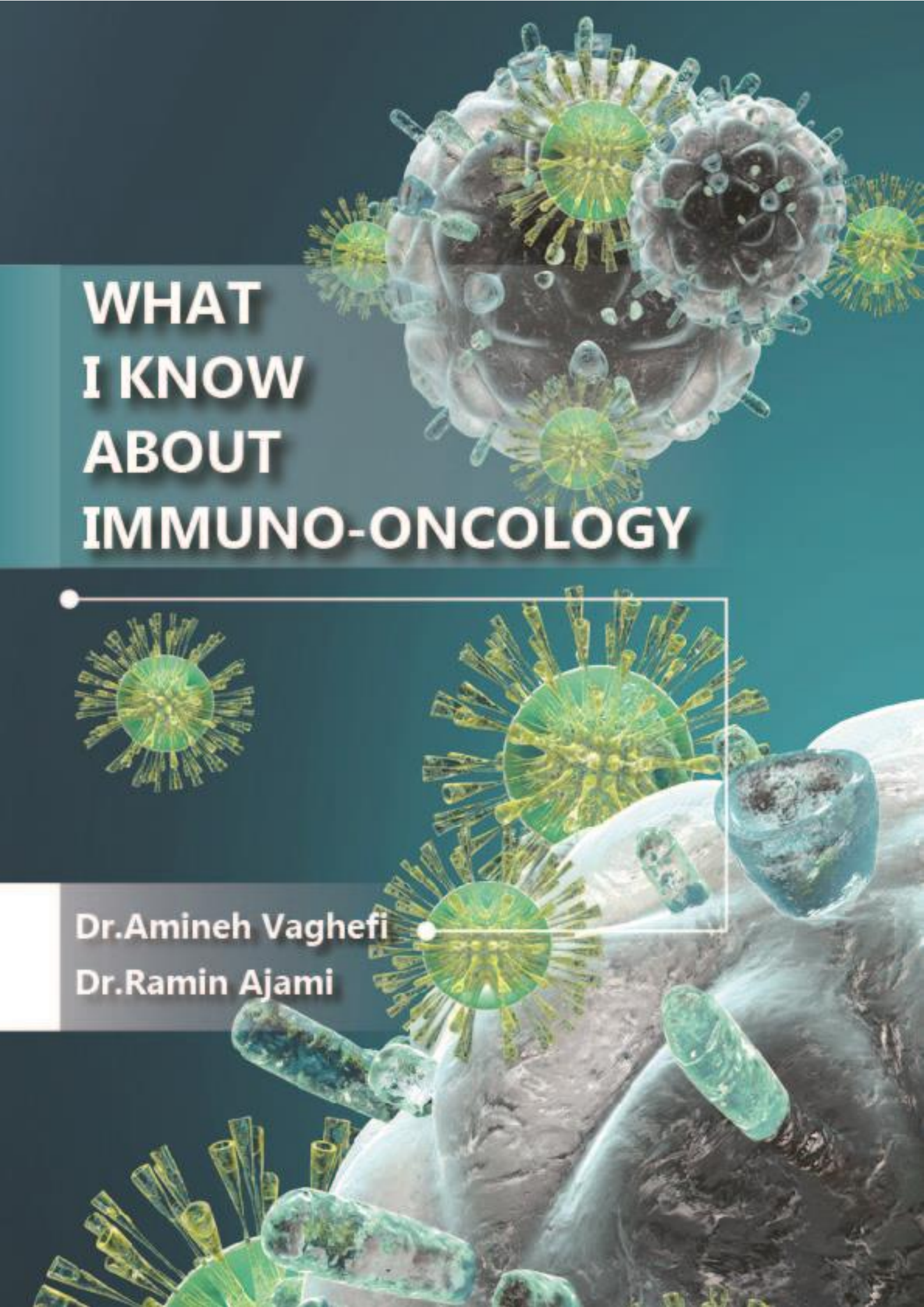
WHAT I KNOW ABOUT IMMUNO-ONCOLOGY



Dr.Amineh Vaghefi
Dr.Ramin Ajami

July 2022

Contents

Preface

This is Dr. Amineh Vaghefi. I am a pathologist with a great desire and passion for reaching new heights of understanding in the field of cancer genetics. Immuno-Oncology is one of the most exciting parts.

The interplay between tumors and their immunologic microenvironment is complex and challenging to decipher, but its understanding is of seminal importance for developing novel prognostic markers and therapeutic strategies.

This manuscript offers a quick review of all the aspects of immuno-oncology one would need to know, from the general principles to the latest advances. The book will serve as a valuable guide for medical students, general practitioners, pathologists, oncologists, etc. or anyone interested in cancer immunology.

This manuscript has been reviewed by Dr Ramin Ajami MD MSc MBA PgDip Oncology.

Should you have any questions, please feel free to contact us.

Dr. Amineh Sadat Vaghefi MD
Clinical and Surgical Pathologist
Fellowship of Cancer Genetics

Ramin Ajami MD MSc MBA PgDip Oncology
Medical Oncologist
Head of RD Medical Services, Oncology Department HCA UK
Guy's and St. Thomas' Hospital, NHS Trust, England

Overview

Our health is frequently threatened by external dangers, like bacteria, viruses, and fungi, as well as internal threats, like tumors. The immune system consists of molecules, cells, and tissues that act together to protect us from these threats.

There are two main branches of the immune system. Innate immunity forms our first line of defence and consists of physical barriers like the skin and cells, such as neutrophils and macrophages. These defences are already active before threats arise and also react quickly to new hazards, but are not very specific to any particular threats.

Adaptive immunity includes B cells that produce antibodies and T-Cells that kill infected cells. This response takes longer to develop, but is much more specific and powerful than the innate response. The adaptive response also remembers threats it has seen before and can act more quickly and efficiently against them if they appear again.

The two responses work together to keep us healthy by fighting off harmful molecules and microbes and, perhaps, surprisingly, by trying to protect us from cancer.

How can we work with our immune system's natural abilities in order to develop novel therapies to treat and potentially even cure cancer?

There are many different types of cancers, and the risk of developing each type can be influenced by factors like age, genetics, environmental exposure, and lifestyle. However, all cancers involve uncontrolled replication and spread of our own cells.

As we grow, each cell in our body divides a finite number of times. As cells age, they accumulate changes in their DNA sequences known as genetic mutations. Some of these mutations may allow the cells to continue dividing in an uncontrolled fashion, prevent them from dying when they should or destabilize the genome and increase the rate of mutation. The genetic changes that occur in each individual's cancer can be very different, leading to changes in how quickly cancer spreads and how susceptible the tumor is to treatment.

As cancer grows, there can be a number of effects on the body. These effects begin locally when the tumor interferes with the function of organs and tissues. As the tumor continues to grow, some cancerous cells can enter the blood or lymphatic circulation, spread elsewhere in the body, and establish new tumors in a process called "metastasis". In metastatic cancers, additional life-threatening effects arise as the growing tumors impede the function of other organs.

Due to cancer's high incidence and mortality rate, extensive research has gone into developing treatments to combat it. Removing the tumor surgically, also known as resection, or killing the cancer cells with high doses of radiation can effectively treat tumors that are concentrated in a signal area, but are generally ineffective against cancers that have already metastasized.

Chemotherapy drugs are toxic to cancer cells and generally act throughout the entire body, but often have serious side effects as the drugs can kill both cancerous and non-cancerous cells.

While conventional treatments help many patients, their effects are sometimes limited. So new therapies are needed to improve our ability to treat cancer when it arises.

One reason individuals have such varied responses to cancer treatments is that cancer is a heterogeneous group of afflictions that can affect any tissue of the body and have a range of underlying molecular causes. Even a single tumor can contain a heterogeneous mix of cells that respond differently to a treatment. One way to improve outcomes is to utilize treatments that focus on the underlying biology of the tumor.

Development of precision therapies that act against specific genetic mutations has allowed more precious targeting of cancer cells and has dramatically reduced adverse side effects. However, there are many cancers for which precision therapies are not available. Furthermore, cancers often develop resistance to the existing treatments when the target genes mutate further.

In some cases, immunotherapies provide a new option for treatment. Due to the presence of mutations, cells in developing cancers can produce abnormal molecules that can be seen as foreign by the immune system, which then attempts to destroy the nascent tumor as it would infection. However, tumor can often evade this response, so this process alone generally fails to stop tumor growth. Immunotherapy treatments, such as checkpoint blockade and CAR T-Cell therapy, can increase the immune response and help overcome this immune evasion.

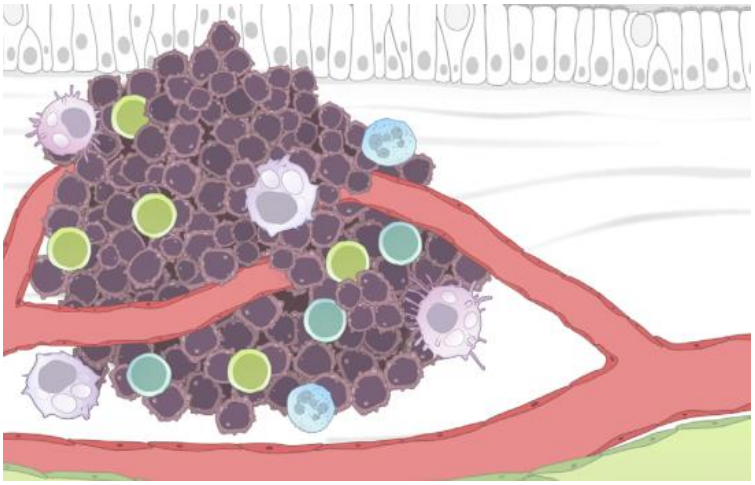
These types of treatments have proven very effective in a significant fraction, albeit a minority of patients with otherwise resistant diseases.

There are still obstacles to overcome, including an increased range of diseases against which these effective treatments reduce adverse side effects, but research to create and improve immunotherapies that can be used alone or in combination with existing therapies shows excellent promise. While no single treatment will be effective for all cancers, we can harness the anti-tumor activity of the immune system in multiple ways to create novel therapies that can treat and potentially even cure various cancers.

Basic Tumor Immunology

1) Biology of Cancer

In the epithelial tissue, a single cell acquires mutations, possibly in genes that regulate cell growth and as these mutations accumulate, the cell starts to proliferate. It loses its anchoring to its surroundings and starts to multiply as a tumor. As it grows, it produces some growth factors that allow small blood vessels to invade the tumor and provide it with nutrients. This process is called "angiogenesis."

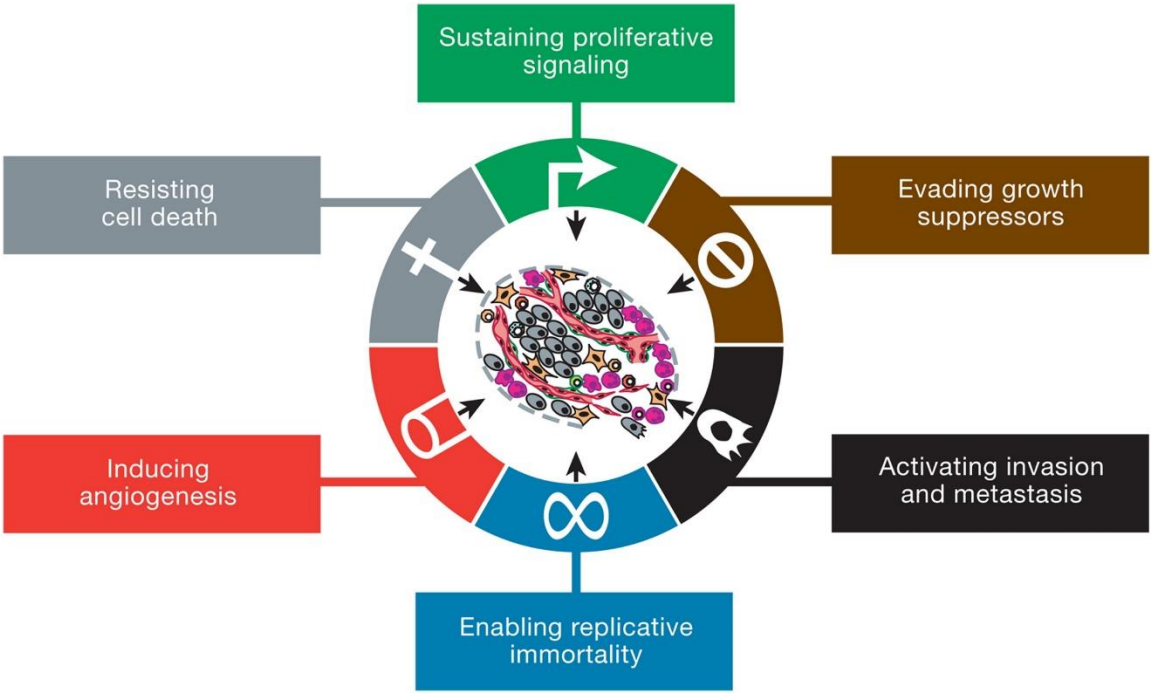


As the tumor continues to grow, sometimes the tumor will invade surrounding tissues, or it might be able to cross into either a blood vessel or perhaps into a lymphatic.By doing so, it can spread, and this process of spreading is known as "metastasis."

So by metastasis through the blood, a tumor might reach other organs and you could have secondaries of a tumor in the liver, or in the kidney, or the lungs, for instance, or even in the brain. And sometimes the tumor will spread through lymphatics to the draining lymph node, and you can see an enlargement of lymph node in the vicinity of the tumor.

Since 15 years ago, the general view of tumorigenesis was that it involved these properties that basically affected the growth of the cell and its migration, and the immune system was not brought into the picture.

Doug Hanahan and Bob Weinberg describe certain properties of tumors, and they call these, the six hallmarks of cancer, which include evasion of growth suppressors, the activation of invasion and metastasis, the enabling of replicative immortality, the induction of angiogenesis, the resisting of cell death, and sustaining proliferative signaling.



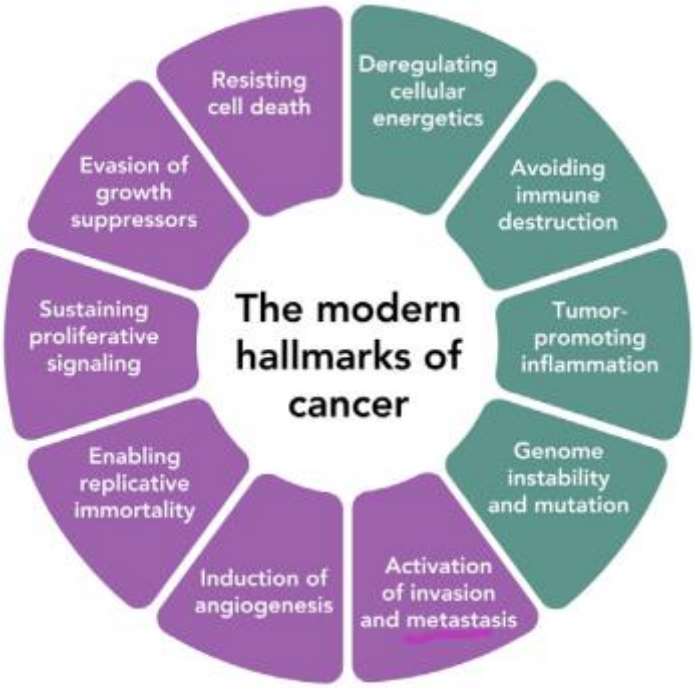
However, it was recognized that sometimes tumor seem to grow in the context of inflammation. It's also been recognized that often,they have a better prognosis when tumors are infiltrated by immune cells.

We know that tumors are infiltrated by immune cells, and that sometimes the inflammatory cells provide mutagens and drive the growth of the tumor by creating more mutations. So in this situation, one could think of inflammatory cells as pro-tumorigenic.

However, there are many other contexts in which immune cells come into the tumor. Sometimes they would have eliminated the tumor, but we would not have seen it, which is called "immune surveillance."

Nevertheless, the immune cells are induced on other occasions and become exhausted and effete, where the tumor grows.

So today, when we think about the cardinal signs of cancer, we include among these cardinal features of cancer a few aspects of the interaction of the immune system with the tumor.



So, on the one hand, the tumor has survived because it has acquired the ability to avoid immune destruction. On the other hand, sometimes the tumor has grown because immune cells have created the environment for tumor growth.

We now think of the biology of cancer as including aspects controlling cell growth, angiogenesis, and metastasis, but also we think about the interplay between the immune system and the tumor, which could work in two directions. The immune system could have induced the tumor, but the immune system also has the power to help us get rid of the tumor.

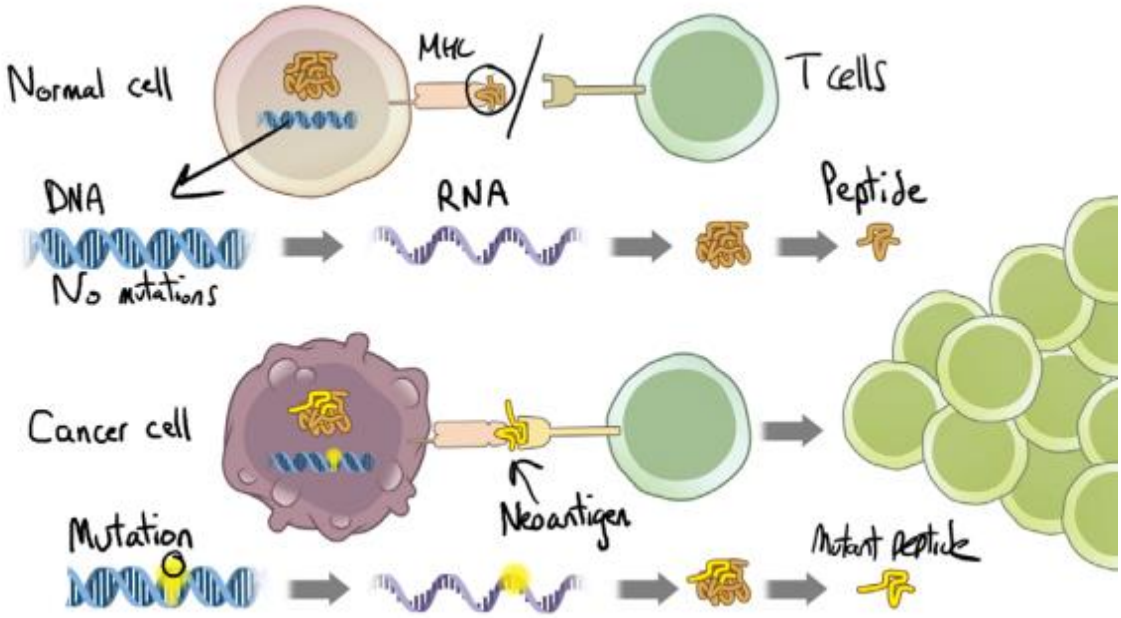
2) Immune Recognition

The immune system uses antibodies and T-Cells to recognize and kill cancer cells. Antibodies are made by B cells and their progeny, called plasma cells and they will recognize any chemical form of antigen that a tumor can make, as long as it's on the surface of a tumor cell. Antibodies cannot get inside and see antigens inside the cell.
CD8-positive T-Cell will recognize peptide fragments of proteins made inside a tumor cell and placed on an HLA molecule.
CD4-positive helper T-Cells will recognize peptide fragments of proteins taken up from the cancer cell by another cell, such as an antigens-presenting cell.

So how does the immune system control cancers?
How do the antibodies and T-Cells impact tumor growth?

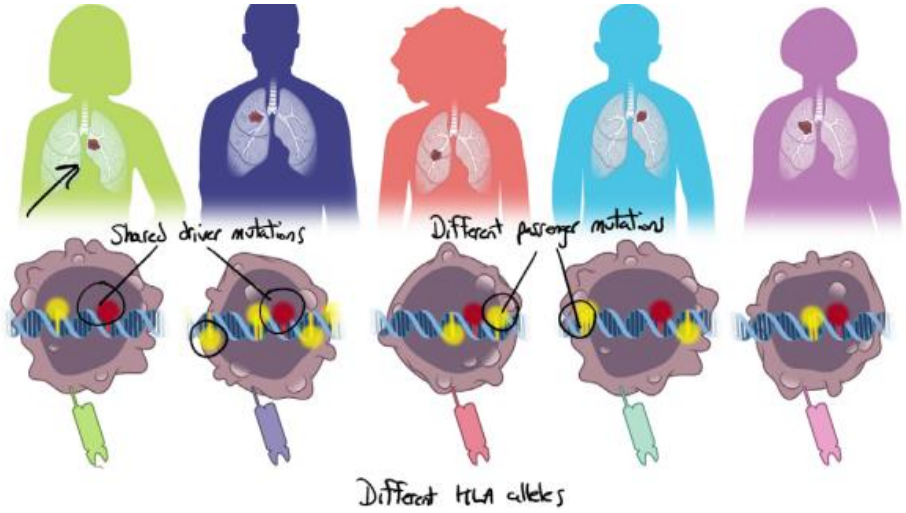
For antibodies, the mechanism by which they can kill tumor cells includes the same mechanisms used to fight infections. These include: 1- the activation of the complement system, leading to lysis of the tumor cell or opsonization, that is, the tagging of the surface of the tumor cell with the complement protein, so that they are eaten by phagocytes, such as macrophages, or 2- by targeting tumor cells with the antibodies in a way that another type of killer cells , called NKC, can see the antibody bound to the tumor cell and prompt killing. In all cases, the outcome is dead tumor cells.
CD8-positive cytotoxic T-Cells kill cancer cells by directly releasing cytotoxic substances that they store in granules, called perforins and granzymes. These will lead to the apoptotic death of the cancer cell.
CD4-positive T-Cells recognize peptide fragments of proteins that have been taken up by a macrophage. They can release cytokines that will work back on the macrophage and activate it to be a better killer of ingested tumor cells.

The nature of the antigens: Here, the focus is on T-Cells and the antigens they see; as we know, T-Cells are the essential effector mechanisms by which the immune system controls cancer.
We will also focus on DNA and mutations made in encoded genes because the T-Cell will see mutated proteins.
In a normal cell, there will be relatively few mutations so that when the genes are encoded into RNA and translated into proteins, the generated peptides that may be placed on an MHC molecule are self-peptides. We will normally not respond to our healthy self-peptide because of tolerance mechanisms, and the immune system is trained not to respond to self-antigens.
However, there are many mutations in a cancer cell, some of which are important to drive cancer development, and some are not. These mutations are new to the cancer cell and not present in normal cells. The peptides generated from mutated proteins put on HLA molecules will be seen by T-Cells because we are not tolerant to these so-called neoantigens, generating a T-Cell response.



So even in the normal case of ageing, with toxins in our environment, exposure to low levels of radiation exposure, and even mistakes made in DNA repair, a vast amount of mutations are generated in cells, most of which are harmless, and if they are harmful, the cell will probably die. However, occasionally, mutations in a gene, such as an oncogene or a tumor suppressor gene, contribute directly to the transformation of the cell into a cancer cell. These are so-called driver mutations. They deregulate cell growth, death and metabolism. These driver mutations will lead to increased genomic instability of cancer, which will generate even more mutations that are not necessarily required for the malignant phenotype, called passenger mutations.

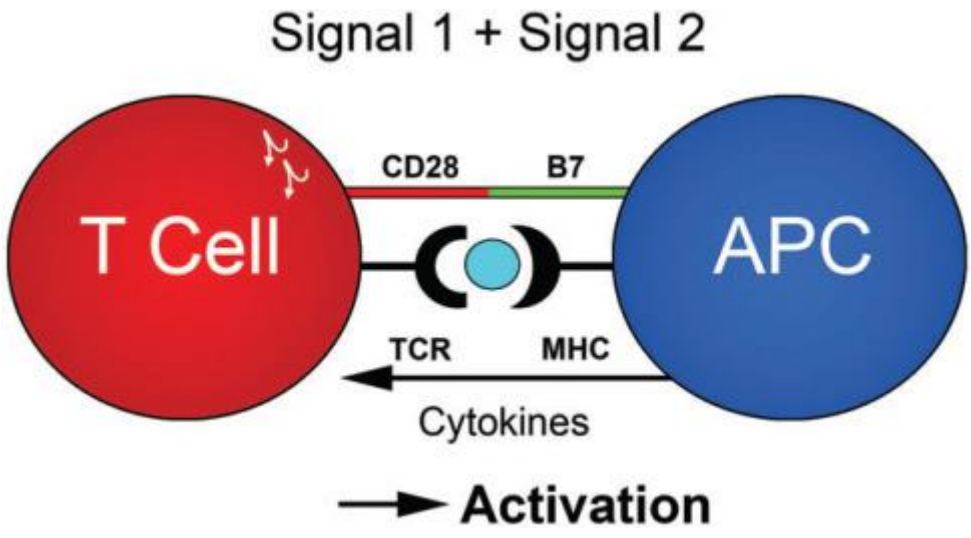
The T-Cell's response to cancer is mainly for these passenger mutations, simply because they outnumber the driver mutation. T-Cells could respond to the neoantigens that are part of a driver mutation.
If we look at a bunch of different tumors from the same organ from different people for example, lung cancer, every one of these tumors will be immunologically different. There may be some common driver mutations in genes that are frequently associated with cancer development, in the case of lung cancer, KRAS or EGFR. But the passenger mutations are generally random and have nothing to do with the phenotype, and they differ for each patient.



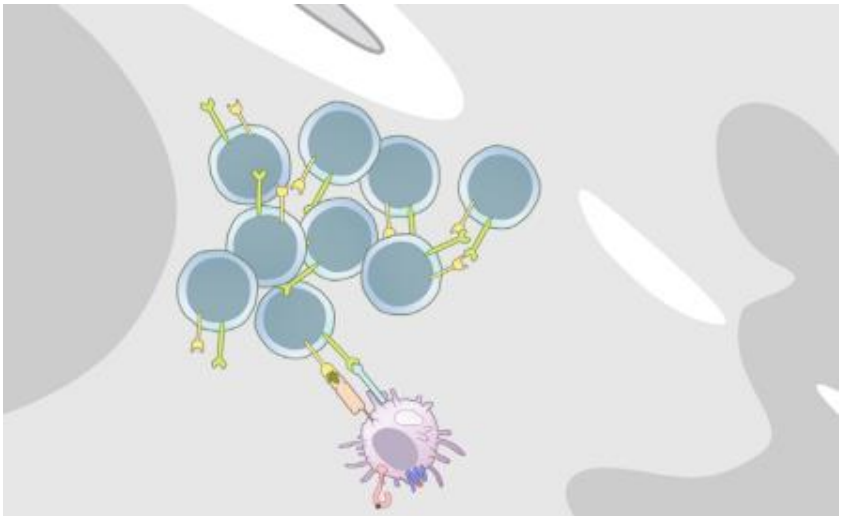
Furthermore, each patient probably has different MHC or HLA molecules because HLA molecules are highly polymorphic. Unless identical twins, they are almost sure to have different alleles in the HLA locus and since the peptides bind to HLA alleles, the combination of neoantigens being different and HLA alleles being different means that every different lung cancer in all these patients will be different in terms of what it is showing to the immune system. So this terrific heterogeneity among cancers in terms of the antigens they show to the immune system is significant when considering certain types of immunotherapy.

3) Induction of the CD8+ T Cell Response

Like any other tissues, tumors will be infiltrated by sentinel cells called dendritic cells. They named such due to their long dendrites, expressing both MHC class I and MHC class II molecules on their surface.
The MHC class I molecules can activate CD8+ T-Cells and MHC class II molecules can activate CD4+ T-Cells. The tumor has within it mutant proteins. These mutant proteins might be in any gene, and the proteins, which are now mutated and yield peptides, which can bind to this individual's MHC class I or class II, are called neoantigen peptides because they can prime the immune system since they are novel. They are created by mutation.
One more thing is that the tumor cells, as they die, they release DAMPs, Damage-Associated Molecular Patterns, molecules that can activate this dendritic cell to express on its surface, a molecule called B7, which is an example of a co-stimulatory ligand. The activated dendritic cell also expresses a new molecule on its surface, a chemokine receptor called CCR7, which draws the dendritic cell into a lymphatic and, finally, to the T-cell zone of a lymph node. So the dendritic cell has acquired the ability to give two signals and also has acquired the ability to migrate. This dendritic cell then migrates through the lymphatic and arrives at a lymph node through an afferent lymphatic to the T-cell zone lymph node. There, a T-Cell recognizes the peptide presented by dendritic cells. T-Cell also has CD28, which recognizes B7. This is the co-stimulatory receptor, CD28, and it will provide the second signal for the activation of this T-Cell. In summary, activating CD8+ T-Cells requires two signals from antigen-presenting cells, binding their T-Cell receptor (TCR) to the appropriate peptide presented on MHC I and co-stimulation by B7 binding to CD28.

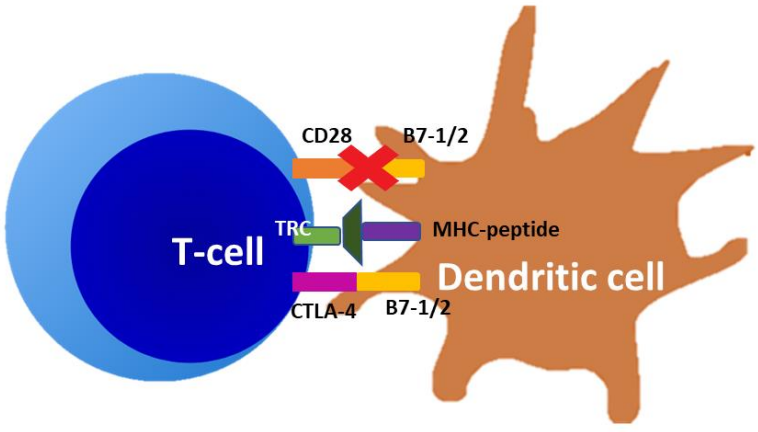


Keep in mind there's a unique T-Cell that can recognize the peptide, which is related to the neoantigen derived from the tumor. As a result, tumor-specific T-Cell starts to proliferate. Some of these T-Cells can leave the lymph node, go out through an efferent lymphatic, go through the blood, and go back to the tumor.



Then what will happen?

Some of these T-Cells, which proliferate in this expanded clone, emigrate through an efferent lymphatic and go to the tumor site. But, even as this is happening, it is time to dampen the immune response. The activated T-Cells that have yet to leave the lymph node might induce on their surface a molecule called CTLA-4, which has a very high affinity for B7. And it can, therefore, displace CD28 and prevent CD28 from receiving signals from B7. As a result, the ability to provide signal two. And now we have the B7 molecule that you can see over here. The B7 is now bound to CTLA-4 and not to CD28. As a result, we will attenuate this clonal expansion process and the immune system is dampened.



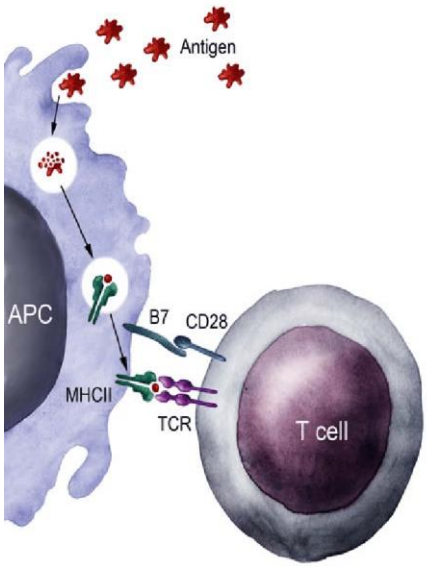
Nonetheless, some of the already activated cells have left the efferent lymphatic, into the bloodstream and to a distant blood vessel, and in a post-capillary venule at the tumor site, they emigrate out of the blood vessel into the tumor. These tumors specific CDB+ T-Cells can kill tumor cells.

In parallel, dendritic cells activate their cognate CD4+ T-Cells in a lymph node through MHC class ll molecules. These cells also emigrate into the tumor and work together to cause the elimination of the tumor.

4) Induction of the CD4+ T Cell Response

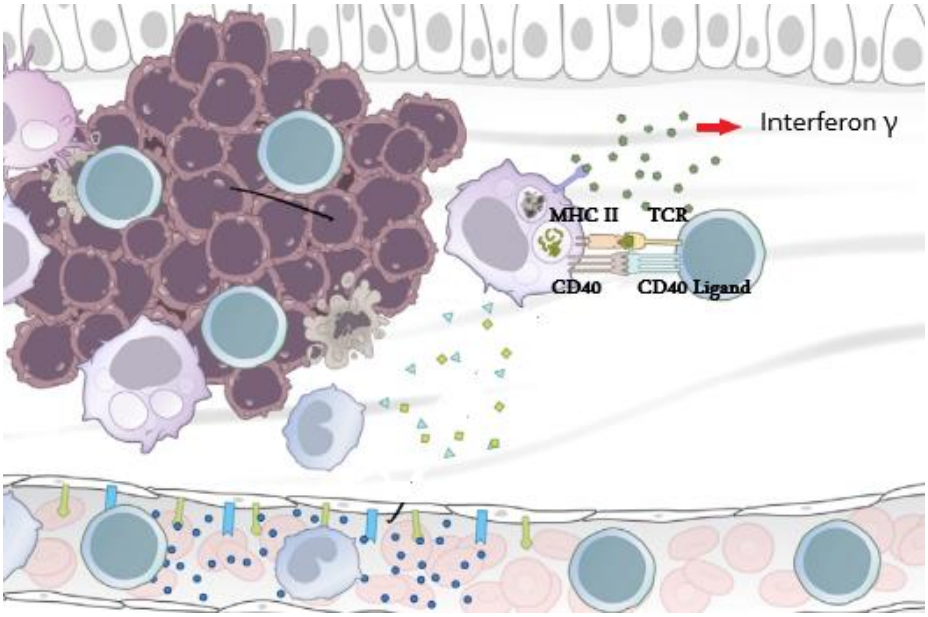
Although CDB+ cytotoxic T lymphocytes appear to be the most important effector cells in anti-tumor responses, there is much evidence that CD4 helper T-Cells contribute.
In particular, they may contribute in the setting of certain types of immunotherapy now in the clinic. Therefore, we will talk about how CD4+ T-Cells get activated initially by tumor antigens and how they get to the tumor and help in killing the tumors.

First, in any T-Cell response, it is important to note that the protein is the type of antigen. In particular, we are talking about mutated proteins made by dying tumor cells that may be released, and they have to be taken up by professional antigen-presenting cells called dendritic cells. When taken up, they are processed into peptides that will bind to class ll MHC molecules.
To be recognized by the immune system, this peptide has to be a neoantigen, a tumor peptide with a mutation in the peptide.
In addition to the generation of neoantigen, tumor cells will also release substances as they die, or native tissue cells that are injured, called damage-associated molecular patterns, or DAMPs.
These are signals to the innate immune system, which will bind to receptors, such as toll-like receptors or other innate immune receptors, and generate activation signals for the dendritic cell to express other molecules also important for the T-Cell response.
These include molecules called Co-stimulators, the most important of which are B7 molecules.
Also, chemokine receptors, called CCR7, will recognize or respond to chemokines that direct dendritic cells into draining lymphatics. They migrate to a region of the lymph node called T-Cell zone.
T-Cell zone is where native recirculating T-Cells of the CD4+ or CD8+ type will migrate into the lymph node through the blood, get out into this region because of the same chemokine receptor on the naive T-Cell. So the dendritic cell and random mix of circulating naive T-Cells will find themselves in the same place in the lymph node.
If the naive T-Cell happens to have a receptor specific for the tumor peptide, that mutant peptide on a class ll MHC molecule, it can then get one of two important signals for activation, and that is antigen recognition.
In addition, the B7 molecule that we said was induced on the dendritic cells by the DAMPs will bind to a molecule called CD28 on the naive T-Cell, giving signal 2. Those two signals will be sufficient to drive the clonal expansion of these T-Cells and their differentiation into helper T-Cells. So these are now CD4+ helper T-Cell.



Many thousands are generated from just a few naive T-Cells. These helper T-Cells must get out of the lymph node and travel to the tumor to do any good. So they leave through efferent lymphatics, and eventually, the lymphatic will drain into the bloodstream.
In the bloodstream, they recirculate through the body, and some enter the tumor site.

Why does the helper T-Cell leave the blood circulation at the site of the tumor?
It is because the tumor has many stimuli from the innate immune system. Those stimuli will also activate the endothelial cells to become sticky for these circulating leukocytes, and they will express adhesion molecules and chemokines, allowing the T-Cell to leave and get out into the tumor site.
Once in the tumor site, this CD4+ helper T-Cell, with that T-cell receptor specific for the tumor peptide. The tumor macrophage can take up the proteins locally from the tumor and generate the peptide MHC class II complexes. That signal 1 is sufficient and unnecessary to have signal 2 or co-stimulation to activate the helper T-Cells.
So the T-helper cell, on getting this signal from the T-Cell receptor, will trigger the production of cytokines such as interferon-gamma, which will bind to cytokine receptors back on the macrophage and contribute to the activation of the macrophage.

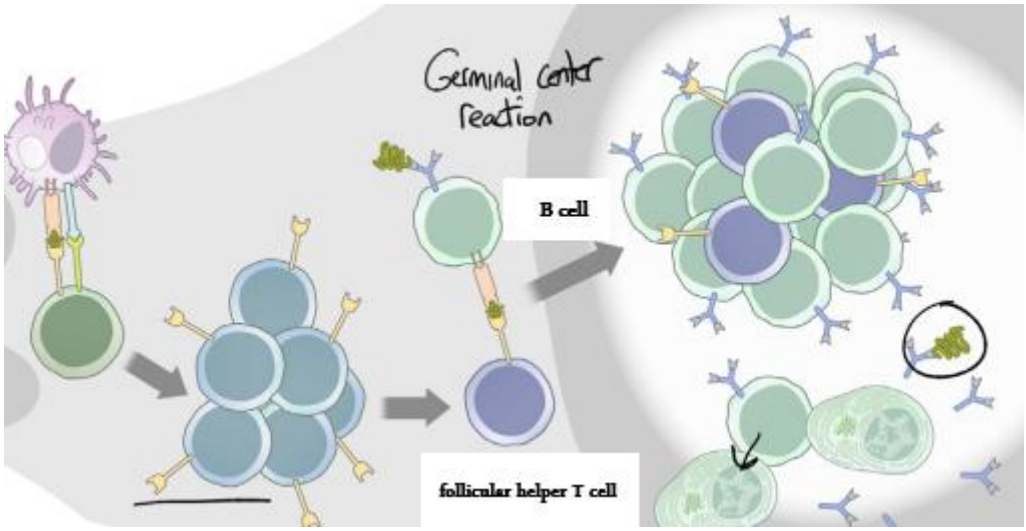


In addition, the signals from the T-Cell receptor will upregulate a molecule called CD40 ligand, which binds to CD40 on the macrophage. That is another signal that helps activate the macrophage. So this very activated macrophage will now be able to kill the tumor cells efficiently. But importantly, also produce cytokines such as TNF and IL-1 that further increase the ability of leukocytes to leave the circulation at this site because of upregulation of adhesion molecules and chemokines. So we get lots of T-Cells, and lots more of circulating monocytes and macrophages, which increases the tumor's inflammatory milieu and leads to tumor destruction. CD4+ helper T-Cells in addition to working through macrophages, can also help the CD8+ CTL responses that are important for tumors.

They do this back in the secondary lymphoid organ, where the dendritic cell presents the peptide class I antigen to the naive CD8+ T-Cell.

At the same time, that dendritic cell can take another tumor antigen and make a peptide class II antigen complex, which will activate this helper T-Cell that has been generated previously. The cytokines that the helper T-Cell produces, including one called IL-2, will contribute to the clonal expansion of the CD8+ T-Cell and its differentiation into cytotoxic T lymphocytes. So CD4+ helper T-Cells can induce CD8+ T-Cell responses against tumor.

Finally, helper T-Cells can also help B cells make good antibodies. They do this by differentiating into something called a follicular helper T-Cell. So of that expanded clone of helper T-Cells that we discussed, most leave the lymph node, but some stay around. They turn into these follicular helper T-Cells that help B cells make good antibodies that bind with high affinity and have B cell memory and long-lived plasma cell generation. This process is called the germinal center reaction.



We know that patients with tumor make these high-affinity antibodies, and they have long-term memory, indicating that there was help from T-Cells. We do not know yet whether those antibodies are essential in the natural immune response against the patient's tumor.

5) The Immune Response of NK Cells

Natural killer cells, a type of innate immune cell found in the blood and some tissues, can function as an anti-tumor effector cells. Natural killer cells are not lymphocyte, but are part of a family called innate lymphoid cells.

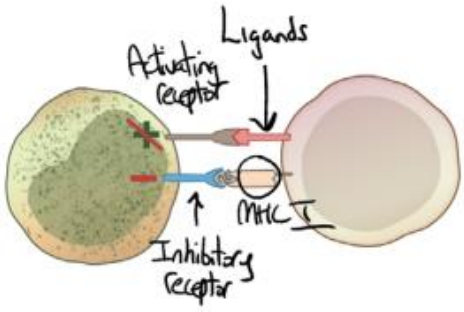
They look like lymphocytes and are often called large granular lymphocytes because they have small cytoplasmic granules that can be seen on blood smears. They represent 5% to 10% of the lymphocyte-like cells in the blood, but importantly, they don't express T-Cell receptors or immunoglobulins.

They are not part of the adaptive immune system and use other mechanisms to recognize antigen and are part of a larger family of innate lymphoid cells. So what do they do?

Natural killer cells recognize, in a variety of ways, abnormal cells, such as virally infected cells or tumor cells. When activated, they act just like a cytotoxic T lymphocyte. They release granule contents with perforin and granzymes that enter the targeT-Cell and induce apoptotic cell death.

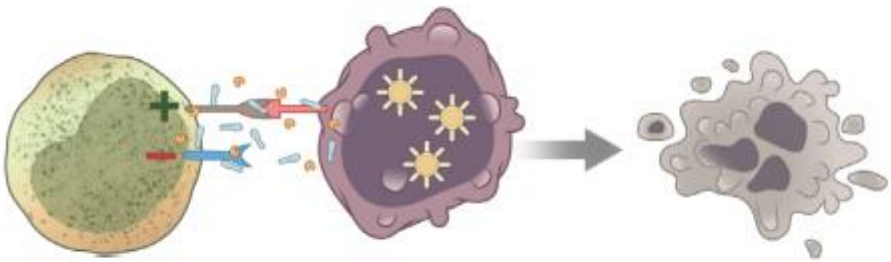
This is the same mechanism the cytotoxic T lymphocytes use, but recognition events are different. Natural killer cells also produce gamma interference, an inflammatory cytokine that activates macrophages and is good for anti-tumor immunity. Macrophages can also eat up dead cells.

The natural killer cells have two general types of receptors on their cell surface. One set of receptors are activating receptors, and they can recognize a variety of ligands, some of which may be expressed at low levels on normal cells. That is a dangerous thing because we do not want the natural killer cell to be activated to kill normal cells, but this is prevented by the presence of inhibitory receptors. A family of them, among others, are called KIRs, or killer IG-like receptors. What KIRs recognize are MHC class I molecules, which are present on all normal nucleated cells. Remember that MHC molecules are also what T-Cell receptors see, but the T-Cell receptors are highly specific for a particular peptide in a particular allele of class I MHC whereas natural killer cells broadly recognize many different class I molecules. So basically, it is a test of whether the cell is healthy or not because unhealthy cells may lose MHC class I. So the inhibitory signals generated by this receptor will block the activating receptors and leave the normal cells alone.



When cells become transformed into cancers, one of the ways that they can evade adaptive immunity—in particular, evade T-Cell recognition—is to lose expression of class I MHC.

This allows the NK cell to become activated because you no longer have an inhibitory signal and possibly increased expression of activating ligands—that go along with the cancer phenotype. So thaT-Cell is uninhibited and can lead to granule exocytosis and apoptotic killing of the cancer cell.



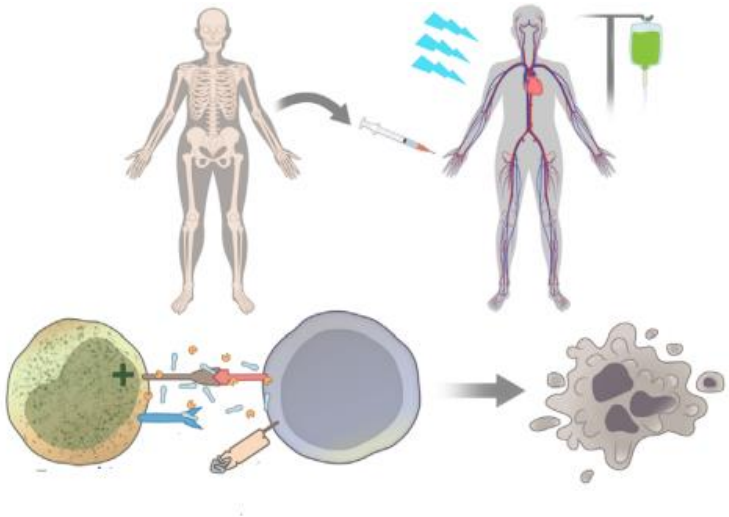
When do NK cells play a role in anti-tumor immunity?

One case is possibly the case of viruses that cause cancer. We know that NK cells are important for antiviral immunity because rare immunodeficiencies in NK cells lead to severe viral infections. Some viruses can cause cancers. Because of the cancer transformation or by mechanisms cancer by the virus alone, that can lead to loss of class I MHC, therefore loss of inhibitory signals and release of the general contents and apoptotic death of the cell.

Another case where NK cells may become important in anti-cancer therapy is for patients that are being treated by a type of treatment developed over the last few years called checkpoint blockade, largely targeting T-Cells. So checkpoint blockade is the delivery of monoclonal

antibodies against molecules that inhibit T-Cells, and one those molecules is PD1, but PD1 is also expressed on NK cells and provides inhibitory signals, sort of like the KIRs do, which will block the activating receptor's efficacy. So with PD1 blockade, you no longer get PD1 inhibition and the NK cell can activated to kill the targeT-Cell. Therefore, patients receiving so-called checkpoint blockade therapy with anti-PD1 may be benefiting not just by activating T-Cells, but also by activating NK cells. This checkpoint blockade will be later discussed in detail.

Another particular case where NK cells have been shown to be very important in anti-tumor immunity is in the case of certain types of leukemias. Leukemias are malignant tumors of bone marrow-derived cells that circulate leukocytes throughout the body. Some leukemias can only be cured by irradiating and giving chemotherapy to the patient to kill all the leukemic, but also, unfortunately, all the patient's own normal hematopoietic cells. Therefore, after this treatment, the patient has to be rescued by bone marrow transplantation from a donor.If the donor is what we call MHC haploidentical—such as would be the case of a parent giving bone marrow to a child—and the bone marrow sample has NK cells in it, then there can be a very effective treatment that involves killing most of the cells by the chemotherapy and radiation and then the residual cell that is left over by NK cell-mediated killing.



How does this work?

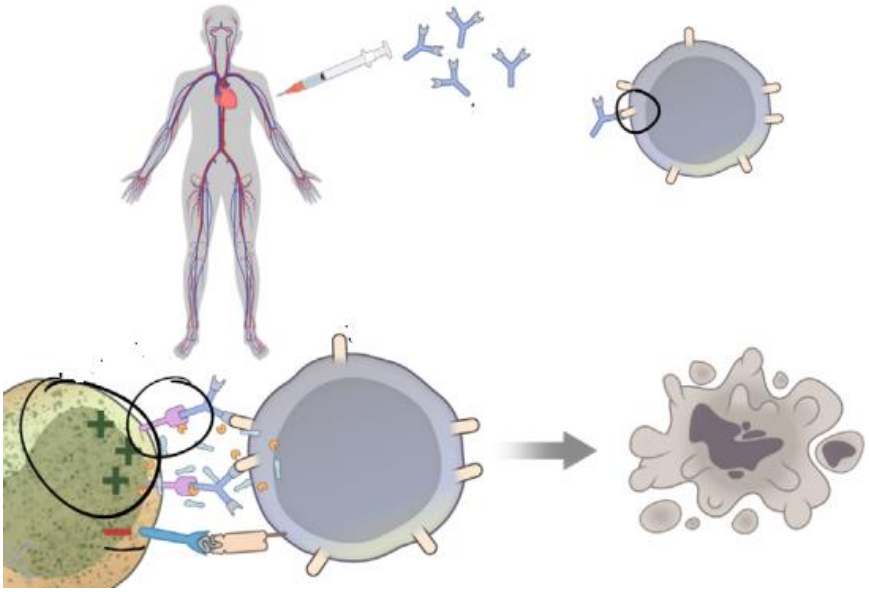
If there are still residual leukemic cells, they will have the patient's own MHC class I. But the donor NK cells, even though they have inhibitory receptors that can recognize class I in the donor, will not recognize the patient's MHC class that differ from the donors. And therefore, there will not be negative signals, and the activating signals can lead to successful activation of the cell and killing of the tumor. And this is called NK-mediated graft-versus-leukemia effect.

A final example where NK cells play a role in anti-tumor immunity is in monoclonal antibody therapy, where the monoclonal antibodies are used as drugs because they are specific for certain tumor antigens. There are various tumors for which this type of treatment is given.

How does the monoclonal antibody lead to the killing of the tumor cell?

There are multiple mechanisms but one is actually by activating NK cells. So it turns out that NK cells have among their activating receptors some that are what are called Fc receptors that bind

immunoglobulins at the tail end of the molecule. And what the monoclonal antibodies that are given to the patient do is they bind the target antigen by one end, and the other end binds to the activating receptors in the NK cell. That generates these activating signals; even though there may be inhibitory signals, they are overshadowed by the activating signals, and that can lead to the killing of thaT-Cell. This is called antibody-mediated or antibody-dependenT-Cellular cytotoxicity, and it is the mechanism by which several different monoclonal antibodies against tumor antigen work in vivo.



6) Evasion of the T Cell Response

Now, some tumors are infiltrated by T-Cells and by other immune cells, and the types of T-Cells include effector T-Cells, which try to kill the tumor; exhausted T-Cells, that we'll talk about in a minute; and regulatory T-Cells, which can inhibit effector T-Cells. Tumors also have macrophages, dendritic cells, and other cells, but we will focus on the evasion of T-Cell immunity.

Regarding tumor immunity, we have discussed before that dendritic cells might capture antigen from a tumor. The dendritic cells will then go to a lymph node. They find a cognate T-Cell, which recognizes the tumor neoantigen peptide. The T-Cells is then activated. Effector T-Cells leave the lymph node. And they go through the circulation, through post-capillary venules, and thus into the tumor site, where this tumour invasion by effector T-Cells might lead to the apoptotic death of the tumor.

Now obviously, this is the ideal scenario. We would call this immune surveillance if it did happen all the time. Perhaps this does happen, and some tumors are eliminated in many people by immune surveillance. However, when we do see a tumor, clearly, it has evaded immune surveillance, and it has evaded the immune system.

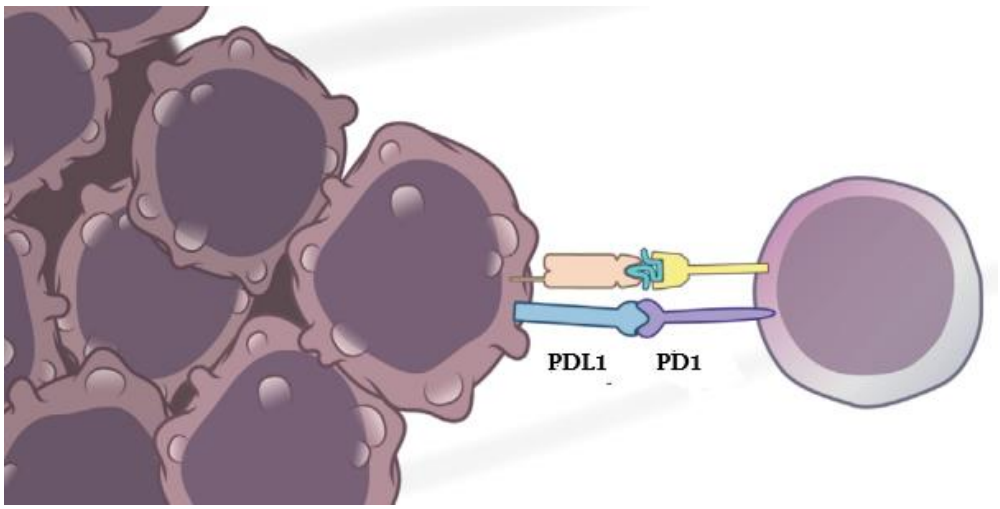
And what we're going to talk about are the three main mechanisms by which the tumor might evade adaptive immunity and T-Cells. And one mechanism is if you consider that a tumor cell is going to be recognized by a T-Cell because it presents a peptide on MHC class I, and this recognition of MHC class I on the tumor is essential to a T-Cell being able to kill it.

A straightforward mechanism before the tumor is wholly killed would be for the tumor to lose, to become invisible to the immune system by losing its MHC class I molecules. So normally, a tumor like any other cell might have MHC class I molecules, these MHC class I molecules allow the presentation of this neoantigen peptide.

The tumor has a program by which it shuts off or loses the expression of MHC class I, and basically becomes invisible to T-Cells. This would allow the tumor to be perhaps invaded by effector T-Cells, but the effector T-Cells would not be able to see the peptide on the tumor cell and thus kill it. This is a straightforward mechanism of immune evasion, just by the loss of expression of MHC class I. Effector T-Cells can come into the tumor, but they cannot kill it if the tumor cells do not express MHC class I.

The other mechanism that can lead to immune evasion is that effector T-Cells might enter the tumor. They are ready to kill the tumor. However, somehow in the tumor environment, these effector T-Cells are told to lie down and not kill the tumor cell. This process of causing the effector cell to become unable to kill is called the induction of T-Cell exhaustion. How exhaustion occurs is presumably because, in the vicinity of the tumor, or at the tumor site, the T-Cells that can recognize MHC class I and peptide on the surface, neoantigen peptide. This recognition event allows repeated activation of the T-Cell, and in the milieu of the tumor, this repeated activation might induce the state of exhaustion, which may then be maintained by a molecule called PD-L1, which might be expressed on the tumor cell and then ligates an inhibitory receptor

on the T-Cell called PD-1. PD-1 on the T-Cell will maintain or help induce the final stages of exhaustion in the T-Cell.



Also, we could have PD-L1 not expressed on the tumor itself but perhaps on a dendritic cell. So again, we have the dendritic cell expressing PD-L1 and PD-1 on the T-Cell; that is the interaction which might help maintain the state of exhaustion.

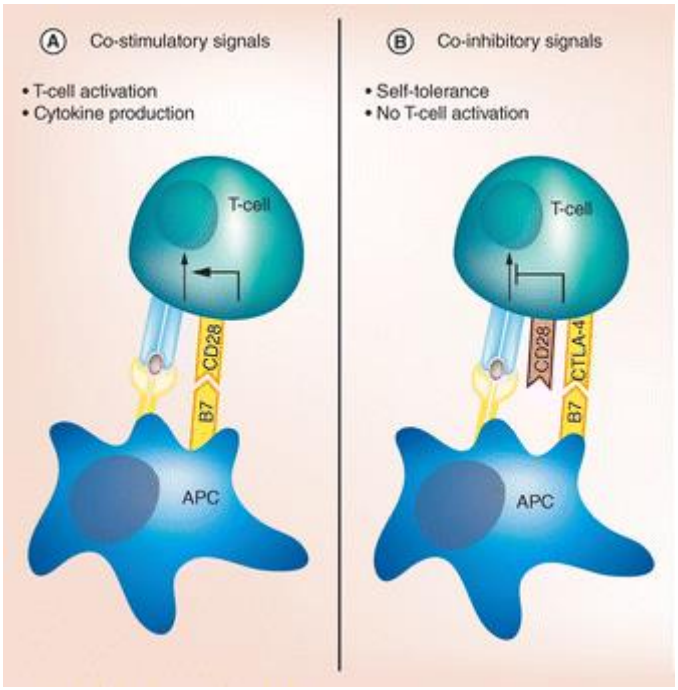
The exhausted T-Cell can recognize its targets but cannot kill. This mechanism of evasion tells us that there are T-Cells that can recognize a tumor, but they cannot kill the tumor.

Another scenario that we should consider is that we have a lot of effector cells T-Cells entering the tumor, but then the milieu of the tumor allows the induction of T-Cells called regulatory T-Cells. And the regulatory T-Cells, or Tregs, will also come into the tumor. And they are going to dominantly inhibit the effector T-Cells.

Even though the effector T-Cells have the ability to kill, because they are being told by Tregs not to do so, the tumor then survives. This is another mechanism of immune evasion.

The regulatory T-Cells work through the secretion of cytokines, like IL-10 and TGF-beta, making the effectors quiescent and the tumor persists. One of how regulatory T-Cells work, other than by secreting inhibitory cytokines, is by expressing large amounts of an inhibitory receptor called CTLA-4. These very high levels of CTLA-4 on a regulatory T-Cell can allow the regulatory T-Cell actually to denude a dendritic cell of B7. So usually, dendritic cells express B7. Normally, B7 is going to activate CD28. However, if this dendritic cell was to be seen by a regulatory T-Cell, with large amounts of CTLA-4, then it would be stripped of its B7, which will be taken up by the regulatory T-Cell, ingested and degraded.

As a result, the dendritic cell, now lacking B7, cannot provide a second signal for the activation of effector T-Cells. So regulatory T-Cells, either by making cytokines or by their ability to strip dendritic cells of B7, can now cause immune system inhibition and evasion of immunity.



As a result, the tumor keeps growing, and the tumor keeps growing because either the tumor lost class I or it had the induction of exhaustion, or perhaps we had a lot of regulatory T-Cells, which inhibit the immune system.

7) Evasion of Other Immune Responses

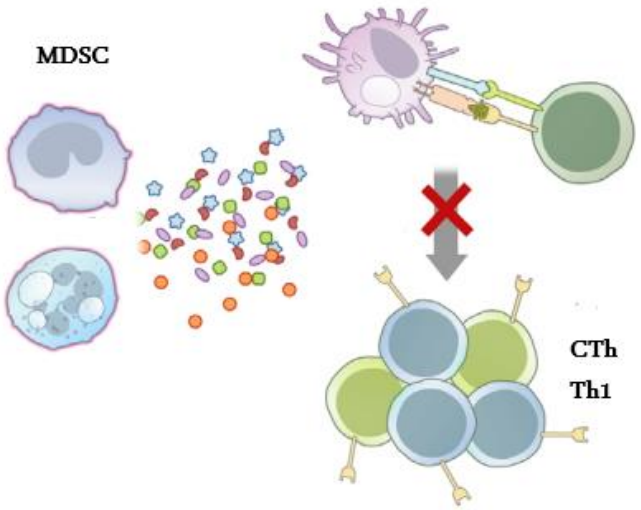
In this chapter, we will talk about immune evasion strategies by tumor, beyond T-Cells checkpoint pathways. Tumors express antigens seen by the immune system, including T-Cells. And T-Cell responses are often generated. Yet they are usually inadequate for eliminating the tumors. Although tumors do use checkpoint blockade pathways, such as the molecules PD-1 and CTLA-4, to evade T-Cell responses – and these have been successfully targeted for immunotherapy – there are many other ways that tumors evade the immune system.

Looking at the solid tumors in most patients and the types of cells present, one would think there is a wide variety of leukocytes that should be adequate to control tumors. These include neutrophils, macrophages, and dendritic cells that should be able to activate T-Cells. There is also a rich vasculature of tumor vessels which, on the one hand, could promote tumor growth by providing nutrients and be a conduit for the delivery of all these leukocytes into the tumor to help fight them.

So why then do all these cellular responses fail to eradicate tumors?

If we look at the leukocytes within tumors, we find that many of them have an immunosuppressive phenotype. Some cells look like the effector T-Cells we described that should be able to fight tumors, but they are different in important ways.

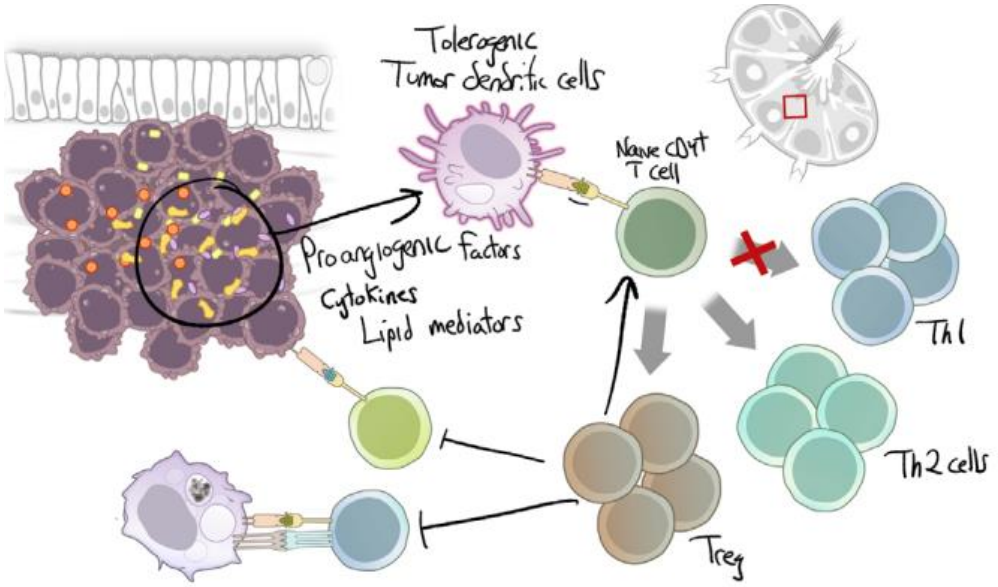
There are the myeloid-derived suppressor cells, tumor dendritic cells, and tumor-associated macrophages that we will explore in a little more detail. The myeloid-derived suppressor cell, or MDSC, is a cell that looks like a normal myeloid cell – either a monocyte or a neutrophil. There are two main types. There is the PMN, or neutrophil-like MDSC, and there is the monocytic MDSC. These cells will make a variety of substances that will largely inhibit the ability of dendritic cells to activate T-Cells to generate good effector T-Cells that can kill tumors, such as cytotoxic T lymphocytes or gamma interferon Th1 cells.



These myeloid-derived suppressor cells are found in secondary lymphoid organs, as well as within the tumor itself. It's important to note that these MDSCs are not unique to the tumor situation but are also present in patients with chronic infections that cannot clear infections. The MDSCs, besides directly blocking T-cell activation, will also secrete proangiogenic factors. That will increase the ability of the tumor to supply itself with a rich vasculature, promoting its growth.

Tumor DCs are generated in response to various factors within the tumor, made by tumor cells themselves but also by some of the non-malignant stromal cells. These factors include proangiogenic factors, cytokines, lipid mediators that work on conventional DCs that should be able to generate good T-Cells but change them into a phenotype that is co-called tolerogenic or immunosuppressive tumor DC.

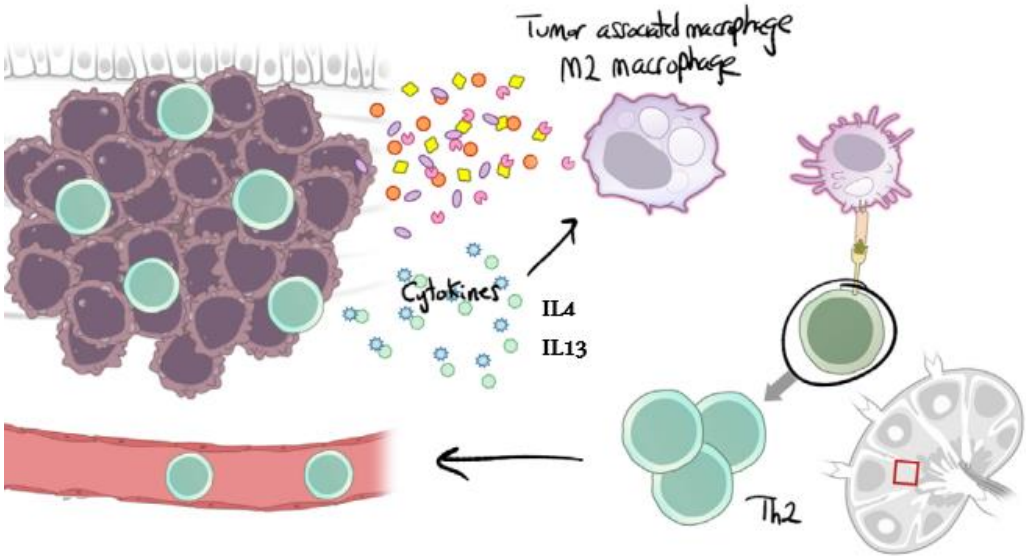
So what does this tumor DC do that is different from the type of DC that could be helpful. Remember that the dendritic cell presents tumor antigen to a naive CD4 positive T-Cell, Th1 cells that make gamma interferon that activate macrophages. But there is something about the tumor DC that blocks this pathway of activation and steers differentiation of the T-Cells into Th2 cells that make different cytokines that are bad for the patients—good for the tumor—and regulatory T-Cells. Regulatory T-Cell's job is to inhibit effector T-Cell responses, such as blocking CTL activation or Th1 activation at the tumor site. But also to work back and further enhance the generation of more Th2 cells and Tregs. Though overall, tumor DCs are anti-inflammatory, pro-tumor.



What about tumor-associated macrophages? Where do these macrophages come from?

Well, go back to the draining lymph node, where the naive CD4+ T-Cell has generated into or turned into Th2 cells because of the tumor DC. Those Th2 cells go back into the tumor where they secrete cytokines that they are very good at secreting, including interleukin 4 and 13. And they will work on the tumor's macrophages to change into a phenotype sometimes referred to as the M2 macrophage. So the tumor-associated macrophage has this M2 phenotype, and these macrophages secrete many factors that, again, pro-tumor, anti-patient. There are growth factors

for blood vessels, and there are factors that promote the malignanT-Cell growth directly, there are immunosuppressive cytokines, as well as enzymes – proteases – that cause tissue remodelling and allow the tumor to spread in the tissue.



If we put all these together and look at what's going on in the tumor, we have something that's referred often to as the tumor microenvironment. The tumor microenvironment is a very complex mixture of cells, molecules, matrix, and physical-chemical properties. It includes the tumor cells, but it also includes non-transformed cells, such as fibroblasts and other stromal cells, as well as the infiltrating immune cells we talked about – the MDSCs, the tumor-associated macrophages, and the tumor-associated or tumor DCs which generate more Tregs and Th2 cells and leave the Th1 cells at a barrier between the tumor and environment and not in the tumor.

In addition, all these cells secrete cytokines, growth factors, and lipid mediators and modify the extracellular matrix. The tumor microenvironment also is a very acidic environment with a lot of lipid accumulation and low oxygen content. They're hypoxic and ultimately end up with enhanced tumor blood vessels and reduced extracellular matrix synthesis, so the tumor can grow, spread, and get into blood vessels where it can metastatise.

These various aspects of the tumor microenvironment that contribute to tumor evasion are incompletely understood and are the subject of active research.

Checkpoint Blockade

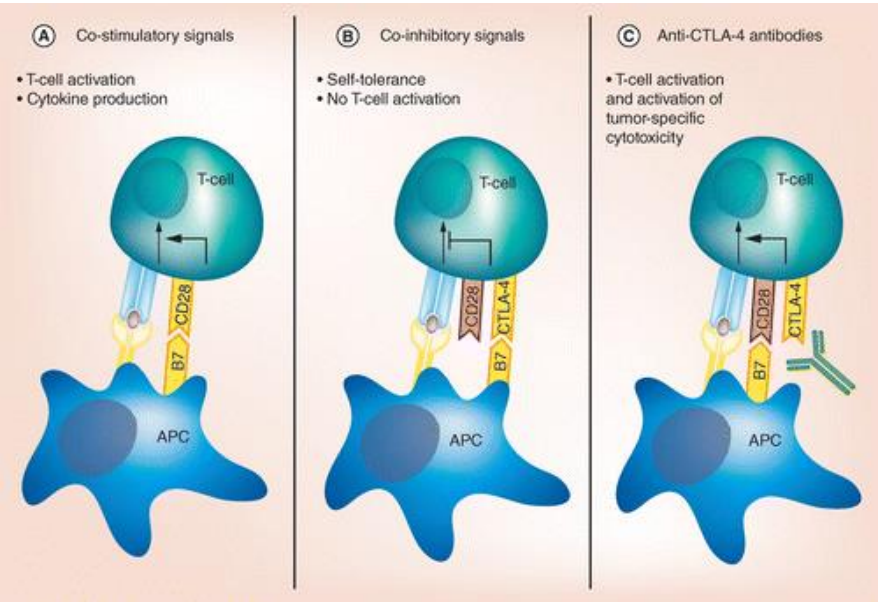
1) Mechanisms of Checkpoint Blockade

Here, we're going to discuss about immune checkpoint blockade, a form of therapy that can result in cure for cancer. Here, we're going to discuss about immune checkpoint blockade, a form of therapy that can result in cure for cancer.

As we discussed in the previous section, the tumor will release mutated proteins. These proteins are taken by dendritic cells, which will then process proteins into neoantigen peptide that will be presented on MHC class I or MHC calss II molecules. The dendritic cells are also then activated by DAMPs. The DAMPs will induce the expression of B7 as well as CCR7, which is a chemokine receptor. These dendritic cells are then going to migrate carrying on the surface B7.

Go to the draining lymph node, go to the T-Cells zone, and they're going to activate a cognate T-Cell that happens to recognize the neoantigen peptide. The delivery of both signal 1, through the T-Cell receptors, and signal 2, through CD28, is crucial for T-Cell activation.
The T-Cell expands and we have this effector clone that's expanded.

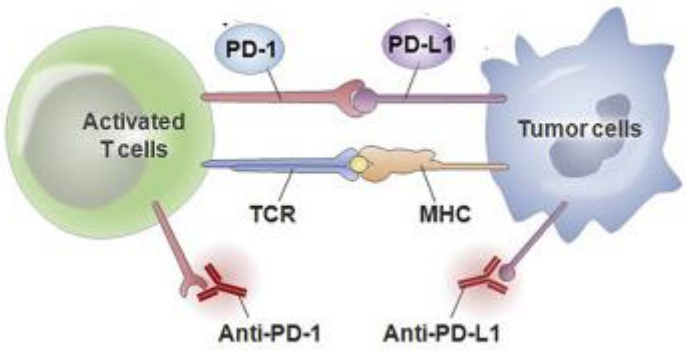
Activated T-Cells are then going to express CTLA-4, an inhibitory receptor. So CTLA-4 can bind to B7 and then by doing, it's going to prevent the ligation of CD28 by B7.
As a result, the T-Cell receives signal 1, does not received signal 2, and the activation of the clone is attenuated. So eventually the clone will decline in size and this is one of the functions of CTLA-4 in dampening immunity. If one now chose to give a patient with a tumor an antibody to CTLA-4, the antibody to CTLA-4 would enter the lymph node, and in the lymph node this antibody will now bind to CTLA-4, block it from binding to B7, and allow the ligation of CD28 by B7.



As a result, the T-Cell is going to be activated in an extremely thorough fashion, the clonal expansion will not be attenuated, and we have a large clone of T-Cells that are tumor antigen specific. And these T-Cells will now leave the lymph node, go through the efferent lymphatic, go through the bloodstream, enter the tumor site, and at the tumor site they're going to cause apoptosis of the tumor. This is what might happen if we use checkpoint blockade.

The antibodies to CTLA-4 can work in a different way as well.
So if you think about a tumor, the tumor might also contain cells called regulatory T-Cells. So Tregs can dominantly inhibit effector T-Cells. And Tregs also express large amounts of CTLA-4 on the surface. And the CTLA-4 in the Treg can capture B7 from a dendritic cell, take this B7, strip it away, and cause the dendritic cell to be unable provide signal 2.
As a result, using an antibody to CTLA-4 blocks CTLA-4, prevents it from capturing B7, and allows dendritic cells to more properly activate T-Cells. Providing both signal 1 and signal 2 without the loss of the B7 that the presence of CRLA-4 on the Treg would entail. Finally, we now have more effector T-Cells which can now damage the tumor and cause it to go away.

There's also another mechanism by which tumor immunity might be constrained which involves the conversion of effector T-Cells into effete T-Cells called exhausted T-Cells. Exhausted T-Cells are T-Cells that can recognize the tumor neoantigen, but have been induced by repeated signaling to become cells no longer want to kill. So the tumor has these effector T-Cells that have now become exhausted, which can recognize the tumor antigen, but nothing is happening in terms of elimination of the tumor. One of the mechanisms by which exhaustion is maintained, is by a signal given to the T-Cell through a molecule called PD-1.

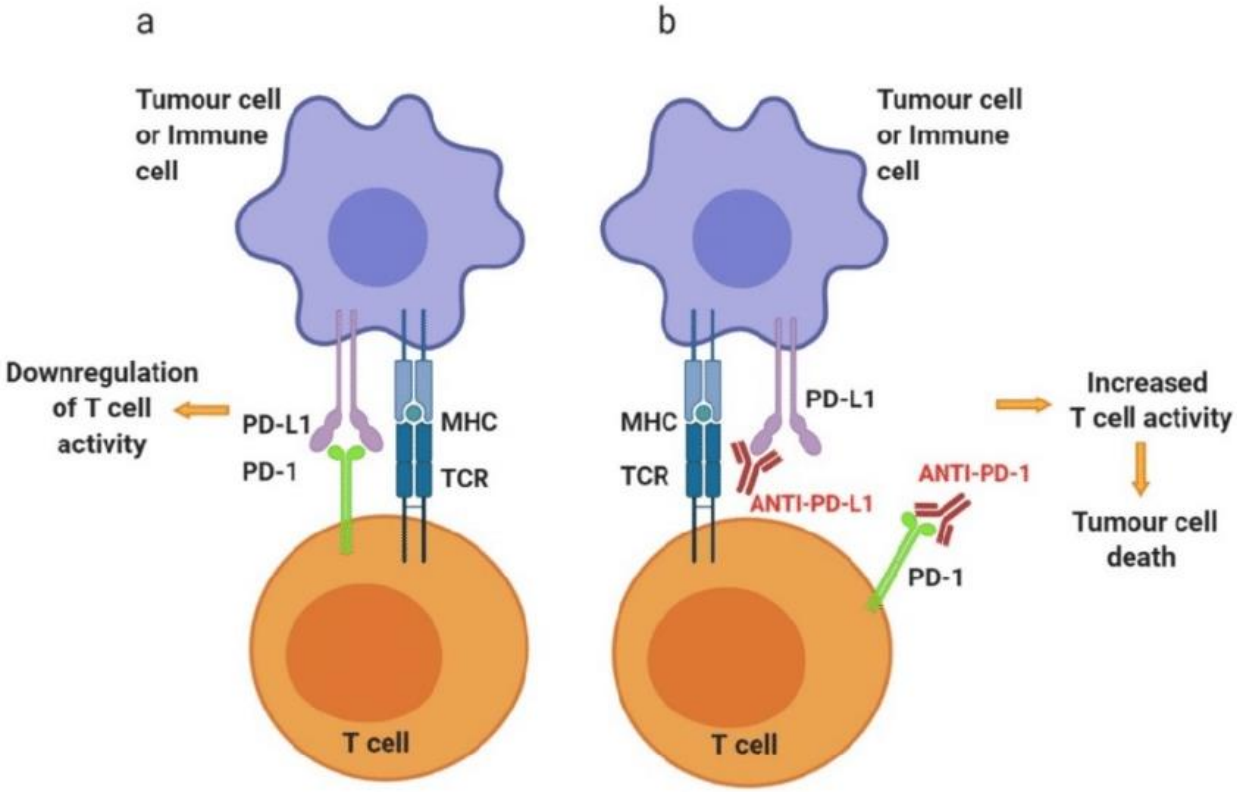


Tumor present its neoantigen peptide on MHC class l. This act with the T-Cell receptor. However, the tumor might also present on its surface a molecule called PDL-1. And PD-L1 can ligate PD-1 on the T-Cell, helping to maintain an exhausted state in this T-Cell that was once an effector. As a result, we have exhausted T-Cells maintained by this PD-L1/PD-1 interaction and this offers us an opportunity for therapy where we could use an antibody to PD-1 or an antibody to PD-L1 and thus block this inhibitory signaling that is maintaining exhaustion.
As a result, exhausted T-Cells now become activated T-Cells. And they are now able to see tumor and also to kill it. So checkpoint therapy blocking either PD-1 or blocking PD-L1.

This type of therapy is capable of reversing exhaustion, reactivating effector T-Cells, and causing the elimination of a tumor.

2) Tumor Responses

Monoclonal antibodies against inhibitory molecules expressed on T-Cells, including monoclonal antibodies specific for CTLA-4 or PD-1 or PD-L1, are infused into patients.

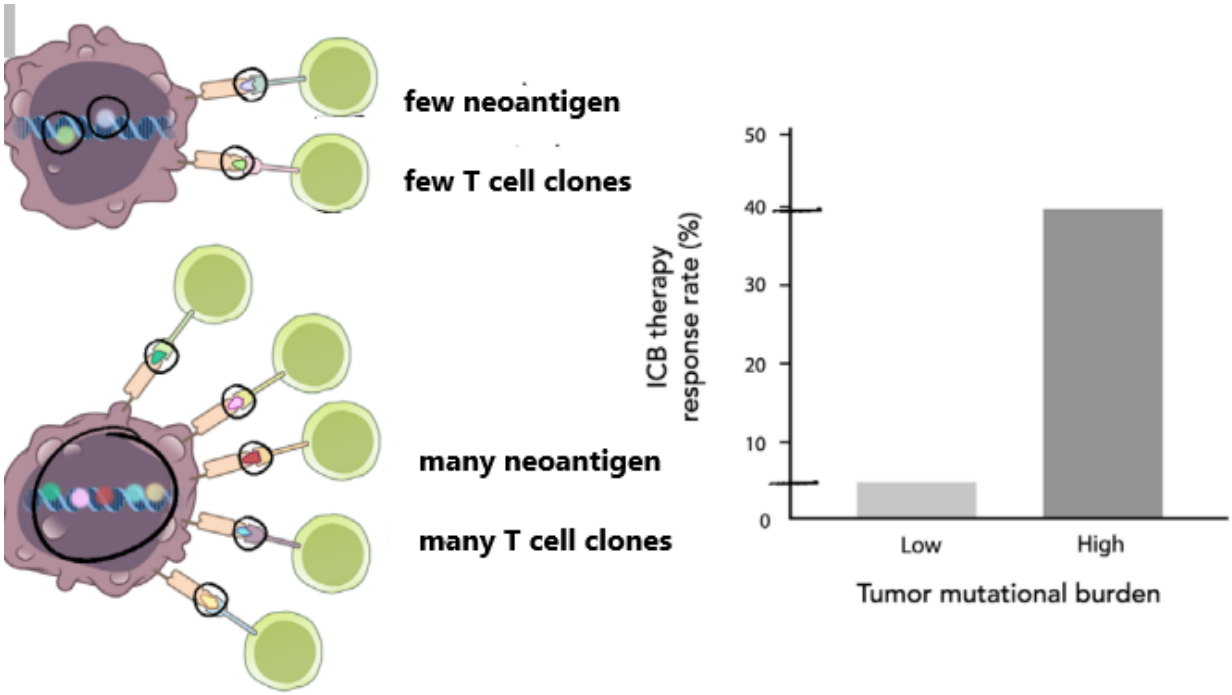


The monoclonal antibodies get out of the circulation at sites where there are tumors. They block the molecules that inhibit T-Cells that impair their ability to fight tumors.
The application of this monoclonal antibody will release inhibition of T-Cell to become activated. That will allow the cell to kill tumor cells. This leads to death of a lot of tumor cells as well as an increase in the number of the T-Cells that can fight the tumor.

Checkpoint inhibition, or ICB therapy, has been remarkably successful in treating tumors that were otherwise lethal by any conventional cancer therapy methods before they were evolved. There are many types of tumors now that are approved targets for checkpoint inhibition involved in all sorts of systems in the body including very common cancers, such as lung cancer and breast cancer. These checkpoint inhibitors will work to different degrees of success in these different tumors. But overall, as more and more clinical trials are being done against different tumors, there are more and more approvals of types of tumors that are treated.

So how do we identify which patients will respond?
And importantly, can we make patients who don't respond into responders?

These are the questions that are the subject of a lot of clinical research at this time. But we have made some progress in answering parts of these questions. One of the first things that was found in regard to who will respond is that tumors with more mutations are found to more likely respond to ICB therapy than tumors with low amounts of mutations.
So if we take a tumor with just two mutations, shown in below theoretical scheme, there will be maybe only two T-Cell clones that can see peptides from those proteins encoded by those mutations and only a few of those clones then can respond to ICB therapy but if a tumor has a high mutational burden, there will be many neoantigens that it can express. And therefore, many T-Cell clones can respond. Therefore, there are many T-Cell clones that can also respond to checkpoint inhibition and become activated. So in this theoretical plot, we see if a tumor has a low mutational burden, a very small number of those tumors will respond to ICB. But tumors with high mutational burdens, will show better response.



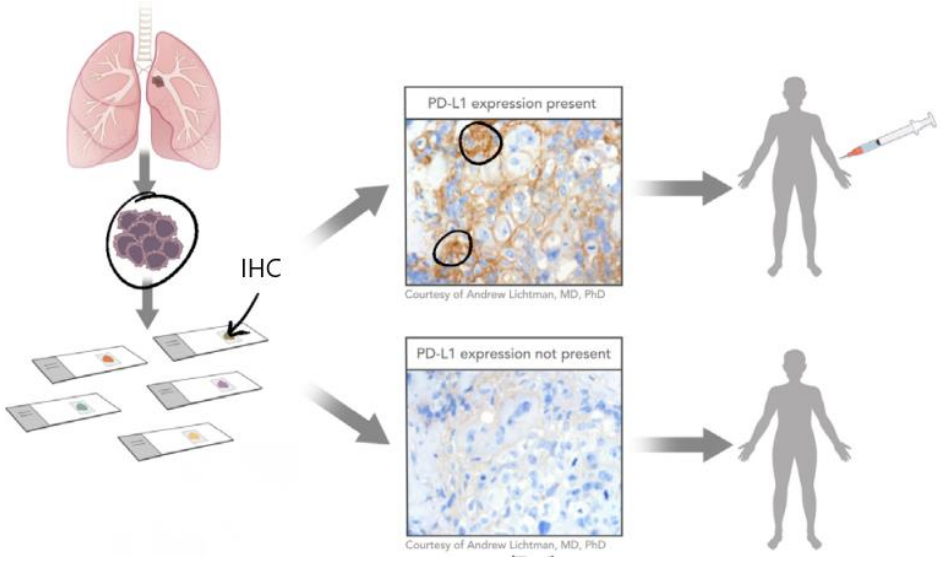
So tumor from kidney, colon, lung, liver, or skin, or any other tissue, would be biopsied. The DNA would be sequenced (by PCR method) to look for mismatch repair gene mutation or another marker of mismatch repair called microsatellite instability, MSI or the tumor tissue can be checked for mismatch repair (MMR) protein expression by immunohistochemistry (IHC) method.

If mismatch repair mutations or absence of MMR protein expression are found, then we know those tumors are going to have a high mutational burden. They can be treated with ICB therapy and most likely will respond. If there is not mismatch repair mutation, then those tumors would not be treated, and other treatments would be followed.

This represents a difference in the way traditional oncology and pathology has progressed in that the type of treatment depended on the tissue source of the tumor. Each tumor may have a different type of treatment. Here doesn't matter about the tissue source or the histologic appearance. It's just the presence of mismatch repair that the determines the therapy.

You can get more information about MMR machinery through the below links:
What is microsatellite instability or MSI?
MMR by IHC vs MSI by PCR

Another way of detecting which tumors will respond is to look for the expression of the molecules that are being targeted of the therapy. So for some cancers, detection of PD-L1 expression on the tumor cell is a prerequisite for using anti-PD-1 or anti-PD-L1 therapy. This is true for lung cancers.
For instance, in the case of lung cancers of a certain type, biopsies will be performed. The biopsies will be stained by a method called immunohistochemistry where antibodies specific for PD-L1 are applied to the tissue on the slide, the presence of the antibody bound will be detected by a chemical reaction shown as the brown stain. If the tumor expresses PD-L1, then that is a pathway by which the tumor is evading the immune system and those patients would be treated by ICB therapy, either the anti-PD-1 or anti-PD-L1 antibody. In PDL1 negative cases, other treatments will be chosen.



Similar to PD-L1 expression, the presence of evidence of gamma interferon signalling in tumor cells appears to be a marker for being able to respond to anti-PD-1 therapy. The reason is that gamma interferon, produced by the tumor-specific T-Cells, will signal into the tumor cell and generate intermediates that can be detected biochemically, leading to PD-L1 gene expression. So looking for these signalling intermediates in a cell is a way of identifying a tumor cell that will respond. We have discussed that most tumor patients do not respond to PD-1 or CTLA-4 targeted therapy. Some features of tumors predict which one will respond. Those include a high neoantigen burden, especially if there are mismatch repair enzyme defects due to mutations in

those genes, or the expression of PD-L1 or interferon-gamma signalling by some cancers, indicating that's a pathway the tumor uses to evade immunity.

Undoubtedly, we need to find more responders and many more ways of treating the tumors. One way is to expand the number of ICB targets, that is, by finding other inhibitory molecules that are inhibiting T-Cells and trying to block them. We also need to better understand the mechanisms of resistance or loss of responsiveness after treatment so that those mechanisms can be dealt with in a way that will lead to more patient responses.

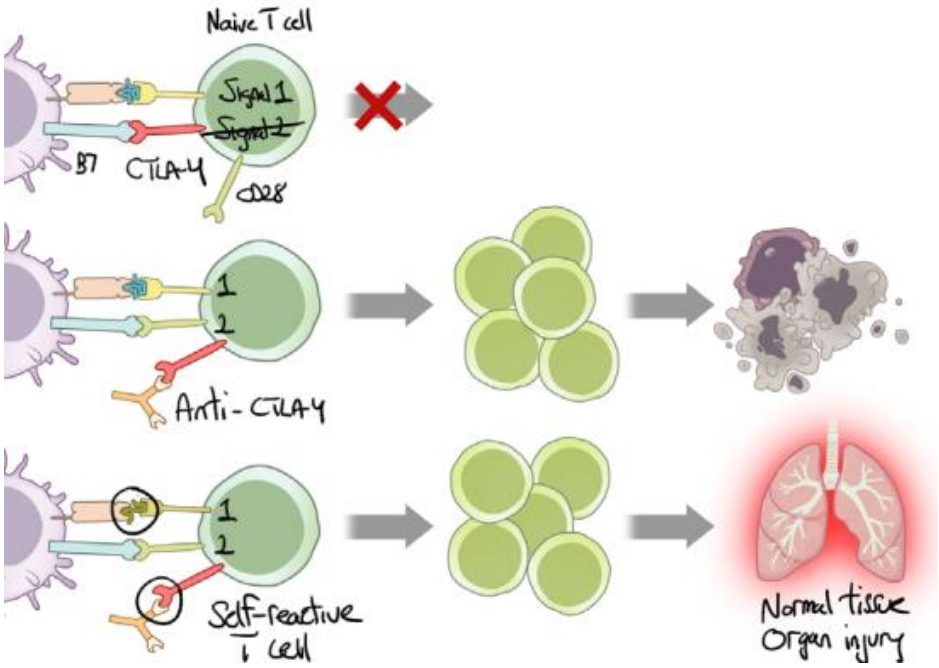
3) Complications of Checkpoint Blockade

Here we will talk about some of the immune-related complications associated with ICB therapy. ICB therapy is the use of blocking antibodies that are specific to proteins that inhibit T-Cells, such as PDL1 or CTLA4. These antibodies will release inhibition and increase the efficacy of T-Cells in killing cancer. They are in clinical use to treat a wide variety of tumors.
As currently practised, ICB therapy has two major drawbacks. One of these is that only a minority, about 14 percent of patients, clinically benefit. The other is that many patients will have adverse side effects related to dysregulation of inflammatory immune processes that damage normal tissues, not just the tumor. These are called Immune-Related Adverse Effects or irAEs. Any organ or tissue in the body can be affected by these irAEs. The most common is skin with many different rashes, which are rarely serious but can be. The most common ones that can cause severe reactions are pneumonitis and colitis. Overall, the lung and colon inflammation associated with this therapy is not severe enough to cause death.

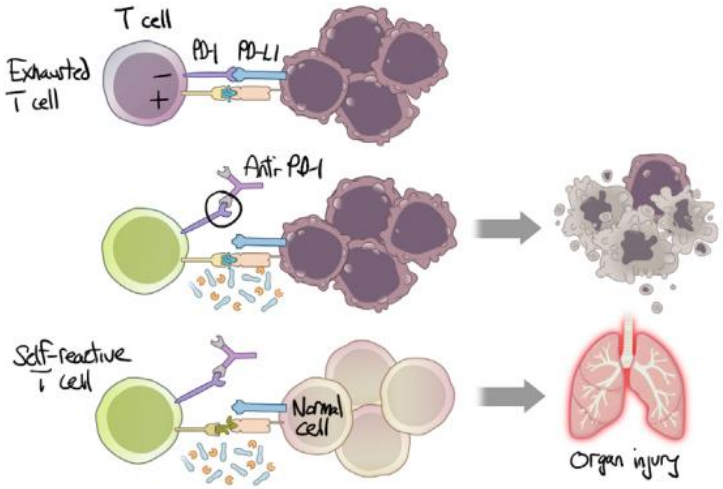
Endocrine organs are often involved, including the thyroid gland, the anterior hypophysis or anterior pituitary gland, and the pancreatic islets that produce insulin. The damage to these sites leads to ineffective hormone production and hormone deficiency.

A rare side effect in this family of irAEs is myocarditis or inflammation in the heart. It is rare but very lethal. More than 50% of patients with ICB myocarditis die. Very few lymphocytes are present in a normal heart between the muscle fibres. In ICB myocarditis, there are lots of lymphocytes that are activated and causing damage in the heart that can lead to fatal arrhythmias and other destruction of tissue in the heart.

Let's look at how ICB therapy can lead to these immune-related adverse events.
Remember that CTLA-4 and PD-1 or PD-L1 are two clinically targeted pathways. CTLA-4 binds to B7, and therefore blocks B7's ability to bind to CD28, which means that there is no co-stimulation or "signal two". Signal one that is, antigen recognition, is intact. But without co-stimulation, body cannot activate naive T-cells and will not get an anti-tumor response. However, with anti-CTLA-4 ICB therapy, CTLA-4 is blocked, so now CD28 can bind to B7, and signal two is generated. The antigen gives signal one, and naive T-Cells are activated to form effector cells that can go on to kill the tumor.
The problem is that this pathway with CTLA-4 is needed to prevent our T-Cells, specific for our tissues, from activating and causing autoimmunity. With the blockade of CTLA-4, effector T-Cells specific for the normal tissue are developed and get into tissues and cause organ injury.



For anti-PD-1 therapy, a similar story exists, PD-1 is directly inhibiting the T-Cell by signalling into the T-Cell, so although the antigen is giving positive signals, PD-1 is giving negative signals, mainly when it binds the PD-L1 molecule on a tumor cell.
These effector T-Cells with lots of PD-1 inhibition are called exhausted T-Cells. With anti-PD-1 therapy, you block the PD-1, and it can no longer bind PD-L1. There is no more negative signal, and the T-Cell can be activated and go on to kill the tumor. As is the case for CTLA-4 blockade, with anti-PD-1, effector T-Cells specific for normal tissues can get activated and allow them to kill normal cells and cause organ injury and disease.



That's the fundamental basis for ICB-related adverse immune effects.

One specific example may be when the target of a T-Cell attacking cancer is a molecule that's present both in cancer and normal tissues. So when an effector T-Cell-specific for the tumor antigen is activated by anti-PD-1 therapy, the tumor can get killed, but the T-Cells can also respond to that same protein on normal cells and cause damage.
An example of this is the phenomenon of vitiligo in ICB-treated melanoma patients. Melanoma is a malignancy of melanocytes, the skin cells that have pigment. When ICB therapy is given to melanoma patients, they often get this loss of pigmentation in the skin.

How do we deal with these immune-related adverse events?
If the reactions are severe enough, and could theoretically be lethal, then ICB therapy must be stopped. For cases that are not so severe, it is possible to try to dampen, quickly, the inflammatory events caused by the checkpoint blockade. High doses of corticosteroids, which are anti-inflammatory medicines, are used in a variety of cases, such as myocarditis and insulitis. Drugs that inhibit T-Cell responses that were developed for other diseases, like autoimmune diseases or transplant rejection, can be applied to these patients. For patients who have lost the function of endocrine organs, hormone replacement therapy can be lifesaving.

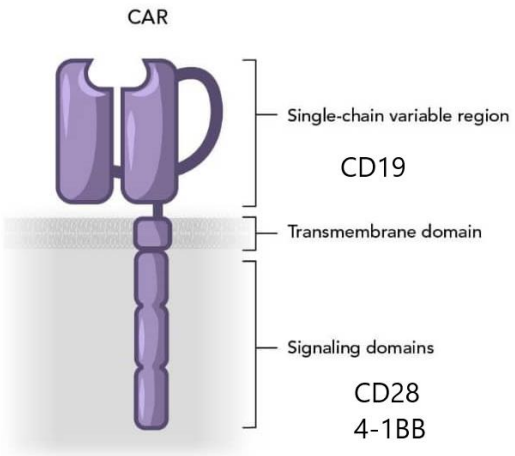
CAR T Cells and Other Novel Immunotherapies

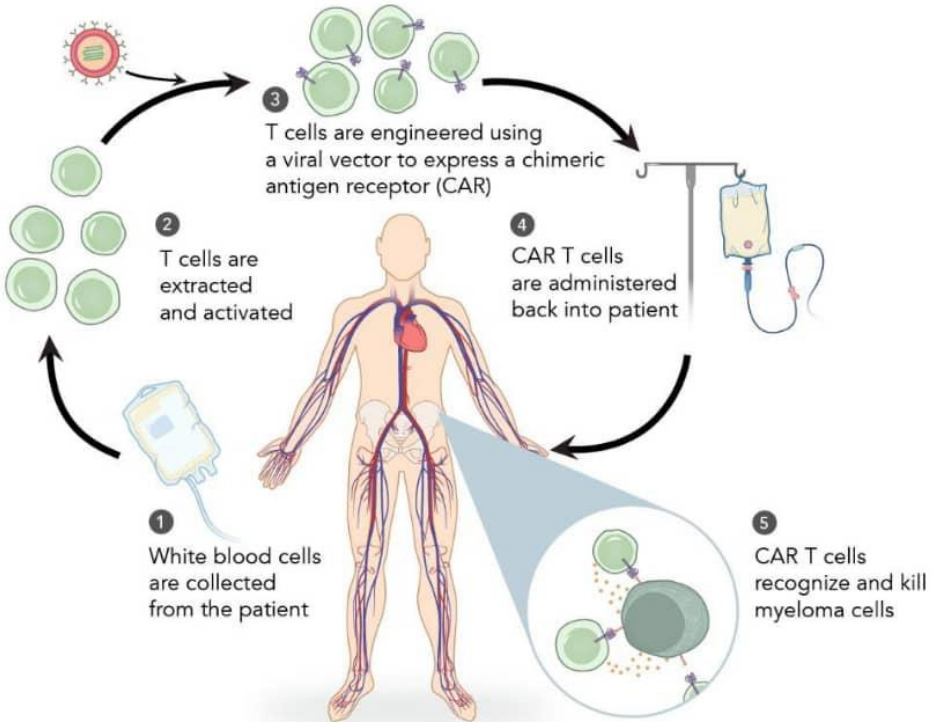
1) Basics of CAR T Cells

In this chapter, we will discuss a novel type of immunotherapy called CAR T-Cell therapy, where the CAR stands for chimeric Antigen Receptor.
In this therapy, engineered T-Cells from a patient are put back into the patient's bloodstream, where they can attack a malignancy, usually a hematopoietic malignancy.

CAR T-Cells are genetically modified cells created from T-Cells taken from the patient's own body. A patient's own cells are used because the HLA proteins displayed on T-Cells from another person would not match those recognized by the patient's immune system. Due to this difference, the infused T-Cells would be seen as foreign by the patient's immune system and killed before they could effectively treat cancer.

Before CAR T-Cells can be produced, a CAR gene must be created in the laboratory. These genetic constructs are built by fusing a genetically engineered antigen recognition portion of an antibody, known as a single-chain variable fragment (scFV), to a transmembrane domain and a series of intracellular signalling domains. The CARs approved for treating leukaemia and lymphoma have scFvs that recognize CD19 on the surface of B cells, but other targets are in development. The transmembrane domain embeds the protein in the cell membrane, allowing it to recognize antigens outside the cell while also sending stimulatory signals inside the cell. The intracellular signalling domains are various domains taken from the T-Cell receptor complex and co-stimulatory receptor molecules. These domains allow the CAR T-Cell to be activated by the same signals generally provided by peptide-MHC complex binding to TCRs and co-stimulatory molecules binding to receptors on the T-Cell. These domains are taken from proteins such as CD28 and 4-1BB, though the exact domains included depending on the CAR. Once the CAR gene is created, it is packaged into a lentivirus that can infect the patient's T-Cells and cause them to express the CAR. These viruses are similar to the human immunodeficiency virus (HIV). Still, they have been stripped of the machinery needed to replicate, so they cannot infect the patient after the T-Cells have been prepared.





Once the CAR gene has been created and packaged into a lentivirus, it must be inserted into the patient's T-Cells genome. First, white blood cells from the patient are collected by a process called leukapheresis, and the T-Cells are isolated. These T-Cells are then transduced, meaning they are infected with the lentivirus, which inserts the CAR gene into their genomes, allowing it to be expressed. The T-Cells that are transduced include both CD4+ and CD8+ T-Cells, though the main goal is to transduce CD8+ T-Cells that can then kill cancer cells. These modified T-Cells continue to express their native T-Cell receptor (TCR), which is likely irrelevant for treating the tumor. They also express the CAR specific for an antigen on the tumor cells. The transduced T-Cells are then expanded in culture to create many additional copies. Several tests are performed on the cells to ensure that a sufficient number of CAR-expressing T-Cells have been created and that the cells are free from potentially harmful contaminants. The CAR T-Cells are then infused back into the patient. They will circulate in the blood and leave at sites wherever there are tumors; they can recognize and destroy cancer cells bearing the Car-recognized antigen. For instance, a cytotoxic T lymphocyte will release granules that will kill the targeT-Cell, perforin and granzymes typical of CTLs. Also, the T-Cells will expand in number and produce inflammatory cytokines that contribute to the anti-tumor effect and some of this therapy's toxicities.

Genetically modified T-Cells expressing chimeric antigen receptors, or CARs, have been created to combat various cancers—the CARs approved for leukaemia and lymphoma target CD19 on B cells. However, CARs targeting other molecules are in development. Before creating these therapies, diseases such as acute lymphocytic leukemia and multiple myeloma relied on chemotherapy, radiation therapy, and bone marrow transplants for treatment, leaving many

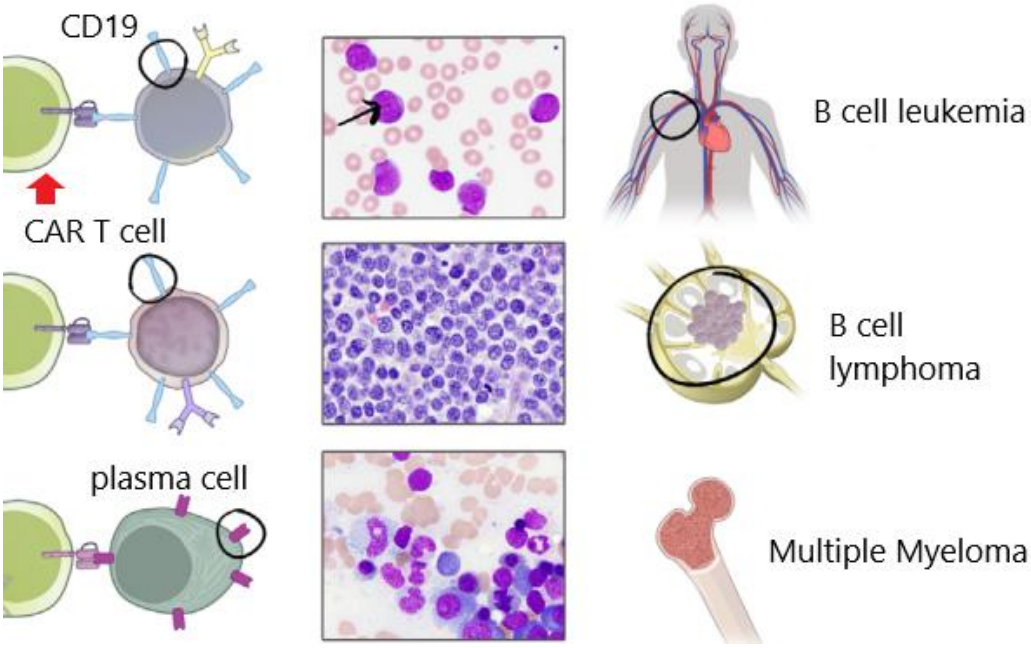
patients for whom these treatments did not work without other options. Now, some patients treated with CAR T-Cells have experienced long-term cancer remission, showing promise for treating other types of cancer in the future.

2) Clinical use and limitations of CAR T cell therapy

The first type of tumor successfully treated with CAR T-Cell therapy was a B-cell leukaemia, a malignant tumor of B cells that arises in the bone marrow. Patients produce many of these malignant B cells in the circulation, called acute lymphoblastic leukaemia. The B cells in this tumor express an antigen on their surface called CD19, which is also present on normal B cells. The first CARs developed to treat this tumor are specific for CD19.
Most children with ALL, about 80%, can be cured with a rather intensive type of therapy that involves bone marrow transplantation. However, some patients can never get bone marrow transplantation for various reasons, or some who have been treated with bone marrow transplantation relapse. There has been no treatment or cure for those patients and kids; almost all died of the disease. Many of those patients can be cured with CAR T-Cell therapy.

The second type of tumor that has been approved as a target for CAR T-Cell therapy is lymphoma, a solid tumor of B cells present in secondary lymphoid organs and other tissues as well, and again, these are B-cell-derived. These lymphomas express the same CD19 expressed on the leukemic cells, and the CAR T-Cells specifically for CD19 have been approved for this range of diseases. Although the success rate is a little less than that for ALL, but is a true progress in treating this type of tumor.

The third type of tumor, also of B cell lineage, is called multiple myeloma. It is a tumor of plasma cells, the antibody-secreting immune system cells. CAR specific for another surface antigen present on myeloma cells and normal plasma cells have been developed, and very successful clinical trials have been conducted. This is another therapy that we will see in the clinic soon.



One of the major limitations of CAR T-Cell therapies is that this therapy is pretty limited to the number of hematologic malignancies. The more common cancers, the solid tumors of the lung, colon, breast, liver, or kidney, seem resistant to CAR T-Cell therapy in clinical trials.
Partly, there seems to be challenging to get the CAR T-Cells to migrate to and survive within the tumors.

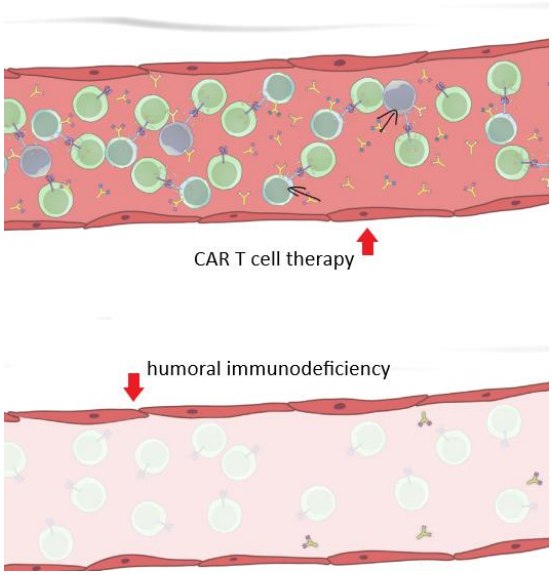
Another limitation is that the range of antigens for which the CAR is specific is limited. It is difficult to find antigens that target tumor cells but not normal cells, especially for common solid tumors. CAR T-cell therapy could cause much destruction of normal tissue if it shares antigens between tumor and normal cell.

A third limitation is the fact that CAR T-cells, like other T-Cells, can become exhausted.
They can upregulate PD-1, and the tumors can express PD-L1. This can inhibit the actual signalling of the CAR so that the T-Cell can no longer kill the tumor cell.

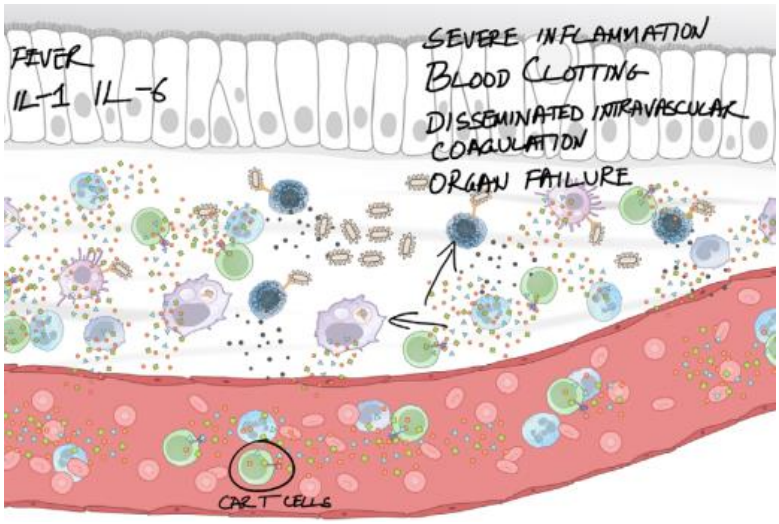
Finally, CAR T-Cell therapy is a highly personalized type of therapy. Each time there is a cancer patient, their T-Cells must be transduced by a virus, turned into CARs, and infused back into the patient. One T-Cell donor cannot give CAR T-Cells to other patients because these T-Cells would be seen as foreign and treated as an allograft that will be rejected immunologically. So this is an expensive therapy that must be repeated each time for each patient.

3) Complications of CAR T Cell Therapy

While car T-Cell therapy can be excellent and lead to the complete elimination of leukaemia, sometimes there can be consequences that can be life-threatening.
For example, in treating B cell leukaemia, once CAR T-cells are infused into the patient, they recognize the leukaemia cells through CD19, induce apoptosis of the B and thereby the consequent clearing of the leukaemia leading to a complete cure in many patients.
Now one of the consequences of such therapy is that the antibody to CD19 ,not only recognizes leukemic, cells but recognizes normal B cells. There are lots of cells in the blood, including normal B cells, and lots of B cells in lymph nodes and everywhere. However, the CAR T-Cells can hunt down these B cells, whether they are leukemic. As a result, CAR T-Cell therapy can completely eliminate B cells in the treated patient.
So essentially, CAR T-Cell therapy, when used for B cell leukaemia, will inevitably cause an immunodeficient state, a humoral immunodeficiency.
This can be treated because we can give antibodies back to the patient, infuse antibodies every few weeks, restore the antibodies that the patient was missing after CAR T-Cell therapy and hold infections at bay.



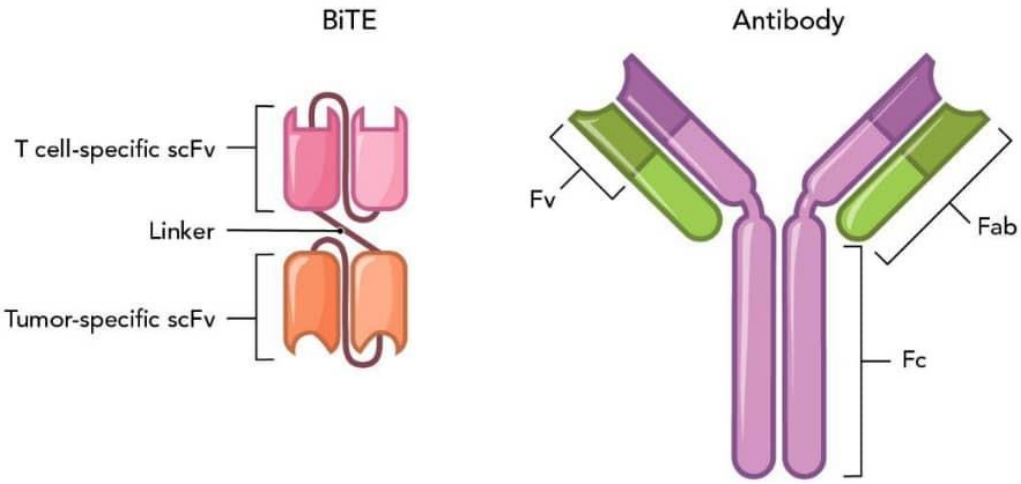
There is another consequence of CAR T-Cell therapy. When CAR T-Cells are infused into a patient's blood, lots of B cells are around, and lots of activated CAR T-Cells are now going to release cytokines. So the blood is filled with cytokines, and this cytokine release syndrome often called cytokine storm, can cause shock, drop in blood pressure, fever, and can be life-threatening.
TNF IL-1 and IL-6 are the major cytokines. There may be a high fever due to induction of severe inflammation, blood clotting, disseminated intravascular coagulation and organ failure. Fortunately, this syndrome can be avoided or treated early when it is recognized by using antibodies to inflammatory cytokines. These antibodies are used for other purposes in medicine, and they can reverse this syndrome of cytokines storm in patients who, unfortunately, have entered this phase.



There is another consequence of CAR T-Cell therapy that's fortunately very rare. So while cytokines go to the brain and cause fever, they can cause encephalopathy that can occur with the swelling of the brain. Fortunately, it is extremely rare, poorly understood, tough to reverse and can also lead to death.

4) What are BiTEs or bi-specific T cell engagers

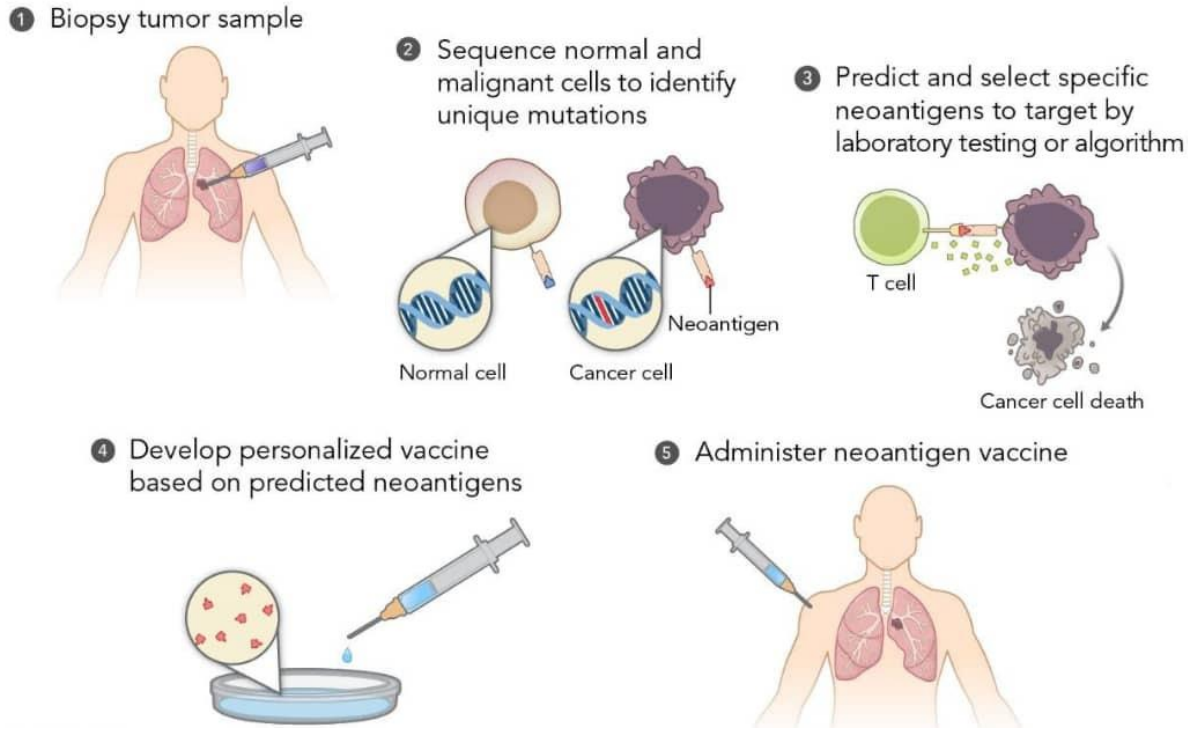
Bispecific T-Cell engagers (BiTEs) consist of two single-chain variable fragments (scFvs) that bind different antigen targets. One is specific for a tumor antigen, such as CD19, while the other is specific for a part of the T-Cell receptor complex needed for T-Cell activation, such as CD3. These two fragments are connected by a linker, creating a single protein injected into the patient. When the BiTE binds both targets, the cancer cell is brought into close proximity to the T-Cell. This proximity is not enough to cause a T-Cell response, but the binding of the BiTE to CD3 on the T-Cell provides stimulatory signals to the T-Cell. This stimulation leads to the release of perforin and granzymes by the T-Cell, and tumor cell death by apoptosis.



Following administration, BiTEs do not last very long in the body. This is partly because BiTEs consist only of single-chain variable fragments, or scFvs of antibodies, which are part of the antigen-binding region, or Fab. Therefore, scFvs completely lack the fragment crystallizable, or Fc, regions of antibodies, which includes domains that protect antibodies from degradation. Additionally, in contrast to living therapies such as CAR T-Cell therapy, BiTEs do not proliferate following administration. The short period that BiTEs persist in circulation allows greater control over the dosing of the BiTEs, but it also requires repeated therapy administration.

5) Neoantigen Vaccine Production

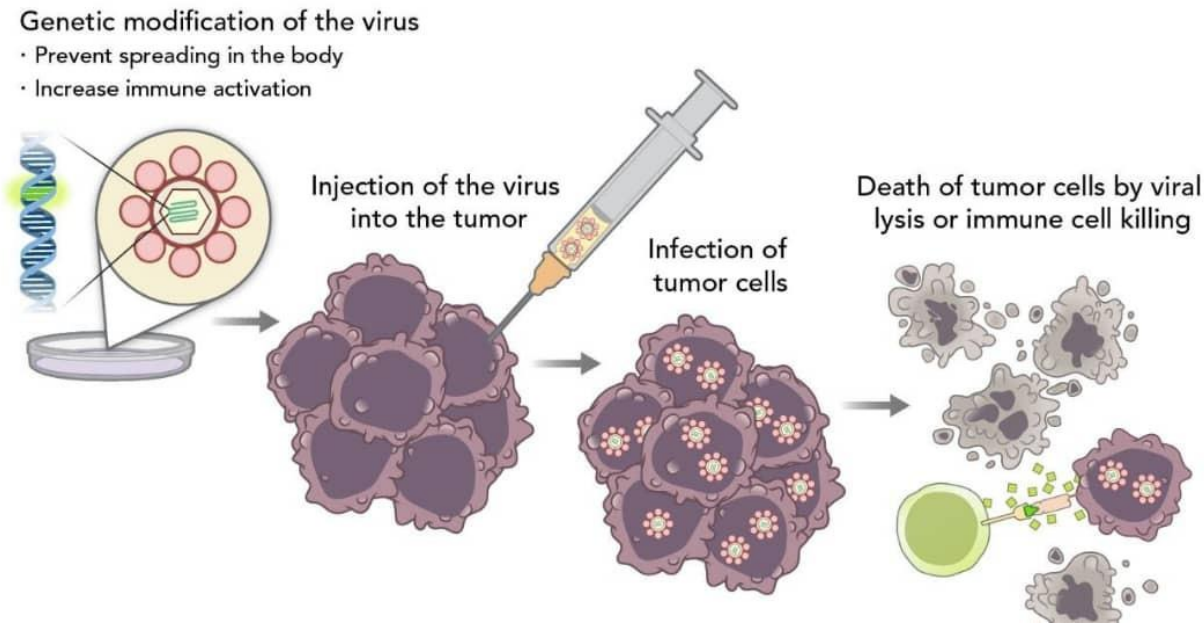
Neoantigen vaccines are highly personalized, as they are designed for each individual based on the specific mutations present in his or her cancer. The steps of this process are summarized below.



Tumor vaccines consist of tumor antigens and immune-activating adjuvants that can create the T-cell response that did not usually occur in the patient. Vaccination with tumor antigens can be used alone with ICI , to increase the efficacy of checkpoint blockade therapy.

6) Oncolytic Viruses

Many viruses, in their lifecycle, kill the cells they replicate inside. Oncolytic viruses are specially designed and administered to kill cancer cells without creating a risk for the rest of the body. The viruses may be modified to stop cancer cells from spreading in the body, requiring them to be injected at the tumor site. The process is summarized below



Lots of different viruses and virus families have been attempted. There is one that has gone into clinical use, which is a herpes virus, a genetically modified herpes virus that targets tumors by direct injection of the virus into the tumor bed.
Tumors then get infected by the virus and get killed by viral replication. But we could further genetically engineer the viruses not just to go through their lytic tumoricidal activity but also to express other proteins by putting in genes that will enhance not only the killing ability but perhaps the immune response to the tumor, for example, by making some cytokine that would enhance recruitment of lymphocytes to the tumor.

7) Combination therapy

Checkpoint blockade can be combined with chemotherapy, targeted therapy, and/or radiation. They can be used with other checkpoint inhibitors beyond PD1 and CTLA4.

The general concept is that radiation or chemotherapy is breaking up tumors and exposing tumor antigens, which will then be more exposed to the immune system and then the checkpoint inhibitor will be able to release the brakes on the T-Cells that see those antigens.

The other general theoretical approach is to target two different ways the tumor is evading immunity or otherwise having a malignant phenotype.

One of the risks of employing two different ways of enhancing immune responses to the tumor, is the increased risk of unwanted immune responses. The autoimmune or immune-related complications of using one checkpoint inhibitor may be doubled in combination therapy. That has already been observed in the limited amount of combined checkpoint inhibition in clinical use like CTLA-4 plus PD-1 targeting has a higher toxicity than either one alone.