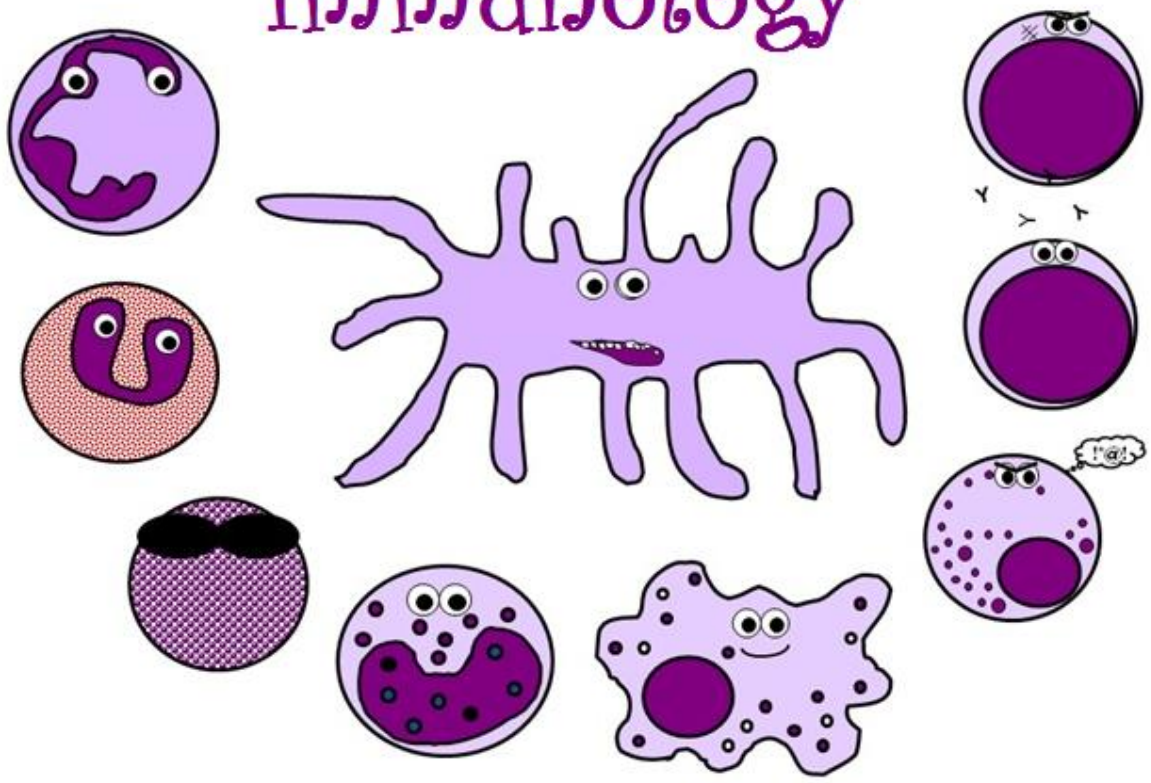
Immunology



Sheet Slides

Number: 24

Subject: Cancer and The Immune System

Done by: Reem Akiely

Corrected by: Asma' Al-Kilani

Doctor: Issa Abu-Dayyeh

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**** This sheet was written according to the recording that belongs to section 2.**

**** All the content of the slides of this lecture is included within this sheet** *(except for the figure in slide 15).*

❖ Topics of this lecture:

* Introduction and Epidemiology of cancer.
* Classification of cancer.
* Immune surveillance against cancer.
* Cancer Immunotherapy.
* Cancer Vaccines.

بعتذر مسبقًا على عدد صفحات الشيت..

** في عدد كبير من الصور، بالإضافة لمعلومات مكررة (من محاضرات اميونولوجي سابقة، و من محاضرات باثولوجي، ولكن كتابتها كانت لازمة لأنها ذُكِرت في المحاضرة).

❖ Introduction and Epidemiology

- Cancer is one of the diseases that are becoming more and more significant these days. It is necessary to always remember that the immune system plays a key role in dealing with cancerous cells in an attempt to protect the human body.
- It is expected that the use of chemo- and radio-therapy will decline in the upcoming years, due to the evolution of immunotherapy that constitutes, nowadays, an important method for cancer treatment.

I. Cancer Epidemiology

Figure (1) shows the top leading cancers for both sexes in the US.
Source of the data: Centers for Disease control and prevention (CDC), US., 2014
Although the data in the figure is for the US population, the numbers concerning the whole world population – including Jordan- are close.

- The leading cancers in males are a bit different from those in females:
  - The most common cancer type in males is prostate cancer (it constitutes approximately 27% of total cancer incidences in males). On the other hand, the most common cancer type in females is breast cancer (29% of total cancer incidences in females).
  - Lung cancer comes in the second place, and is followed by colorectal cancer in the third place. (in both; males and females)
  - Other types of cancers like lymphomas and leukemias are less common compared to the three types mentioned above.

- The most important thing to say is that nearly **one in three** people will get cancer in their lifetime. *Statistics in the UK even predict that in 5 to 7 years from now, we will start saying that **one in two** people will be diagnosed with cancer at some point in their lives.*
  ➔ The number of cancer incidences is increasing. That's why understanding the basics behind cancer development and its relation to the immune system is a must.

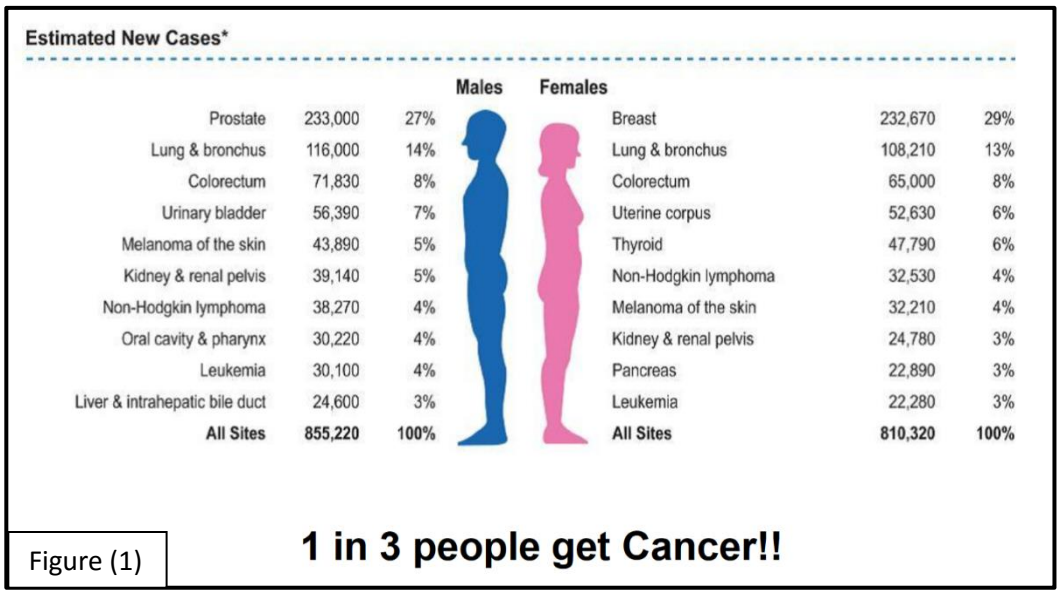


Figure (1)

Breast cancer constitutes nearly one third of total cancer incidences in females. That's why breast cancer awareness campaigns are organized very often.

- Cancer is the second leading cause of death worldwide.
  First leading cause of death: Heart disease.
  Third: chronic respiratory diseases.
- It is important to emphasize the fact that cancer is not a single disease, it's a group of diseases. Some principles related to cancer are seen in all types. However, every cancer type has its own unique features, affected tissues, mode of expansion, …etc.
  ➔ This makes dealing with cancer much harder.
- It is hard to group all cancer types together and put a single plan for treatment. However, scientists hope that discovering the common things between all types might make some cancer therapy plans effective against many types of cancer.
  (this principle might be useful in cancer immunotherapy plans)

II. Cancer is a control system problem

- Cancer is a problem of cell division.
- Two major types of genes are responsible for cell division control: *(figure (2))*

(1) Proto-oncogenes

- A gene, which when mutated, can cause a cell to proliferate inappropriately is called "proto-oncogene", and *the mutated version is called an oncogene.*
- **proto-oncogenes** (the normal versions) have a role in regulating the cell cycle.
- An **oncogene** drives cells to divide in an uncontrollable fashion, and thus accelerates the occurrence of cancer.

(2) Tumor Suppressor genes

- These are genes that function as suppressors of abnormal growth. In other words, they help safeguard against uncontrolled cell growth.
- If a mutation that occurs in a tumor suppressor gene renders it inactive, the outcome will be an uncontrollable quicker proliferation of cells. This scenario is seen in many types of cancer.

- The role of tumor suppressor genes is noticed when a cell starts to become abnormal due to DNA damage. *(figure (3))*
- The most important tumor suppressor gene is the p53 gene. More than 50% of cancers have a mutated p53.
  (this is one of the common mechanisms)
- When DNA damage occurs, p53 causes the cell to be arrested in the G_1 phase of the cell cycle *(see the extra figure #1),* in an attempt to fix the damage.
- p53 allows time for DNA repair mechanisms to work. If DNA repair attempts succeed in fixing the damage, the cell cycle will be resumed. However, if DNA repair mechanisms fail, the cell will be pushed towards apoptosis.

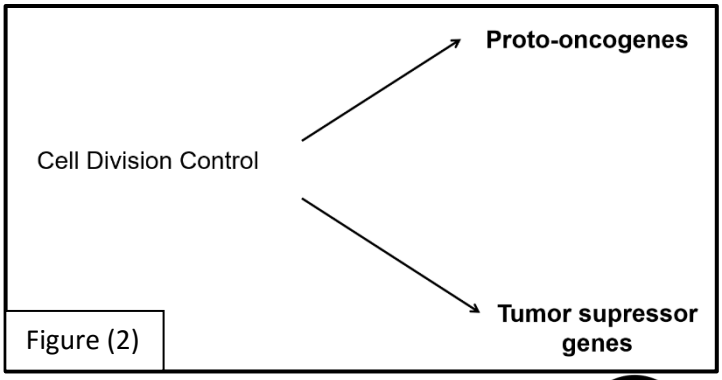


Figure (2)

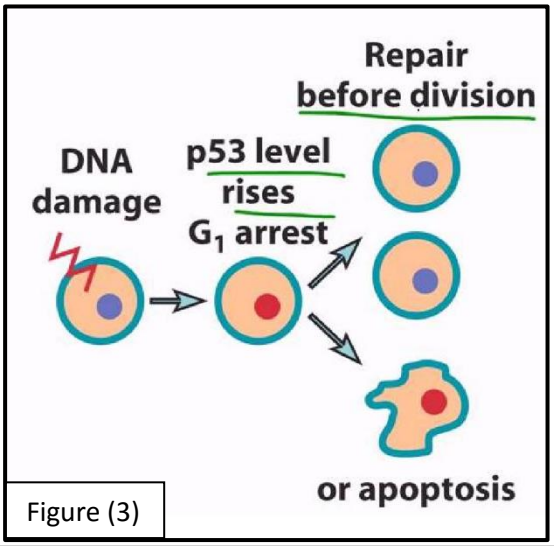
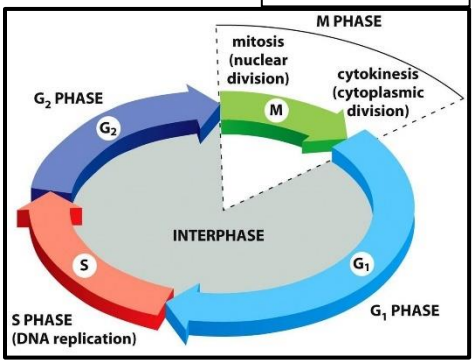


Figure (3)

Extra figure #1



- **Note**: when p53 knockout experiment was carried out in mice, scientists found out that mice lacking p53 die of cancer in few months.

- Look at *figure (4)*, consider the proto-oncogene as the accelerator of the car, and the tumor suppressor gene as the brake.

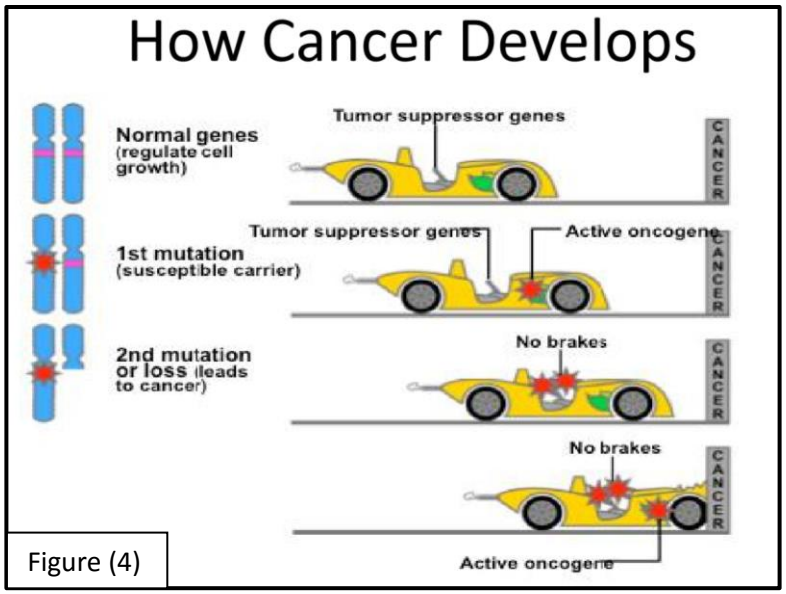


Figure (4)

- According to the figure *(but this is not always the case as we will see in few seconds)*: two types of mutations were needed to develop cancer;
  - a mutation that affects the proto-oncogene resulting in an Oncogene.
  - a mutation that affects the tumor suppressor genes resulting in an inactive tumor suppressor gene.

  (the outcome of both is an uncontrolled growth, and eventually cancer)

Question: for cancer to develop, is it a must that the mutations taking place should affect both, proto-oncogenes AND tumor suppressor genes?

Not necessarily. In some cancers, the main cause is an oncogene, and in others, the main cause is a mutation in a tumor suppressor gene. However, many -not all- cancers end up having both types mutated.

Hereditary Retinoblastoma represents an example in which a defective tumor suppressor gene is enough to cause cancer:

> These patients are born with one defective copy of the Rb gene (retinoblastoma gene, a tumor suppressor gene). They eventually get a second mutation (in the other copy of the gene) and develop retinal tumors early on.

III. Cancer is a multi-step process

- Very few cancers can arise from only one mutation (an example is hereditary retinoblastoma as mentioned previously).
- In most cases of cancers, we would be talking about a multi-step process. And in this case, we consider cancer a disease of aging.
  - Most -not all- cancers are seen in older individuals (above 50 years). Why? Because cancer is usually a multi-step process, meaning that an individual has to accumulate certain types of specific mutations throughout his life, in order for cancer to develop. (on average, 4 to 7 mutations are required). *For example, an individual might develop one significant mutation every 10 years, and thus develop cancer at the age of 50 (this is just an example mentioned by the doctor. It does not mean that this is always the case).*
- Don't forget that mutations occur naturally every day during DNA replication. The cells have repair mechanisms for mutations that occur (These include certain enzymes that detect and correct mistakes that took place). However, some mutations escape the repair mechanisms and might favor the development of tumors if important genes are involved.
- Certain groups of people are at higher risk of developing cancer compared to other individuals. For example, people who are exposed to more mutagenic factors like radioactive substances and heavy metals are predisposed to faster accumulation of mutations and thus they are more predisposed to cancer.

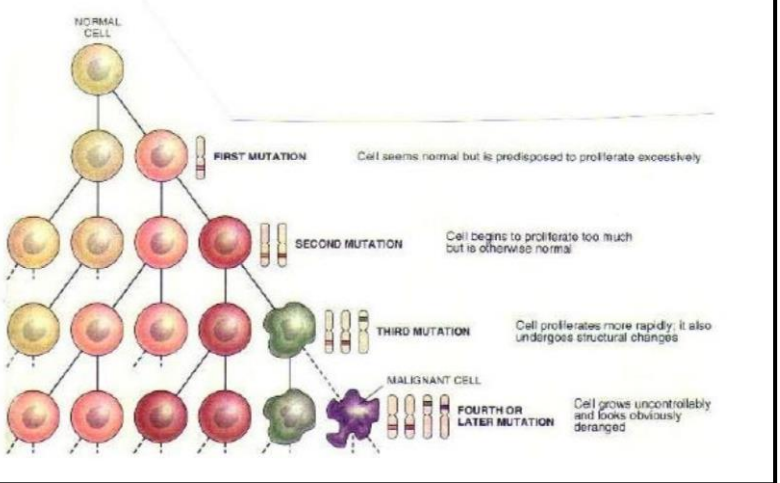


Figure (5)

Radioactive elements have disastrous effects on the human body, including cancers. That's why individuals who are exposed to higher amounts of radiation (for research purposes for example) should be properly protected using specific equipment and suits.

It is believed that Marie Curie, who conducted pioneering research on radioactivity, developed cancer due to her exposure to radioactive elements. She died of aplastic anemia caused by radiation exposure.

- Cancer cells mutate a lot and are genetically **unstable** compared to normal cells.
  When a cytotoxic T lymphocyte (CTL) develops against a certain cancerous cell, and then this cell undergoes division with high rate of mutations, the result will be a change in the cell's antigenic properties, and this will be misleading to the immune system, because new immune cells will be needed to recognize the new properties so that an immune response can be elicited.

  ➔ **Cancer cells are one step ahead of the immune system, due to the high mutagenesis rate.**

We took in the previous lecture that HIV is always one step ahead of the immune system, because it has the ability to mutate its antigens and confuse B and T cells. A similar concept exists in cancer.

❖ Classification of cancer

I. According to the tissue from which the cancer originates.

- **Carcinoma**
  - Derived from epithelial cells, which line surface of skin and organs, digestive tract, airways and mammary ducts
  - Most common cancer type (89-90% of all reported cases)
- **Sarcoma**
  - Derived from mesenchymal tissue – muscle, bone, cartilage, fat, connective tissues
- **Hematopoietic**
  - Leukemia – derived from white blood cells or their precursors
  - Lymphoma – involves cells of the lymphatic system
  - Myelomas – involves white blood cells responsible for the production of antibodies (B lymphocytes or B-cells)

Few notes:

- Leukemias develop in the lymphatic tissue. There are many types of leukemias. The most important are:
  1) *ALL (Acute Lymphoblastic Leukemia)→ mainly seen in children.*
  2) *AML (Acute Myeloid leukemia)→ seen in middle aged individuals.*
  3) *CLL (Chronic Lymphocytic Leukemia) → seen in people above 50.*

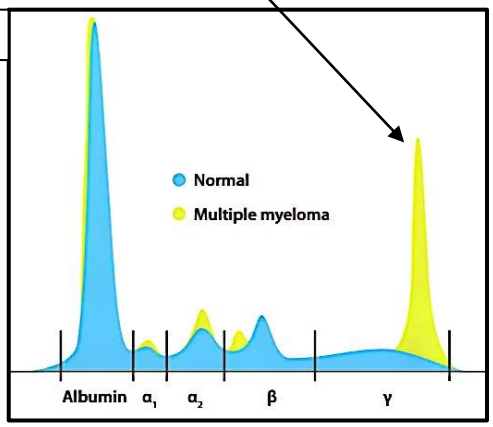
Chronic Lymphocytic Leukemia:

- CLL is seen quiet often.
- It comprises most of the diagnosed cases of leukemia. It is mostly an incidental finding. *For example, a person above 50 might be getting his routine checkup when the lab technician notices a high white blood cell count in his CBC (and upon examination, the cells were mostly mature lymphocytes), and further tests like flowcytometry revealed that this patient has CLL.*

Myelomas:

- Involve B cells that are capable of producing antibodies. (plasma cells).
- Myeloma results in the production of a monoclonal antibody that will overwhelm the bone marrow leading to bone lesions.
- Diagnosis: the presence of the M -band in protein electrophoresis. *(see extra figure #2)*

Extra figure #2



II. Another classification for human tumors (spontaneous Vs. Virus induced)

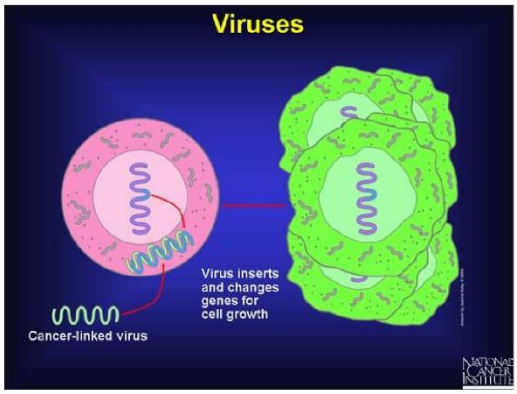
(1). Spontaneous Tumors

- Most human tumors are spontaneous, because they arise when a single cell accumulates a collection of mutations that causes it to develop cancerous properties.
  (without the need of a viral infection to cause cancer)
- In this case, the presence of one cancerous cell is enough to produce a cancer tissue.
- It's very important to always remember the previous statement because the success of cancer therapy depends on whether all cancerous cells have been eliminated or not. If one cell survives after therapy (chemotherapy, radiotherapy, or surgery), it would be sufficient for cancer recurrence.
- Causes:
  - Natural process (every cell division in our bodies introduces more mutations to the genome)
  - Radiation
  - Smoking → it plays a major role in development of lung and laryngeal cancers.
  - Fatty diet → may increase the risk of cancer, especially cancers of the GI tract.
  - Others.

(2). <u>Virus-induced tumors</u>

- In some tumors, a viral infection can help accelerate the process of cancer development, by interfering with cell's safeguard systems.
  Here, we are mostly talking about viruses that are capable of establishing chronic infections, not acute.

Figure (6)



- Examples:
  - **Cervical cancer**
    - Related virus: Human Papilloma Virus (HPV).
    - <u>Important note</u>: not all incidences of high risk HPV infection lead to cervical cancer in females. (1% of cases lead to cervical cancer).
      However, almost all females who develop cervical cancer appear to be positive for high risk HPV.
  - **Liver cancer**
    - Related viruses: hepatitis B and hepatitis C viruses.
    - Hepatitis B and hepatitis C viruses have the ability to induce liver fibrosis, cirrhosis, and liver cancer.

Strains of HPV fall into two main categories:

1) <u>High risk strains</u>
   Can cause cervical cancer.
   These include HPV types **16 and 18**
2) <u>Low risk strains</u>
   Do not cause cancer but can cause genital warts in both; males and females.
   These include HPV types **6 and 11.**

Note: Dr. Issa said that there are also "intermediate" risk strains.

❖ <u>Immune Surveillance Against Cancer</u>

What are the main immune cells that have a role in immune surveillance against cancer cells?
From the innate immune system: dendritic cells, macrophages, and natural killer cells.
From the adaptive immune system: Cytotoxic T lymphocytes.

Note: Dendritic cells are very efficient antigen presenting cells. If dendritic cells -that are capable of capturing cancer antigens and presenting them properly to the adaptive immune system- are not preset, we will have a big problem.

Note 2: the following discussion will be mainly concerning the three types of cells that are involved in <u>fighting and killing cancerous cells</u>. These three are: macrophages, NK cells, and CTLs.

I. The role of macrophages

- Macrophages play an important role in fighting solid tumors, because they are found in the tissues. (other cells like NK cells are mainly found in the circulation).
  - ➔ The presence of macrophages in the tissues makes them very important in the initial recognition and fight against cancer.
- Macrophages are able to secrete many cytokines including Tumor necrosis factor (TNF) which has the ability to cause the death of tumors.

- An evidence that shows the significant role played by macrophages in fighting solid tumors:
  Nowadays, there are certain methods for cancer therapy that rely on the activation of macrophages. One example is the use of BCG in the treatment of superficial (non-invasive) bladder cancer.
  Several patients with certain types of bladder cancer were treated using this method. The results were actually promising.
  - Use of BCG in bladder cancer therapy:
    Injecting BCG (Bacillus Calmette- Guerin), which is a strain of bacteria, into the bladder.
    - ➔ The entry of this bacteria to the bladder stimulates macrophages.
    - ➔ The hyperactivation of macrophages in the site of tumor in the bladder causes the tumor to regress and diminish.

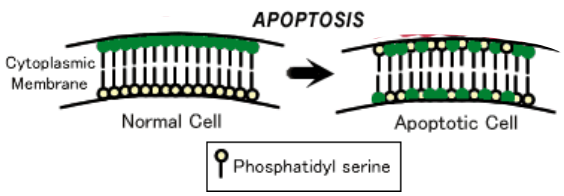
Additional piece of information:

(not mentioned by Dr. Issa)

Bacillus Calmette–Guérin (BCG) is a live attenuated strain of *mycobacterium bovis* (BCG is a type of vaccines).

The BCG vaccine is primarily used in treatment of tuberculosis but it is also an effective treatment for some non-invasive bladder cancers.

- Macrophages have versatile receptors that allow them to differentiate normal cells from abnormal ones. Of these receptors are certain receptors that recognize phosphatidylserine.
  - ➢ Phosphatidylserine is a component of the plasma membrane, but usually, it faces the interior of the cell when the cell is healthy.
    - ➔ When macrophages do not see phosphatidylserine on the outer surface of the cell, this represents a signal that informs the macrophage that the cell encountered is healthy (not dying, nor becoming abnormal).
  - ➢ When the cell becomes apoptotic or abnormal, some phosphatidylserine molecules will become exposed on the outer surface of the cell.



➔ When macrophages see the phosphatidylserine molecules, this represents a signal that tells the macrophage that the cell encountered is becoming old (senescent), dying, or becoming cancerous.

Note: the previous mechanism is also used in the spleen to filter the cells and differentiate between old cells and healthy new ones.

Note 2: macrophages have other receptors that can differentiate between normal and abnormal cells.

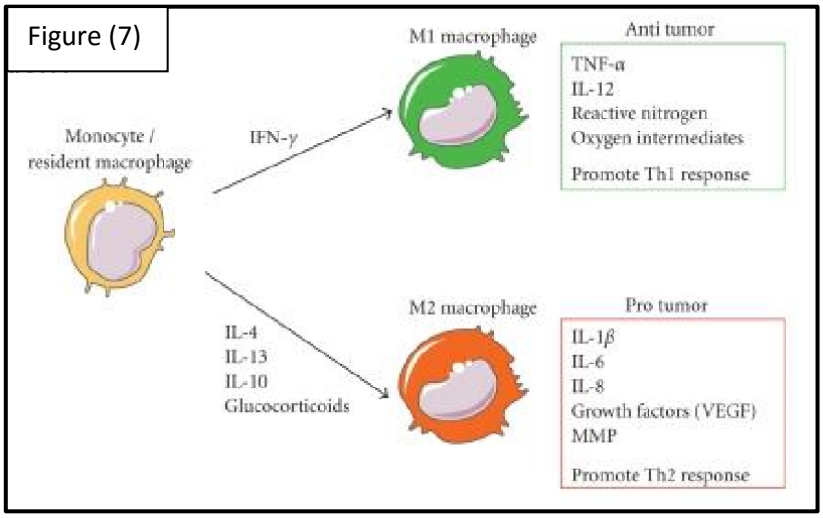
Polarization of macrophages

- In previous lectures, we talked about polarization of T helper cells. A T helper cell will either become a T_{h1} or T_{h2} based on the cytokine profile present. A similar principle applies in the case of monocytes.
- The cytokines present in the environment surrounding a naïve monocyte determine whether a naïve monocyte will become an M1 or M2 macrophage.
  Figure (7)
  a. In the presence of IFN-γ, a naïve monocyte will become an **M1 macrophage.**
  M1 macrophages produce TNF-α that is very important in fighting tumors.
  M1 macrophages also produce IL-12.
  (IL-12 produced by M1 macrophages can drive a Th_1 response. Th_1 will induce polarization of monocytes towards M1 (both cells help each other)).

Note that the cytokine profile that gives Th_1 is similar to what gives the M1 macrophage, and the profile that gives Th_2 is similar to what gives M2 macrophages.

Note: studies have revealed that in individuals whose immune system responds very well to cancer and is capable of fighting it, the macrophages are mostly polarized towards the M1 type.

Figure (7)



b. In the presence of anti-inflammatory cytokines like IL-4, IL-13, IL-10, a naïve monocyte will become an **M2 macrophage**.
M2 macrophages promote a Th_2 response
➔ Thus, promote an immuno-suppressive environment
➔ This explains why if we use flow cytometry (using specific markers) to find out whether the macrophages in a tumor tissue are of the M1 or M2 type, the result will be that most macrophages are of the M2 type.

Question: how does the immuno-suppressive environment that is generated by M2 macrophages aid in the development of cancers?

If a cancerous cell arises in this tissue -where immunity is suppressed-, immune cells like CTLs that come to this location in an attempt to kill the cancerous cells will find M2 macrophages and an anti-inflammatory environment.

→ CTLs will be blocked (their actions will be restrained due to the surrounding environment), and the tumor will grow.

Note: converting M2 macrophages into M1 macrophages is one of the cancer therapy plans that scientists are thinking of nowadays. *(similar to the concept of shifting the Th_2 response to a Th_1 response in an attempt to treat asthma, as mentioned in previous lectures).*

Note 2: M1 and M2 are **NOT** the only types of macrophages found in the human body.

Things in immunology are never black and white. Gray is always present.

II. The role of NK cells

- NK cells are licensed to kill.
  (NK cells are one of few types of cells in our bodies that are able to take the decision of killing a cell straight away without a prior authorization from other cells. *Other immune cells mostly need two signals and the authorization from other cells to tell them to go ahead and fight certain cells*).
- How can the NK cell recognize its targets?
  - NK cells have activating receptors that recognize abnormal proteins and components.
  - NK cells also have inhibitory receptors that check for the presence of MHC class I molecules. *(if MHC I is present, the NK cell will be inhibited and will not kill the cell).*

Question: Many tumors decrease the expression of MHC class I molecules on the surface. What is the significance of this?

To escape killing by **CTLs**

(the answer is not "to escape killing by NK cells". Please don't get confused and read the following carefully).

If a cytotoxic T lymphocyte did not recognize its antigen presented on an MHC class I molecule, it will not be able to kill the cancerous cell. This is why tumor cells reduce the expression of MHC I molecules (it's an attempt to avoid recognition and killing by CTLs).

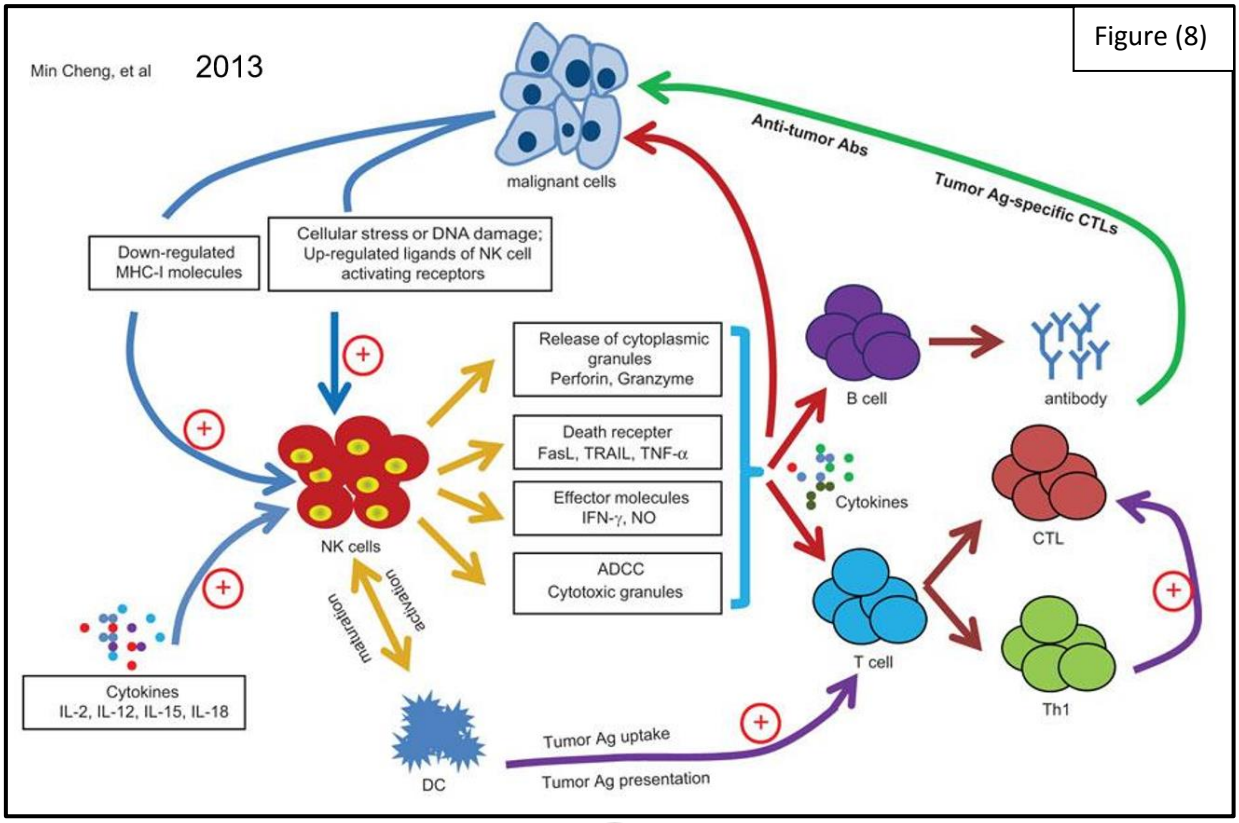
However, due to the decreased expression of MHC I molecules, NK cells will get activated and fight the cancerous cell.

Conclusion:

The results of reduced expression of MHC I on tumor cells:

a) Escape recognition and killing by **CTLs**.
b) Become susceptible for recognition and killing by **NK** cells.
(some sort of compensation)

Figure (8) (about NK cells) is very important. *Kindly read the text that follows, step by step, so as not to get lost.*



- There are three major ways to activate NK cells
  1) Certain antigens produced by cellular stress or DNA damage.
  These will be on the surface of the malignant cell and will give an activation signal when they bind to NK cell activating receptors.
  2) Down-regulation of MHC I molecules.
  3) Certain cytokines in the environment can induce the activation of NK cells. *(according to the figure, these include; IL-2, IL-12, IL-15, IL-18)*

- Once NK cells are activated, they use their methods of killing that include:

  a) Release of cytoplasmic granules that contain preforin and granzyme B.

  b) Death receptor mechanism
  NK cells express FasL on their surfaces.
  And many tumor cells express Fas (a death receptor).
  ➔ Fas-FasL binding will cause the death of the cancerous cell by apoptosis.

  NK cells also express TRAIL and TNF-α on their surface.

  Note that the Ligand is expressed on the surface of the NK cell, while the corresponding Death receptor is expressed on the surface of the tumor cell.

  c) NK cells can produce certain cytokines (like IFN-γ), and nitric oxide (NO).
  - IFN-γ → activates macrophages and other immune cells. Moreover, it drives a Th_1 response to aid in fighting cancer.
  - NO → has a direct toxic effect, because it's a very aggressive free radical that can cause the death of the cell.

  d) ADCC (Antibody-Dependent Cellular Cytotoxicity)
  In response to cancerous growth, immune cells (like CTLs, Th cells, and B cells) get activated.
  ➔ Activated B cells produce antibodies that can bind tumors.
  ➔ Antibodies that are attached to malignant cells result in the activation of NK cells that will become able to kill the malignant cell.
  (the concept of ADCC was discussed in lecture 4)

A quick reminder:

The release of perforin and granzyme-B by exocytosis as a method for killing is seen in both; **NK cells and CTLs**.

Perforin → creates pores in the plasma membrane.

Granzyme B → induces the death of the cell via caspase dependent and independent pathways.

Another quick reminder:

Death receptors are cell surface receptors that transmit apoptotic signals initiated by specific ligands such as FasL (Fas ligand), TNF-α, and TRAIL.

Note

TRAIL: TNF-Related Apoptosis-Inducing Ligand. It's a ligand that is capable of initiating apoptosis through binding to its receptor.

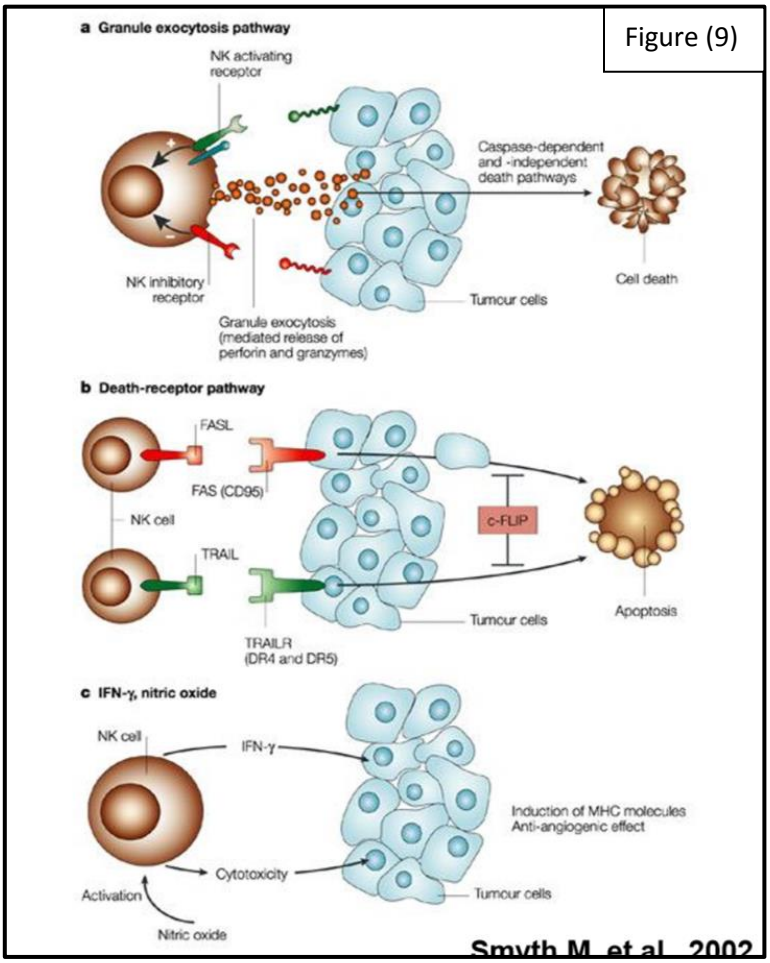
- <u>The results of activation of NK cells:</u>
  - Direct killing of malignant cells using the previous mechanisms.
  - Cytokines released by activated NK cells result in:
    1. Activating B cells to produce antibodies.
    2. Activating Th cells that will differentiate into Th_1
       → Th_1 can activate CTLs
       → CTLs can kill malignant cells

- <u>An additional INDIRECT action of NK cells against tumor cells:</u>
  NK cells can activate dendritic cells to become better antigen presenters.
  (without NK cells, dendritic cells would be less efficient in antigen presentation).

Figures (8) and (9) should be clear at this point.

<u>One final note about NK cells:</u>
According to the previous discussion, NK cells appear as very powerful cells. Can you think of a factor that limits their powers?
Answer: the trafficking pattern.
NK cells are mostly found in the circulation, so the major challenge facing them is <u>leaving the circulation and exiting to the tissue</u> in order to be able to fight solid tumors.

Figure (9)



III. The role of CTLs

- It's important to remember that: for a CTL to be active, it needs the Th cell to be active.
- The problem we face when we talk about the role of CTLs in fighting cancer is that naïve CTLs do not normally go to tissues (because they follow the normal trafficking pattern so it's a little bit hard for them to reach the site of the tumor). Moreover, even if CTLs go to tissues, they do not get co-stimulated, because tumors develop many mechanisms to suppress the function of CTLs. *(immune cells at the cancer site will further suppress CTLs as we will see in the following section).*

After discussing the role of immune cells in fighting cancer. It is now important to mention the mechanisms that enable cancer to beat the immune system and establish itself in the human body: *(figure(10))*

(A). Cancer cells express PD-L1 (PD-1 ligand) and CTLA-4 ligand on their surfaces.
When these ligands bind to their receptors on the T cell (PD-1 and CTLA-4, respectively), the T cell will be inhibited.
Most -not all- tumors express these ligands to block tumor specific cytotoxic T cells.

(B). The presence of high amounts of MDSCs in tumors.

- MDSCs: Myeloid Derived Suppressor Cells.
- MDSCs are myeloid cells that are not fully differentiated.
- These cells are found in high amounts in tumors.
- They express PD-L1 on their surfaces. These cells also produce IDO and Arginase.
  - IDO: Indole amine 2,3 dioxygenase → *it has an immuno-suppressive role but Dr. Issa did not mention its exact function.*
  - Arginase → cleaves arginine (catalyzes its hydrolysis)
    Arginine is very crucial for NO synthesis by iNOS *(inducible NO synthase)*
    By depleting arginine using arginase, MDSCs suppress the ability of other cells to produce NO.

(C). Tumor cells themselves can produce certain cytokines. Of these:

- IL-6 and IL-10 → interfere with the maturation of dendritic cells and macrophages. If dendritic cells and macrophages do not mature, proper antigen presentation will not take place.
- VEGF (Vascular endothelial growth factor) → important for angiogenesis.

<u>Note</u>: Angiogenesis (the growth of blood vessels from pre-existing vasculature), is very important for the survival of tumor cells.

(D). Activation of regulatory T cells (TReg cells)

This occurs as a result of the release of <u>TGF- β</u>, mostly by the APCs in the area.

TGF- β is important for the differentiation of TReg cells that suppress the immune system.

Remember that we also said in previous lectures that the TReg cells themselves can also produce TGF- β.

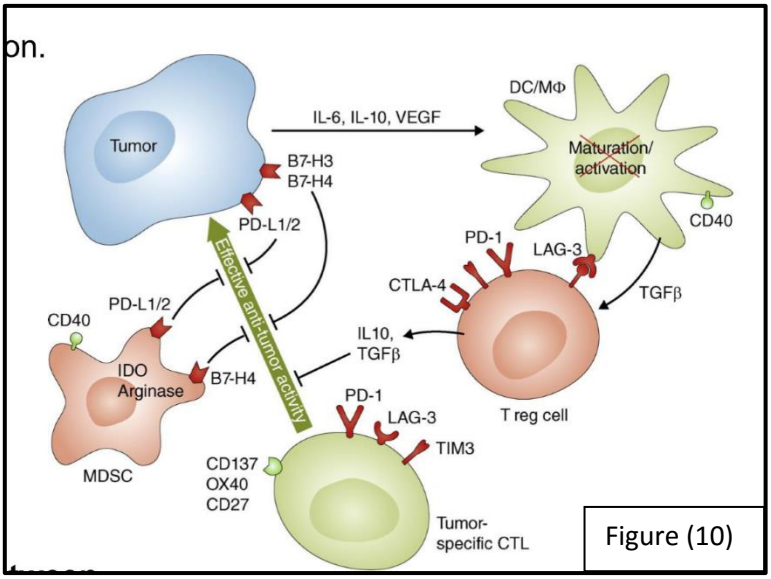


Figure (10)

Dr. Issa briefly revised the levels of peripheral tolerance *(from lecture 14)*:

- Naïve lymphocytes follow regular trafficking patterns, so they do not exit to tissues normally.
- If they reach the tissues, TReg cells can suppress their action.
- Even if TReg cells fail to suppress the action of these lymphocytes, the lymphocytes will not be co-stimulated *(lack of co-stimulatory signal in tissues will lead to anergy and death).*
- Even if they manage to get co-stimulation, they will express Fas and FasL and kill each other by apoptosis.

Mechanisms of peripheral tolerance aim to <u>protect us from autoimmune diseases</u>. But everything comes with a price. The price here is that <u>we are more predisposed to cancer</u> because cancer cells utilize those mechanisms to suppress immunity so that the tumor can establish itself in the human body.

Most, if not all, solid tumors are surrounded by immuno-suppressive environment. This is why the immune system fails to beat cancer.

The previous discussion explains the following statement mentioned in the slides:

"There's a serious conflict between tolerance preservation to self, and the need to provide surveillance against tumors arising in tissues".

❖ Cancer Immunotherapy

I. CTLA-4 targeted immunotherapy

Figure (11)

- Before treatment: When the effector T cell encounters the cancer cell, the T cell receptor will recognize its antigen (this is the first signal).
  However, instead of receiving a co-stimulation signal as the second signal, the T cell will receive a suppressive signal when CTLA-4 on the T cell binds to its ligand on the cancerous cell → thus, preventing the effector T cell from killing the cancerous cell.
- CTLA-4 targeted immunotherapy is based on the use of monoclonal antibody against CTLA-4:
  - The monoclonal antibody binds to CTLA-4
  - The result is blocking the ability of the cancer cell to establish the (CTLA-4 – ligand) interaction.
  - The ability of the cancer cell to suppress the T cell function declines.
  - The effector T cell will receive the proper activation signal and therefore, attack the tumor.
- Again, every benefit has a price. The use of this therapy makes the individual more predisposed to autoimmune diseases.

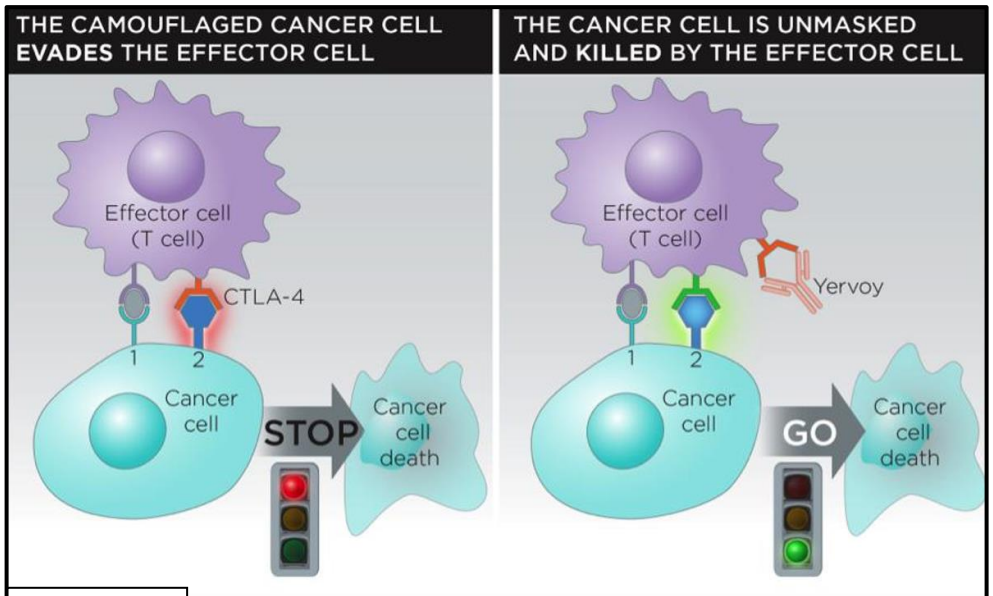


Figure (11)

II. PD-1 targeted immunotherapy

Figure (12)

- Many -not all- tumor cells express PD-L1.
  Similarly, many -not all- T cells express PD-1
  ➔ For the previous reason, histopathologic examination of biopsies from tumors is necessary if we want to use immunotherapy.
  (If the tumor expresses PD-L1 and the immune cells of the patient express PD-1, the patient would be a good candidate for immunotherapy. Otherwise, PD-1 targeted immunotherapy will be completely useless).

- The same principle of CTLA-4 targeted immunotherapy applies here:
  The used monoclonal antibody binds to PD-1 (on the T cell), or to PD-L1 (on the tumor cell), thus preventing the tumor from giving an inhibitory signal to the T cell.
  ➔ T cells are allowed to kill the tumor.

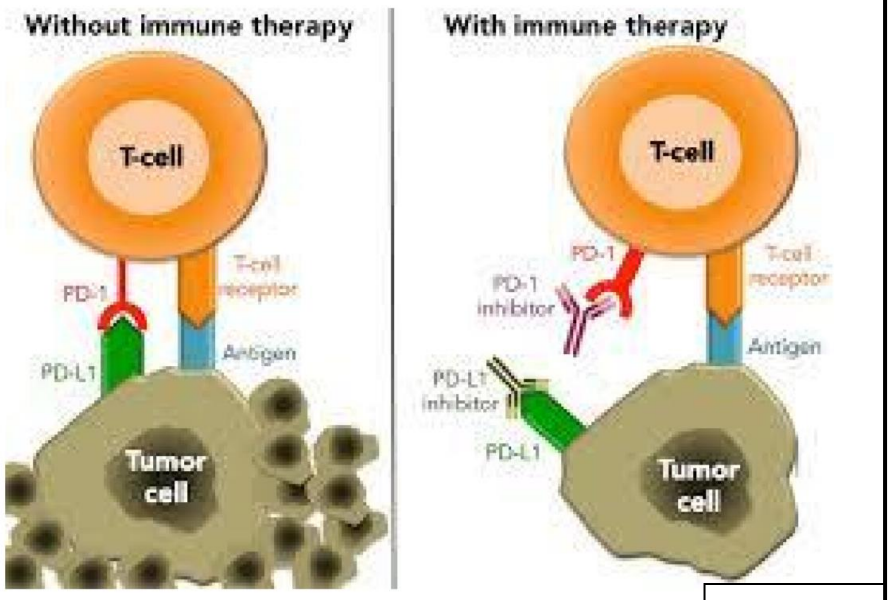


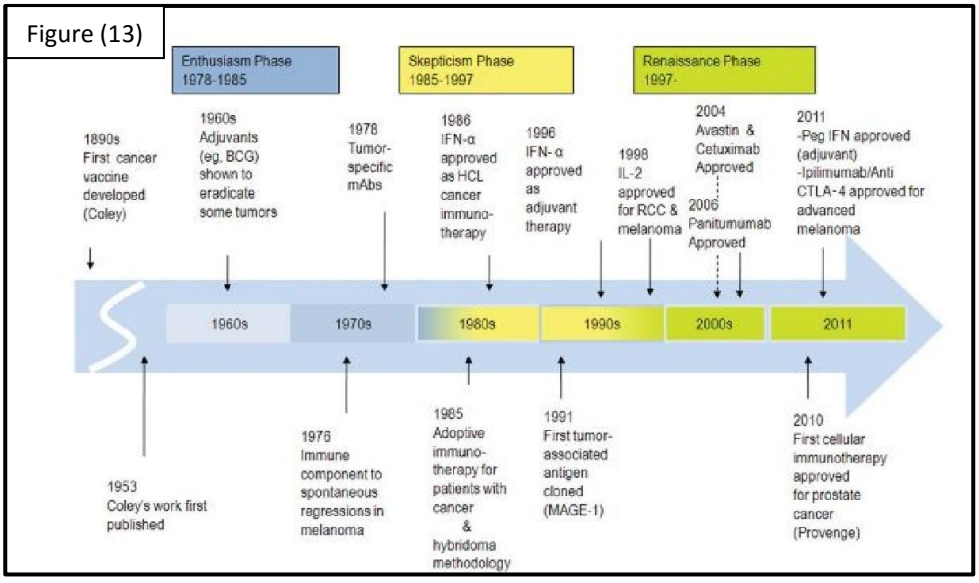
Figure (12)

Note: King Hussein Cancer Center is currently using immunotherapy (CTLA-4 targeted and PD-1 targeted) in treatment of many cancer patients, especially in melanomas, lung cancers, and colon cancers.

III. Cancer immunotherapy over the years *(figure (13))*

- The concept of cancer immunotherapy is not new. In fact, it was introduced many decades ago.
  - In the 19th century, attempts for making cancer vaccines were initiated, but these studies failed.
- In the late 1970s, an **Enthusiasm Phase** began (1978-1985):
  - Scientists found out that immunotherapy has promising potentials. This was due to the observed role of BCG as an adjuvant (booster) of the immune response, in patients with bladder cancer. *(when BCG is given, the immune system becomes more activated and more efficient in fighting cancer).*
  - This discovery lead to more and more clinical trials to enter the "skepticism phase".
- The **Skepticism phase** (1985-1997): big clinical trials were held. Unfortunately, most of them ended with failure.
  - For example, scientists tried inserting certain antigens to activate T cells (or isolate the T cells from the body and activate them against tumor cells and then put them back), but the result was that these cells were not able to kill the tumor.
    Why? Because at that time, the role of PD-1 and CTLA-4 was not yet discovered.
  - At this stage, immunotherapy was considered to be not useful, and the main focus stayed on surgery, chemotherapy, and radiotherapy.

Figure (13)



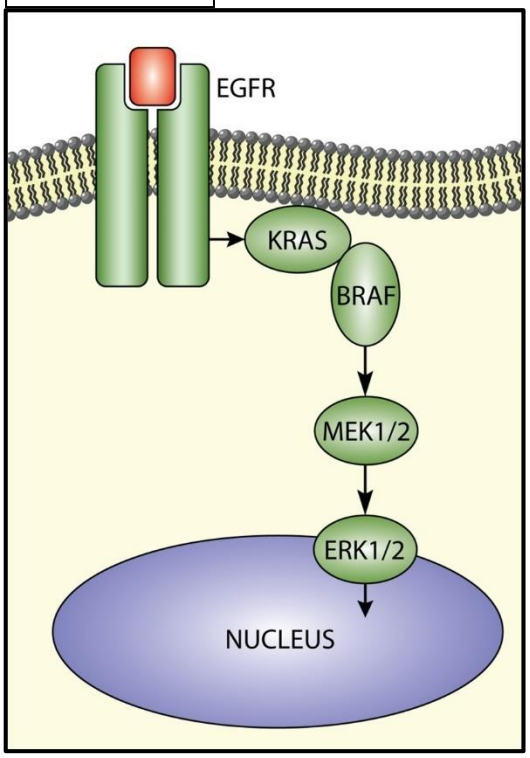
- In the late 90s, the ability of manufacturing monoclonal antibodies represented a breakthrough in the field of immunotherapy, and the "**Renaissance phase**" began.

- The use of monoclonal antibodies played a key role in improving the situation of many cancer patients. For example:
  - In 2004, the use of "Avastin" (a monoclonal antibody against VEGF) was approved.
  - Other examples are Cetuximab and Panitumumab (antibodies against EGFR).

EGFR:

- "Epidermal Growth Factor Receptor" is a receptor present on the cell surface.
- EGFR signaling pathway **promotes cell proliferation**.
  When the receptor binds to its ligand, the cell will proliferate faster through a cascade of signaling molecules that include KRAS and BRAF.
  (extra figure #3)
- *Immunotherapy against EGFR can be useful for treating certain cases of colorectal cancer.*
- For immunotherapy against EGFR to succeed, the downstream molecules (including KRAS and BRAF) must be of the wild type *(not mutated)*.
  Why?
  Because if one of the downstream molecules is mutated (KRAS for example), the cell will continuously divide, even if EGFR engagement to its ligand did not take place, so blocking EGFR will be useless (because EGFR is upstream of the mutated KRAS).
  On the other hand, if the patient's downstream molecules are of the wild type, blocking EGFR will result in stopping the proliferation of the colon cancer cells.

Extra figure #3



- Nowadays, genetic profiling for cancer patients is extremely important.
  (genetic profiling of tumors means sequencing the whole genome of the tumor to reveal the genes that are mutated and the ones that are of the wild type).
  "Every cancer is unique in its mutations and presentation"

  → Accordingly, **personalized cancer therapy** should be used.

❖ Cancer Vaccines

Two main types; preventive and treatment vaccines

I. Preventive vaccines

These vaccines are mainly aggainst viruses that can induce cancer.

Examples : Hepatitis B vaccine, and HPV vaccine.

(A). Hepatitis B vaccine (since 1982) is highly effective.

This vaccine is usually given to indiveduals working in health sectors who are at higher risk of getting the infection compared to others. The vaccine is given not only to prevent cancer, but also to prevent hepatitis B infection in the first place.

(B). HPV vaccine is extremely important in preventing cervical cancer in females.

- Unfortunately, because HPV infection is transmitted sexually, and because the vaccine is more effective when given to females at young ages *(the best age group: 5-18 years)*, HPV vaccine faces a huge resistance from families in Jordan.
  - ➔ Awarness in Jordan should be raised because this vaccine is very important, and at some point sooner or later, this young female will most probably become sexually active when she gets married. So why not protect this female from the posibility of developing HPV-induced cervical cancer through a simple preventive measure which is giving her the HPV vaccine at a young age.
- Nowadays, two companies manufacture the HPV vaccine.
  1) "GSK" produces HPV vaccine against HPV16 and 18 (these two strains are high risk strains that are responsible for more than 70% of incidences of HPV-caused cervical cancer).
  2) "Merck" produces a vaccine that includes also low risk strains; HPV6 and 11 (in addition to the high risk strains).

Question: why do you think low risk strains were included in the vaccine although they do not carry the risk of causing cervical cancer?

Giving the HPV vaccine to males has the same importance as giving it to females (because HPV infection is transmitted sexually).

Males can transmit the HPV infection, but of course without the risk of developing cervical cancer. For this reason, most males reject the idea of taking HPV vaccine that includes the high risk strains (because no benefit will be obtained).

However, since low risk HPV strains are associated with genital warts (in both; males and females), including them in the HPV vaccine encourages men to take the vaccine for the purpose of protection against genital warts. *(it was some sort of bribe for males to start getting vaccinated).*

II. Treatment vaccines

- Treatment vaccines are important to boost the immune system -using cancer antigens- to attack tumors.
- Although they seem useful, treatment vaccines are mostly used in research clinical trials only.
- Recently, the FDA approved Sipuleucel-T for men with metastatic prostate cancer.

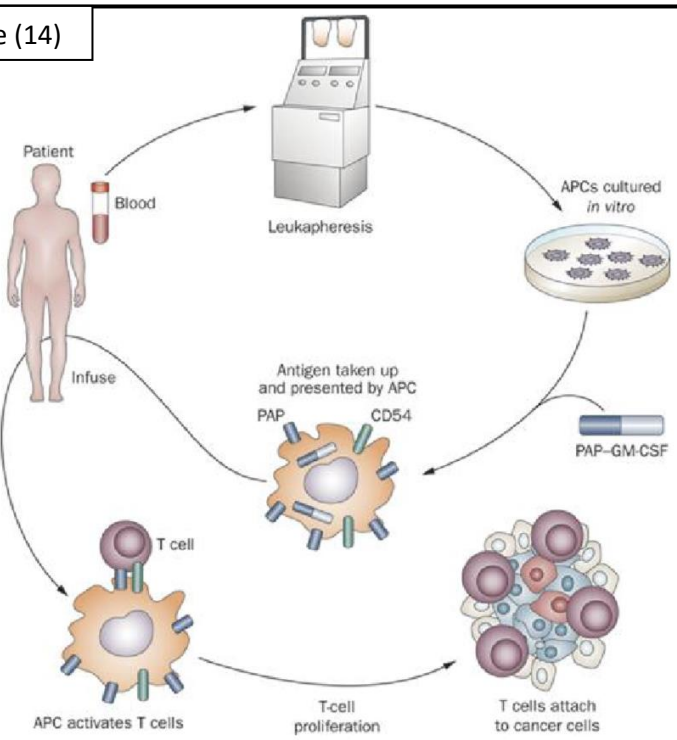
Sipuleucel-T

- It's an example of treatment vaccines for cancer.
- What might be interesting to know is that one of the people who participated in developing this vaccine developed prostate cancer in his lifetime and he used the technique to treat himself. The Sipuleucel-T method succeeded in improving his condition and prolonging survival.

- The treatment method *(see figure (14)):*
  - A blood sample is withdrawn from the patient who has prostate cancer.
  - Using leukapheresis, we separate the WBCs, and isolate the APCs (mainly, the patient's dendritic cells).
  - As we said earlier, the dendritic cells will be paralyzed and not efficient in antigen presentation *(due to the surrounding immuno-suppressive environment that blocks their action).*
  - We culture the dendritic cells in a dish and expose them to a recombinant protein that has two main parts:
    (1) PAP (prostate acid phosphatase): a prostate antigen found in more than 95% of prostate cancers.
    (2) GM-CSF: a growth factor that is important for the proliferation and maturation of dendritic cells.
  - By using this recombinant protein, we will be allowing dendritic cells to become very efficient APCs for the antigens of prostate cancer, and the dendritic cells will be in a high maturity state (ready to enter the body and activate T cells).
  - We inject the dendritic cells back to the same patient:
    →Dendritic cells will activate T cells (helper and cytotoxic T cells).
    →T cells will attack the tumor efficiently and kill it.

- *The use of the same principle for other cancer types is very promising and will probably be seen more often in the future.*

Figure (14)



I apologize for any mistake I made.

Wish you all best of luck :D