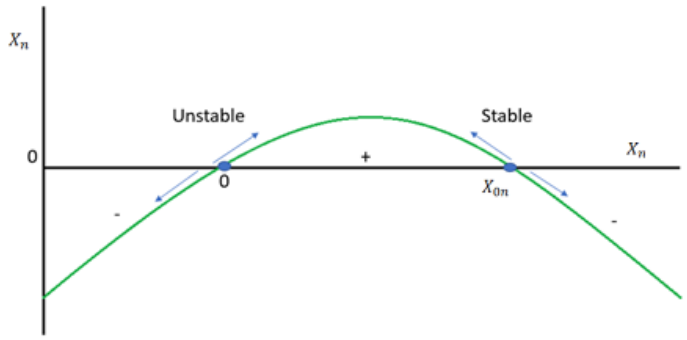


Figure 17. A phase plot of equilibrium points

The difference between stable and unstable equilibria is in the slope of the line on the phase plot near the equilibrium point. Stable equilibria are characterized by a positive slope (positive feedback). The fundamental existence and uniqueness theorem states that only one solution can pass through a given point in the ty-plane. Thus, a solution that starts in the interval $0 < < X_{0n}$ remains in the interval for all time, and similarly for a solution that starts in the interval $X_{0n} < X_n < \infty$. K is the upper bound that is approached, but not exceeded, by growing populations starting below this value. Thus, it is natural to refer to K as the saturation level or carrying capacity for giving species. In many cases especially in non-linear cases we are not able to find the exacts solutions to the differential equations, therefor the stability analysis is useful to determine the asymptotic behavior of such solutions. Other methods can be used to estimate such behavior.

A logistic differential equation is used to study the growth of tumors [44]. Denoting with $C(t)$ the size of the tumor at time t, is governed by:

$$X'_n(t) = k\left(1 - \frac{X_n}{X_{0n}}\right)X_n,$$

Where $k\left(1 - \frac{X_n}{X_{0n}}\right)$ is the proliferation rate of the tumor.

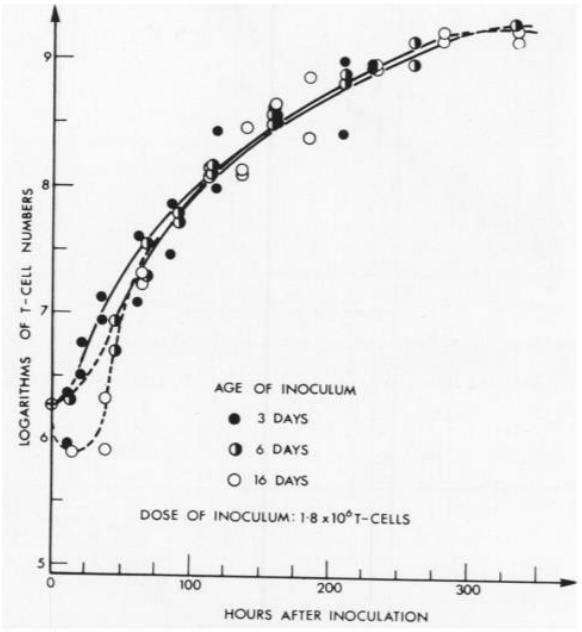


Figure 18. Plot of the cancer growth from one of the first articles published of growth modeling in 1964. Extracted from: [18].

Generalized logistic curve or Richards differential equation (RDE) is originally developed for growth modelling in the early stages of 1960s [45] originally developed for growth modelling, as an extension for the logistic functions, allowing us for more flexible S-shaped curves.

$$X'_n(t) = k\left(1 - \left(\frac{X_n}{X_{0n}}\right)^v\right)X_n\,,$$

where $k > 0$, K is the carrying capacity, and $v > 0$ affects near which asymptote maximum growth occurs. $v = 1$ gives us the classical logistic differential equation as above.

The first article using this type of model in tumor growth was [46] introducing the Gompertz model, a model used to describe tumor dynamics as a type of mathematical model for a time series, where the growth is slowest at the end of a time period.

$$X'_n(t) = X_n(a - b\,(\ln C)),$$

with the solution

$$X_n(t) = e^{(\ln C_0 - \frac{a}{b})e^{-bt} + \frac{a}{b}}\,.$$

The function $C(t)$ has the plateau cell number which is reached at large values of b and the parameter a is related to the initial tumor growth rate.

There is no common point of view on how the tumor growth should be modeled because the data vary for different types of cancer and different stages of the disease. Guiot et al.2006 [47] describe the "universal law" for the growth of many organisms under normal conditions. The law states the total body mass m grows with rate

$$\frac{dm}{dt} = am^p\left(1 - \left(\frac{m}{M_0}\right)^{1-p}\right),$$

where the exponent p ranges from 2/3 to 1 and M_0 is the maximum size of the organism. This growth law combines both power and exponential growth and is used by Friedman et al. [40] to discuss the cancer tissue growth. They use the value $p = 3/4$ and report that the choice of slightly different values of p does not significantly influence the conclusion.

Basic ideas of biochemical modeling

In this next section we want to discuss some mathematical principles useful for modeling of cytokines by using the basic ideas of mathematical modeling in biochemistry. We know that in human body cytokines are changing on the short time- scale comparing to the cells for example cancer cells or bystander cells. On the short time- scale, the conversion of cytokines comes rapidly to equilibrium and we can make use of the fact that the equilibrium is maintained to relate the concentrations of cytokines. On the longer time- scale, the equilibrated pool of cytokines slowly changes (the rapid equilibrium assumption). By assuming that the change of cytokines is always at the equilibrium state, we use the equilibrium to

simplify the dynamic description of the system. By quasi-steady state assumption, we separate the time- scales. Following this idea, we replace the original differential equation- based description with an algebraic description which means that we equate the right hand- sides of the differential equations describing the dynamics of cytokines to zero. The change of IL-6 is noticeable comparing to the change of other cytokines in this stage of CRC [Dr. Kersten`s educated guess experience].

In order to write the system of differential equations describing the cytokine dynamics in the human body we used the simple principle of modeling the biochemical reaction. Biochemical network are open systems that change material with the outside environment and reach a 'steady state that involves a steady flow through the network. This state is called a dynamic equilibrium. This diagram shows an open network with a group of molecules that can undergo the following chemical reactions

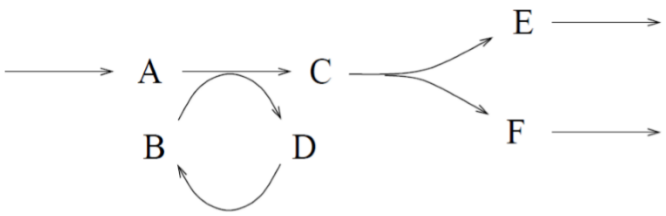


Figure 19. Schematic description of a biochemical reaction.

$$\rightarrow A + B \rightarrow C + D,$$

$$D \rightarrow B,$$

$$C \rightarrow E + F,$$

where B and D form a feedback loop. We consider now an open network involving two reactions, production and decay:

$$\underset{\rightarrow}{k_0}\ A\ \underset{\rightarrow}{k_1}.$$

Letting $a(t)$ denote the concentration of A at time t, the reaction dynamics are described by:

$$rate\ of\ change\ of\ [A] = rate\ of\ production\ [A] - (rate\ of\ decay) of\ [A]$$

which leads to the model

$$\frac{d}{dt}\big(x_n(t)\big) = k_0 - k_1 x_n(t)$$

To find the steady- state (rate of production = rate of decay), we solve the algebraic equation

$$k_0 - k_1 x_n^{ss} = 0, \text{ or}$$

$$x_n^{ss} = \frac{k_0}{k_1}.$$

The solution of the DE is:

$$x_n(t) = De^{-k_1 t} + \frac{k_0}{k_1}.$$

The constant D depends on the initial concentration, it can be shown to equal

$$D = A_0 - \frac{k_0}{k_1}.$$

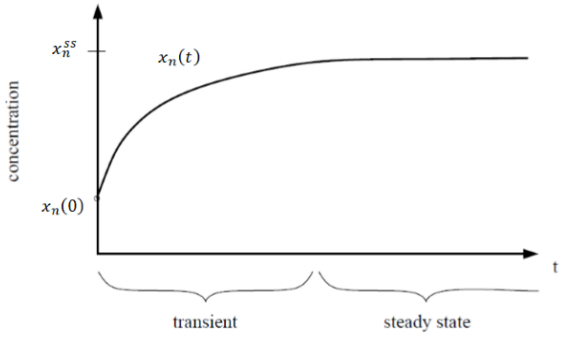


Figure 20. Transient and steady-state behavior. The transient occurs while the concentration relaxes to its steady-state value.

We also consider a closed system consisting of a single reversable reaction:

$$A \underset{\rightarrow}{\overset{k_+}{}} B, \quad B \underset{\rightarrow}{\overset{k_-}{}} A,$$

or, more concisely,

$$A \underset{k_-}{\overset{k_+}{\leftrightarrow}} B.$$

We have the system:

$$\frac{d}{dt}\big(x_n(t)\big) = k_- b(t) - k_+ x_n(t),$$

$$\frac{d}{dt}\big(b(t)\big) = k_+ x_n(t) - k_- b(t).$$

This system of linear DEs and such systems can be easily solved.

The exponential relaxation exhibited by the examples represented before is characteristic of linear system. Nonlinear models exhibit a wide range of behaviors, and do not typically admit explicit solutions such as the concentrations formulas above. Differential equation models of biochemical and genetic systems are invariably nonlinear. Often it is necessary to resort to numerical simulations to investigate the behavior of these systems. Numerical simulations do not generate continuous curves. They produce approximate values of the solution at a specified collection of time-points (experimental time-series).

When constructing a dynamic model, one must decide which time- scale to address. This choice is typically dictated by the time scale of the relevant reactions and process. For the simple examples considered above, the time scale (time constant) could be deducted from the reaction system. For nonlinear processes, characteristic time scales are not so neatly defined. Biological process take place over a wide range of times scales. Consider, for example a genetic network that generate a circadian rhythm. A model of this network will describe oscillator behavior with a period of roughly 24 hours, and so will incorporate process acting on the time- scale of hours. In order to describe the cytokine dynamics, we use the linear equations and so we can simplify the system by reducing differential equations to algebraic equations which allows us to investigate the system numerically.

Deterministic models of cancer

Our work on the model was very much influenced by important contributions to cancer research made by professor Avner Friedman and his collaborators. His work has always been an inspiration when we were building our model. Two articles that were particularly useful for this thesis deal with pancreatic cancer [40] and chronic pancreatitis [41], and Professor Friedman primarily used partial differential equations for mathematical modeling of cancer based on his extensive research experience with such equations. Using partial differential equations accounts for spatial dynamics which is important for almost all types of cancer. In [48] partial differential equations are used with the term $\nabla(uX)$ describing direct movement of cells with a velocity $u = (r, t)$, where r is the distance and t is time.

For our model where the cancer is already at an advanced stage, we concentrate our attention on the interactions between the cells and thus we are primarily interested in temporal dynamics described by ordinary differential equations.

Designing our equations, we analyzed different ways of describing the terms for proliferation and inhibition. Friedman et al. [48] explained that “enhanced proliferation of cancer cells is associated with activation of certain intracellular pathway. Associating this activation with a protein X, we take the enhancement rate to be proportional to

$$\frac{\mathrm{X}}{\mathrm{K_X} + \mathrm{X}}$$

to reflect the limited recycling time of active X, where $\mathrm{K_X}$ ii the half-saturation value of X, taken as the steady state concentration of X in healthy individuals. Inhibition by a protein X will be represented by the factor

$$\frac{1}{1+\mathrm{X/K}}$$

We also assume linear natural death/degradation for all proteins and cells". They also "assume the logistic growth of species of cells X is limited by the presence of another competing (for space) species Y, its growth is then proportional to

$$\mathrm{X}\left(1-\frac{\mathrm{X}+\varepsilon\mathrm{Y}}{\mathrm{C_M}}\right)$$

for some $0 < \varepsilon \leq 1$".

In this article they also have estimates for many parameters which are important for testing models. Unfortunately, these parameters do not necessarily fit our model because they reflect the influence of intrinsic factors of colorectal cancer. The way they are obtaining their clinical data is interesting and can be used in our future work. The following example is taken from Chou and Friedman`s book on mathematical biology [49]. Equations (*):

$$\frac{dC}{dt} = \lambda_C C\left(1-\frac{C}{C_0}\right) - \mu_C T C,$$

$$\frac{dM_1}{dt} = k_1 - \gamma M_1 \frac{C}{K_1+C} - \mu M_1,$$

$$\frac{dM_2}{dt} = \gamma M_1 \frac{C}{K_1+C} - \mu M_2,$$

$$\frac{dT}{dt} = K_T \frac{M_1}{K_2+M_2} - \mu_T T.$$

This simplified model of cancer immune interactions helps us to illustrate our case. We present the MATLAB code for numerical simulations of the system (*) in Figure 21. We plotted solutions of the system (*) with the logistic growth for cancer cells in Figure 22. In Figure 23 we model a modified system (*) with a logistic growth replaced with the power growth.

$$C^p\left(1-\left(\frac{C}{C_0}\right)^{1-p}\right).$$

We observe a noticeable difference in the behavior of cancer cells and T cells with monotone growth for both without bumps as in the case of logistic growth. We believe that without cancer treatment this behavior is more realistic for the model of detrimental inflammation in the late stage of colorectal cancer and we use power function in equation (1). In Figure 24, we plotted the graphs for the density of the cancer cells with the model using power function for different values of $p \in \left\{\frac{2}{3}, \frac{3}{4}-\frac{1}{50}, \frac{3}{4}, \frac{3}{4}+\frac{1}{50}, 1\right\}$. As noted by Friedman et al. [40], the

difference in the behavior of the values of p close to $3/4$ the behavior does not differ much, and we plan to use $3/4$ in our future work.

```
function dz = fun_cancer_immune(t,z)
global lambda_c C_0 mu_c k_1 gamma K_1 K_2 k_T mu mu_T
dz = zeros(4,1);
C = z(1);
M_1 = z(2);
M_2 = z(3);
T = z(4);
dz(1) = lambda_c*C*(1-C/C_0)-mu_c*T*C;
dz(2) = k_1-gamma*M_1*C/(K_1+C)-mu*M_1;
dz(3) = gamma*M_1*C/(K_1+C)-mu*M_2;
dz(4) = k_T*M_1/(K_2+M_2)-mu_T*T;
end
```

```
>> % Example of computing a mathematical model in biology in Matlab
% It will generate 4 curves corresponding to equations (*)
clear all
close all
global lambda_c C_0 mu_c k_1 gamma K_1 K_2 k_T mu mu_T p
%% parameters
lambda_c=10^(-2);
mu_c=10^(-5);
C_0=10^6;
mu=0.3;
k_1=3000;
gamma=200;
mu_T = 0.2;
k_T=3300;
K_1=0.05*C_0;
K_2=10^5;
%% initial conditions
C=10^2;
M_1=5*10^4;
M_2=0;
T=0;
z_ini=[C M_1 M_2 T];
>> tspan=[0,60];
>> %% ODE solver
>> [t,z]=ode15s('fun_cancer_immune',tspan,z_ini);
>> %% Plot
>> tvec={'C','M','M_2','T'};
>> for i=1:4
subplot(2,2,i)
plot(t,z(:,i)), hold on
xlabel('t'), ylabel(tvec(i))
end
```

Figure 21. Example of the code in MATLAB used for numerical simulation of a mathematical model of cancer described by equations (*)

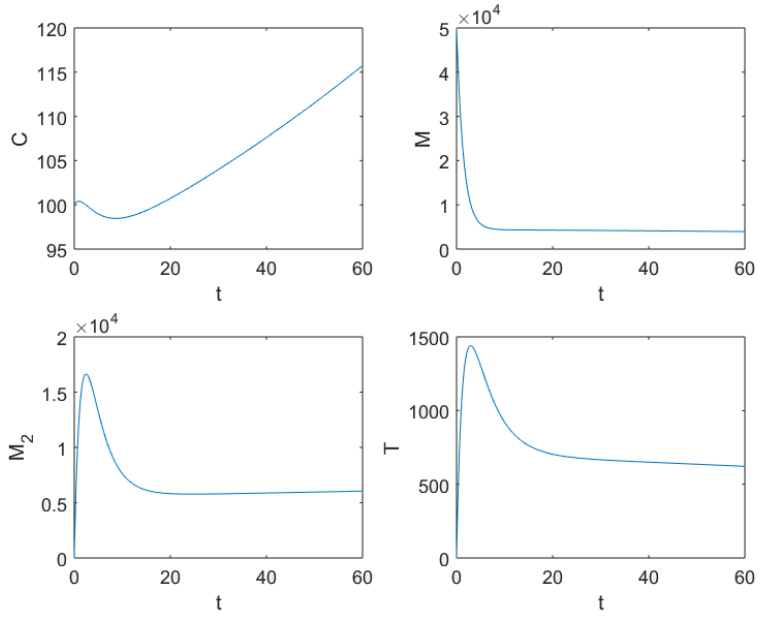


Figure 22. Four MATLAB plots resulting from numerical computation for equations (*)

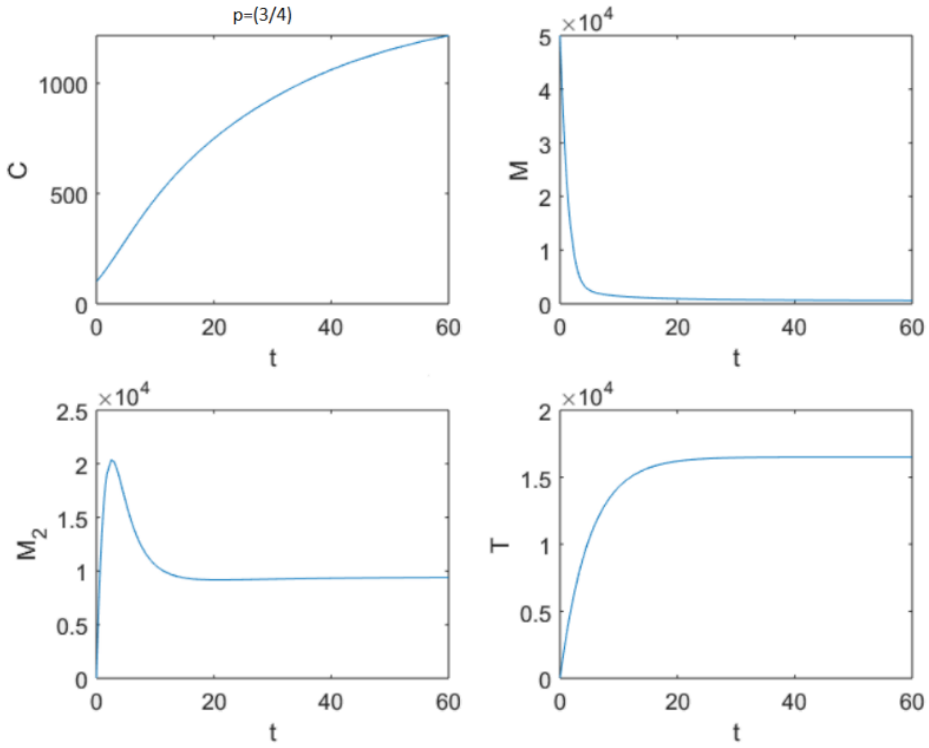


Figure 23. Four MATLAB plots resulting from numerical computation for modified equations (*) for p=3/4

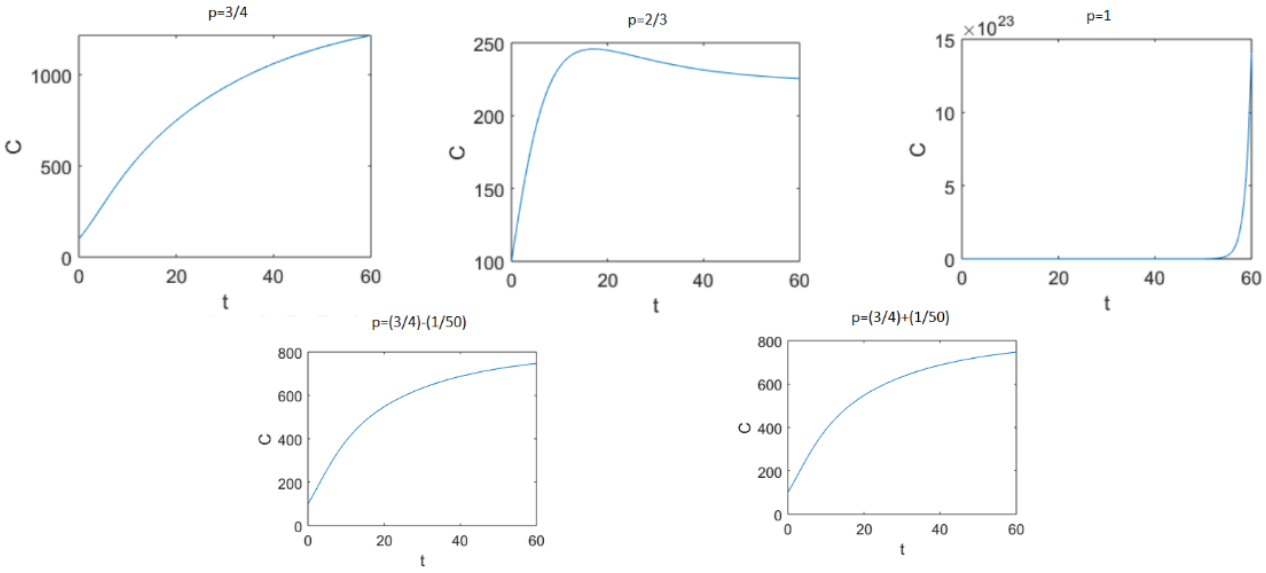


Figure 24. Five MATLAB plots of the density of cancer cells for different p values.

There are several ways of describing the cancer cell growth in the literature. Some authors suggest using the power law growth which has shown to be a good fit for breast cancer in which case the tumor growth is modeled by the equation

$$\frac{dm}{dt} = am^p,$$

describing well tumors of clinical size. Power law [50] does not model well the growth of very small tumors and it has been shown that large slow growing tumors are better described by exponential growth, Skipper [51]:

$$\frac{dm}{dt} = bm\,.$$

The equation for the growth of cancer cells used by Friedman et al. [40] has two main components corresponding to proliferation and removal:

$$\frac{dC}{dt} = \overbrace{[k_c + \mu_c P]C^p\left(1 - \left(\frac{C}{C_0}\right)^{1-p}\right)}^{cancer\ growth} - \overbrace{\frac{\lambda_c}{k_c + I_{10}}CT}^{removal\ dependet\ on\ T}\quad,$$

where the cancer growth rate is the sum of basal growth rate k_c, and the enhancement by pancreatic stellate cells (PSCs), $\mu_c P$. The maximum cancer density is C_0. In their model, IL-10 can reduce the ability of cytotoxic CD8+ T cells (CTLs) in killing cancer cells, and it is assumed that the removal of cancer cells is a decreasing function of IL-10.

The first term on the right-hand side (RHS) models cancer growth. The second term models the removal of cancer cells by T cells. PSCs can be activated by cytokines and activated PSCs can secrete more cytokines. Compared to cancer cells, PSCs are sparser in the pancreas ducts, and their growth is modelled using a logistic function:

$$\frac{dP}{dt} = \left(k_P + \frac{\mu_P T_\beta}{k_P + T_\beta}\right) P \left(1 - \frac{P}{P_0}\right) - \lambda_P P.$$

In the equation the basal growth rate in the absence of TGF-beta

$$k_p P \left(1 - \frac{P}{P_0}\right),$$

and the TGF-beta induced growth rate

$$\frac{\mu_p T_\beta}{K_p + T_\beta} P \left(1 - \frac{P}{P_0}\right),$$

is the TGF-beta induced growth rate, and $\lambda_P P$ is the death rate of PSCs. The term

$$\frac{\mu_p T_\beta}{K_p + T_\beta},$$

is used to model the saturation limited effect of TGF-beta.

PSCs are the resident myofibroblast-like cells in the pancreas, playing important role in the cancer growth process. In our case non-immune bystander cells that also include the cancer associated fibroblasts (CAFs) are playing less significant role because we model the late stage of colorectal cancer where their influence can be incorporated in the equation for cancer cells in the form of the term $0.1C$ as suggested by clinical experience of Dr. Kersten.

Cytokines play a very important role in our model because they serve as communication means for the cell's crosstalk. The dynamics of cytokine is complex and includes interaction between pro -inflammatory and anti-inflammatory cytokines which mutually influence their proliferation. To capture the rate of interactions between cytokines Hill functions [52] are used of the form:

$$h(x) = \frac{x^m}{k^m + x^m}.$$

In this paper a simple framework for cytokine dynamics is presented in the form of two differential equations for the variables p and a which stand for pro- and anti-inflammatory cytokines:

$$\frac{dp}{dt} = \left(C_0 + C_1 \frac{p^n}{C_2^n + p^n}\right) \frac{C_3^n}{C_3^n + a^n} - d_p n,$$

$$\frac{da}{dt} = C_4 \frac{p^n}{C_5^n + p^n} - d_a a.$$

In our model we do not discuss this type of dynamics at the molecular level, but we acknowledge the double-faced role of the cytokines IL-4, IL-6, IL-10, IL-13, TGF-beta and PD-L1. In Figure 4 we see that cytokines EGF plays only pro-inflammatory role and IL-1, IL-2, IL-8, IL-12, TNF-alpha, INF-gamma have only anti-inflammatory effect.

In our model we use the same principle to design the equations for the cytokines assuming for simplicity that they are produced by relevant cells at the constant rate and are removed at constant rate due to a combination of factors as in Friedman et al. [13]. All equations for cytokines include the natural removal terms like $\delta_{I_k} I_k$ in addition to removal terms which corresponds to the inhibitions by the cells. For example, the term

$$-\left(\delta_{I_\gamma}^{(2)} + \delta_{I_\gamma}^{(5)} + \delta_{I_\gamma}^{(28)}\right) I_\gamma C,$$

in the equation for I_γ corresponds to the inhibition of I_γ by cancer cells associated with the interactions labeled as 2, 5 and 28 in figure 6.

We model the dynamics of cytokine growth with the differential equations describing the growth rate of every cytokine with at most three contributing parts: cytokine production and proliferation (cell talk) influenced by cells, inhibition by cells and cytokine`s natural degradation at a constant rate. We use the notation λ for the coefficients engaged in the proliferation and contributing to the cell cross talk and μ inhibition by the cells also due to the cell cross talk and δ for natural degradation rates. In our case most cytokines are produced by several cells. For the design of analysis of our model we follow the idea of Friedman et. al [40] assuming that the proliferation and degradation of cytokines occur on a time scale of seconds to hours compared to the growth of cancer cells on a time scale of months to years which allows us to use quasi-steady-state approximations for cytokine concentrations replacing differential equations for cytokines with algebraic equations relating cytokine levels to the density of cells. However, we do not also exclude the possibility that we may need to allow faster rate of change for IL-6 which is the most important cytokine for our discussion because it is linked to CRP that is a fast marker of patient`s condition due to inflammation. In that case the differential equations for IL-6 will be used.

Model development

At the beginning of the thesis we described the influence and interplay between the extrinsic and intrinsic factors in the CRC microenvironment. We looked at the most important parameters we had to include into the model from the three hallmarks, based on the research reported in the literature [ref], educated guesses and clinical experience of the research team of Dr. Kersten. These articles described different models for different cancer types but at early stages. Many papers discussed cancer models mechanistically not reflecting important medical aspects and very little was known about the influence of inflammation at the advanced stages of cancer. In our search for the closest mathematical description of CRC we had to study articles describing pancreatic cancer [40], and chronic pancreatitis [41]. In [40] the most important component for us was the equation for the cancer growth whereas in [41]

we paid particular attention to how the inflammation processes were modeled. We reflected about possibilities of using these ideas in our situation. We attempted to describe the detrimental inflammation in the late stage of colorectal cancer were the tumor is metastasized, the levels of inflammation are high and chronically self-perpetuating, the anti-inflammatory factors (macrophages, neutrophils and T helper cells) are suppressed and the pro-inflammatory effects dominate.

The whole process of the model design has been strongly motivated by the research about the correlation between IL-6 and CRP. Based on this experience, Kersten proposed the list of variables deemed to be most important for our model. In the work with metastatic colorectal cancer, macrophages play an important role. Macrophages are characterized by plasticity which is related to the polarization between two extreme classes of macrophages of M1 (anti-tumor) phenotype and M2 (pro-tumor) phenotype and possibility of "reeducation" of macrophages to an opposite phenotype. In our case the transition from M2 to M1 is not yet clinical confirmed for CRC, the levels of M1 are low and M2 are high. The effect of plasticity was described in several articles related to beneficial inflammation or early stages of cancer. Further discussions also brought up other important biomarkers including cancer associated fibroblasts (CAFs) as a link to IL-6 and cancer, along with EGF as a link to the intrinsic factors. Later, we incorporated neutrophils and T helper cells which also possess the plasticity property leading to the polarization between N1 and N2, as well as between Th1 and Th2, as mentioned in [53].

The first schematic diagram for our model was made [fig x] based on the separation of the cancer cell extrinsic factors into two groups: augmenting cancer cell proliferation and cancer cell removal. The idea of describing the microenvironment of cancer cell intrinsic factors of beneficial inflammation in end stage CRC has just started to develop based on the work reported in [48].

We have been working on the development of the model all the time and even at the time of submission of this thesis we still see it as a work in progress with more and more modifications based on new discoveries from the references or new ideas triggered by the repeated discussions of the model. At the final stage of the development of the diagram we keep the structure with a proliferation and cancer cell removal parts concentrating our attention on the interaction between the cells with the cytokines as the communication means for the cell crosstalk. A new part describing the non-immune bystander cells was added including the surrounding nearby cells and cancer associated fibroblasts. The role of EGF as an intrinsic factor has now more correctly been changed to a tumor promoting cytokine. To simplify already complex model, we are not considering further cancer cell intrinsic factors at all in the new diagram presented in Figure 6.

Based on our analysis of the clinical picture of the colorectal cancer with detrimental inflammation, the "simplest" mathematical model must include the so-called MNT factors (macrophages, neutrophils and T cells). This is because the combined effect of the

inflammation and tumor influences the phenotype of macrophages (M1 → M2), neutrophils (N1→ N2) and T cells (Th1 → Th2). In our full model we are only looking at the microenvironment cancer cells extrinsic factors like the macrophage phenotype M1 and M2, Neutrophils phenotype N1 and N2, T helper cells type Th1/CD8 + T cell and Th2/Treg, non-immune bystander cells/source, promoters and inhibitors. There are also 11 important cytokines responsible for the intracellular communication – cell crosstalk.

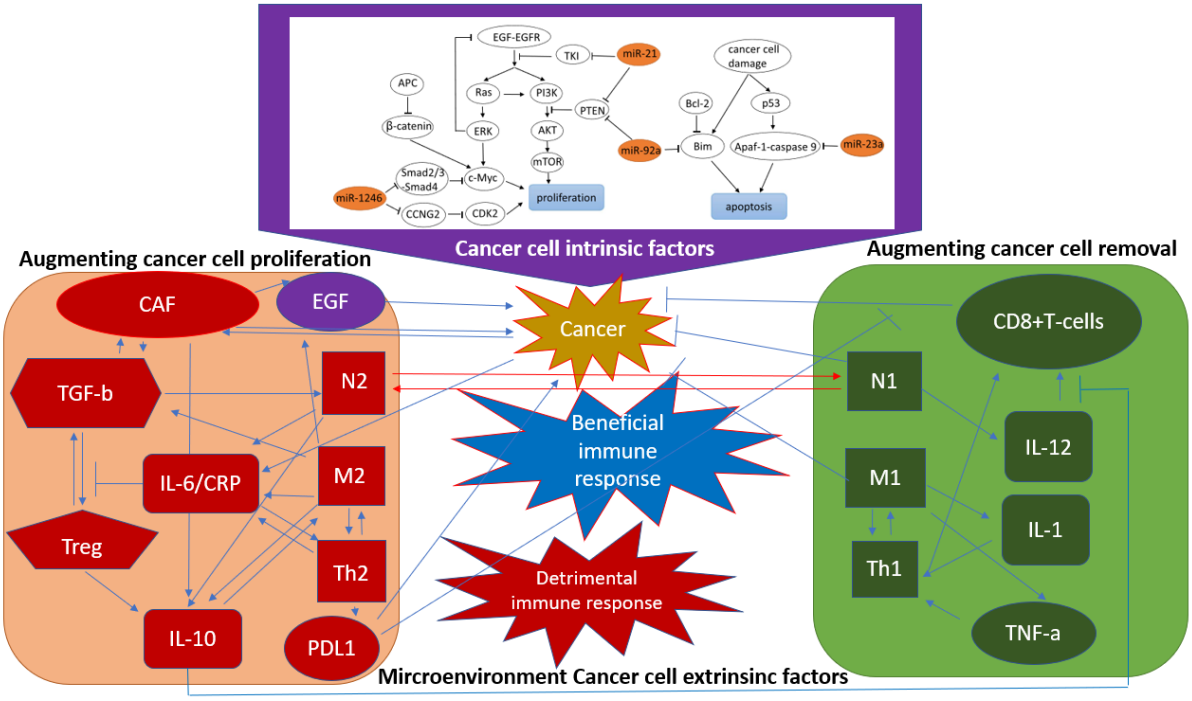


Figure 25. First model suggested at the very start of the work on the thesis

This full model would be too complex to study at this stage. We are aware of the fact that this diagram would not include all promoters neither all inhibitors of cancer. The components named “other promoters” and “other inhibitors” in our diagram stand for the factors not included in the current model or even not known to us at the moment and are left for the later work.

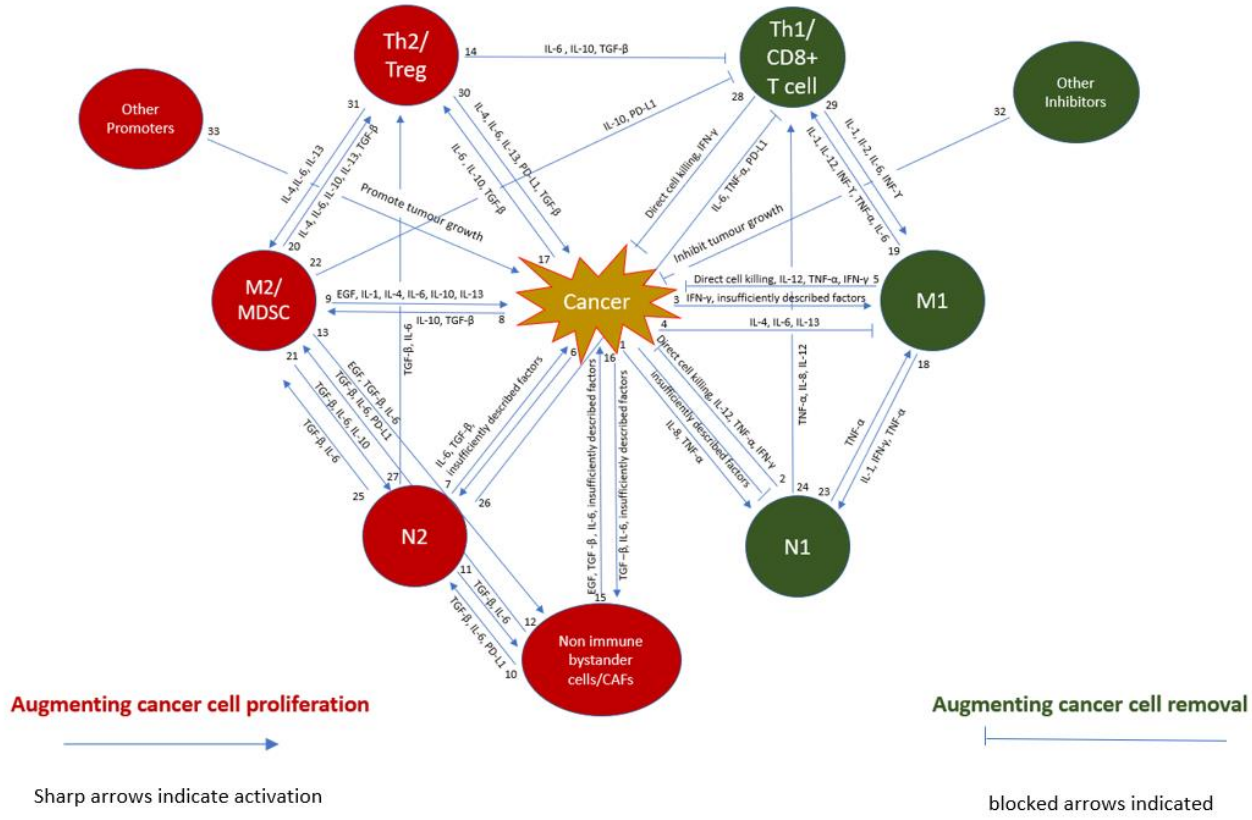


Figure 26. The newest model with indications

Our final diagram describes five cells promoting tumor growth marked red and four cells contributing to the inhibition of the tumor growth marked green. The communication between the cells is modeled as a cross talk assisted by 11 cytokines and helping us to describe interactions at the cell level. As we see in the diagram the polarization part is no longer present because there is no proven effect of cell reeducation at this stage of colorectal cancer but there is a noticeable influence of the pro-inflammatory part of macrophages, neutrophils and T helper cells. We assume that at this point they are not polarized back to their M1 stage [54]. The anti-inflammatory part of macrophages, neutrophils and T helper cells is still present in small amounts and function as inhibitors in cancer cell removal.

Differential equations describing the model

Our model consists of 22 equations, where 13 equations describe the concentration of the cytokines, one for CRP linked to IL-6, and eight for the density of cells. The first equation describes the rate of change of the density of cancer growth and reflects their interactions with other cells orchestrated by the cytokines. The design of these equations was inspired by the corresponding equations in two articles dealing with the pancreatitis cancer [40] and chronic inflammation in pancreatitis [41]. There is certain similarity between the pancreatic

cancer and the end stage of the colorectal cancer but the dynamics of these two cancers is still quite different. We designed the equation for the cancer cells to fit our situation and factors and we use the values of the parameters from these articles as the first approximation in our model. Calibration of parameters, adjustment of the measurement units and model testing are left for the future work.

The equations for cells are in the form of an ordinary differential equation:

$$Rate\ of\ change\ of\ the\ density\ of\ cells\ (X_n) = \underbrace{q_{X_n}}_{source} + \underbrace{k_{X_n}X_n}_{proliferation} - \underbrace{\delta_{X_n}X_n}_{removal},$$

where some terms may be missing or may have a more complex structure.

In our model, we include the following variables for cells and cytokines presented in the diagram in Fig. 6:

- Density of bystander cells: B
- Density of cancer cells: C
- Density of macrophages M1: M_1
- Density of macrophages M2: M_2
- Density of neutrophils N1: N_1
- Density of neutrophils N2: N_2
- Density of T helper cells Th1: T_1
- Density of T helper cells Th2: T_2
- Concentration of EGF: E
- Concentration of PDL1: P
- Concentration of TNF-α: T_α
- Concentration of TGF-β: T_β
- Concentration of INF-ϒ: I_γ
- Concentration of IL-1: I_1
- Concentration of IL-2: I_2
- Concentration of IL-4: I_4
- Concentration of IL-6: I_6
- Concentration of IL-8: I_8
- Concentration of IL-10: I_{10}
- Concentration of IL-12: I_{12}
- Concentration of IL-13: I_{13}

As suggested by Friedman et.al [40], cancer growth is promoted through various cytokines and is represented by a basal growth rate q_c and the enhancement by k_cB . The proliferation rate is influenced by the density of eight cytokines. In a logistic function the maximum cancer cell density is denoted by C_0. We assume that the rate of removal of cancer cells is a decreasing linear function depending on density of three associated cytokines. Based on this

consideration, the evolution of the density of cancer cells can be described by following equation:

$$(1)\ \frac{dC}{dt} = q_c + k_c B + \overbrace{[\left(\lambda_{I_1}^{(9)} + \lambda_{I_1}^{(30)}\right) I_1 + \left(\lambda_{I_4}^{(9)} + \lambda_{I_4}^{(30)}\right) I_4 + (\lambda_{I_6}^{(7)} + \lambda_{I_6}^{(9)} + \lambda_{I_6}^{(15)} + \lambda_{I_6}^{(30)}) I_6 + \lambda_{I_{10}}^{(9)} I_{10} + (\lambda_{I_{13}}^{(9)} + \lambda_{I_{13}}^{(15)}) I_{13}}^{Proliferation}$$
$$\overbrace{+ \left(\lambda_{T_\beta}^{(7)} + \lambda_{T_\beta}^{(15)} + \lambda_{T_\beta}^{(30)}\right) T_\beta + \lambda_P^{(30)} P + (\lambda_E^{(9)} + \lambda_E^{(15)}) E\ ]}\ \overbrace{C^p \left(1 - \left(\frac{C}{C_0}\right)^{1-p}\right)}^{generalized\ logistic\ growth}$$
$$- \overbrace{\left(\delta_C + \left(\mu_{T_\alpha}^{(2)} + \mu_{T_\alpha}^{(5)}\right) T_\alpha + \left(\mu_{I_{12}}^{(2)} + \mu_{I_{12}}^{(5)}\right) I_{12} + \left(\mu_{I_\gamma}^{(2)} + \mu_{I_\gamma}^{(5)} + \mu_{I_\gamma}^{(28)}\right) I_\gamma\right) C}^{Removal},$$
$$\frac{2}{3} < p < 1.$$

In equation (1), the cytokines promoting tumor growth are: IL-1, IL-4, IL-6, IL-10, IL-13, TGF-beta, PD-L1 and EGF which corresponds to the interactions between the cells illustrated in the diagram Figure 6. The parameter q_c is the source for cancer cells coming from bone marrow, $k_c B$ is the term associated with the contribution of bystander cells to the tumor growth. The removal part includes the natural decay of cancer cells with the coefficient δ_C and it also involves the cytokines inhibiting cancer: TNF-alpha, INF-gamma and IL-12.

The inclusion of at term associated with non-immune bystander cells/CAFs is suggested by Dr. Kersten's professional experience and has an element of educated guess in what concerns the percentage of such cells around tumor. This term accommodates all surrounding cells like blood cells, the cancer associated fibroblasts, some components of microenvironment and comprises a less significant part corresponding to about 10 % of the tumor. This term is likely to appear in the equation (1) as 0.1C in our further work.

$$(2)\ \frac{dB}{dt} = q_B + \overbrace{\left(k_B + (\lambda_{T_\beta}^{(11)} + \lambda_{T_\beta}^{(13)} + \lambda_{T_\beta}^{(16)}) T_\beta + (\lambda_{I_6}^{(11)} + \lambda_{I_6}^{(13)} + \lambda_{I_6}^{(16)}) I_6 + \lambda_E^{(13)} E\right)}^{Proliferation} \overbrace{B\left(1 - \frac{B}{B_0}\right)}^{logistic\ growth} -$$
$$\overbrace{\delta_B B}^{removal}$$

Equation (2) for non -immune bystander cells/CAFs in our model is an analogue of equation for pancreatitis stellate cells (PSCs) in [40] with appropriate modifications corresponding to fig.6. The proliferation of non-immune bystander cells/CAFs is affected by the cytokines TGF-beta, IL-6 and EGF.

Friedman et. al [40] describe that macrophages M1 and M2 can be attracted to the site of cancer, undergo apoptosis and switch the type. In their work the transition from M1 to M2 is mediated by cytokines TGF-Beta, IL-6, MCSF and GMCSF. At an early stage of cancer macrophages M1 can polarize into M2, and vice versa. The equations in [40] assume the form

$$\frac{dM_1}{dt} = \overbrace{\widehat{k_1}}^{influx\ rate} - \overbrace{\widehat{\lambda_1 M_1}}^{death\ rate} + \alpha M_2 - \overbrace{\widehat{(\alpha_1 T_\beta + \alpha_2 I_6 + \alpha_3 S + \alpha_4 G) M_1}}^{Transition\ rate\ M_1 to\ M_2}$$

$$\frac{dM_2}{dt} = \overbrace{\widehat{k_2}}^{influx\ rate} - \overbrace{\widehat{\lambda_2 M_2}}^{death\ rate} - \alpha M_2 + \overbrace{\widehat{(\alpha_1 T_\beta + \alpha_2 I_6 + \alpha_3 S + \alpha_4 G) M_1}}^{Transition\ rate\ M_1 to\ M_2}$$

The equation for anti-tumour macrophage M1 is assuming a logistic growth $\left(1 - \frac{M_1}{M_{01}}\right)$ where M_{10} is the maximal macrophage density for M1. The proliferation of the macrophage M1 is based on the influx with the rate q_{M_1} and is influenced by the cytokines IL-1, IL-2, IL-6, INF-gamma, TNF-alpha Y. Lin [41] , F.J.V Dalen [48]. The removal is also based on a death rate constant δ_{M_1} due to the cytokines inhibiting macrophage M1, like IL-4, IL-6, and IL-13.

(3) $\frac{dM_1}{dt} =$

$$q_{M_1} + \overbrace{\left(k_B + \lambda_{I_1}^{(29)} I_1 + \lambda_{I_2}^{(29)} I_2 + \lambda_{I_6}^{(29)} I_6 + (\lambda_{I_\gamma}^{(3)} + \lambda_{I_\gamma}^{(29)}) I_\gamma + \lambda_{T_\alpha}^{(23)} T_\alpha\right)}^{Proliferation} \overbrace{M_1 \left(1 - \frac{M_1}{M_{01}}\right)}^{logistic\ growth} - \overbrace{(\delta_{M_1} + \mu_{I_4}^{(4)} I_4 + \mu_{I_6}^{(4)} I_6 + \mu_{I_{13}}^{(4)} I_{13}) M_1}^{removal}$$

The equation for the macrophage M2 is designed in a similar manner under the assumption of a logistic growth $\left(1 - \frac{M_2}{M_{02}}\right)$ where M_{20} is again the maximal macrophage density for M2. The contributions to the proliferation of the macrophage M2 come from the influx term q_{M_2} and from the cytokines IL-4, IL-6, IL-10, IL-13, TGF-beta and PD-L1 linearly. The removal is only due to the natural decay term $\delta_{M_2} M_2$ and in this case there are no cytokines involved in the inhibition of M2.

(4) $\frac{dM_2}{dt} = q_{M_2} +$

$$\overbrace{\left(k_{M_2} + \lambda_{I_4}^{(31)} I_4 + (\lambda_{I_6}^{(12)} + \lambda_{I_6}^{(25)} + \lambda_{I_6}^{(31)}) I_6 + \lambda_{I_{10}}^{(8)} I_{10} + \lambda_{I_{13}}^{(31)} I_{13} + (\lambda_{T_\beta}^{(8)} + \lambda_{T_\beta}^{(12)} + \lambda_{T_\beta}^{(25)}) T_\beta + \lambda_P^{(12)} P\right)}^{Proliferation}$$

$$\times \overbrace{M_2 \left(1 - \frac{M_2}{M_0 2}\right)}^{logistic\ growth} - \overbrace{\delta_{M_2} M_2}^{removal}$$

We model four equations for neutrophils N1 and N2, T helper cells Th1 and Th2 similarly assuming that their dynamics is as for the macrophages. The proliferation of the anti-inflammatory neutrophils N1 is promoted by the cytokines IL-1, IL-8, INF-gamma, and TNF-alpha as stated in R. Grecian [11], X. Wang [12] and L. Wu [16] .

(5) $\frac{dN_1}{dt} = q_{n_1} + \overbrace{\left(k_{N_1} + \lambda_{I_1}^{(18)} I_1 + \lambda_{I_8}^{(1)} I_8 + \lambda_{I_\gamma}^{(18)} I_\gamma + (\lambda_{T_\alpha}^{(1)} + \lambda_{T_\alpha}^{(18)}) T_\alpha\right)}^{Proliferation} N_1 \overbrace{\left(1 - \frac{N_1}{N_{01}}\right)}^{logistic\ growth} - \overbrace{\delta_{N_1} N_1}^{removal}$

The cytokines IL-6, IL-10, TGF-beta, and PD-L1 promote the proliferation of pro-inflammatory neutrophils N2 as stated in R. Grecian [11], X. Wang [12] and L. Wu [16].

(6) $\frac{dN_2}{dt} = q_{n_2} +$

$$\overbrace{\left(k_{N_2} + (\lambda_{I_6}^{(10)} + \lambda_{I_6}^{(21)})I_6 + \lambda_{I_{10}}^{(21)} I_{10} + (\lambda_{T_\beta}^{(10)} + \lambda_{T_\beta}^{(21)})T_\beta + \lambda_P^{(10)} P\right)}^{Proliferation} \overbrace{N_2\left(1 - \frac{N_2}{N_{02}}\right)}^{logistic\ growth} - \overbrace{\delta_{N_2} N_2}^{removal}$$

We decided to combine Th1 and CD8 + T cells in one common class Th1/CD 8 +T cell denoting it Th1 because they both influence the same cytokines contributing to direct removal of cancer cells. The cytokines that promote the proliferation of Th1 are IL-1, IL-6, IL-8, IL-12, INF-gamma, and TNF-alpha. Here we also assume a logistic growth. The cytokines that are involved in the removal of Th1 are IL-6, IL-10, TNF-alpha, TGF-beta and PD-L1. Here IL-6 contributes both to pro- and anti-inflammatory effect which emphasizes the important role of IL-6 we wish to acknowledge in our model.

(7) $\frac{dT_1}{dt} = q_{T_1} +$

$$\overbrace{\left(k_{T_1} + \lambda_{I_1}^{(19)} I_1 + \lambda_{I_6}^{(19)} I_6 + \lambda_{I_8}^{(24)} I_8 + (\lambda_{I_{12}}^{(19)} + \lambda_{I_{12}}^{(24)})I_{12} + \lambda_{I_\gamma}^{(19)} I_\gamma + (\lambda_{T_\alpha}^{(19)} + \lambda_{T_\alpha}^{(24)})T_\alpha\right)}^{Proliferation} \overbrace{T_1\left(1 - \frac{T_1}{T_{01}}\right)}^{logistic\ growth} -$$

$$\overbrace{(\delta_{T_1} + (\mu_{I_6}^{(14)} + \mu_{I_6}^{(26)})I_6 + (\mu_{I_{10}}^{(14)} + \mu_{I_{10}}^{(22)})I_{10} + \mu_{I_\alpha}^{(26)} T_\alpha + \mu_{I_\beta}^{(14)} T_\beta + (\mu_P^{(22)} + \mu_P^{(26)})P)T_1}^{removal}$$

We do the same combining Th2 and Treg in one common class of Th2/Treg in equation (8). Here we also use the notation Th2 for the same reason that both Th2 and Treg are influencing the same cytokines IL-4, IL-6, IL-10, IL-13 and TGF-beta. We also assume logistic growth and that the term $\lambda_{T_2} T_2$ corresponds to the removal of Th2/Treg.

(8) $\frac{dT_2}{dt} = q_{T_2} +$

$$\overbrace{\left(k_{T_2} + \lambda_4^{(20)} I_4 + \lambda_{I_6}^{(17)} + (\lambda_{I_6}^{(20)} + \lambda_{I_6}^{(27)})I_6 + \left(\lambda_{I_{10}}^{(17)} + \lambda_{I_{10}}^{(20)}\right) I_{10} + \lambda_{I_{13}}^{(20)} I_{13} + (\lambda_{T_\beta}^{(17)} + \lambda_{T_\beta}^{(20)} + \lambda_{T_\beta}^{(27)})T_\beta\right)}^{Proliferation}$$

$$\times \overbrace{T_2\left(1 - \frac{T_2}{T_{02}}\right)}^{logistic\ growth} - \overbrace{\delta_{T_2} T_2}^{removal}.$$

For simplicity, we assume that the cytokines are produced by cells like bystander cells, macrophages, neutrophils, cancer cells or T cells at constant rates proportional to the density of cells. They undergo a natural decay with constant rates. We use the notation λ`s for the proliferation depending on the density of cells, μ`s for the inhibition terms depending on density of the cells, and the δ's are the degradation rates of the cytokines. We are working with nine cytokines; all differential equations have the following form:

$$Rate\ of\ change\ of\ the\ density\ of\ cytokines\ (x_n) = \overbrace{k_{x_n}(cell\ source)}^{proliferation\ depending\ on\ relevant\ cells} - \overbrace{\delta_{x_n} x_n}^{degradation}.$$

In the equations for cytokines, the degradation term is described by the removal term; $\delta_{I_k} I_k$

TNF-alpha is promoted by cancer cells, macrophages M1 and neutrophils N1. On the other hand, it is inhibited by neutrophils N2, N1 and M1, where M1 and N1 contribute both positively and negatively through different biochemical links between the cells. For example, positive contribution of M1 comes from relation (18) in the diagram 6 and negative contribution results from relation (5).

$$(9)\ \frac{dT_\alpha}{dt} = \lambda_{T_\alpha}^{(1)} C + \lambda_{T_\alpha}^{(18)} M_1 + \lambda_{T_\alpha}^{(23)} N_1 - \delta_\alpha T_\alpha - \mu_{T_\alpha}^{(2)} N_1 - \mu_{T_\alpha}^{(5)} M_1 - \mu_{T_\alpha}^{(26)} N_2.$$

In contrast to the equation describing TNF-alpha with the double-faceted role of macrophages M1 and neutrophils N1 the equation for TGF-beta reflects only promotion by neutrophils N2, non- immune bystander cells/CAFs, Th2/Treg, macrophages M2, and cancer cells.

$$(10)\ \frac{dT_\beta}{dt} = (\lambda_{T_\beta}^{(7)} + \lambda_{T_\beta}^{(11)} + \lambda_{T_\beta}^{(25)}) N_2 + (\lambda_{T_\beta}^{(10)} + \lambda_{T_\beta}^{(12)} + \lambda_{T_\beta}^{(15)}) B + \lambda_{T_\beta}^{(30)} T_2 + (\lambda_{T_\beta}^{(13)} + \lambda_{T_\beta}^{(20)} + \lambda_{T_\beta}^{(21)} + \lambda_{T_\beta}^{(27)}) M_2 + (\lambda_{T_\beta}^{(8)} + \lambda_{T_\beta}^{(16)} + \lambda_{T_\beta}^{(17)}) C - \delta_\beta T_\beta.$$

The proliferation of INF-gamma receives contributions from the cancer cells, microphages M1, and Th1/CD8 + T cells. Here macrophages M1 also have the double-faceted role working both as promoters and inhibitors together with N1 and Th1.

$$(11)\frac{dI_\gamma}{dt} = \lambda_{I_\gamma}^{(3)} C + (\lambda_{I_\gamma}^{(18)} + \lambda_{I_\gamma}^{(19)}) M_1 + \lambda_{I_\gamma}^{(29)} T_1 - \delta_\gamma T_\gamma - \mu_{I_\gamma}^{(2)} N_1 - \mu_{I_\gamma}^{(5)} M_1 - \mu_{I_\gamma}^{(28)} T_1.$$

Interleukin-1 is produced by macrophages M1, Th1/CD8 + T cells, and macrophages M2. There is no inhibition of IL-1.

$$(12)\frac{dI_1}{dt} = (\lambda_{I_1}^{(18)} + \lambda_{I_1}^{(19)}) M_1 + \lambda_{I_1}^{(29)} T_1 + \lambda_{I_1}^{(9)} M_2 - \delta_{I_1} I_1.$$

Interleukin-2 is produced by Th1/CD8 + T cells.

$$(13)\frac{dI_2}{dt} = \lambda_{I_2}^{(29)} T_1 - \delta_{I_2} I_2$$

Interleukin-4 is produced by macrophage M2 and Th2/Treg. The cytokine is also inhibited by the cancer cells.

$$(14)\ \frac{dI_4}{dt} = (\lambda_{I_4}^{(9)} + \lambda_{I_4}^{(20)}) M_2 + (\lambda_{I_4}^{(30)} + \lambda_{I_4}^{(31)}) T_2 - \delta_{I_4} I_4 - \mu_{I_4}^{(4)} C$$

Interleukin-8 is produced by the cancer cells, and neutrophils N1.

$$(15)\frac{dI_8}{dt} = \lambda_{I_8}^{(1)} C + \lambda_{I_8}^{(24)} N_1 - \delta_{I_8} I_8$$

Interleukin-10 is produced by the cancer cells and macrophages M2. At the same time macrophages M2 act as the double-faceted manner inhibiting the production of IL-10. In this situation Th2/Treg are also contributing to the inhibition of the cytokine.

$$(16)\ \frac{dI_{10}}{dt} = \left(\lambda_{I_{10}}^{(8)} + \lambda_{I_{10}}^{(17)}\right) C + (\lambda_{I_{10}}^{(20)} + \lambda_{I_{10}}^{(21)}) M_2 - \delta_{I_{10}} I_{10} - \mu_{I_{10}}^{(22)} M_2 - \mu_{I_{10}}^{(14)} T_2.$$

Interleukin-12 is produced by macrophages M1 and neutrophils N1, and these cells have also the two-sided behavior producing and inhibiting IL-12 at the same time.

(17) $\frac{dI_{12}}{dt} = \lambda_{I_{12}}^{(19)} M_1 + \lambda_{I_{12}}^{(24)} N_1 - \delta_{I_{12}} I_{12} - \mu_{12}^{(2)} N_1 - \mu_{12}^{(5)} M_1.$

Interleukin-13 is produced by the same cells as IL-4; macrophage M2 and Th2/Treg and inhibited by the cancer cells.

(18) $\frac{dI_{13}}{dt} = (\lambda_{I_{13}}^{(9)} + \lambda_{13}^{(20)}) M_2 + (\lambda_{I_{13}}^{(30)} + \lambda_{I_{13}}^{(31)}) T_2 - \delta_{I_{13}} I_{13} - \mu_{I_{13}}^{(4)} C.$

Interleukin-6 is produced by all the cells and is functioning both as a promoter and inhibitor of the tumor. This understates IL-6 as an important biomarker.

(19) $\frac{dI_6}{dt} = \lambda_{I_6}^{(16)} C + \left(\lambda_{I_6}^{(7)} + \lambda_{I_6}^{(11)} + \lambda_{I_6}^{(25)}\right) N_2 + (\lambda_{I_6}^{(9)} + \lambda_{I_6}^{(13)} + \lambda_{I_6}^{(21)}) M_2 + (\lambda_{I_6}^{(30)} + \lambda_{I_6}^{(31)}) T_2 + \left(\lambda_{I_6}^{(10)} + \lambda_{I_6}^{(12)} + \lambda_{I_6}^{(15)}\right) B + \lambda_{I_6}^{(29)} T_1 + \lambda_{I_6}^{(19)} M_1 - \delta_{I_6} I_6 - \mu_{I_6}^{(4)} C - \mu_{I_6}^{(26)} N_2 - \mu_{I_6}^{(14)} T_2.$

The concentration of CRP is 2/3 of the concentration of IL-6, based on correlation data [26].

(20) $\frac{dCRP}{dt} = \frac{2}{3} * \frac{dI_6}{dt}.$

EGF is produced by macrophage M2 and non-immune bystander cells/CAFs.

(21) $\frac{dE}{dt} = (\lambda_E^{(9)} + \lambda_E^{(13)}) M_2 + \lambda_E^{(15)} B - \delta_E E.$

PD-L1 is produced by Th2/Treg and non-immune bystander cells/CAFs. The cytokine is also inhibited by M2 and by neutrophils N2.

(22) $\frac{dP}{dt} = \lambda_P^{(30)} T_2 + \left(\lambda_P^{(10)} + \lambda_P^{(12)}\right) B - \delta_P P - \mu_P^{(22)} M_2 - \mu_P^{(26)} N_2.$

The list of parameters is taken from three articles given in the references and is not complete due to the lack of real data. Most of parameters are originally estimated or fitted to the pancreatic cancer. For the future we wish to do the fitting of the model based on data for detrimental inflammation in late stage CRC. The problem is that there is currently little available data in this field at this stage. Further research is needed when looking at the parameters which can be completed both by literature review and participating in the analysis of the experimental research.

Table 2. Parameters for the cell sources

Parameters for sources	Description	Value
q_c	Bone marrow coefficient [Educated guess and experience]	$7.5x10^{-2}\ cells^{1/4} mL^{-1/4}$[40]
q_B	Source for bystander cells	

q_{m_1}	Source for macrophages M1	
q_{m_2}	Source for macrophages M2	
q_{n_1}	Source for neutrophils N1	
q_{n_2}	Source for neutrophils N2	
q_{t_1}	Source for T-helper cells Th1	
q_{t_2}	Source for T-helper cells Th2	

Table 3. Parameters for the cell proliferation

Parameters for cell proliferation	Description	Value
$k_c 0.1C$	Sum of basal growth rate of cancer [*Enhancement by CRC*]	$20q_c/B_0[40]$ $0.2\ per\ day[40]$
k_B	Basal growth rate of non-immune bystander cells/CAFs [*Enhancement by non-immune bystander cells/CAFs*]	$20\ q_B[40]$
k_{M_1}	Basal growth rate of M1 [I*nflux rate of M1]*	$1\ [41]$
k_{M_2}	Basal growth rate of M2 [I*nflux rate of M2]*	$1\ [41]$
k_{N_1}	Basal growth rate of N1	
k_{N_2}	Basal growth rate of N2	
k_{T_1}	Basal growth rate of Th1	
k_{T_2}	Basal growth rate of Th2	

For the future research these lumped parameters can be used when comparing the outcome from the system to the real data.

Table 4. Parameters for the cytokines lumped concentrations

Parameters for lumped concentrations	Description	Value
K_E	Lumped concentration of EGF in tissue [*Activation rate of EGF*]	$1.1741x10^{-3}\ day^{-1}g\ /cm^3$ [48]
K_P	Lumped concentration of PD-L1 in tissue	
K_{T_α}	Lumped concentration of TNF-alpha in tissue	$3x10^{-11}\ g/cm^3$[41]
K_{T_β}	Lumped concentration of TGF-beta in tissue *[Concentration of TGF-beta in tissue]*	$8x10^{-7}\ g/cm^3$[41]
K_{I_γ}	Lumped concentration of INF-gamma in tissue	
K_{I_1}	Lumped concentration of INF-gamma in tissue	
K_{I_2}	Lumped concentration of INF-gamma in tissue	
K_{I_4}	Lumped concentration of IL-4 in tissue	
K_{I_6}	Lumped concentration of IL-6 in tissue	
K_{I_8}	Lumped concentration of IL-8 in tissue	
$K_{I_{10}}$	Lumped concentration of IL-10 in tissue	
K_{12}	Lumped concentration of IL-12 in tissue	
K_{13}	Lumped concentration of IL-13 in tissue	

Table 5. Parameters for the density of cells

Parameters for density of cells	Description	Value
C	Density of cancer cells	
C_0	Maximum cancer cell density	$10^6 cells/mL[40]$
B	Density of bystander cells (10 % of total cell mass inn CRC)	$10x10^{-3}\ g/mL$ [41], Dr. Kersten`s intelligent guess and experience
B_0	Maximum bystander cell density	$10^5 cells/mL[40]$
M_1	Density of M1 cells	
M_{01}	Maximum M1 cell density	
M_2	Density of M2 cells	
M_{02}	Maximum M2 cell density	
N_1	Density N1 cells	
N_{01}	Maximum N1 cell density	
N_2	Density of N2 cell	
N_{02}	Maximum N2 cell density	
T_1	Density of Th1 cells	
T_{01}	Maximum Th1 cell density	
T_2	Density of Th2 cells	
T_{02}	Maximum Th2 cell density	

Table 6. Parameters for concentrations for cytokines

Parameters for concentrations for cytokines	Description	Values
E	Concentration of EGF	
P	Concentration of PDL1	
T_α	Concentration of TNF-α	
T_β	Concentration of TGF-β	
I_γ	Concentration of INF-ϒ:	
I_4	Concentration of IL-4:	
I_6	Concentration of IL-6	
I_{10}	Concentration of IL-10	
I_{12}	Concentration of IL-12	
I_{13}	Concentration of IL-13	
I_{14}	Concentration of IL-14	

Table 7. Parameters for the promoters of cytokines

Parameters of promoters	Description	Value
$\lambda_{I_1}^{(9)}$	Coefficient of IL-1 crosstalk between M2/MDSC and Cancer cells	
$\lambda_{I_1}^{(18)}$	Coefficient of IL-1 crosstalk between M1 and N1	
$\lambda_{I_1}^{(19)}$	Coefficient of IL-1 crosstalk between M1 and Th1/CD8+ T cells	
$\lambda_{I_1}^{(29)}$	Coefficient of IL-1 crosstalk between Th1/CD8+ T cells and Cancer cells M1	

$\lambda_{I_1}^{(30)}$	Coefficient of IL-1 crosstalk between Th2/Treg and Cancer cells	
$\lambda_{I_2}^{(29)}$	Coefficient of IL-2 crosstalk between Th1/CD8+ T cells and Cancer cells M1	
$\lambda_{I_4}^{(9)}$	Coefficient of IL-4 crosstalk between M2/MDSC and Cancer cells	
$\lambda_4^{(20)}$	Coefficient of IL-4 crosstalk between M2/MDSC and Th2/Treg	
$\lambda_{I_4}^{(30)}$	Coefficient of IL-4 crosstalk between Th2/Treg and cancer cells	
$\lambda_{I_4}^{(31)}$	Coefficient of IL-4 crosstalk between Th2/Treg and M2/MDSC	
$\lambda_{I_6}^{(7)}$	Coefficient of IL-6 crosstalk between N2 and cancer cells	
$\lambda_{I_6}^{(9)}$	Coefficient of IL-6 crosstalk between M2/MDSC and Cancer cells	
$\lambda_{I_6}^{(10)}$	Coefficient of IL-6 crosstalk between Non-immune bystander cells/CAFs and N2	
$\lambda_{I_6}^{(11)}$	Coefficient of IL-6 crosstalk between N2 and non-immune bystander cells/CAFs	
$\lambda_{I_6}^{(12)}$	Coefficient of IL-6 crosstalk between non-immune bystander cells/CAFs and M2/MDSC	
$\lambda_{I_6}^{(13)}$	Coefficient of IL-6 crosstalk between M2/MDSC and non-immune bystander cells/CAFs *(Activation rate of IL-6 due to bystander cells)*	$7x10^{-11}\ day^{-1}$ [41] $6.7x10^{-3}\ day^{-1}$ [41]
$\lambda_{I_6}^{(15)}$	Coefficient of IL-6 crosstalk between non-immune bystander cells/CAFs and cancer cells	
$\lambda_{I_6}^{(16)}$	Coefficient of IL-6 crosstalk between cancer cells and non-immune bystander cells/CAFs	

$\lambda_{I_6}^{(17)}$	Coefficient of IL-6 crosstalk between cancer cells and Th2/Treg	
$\lambda_{I_6}^{(19)}$	Coefficient of IL-6 crosstalk between M1 and Th1/Cd8 + T cells	
$\lambda_{I_6}^{(20)}$	Coefficient of IL-6 crosstalk between M2/MDSC and Th2/Treg	
$\lambda_{I_6}^{(21)}$	Coefficient of IL-6 crosstalk between M2/MDSC and N2	
$\lambda_{I_6}^{(25)}$	Coefficient of IL-6 crosstalk between N2 and M2/MDSC	
$\lambda_{I_6}^{(27)}$	Coefficient of IL-6 crosstalk between N2 andTh2/Treg	
$\lambda_{I_6}^{(29)}$	Coefficient of IL-6 crosstalk between Th1/CD8+T cell and M1	
$\lambda_{I_6}^{(30)}$	Coefficient of IL-6 crosstalk between Th2/Treg and cancer cell	
$\lambda_{I_6}^{(31)}$	Coefficient of IL-6 crosstalk between Th2/Treg and M2/MDSC	
$\lambda_{I_8}^{(1)}$	Coefficient of IL-8 crosstalk between cancer and N1	
$\lambda_{I_8}^{(24)}$	Coefficient of IL-8 crosstalk between N1 and Th1/CD8+Tcell	
$\lambda_{I_{10}}^{(8)}$	Coefficient of IL-10 crosstalk between cancer cell and M2/MDSC	
$\lambda_{I_{10}}^{(9)}$	Coefficient of IL-10 crosstalk between M2/MDSC and cancer cell [*Production rate of IL-10 due to macrophage M2]*	$6.67x10^{-3}day^{-1}$ [48]
$\lambda_{I_{10}}^{(17)}$	Coefficient of IL-10 crosstalk between cancer and Th2/Treg	

$\lambda_{I_{10}}^{(20)}$	Coefficient of IL-10 crosstalk between M2/MDSC and TH2/Treg	
$\lambda_{I_{10}}^{(21)}$	Coefficient of IL-10 crosstalk between M2/MDSC and N2	
$\lambda_{I_{12}}^{(19)}$	Coefficient of IL-12 crosstalk between M1 and Th1/CD8+T cell	
$\lambda_{I_{12}}^{(24)}$	Coefficient of IL-12 crosstalk between N1 and Th1/CD8+ T cell	
$\lambda_{I_{13}}^{(9)}$	Coefficient of IL-13 crosstalk between M2/MDSC and cancer	
$\lambda_{I_{13}}^{(15)}$	Coefficient of IL-13 crosstalk between non-immune bystander cells/CAFs and cancer	
$\lambda_{I_{13}}^{(20)}$	Coefficient of IL-13 crosstalk between M2/MDSC and Th2/Treg	
$\lambda_{I_{13}}^{(31)}$	Coefficient of IL-13 crosstalk between Th2/Treg and M2/MDSC	
$\lambda_{T_\alpha}^{(1)}$	Coefficient of TNF-alpha crosstalk between cancer and N1	
$\lambda_{T_\alpha}^{(18)}$	Coefficient of TNF-alpha crosstalk between M1 and N1 [*activation rate of TNF-alpha due to macrophage M1]*	$3x10^{-2}\ day^{-1}$ [48]
$\lambda_{T_\alpha}^{(19)}$	Coefficient of TNF-alpha crosstalk between M1 and Th1/CD8+ T cell	
$\lambda_{T_\alpha}^{(23)}$	Coefficient of TNF-alpha crosstalk between	
$\lambda_{T_\alpha}^{(24)}$	Coefficient of TNF-alpha crosstalk between	
$\lambda_{T_\beta}^{(7)}$	Coefficient of TGF-beta crosstalk between N2 and cancer cells	

$\lambda_{T_\beta}^{(8)}$	Coefficient of TGF-beta crosstalk between M2/MDSC and Cancer cells *[Activation rate of TGF-beta due to macrophage M2]*	$1.5x10^{-2}\ day^{-1}$ [48]
$\lambda_{T_\beta}^{(10)}$	Coefficient of TGF-beta crosstalk between Non-immune bystander cells/CAFs and N2	
$\lambda_{T_\beta}^{(11)}$	Coefficient of TGF-beta crosstalk between N2 and non-immune bystander cells/CAFs	
$\lambda_{T_\beta}^{(12)}$	Coefficient of TGF-beta crosstalk between non-immune bystander cells/CAFs and M2/MDSC	
$\lambda_{T_\beta}^{(13)}$	Coefficient of TGF-beta crosstalk between M2/MDSC and non-immune bystander cells/CAFs	
$\lambda_{T_\beta}^{(15)}$	Coefficient of TGF-beta crosstalk between non-immune bystander cells/CAFs and cancer cells [*Activation rate of TGF-Beta due to bystander cells]*	$6.7x10^{-3}\ day^{-1}$ [41]
$\lambda_{T_\beta}^{(16)}$	Coefficient of TGF-beta crosstalk between cancer cells and non-immune bystander cells/CAFs	
$\lambda_{T_\beta}^{(17)}$	Coefficient of TGF-beta crosstalk between cancer cells and Th2/Treg	
$\lambda_{T_\beta}^{(20)}$	Coefficient of TGF-beta crosstalk M2 and Th2/Treg	
$\lambda_{T_\beta}^{(21)}$	Coefficient of TGF-beta crosstalk between M2/MDSC and N2	
$\lambda_{T_\beta}^{(25)}$	Coefficient of TGF-beta crosstalk between N2 and M2/MDSC	
$\lambda_{T_\beta}^{(27)}$	Coefficient of TGF-beta crosstalk between M2/MDSC and N2	
$\lambda_{T_\beta}^{(30)}$	Coefficient of TGF-beta crosstalk between Th2/Treg and cancer	

$\lambda_{I_\gamma}^{(3)}$	Coefficient of INF-gamma crosstalk between cancer and M1	
$\lambda_{I_\gamma}^{(29)}$	Coefficient of INF-gamma crosstalk between Th1/CD8+ T cell and M1	
$\lambda_{I_\gamma}^{(18)}$	Coefficient of INF-gamma crosstalk between M1 and N1	
$\lambda_{I_\gamma}^{(19)}$	Coefficient of INF-gamma crosstalk between M1 and Th1/CD8+ T cell	
$\lambda_{P}^{(10)}$	Coefficient of PD-L1 crosstalk between non-immune bystander cells/CAFs and N2	
$\lambda_{P}^{(12)}$	Coefficient of PD-L1 crosstalk between non-immune bystander cells and M2/MDSC	
$\lambda_{P}^{(30)}$	Coefficient of PD-L1 crosstalk between Th2/Treg and cancer	
$\lambda_{E}^{(9)}$	Coefficient of EGF crosstalk between M2/MDSC and cancer	
$\lambda_{E}^{(13)}$	Coefficient of EGF crosstalk between M2/MDSC and non-immune bystander cells/CAFs	
$\lambda_{E}^{(15)}$	Coefficient of EGF crosstalk between non-immune bystander cells/CAFs and cancer	

Table 8. Parameters for the inhibitors of the cytokines

Parameters for the inhibitors	Description	Values
$\mu_{I_4}^{(4)}$	Coefficient of IL-4 crosstalk between cancer and M1	
$\mu_{I_6}^{(4)}$	Coefficient of IL-6 crosstalk between cancer and M1	

$\mu_{I_6}^{(14)}$	Coefficient of IL-6 crosstalk between Th2/Treg and Th1/CD8+ T cell	
$\mu_{I_6}^{(26)}$	Coefficient of IL-6 crosstalk between N2 and Th1/CD8 + T cell	
$\mu_{I_{10}}^{(14)}$	Coefficient of IL-10 crosstalk between Th2/Treg and Th1/CD8+ T cell	
$\mu_{I_{10}}^{(22)}$	Coefficient of IL-10 crosstalk between M2/MDSC and Th1/CD8+ T cell	
$\mu_{I_{12}}^{(2)}$	Coefficient of IL-12 crosstalk between N1 and cancer l	
$\mu_{I_{12}}^{(5)}$	Coefficient of IL-12 crosstalk between M1 and cancer	
$\mu_{I_{13}}^{(4)}$	Coefficient of IL-13 crosstalk between cancer and M1	
$\mu_{T_\alpha}^{(2)}$	Coefficient of TNF-alpha crosstalk between N1 and cancer	
$\mu_{T_\alpha}^{(5)}$	Coefficient of TNF-alpha crosstalk between M1 and cancer	
$\mu_{T_\alpha}^{(26)}$	Coefficient of TNF-alpha crosstalk between N2 and Th1/CD8 + T cell	
$\mu_{I_\beta}^{(14)}$	Coefficient of TGF-beta crosstalk between Th2/Treg and Th1/CD8+ T cell	
$\mu_{I_\gamma}^{(2)}$	Coefficient of INF-gamma crosstalk between N1 and cancer	

$\mu_{I_\gamma}^{(5)}$	Coefficient of INF-gamma crosstalk between M1 and cancer	
$\mu_{I_\gamma}^{(28)}$	Coefficient of INF-gamma crosstalk between Th1/CD8 + T cell and cancer	
$\mu_P^{(22)}$	Coefficient of PD-L1 crosstalk between M2/MDSC and Th1/CD8+ T cell	
$\mu_P^{(26)}$	Coefficient of PD-L1 crosstalk between N2 and Th1/CD8 + T cell	

Table 9. Parameters for the removal of the cells and cytokines

Parameters of removal	Description	Value
δ_C	Death rate of cancer cells [*maximum rate of CTL in killing cancer cells]*	$10^{-7} mL\ per\ cell\ per\ day$[40]
δ_B	Death rate of non-immune bystander cells/CAFs [*half-life of bystander cells in 2-4 days]*	$0.25\ per\ day$[40]
δ_{M_1}	Death rate of macrophage M1	$0.02\ day^{-1}$ [41]
δ_{M_2}	Death rate of macrophage M2	$0.015\ day^{-1}$ [41]
δ_{N_1}	Death rate of neutrophils N1	
δ_{N_2}	Death rate of neutrophils N2	
δ_{T_1}	Death rate of T helper cell 2	
δ_{T_2}	Death rate of T helper cell 2	
δ_{T_β}	Degradation rate of TGF-beta	$3.33x10^2\ day^{-1}$ [48]

δ_{T_α}	Degradation rate of TNF-alpha	$55.45x10^{-1}\ day^{-1}$ [41]
δ_E	Degradation rate of EFG	$0.8318\ day^{-1}$ [48]
δ_P	Degradation rate of PD-L1	
δ_{I_γ}	Degradation rate of INF-gamma	
δ_{I_1}	Degradation rate of IL-1	
δ_{I_2}	Degradation rate of IL-2	
δ_{I_4}	Degradation rate of IL-4	
δ_{I_6}	Degradation rate of IL-6	$0.173\ day^{-1}$ [41]
δ_{I_8}	Degradation rate of IL-8	
$\delta_{I_{10}}$	Degradation rate of IL-10	$16.64\ day^{-1}$[48]
$\delta_{I_{12}}$	Degradation rate of IL-12	
$\delta_{I_{13}}$	Degradation rate of IL-13	

Discussion and future work

The immune system can be engaged in tumor promotion via several mechanisms. Interleukin-6 is thought to be one of the critical elements is chronic inflammation. Mathematical modelling may shed the light on these tumor-immune interactions by testing different hypotheses in silico. In this work we employ mathematical modelling to describe detrimental inflammation using IL-6 as one of the parameters describing the CRC at the late stage colorectal cancer (IsCRC). From diagnosis of end stage of colorectal cancer, the incorporate assumption healthy person that tolerates optimal therapy has an ideal survival of 25 month. We will develop a model of two sets of parameters (green and red) that increase(red) or decrease(green) fig [3]. Our optimal goal is to estimate life expectancy of the patients in relation with Dr. Kersten`s experience.

Another interesting future task will be to extend this work suggesting an equation describing the dependence of CRC of several important hallmarks of cancer, like angiogenesis, apoptosis, proliferation, metastasis. In the future, we want study the impact of anti-IL-6 on the density of cancer cells, thereby exploring the potential of anti-IL-6 treatment as possible anti-CRC therapy.

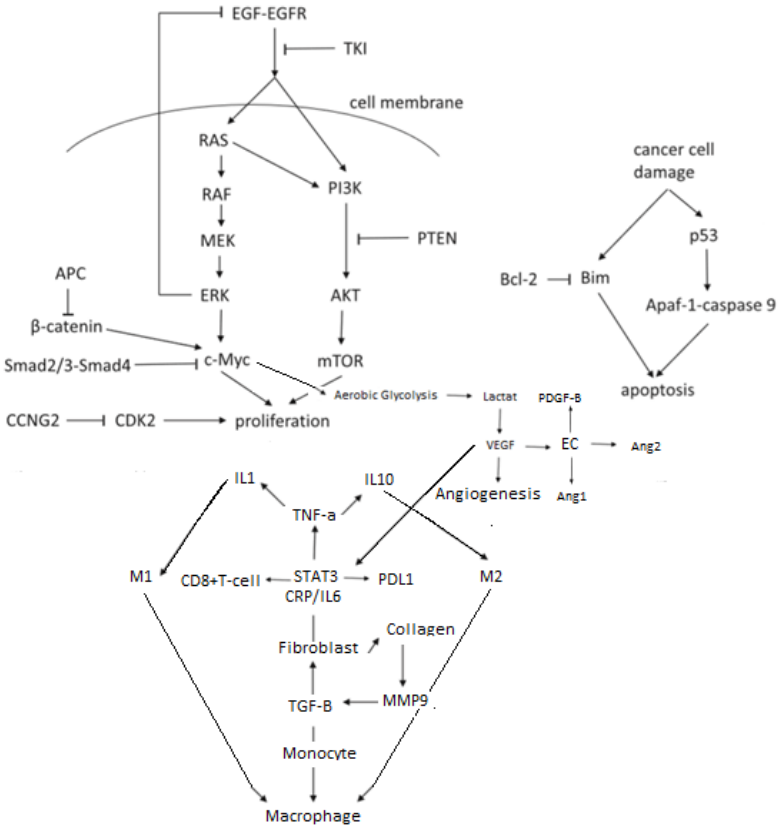


Figure 27. The interaction diagram we created for the five hallmarks

In our opinion, for late stage colorectal cancer with detrimental inflammation, the mentioned five hallmarks of cancer are of particular relevance. Our ultimate and very ambition goal is to model these five hallmarks mathematically in one model describing the development of CRC over time.

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