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Stavanger, May 2023

Arelis Heredia

Abstract

This thesis is a theoretical research of about the use of Lipid Nanoparticles in cancer treatments. In order to show the functionality and benefits as an alternative and/or complementary process to the ones used right now, such as chemotherapy, surgery, hormonal therapy, and immunotherapy. Lipid Nanoparticles is a specific treatment, which main objective is to distribute the drug throughout liposomes, vaccines therapies with the mRNA, looking forward the reduction of collateral effects of other aggressive treatments that impact not only the tumour but the rest of the body and causing weakness in the cancer patient organism.

Lipid Nanoparticles are used in genetic therapy treatments, which are part of active specific treatments.

In order to understand why to use this new technology in therapies, it is quickly explained the types of cancer and their evolution to attempt to find a fast way to fight this illness and at the same time, how it is attacked the patient immune system.

This research may be taken as a theoretical base for future practical investigations at lab levels, in order to deep further in the functionality of lipid nanoparticles; where the benefits and consequences of the use of new technology strategies may be shown.

Selected abbreviations

ALL	Acute Lymphoblastic Leukaemia
AML	Acute Myeloid Leukaemia
APCs	antigen-presenting cells
Arg	Arginine
CLL	Chronic Lymphocytic Leukaemia
CML	Chronic Myeloid Leukaemia
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
ECM	Extracellular matrix
FDA	Food and Drug Administration
GS	Growth signals
HIF	Hypoxia and Inducible transcription factor
LDC	lipid drug conjugate
LN	Lipid Nanoparticles
MDSC	Myeloid derived suppressor cells
NHL	Non- Hodgkin Lymphoma
NK-cells	Natural killer cells
NLC	nanostructured lipid carrier
nm	nanometers
NP	Nanoparticles
PGE	polyethylene glycol
PLN	polymer-lipid hybrid nanoparticle
RB	retinoblastoma-associated
RT	Radiotherapy
SLN	Solid lipid nanoparticles
TME	Tumour microenvironment
Trp	Tryptophan
TSP-1	thrombospondin-1
VEGF	vascular endothelial growth factor

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1. Introduction

Cancer is an ancient disease of mankind. The first written evidence of this disease can be found in the Edwin Smith papyrus, which is dated back or around 3000 BC in the ancient Egypt (Herndon, 2022); in which it is written eight different cases of tumors and breast ulcers, and a corresponding palliative treatment which was called fire drill that was used to cauterize ulcers and tumors with no other post treatment as there was not known any other way to fight cancer or disease condition (Herndon, 2022). Among fossilized bone tumors are some of the earliest evidences of cancer we have found, in human mummies in ancient Egypt, recorded in ancient manuscripts (Herndon, 2022).

Some term, such as cancer, carcinoma and oncology refer to or are dated back from Latin and old Greek. Today, the terminology oncology refers to the study of cancer.

The Greek physician Hippocrates (460-370BC),who is considered the "Father of Medicine", was the one who called the disease, cancer. In order to describe a non-ulcer forming and ulcer-forming tumors Hippicreates used the terms *carcinos* and *carcinoma*. These two words refer to crab in Greek, the finer-like spreading projection from a cancer which to him, it reminded the shape of a crab. According to Hippocrates, the human health was influenced by four fluids in the body: blood, phlegm, yellow bile, and black bile; as he believed that if there was an imbalance of these four fluids, this would cause the illness, because the individual or organism having too much black bile in a part of the body would develop the illness in that body area. Because of this, for the following 1,400 years people believed that cancer was caused by having too much black bile. Then, in ancient Rome, the Roman physician Celsus (25 BC- 50AD), was the one who translated the Greek term to *cancer* which is the Latin word for crab. Galen (130-200AD) was another Greek physician, who used the term *oncos*, meaning swelling in Greek in order to describe tumors. Now a day, the crab analogy of Hippocrates and Celsus is still used to describe malignant tumors and Galen's term is the root for the word oncology (Herndon, 2022)

At the beginning of the Renaissance, during the 15th Century, scientists developed a greater and better understanding of the human body; taking as a reference the work of Leonardo da Vinci who dissected cadavers for artistic and scientific purposes (American Cancer Society, 2018).

Men, in their insatiable search for treatments to eradicate this disease have developed and used different types of treatments, such as chemotherapy, radiotherapy, drug use, surgery, target therapy, hormonal therapy, transplants and the newest one or lately, the immunotherapy (American Cancer Society, 2014).

Chemotherapy is the use of anti-cancer drugs to destroy cancer cells (Cancer Research UK, 2020). Radiotherapy is a type of treatment for reducing the change for cancer coming back, but it can also be helpful throughout symptoms relieve (Cancer Research UK, 2020). Surgery is the removal of tissues from the body. It is one of the most used treatments for many cancers (Cancer Research UK, 2022). Target therapy is the foundation of precision medicine, which targets

proteins that control the growth, division and spreading of cancer cells (National Cancer Institute of USA, 2022). Hormonal therapy is for slowing down or stopping/blocking cancer cells growth that uses hormones (Cancer Research UK, 2021).

The use of transplants are usually treatments for leukaemia, lymphomas, and myeloma. This is because the transplants are in form of stem cells or bone marrow. Stem cells and bone marrow transplants are collected from the bloodstream for replacing the blood cells that are destroyed by high doses of chemotherapy or radiotherapy (Cancer Research UK, 2022).

At last immunotherapy helps the immune system for detecting cancer cells, for a better attack by recognising these abnormal cells (Cancer Research UK, 2021).

In the first part of this thesis, different types of cancer are presented as well as their development and propagation via methastasis. I will also describe their effect on the body immune system and the current different treatments. In the second part, the use of lipid nanoparticles as cancer treatment or as cancer vaccine.

2. Cancer

Cancer is defined as a disease in which cell division is uncontrolled and can lead to invasion of nearby tissues. These events, also called malignancies, usually happen by the lymph or blood systems. As cancer cells can break away from a tumour, they travel to other parts of the body using the blood or lymph system as a method of spreading itself. Normally most of the escaped cancer cells die or get killed before they can settle down in a new area. But it may come that one or two cells survive and settle down in new areas, beginning to grow and forming new tumours causing metastasis (American Cancer Society, 2022). Fig.1 shows the difference between normal cell and cancer cells.

The human body is made of trillions of cells, and it is always producing new cells for replacing the cells that naturally die. Cancer can also be described as a cellular change in one cell or in a group of cells, such as dramatic changes in the process of cell division. As a matter of fact, the genes are responsible for how the cells grow and divide.

In the case of a change in the gene, this means a change in the DNA sequence of an organism such as deletion, duplication, inversion, insertion, or translation during a cell divides, the term used is called a mutation. (Fig 2) (Daniel A. Gilchrist, 2023, NIH) During a mutation a cell or a group of cells can start growing and multiplying too fast creating a lump also called a tumour. There are benign and malignant tumours. Benign means it's not cancer. And all kinds of tumours can start anywhere in the body. For malignant tumours that develops cancer are named for the part of the body it stared. (American Cancer Society, 2022), (Cancer Research UK, 2020)
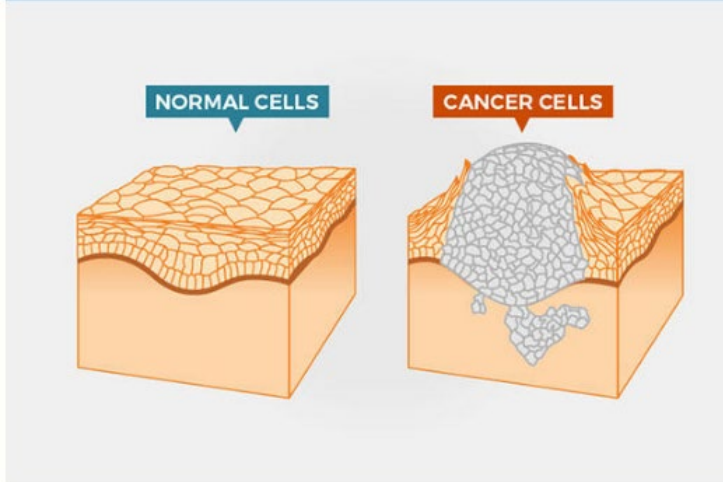
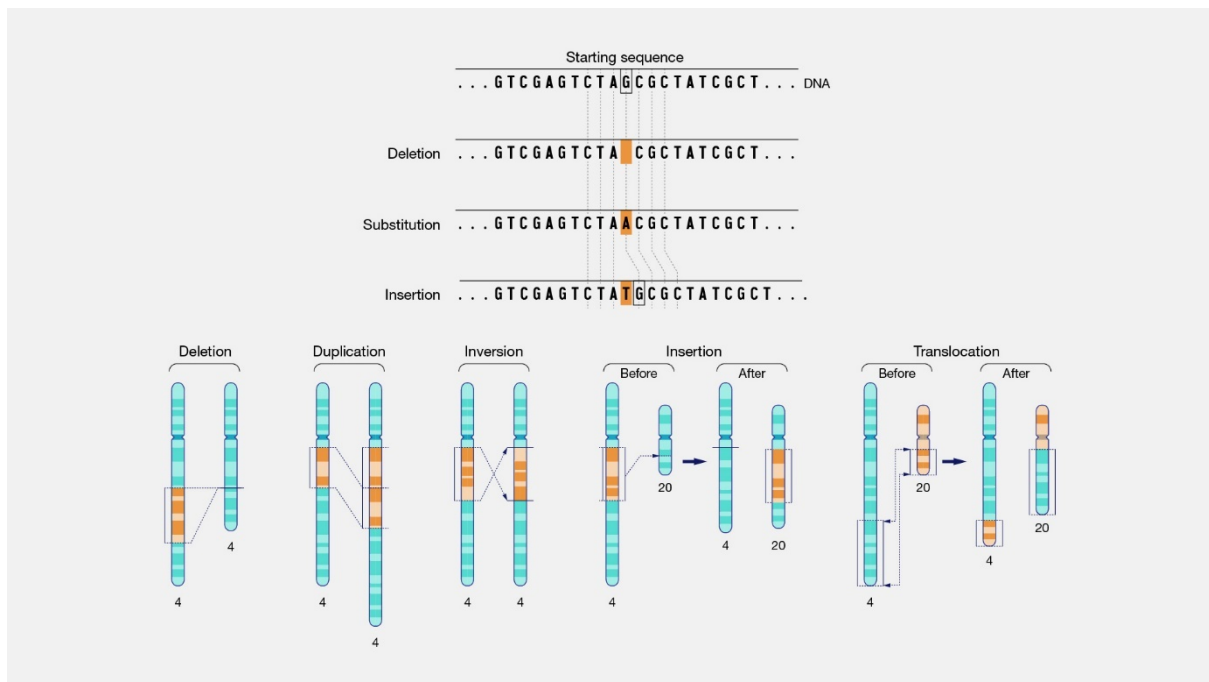*Figure 1 Difference between normal cells and cancer cells*

Figure 2 Gene mutation

The differences between Normal and Cancer cells are described as following from the US National Cancer Institute:

- Normal cells proliferate only when they receive a signal to grow while cancer cells proliferate in absence of such signal.
- Normal cells stop dividing or die when they process and respond to specific molecular signals while cancer cells ignore such signals.
- Normal cells stop dividing if the encounter other cells in close proximity and do not move around them. Cancer cells have no contact growth inhibition and can invade nearby cells travel to other parts of the body.
- Immune system eliminates damaged or abnormal cells while cancer cells hide from the immune system.

- Cancer cells can display molecular signals that induce immune cells to protect the tumour and allow it to grow.
- Cancer cells apply multiple changes in their chromosomes, including duplications and deletions of chromosome parts. Some cancer cells have even duplicated the normal number of chromosomes.
- Cancer cells produce energy via different sources and grow faster than normal cells.
- Cancer cells induce blood vessels to grow toward tumours, Neo vasculature supplies tumours with oxygen and nutrients and removing its waste.

2.1 Types of cancer

Cancer is divided in six different main groups: Carcinomas, Sarcoma, Leukaemia, Multiple Myelomas & Lymphoma and Central Nervous System cancers. (Warrier S. et al, 2021)

Carcinoma Cancers are the ones starting in the skin or in the tissues that covers or protect the internal organs. Carcinomas are formed by epithelial cells which can be divided into different types (Fig. 3-7): squamous carcinoma, adenocarcinoma, transitional carcinoma, and basal cell carcinoma. (Cancer Research UK, 2020)

Figures 3, 4, 5, 6 and 7 shows the different types of cancers in epithelial tissues.
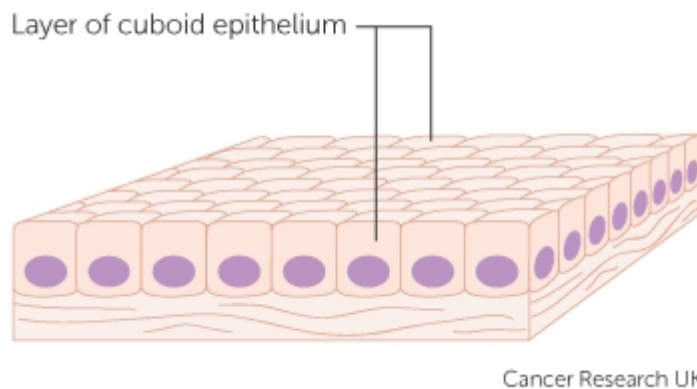

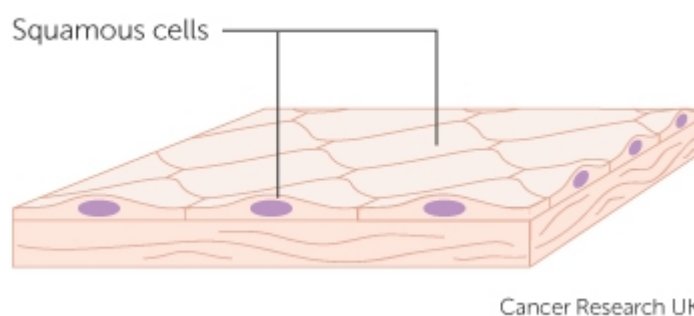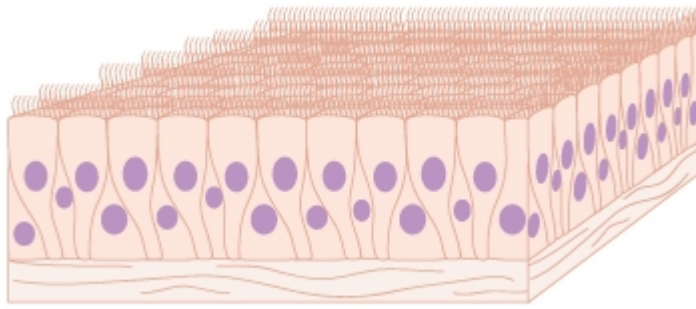*Figure 3 Epithelial tissues, they cover the inside and outside of the body*

Figure 4 Squamous cells carcinoma, stars in the flat fish scale looking squamous cells. That are surface covering cells, covering the skin, or lining the throat or oesophagus

Adenomatous cells

Cancer Research UK

Figure 5 Adenomatous cells, are glandular cells that produce fluids to keep tissues moist. The Adenocarcinoma stars with these cells
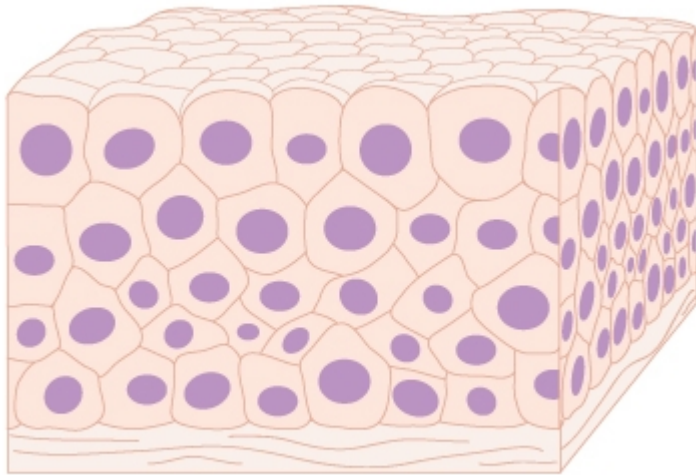
Cancer Research UK

Figure 6 Transitional cell carcinoma, these epithelial tissues are called transitional epithelium or urothelium. These cells can be found in the lining of the bladder, uterus, and parts of the kidneys
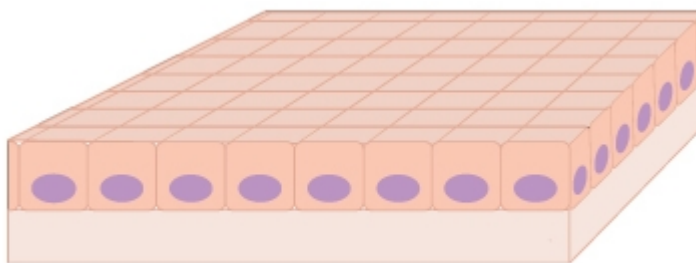
Cancer Research UK

Figure 7 Basal cells carcinoma, cells that lines the deepest layer of the skin.

Carcinomas are the most common type of cancer, making about 80-90% of the cancer cases (Warrier S. et al, 2021).

Sarcomas are cancers which starts developing on the bones, muscles, blood vessels, fat, cartilage, or any other connective tissue. Within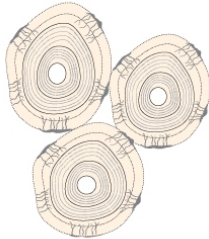 sarcomas are there two main types: osteosarcoma and soft tissue sarcoma (Cancer Research UK, 2020). Osteosarcoma also known as bone cancer, start in the bone cells called osteocytes, which are shown in Fig.8
Meanwhile in a minor and rare cases there are found soft tissue sarcomas. These typically develop in cartilage or muscles. (Fig. 9 and 10) (Cancer Research UK, 2020)

Figure 8 Osteocytes
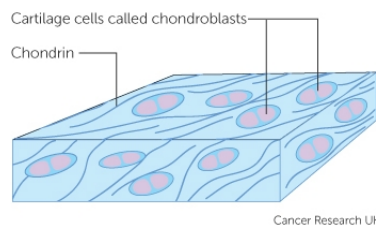
Figure 9 Cartilage cells
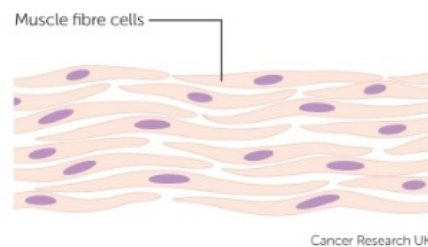
Figure 10 Muscle cells

The next three types of cancer are related so called blood cancer because they start in the blood cells, these are leukaemia, lymphoma, and multiple myeloma. And how can they be separated? Well, the answer lies in which cells are affected first. In the case of a leukaemia, it starts normally in the bone marrow, for a lymphoma the cells affected are in the lymph nodes, and for the multiple myeloma are the plasma cells affected creating antibodies to fight the disease. (Susan Bernstein, 2021)

Leukaemia are the ones arising in the blood system. This cancer doesn't create enlarged mass of cells, instead there is an increase production of white blood cells in the bone marrow, which builds up in the blood. This growth causes difficulties for the normal blood cells, such as getting the oxygen to the body tissues. There are different types of Leukaemia, depending both on how quickly the disease escalates and which cells it arises first. The diseases can either be acute or

chronic, likewise it can start in either the lymphocytes (B-cells or T-cells), or it can also start in the myeloid lineage (e.g. plasma cells).

Creating therefore acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), chronic lymphocytic leukaemia (CLL), and chronic myeloid leukaemia (CML). All these types apply also to children. (Cancer Research UK, 2022 and National Cancer Institute, 2021)

White blood cell
(leucocyte)

Cancer Research UK
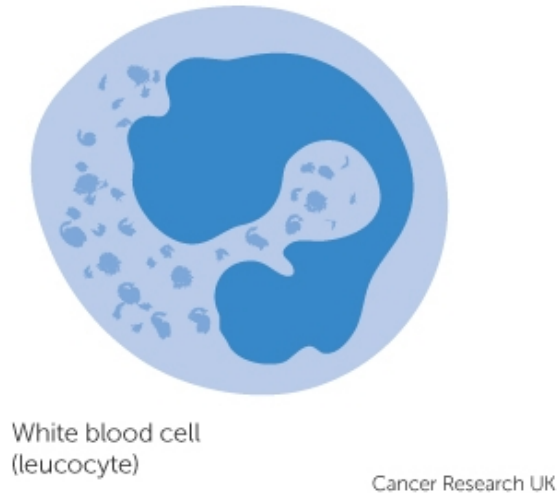
Figure 11 Leucocyte

Multiple Myelomas & Lymphomas are the ones which uncontrolled proliferation of immune cells. Since both lymphomas and multiple myelomas arise in the immune system there are still two different types of cancers. In fact, the difference lies on which type of blood cells the cancer begins in. Lymphoma starts either in the lymph glands or the lymphatic organs. Therefore, this disease begins in the lymphocytes including T-cells and B- cells. (Cancer.net, 2021) There are two main types of lymphoma: Hodgkin Lymphoma and Non- Hodgkin Lymphoma (NHL). Hodgkin Lymphoma or Hodgkin's disease begins in the B-cells and contains abnormal cells called Reed-Sternberg cells, and it affect most commonly the lymph nodes in the neck, but the disease can start in any of the lymph nodes of the body. (Cancer Research UK, 2020) The NHL is a term that covers a large group of cancer in the lymphatic system that grows quickly or slowly in the lymphocytes. NHL starts most common in the lymph nodes, liver, spleen, or bone marrow, but it may be also found in the stomach, intestines, skin, thyroid gland, brain, or any other part of the body. (Cancer.net, 2021) Fig 11 features a lymphocyte.

Conversely Multiple Myelomas develops within the plasma cells. Taking into consideration that every hematopoietic cell is created in the bone marrow, an abnormal growth in any blood cell can suppress the growth of the other cells in the bone arrow. This can cause anemia due to a lack of red blood cells, excessive bleeding from cuts to the skin caused by shortage of platelets, or the increasing risk of infection due to lacking leucocytes. (Cancer.net, 2021) Fig 12 shows a plasma cell.
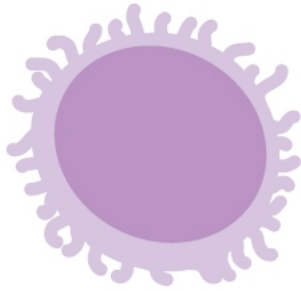
Lymphocyte

Cancer Research UK

Figure 12 Lymphocyte cells

Plasma cell
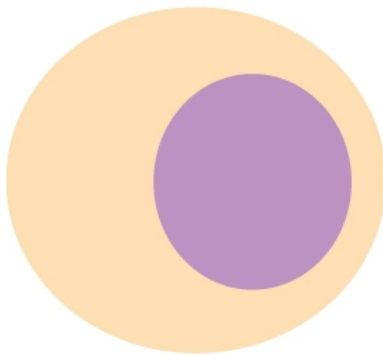
Cancer Research UK

Figure 13 Plasma cells in meyloma

Finally Central Nervous System Cancers are the ones arising in the spinal cord and brain tissues. It is well known that the brain controls our human body by sending electrical messages along never fibres. These fibres connect with the spinal cord which also takes messages from the body to the brain. The human brain is built up of billions of nerve called neurones. But it also contains other types of cells that are special connective tissues and support the neurones called glial cells (Fig 14). A glioma is the most type of brain tumour that develops in the glial cells. (Cancer Research UK, 2020)
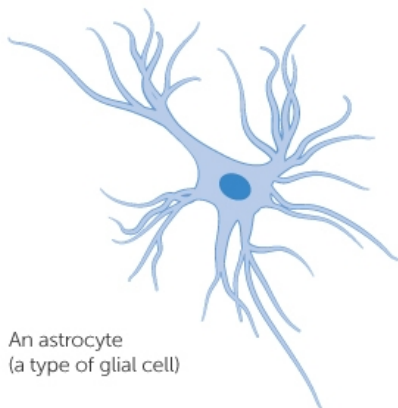
An astrocyte
(a type of glial cell)

Cancer Research UK

Figure 14 Neurones- glial cells

Cancers are also defined by their originating tissue. These localizations are divided in different group, which are the following:

AIDS-Related, Breast, Digestive/ Gastrointestinal, Endocrine and Neuroendocrine, Eye, Genitourinary, Germ Cell, Gynecologic, Head and Neck, Hematologic/ Blood, Musculoskeletal, Neurologic, Respiratory/ Thoracic and Skin.

Some of the most common Cancer types are Bladder Cancer, Breast Cancer, Colon and Rectal Cancer, Endometrial Cancer, Kidney Cancer, Leukemia, Liver Cancer, Lung Cancer, Melanoma, Non-Hodgkin Lymphoma, Pancreatic Cancer, Prostate Cancer, and Thyroid Cancer. (The National Cancer Institute of USA)

Not all tumours are cancers, some of these tissue changes even though they are not cancer, they may develop into cancer if they are not treated on time. Some examples of these tissue changes that gets monitored considering that they may develop into cancer are Hyperplasia, Dysplasia, Carcinoma in situ.

- Hyperplasia
  It happens when cells inside a tissue multiplies or grows faster than normal and there is build-up of cells.
- Dysplasia
  The term Dysplasia defines a more advance condition of hyperplasia. In this the cells look abnormal under a microscope and there is also an even larger build-up of cells.
- Carcinoma in situ
  Dysplasia can further develop into a Carcinoma which is also called stage 0 cancer. A group of abnormal cells are only found in the place where they formed and hasn't invade nearby tissue yet. Even so the possibility of this becoming malignant tumours is higher. In this condition the group of abnormal cells looks like cancer under the microscope.

In Fig. 15 and 16 it may be seen Normal cells, the Hyperplasia, the Dysplasia, the Carcinoma in situ and the cancer cells.
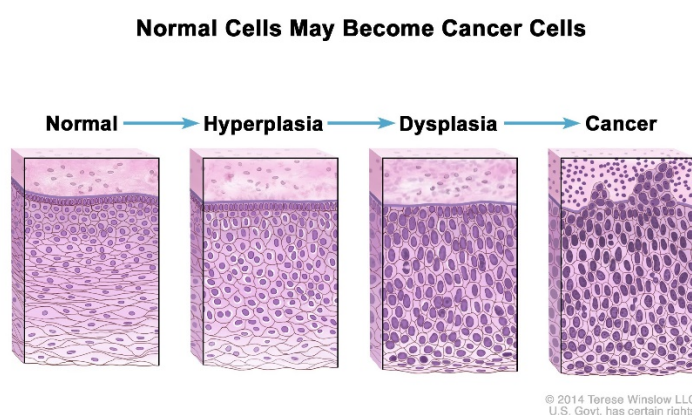
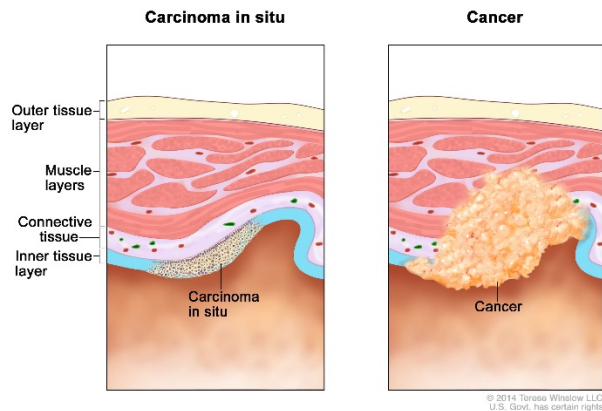*Figure 15 Diagram of Normal cells, Hyperplasia, Dysplasia and Cancer*

Figure 16 Diagram of Carcinoma in situ

As the malignant tumour grows it creates its own environment called the tumour microenvironment. Which refers to the cellular environment that the tumour exists in human body. Including the blood vessels, fibroblast, immune cells (lymphocytes, bone marrow - derived inflammatory cells from the innate immune system) that interacts with its surrounding and the extracellular matrix.

2.2 Cancer Hallmarks

In the paper "*The Hallmarks of Cancer*" by Hanahan and Weinberg in 2000, the authors proposed that the hallmarks were defining the way normal cells evolving progressively to a neoplastic state (Douglas Hanahan, 2011). In other words, Cancer hallmarks is a tool for a set of capabilities acquired during tumour development (Douglas Hanahan, 2021).

Our understanding in the common traits and in rational drug design for cancer comes from the description of hallmarks of cancer by Hanahan and Weinberg. (In the year 2000 Hanahan and Weinberg attempted to organize the biology of cancer and its dense complexities into six major hallmarks. These being the following ones: self-sufficiency in growth signals, insensitivity to anti-growth signals, evading apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. (Douglas Hanahan, 2011)

In 2011 was there an update of the article "Hallmarks of Cancer" published in the year 2000 by Hanahan and Weinberg. Here below I describe these eight hallmarks as they are mentioned in the paper "*Hallmarks of Cancer: New Dimensions*" (Douglas Hanahan, 2021):

- **Sustained proliferative signaling**
  Normal cells regulate the production of their growth signals (GS) by maintaining homeostasis of the cells number and normal tissue architecture. However, this control is impaired in cancer cells, and the ability of sustained proliferation can be achieved in several two ways: cells may respond to the production of growth factor ligands. This is due to the expression of cognate receptors.
  The second way is by sending signals to stimulate normal cells in the tumour associated stroma, causing the procreation of growth factor which cancer cells supplies. (Douglas Hanahan, 2011)

- **Evading growth suppressors**

  For cancer cells it is necessary to avoid antigrowth signal to thrive. These signals negatively regulate cell proliferation and are linked to tumour suppressor genes. (Douglas Hanahan, 2011) Two of the most known tumour suppressor genes are RB (retinoblastoma-associated) and TP53 proteins.

  The RB proteins react to signals from the extracellular and intracellular sources, which then decides whether should a cell proceed through its growth-and division cycle. (Douglas Hanahan, 2011)

  Meanwhile through receiving inputs from abnormality and stress sensors in the intracellular system of the cell the protein TP53 is activated. This protein can call a halt in the cell cycle progression until the system is normalized. This means that if the damage to the genome is excessive, or if the levels of nucleotide pools, growth-promoting signals, glucose, or oxygenation is suboptimal will TP53 act immediately. Even though TP53 calls on halt, doesn't mean that the problem can be fixed. In these cases, is apoptosis triggered by TP53. (Douglas Hanahan, 2011)

- **Resisting cell death**

  One of the defence mechanisms of the body against cancer cells is apoptosis which is triggered in response to various physiologic stressors during tumorigenesis (Douglas Hanahan, 2011)

  At the beginning of the last decade, it was very appreciated the structure of the apoptotic machinery and programs, all well as the strategies used by cancer cells to evade these pathways. (Douglas Hanahan, 2011)

- **Enabling replicative immortality**

  A need for cancer cells forming tumours is an unlimited replicative potential. The majority of cells are able to pass through a limited number of growth-and-division cycles. This limitation has been associated with two distinct barriers to proliferation: senescence, a typically irreversible entrance into a nonproliferative but viable state, and crisis, which involves cell death. (Douglas Hanahan, 2011)

  There is evidence indicating that telomeres protects the ends of the chromosomes and are involved centrally in the capability for unlimited proliferation.(Blasco, 2005, Shay and Wright, 2000)

- **Invasions and metastasis**

  Metastasis develops when one or more cancer cells from the primary tumour move outwards toinvade other tissues, traveling distances that can either be short, or long depending on where the new colony of cancer cells successfully migrated. Causing a 90% of human cancer death in patients. (Douglas Hanahan, 2000) Invasions and metastasis are complex processes in a cascade of changes in the cell. Those changes are the following: local invasion progression, intravasation into nearby blood and lymphatic vessels, transit of cancer cells through the lymph and blood system, escape these cancer cells into distant tissues, forming of micro metastases, and grow of lesions.

- **Inducing angiogenesis**

Tumours as well as normal cell require nutrients and oxygen in order to grow. Just like normal healthy cells, cancer cells need to eliminate waste and carbon dioxide. (Douglas Hanahan, 2011) Angiogenesis is the cell process which consists in migration endothelial assembly from existing vessels. This is process is present during embryogenesis, via remodelling and expansion of primitive vascular network and it is also a process during wound healing and postnatal events (Fouad & Aanei, 2017).

The regulation of angiogenesis depends on some molecules such as anti and pro-angiogenic factors, causing an angiogenic switch. (Mathonnet et al, 2014, p.4189-4196) These regulators in the angiogenic switch are also known as inducers and inhibitors such asthe vascular endothelial growth factor-A (VEGF-A) and thrombospondin-1 (TSP-1) (Douglas Hanahan, 2011). VEGF signalling is rather complex and involves three receptor tyrosine kinases (VEGF-1-3). The VEFG receptors are illustrated in figure 17.(Ferrara, 2009, Mac Gabhann and Popel, 2008, Carmeliet, 2005)
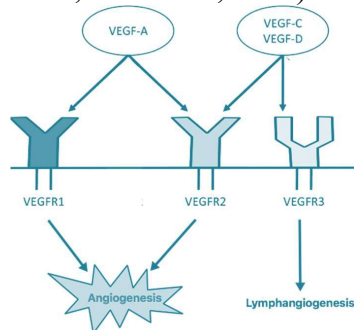
Figure 17 VEGF reseptors

A tumour develops fast, leading to an insufficiency of nutrients and oxygen supply for the cancer cells. The terminology of this low oxygen condition is hypoxia, which often leads to cell death in the central core of the tumour (Lenihan, C. R. and Taylor C. T., 2013). Eventually the hypoxia-inducible factor (HIF) will the activated in turn of the hypoxic environment. The activation of HIF will signal a pathway because of the lack of molecular oxygen, while the hydroxylation of HIF is preventing by prolyl-4-hydroxylase. HIF is also a key transcriptional regulator of many genes, including VEGF. (Takahashi H., and Shibuya M., 2005; Pugh C. W., and Ratcliffe P. J., 2003) Figure 18 shows how HIF is a key transcriptional regulator.
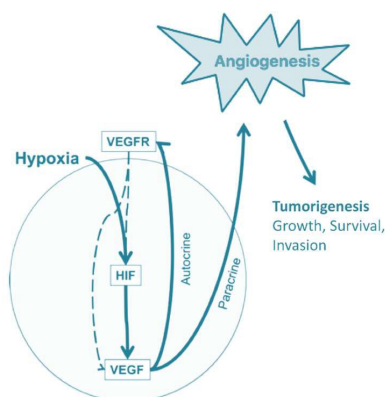
Figure 18: Hypoxia related vascular endothelial growth factor (VEGF) transcription. In low oxygen

- **Reprogramming of energy metabolism**

Normal cells process glucose under aerobic conditions. This process first produces pyruvate via glycolysis in the cytosol, afterwards leads to carbon dioxide inside the mitochondria. Meanwhile under anaerobic conditions little pyruvate is dispatched to the mitochondria due to the preference of glycolysis process (Douglas Hanahan, 2011).

By changing the tumour microenvironment (TME) there is a high alteration rate of cancer cells in the metabolic mechanism of glycolysis or amino acid metabolism.
Even in the presence of sufficient oxygen and low rate of oxidative phosphorylation do tumours and cancer cells have an increase of glucose uptake and lactate production.

In the amino acid metabolism tryptophan (Trp) and arginine (Agr) are considered to provide kye nutrients in TME. Arg is a catalysator that have been linked to suppression of antitumour immunity, while Trp attenuates antitumour immunity in primary tumours and the neighbouring tumour lymph nodes. (Ravi. S, et al., 2022)
Fig 19 shows the nutritional competition between tumour cells and immune cells inside a tumour. Which is a deficiency in glucose, glutamine, a couple of amino acids, and fatty acids that affect the function of immune cells.

Figure 19 Nutritional competition of tumour cells and immune cells in a tumour

- **Evading Immune Suppression**

Tumour escapes immune destruction by several mechanisms.
Myeloid derived suppressor cells (MDSC) are converted immature myeloid cells that are derived factors of tumours suppressing antitumour immune response. The TME is a permissive microenvironment and the creation of cancer cells recruiting and educating immune cells such as NK cells, regulatory T cells, dendritic cells, granulocytes, macrophages, and MDSC.

The extracellular matrix (ECM) can impair antigen presenting cells and inhibit T-cell activation suppressing T-cell function against tumour. Commensal microbiota is also reported to have a role in impairing antitumor immunity. (Ravi. S, et al., 2022)

2.3 How does cancer affect our immune system?

The immune system is triggered by diseases-causing microorganisms, *i.e.* pathogens, but it can also kill abnormal cells of the body. It is well known by now that the immune defence of our bodies consists of two types: the innate immune system, and the adaptive immune system. Both of these systems the innate and adaptive immune system have established roles in the host defence from pathogens or any abnormality through various mechanisms, which are raising an unprecedented development in the immunotherapies. (Longzheng et al, 2021) The difference between the innate and adaptive immune system is shown in table 1. And in fig 20 is an overview of both the innate and adaptive immune system.

Table 1 Innate and adaptive immune system

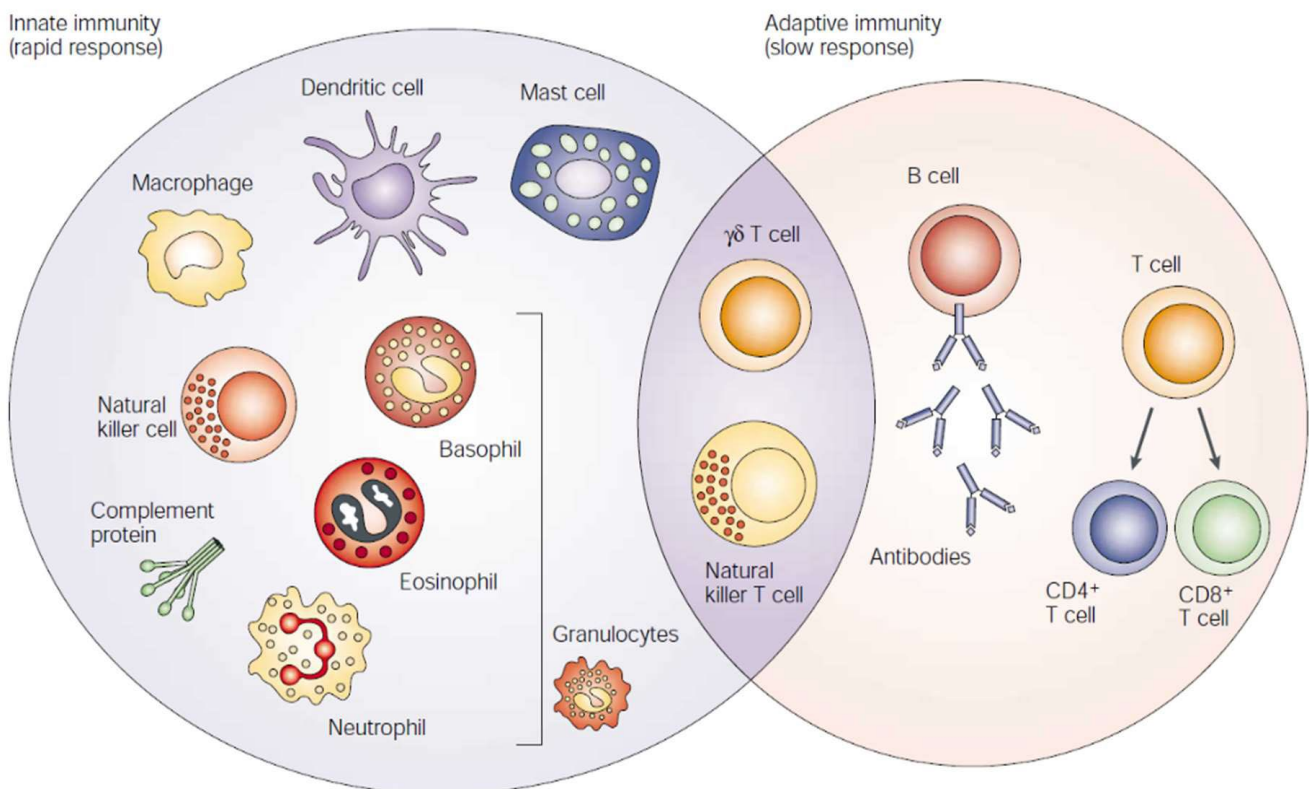|	Innate	Adaptive
Specificity	- Nonspecific - Present at all time - Reacts to all foreign pathogens	- Specific - Requires activation - Direct response to triggering pathogen
Response time	- Immediate reaction - General defence	- Delayed reaction - Specific defence
Memory	Absent Same response with repeated exposure to same pathogens	Present Antibody development Provides retained immunity to repeated exposure to same pathogens
Cell components	- Macrophages - Dendritic cells - Phagocytes - Neutrophils - Natural killer cells (NK-cells)	- T lymphocytes (T-cells) - B lymphocytes (B-cells)

Source: (Longzheg et al, 2021)

*Figure 20 **An overview of the immune system**: on the left side is the innate immune system and on the right side is the adaptive immune system*

How can the immune system recognize, fight, and ultimately destroy malignant cells? And how do cancer cells evade the immune system and expand? The recognition of cancer cells by the immune system is because, of the biochemical differences in cancer cells, that are formed by "self" cells. In a dynamic period of immunoediting, in which initially immune cells can destroy tumour cells, but eventually there is a time when cancer cells through various mutation and mechanism can evade elimination of the immune system. The current term is *Immunoediting* including all phases of cancer and immune system interaction beyond immunosurveillance. (Abbott M. and Ustoyev Y., 2019)

The immunoediting is a hypothesis which is composed of three phases (Fig 21). The first phases is elimination. Cells that aren't normally repaired by the inherent genetic DNA repair mechanisms either become malignant or potentially malignant can be identified and killed by the immune system. Then the innate immunity follows by presentation to tumours antigens, dendritic cells and the develops to tumour specific CD4+ and CD8+ T-cells allowing the destruction of cancer cells. Immunosurveillance is widely considered to be the phase of undetectable and early tumour development. (Abbott M. and Ustoyev Y., 2019)

Equilibrium is the second phase, in this phase the tumour cells that survived to be destroy by the immune system during elimination are unable to progress. These tumour cells continue to coexist with the immune system, and the equilibrium phase it thought to be the longest out of all three phases, and it could theoretically last for years. (Abbott M. and Ustoyev Y., 2019)
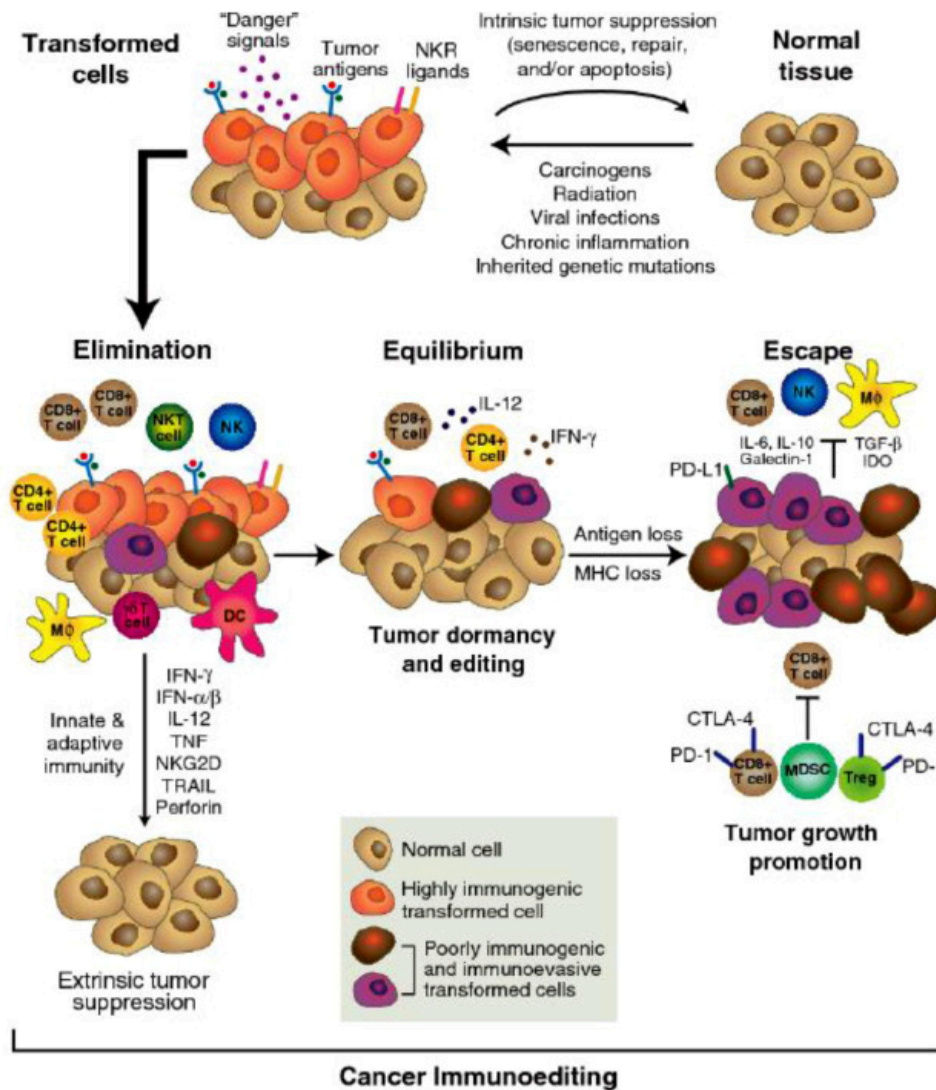
Figure 21 Cancer Surveillance and Immunoediting.

Following equilibrium is the third and final phase of immunoediting called escape. In this last phase cancer cells grow and can become metastasis, caused by the lack of control and elimination by the immune system. During scape the body immunity is overwhelmed and can no longer retain the growth of malignant cells. Allowing multiple mechanisms for tumour cells to evade elimination, this includes suppression of the immune system on the tumour cell itself or by genetic acquisition that allows immune suppression. (Abbott M. and Ustoyev Y., 2019)

One of such mechanism is that cancer cells have the ability to express immune checkpoints (molecules that acts as a brake on the immune system, by stopping T cells from mounting a full immune response) molecules on their surface, like those found on normal cells, thus evading an immune system attack by suppressing the T cell at the immune checkpoint. This ability is considered to be one the cancer hallmarks. (Abbott M. and Ustoyev Y., 2019) Which I have already mention in text above before. When I talk about the cancer hallmarks. So then how does immunotherapy treat cancer?

2.4   Cancer treatment

There are many methods for cancer treatment. Depending on the type of cancer and at what stage it is advanced will determinate the method. Treatment options can be chemotherapy, surgery, radiation therapy, hormonal therapy, targeted therapy including immunotherapy. And in some cases, the doctor will give a treatment plan that may use a combination of the treatment methods to have maximum effectives for the treatment. Because there is no particular method or technique for treating cancer. (Wang J., Lei F., and Han F., 2018)

The required goal of any treatment plan is a complete removal of the cancer tissue without causing damages to the normal tissue nearby. Removing completely a tumour is limited due to metastasis. The procedures for treatments such as chemotherapy and radiotherapy have negative side effects on the healthy tissues. The basic purpose of a cancer treatment plan is to have cure for the cancer and, if by any means a complete cure is not possible, the treatment plan should be to suppress the cancer to a subclinical state and maintaining the normal state for the subject to lead a normal quality of life. (Wang J., Lei F., and Han F., 2018)

Understanding how cancer work in the human body have increase in many ways the advances on cancer treatments with the help of technology. Various cancer treatments methods have been practiced in the past such as surgery, but also many innovative methods like targeted therapy, are being practiced today. Since new information and understanding of biological process of cancer tissues are emerging regularly, the development and modification of new treatment procedures and plans have to increase the effectiveness and precision of the treatment, resulting in the survivability and improving the quality of life of the patients. Cancer can be treated by surgery, chemotherapy, ionizing radiation therapy, hormonal therapy, targeted therapy etc. (Wang J., Lei F., and Han F., 2018)

2.4.1 Types of treatment

- **Chemotherapy:**
  Chemotherapy is the most common treatment use against cancer, and its procedures by the use of drugs, usually known as anticancer drugs or cytotoxic. These molecules kill cancer cells throughout the body by interfering with the growth of the tumour. (Wang J., Lei F., and Han F., 2018)
  In general, the chemotherapy is considered as an effective method for treating cancer. However, it can cause severe side effects as they can destroy either healthy cells or tissues. The side effects caused by chemotherapy are depending upon the of drug being used for the treatment, the type of cancer and its location, and also on the individual patient's response to the chemotherapy treatment. The side effect on the patient vanishes once the treatment process is completed, and it is not related to the effectiveness of the treatment. (Wang J., Lei F., and Han F., 2018)
  When a patient is going throught chemotherapy, it is often prescribed in either measured doses, or in a time lapse with a specific number of intervals, allowing some time for the repair of normal cells. (Siegle, Deepa, Alhmedin, 2012, Wang J., Lei F., and Han F., 2018)
  Some of the side effects with this treatment are anemia, appetite loss, diarrhea, flu-like symptoms, fatigue, hair loss, nausea and vomiting, and pain among others. (National Cancer Institute, 2022)

- **Radiotherapy:**

For this cancer treatment it is use high doses of energy radiation to destroy the tumour tissues and killing cancer cells.

Along with chemotherapy, radiotherapy (RT) is also a common method of cancer treatment in patients. It is applied for approximately 60% of all newly diagnosed patients as a frontline therapy. The side effects with RT are fewer than in chemotherapy, and it shows better effects on the control of local tumour. (Magdalena J. et. al, 2019)

As mentioned before there are many types of cancers such as brain cancer, breast cancer, cervix cancer, larynx cancer, liver cancer, lung cancer, pancreas cancer, prostate cancer, skin cancer, stomach cancer uterus cancer, and so on radiation therapy is quite commonly used to treat these types of cancers. (Wang J., Lei F., and Han F., 2018)

RT can also be a complementary therapy to prevent recurrence. Patients with advanced cancers have a routine with RT to reduce the symptoms and increase their quality of life. The most effective way to use RT is by having a solid tumour, interpreting its effectiveness as a consequence of better local tumour control and reduction of the spread of the disease. (Magdalena J. et. al, 2019)

Fatigue is a really common side effect of radiotherapy.

- **Surgery:**

The oldest oncological discipline is surgery, dating back thousands of years. The high mortality and morbidity due to the low rates of cure of surgery prior to the advent of anaesthesia and antisepsis for 150 years ago, were only to the patients that were brave, desperate, or ill-advised. However, since then, cancer surgery has flourished, driven by relentless technical innovation and research. (Wyld, L. et. al., 2015)

The procedure for a surgery of cancer is commonly practiced on non-haematological cancer. Through surgery, the cancer tissues are removed and can give a completely or partially cure to the disease. If there are side effects with this treatment, are they depending on the type of cancer, and the health condition of the patient. The completely removal of the tumour will be impossible if the cancer has metastasized. Cancer surgery can be done to cancers that are localized and have a small size tumour. (Wang J., Lei F., and Han F., 2018)

The pathologist by carrying out biopsy, can assessed if there are any chances of reoccurrence of the cancer. This is achieved by analyzing the surgically removed tissues and comparing it to the healthy tissues. With this analysis can the stage of cancer be investigated by the end of the evaluation of the presence of healthy or cancerous tissues. Other treatment procedures, such as chemotherapy, can be done before or after that the surgical procedure is carried out to remove the affected tissues. (Wang J., Lei F., and Han F., 2018)

- **Hormonal therapy:**

In the 1940s was the concept of hormone therapy born as a treatment for prostate cancer. (Student S. et. al, 2020)

Certain types of cancer depend on hormones to grow and spread, hormone therapy fights by changing the amount of hormones in the body. This treatment is used for treating cancers of prostate, reproductive system, and breast. The side effects depend

on the type of cancer, age, sex, and the type of drug used in the treatment. (Wang J., Lei F., and Han F., 2018)

- **Immunotherapy:**
Immunotherapy is a cancer treatment that helps the immune system to fight and prevent cancer. This method is also known as biologic therapy, in where the patient's disease fighting mechanism gets stimulated to fight the cancer. Immunotherapy is achieved by training the immune system to recognize and attack the cancer cells. And there is a high number of research that have been carried out to treat cancer with this method, such as monoclonal antibodies that block specific protein function through binding to cancer cells. This method of treatment is safe and does not have any major side effects. (Wang J., Lei F., and Han F., 2018)

One goal that immunotherapy have is to balance the immune system to eradicate cancer cells, while not producing unchecked autoimmune inflammatory responses resulting in therapeutic limitations of the treatment.
As I mentioned earlier the immune system have to ways of responses. The innate immune system which releases cytokines that recruit immune cells to begin the non-specific immune response, and the adaptive immune system have which gives a specific immune response. Giving this immune response, makes the adaptive immunity better for defeating cancer due to the ability to specifically target non-self-antigens. (Abbott M. and Ustoyev Y., 2019)

In result from this understanding, there have been several methods of immune therapy developed, including vaccination, monoclonal antibodies, and checkpoint inhibitors. Each of these methods mentioned above are categorized and seeks to increase immune function and differs by various mechanisms of action. Immunotherapies are generally divided into active and passive immunotherapies. (Abbott M. and Ustoyev Y., 2019)

Active immunotherapy is the stimuli of an immune response, immune memory, and lasting response, as an example we have oncolytic vaccines. Meanwhile passive immunotherapies include monoclonal antibodies but require a regular administration because the production is specific but often short-lived responses. Finally, there can be a delay in clinical and radiologic response to immunotherapy. The reason behind this is because the time it takes, not only for an immune response to occur, but also for the destruction of a tumour by the T cells. (Abbott M. and Ustoyev Y., 2019)
Maybe the only negative side of this treatment is that there are evidence of tumours becoming resistant to immune therapy over time. Thereby trials to develop therapies to combat immunotherapy resistance are ongoing. (Abbott M. and Ustoyev Y., 2019)

- **Targeted therapy:**

Targeted therapy or molecular targeted therapy refers to the use of drugs or other substances that targets specific molecules. Causing the block of growth and spreading of cancer cells. The concept for targeted therapy was derived from the idea of "magic bullet" which was first expatiated by Paul Rich in late 1800 (Lee Ting Y., Tran Jer Li, Chern Ein Onn, 2018)

Targeted treatment uses specific agents for the deregulated proteins of cancer cells.

These small, targeted molecules delivering drugs are generally inhibitors of enzymatic domains on the mutated, overexpressed, or otherwise critical proteins within the cancer cells. These agents used in molecular targeted therapy are classified into small molecules, monoclonal antibodies, and immunotherapeutic cancer vaccines. (Wang J., Lei F., and Han F., 2018)

Since targeted therapy consist in using drugs on a molecular level, it's needed the approvement by the Food and Drug Administration (FDA). Something that this treatment has the approvement on many molecular targeted therapies.
In addition, there is remarkable clinical success in the treatment of a myriad of cancer types, such as breast, leukaemia, colorectal, lung and ovarian cancers. Furthermore, there are some advantages as well as limitations. (Lee Ting Y., Tran Jer Li, Chern Ein Onn, 2018)

2.4.2 Drugs

There are many different drugs approved by the Food and Drug Administration (FDA) as treatment for cancer. This is because there are different types of cancer, and as such the treatment has to be different. In the tables 2-4 will there be shown an overview of the nanodrugs that are approved, some examples of targeting ligands, and an overview of nanoparticles platform for drugs delivery system.

*Table 2 **Overview of approved anti-cancer nanodrugs***

Name	Formula	Approved indication(s)
DaunoXome	Liposomal daunorubicin	HIV-related Kaposi sa
Caelyx, Doxil	Pegylated liposomal doxorubicin	Breast, Ovarian ca, Kaposi sa, Multiple myeloma
DepoCyte	Liposomal cytarabine	Lymphomatous meningosis
Oncaspar	PEG asparaginase	Acute lymphoblastic leukemia
Abraxane	Albumin-bound paclitaxel	Breast, Pancreas ca, NSCLC
Myocet	Liposomal doxorubicin	Breast, Ovarian ca, Kaposi sa, Multiple myeloma
Marqibo	Liposomal vincristine	Acute lymphoblastic leukemia
Genexol	Paclitaxel loaded polymeric micelle	Breast, Ovarian ca, NSCLC
Onivyde	Liposomal irinotecan	Pancreas ca
Kadcyla	Trastuzumab linked to emtansine	HER2+ breast ca
Mepact	Liposomal mifamurtide	Osteosarcoma
Gliadel Wafer	Carmustine in poliferosan 20	High grade glial tumours- local therapy

ca - carcinoma, sa - sarcoma
*Source: (*Kopeckova K. et. al, 2019)

Table 3 Examples of targeting ligangds

Type of ligand	Ligand	Receptor	Cancer
Antibodies	Trastuzumab	Her2/neu	Breast, gastric, lung ca
	Rituximab	CD20	B-cell NHL and leukemia
	Anti-CD19	CD19	B-cell NHL and leukemia
Aptamers	Pegaptanib	VEGF receptor	Different cancers
	A10 aptamer	PSMA	Prostate ca
	RGD	Integrin receptors	Different cancers
Peptides	ATWLPR	VEGF receptor	Different cancers
	Vasoactive intestinal peptide	VAP receptor	Different cancers
	Lyp-1	P32 receptors	Different cancers
Proteins	Transferrin, Ferritin	Transferrin receptor	Different cancers
	LHRH	LHRH receptor	Breast, ovarian, prostate ca
	Folic acid	Folate receptor	Different cancers
Small molecules	Galactose	Asialoglycoprotein receptor	Hepatocellular ca
	Biotin	Biotin receptor	Different cancers
	Mannose	MRC1 mannose receptor	Different cancers

ca - carcinoma
*Source: (*Kopeckova K. et. al, 2019)

Table 4 Overview of nanoparticles platform for drug delivery system

Composition	Particle type	Size (nm)	Properties
Polymer		10-1000	Biodegradable
Poly (amidoamine)	Dendrimer	1-100	Biocompatible
Lipid	Liposomes, micelles	15-1000	Biocompatible, carry hydrophobic drugs, biodegradable
Gold	Spheres, rods, shells	10-100	Biocompatible
Silica	Spheres, rods, mesoporous	10-100	Biocompatible
Carbon	Nanotubes, buckyballs, graphene, nanodiamonds	*	Biocompatible

*Carbon nanotubes- diameter 10-100 nm, length < 100 μm,
nanodiamnonds - ~5 nm.*
*Source: (*Kopeckova K. et. al, 2019)

Drug as a possible cancer treatment
The history of drugs as a mean for cancer treatment dated back to the nineteen hundreds or nineteen century and since their first uses, they were designed to fight specific areas or targets, where there was not any difference if they were synthetically or natural products designed (Jones, 2014). For him, the purpose of the drug is to be able to deliver a specific antibody or enough antibodies that could be able to attack or fight a specific cancer cell. In addition, he also mentions that drugs use promises a very high indication of success as many plants and bacteria are able to produce toxins which are already proven to be successful in fighting or attacking cancer cells.

It is very important to mention that according to Arrondeau, et. al (2010), in order for any anticancer drug to be approved for its use; these steps have to be followed:

1. The first phase or step is to make sure or represent that the drug may be administrated to human beings. In this phase, the goal is to find out the toxicity and dose which is recommended.

2. The second phase or step is to observe and study the anti-tumor or anti-cancer cells activities in the specific cancer cells or tumor cells.

3. The third phase or step is to compare and evaluate the efficiency of the new drug treatment with regular treatment or care or previous drug treatment.

3. Using Lipid nanoparticles as a "magic bullet"

Chemotherapy can lead to a high toxicity, and thereby limiting the amount of drug the patient needs. As a result, not all the tumour tissue may be exposed to the drug as a lethal dose. The level of toxicity can decrease using nanocarriers or nanoparticles (NP) to deliver the drug targeting the tumour. These NP can be liposomes and micelles, in their small size (~ 100nm or less) (David R. Khan, 2010). So other words the use of NP is a form of targeted therapy. The following points will have the definitions of lipids, nanoparticles, lipid nanoparticles, how we can use these molecules as a cancer treatment, and the formation and structure of Lipid Nanoparticles (LNPs).

There are two ways of using NPs, one is by passive targeting and the other is by active targeting. These concepts will be discussed later.

3.1. Lipid definition

It is commonly known as fat but chemically, it is defined as a substance which it is not soluble in water, but it is soluble in chloroform, alcohol, and ether. For living cells, they are a very important component. Lipids, carbohydrates, and proteins are the most important components of living cells for animals and plants. A body may easily store lipids, Cholesterol and triglycerides are lipids and may be considered as fuel and a very important part of cell's structure. (Davis, 2021)

It may also be said that the body uses Lipid to store energy and for insulation.

Lipids have different and very large groups of organic molecules which may include fatty acids (unsaturated or saturated carboxylic acid with a aliphatic chain which is very long such as oleic, palmitoleic and linolelaidic acids), sterols (steroids alcohol such as testosterones, bile acids and cholesterols), waxes (cetyl palmitate), diglycerides, phospholipids, triglycerides, monoglycerides, soluble Vitamins (K, E, D and A). For them, lipids are well known as biological systems which store energy and also act have functions in cell structural membrane's structural components and signaling. (Rajabia and Moursa, 2016)

3.2. Nanoparticles definition

When the nanotechnology is applied in the medicine, it becomes as nanomedicine, and it is a new tool used to treat cancer which gives the benefit of a more effective therapy and less toxic and invasive for patients. (Rajabi and Mousa, 2016),

In general, are nanoparticles small molecules with sizes in range from 1 nm to 1μm, for biological purposes. However, the size usually varies between 10 and 800 nm. Nanomedicine is aiming for a development of novel imaging and therapeutic agents with enhanced efficacy, improved safety, and lower toxicity. (Jovčevska I., and Muyldermans S., 2020)

Nanomedicine is constantly in development through the fields of nanodiagnosis and nanotherapy. Nanodiagnosis stands for the planned use of devices with at least one dimension in the nanometre scale for detecting events that occur at a molecular level. (Jovčevska I., and Muyldermans S., 2020)

3.3. Lipid Nanoparticles

Lipids are recognized as vehicles for the delivery of active ingredients through different routes of administration because of their biocompatibility and biodegradability (Rosiaux et al., 2014). Liposomes are spherical structures and one of the oldest, while being still promising drug carriers. The structure is made of a hydrophilic core surrounded by a bilayer of some amphiphic lipid materials, normally phospholipids. The size of the liposomes is in the range of 25nm-2.5µm with one or several bilayer membranes. (Alvi M. and Hamidi M., 2019)

There are some major disadvantages using liposomes for drug delivery, including the lack of affordable preparation methods, low degree of drug loading capacity and stability, and rapid decomposition in the human body before the therapeutic effect can be achieved.

LNPs are mainly distributed in four types, solid lipid nanoparticles (SLN), nanostructured lipid carrier (NLC), lipid drug conjugate (LDC) and polymer-lipid hybrid nanoparticle (PLN). In 1993.1996 was the first generation of LNPs introduced as SLNs. (Alvi M. and Hamidi M., 2019)

3.4. Formation and structures of Lipid Nanoparticles

Originally were LNPs designed to deliver siRNAs, but recently these molecules have been applied for the delivery of mRNA and present the most clinical-translatable non-viral delivery vehicles. The composition of the LNPs is mainly of an ionizable amino-lipid-like molecule, cholesterol, a helper phospholipid, and lipid-anchored polyethylene glycol (PEG). (Miao L. and Huang. L., 2021) In fig 22 you can see the components of a LNP, and the various hydrocarbons chains ligands.

The ionizable lipid is an amphipathic structure with a hydrophilic headgroup, which contains one or multiple ionizable amines, hydrocarbon chains are capable of promoting self-assembly, and with a linker that connects the headgroups with the hydrocarbon chains. The design of an ionizable lipid is to acquire positive charge by protonation of the free amines at a low pH for two main purposes:

1. During the preparation of LNPs, the encapsulation of the negatively charged mRNA via electrostatic interaction is facilitate by the positively charged lipids.

2. The release of mRNA from both LNPs and endosome can be caused by the possible interaction of the positively charged lipid with the ionic endosomal membrane, which facilitate the membrane fusion and destabilization. All of this occur in the acidic endosomal microenvironment upon intracellular delivery of LNPs. At the physiological pH, the ionizable lipid remains neutral, improving stability and decreasing systemic toxicity. (Miao L. and Huang. L., 2021)
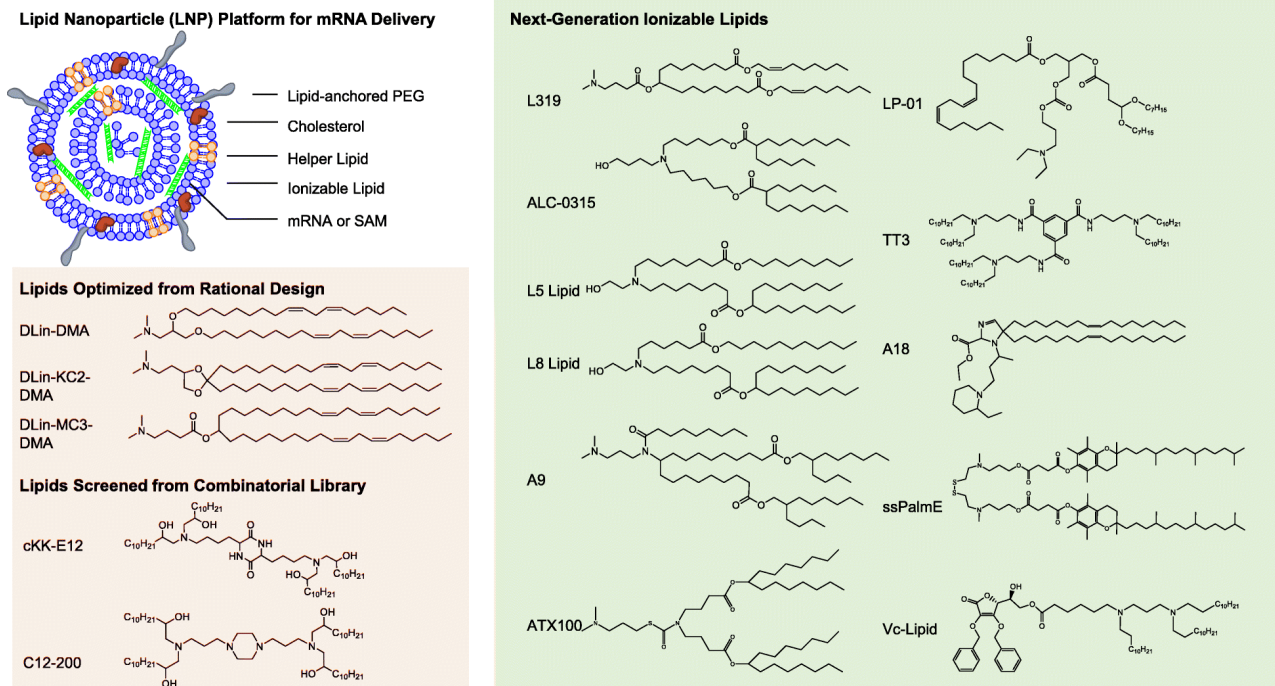

Figure 22 Representative LNP structure and ionizable lipids used in preclinical research and clinical trials, (Miao L. and Huang. L., 2021)

Liposomes encapsulates drugs due to an internal aqueous core, which is surrounded by a phospholipid bilayer. The use of phospholipids is ideal as it relates to the biocompatibility of these nanocarriers. This aqueous core suites perfectly for hydrophilic drug delivery, while the encapsulation of hydrophobic chemotherapeutics is caused by the phospholipid bilayer. Figure 23 illustrated the interior of a liposome. (New, 1990; Khan et al., 2008)
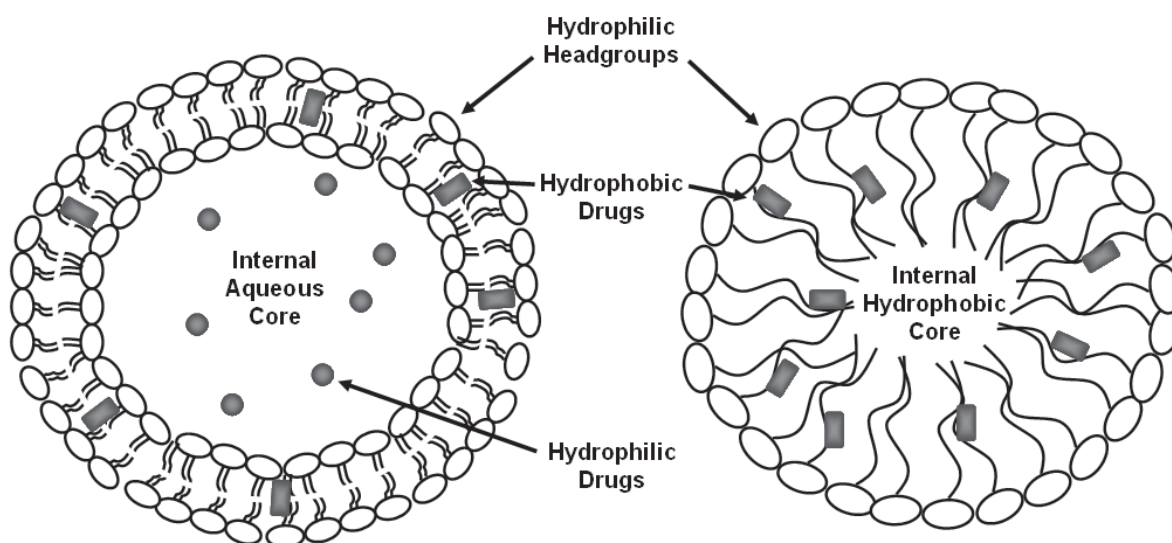
Figure 23 Structure of liposomes, with both hydrophilic and hydrophobic drugs. (David R. Khan, 2010)

During the preparation method could it be possible for a practically acceptable drug encapsulation by using SLN instead of liquid lipids, due to that SLN have a high stability at a physiologic temperature of 37 °C in the reticuloendothelial system.
These structures: SLN, NLC, LDC and PLNs have other advantages including an easy large-scale production, simple sterilization, suitable bioavailability, biocompatibility, biodegradability, controlled release of drugs, higher shelf life, efficient drug targeting, and improved drug absorption and dissolution. (Alvi M. and Hamidi M., 2019)

Another type of LNPs are NLCs, which without a perfect crystalline stricture still have both solid and liquid lipids in their composition. An advantage of this system over the use of SLNs is a higher capacity to encapsulate a wide range of drugs with solubility in the liquid and solid phases of lipids. With respect to loading of hydrophilic drugs both SLNs and LNPs have limited capabilities. (Alvi M. and Hamidi M., 2019)

The result of a new nanostructure named LDC, is a conjugation of hydrophilic drugs with hydrophobic molecules due to covalent bonds and salt formation. There are some drugs that are sensitive for the acidic condition of the stomach, so LDCs can be used for these drugs. And for an even better loading of hydrophilic drugs, were PLNs introduced as a linkage between ionic polymers and hydrophilic drugs such as gemcitabine. Within a core-shell structure can several polymers such as polycaprolactone and polylactic-coglycolic acid be utilized for conjugation with drugs. (Alvi M. and Hamidi M., 2019)

3.5. Nanoparticles as treatment

As mentioned earlier in the thesis, the use of nanoparticles as cancer treatment is a form of targeted treatment. Targeted treatment can be used in two ways, passive targeting, or active targeting.

3.5.1. Passive targeting

The basis for the passive targeting of the tumour tissues by liposomes is mainly, the different pore sizes between the endothelial cells of the tumour microvasculature compared to the "tighter" structures found in normal capillaries. For an ideal targeting goal would be achieved, the preparation of liposomes has to be with such a size that allows them to extravasate in the tumour tissues, while also prohibiting the carries to exit through the capillaries in normal tissues. Fig. 24 shows how liposomes target cancer tissues in both active and passive targeting. (Alvi M. and Hamidi M., 2019)

The accumulation of nanoparticles in tumours is caused by the enhanced permeability and retention effect, which is due to angiogenic processes that produce highly permeable blood vessels in tumours and their characteristic abnormal lymphatic drainage that leads to accumulation of the nano-scale particles un them, thereby allowing cytotoxic drugs to be release close to the tumour cells. (Pérez-Herrero E. and Fernández-Medarde A., 2015)
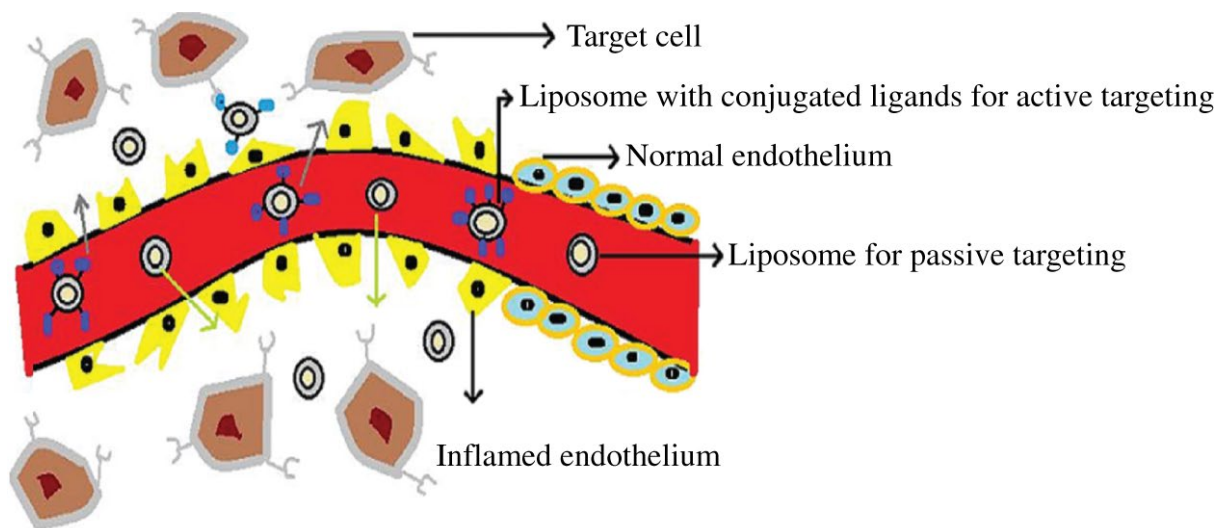

Figure 24 Passive and active targeting of cancer cells for drug targeting by liposomes (Alvi M. and Hamidi M., 2019)

3.5.2. Active Targeting

To achieve active targeting, a variety of ligands are utilized to exploit any specific antigens expressed by cancer cells (Alvi M. and Hamidi M., 2019). This expression of antigens increases the cytotoxicity of anticancer agents in tumours and avoiding most of the side effects, since the exposure of healthy cells to the drug is minimized. In the surface of polymer nanoparticles is it found the antigens which by the functionalization do it not only provide active targeting characteristics to the particles, but also improve the therapeutic efficacy of cytotoxic drugs and overcome the multidrug resistance.

However, there are several ways a drug carrier can target actively a specific site of body (Fig 25).
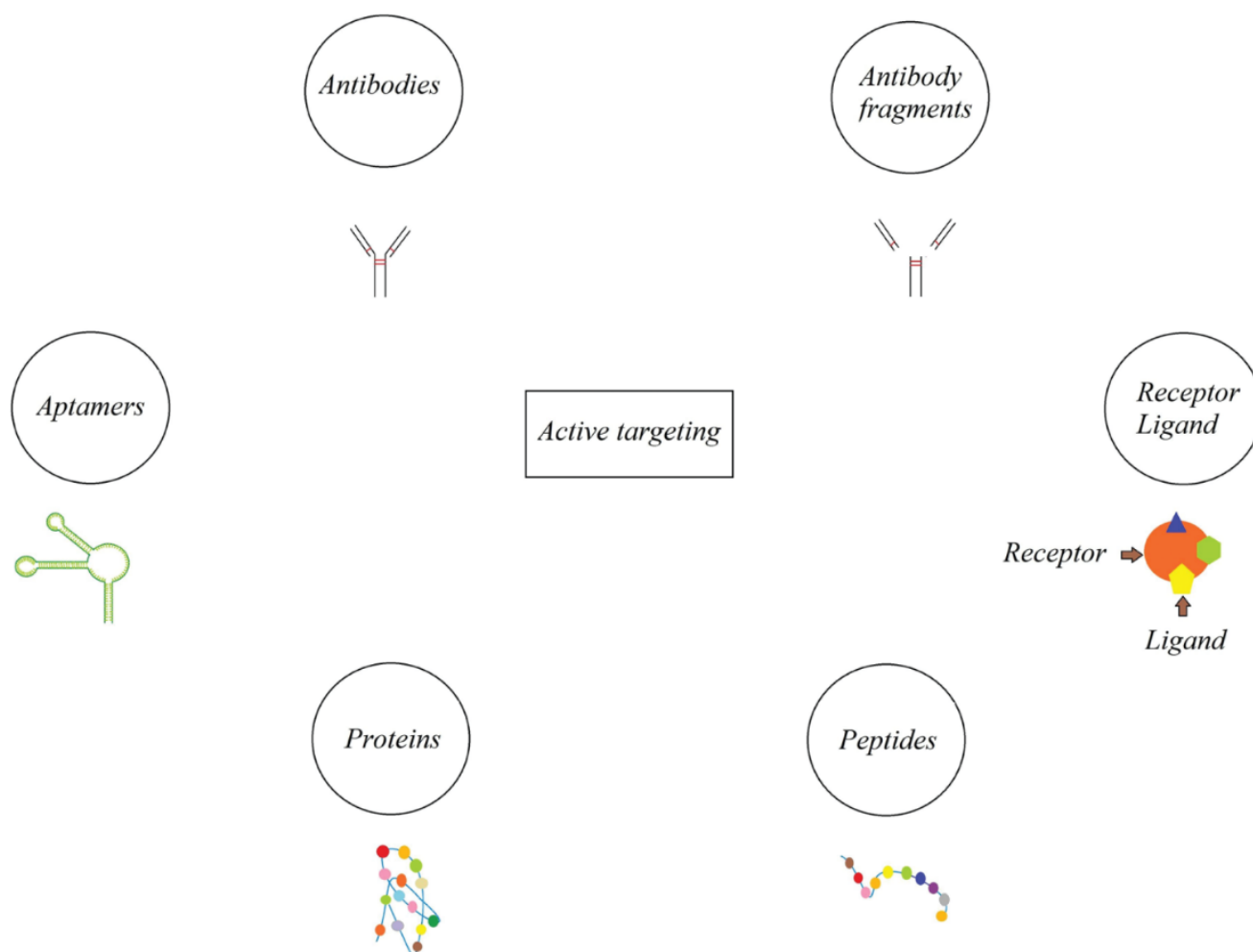
Figure 25 Different approaches of active targeting by liposomes in drug delivery system (Alvi M. and Hamidi M., 2019)

3.5.3. Application

3.5.3.1.   Gene Therapy and CRIPS/CAS9

Gene therapy consist in introducing genetic material of either DNA or RNA into cancerous cells to destroy or inhibit their growth. The method of performance to gene therapy is by either replacing mutated tumour suppressor gene with normal gene to restore their function, causing the inhabitation of expression of oncogene by introducing a genetic material like siRNA, or the stimulation of the immune response by antisense oligonucleotide, inhibiting tumour-associated angiogenesis process to sensitize the cancer cells towards cancer treatments (National Cancer Institute, 2013).

Gene therapy is used in immunotherapy, oncolytic virotherapy, microenvironment modulation, RNA interference, targeted genomic interventions, gene editing and gene transfer (Yeuan T. L., Yi J. T., Chern E. O., 2018)

The most recent genome editing tool used in gene therapy es a novel technique called "Clustered Regularly Interspaced Palindromic Repeats" (CRISPR) or CRISPR associated nucleased 9 (CRISPR-Cas9). Jinek and colleagues were the first to identify the potential use of CRISPR-Cas9 to be used in genome editing, through an elaborate study on site specific

cleavage ability of Cas9 endonuclease to introduce targeted double-strand breaks in *Streptococcus pygene bacteria* (Jinek et al., 2012, Yeuan T. L., Yi J. T., Chern E. O., 2018).

In general, CRISPR spacer sequence is transcribed into a short RNA sequence which acts as a guide and matched to the targeted mutated gene sequence. In the meantime, will Cas9 bind to and excise mutated DNA sequences, causing a particular gene to be silence. In a recent study was reported the successful use of CRISPR-Cas9 to introduce a gene-encoding for prodrug-converting enzyme herpes simplex virus type 1 thymidine kinase to replace a mutated DNA of the cancer fusion gene with a suicide gene that led to cell death of the human prostate cancer and hepatocellular carcinoma cells (Chen et al., 2017, Yeuan T. L., Yi J. T., Chern E. O., 2018). Figure 26 illustrate the components delivery methods of using CRISPR-Cas9
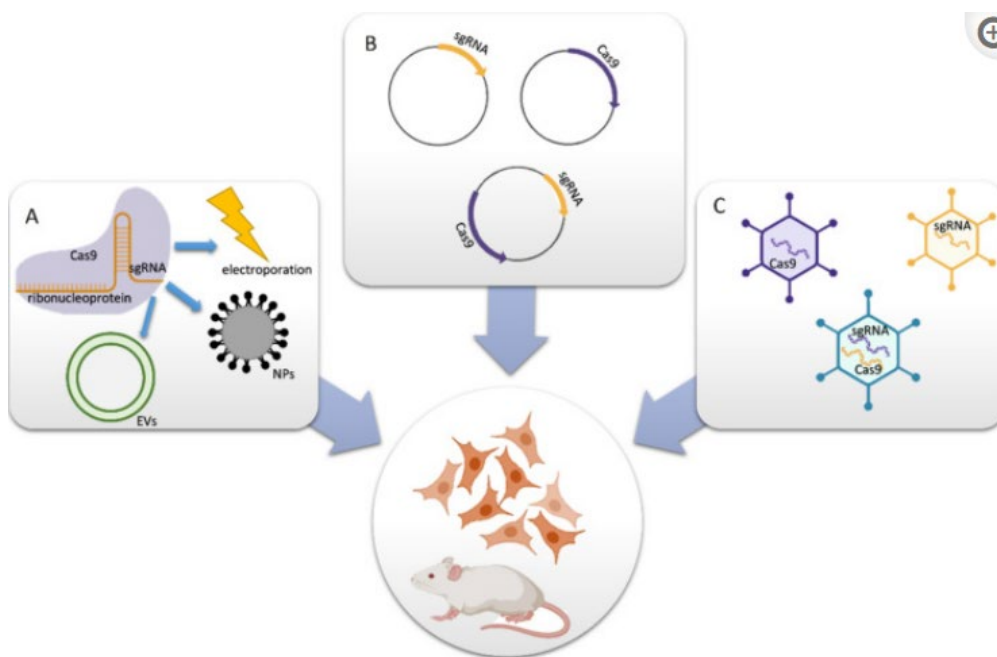

Figure 26 Fig.1. CRISPR/CAS9 components delivery method. (A) Cas9 proteins and sgRNA forms and ribonclousprotein (RNP) complex packaged in extracellular vehicles (EVs), nanoparticles, or electroporated directed into model organism or cells. (B) sgRNA and/or Plasmids expressing Cas9 transfected into cells. (C) Viral vectors encoding sgRNA and/or Cas9 deliver in vivo or in vitro

3.5.3.2.   Therapeutic and mRNA vaccines

The immune-mediated anitumour response is the target of therapeutic cancer vaccines. This response is divided in two main types: patient-specific and patient.nonspecific. Vaccines with patient-specifitivety are generated from patient's tumour cells, while patient-nonspecific vaccine are generated by inducing a generalized immunologic response, which may have in a minority of patients an antitumour effect. Therapeutic cancer vaccines also target specific tumour-associated antigens, through stimulated T-cells, in the context of a peptide-major histocompatibility complex to induce antitumour immune response (Yeuan T. L., Yi J. T., Chern E. O., 2018).

mRNA transfection into dendritic cells for adoptive transfer was the first mRNA based therapeutic cancer vaccine entering clinical trial. Therapeutics still account for the majority of mRNA cancer vaccines in clinical trials eventhough they are DC-based mRNA vaccine, in vivo

transcription mRNA-based immunotherapies are delivered by non-viral vectors, extensively explored recently as a result of the promising antitumour outcomes collected from preclinical studies, with CureVac, BioNTech and Moderna as poneers in the campaign. (Miao L. and Huang. L., 2021)

The delivery system for these mRNA-based cancer vaccines includes lipid polyplexes, LNPs or protamine. These vaccines have been applied to treat less accessible, aggressive, and metastatic solid tumours, including non-small cell lung cancer, colorectal carcinoma, melanoma, etc. (Miao L. and Huang. L., 2021)

Figures 27 and 28 are diagrams of mRNA translation and the components of a cell

There have been expedited advances in the drug delivery system through preclinical development of mRNA therapeutics, providing the basis for mRNA as a new class of drug as shown in figure 29. (Hou X., Zaks T., Langer R., Dong Y., 2021)
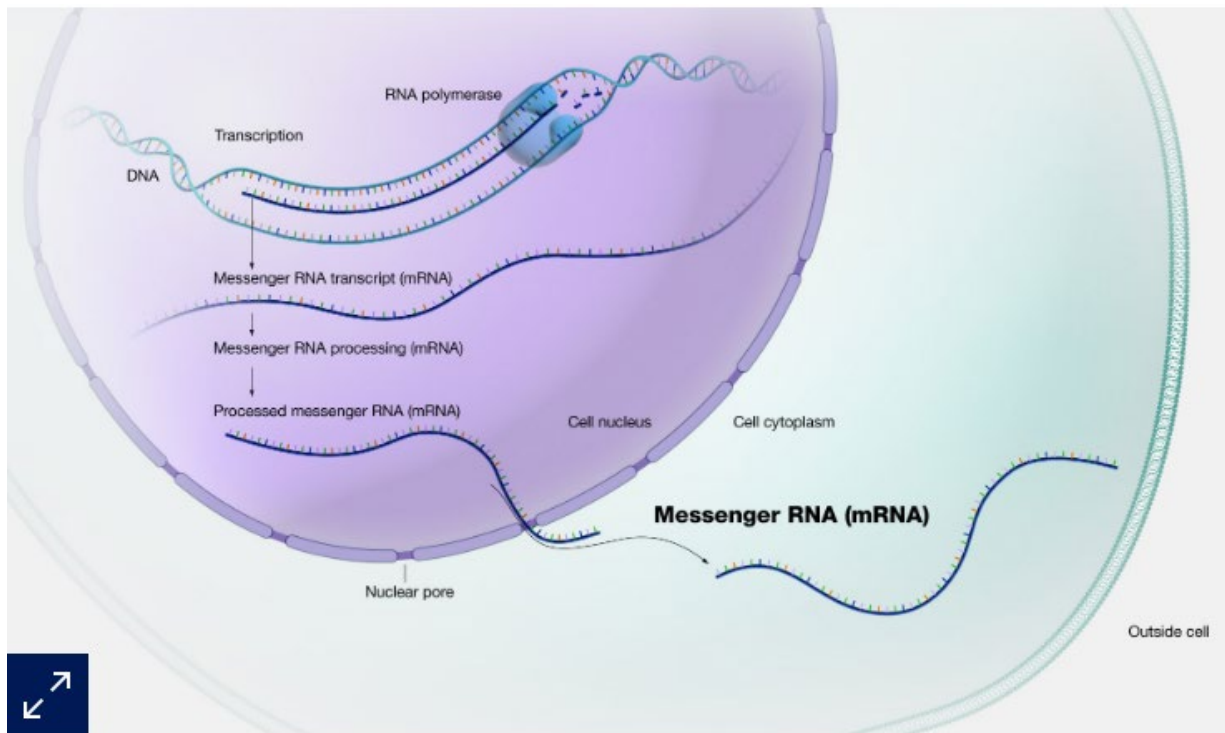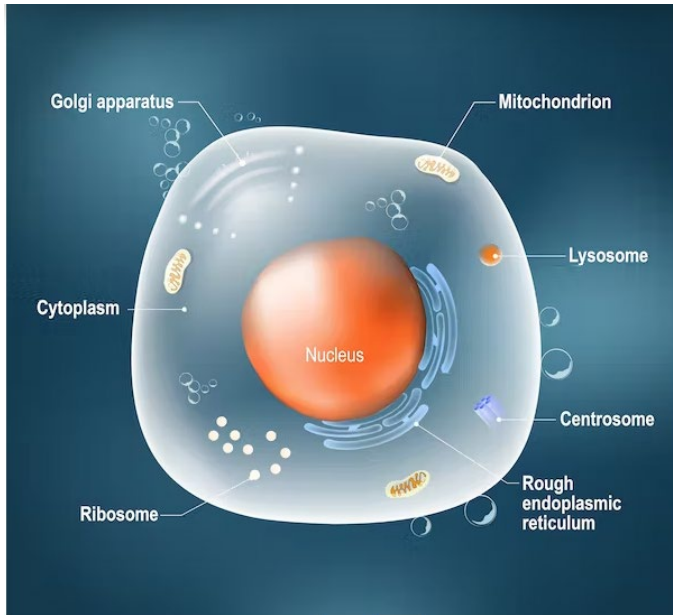

Figure 27 mRNA delivery prosses and translation

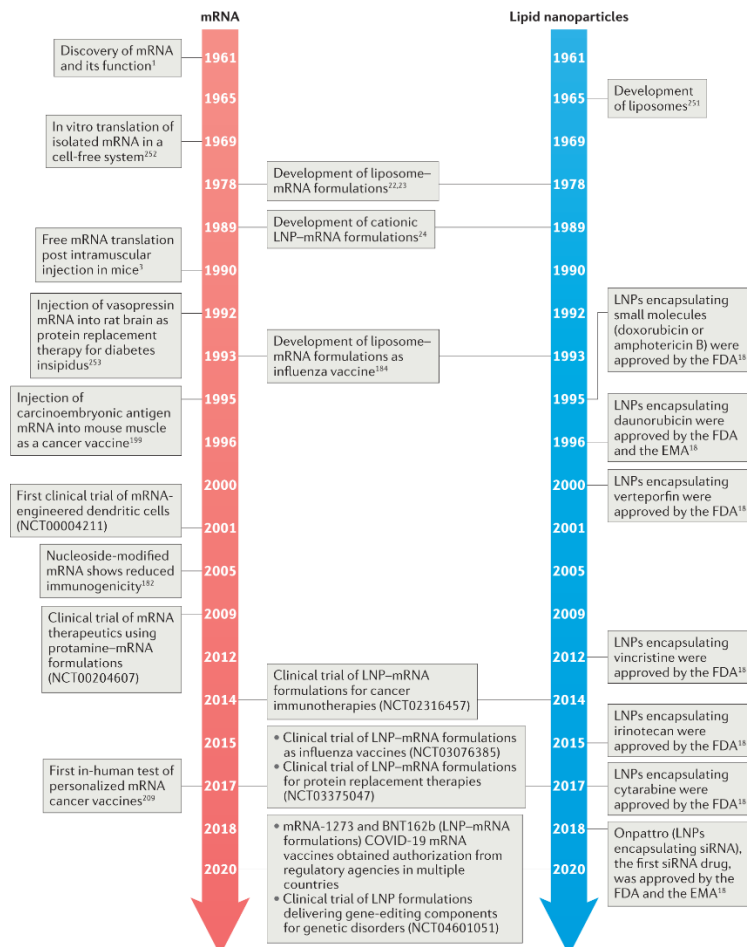*Figure 28 Cell elements*

Figure 29 Timeline of some key milestones for mRNA and lipid nanoparticle development (Hou X., Zaks T., Langer R., Dong Y., 2021)

4. Discussion

Over time through the decades the technology for cancer treatment have had an enormous advance. And in the last decades we have improved our understanding of the process of carcinogenesis, cancer biology and the tumour micro-environment. (Wang J., Lei F., and Han F., 2018)

Some cancer treatments have severe side effects on the patients, such like chemotherapy and radiotherapy, even though these treatments are verry efficient. That's why recently scientists are approaching cancer with other treatments like immunotherapy, hormonal therapy, or targeted therapy, by developing and modifying the cancer treatment procedures and plans to increase the effectiveness and precision of the treatment, causing the survivability of the patients and improving their quality of life. (Wang J., Lei F., and Han F., 2018)

The side effects of any cancer treatment occur when the healthy or organs are affected by the treatment. Some of these side effects can be seen in radiotherapy such as fatigue, but also in chemotherapy like anemia, appetite loss, diarrhea, fatigue, hair loss among other.

Along with chemotherapy, surgery and radiation have immunotherapy and targeted therapies become pillars of cancer therapy. There are ongoing studies to prevent and combat immunotherapy resistance, by combining other therapies with immunotherapy. The studies also help to determinate how to make a tumour "immune hot" so that there is a better response to immunotherapy. (Abbott M. and Ustoyev Y., 2019)

The reason why immunotherapy is not yet a cure-all for cancer, is because not all patients survive it and there are some tumours that do not response to the treatment. However, there is promise for further development and efficacy in the future. (Abbott M. and Ustoyev Y., 2019)

Within the targeted therapy the use of nanomedicine such as lipid nanoparticles, gene therapy, therapeutic vaccination and nanoparticles drug delivery is really common in the last decades. There have been limitations for the development of cancer vaccines caused by the lack of understanding on how to immunize patients to achieve a response of cytotoxic T-cell as well as circumvent and incapacitate the TME for an anti-tumour response. Clinically relevant for tumour death before they can occur. (Abbott M. and Ustoyev Y., 2019)

In immunotherapy mRNA vaccines have become a promising platform for cancer treatment. During vaccination, naked or vehicle loaded mRNA vaccine efficiently express tumour antigens for antigen-presenting cells (APCs), facilitating the activation and stimulation of the innate/ adaptive immune system. mRNA cancer vaccines have a high potency, meaning that they can be used in other conventional vaccine platforms. In addition to a safe administration, rapid development potentials, and cost-effective manufacturing. Still there are some limitations in the applications, due to instability, innate immunogenicity, and inefficient in vivo delivery. (Lei Miao, Yu Zhang and Leaf Huang, 2021)

mRNA has shown therapeutic potential in a range of applications, including viral vaccines, protein replacement therapies, cancer immunotherapies, cellular reprogramming, and genome editing. By using mRNA delivery as a treatment this technology have given good results treating the COVID-19 pandemic and may be used as a cancer treatment as it was already developed the spleen-targeted DOTMA-mRNA lipoplexes (RNA-LPX) for a systematic

cancer vaccine. Using the same formulation, for the autoimmune encephalomyelitis treatment a mRNA vaccine was developed. (Hou X., Zaks T., Langer R., Dong Y., 2021)

Two of the major concerns about CRISPR-Cas9 therapeutics are potential toxicity and immunogenicity as an evaluation of potential therapies using cLNPs for cancer. The encapsulation of the Cas9nuclease remains a challenge in both viral and nonviral delivery system due to its large size. Several approaches have been used to overcome the obstacle of delivering the large Cas9 nuclease as nucleic acid or protein for gene editing in the liver or locally for treating genetic disorders. (Daniel R. et. al, 2020)

5. Conclusion

Through time cancer treatment have been develop, as well for a global cure for the disease with the technological advances. As stated earlier in the thesis there are several types of cancer, which leads to several types of treatment. Again, some of the most common cancers are bladder cancer, prostate cancer, breast cancer, leukaemia, liver cancer, lung cancer. And the most common treatments are chemotherapy, radiotherapy, immunotherapy, hormonal therapy and recently target therapy.

Within the target therapy we have the use of LNP is versatile with the use of SLN, liposomes, gene therapy and mRNA delivery through either active targeting or passive targeting.

Solid lipid nanoparticle, although in its nascent stage, has a great potential to cure the cancer, with least side effects. It is the technology that will grow in years to come, and probably, the human race will have a 100% cure to cancer. (Mathur V. et. al, 2010)

In conclusion, mRNA is a powerful and versatile cancer vaccine platform. Its successful development towards clinical translation will remarkably strengthen our ability to combat cancers. Future investigations should continue focusing on (but not limited to) understanding and utilizing the paradoxical inherent innate immunity of mRNA, improving the efficiency of antigen expression and presentation by designing advanced and tolerable delivery systems, and modifying mRNA structures to achieve extended and controlled duration of expression. (Miao L. et. al, 2021)

6. Future research

The study of tumor microenvironment, its cellular and molecular components, and how they affect tumor progression, are emerging topics in cancer research. The factors released by the tumor cells themselves, in particular pro/anti-inflammatory molecules or pro/ anti-angiogenic mediators that contribute in creating an environment, mostly affect or not the tumor. The events and molecules involved in this cross talk within the tumor microenvironment have emerged as attractive targets in anticancer therapeutic treatments. The stroma surrounding the cancer cells plays an important role to study the development, progression and the behavior of the tumor.

Still some hurdles for nanodrugs have to be overcome. Most of these drug systems have undergone some in vitro and in vivo testing. However, we await the data from more clinical trials with nanodrugs.

SLN constitute an attractive colloidal drug carrier system due to successful incorporation of active compounds and their related benefits. Although most of the technologies have focused on the delivery of single chemotherapeutic agents to the tumors, it is increasingly becoming clear that an integrative approach may work better than a reductionist approach. Nanotechnology platforms can provide the unique niche within this space by enabling multimodal delivery with a single application. Although SLN's may be used for drug targeting, when reaching the intended diseased site in the body the drug carried needs to be released. So, for drug delivery biodegradable nanoparticle formulations are needed as it is the intention to transport and release the drug in order to be effective. (Mathur V. et. al, 2010)

With the recent approval of two mRNA LNP vaccines to prevent COVID-19, mRNA vaccines are experiencing a considerable burst in preclinical and clinical research in both cancer and infectious disease fields. The challenges of developing cancer vaccines versus infectious disease vaccines lie in: firstly, most infectious disease vaccines are prophylactic, whereas cancer vaccines are therapeutic. The cases for preventive cancer vaccines are rare with only two FDA approved such vaccines, and these two vaccines are applied to prevent virus-induced malignancies (HPV and HBV). (Lei Miao, Yu Zhang and Leaf Huang, 2021)

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