University of Camerino

School of Science and Technology

MSc in Computer Science (LM-18)

Obesity Paradox: an Agent-Based Modeling Approach

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1. Introduction

The Obesity Paradox:
Obesity is associated with an increased risk of developing clear cell renal cell carcinoma (ccRCC) but, paradoxically, obesity is also associated with improved oncological outcomes in this cancer. (Sanchez et al. 2019)

This project is an attempt to model the immune system response against Renal Cell Carcinoma by means of Agent Based Modeling (ABM).

It also aims to simulate the tumour-microenvironment in both cases of lean (lower BMI) and obese (higher BMI) patients in order to verify the improved outcomes observed in the literature.

The project was developed using Repast Simphony 2.8, a Java-based modeling system for ABM and simulation.

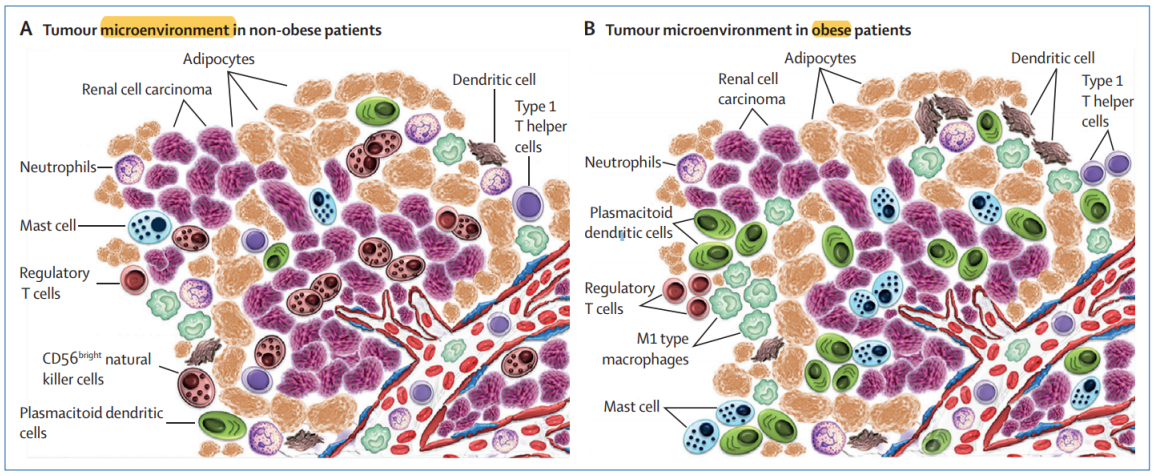

Figure 1.1: Perinephric tumour microenvironment in non-obese vs obese patients with renal cell carcinoma

2. Cells Overview

2.1 Immune

The immune system is a network of biological processes that protects an organism against disease. It detects and responds to a wide variety of pathogens, from viruses to parasitic worms and cancer cells, distinguishing them from the organism's own healthy tissue.

Many species have two major subsystems of the immune system:

- **Innate immune system** provides a preconfigured response to broad groups of situations and stimuli
- **Adaptive immune system** provides a tailored response to each stimulus by learning to recognize molecules it has previously encountered

Both use molecules and cells to perform their functions.

2.1.1 T Cells

T cells are so called because they are predominantly produced in the thymus, where they develop and then egress, moving to secondary lymphoid organs (SLO: spleen and **lymph nodes**). They recognise foreign particles (antigen) by a surface expressed, highly variable, T cell receptor (TCR). Unlike antibody, the TCR cannot bind antigen directly. Instead it needs to have broken-down peptides of the antigen 'presented' to it by an antigen presenting cell (APC). Professional antigen-presenting cells (APC) are primarily dendritic cells, macrophages and B cells.

The molecules on the APC that present the antigen are called major histocompatibility complexes (MHC). There are two types of MHC: MHC class I and MHC class II.

There are also two major types of T cells: the helper T cell (also called CD4+ T Cells) and the cytotoxic T cell (also called CD8+ T Cells). As the names suggest helper T cells 'help' other cells of the immune system, whilst cytotoxic T cells kill virally infected cells and tumours. MHC class I presents to cytotoxic T cells; MHC class II presents to helper T cells. [Hus]

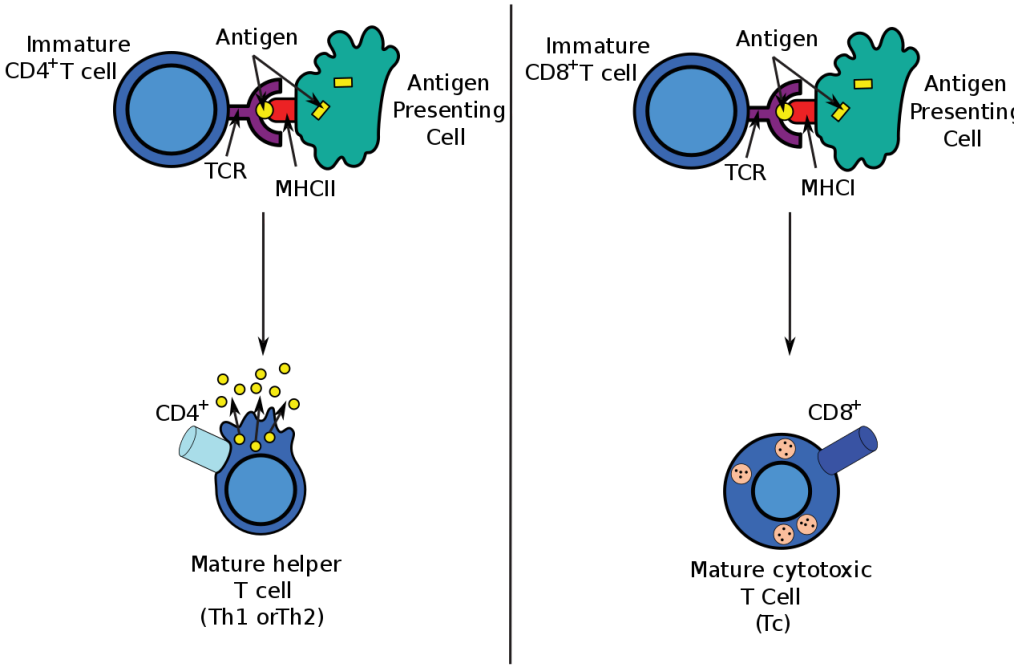

Figure 2.1: T Cells differences and activation [Com20].

CD4+ T Cells

Helper T Cells (also called CD4+ T Cells) 'help' other cells of the immune system by releasing cytokines, small protein mediators that alter the behavior of target cells that express receptors for those cytokines [Wik20c]. These cells are so called because they express the surface protein CD4. Upon activation they are able to differentiate themselves (via autocrine signaling) into either Th1, Th2, Regulatory T cells and other kinds of cells.

Activation of naive helper T cells The activation of naive T cells is commonly explained in terms of the 3-signal model, which means that it has to undergo 3 phases in order to be activated.

- **Activation (signal 1):** During an immune response, professional antigen-presenting cells (APCs) endocytose antigens (typically bacteria or viruses), which undergo processing, then travel from the infection site to the lymph nodes. Typically, the APC responsible is a dendritic cell. Once at the lymph nodes, the APCs begin to present antigen peptides that are bound to Class II MHC, allowing CD4+ T cells that express the specific TCRs against the peptide/MHC complex to activate. [Wik20c] [Cav]
- **Survival (signal 2):** Having received the first signal, the naïve T cell must activate a second independent biochemical pathway, known as Signal 2. This verification step is a protective measure to ensure that a T cell is responding to a foreign antigen. Once the naïve T cell has both pathways activated, it can activate instead of undergoing anergy. The second signal is then obsolete; only the first signal is necessary for future activation. This is also true for memory T cells, which is one

example of learned immunity. Faster responses occur upon reinfection because memory T cells have already undergone confirmation and can produce effector cells much sooner. [Wik20c] [Cav]

- **Differentiation (signal 3):** Once the two-signal activation is complete the T helper cell then allows itself to proliferate. It achieves this by releasing a potent T cell growth factor called interleukin 2 (IL-2) which acts upon itself in an autocrine fashion. The Th cells receiving both signals of activation and proliferation will then become Th0 cells (T helper 0) cell that secrete IL-2, IL-4 and interferon gamma (IFN-γ). The Th0 cells will then differentiate into Th1 or Th2 cells depending on cytokine environment. Interferon gamma drives Th1 cell production while IL-10 and IL-4 inhibit Th1 cell production. Each one of these cells performs a specific task in the tissue and in developing further immune responses. [Wik20c] [Cav]

The resulting cell population moves out to the site of the infection or inflammation in order to deal with the pathogen. Other cells present at the tissue site of inflammation– such as neutrophils, mast cells, and epithelial cells – can also release cytokines, chemokines, short peptides and other molecules which induce further activation and proliferation of the T cells [Cav].

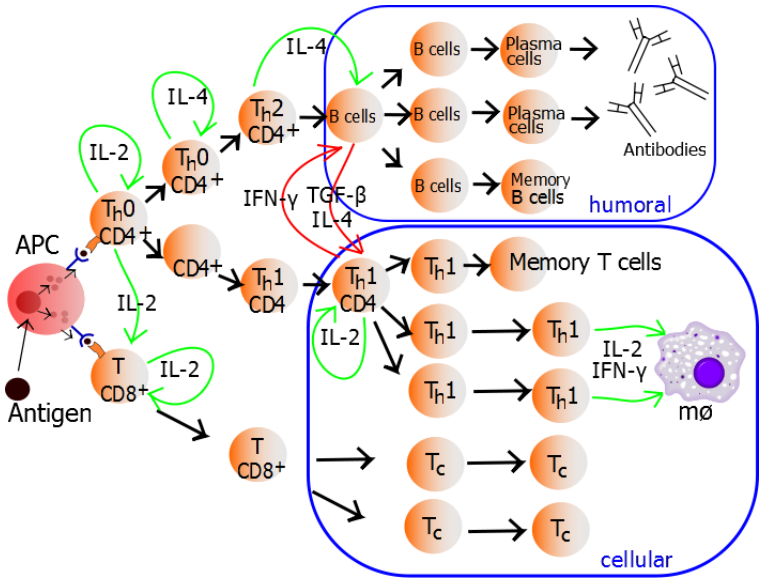

Figure 2.2: Differentiation of CD4+ T cells

Th1/Th2 dichotomy:

Th1 cells: Th1 cells mostly produce IFN-γ, interleukin (IL)-2, and TNF-β. [Rom99] These cytokines are able to activate and maximize the killing efficacy of the macrophages and the proliferation of CD8+ T Cells. [Wik20c]

This kind of Helper T Cells also enhance the production of opsonizing, and some kind of antibodies involved in antibody-dependent cell cytotoxicity. [MC89] [Pre+91]

	Type 1/T_h1	Type 2/T_h2
Main partner cell type	Macrophage, CD8+ T cell	B-cell, eosinophil, mast cell
Immune stimulation promoted	Cellular immune system. Maximizes the killing efficacy of the macrophages and the proliferation of cytotoxic CD8+ T cells. Also promotes the production of IgG, an opsonizing antibody.	Humoral immune system. Stimulates B-cells into proliferation, to induce B-cell antibody class switching, and to increase neutralizing antibody production (IgG, IgM and IgA as well as IgE antibodies).

Table 2.1: Th1/Th2 dichotomy

Th2 cells: Th2 cells are responsible for helping the humoral immune response enhancing mast cell differentiation, B Cells activation and eosinophil growth and differentiation factors.

Other than this they are responsible for phagocyte-independent protective responses. In fact Th2 cells play an important down-regulatory role in dampening phagocyte-dependent responses when they become dangerous for the host (for example in chronic inflammatory responses). [Rom99]

Treg cells: Regulatory T cells (also called Tregs) are T cells which have a role in regulating or suppressing other cells in the immune system. Tregs control the immune response to self and foreign particles (antigens) and help prevent autoimmune disease. Tregs produced by a normal thymus are termed 'natural'. Treg formed by differentiation of naïve T cells outside the thymus, i.e. the periphery, or in cell culture are called 'adaptive'.

Tregs suppress activation, proliferation and cytokine production of CD4+ T cells and CD8+ T cells, and are thought to suppress B cells and dendritic cells. [Mon]

CD8+ T Cells

CD8+ T Cells (also called Cytotoxic T Cells) recognise peptides presented by MHC Class I molecules. Antigens are presented to the MHC Class I molecule during T cell/antigen presenting cell interactions. This means that APC cells – like Dendritic cells, Macrophages, ecc. . . – are able to activate this kind of T Cells.

CD8+ T Cells are very important for the immune response against intracellular pathogens and for tumour surveillance. [Wis]

When a CD8+ T cell recognises its antigen and becomes activated, it has three major mechanisms to kill infected or malignant cells.

- **Secretion of Cytokines:** The first is secretion of cytokines, primarily TNF-α and IFN-γ, where the latter activates macrophages. [Wis]
- **Secretion of Cytotoxic Granules:** The second major function is the production and release of cytotoxic granules. These granules, also found in NK cells, break

through the membrane of the target cell and shut the production of viral proteins (triggering the caspase cascade), resulting in apoptosis of the target cell. The cytotoxic granules are released only in the direction of the target cell, to avoid damage to healthy surrounding tissue. [Wis]

- **Destruction via Fas/FasL interactions:** The third major function of CD8+ T cell destruction of infected cells is via Fas/FasL interactions. Activated CD8+ T cells express FasL on the cell surface, which binds to its receptor, Fas, on the surface of the target cell. This binding causes the activation of a series of interactions that leads to the activation of the caspase cascade, which also results in apoptosis of the target cell. Because CD8+ T cells can express both molecules, Fas/FasL interactions are a mechanism by which CD8+ T cells can kill each other, called fratricide, to eliminate immune effector cells during the contraction phase at the end of an immune response. [Wis]

CD8+ T cells are able to release their granules, kill an infected cell, then move to a new target and kill again, often referred to as serial killing. [Wis] This is also possible because CD8+ T Cells can induce infected cells to release a protein that is able to stimulate the activation of the CD8+ T Cell itself.

CD8+ T Cell Activation The presentation of the antigen by MHC Class I molecule to the T cell antigen receptor (TCR) on CD8+ T cells is not enough to activate this kind of cell.

In fact we have to consider the 2-signal model for CD8+ T cell activation.

- **First Signal:** The antigen is presented by APC's MHC Class I molecule to the TCR on CD8+ T cells.
- **Second Signal:** some APC's proteins (CD80 or CD86) are presented to a specific molecule (CD28) on the Cytotoxic T Cell. This proteins are costimulators and help the T cell to activate. This second signal can be assisted or replaced by stimulating the CD8+ T cell with cytokines released from T helper cells.

A simple activation of naive CD8+ T cells requires the interaction with professional AP cells, mainly with matured dendritic cells. [Wik20b] To generate longlasting memory T cells and to allow repetitive stimulation of cytotoxic T cells, dendritic cells have to interact with both, activated CD4+ helper T cells and CD8+ T cells. [Hiv+12] [Hoy+14] During this process, the CD4+ helper T cells "license" the dendritic cells to give a potent activating signal to the naive CD8+ T cells. [Lan98]

Furthermore, maturation of CD8+ T cells is mediated by CD40 signalling. [Ben+98] Once the naïve CD8+ T cell is bound to the infected cell, the infected cell is triggered to release CD40. [Ben+98] This CD40 release, with the aid of helper T cells, will trigger differentiation of the naïve CD8+ T cells to mature CD8+ T cells. [Ben+98]

CD4+/CD8+ Ratio

The CD4+/CD8+ ratio in the peripheral blood of healthy adults and mice is about 2:1, and an altered ratio can indicate diseases relating to immunodeficiency or autoimmunity [Wik20a]. An inverted CD4+/CD8+ ratio (namely, less than 1/1) indicates an impaired immune system. [MS17] [Aie+19] [Tur16]

Obesity and dysregulated lipid metabolism in the liver leads to loss of CD4+, but not CD8+ cells. Regulatory CD4+ cells decline with expanding visceral fat, whereas CD8+ T-cells increase. [Kr14]

2.1.2 Mast Cells

Mast Cell is a migrant cell of connective tissue that contains many granules: specifically, it is a type of granulocyte derived from the myeloid stem cell that is a part of the immune and neuroimmune systems.

Mast Cells are best known for their activity in allergic reactions, but they have been involved in different physiological and pathological conditions, like in renal cell carcinoma where they can promote angiogenesis.

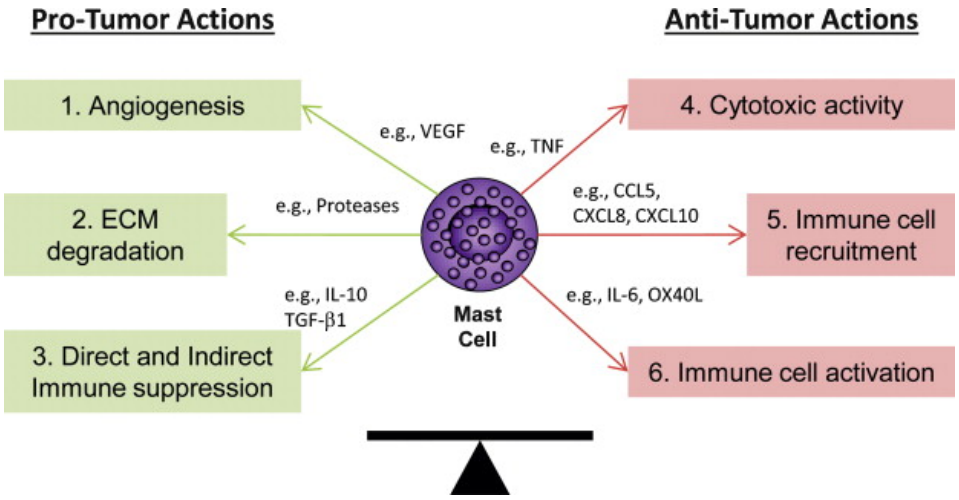

Figure 2.3: Mast cell effects

Mast Cells may mobilize and modulate the activity of T cells, regulatory T (Treg) cells, antigen-presenting cells (APC) and myeloid-derived suppressor cells (MDSCs) with their soluble mediators or through cell-cell contact.

Interacting with other immune cells, Mast Cells may regulate both innate and adaptive immunity, tuning the host responses toward developing cancers and influencing the clinical outcome of several tumors.

During degranulation, Mast Cells release a large variety of mediators that could intervene in the immune response in an unpredictable manner:

- **Heparin**
- **Histamine**
- **Cytokines**
- **Adenosine triphosphate**

Also, the context and the timing of Mast Cells activation play a fundamental role on the positive or negative effect of Mast Cells on tumor growth and progression [Wik].

2.1.3 Dendritic Cells

Dendritic cells (DCs) are responsible for the initiation of adaptive immune responses and hence function as the sentinels of the immune system. DCs are bone marrow (BM)-derived leukocytes and are the most potent type of antigen-presenting cells. They can also be propagated in vitro from BM and blood using various combinations of growth factors.

Since DCs have numerous cytoplasmic processes, they have a high surface area permitting intimate contact with a large number of surrounding cells, e.g. T cells, natural killer cells, neutrophils, epithelial cells etc. For instance only one mature DC (mDC) is required to stimulate 100–3000 T cells.

DC precursors migrate from the BM through the blood stream to almost every non-lymphoid tissue, where they reside in an immature state (iDC).

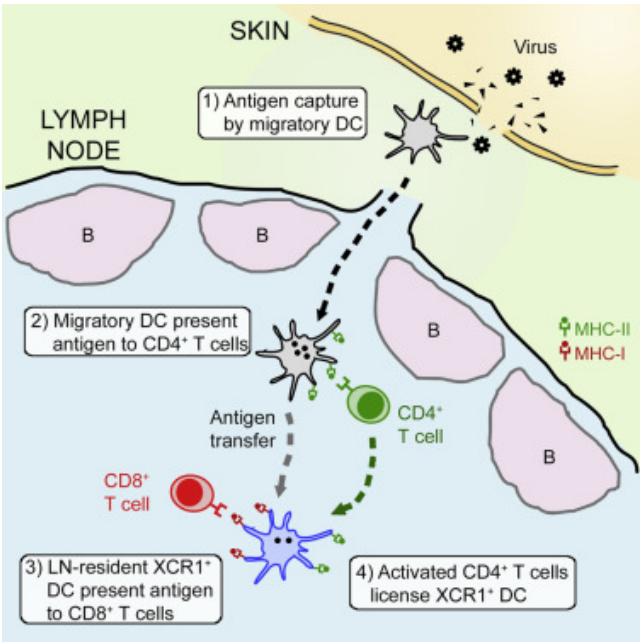

Figure 2.4: Dendritic cell moving into Lypmh node

During pathogen invasion, resident iDCs detect intruders via pattern recognition receptor (e.g. TLRs) capture antigens and quickly leave the tissue. They crawl through the cells, cross the endothelium of lymphatic vessels and migrate to the draining lymph nodes (LN).

During their migration from the peripheral tissues, DCs undergo phenotypical and functional maturation. After reaching the subcapsular sinus of the LN, DCs move to T-cell zones. Here, the interdigitating DCs are actively involved in the presentation of antigens to T cells [Rog].

Plasmacytoid Dendritic Cells

Plasmacytoid dendritic cells (pDCs) are a rare type of dendritic immune cell that are known to secrete large quantities of type 1 interferon (IFNs) in response to a viral infection. They circulate in the blood and are found in peripheral lymphoid organs.

Upon stimulation and subsequent activation of Toll-like receptor, these cells produce

large amounts of type I interferon, which are critical anti-viral compounds mediating a wide range of effects and induce maturation of the pDC.

For example, the secretion of type 1 interferon α triggers natural killer cells to produce IFNγ while also activating the differentiation of B cells. In addition, they can produce cytokines as well, helping to recruit other immune cells to the site of infection.

Other than conducting antiviral mechanisms, they are capable of activating other immune cells thus they serve as a bridge between innate and adaptive immunity [Pla].

2.1.4 Natural Killer Cells

Natural Killer (NK) Cells are lymphocytes in the same family as T and B cells, as cells of the innate immune system they are classified as group I Innate Lymphocytes (ILCs) and respond quickly to a wide variety of pathological challenges.

NK cells are best known for protecting against disease and killing virally infected cells, and detecting and controlling early signs of cancer.

NK cells kill tumour cells without any priming or prior activation (in contrast to cytotoxic T cells). They are named for this natural killing. Additionally they secrete cytokines such as IFN-γ and TNF-α, which act on other immune cells like Macrophage and Dendritic cells to enhance the immune response.

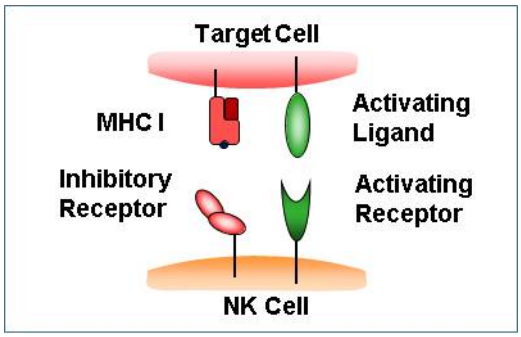

Figure 2.5: NK cell receptors

Whether or not the NK cell kills these cells depends on a balance of signals from activating receptors and inhibitory receptors on the NK cell surface.

Activating receptors recognise molecules that are expressed on the surface of cancer cells and infected cells, and switch on the NK cell. Inhibitory receptors act as a check on NK cell killing.

Most normal healthy cells express MHC I receptors which mark these cells as self. Inhibitory receptors on the surface of the NK cell recognise cognate MHC I, and this switches off the NK cell, preventing it from killing. Cancer cells and infected cells often lose their MHC I, leaving them vulnerable to NK cell killing.

Once the decision is made to kill, the NK cell releases cytotoxic granules containing perforin and granzymes, which leads to lysis of the target cell [Eis].

2.1.5 Macrophage

Macrophages are specialised cells involved in the detection, phagocytosis and destruction of bacteria and other harmful organisms [Mac].

The way they interact with the immune system depends on their polarization:

- **M1** promote inflammation and immune response (they are activated by the release of IFN-γ and once active release IFN-γ and TNF-α)
- **M2** suppress inflammation and immune response (they are activated by the release of IL-10 and TGF-β and once active release IL-10, TGF-β, and TNF-α

2.1.6 Immune Cells Percentage

Some percentage of Immune Cells within the peripheral blood in healthy adults [mil]:

Cell type	Percentage among PBMCs (range in healthy individuals)
CD4+ T cells	25%–60%
CD8+ T cells	5%–30%
B cells	5%–10%
NK cells	10%–30%
Monocytes	5%–10%
Dendritic cells	1%–2%

Table 2.2: Immune Cells Percentage

2.2 Not Immune

They are cells that are not part of the immune system.

2.2.1 Tumor - Renal cell carcinoma

Tumor indicates a mass of tissue that grows in excess and uncoordinated with respect to normal tissues, and which persists in this state after the cessation of the stimuli that induced the process.

Tumor cells are cells whose DNA has been modified, resulting in a change of the cell's behavior.

Tumors need a dedicated blood supply to provide the oxygen and other essential nutrients they require in order to grow. Tumors induce blood vessel growth (**angiogenesis**) by secreting various growth factors and proteins. Unlike normal blood vessels, tumor blood vessels are dilated with an irregular shape.

A tumor cell can also implement strategies for not being recognized by the immune system's cells: for instance, it can hides MHC class I molecules. In This way, cells of the immune system treat a tumor cell as a self one and no action is performed against it.

2.2.2 Adipocyte

Adipocytes, also known as lipocytes or fat cells, are the cells that primarily compose adipose tissue, specialized in storing energy as fat [Fat].

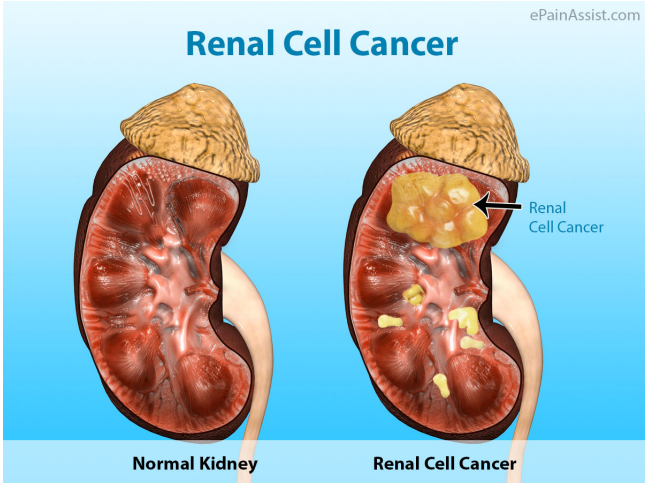

Figure 2.6: Representation of renal cell carcinoma

Adipocytes are derived from mesenchymal stem cells which give rise to adipocytes through adipogenesis. Adipocytes can also form osteoblasts, myocytes and other cell types.

In obese patients these cells are present in greater quantities.

3. Implementation

In this chapter we will present how we implemented the cells described above and how we represented the environment.

The system is not deterministic:

- Random positioning of agents
- Agents may expose a random variety of effects
- Agent actions have a probability of success/failure

The Random Number Generator depends on a seed value, it is taken as input.

3.1 Environment

The environment where the agents act is represented both by a 2-dimensional and a 3-dimensional grid.

It is mainly characterized by the Body Mass Index (BMI): its value (higher in obese individuals and lower in lean ones) will change the initial state of the model (the number of cells) and also the behavior of the agents.

The environment is also characterized by one blood vessel in order to simulate the tumor angiogenesis.

3.1.1 Body Mass Index - BMI

The body mass index (BMI) is a biometric data, expressed as the ratio between weight and square of the height of an individual and is used as an indicator of the state of ideal weight.

It is the main factor to be taken into account in this project because its value affects the number and proportion of initial immune cells:

- More Dendritic and Plasmacytoid Dendritic Cell and Mast Cell in obese individuals
- More Natural Killer Cell in non-obese individuals
- Increased numbers of M1 Macrophage and T Cells in perinephric fat in obese individuals

Its value could also change the effects of some immune cells.

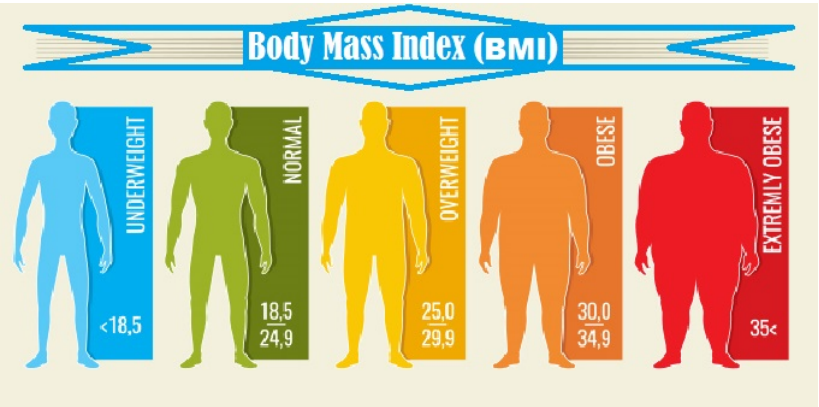

Figure 3.1: Representation of the scale of the body mass index

3.1.2 Blood Vessel

The blood vessels are the components of the circulatory system that transport blood throughout the human body.

These vessels transport blood cells, nutrients, and oxygen to the tissues of the body. They also take waste and carbon dioxide away from the tissues.

Blood vessels are needed to sustain life, because all of the body's tissues rely on their functionality.

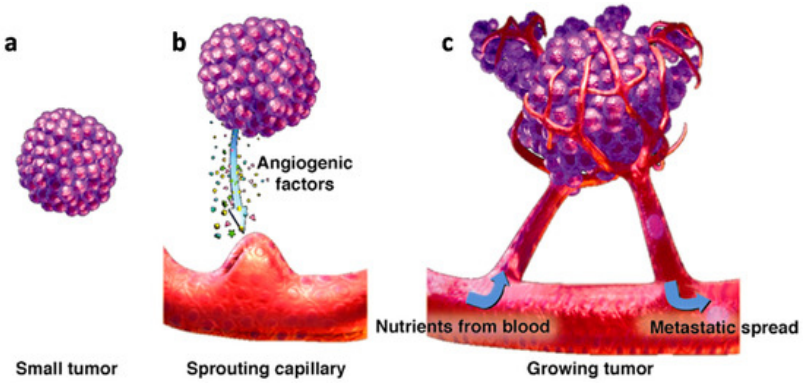

Figure 3.2: Tumor angiogenesis

The blood vessel can be the target of tumour angiogenesis, in which case tumour grows in an uncontrolled way.

3.1.3 Lymph Node

Organ of the lymphatic adaptive immune system and major sites of lymphocytes that include B and T cells.

Lymph nodes are important for the proper functioning of the immune system, acting as filters for foreign particles including cancer cells, but have no detoxification function.

Once the Dendritic cell has reached the Lymph Node, carrying the antigen with it, if there is a T cell with the appropriate T cell receptor, it will be activated.

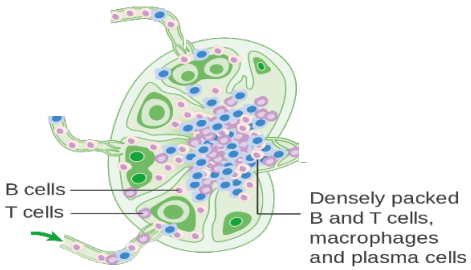

Figure 3.3: Lymph Node representation

3.2 Agents

In this case the agents are mainly represented by tumor cells and cells of the innate immune system and the adaptive immune system.

There are also other cells which have been useful in modeling the behavior of the immune system and which we will discuss briefly.

3.2.1 Tumor - RCC

This class represent a Renal Cell Carcinoma: the main behaviour is duplication, which can be increased by having access to the blood vessel (angiogenesis).

Through input parameters, we can decide the number of ticks to wait for the next tumor expansion and how much it can expands (”reproTime” and ”reproFactor” variables).

The main method ”grow()” check if the time to expand has arrived or if there is a Mast Cell that is pro-tumor in its neighborhood: if one of these conditions is satisfied, the method ”reproduce()” is invoked to create new tumor cells, depending on ”reproFactor”.

A tumor cell implement also a strategy for not being recognized by the immune system’s cells: when a tumor grows it can mutate and with a certain probability it changes its ”self” variable to true. This behavior simulates the hiding of MHC class I molecules. In This way immune system’s cells treat a tumor cell as a self one and no action is performed against it.

Another characteristic of the tumor is to disable the effects of Tcells, and this is done by setting their ”active” variable to false, with a certain probability.

3.2.2 Cell

Cell class is the root of all the hierarchical structure of classes. This class specifies the lifespan of a cell, the current age and the fact that it can die. Moreover, it specifies if the cell is foreign or not through the attribute ”self”.

3.2.3 NotImmune

NotImmune class is just a “tag” to recognize cells that are not part of the immune system.

3.2.4 Immune

Immune class is a “tag” to recognize cells that are part of the immune system.

It is characterized by the ”active” attribute (false by default) which is used to check if the cell’s effect can be activated.

When it is not active, a cell by default executes the ”actIfNotActive()” method, looks for a tumor that is close to it and eventually becomes active. Instead, when it is active, each immune cell has a different behaviors or effects and has to override the method ”actIfActive()”.

By default, each immune cell implemented a random movement to a nearby empty cell.

3.2.5 Tcell

Tcell class is just a ”tag” to represent those cells that are part of the T-cell family.

3.2.6 CD8 Cell

Like all other immune cells, this cell wander within the grid until either it is activated by an APC cell or until it bumps into an RCC cell. Upon activation it moves towards the nearest RCC cell that is able to detect (not self).

When it reaches the RCC cell it has a certain probability to destroy the malignant cell.

3.2.7 CD4 Cell

This is the representation of a CD4+ Naive T Cell. This kind of cell can be activated from any APC, such as Dendritic Cells, Macrophages, B cells and so on.

Upon activation this cell can only differentiate either into Th1 or Treg.

3.2.8 Th1 Cell

This cell represent one of the differentiation of a CD4+ T Cell, a Th1 Cell. They release IFN-γ and TNF-β, the first triggers the activation of macrophages and the second triggers the activation of CD8+ T Cell. This has been simulated (implemented) defining 2 methods:

- **releaseIFNGamma:** simulates the release of IFN-γ by activating all the macrophages that live within a certain radius of distance from the actual Th1.
- **releaseTNFBeta:** simulates the release of TNF-β by activating all the CD8+ T cells that live within a certain radius of distance from the actual Th1.

The radius of distance is calculated based on the dimension of the grid.

After the release of the cytokines this cell is deactivated and moves randomly like any other immune cell.

3.2.9 Th2 Cell

We have not provided an implementation for Th2 cells because they express a range of cytokines that influence B-cell differentiation and more in general, they mostly influence cells that we have not implemented.

In fact, Th2 cells are mostly useful in case of parasites infection.

Nonetheless, since they are still part of the immune system and they could affect other cells, this kind of cells should be implemented in future developments.

3.2.10 Treg Cell

Tregs suppress activation, proliferation and cytokine production of CD4+ T cells, CD8+ T cells and other immune cells.

They do this by secreting TGF-β that suppress proliferation and differentiation, and similarly IL-10 that is able to suppress cytokine synthesis of IFN-γ, TNF-α and other cytokines, produced by cells like macrophages and some kind of T helper cell.

This has been simulated (implemented) defining 2 methods:

- **releaseTGFbeta:** deactivates and decreases the growth factor of every T Cell that live within a certain radius of distance from the actual Treg Cell. In the same way deactivates also M2 cells.

- **releaseIL10:** deactivates every M1, M2, Th1, Dendritic and Plasmacytoid Dendritic Cell that live within a certain radius of distance from the actual Treg Cell.

3.2.11 NK Cell

If this innate immune cell is not active, it moves randomly by default in search of a tumor cell.

When a natural killer cell encounters a tumor it becomes active and tries to kill it with a certain probability ("killProb" taken as input), regardless of the presence of the MHC molecule.

They are also able to stimulate the activation of macrophages and dendritic cells by setting their "active" variable to true, simulating the release of IFN-γ and TNF-α.

3.2.12 Dendritic Cell

When it is not mature, this cell moves randomly by default in search of a tumor cell.

Instead it moves in the direction of the lymph node (outside the grid) to present the antigen once it has discovered a tumor cell and has become mature. On the way to the lymph node, if it encounters a tcell it is also able to activate its effect.

When the dendritic cell is on the edge of the grid, to simulate the reaching of the lymph node and the release of the Tcells, a number of CD8 and CD4 are introduced into the grid (it depends on their ratio).

After the release of the tcells, the dendritic cell becomes immature and starts to move again randomly.

Plasmacytoid Dendritic Cell

This cells behave in the same way as dendritic cells, but they are also able to activate new natural killer cells, to simulate the release of type 1 interferon α.

3.2.13 Macrophage Cells

Macrophages move randomly and phagocytate nearby Dead Cells.

Only M1 and M2 polarization are implemented, and are themselves a simplified view of macrophage polarization.

M1 Cell

M1-polarized macrophages are activated by the release of IFN-γ and once active release IFN-γ and TNF-α, stimulating the activation of NK cell, Dendritic cell and other M1 by setting their "active" variable to true.

M2 Cell

M2-polarized macrophages are activated by the release of IL-10 and TGF-β and once active release IL-10, TGF-β, and TNF-α, inhibiting the activation of TCell, Dendritic and M1 by setting "active" to false and stimulating other M2.

3.2.14 Mast Cell

Mast cells can move randomly in search of cancer cells.

When it is close to a tumor cell, it can have either a pro-tumor or anti-tumor effect: if it is pro-tumor cell it promotes tumor growth, otherwise it can activate the effect of nearby Tcells, to simulate the release of mediators in its granules.

3.2.15 Lymph Node

Class used to represent a Lymph node, where there are the Tcells that must be released once the Dendritic cell has reached it, carrying the antigen with it.

It is represented as an agent that is outside the grid, in particular it is placed in one of these points: above/below/left/right the grid.

3.2.16 Adipocyte

Adipocyte class is a dummy agent, just a placeholder for cells (fat) that composes the kidney itself and they do not perform any action.

3.2.17 Blood Cell

This class is used to represent the blood: in particular it is used to depict only one blood vessel that is directly modeled in the environment (the other ones are supposed to be outside the grid).

These cells can be the target of tumour angiogenesis, in which case tumour growth is increased and new blood cells are created.

3.2.18 Dead Cell

DeadCell class is used to represent a dead cell and it can not perform any action.

A cell reaches the dead state if it has exceeded its maximum age or if it has been killed by an immune cell in order to simulate apoptosis effect.

3.2.19 Empty Cell

This class was simply implemented to simulate empty spaces and to allow cells to move around.

4. Repast

As said before, the project was developed using Repast Simphony 2.8, a Java-based modeling system for ABM and simulation.

4.1 Why Repast?

Our first experience with Agent-Based Modelling was done in NetLogo implementing the famous Game Of Life by John Conway. While NetLogo can be a user-friendly platform to start with, it starts showing less flexibility as the project complexity increases; for this reason we decided to use Repast Simphony to develop our project.

As well as for NetLogo, we also started with the same Game Of Life example in Repast. In this case we decided to take a look at the implementation of the game that was already present in the Repast Simphony Models Repository, to get the hang of how the environment and the agents are modelled.

We then looked at how agents can be placed in the environment, how they can interact with each other and with the environment itself and how different types of environment can be shaped.

In all these cases and more, Repast has proved to be pretty flexible as we kept adding more and more complexity.

The next step was modelling the same Game Of Life in a three-dimensional grid and understanding how much effort was needed to do that; we found out that most of the code remained unchanged.

From this point on, we moved on modelling the Obesity Paradox in a two-dimensional grid and then eventually adapt it to a three-dimensional grid environment.

After successfully implementing the project in 2D and adapting it to 3D, we decided to put all the modelled cells and some utility class in a common package and the 2D and 3D environments in different packages.

We also created two different scenarios and for each scenario we created a folder with all the configuration files needed for the context, displays, dataset loaders, parameters initialization and so on.

At the end, we played around with the context creator and the batch parameters in order to make batch runs; in particular we made each run end after 100 ticks and we launched 100 runs for each value of BMI. Finally, we took the model that was created (a CSV file) and we imported it into Excel to create meaningful graphs.

4.2 Project Structure

The project has been structured using Java packages, as in figure 4.1.

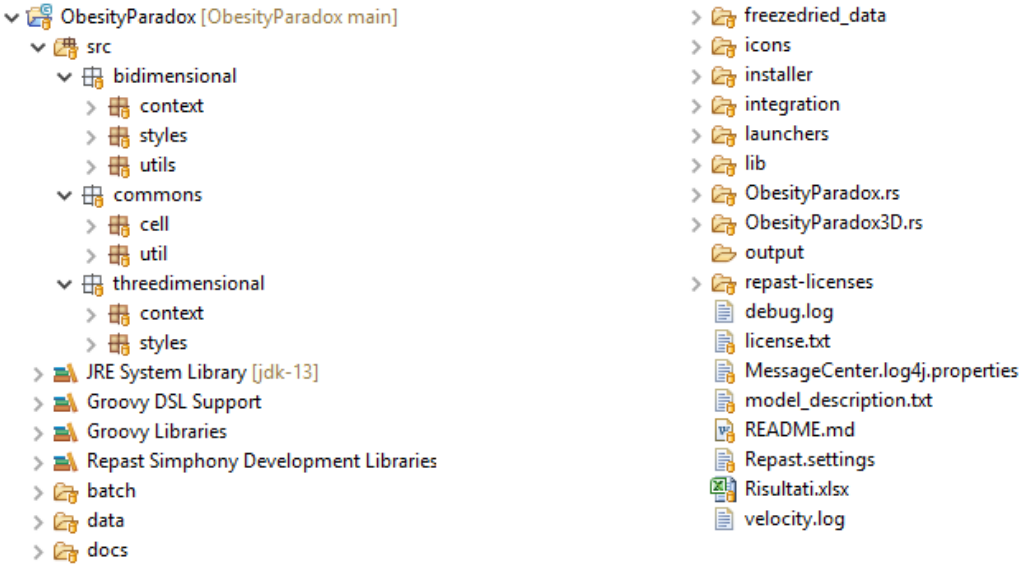

Figure 4.1: Project Structure

From the figure 4.1 we can find what we just said:

- **commons package:** containing all the agent cells and utility functions such as the methods *getNeighbors*, *moveCell*, *replaceCell* and so on
- **two-dimensional and three-dimensional packages:** containing the context creators to respectively create a 2d or 3d environment and the styles that can be applied to the cells
- **ObesityParadox.rs and ObesityParadox3D.rs folders:** containing the scenario definition and the configurations files needed that will be loaded based on the launcher used
- **batch folder:** containing two XML files that will be used to define the parameters for the batch runs

4.3 Environment

The environment wasn't implemented as a single Java Class but was represented as a set of parameters the user can choose the value of:

- **BMI:** an integer between 15 and 40. The number and percentage of initial Cells depend on this value
- **Blood Vessel:** represented as a set of Blood cells positioned in one of the possible orientations of the grid (north / south / west / east or front / back / up / down / left / right)
- **Lymph Node:** represented as a direction towards which some cells will move when active (north / south / west / east or front / back / up / down / left / right)

4.3.1 Parameters

There are also other parameters that influence the proliferation of the tumor and the immune cells and the size of the grid:

- **Width, Height (and Depth):** the length of the dimensions of the grid
- **Mutation Percentage:** the probability of tumor mutation, to simulate the fact that it can hide from the immune system
- **Reproduction Time:** number of ticks to wait for the next tumor expansion
- **Reproduction Factor:** how many copies of itself the tumor creates when reproducing
- **Disable TCell Percentage:** the probability for the tumor to disable nearby TCells
- **Immune Cell Growth:** percentage of the lifespan of an immune cell after which the cell will reproduce

4.3.2 Grid

Grids are a mainstay of agent-based simulation and they can be used to simulate spaces and to create highly structured relationships between agents.

Grids are a 1 or more dimensional data structure (n-dimensional matrix) that is divided up into a number of cells. These cells can be referenced by their integer coordinates.

In our project we decided to implement both a 2-dimensional and a 3-dimensional grid in which the agents can move and interact with each other.

Based on the kind of grid we take into consideration, we can have different type of behaviours and interactions between agents and with the environment itself.

However, both 2D and 3D grids share some similarities:

- **Border Rule:** we chose to use the Sticky Borders rule, which is a border implementation where translations across the border limits are clamped to the border coordinates (e.g. can't move outside of the grid)
- **One agent per cell:** in any given moment, in any cell, there can be at most only one agent
- **Moore Neighborhood:** we used the Moore Neighborhood to express the set of nearby cells an agent can usually interact with

4.3.3 Interaction

Different agents and effects may have different ways to interact with the environment and the other agents in the grid, so we implemented three main types of interaction:

- **Nearby Cells:** as aforementioned, we chose to use Moore Neighborhood over von Neumann Neighborhood, because it allows for more possible interactions, as shown in figure 4.2

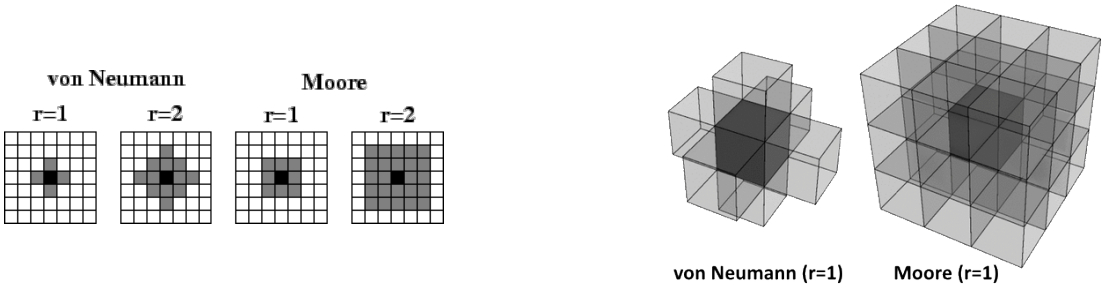

Figure 4.2: von Neumann and Moore Neighborhoods

- **Radius:** an agent or an effect may influence all the cells within a certain radius of action, for example the activation or the inhibition of a certain type of agents, as when mediators or other substances are released. The calculation of the value of the radius is fixed, but the value itself changes based on the size of the grid, according to the following formula:

$$radius = \max(2, \frac{\min(width, height[, depth])}{5}) \tag{4.1}$$

- **The Whole Grid:** an agent or an effect may depend on or influence the whole grid (e.g. spawn new cells, move towards the nearest tumor cell, etc. . .)

4.4 Context Creator

The Context Creator class is what is used in Repast Simphony to build a Context by adding projections, agents and so forth. In our case, in this Java class, we access to the parameters that are defined in the XML configuration file in our scenario folder and based on their values we act accordingly.

In general, we first create a grid with the specified number and length of dimensions, we then proceed to check the blood vessel and the lymph node orientation and make sure they are not the same, otherwise we stop the simulation. Later we create the set of blood cells, so that other cells won't spawn in their places.

After that, we spawn a certain number and types of cells based on percentages and whether they are part of the immune system or not.

As we said, the BMI is the most important factor in the Obesity Paradox, so most of the percentages of cells to spawn vary depending on it, in accordance to two formulas:

- **limitVariableToBMI:** returns a variable that is within the range [minPerc, maxPerc] that is directly related with the given BMI value (e.g. the number of adipocytes increases as the BMI increases)

$$limitedVariable = maxPerc - \frac{40 - bmi}{40 - 15} \cdot (maxPerc - minPerc) \tag{4.2}$$

- **inverseLimitVariableToBMI:** returns a variable that is within the range [minPerc, maxPerc] that is inversely related with the given BMI value (e.g. the number of natural killers decreases as the BMI increases)

$$inverselyLimitedVariable = minPerc + \frac{40 - bmi}{40 - 15} \cdot (maxPerc - minPerc) \tag{4.3}$$

We also take into consideration other factors:

- Apart from blood cells, all the other cells are randomly put in the grid when created
- The number of immune cells can't be more than 10% of the grid's size at tick 0
- The number of each immune cell depends on BMI or on CD4/CD8 ratio
- Renal cell carcinomas occupy 2% of the grid's size at tick 0
- Most actions or effects happen with a certain probability, like NK and CD8 kill probability, which are internally set and may or may not change over time

At the end, we fill the remaining grid spots with empty cells, to allow for movement and replacement of cells.

4.5 Cell Agent

For the modelling of the cells, we used a hierarchical approach, given all the advantages that an Object-Oriented programming language like Java provides.

We created an abstract class called Cell, that all the other cells extend. In this class we put some common properties and methods, like the lifespan, the age of the cell and whether the cell is foreign or not.

A cell can also be either an Immune Cell or a Not Immune Cell. The Not Immune Cell is used more as a tag than as an abstract class with functionalities in common, while the Immune Cell class provides some default implementation for methods like move, proliferate, act.

The scheduling of these and other methods is done via the @ScheduledMethod annotation and a global clock, measured in ticks. We decided to schedule methods like move, act, and others to run once every tick, but we used different priorities to manage the order of execution (higher priority = earlier execution).

Doing things this way, we avoided a lot of duplicated code and we were able to focus on the single behaviours and interactions to implement.

4.6 Cell Style

Reading through the Repast Symphony Reference Manual and looking at some of the example in the Repast Models Repository, we experimented with different styling options and displays for agent visualization:

- **2D display with cells represented as circles:** for each kind of cell, we created an XML file to define the shape and the color of each cell, then in the display XML configuration file, we associated each kind of cell with the corresponding stylesheet (figure 4.3)
- **2D display with an image for each cell type:** for each kind of cell, we created a Java class to define the image to use for visualization, then in the display XML configuration file, we associated each kind of cell with the corresponding style class (figure 4.4)

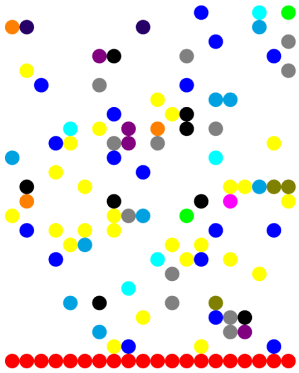

Figure 4.3: 2D Grid, 2D representation of agents with simple circles

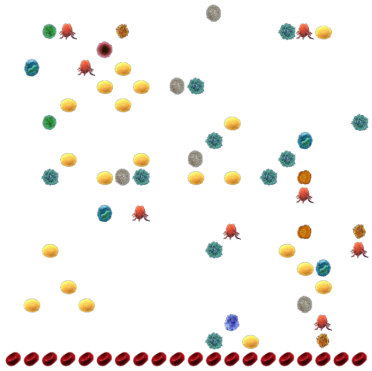

Figure 4.4: 2D Grid, 2D representation of agents with images

- **3D display with cells represented as sphere:** for the three-dimensional visualization, we created a single Java class that based on the type of Cell, load the correct color for the sphere and in the display XML configuration file, we associated the Cell class with this newly created style class, so that the single style class can decide which color to assign (figure 4.5)

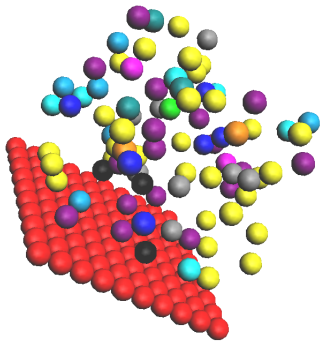

Figure 4.5: 3D Grid, 3D representation of agents with spheres

We decided to use all these three styles of visualizations, but while the first two can be seen when we launch the project with the ObesityParadox scenario folder as an argument, the third one can be seen with the ObesityParadox3D folder argument.

4.7 Time Series Chart

Other than visualization styles and displays, Repast Symphony allows for the creation of Time Series Chart and more. In this case we created only one chart containing all the cells in the grid at each tick, to better see how the environment evolves, but the possibilities are endless.

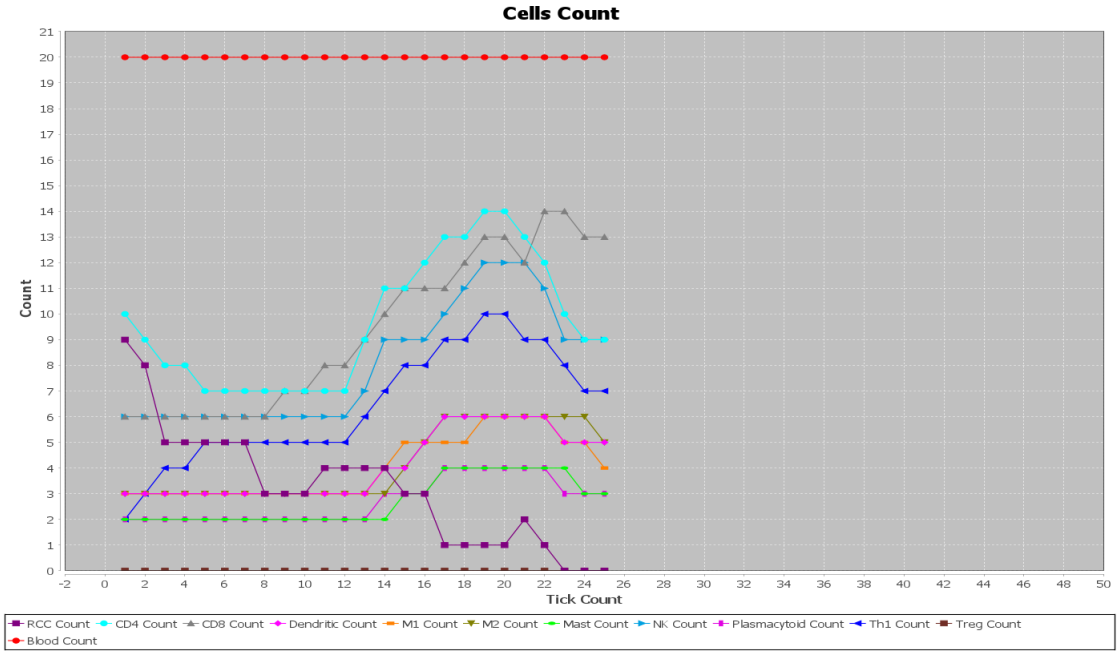

Figure 4.6: Time Series Chart showing the evolution of the cell count after each tick

For example, in this chart, we can see that the tumor has been defeated after 23 ticks and that a high number of CD8 and NK has in fact been produced. Moreover, no angiogenesis has taken place.

4.8 Data Set, Text Sink and Launcher

Repast records model data in memory, in what it calls a Data Set. The content of this Data Set is, however, not in a human-readable format and gets deleted when Repast is closed. To avoid that, we can send the Data Set content to a Text Sink that will direct the data to either the Eclipse program console or to an output file.

Since we wanted to have the model available for later analysis, we dumped the model via a File Sink, that is used to write data to a file.

In Repast Symphony the launchers folder contains the Eclipse launch configuration files that are used to run the Repast model. To simplify the development and the testing phases, we created a launcher for each configuration, both 2D and 3D.

4.9 Batch Runs

The last thing we've seen about Repast is the possibility to do batch runs, that is running the model several times with a different set of parameters each time.

As with many other things in Repast, batch runs can also be configured through an XML file. Differently from other configuration files, the batch_params.xml and batch_params3d.xml files have a syntax that is very similar to the XML files used to define the parameters.

In fact, the batch parameters' names are exactly the same, but the values may be different or even range from a starting value to an ending value; the size of the step to perform can also be defined.

For our example, we let all the parameters keep their default values, but we made the BMI value range from 15 to 40 with an increment of 1 each time, so that we can get more insights on how the BMI influences the outcome of clear cell renal cell carcinoma.

In this configuration file we can also set another value which is the number of runs to perform, while the number of ticks after which we stop the run has to be defined in the Context Creator class.

5. Results

To obtain useful results, batch runs were executed with the following settings:

- 100 runs;
- a single run ends after 100 ticks;
- BMI increasing internally to each run from 15 to 40 with a step of 1.

This made for a total of 2600 runs, which should minimize the impact of potential outliers in the dataset. Furthermore, runs were analyzed after 10, 50, and 100 ticks.

5.1 Data

The data gathered tracks:

- BMI;
- RCC cells;
- CD4 cells;
- Th1 cells;
- Treg cells;
- CD8 cells.

Finding correlations between BMI, the amount of RCC cells, and the amounts of immune cells should be enough to confirm whether the obesity paradox is correctly simulated by the model.

5.2 RCC Count

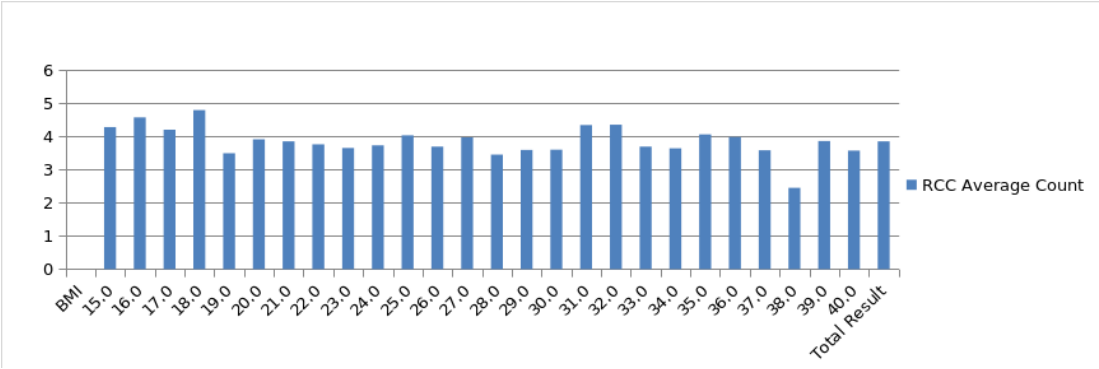

Figure 5.1: 10 ticks

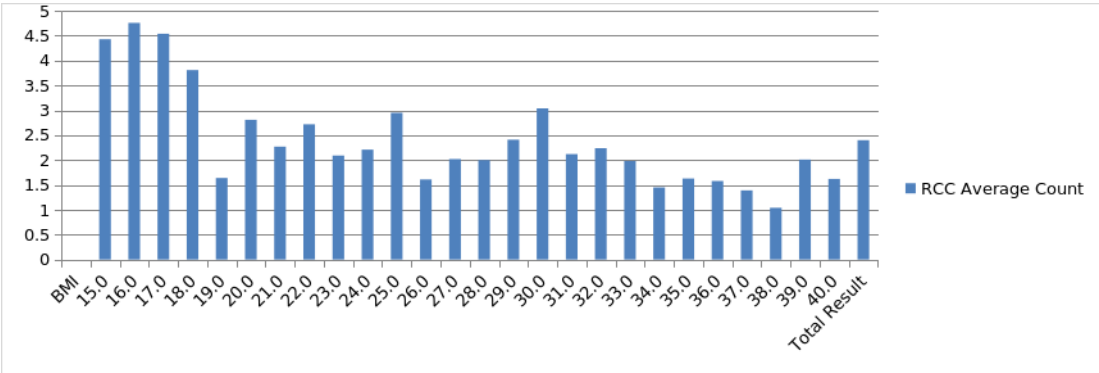

Figure 5.2: 50 ticks

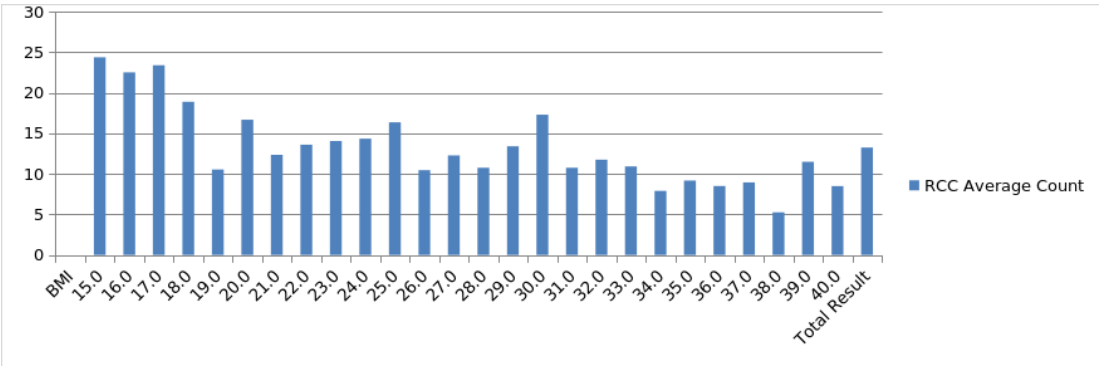

Figure 5.3: 100 ticks

As shown in figures 5.1, 5.2 and 5.3, over longer runs higher BMI seems to correlate with lower RCC counts, leading to a potentially better prognosis.

5.3 Immune Cells Count

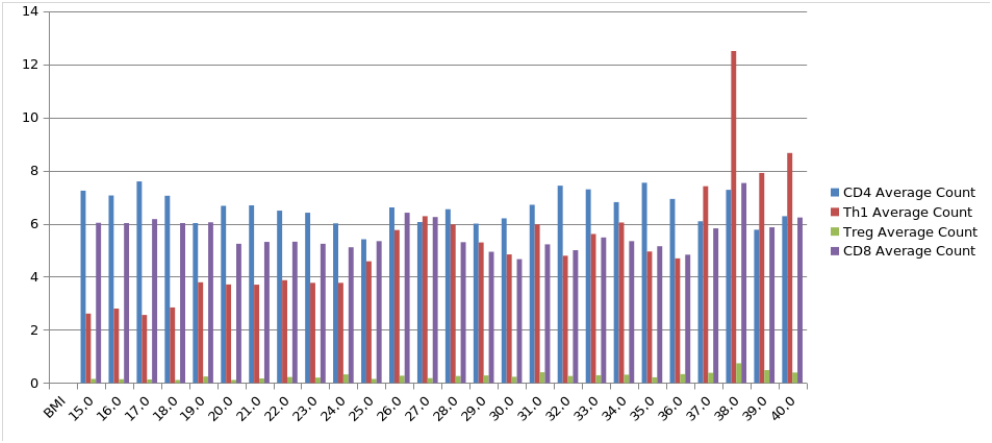

Figure 5.4: 10 ticks

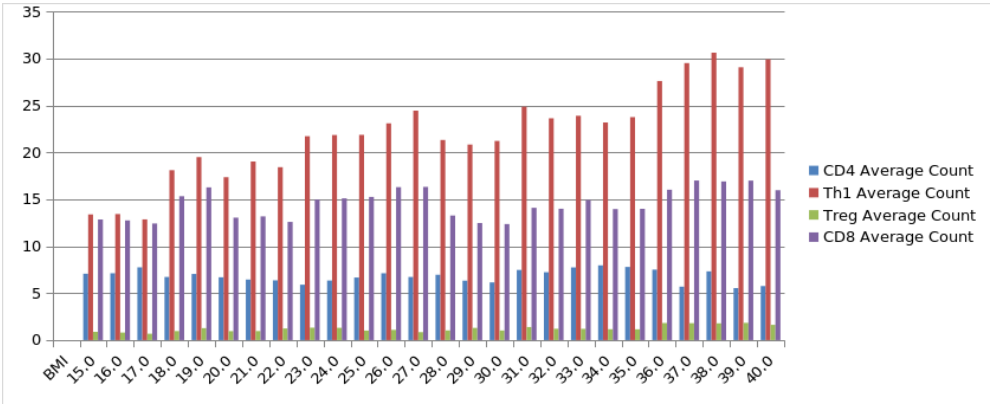

Figure 5.5: 50 ticks

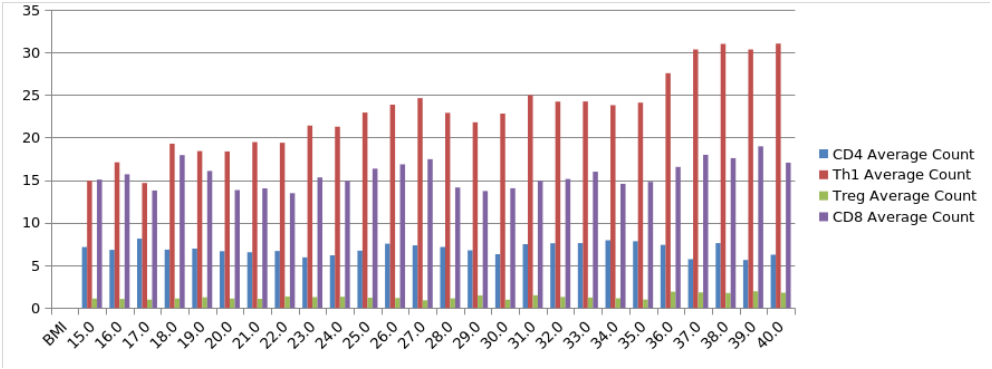

Figure 5.6: 100 ticks

Over longer runs, as seen in figures 5.4, 5.5 and 5.6, it seems that with the current model obese patients get increased amounts of cells promoting inflammation and immune response, which is consistent with the RCC counts results.

6. Conclusion

Summarizing the work done so far we can say that, in the context of the Obesity Paradox, the Renal Cell Carcinoma environment and the main agents have been correctly modelled.
We can in fact see that, at least in a two-dimensional grid environment, some properties hold true on average:

- The number of Renal Cell Carcinomas tends to generally decrease as the BMI value increases
- The ratio between CD4 cells (CD4 + Th1 + Treg) and CD8 cells keeps oscillating between 1.5 and 2.5, which are the boundary values we chose for the CD4/CD8 Ratio
- If we let the run last longer than 100 ticks, when the tumor cells take over, the whole grid is mostly occupied by blood cells (due to angiogenesis)
- If we let the run last longer than 100 ticks, when the immune system gets the upper hand, the number of immune cells is stable over time

In light of this, we can state that we have achieved results that are consistent with the current literature.

6.1 Future works

Even though the system behaves as we expected, there are still some improvements that can be done:

- Add further cells and behaviours, such as B Cells, Memory T Cell, Th2 and opsonizing mechanism
- Add cells speed and improve activation mechanism
- Adjust cells percentage in the 3D context, to have results that are more in line with the 2D context.

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